Vietnamese Journal of Primatology

Cover: Delacour's langur (*Trachypithecus delacouri*). Photo: T. Nadler

Editorial

Vietnam with its outstanding biodiversity, especially with its unique primate fauna, faces unique pressures and threats to these taxa. These creatures need a special effort to preserve them as a valuable part of nature for the future. It is still uncertain whether the endangered primate species of the country have a chance to survive this century. The endangered primates account for 90% of the 25 primate species that occur in the country. 20% of the world's most endangered primates live in the remaining forested areas of Vietnam. Therefore the country has an extremely high responsibility for the conservation of these species.

Unfortunately, the number of active primatologists in Vietnam is more than small and intense work is necessary to strengthen and invigorate primate protection and conservation work. The *Vietnamese Journal of Primatology* tries to contribute to such activities. After the first publication of the journal in 2007 we now present the fifth issue. During the relatively short period for a scientific publication the series has already gained the attention of the international primatological community. The contributions in the journal reflect the increasing activities on primatological work in Vietnam and also the increasing scientific standard.

Important new findings – including the description of new taxa – are published in the journal. We are grateful to all authors and encourage continuing the work and the publications about new findings, techniques and results.

Since beginning the publication of the journal was supported by the German Primate Center. We are grateful for this support which has also enabled us to provide a number of publications which includes colored printings, not common in scientific primatological journals, which can improve the quality on information.

In our own affair we like to inform that the experienced Vietnamese primatologist Mr. Vu Ngoc Thanh will no longer serve as a co-editor for the journal and cede this position to Mr. Van Ngoc Thinh. Thinh studied at the German Primate Centre where he got his PhD. His dissertation was on gibbons in Indochina and he is now working on a conservation project in central Vietnam.

With the publication of the fifth issue of the journal we complete vol. 1 with an index to support the trace of publications, authors and catch-words.

Delacour's langurs (*Trachypithecus delacouri*) in Van Long Nature Reserve. Photos: Tilo Nadler.

Genetic population structure of the critically endangered Delacour's langur (*Trachypithecus delacouri*) in Van Long Nature Reserve, Vietnam

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Key words: Delacour's langur, *Trachypithecus delacouri*, population genetics, conservation genetics, Van Long Nature Reserve, Vietnam.

Summary

Conservation strategies to sustain patterns of genetic variability can be drawn from analysis of genetic structure, which elucidates connectivity or subdivision between (sub) populations. The critically endangered Delacour's langur (*Trachypithecus delacouri*) is an endemic colobine of Northern Vietnam. The Van Long Nature Reserve (VLNR) holds the largest remaining population of the species and it is the only protected area with an increasing population size. To examine the population genetic structure of *T. delacouri* within VLNR (focus area) and neighboring areas, we collected fecal samples and analyzed the hypervariable region I of the mitochondrial D-loop from four locations. In addition we used already existing Delacour's langur sequences to infer the genetic variation within the focus area in relation to the overall genetic diversity. We identified a low overall genetic diversity and even considerably lower one for the focus area. One haplotype was overrepresented indicating a reduced genetic variation within the focus area. The AMOVA and pairwise Φ_{ST} analyses revealed a significant genetic differentiation among the four subpopulations due to dissection within the focus area. This is corroborated by results of the Mantel test, which indicate an effect of isolation by distance. Although, the neutrality tests to explore the demographic history and the mismatch distribution analysis to test for population stability yielded inconsistent results, neither of them indicated population decline. This study gives a first insight into population structure of Delacour's langurs. Thus, finally we evaluated conservation implications specifically for VLNR with regard to the planned reintroduction project of a langur group from the Endangered Primate Rescue Center (EPRC).

Cấu trúc di truyền quần thể của loài Vọc mông trắng (*Trachypithecus delacouri*), một loài linh trưởng đang nguy cấp tại khu bảo tồn thiên nhiên Vân Long, Việt Nam

Tóm tắt

Những kế hoạch bảo tồn nhằm duy trì sự biến thiên đa dạng di truyền của một loài được thiết lập từ những phân tích về cấu trúc di truyền quần thể, yếu tố dùng để giải thích sự liên kết cũng như sự phân chia giữa các tiểu quần thể. Loài vọc mông trắng (*Trachypithecus delacouri*) là một loài khỉ ăn lá đặc hữu của miền Bắc, Việt Nam. Khu bảo tồn thiên nhiên Vân Long là nơi cư trú của một quần thể vọc mông trắng lớn nhất và có kích thước quần thể đang gia tăng. Nhằm xác định cấu trúc quần thể của loài vọc mông trắng trong khu bảo tồn Vân Long và khu vực lân cận (gọi chung là khu vực trọng tâm), chúng tôi đã thu thập mẫu phân để phân tích yếu tố di truyền ty thể. Bốn vị trí của vùng biến thiên mạnh nhất của gen ty thể D-loop đã được phân tích. Bên cạnh đó chúng tôi dùng trình tự mã gen của loài đã có sẵn từ các nghiên cứu trước để suy luận sự đa dạng di truyền của loài tại khu vực trọng tâm trong mối tương quan với sự đa dạng di truyền chung. Chúng tôi nhận thấy sự đa dạng di truyền chung của loài ở mức thấp và mức thấp hơn ở khu vực trọng tâm. Một haplotype đã được tìm thấy ở tất cả các mẫu chứng tỏ có sự suy giảm tính biến thiên di truyền ở quần thể tại khu vực trọng tâm. Khi dùng phân tích phương sai (ANOVA) và so sách từng cặp đôi, chúng tôi phát hiện sự sai khác có ý nghĩa thống kê về mặt di truyền của bốn tiểu quần thể trong khu vực trọng tâm. Sự chia cắt trong phân bố có thể là nguyên nhân dẫn đến kết quả này. Kết quả này cũng tương quan chặt với test Mantel, thường được dùng để chỉ ra ảnh hưởng của sự phân chia do khoảng cách. Mặc dù vậy, khi kiểm tra bằng test đánh giá lịch sử biến động quần thể và phân tích sự phân bố không tương xứng để đánh giá sự ổn định của chủng quần, chúng tôi thu được kết quả mâu thuẫn với nhau. Cả hai không chứng tỏ được quần thể đang suy giảm. Nghiên cứu này lần đâu tiên giúp nhìn thấy rõ hơn cấu trúc quần thể của loài vọc mông trắng. Chúng tôi cũng đánh giá ý nghĩa về mặt bảo tồn về kế hoạch tái thả các cá thể loài này tại trung tâm cứu hộ thú linh trưởng (EPRC) cho khu bảo tồn thiên Vân Long.

Introduction

Loss of genetic diversity is suggested to increase vulnerability to environmental, demographic and stochastic variation. This adversely affects population persistence, evolutionary potential and individual fitness, and consequently increases the probability of extinction (Garner et al., 2005; Frankham, 2010). A key issue of genetic diversity is gene flow realized by dispersal of individuals across landscapes to maintain the genetic connectivity among populations (Lowe et al., 2004). With regard to the rapidly ongoing habitat destruction, conversion and fragmentation due to human activities, estimates of genetic population structure and connectivity are necessary for the development of efficient conservation plans (Vigilant & Guschanski, 2009). In this context, population genetic methods provide a cost effective means of both delineating geographic patterns of molecular variation and inferring processes that generate and maintain those patterns (Austin et al., 2011).

With 25 primate taxa, Vietnam possesses one of the highest primate diversities in Southeast Asia. However, most of these taxa are threatened by extinction (IUCN, 2010) and five species are already listed among the World's 25 most endangered primates (Mittermeier et al., 2009). Among them, the Delacour's langurs (*Trachypithecus delacouri*), an endemic colobine species of Northern Vietnam, which is restricted to limestone mountain ranges in the provinces Ninh Binh, Ha Nam, Hoa

Binh and Than Hoa (20°-21°N, 105°-106°E) (Nadler et al., 2003) (Fig. 1 and 2). Currently not more than 200 individuals exist in probably 10 small and isolated subpopulations, occupying only 400 to 450 km² (Nadler et al., 2008; Nadler, 2010a) (Table 1).
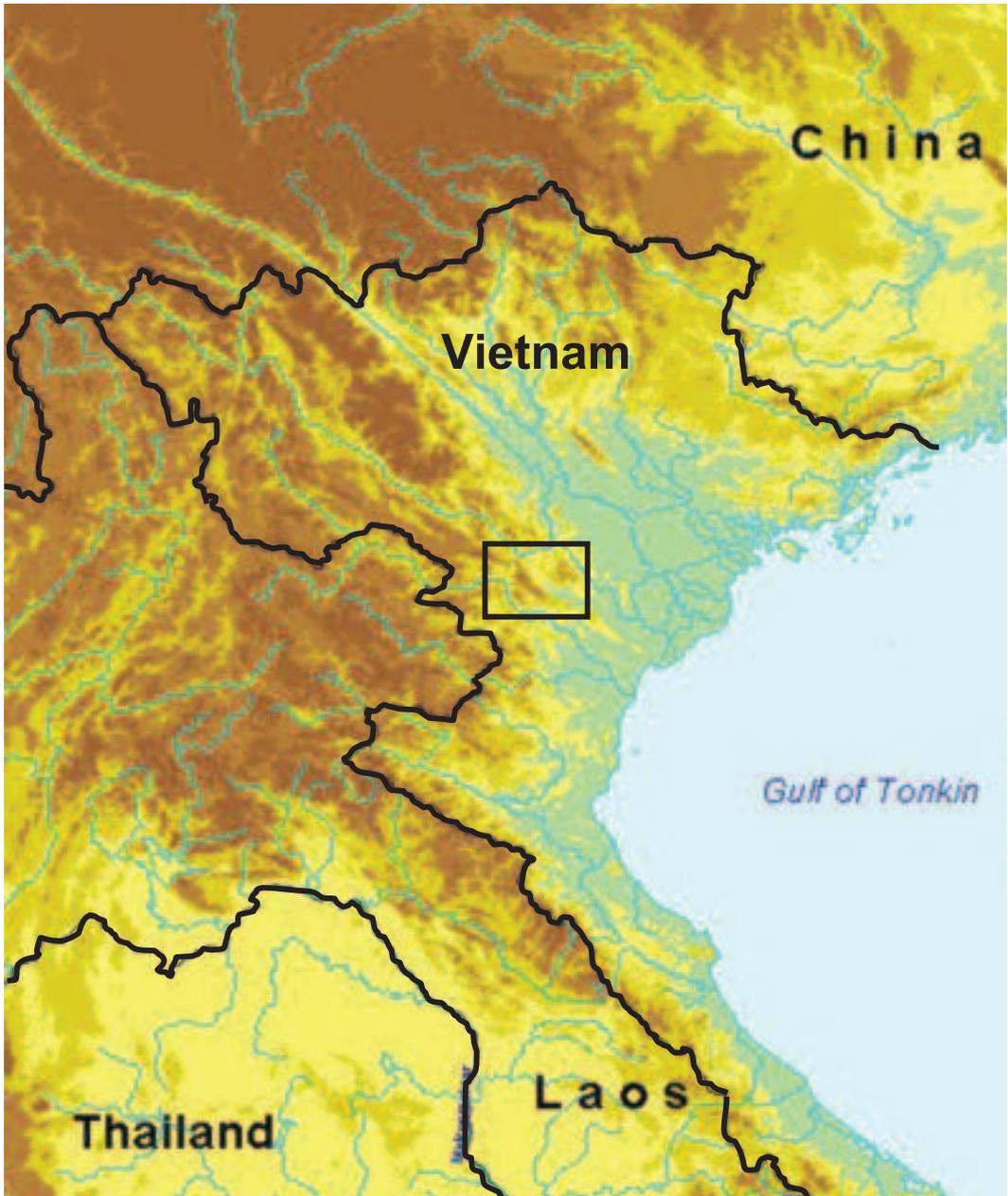
Fig.1. Distribution area of Delacour's langur (*Trachypithecus delacouri*).

Table 1. List of locations of Delacour's langur occurrence and sample sites displayed in Fig. 2.

Location	Site code	Population size / status (Nadler, 2004)	Population size / status and year of record
Hoa Lu Cultural and Historical Site	1	14	< 5 (2009)[2]
Huong Son Mountain (with Chua Thien, CT)	2	27	14-19(2009)[2]
Roc Mountain	3	2	eradicated [3]
Phu Vinh mountainous area	4	15	?
Mai Chau mountainous area	5	15	eradicated [3]
Northern Ba Thuoc mountainous area	6	5	eradicated [3]
Thiet Ong Mountain	7	11	eradicated [3]
Nui Boi Yao mountainous area	8	17-27	?
Thach Than District	9	5	eradicated [3]
Ngoc Son proposed nature reserve	10	9-11	eradicated [4]
Lac Son District, Hoa Binh Province	11	eradicated	eradicated [5]
Ba Thuoc / Cam Thuy Districts, Thanh Hoa Province	12	eradicated	eradicated [5]
Lang Chanh District, Thanh Hoa Province (?[12])	13	eradicated [12]	eradicated [12]
Pu Luong Nature Reserve (north-east & south-west part)	PL	40-45	31-38 (2004)[6]
Cuc Phuong National Park	CP	20-25	8-11 (2008)[7]
Bim Son Mountain	BS	14-18	?
Yen Dong within the Yen Mo limestone mountain ridge	YD	10	4-5(2009)[8]
Kim Bang area - Lac Thuy	KB	20	10-14 (2009)[8]
Van Long Nature Reserve (TOTAL)	VL	(57-67)	(92-105) (2010 / 2011)
Dong Quyen	DQ	40	68-70[9]
Hang Tranh	HT	7	10-15[9]
Gia Vien limestone area (incl. BC, AL, DD, DT)	GV	10-20	-
Gia Vien-Ba Chon	BC		2[10]
Gia Vien-Ao Luon	AL		4[10]
Gia Vien-Dai Dong	DD[1]		8[10]
Gia Vien-Dong Tam	DT[1]		6[11]

[1] belongs to the proposed extension of Van Long Nature Reserve
[2] Le Van Dung & Nadler, 2010
[3] Nadler pers. comm.
[4] Le Trong Dat et al., 2008
[5] Nadler, 2004
[6] Nadler et al., 2004
[7] Luong Van Hao & Le Trong Dat, 2008
[8] Nadler, 2010b
[9] Workman, 2010a
[10] according to own observations and interview information by local people during surveys in 2010
[11] Nadler, pers. comm., and feces samples found during surveys in 2010
[12] the historical occurrence of Delacour's langurs south of the Ma River is not proved, and probably the species never occurred in this area.

Conservation activities with regard to Delacour's langurs, including field surveys, were initiated with the establishment of the Endangered Primate Rescue Center (EPRC) in 1993. The primate
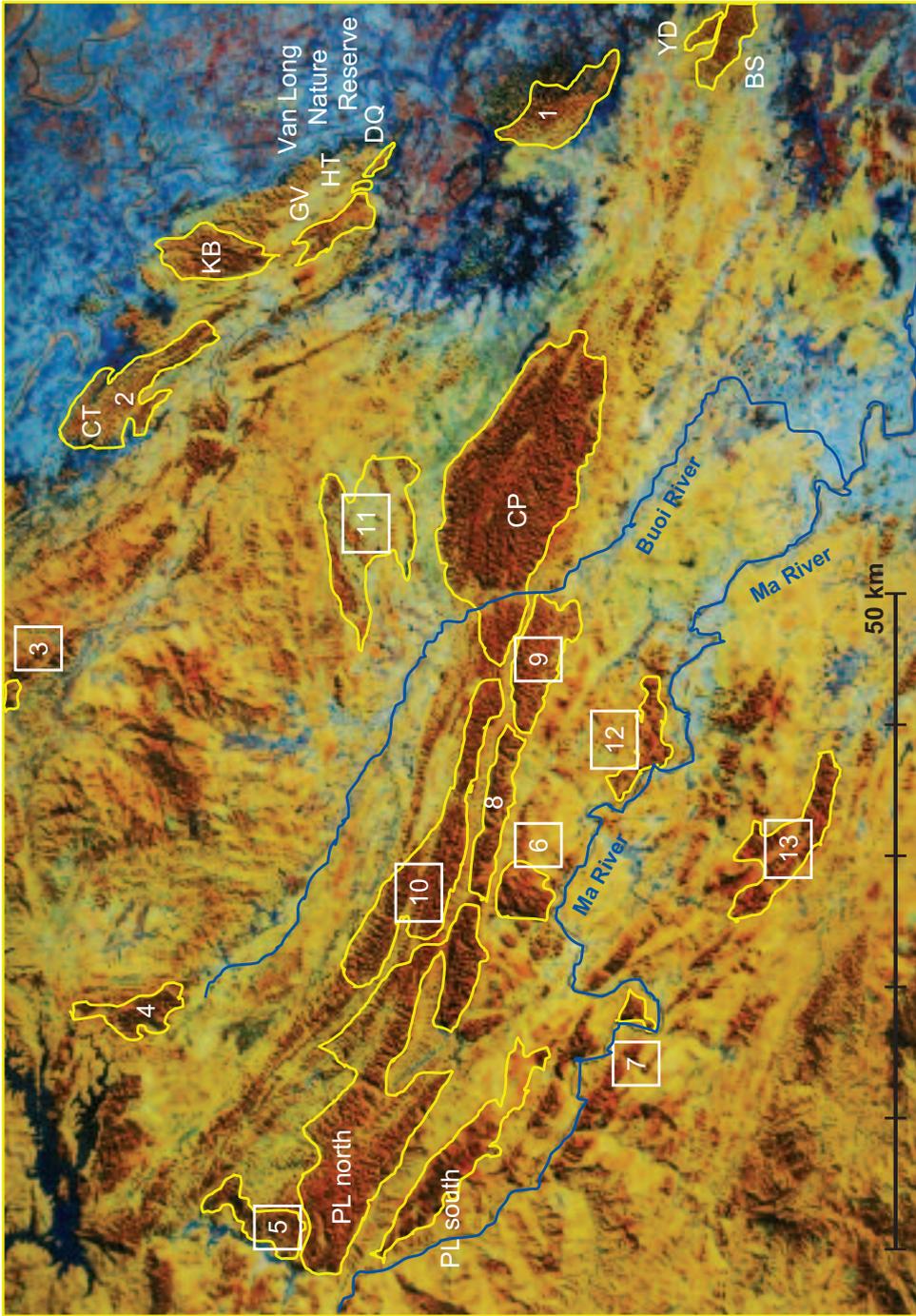
Fig. 2. Overview about the distribution area of Delacour's langurs, showing sites mentioned in the text and listed in Table 1. The species' range is divided in an eastern and a western part by the course of the Buoi River.
Numbers and capital letters represent habitat fragments, where the langurs are expected to occur or are extinct (numbers in square). Locations with capital letters indicate sampling locations: PL (Pu Luong), CP (Cuc Phuong), BS (Bim Son), YD (Yen Dong), CT (Chua Tien), KB (Kim Bang), Van Long Nature Reserve includes GV (Gia Vien limestone area), HT (Hang Tranh) and DQ (Dong Quyen).

center is located in the Cuc Phuong National Park, one of the five reserves where Delacour's langurs occur. The other four reserves are Pu Luong Nature Reserve, Hoa Lu Cultural and Historical Site, Huong Son Cultural and Historical Site, and Van Long Nature Reserve (VLNR). The EPRC is the only facility in the world that breeds Delacour's langurs for reintroduction programs with the aim of sustaining remaining local subpopulations (Nadler, 2004; Workman, 2010a). As a first reintroduction attempt it is planned to release a langur group from the EPRC into the VLNR. The VLNR (Fig. 3) was created in 2001 to protect the habitat of the largest remaining and presumably only viable subpopulation of Delacour's langurs (Nadler 2004; 2010a; Dao Nguyen, 2008). The VLNR-subpopulation at Dong Quyen has increased from 35-40 individuals in 2002 to about 70 individuals in 2008, probably due to an effective conservation management (Nadler 2010a; Workman, 2010a).

Fig.3. Map of the focus area and sampling sites (picture from Google maps, 2011). Black line represents approximately boundary of Van Long Nature Reserve. The focus area comprises subpopulations KB (Kim Bang), DQ (Dong Quyen), HT (Hang Tranh) and GV (Gia Vien limestone area), including BC (Ba Chon), AL (Ao Luon), DD (Dai Dong) and DT (Dong Tam). Colored capital letters display sites from where samples were included in the genetic analyses.

Currently the total population size within VLNR comprises about at least 100 individuals (Table 1). VLNR is dissected into three parts (Nadler et al., 2003; Nadler, 2004) and limestone blasting for cement production restricts habitat use in the eastern part (Nguyen Vinh Thanh & Le Vu Khoi, 2008; Workman, 2010a). It is not known whether genetic exchange still exists among the subpopulations of Delacour's langur of the three parts within the reserve.

The global population of Delacour's langurs dropped from about 300 to 200 individuals within the last decade (Nadler et al., 2003; Nadler, 2010a). The situation would be even worse without the increase of the subpopulation in VLNR. The situation can also deteriorate here if tourism and cement production will increase in the near future as it is planned. A proper management plan for the reserve and the langurs is therefore indispensable. Population genetic data can contribute here substantially, in particular in relation to the proposed reintroductions. Moreover, accurately quantifying genetic diversity is crucial for assessing the viability of small populations in order to design conservation plans to improve genetic diversity (Shirk & Cushman, 2011). However, such data are not available. To attenuate this data deficiency we conducted the first population genetic analysis of Delacour's langurs.

Materials and Methods

Study site

The VLNR (20°21'-20°26'N, 105°48'-105°55'E) (Fig. 3) is located in the Gia Vien District along the northeastern border of Ninh Binh Province, about 85 km from Hanoi. The reserve comprises around 2,600 ha and consists of two major geographic features, karst limestone hills and wetland (Dao Nguyen, 2008). The dominant vegetation on karst is a mixture of mostly evergreen and some deciduous forest consisting of dense irregular patches of trees, shrubs and herbs (Nisbett & Ciochon, 1993; Workman, 2010b).

The VLNR consists of three parts (Fig. 2 and 3): Dong Quyen (DQ) forms the southeastern part of the reserve and to three quarters surrounded by water. DQ is connected with Hang Tranh (HT) only by an 80 m long dam, which the Delacour's langurs avoid to cross, and thus constitutes a barrier between DQ and HT. HT is a small isolated limestone ridge which represents the middle part of VLNR and which is separated by a small unpaved road (between open rock areas degraded vegetation) from the larger western part, Gia Vien limestone area (GV), in particular from Ba Chon (BC), the highest peak inside the nature reserve. The western part of VLNR also includes Ao Luon (AL). Moreover, there are two more forest areas adjacent to the reserve, Dai Dong (DD) and Dong Tam (DT). DD is connected to the Kim Bang (KB) area. However, both are isolated from each other through an unpaved road and human settlements, and are not protected. With the planned extension of the nature reserve Dong Tam will be included.

Sample collection

From March to July 2010, we collected fecal samples for genetic analysis. We mainly focused on the less known western area of the VLNR and adjacent forest regions (DD and DT). We also visited the Kim Bang area (KB) and the Chua Tien (CT) area, which is part of the Huong Son mountain region (Fig. 2). Certain areas were repeatedly visited (HT, BC, AL, DQ, DD, DT). Most samples were collected at langur sleeping sites and places where they were directly observed. Often fecal material was old, dry and indistinguishable from feces of sympatric Assamese macaques (*Macaca assamensis*). We collected 119 fecal samples at several sites of VLNR and neighboring areas (DD and DT), 9 samples at one location in KB, 1 sample in CT and 7 samples at each of two locations in the Bim Son (BS) and Yen Dong (YD) mountain ridges, adding to a total of 143 samples. Samples from the latter two locations were collected by a local research assistant during additional surveys in December 2010. All fecal samples were stored in 90% ethanol or by applying the two-step storage method by Nsubuga et al. (2004).

Laboratory methods

DNA from the fecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) following the given protocol with slightly modifications. Approximately 200 mg of feces were taken and incubated over night in ASL buffer. In the last step, DNA was diluted in HPLC water instead of AE buffer. The hypervariable region I (HVRI, 390 bp) of the mitochondrial D-loop was PCR-amplified with primers 5'-AACTGGCATTCTATTTAAACTAC-3' and 5'-ATTGATTTCACGGAGGATGGT-3' using standard hotstart procedures. PCR conditions were as follows: pre-denaturing at 94°C for 2 min, 50 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min, plus a final extension at 72°C for 5 min. Negative (water) controls were used to check PCR performance and contamination. PCR products were separated on a 1% agarose gel and excised from the gel. PCR products were purified with the standard silica method and sequenced on an ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems) with the BigDye Cycle Sequencing Kit (Applied Biosystems). PCR amplifications were performed at least twice and sequencing was done in both directions to exclude PCR or sequencing errors.

Data analyses

Sequences were checked in 4Peaks (Griekspoor & Groothuis, 2005) and manually aligned in SeaView (Gouy et al., 2010). Haplotype determination was performed in FaBox (Villesen, 2007). We

expanded our data set with already existing sequences from 43 Delacour's langurs (Table 2) and each one of the related species *T. laotum*, *T. francoisi*, *T. poliocephalus* and *T. leucocephalus* deposited at the Indochinese Primate Conservation Genetics Project at the German Primate Centre.

Because the sample sizes of most areas were small and not sufficient to allow population structure analyses (e.g., Pu Luong (PL) with only one sample), the data analyses were performed either for the whole Delacour's langur dataset or for the dataset from only the focus area (KB and VLNR) (Fig. 3).

Genetic variability was estimated by means of haplotype diversity (h) (Avise, 2004; Frankham et al., 2010), nucleotide diversity ($\checkmark$), as average number of nucleotide differences per site between two randomly chosen DNA sequences (Nei & Li, 1979; Nei & Tajima, 1981) and the mean number of pairwise nucleotide differences (k) (Tajima, 1993) using Arlequin 3.5 (Excoffier & Lischer, 2010). Arlequin was further used to perform an analysis of molecular variance (AMOVA; Excoffier et al., 1992) to investigate how genetic variation is distributed among the subpopulations of the focus area. Subpopulations were defined according to Nadler et al. (2003). ſST values among each pair of subpopulations were calculated based on the Tamura-Nei genetic distance method in Arlequin. Statistical significance was assessed with 1,000 permutations.

To depict haplotype relationships and explore the association of haplotype groups with geography, a haplotype network was constructed using minimum spanning tree analysis in Arlequin and HapStar (Teacher & Griffiths, 2011). A Mantel test (Mantel, 1967) was performed to test for isolation by distance (Wright, 1943) within the focus area. Therefore, the correlation between genetic and corresponding linear geographic distances was compared (999 permutations) in GenAlEx 6.4 (Peakall & Smouse, 2006).

To infer the population history of the focus area, the neutrality test was applied to calculate Fu's FS (Fu, 1997) and Tajima's D (Tajima, 1989) in DnaSP 5.0 (Librado & Rozas, 2009) and Arlequin. A mismatch distribution performed in DnaSP was used to test for population stability (or decline) in the focus area.

Divergence ages among haplotypes of *T. delacouri* were estimated with BEAST 1.4.8 (Drummond & Rambaut, 2007). Therefore, a Bayesian MCMC method was used, which employs a relaxed molecular clock approach (Drummond et al., 2006). Furthermore, a relaxed lognormal model of lineage variation and a Birth-Death prior for branching rates was assumed. Since no appropriate fossils are available, we calibrated the molecular clock with divergence ages among limestone langurs as estimated from genetic data (Perelman et al., 2011). The first was the split between the outgroup *T. laotum* and the remaining species 850 thousand years ago (kya) (95% credibility interval: 430-1270 kya) and the second, the split between *T. delacouri* and the clade consisting of the northern limestone langur representatives (*T. francoisi*, *T. poliocephalus*, *T. leucocephalus*) 64 kya (95% credibility interval: 290-990 mya). One replicate was run for 10,000,000 generations with tree and parameter sampling occurring every 1,000 generations. Subsequently, the consensus tree was generated with TreeAnnotator 1.4.8, setting a burin of 25%, a posterior probability of 0.5 (according to 50% majority rule) and a mean node height.

Results

Sequence quality and diversity

We successfully sequenced the HVI region of the mitochondrial D-loop from 42 out of 143 recently collected samples. All other samples revealed no amplification most likely because they were too old, not species-specific or amplified fragments, which turned out to be presumably

pseudogenes. In total, 85 Delacour's langur sequences were available for this study. A sample list with distribution and frequency of haplotypes in the nine sampling sites is summarized in Table 2.

Table 2. List including sampled localities, and the number of sequences and haplotypes. ([1]Sequences from these locations represent the focus area).

Location	N	E	sequences	number of haplotypes	haplotypes (frequency)
Van Long					
Hang Tranh[1]	20°24'	105°52'	20	3	D(16); F(3); G(1)
Dong Quyen[1]	20°22'	105°53'	36	1	D(36)
Ao Luon[1]	20°24'	105°51'	9	2	A(8); F(1)
Dong Tam[1]	20°27'	105°49'	2	2	A(1); F(1)
Kim Bang	20°30'	105°51'	3	1	F(3)
Bim Son	20°04'	105°57'	3	2	D(1); I(2)
Yen Dong	20°05'	105°56'	3	1	H(3)
Cuc Phuong	20°19'	105°37'	3	1	E(3)
Pu Luong	20°32'	105°06'	1	1	B(1)
unknown			5	3	A(1); C(3); D(2)
total			85	9	

For the entire data set, nine haplotypes were found based on 19 polymorphic sites, including only transitions (Table 3). The overall haplotype diversity with h=0.576 is moderate, and the nucleotide diversity with π=0.009 as well as the mean number of pairwise nucleotide difference between sequences (k=3.366) are remarkably low. Although, the focus area comprises 82% of the total sequence data, diversity indices within this region are only around one third to two third as large as for the overall data set (h=0.424; π=0.003; k=1.224). Haplotype D is found with higher frequency in the overall sample set (64%) and is six times as frequent as haplotype A, the second most frequent.

Table 3. Population genetic variables for the whole data set and specifically for the focus area (a), and neutrality test and parameter of mismatch distribution analysis for the focus area (b).
(r: raggedness index; Parameters of demographic expansion: θ_0: population size before expansion; θ_1: population size after expansion; τ: age of expansion in units of mutational time.

	all data	focus area
a)		
sequences	85	70
nucleotide sites	390	390
polymorphic sites	19	7
haplotypes	9	4
haplotype diversity (h±SD)	0.576 ± 0.059	0.424 ± 0.066
nucleotide diversity (π±SD)	0.009 ± 0.005	0.003 ± 0.002
mean no. of pairwise differences (k±SD)	3.366 ± 1.743	1.224 ± 0.788
b)		
Tajima's D (p value)		-0.584 (0.329)
Fu's FS (p value)		1.488 (0.795)
r (p value)		0.401 (0.595)
θ_0		0.857
θ_1		1000
τ		0.254

Genetic structure and demographic history

The AMOVA revealed a relatively high, albeit significant, genetic differentiation among the subpopulations of the focus area with an overall Φ_{ST} value of 0.66 (p<0.001). This indicates that for these four sites around 66% of the mitochondrial variation can be attributed to variation among subpopulation. However, an appreciable amount of variation was also found among individuals within subpopulations (34%) (Table 4).

Table 4. AMOVA (based on Tamura-Nei genetic distance) of the 70 HVRI sequences of the focus area. (Φ_{ST} = 0.664 (p<0.001).

source of variation	d.f.	sum of squares	variance components	percentage of variation
among subpopulations	3	24.345	0.536 Va	66.40
within subpopulations	66	17.898	0.271 Vb	33.60
total	69	42.243	0.807	

The pairwise Φ_{ST} values ranged between 0.16 and 1.00 and all values are statistically significant (p<0.05) indicating that the subpopulations are genetically distinct from each other in varying degree (Table 5). Thereby the lower pairwise Φ_{ST} displays a higher amount of haplotype sharing between two neighboring subpopulations, e.g., Dong Quyen (DQ) shares its only haplotype with Hang Tranh (HT) (Φ_{ST}=0.16), but no haplotype with KB (Φ_{ST}=1.00). A Mantel test yielded a significant positive correlation between genetic and geographical distance within the focus area (Rxy=0.508; p<0.01; data not shown).

Table 5. Pairwise Φ_{ST} estimates (based on Tamura-Nei genetic distance) among the subpopulations of the focus area (DQ (Dong Quyen), HT (Hang Tranh), GV (Gia Vien limestone area) and KB (Kim Bang)). Φ_{ST} values are below diagonal and significance values are above diagonal (*p<0.05, **p<0.01).

	DQ	HT	GV	KB
DQ	—	**	**	**
HT	0.156	—	**	**
GV	0.887	0.516	—	*
KB	1.000	0.642	0.692	—

The haplotype network (Fig. 4) shows a relatively high genetic differentiation between all sampling localities and a more moderate one for the focus area. Geographically, neighboring subpopulations have similar haplotypes (e.g., DQ and HT), separated by two to six substitutions. Similarly, haplotype E (belonging to Cuc Phuong, CP) is closely related to the haplotypes of VLNR.

There is no evidence for population expansion (or decline) within the focus area due to non-significant and near zero results of neutrality tests (Tajima's D=-0.584; p=0.329 and Fu's F_S=1.488; p=0.795) (Table 3). Thus the hypothesis of demographic stability cannot be rejected. Whereas the pattern of the mismatch distribution (Fig. 5) may be indicative for population growth, resulting from a non-significant value of the raggedness statistic (r=0.401, p=0.595) (Table 3).


Fig.4. Minimum spanning tree of all Delacour's langur HVRI sequences. Each node corresponds to one mutation step. Letters represent the given haplotypes and sizes of the circles are proportional to haplotype frequencies. Each haplotype pie chart is color coded according to the proportion found in each sampling region depicted in Fig. 2 and 3. For abbreviations see Table 1.

Fig.4.
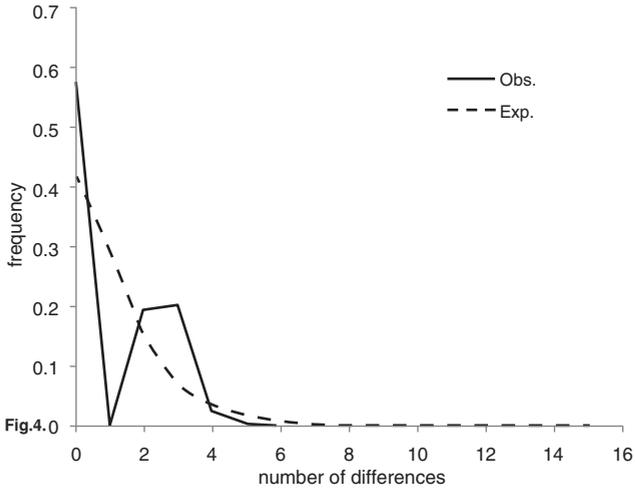
Fig.5. Mismatch distribution of Delacour's langur HVRI sequences of the focus area as determined with DnaSP. Frequency of pairs of DNA sequences plotted over the number of nucleotide site differences.

Divergence times

The estimated divergence time between the two major haplogroups (haplotypes B and C) and (haplotypes A, D, E, F, G, H and I) is 110 thousand years ago (kya) (95% credibility interval (CI)=20-240 kya). This split is temporally similar to the split between *T. francoisi* and *T. leucocephalus* about 130 kya (95% CI=20-260) (Table 6). The divergence between haplotypes B and C occurred 30 kya (95% CI=7-60) and was therefore more recent than between haplotypes G and the remaining haplotypes (A, D, E, F, H and I) within the second haplogroup (60 kya; 95% CI=9-130). The most recent splits are between haplotypes E and H and D and I.

Table 6. Bayesian divergence date estimates in kya. Means and 95% credibility intervals are given. Haplotypes A-I are identical with those of the haplotype network (Fig. 3). ([1]used as calibrations; [2]northern clade consisting of the northern limestone langur representatives: *T. poliocephalus*, *T. francoisi* and *T. leucocephalus*).

	mean [kya]	95% credibility interval (CI) [kya]
T. laotum / other[1]	520	140-870
T. delacouri / northern clade[1*2]	420	100-750
T. poliocephalus / *T. francoisi*+*T. leucocephalus*	200	40-390
T. francoisi / *T. leucocephalus*	130	20-260
B+C / other haplotypes	110	20-240
B / C	30	7-60
G / A+D+E+F+H+I	60	9-130
E / H	9	1-30
D / I	6	0-20

Discussion

Sampling strategy and limitations

Although the majority of colobines have home-ranges of less than 1 km^2 (Newton & Dunbar, 1994). Workman (2010a) observed an average day path length of only 476 m for Delacour's langurs in Dong Quyen. It was difficult to detect them and even more challenging or even impossible to follow them in the karst forests. Therefore, it is also difficult to collect fresh fecal samples. Promising places to find samples might be sleeping sites of the langurs, however, to locate and to reach them is also highly demanding. Hence, most of the samples we collected consisted of old and dry fecal material, and thus, we concentrated on an mtDNA marker, because even low quality samples normally yield enough template DNA for molecular studies.

Genetic diversity, population structure and demographic history

Genetic variation in wildlife is related to population size, and hence species consisting of small populations are suggested to have less genetic variation (Frankham, 1996). Not surprisingly, the overall genetic diversity of Delacour's langurs with a nucleotide diversity of π=0.009 and a haplotype diversity of h=0.576 is relatively low even in comparison to other species with relative small populations, e.g., *Rhinopithecus bieti* (population size≈2000, π=0.036, h=0.945) *R. brelichi* (population size≈800 π=0.014, h=0.457) (Yang et al., subm.) or *Brachyteles hypoxanthus* (population size≈900, π=0.181, h=0.609) (Fagundes et al., 2008). Low nucleotide diversities were also found in island populations as e.g. the Japanese macaques (*Macaca fuscata*) on Yakushima Island (population size=210-388 π=0.002, Hayaishi & Kawamoto, 2006).

In addition, Garner et al. (2005) suggested consistent reduction in genetic diversity in populations that had experienced a demographic bottleneck. Due to the human-induced habitat reduction, fragmentation and strong hunting pressure, it can be assumed that the remaining Delacour's langurs are the survivors of a bottleneck as demonstrated for the orangutan population decline in Eastern Sabah (Goossens et al., 2006). However, the inconsistent results revealed by analyses of the demographic history of the subpopulations within our focus area do not support this assumption, rather indicating population stability. Presumably the very recent expansion in Dong Quyen interfered with the strong overall population decline before, leading to incongruent pattern of the demographic history. Currently Dong Quyen harbors the lion's share of individuals within the focus area, but it consists only of one mtDNA haplotype. This can be expected if a small founder population increased recently without genetic exchange with neighboring populations.

Population structuring (Φ_{ST}=0.66) and an effect of isolation by distance were found within the focus area. Due to maternal inheritance of mtDNA, high levels of mtDNA population subdivision are often the product of female philopatry. Whether males migrate today from one to another subpopulation remains unknown. Before the road between Hang Tranh and Ba Chon was widened, Delacour's langurs were observed crossing the road (Nadler et al., 2003) indicating gene flow among subpopulations in the recent past. Genetic clustering would be assumed for the subpopulations Hang Tranh and Dong Quyen due to the relatively low, albeit still significant, pairwise Φ_{ST} value as it is the case in the study on Bornean orangutans of Arora et al. (2010) and because, Dong Quyen has only one haplotype, which is shared with Hang Tranh. Since it is not clear if Delacour's langurs are able to cross the dam, which constitutes the only connection between the two areas (Nadler et al., 2003), for conservation reasons, Dong Quyen should be treated as an isolated subpopulation.

Implications for conservation

Sodhi et al. (2010) proposed where possible, reforestation, reintroductions and re-establishing connections between severed habitats should be attempted. A first step in case of Delacour's langurs is the planned reintroduction of a group from the EPRC in VLNR. In order to evaluate the feasibility of translocation projects without intervention in the evolutionary process of a species it is indispensable to consider the balance between the risk of outbreeding depression and benefits (higher genetic diversity and fitness) accruing from hybridizing populations (Frankham et al., 2010). Our genetic data provide first insight into the spatial structure of genetic variability and a hint for unproblematic genetic exchange. A geographical subdivision of populations into a western (Pu Luong) and an eastern part (all remaining subpopulations) of the species' range becomes apparent

from the haplotype network and the divergence time estimates, with the Buoi river most likely constituting a dispersal barrier (Fig. 1). Thus, at least the populations within the eastern part of the species' range seem to be small remains of a historically panmictic population as it was suggested for the situation of the Northern Muriqui (Chaves et al., 2011). There a low nucleotide diversity was detected and most haplotypes differed from their nearest neighbor by only one mutation. Such a situation would make translocations within the eastern range of the Delacour's langurs less problematic, especially since the selected individuals for the planned reintroduction consists of descendants of the populations Cuc Phuong and Dong Tam. Given the low genetic diversity within VLNR, the reintroduction will increase the genetic variability as well as the population size, reducing inbreeding and improving the ability of the population to adapt genetically to changing environments (Frankham, 1996). In view of the fact that the current population size is very small and most of the populations consist of only a few individuals without an opportunity for long-term persistence, it should also be considered to support translocations among the eastern and the western part. In particular, when there are larger contiguous forest areas and a better protection within reserves and national parks.

Beside population management by reintroduction, the establishment of migration corridors between the three parts of VLNR would certainly improve the situation as well (Nadler et al., 2003). Moreover, the largest threat to Delacour's langurs today, the quarrying of limestone for cement productions, should be reduced and places for quarrying carefully selected that they do not interfere with Delacour's langurs protection. Especially, stopping the quarrying directly next to VLNR is of particularly interest due to its negative impact on habitat use of the species (Nguyen Vinh Thanh & Le Vu Khoi, 2008; Workman, 2010a). Finally, we suggest an extension of VLNR by including the neighboring areas Dai Dong and Dong Tam.

Acknowledgments

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Mother-infant relationships and infant development in captive grey-shanked douc langurs (*Pygathrix cinerea*)

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Summary

An infant's relationship with its mother influences the development of social bonds throughout the rest of the infant's life, and can have a significant effect on its long-term fitness. The majority of research conducted on this relationship has focused on a limited range of primate taxa. In this study, I observed mother-infant relationships in three dyads of grey-shanked douc langurs (*Pygathrix cinerea*), a critically endangered colobine that is endemic to Vietnam. Such a study broadens our understanding of social relationships amongst primates, which are known to vary significantly among taxonomic groups, and provides information that can improve captive management and potential reintroduction of grey-shanked douc langurs. The three infants varied in age from 7 weeks to 25 months. The two older infants spent significantly more time further away from their mothers than the 7-week old, although all three infants spent a similar amount of time nursing. The majority of the older infants' contact time with the mothers was used for nursing, whereas the 7-week old infant engaged in other activities such as climbing upon and holding onto his mother. There was also evidence of weaning conflict in the oldest infant, who was rejected by his mother more often than the younger two infants. Also, the effect of living without any other infants or juveniles manifested itself in attention-seeking behaviours, such as grabbing at the face and body of the mother, which was particularly prevalent in the middle-aged infant. Suggestions for future studies and their applications to the conservation of grey-shanked douc langurs are discussed, with an emphasis on the potential importance of captive breeding and reintroductions to their survival as a species.

Mối quan hệ Mẹ-Con và sự phát triển của con non trong điều kiện nuôi nhốt ở loài chà vá chân xám (*Pygathrix cinerea*)

Tóm tắt

Mối quan hệ của con non và mẹ có ảnh hưởng quan trọng đến sự phát triển mối quan hệ xã hội của con non trong cuộc đời chúng, và có thể ảnh hưởng đáng kể đến sức khỏe lâu dài của chúng. Trước đây có nhiều nghiên cứu về mối quan hệ này, tuy nhiên chỉ tập trung vào một số loài nhất định. Trong nghiên cứu này, tôi đã quan sát ba cặp mẹ và con của loài chà vá chân xám, một loài khỉ ăn lá đặc hữu của Việt Nam. Nghiên cứu này đã góp phần mở rộng những hiểu biết của chúng ta về mối quan hệ xã hội của các loài thú linh trưởng vốn được biết đến là rất đa dạng. Nghiên cứu cũng đã cung cấp những thông tin cần thiết nhằm nâng cao chất lượng chăm sóc, quản lý các cá thể nuôi nhốt và việc tái thả các cá thể này vào tự nhiên. Ba cá thể con non từ bảy (7) tuần tuổi đến 25 tháng tuổi đã được quan sát. Kết quả cho thấy, hai cá thể nhiều tuổi hơn thường xuyên rời xa mẹ hơn là cá thể 7 tuần tuổi, cho dù cả ba cá thể có

cùng khoảng thời gian bú mẹ. Phần lớn thời gian hai cá thể nhiều tuổi hơn bên mẹ là dành cho việc bú, trong khi cá thể 7 tuần tuổi thường xuyên leo trèo và ngồi trong lòng mẹ. Nghiên cứu này đã thu thập được bằng chứng cho thấy mối xung đột giữa con non lớn nhất và mẹ của nó trong quá trình cai sữa. Đồng thời nghiên cứu cũng ghi nhận các tập tính như cào mặt và cơ thể con mẹ nhằm lôi kéo sự chú ý ở cá thể con non lớn thứ hai. Đây có thể là ảnh hưởng của việc con non phải sống một mình mà không có những cá thể anh em khác. Những gợi ý cho việc tiếp tục nghiên cứu trong tương lai và vận dụng những kết quả vào công tác bảo tồn loài chà vá chân xám sẽ được thảo luận, đặc biệt là chương trình nhân nuôi và tái thả.

Introduction

The relationship between an infant and its mother is one of the strongest bonds in social mammals (Mateo, 2009). It is critical to the infant's immediate survival, and shapes many of its future relationships, which can have a significant effect on long-term fitness (Altmann, 1980; Förster & Cords 2002; Hinde & Spencer-Booth, 1967; Horwich, 1974; MacKinnon, 2007). Primates have a longer period of infancy than all other mammals of similar size (Pereira & Fairbanks, 1993), and rely on their mothers not only for nutrition and protection, but also for transport (Strier, 2000). The mother-infant relationship is therefore particularly influential in a primate's development, and it has been an important focal point in the study of social behaviour.

Many external factors can influence mother-infant relationships, such as group composition and the physical environment, as can life history characteristics such as the length of infancy (Förster & Cords, 2002; Maestripieri, 2009; Nicolson, 1991). The relationship between infants and mothers also varies with taxonomy and body size (Nicolson, 1991; Strier, 2000), and therefore broad generalizations across distant taxa are unreliable. Research on infant development in primates has been biased toward macaques, baboons, and vervet monkeys (Maestripieri, 2009). Aside from the work of Dolhinow on *Semnopithecus* (e.g. Dolhinow 1982; Jay 1965), Asian colobines are a group of primates that have received less attention. Furthermore, few studies of colobine infants have tracked their subjects through to social independence. Instead, many end within the first year of the infant's life, at which point locomotor and nutritional independence has been achieved, but rarely social independence (e.g. Brent et al., 2008; Horwich and Manski, 1975; Rapaport & Mellen, 1990).

This study explores the mother-infant relationship of the grey-shanked douc langur (*Pygathrix cinerea*), an Asian colobine that inhabits evergreen forests in central Vietnam. Although their relationship to other langurs is questioned, doucs are referred to as douc langurs in this paper because this is the term that is most familiar to individuals outside the fields of primatology and systematics. The grey-shanked douc langur was only recently identified (Nadler, 1997), and very little is known about its behaviour and ecology. Research on the grey-shanked douc langur will not only help to fill our current gap in knowledge of infant development in colobines, but is also important for improving our broader understanding of the behaviour of this species. Grey-shanked douc langurs are classified as Critically Endangered (IUCN 2010), and face serious threats from deforestation and hunting (Nadler et al., 2003). Research on their behaviour and life history is necessary to make population viability assessments, and is therefore critical to long-term conservation efforts.

The total population of grey-shanked douc langurs is estimated at fewer than 1000 individuals (Ha Thang Long, 2007). In addition to being rare, douc langurs are almost entirely arboreal, and prefer the upper canopy (Lippold, 1998), making them very difficult to observe in the wild. Studies of captive animals are often used in behavioural research, as they allow for a controlled

environment and remove the difficulties associated with tracking and watching animals in the field. Douc langurs are difficult to maintain in captivity, largely because of their specific dietary needs (Hick 1972; Hill 1972). The Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park, Vietnam, is currently the only facility in the world that houses grey-shanked douc langurs. Most individuals at the EPRC were confiscated from poachers, and in recent years they have been bred at the center. The goal of the center is to reintroduce endangered Vietnamese primates back into their natural habitat. Studies on the social behaviour of these animals may improve the chances of successful reintroductions. A better understanding of infant development, for example, can help us ascertain how best to reintroduce family groups with young offspring. These studies will also help determine the most suitable group sizes and compositions for captive douc langurs, and may inform other colony management decisions such as when to separate offspring from their parents.

I observed the frequency of a wide variety of behaviours (see Appendix) of three mother-infant pairs of grey-shanked douc langurs at the EPRC over a period of six weeks. I also tracked the spatial relations of infants and their mothers. This is the first study on mother-infant relationships in this species, and I compare the observed behavioural patterns to those of other Asian colobines. I also address the potential conservation applications of these results.

Methods

Subjects

From June 23rd to July 25th, 2010, I observed three captive mother-infant dyads of grey-shanked doucs at the EPRC (see Table 1 for information on individuals). The three mothers had been confiscated from poachers, while their infants were born at the center. The three infants were 1.75 months, 15 months and 25 months old at the start of the study. The precise dates of birth for the mothers are unknown.

Table 1. I.D., sex, age, and source of grey-shanked douc mothers and infants at EPRC.

I.D.	Infant Sex	Date of arrival at EPRC (m/d/y)	Birth date (m/d/y)	Age at start of study	Source
Mot1	M	10/19/2006	?/?/2000	10 years	Confiscated
Inf1		05/06/2010	05/06/2010	1.75 months	born EPRC
Mot2	F	11/09/2005	?/?/?	?	Confiscated
Inf2		03/08/2009	03/08/2009	15 months	born EPRC
Mot3	M	12/15/2001	?/?/1997	13 years	Confiscated
Inf3		05/05/2008	05/05/2008	25 months	born EPRC

The study animals lived as family units (parents plus infant) in separate outdoor cages. The Mot1/Inf1 family included a male juvenile (born 10/10/07) that was an older full sibling of Inf1. Each 10 m x 5.5 m x 3.5 m cage was fully enclosed with wire mesh fencing (Fig. 1). The cage floor was concrete, above which three levels of bamboo and wood supports simulated tree branches. Cages were spaced roughly 8 m apart from one another. The douc langurs were therefore able to see human visitors and other monkeys in adjacent cages. Adult males occasionally reacted to sounds made from adjacent cages, while mothers and infants did not appear to alter their behaviour. None

of the individuals appeared to be affected by the presence of visitors. There were 20 staff members at the EPRC who frequently walked along the adjacent footpaths and entered cages to clean them and feed the animals. The center was also open to tourists from 0900-1100 h and 1300-1600 h. During the period of the study, visitor presence was low (5-10 visitors per day).

Fig.1. A typical cage at the Endangered Primate Rescue Center, Vietnam. Photo: Nolan Bett.

The animals were fed three times daily (at 0630-0700 h, 1030-1100, and 1300-1500 h) with bundles of leaves from local tree species. Approximately 10 bundles were spread out in each cage at every feeding. Cage cleaning usually coincided with feeding or occurred shortly before.

Data Collection

I observed the animals six days per week during the study period from 0530-1030 h, and again from 1300-1500 h. A time budget assessment during the week preceding the study determined the douc langurs were most active during these periods. I conducted focal samples of a mother-infant dyad, and made instantaneous records at 20 sec intervals of the behaviour of both the infant and the mother, as well as their proximity. I did not record data when keepers entered the cage, but resumed immediately afterward.

Each of the three dyads was sampled every day: one from 0530-0800 h, another from 0800-1030 h, and the third from 1300-1500 h. The time at which the dyads were sampled was rotated

from one day to the next such that the dyads were observed for the same overall amount of time during each sampling period. I developed an ethogram before starting the behavioural data collection (Appendix). I scored proximity in five categories: 0 m (touching), greater than 0 m and less than or equal to 0.5 m, greater than 0.5 and less than or equal to 1 m, greater than 1 m and less than or equal to 2 m, and greater than 2 m.

Data Analysis

I pooled the observations for each dyad by day, expressing values for individual behaviours as percentages of total daily instantaneous records (expressed as proportion of time). I used these daily proportions to compute mean percentage of time spent on each behaviour. I tested the daily proportions for normality using the Shapiro-Wilk test, and used either ANOVA, (if the distributions were normal) or the Kruskal-Wallis test (if the distributions were not normal) to compare data among subjects/dyads. When the resultant p-value was ≤ 0.05, I used the Tukey HSD test (if distributions were normal) and the post-hoc test provided by Siegel & Castellan (1988; if distributions were not normal) for multiple comparisons between subjects/dyads.

I measured the mothers' rejection rate by dividing the number of times they rejected their infants' attempts to establish nipple contact by the sum of the rejections and acceptances. I considered a series of one or more consecutive sampling points in which the infant was in contact with its mother's nipple as a single nursing bout, and I demarcated each nursing bout as one acceptance from the mother. I therefore assumed that infants did not stop and resume nipple contact between two sample points within a bout. Sample points were recorded at a high frequency, and therefore any gaps in nipple contact between consecutive points would be very brief.

I calculated a weighted average distance between the infants and their mothers by converting proximity categories into a distance index, using the middle value of each proximity range (e.g. 0-0.5 m was averaged to 0.25 m, 0.5-1.0 m was averaged to 0.75 m, and so on). Using the distance index, a mean distance was calculated for each sample day. The mean distances were normally distributed, and I used ANOVA to compare the daily mean distances between mothers and infants in the different dyads. All p-values are two-tailed. Results are displayed as box plots with medians and inter-quartile ranges when non-parametric analyses were used, and as bar charts with means and standard errors when parametric analyses were used. I used the statistical software R (2.11.1) for all analyses.

Results

There was a total of 27,005 sample points over 150 hours of observation, spread evenly across the three dyads. The three infants differed from one another in several aspects of their behaviour. These included the proportion of time spent holding onto, sitting in the lap of, and climbing on their mothers (respectively: Kruskal Wallis; $X^2 = 46.852$, d.f. = 2, $P < 0.001$; $X^2 = 31.276$, d.f. = 2, $P < 0.001$; $X^2 = 51.096$, d.f. = 2, $P < 0.001$). Post hoc comparisons indicated Inf1 spent more time doing each of the three behaviours than Inf2 and Inf3 (Fig. 2a-c). The infants also differed in the proportion of time spent grooming their mothers, sharing food with their mothers, and grabbing at their mothers' faces and bodies (respectively: $X^2 = 19.841$, d.f. = 2, $P < 0.001$; $X^2 = 16.612$, d.f. = 2, $P < 0.001$; $X^2 = 19.048$, d.f. = 2, $P < 0.001$). Post hoc comparisons revealed Inf2 spent more time engaged in each of these behaviours than Inf1 and Inf3 (Fig. 2d-f).
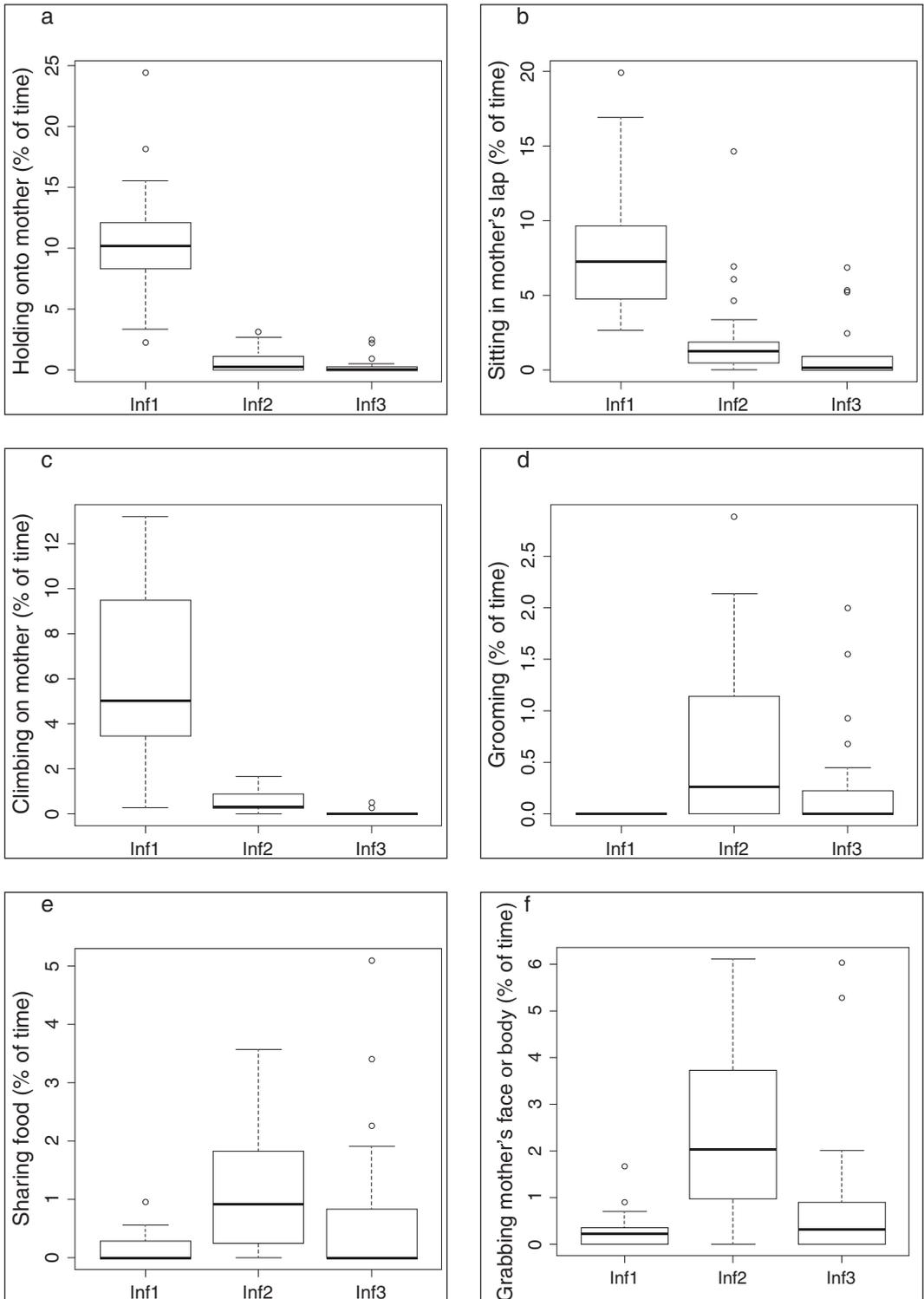
Fig.2. Proportion of time spent by infants a) holding onto their mothers, b) sitting in their mothers' laps, c) climbing on their mothers, d) grooming their mothers, e) sharing food with their mothers, f) grabbing their mothers' face or body.
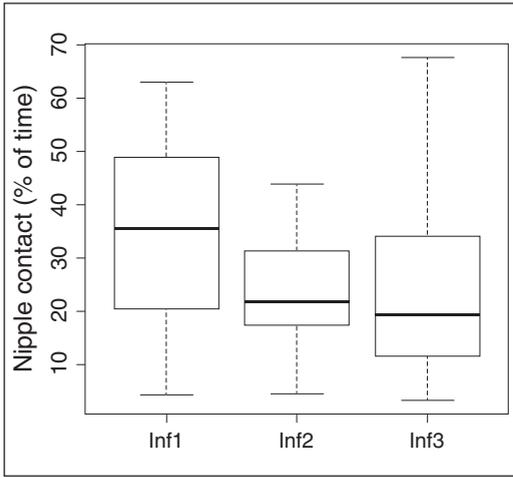
Fig.3. Proportion of time spent by infants in contact with their mothers' nipples.

The three infants did not differ significantly in the amount of time they spent in contact with their mothers' nipples (Kruskal Wallis; X^2 = 5.7926, d.f. = 2, P = 0.055) (Fig 3). This was the most common behaviour for each of the infants: Inf1 spent 36 ± 8% (n = 22, 95% C.I.), Inf2 spent 23 ± 5% (n = 22) and Inf3 spent 27 ± 9% (n = 22).

The three mothers differed in the amount of time they spent physically supporting their infants' weight (ANOVA; F = 74.235, d.f. = 2, P < 0.001), and post hoc comparisons revealed Mot1 spent more time than Mot2 and Mot3 (Fig. 4a). The mothers did not differ significantly in the amount of time they spent grooming their infants (Kruskal Wallis; X^2 = 5.280, d.f. = 2, P = 0.071) (Fig. 4b).
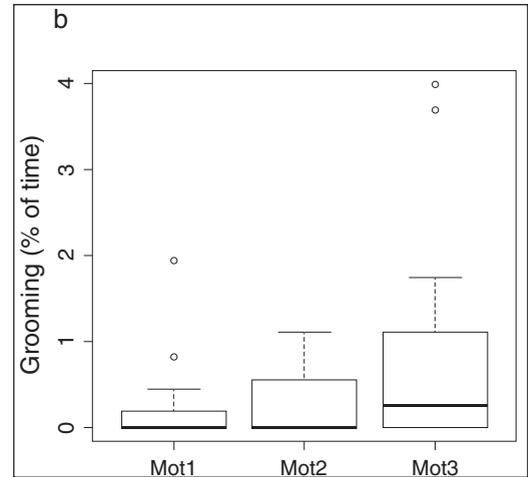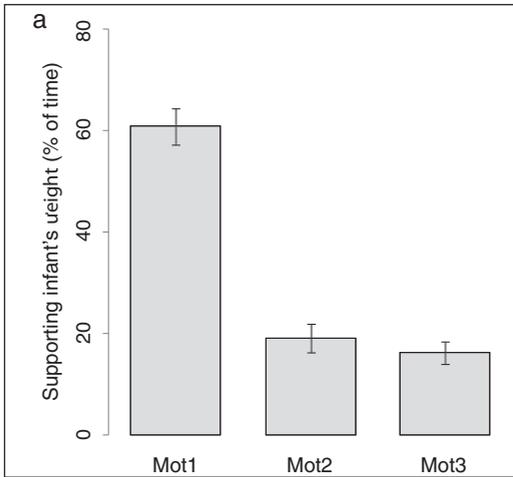

Fig.4. Proportion of time spent by mothers a) supporting their infants' weight, b) grooming their infants.
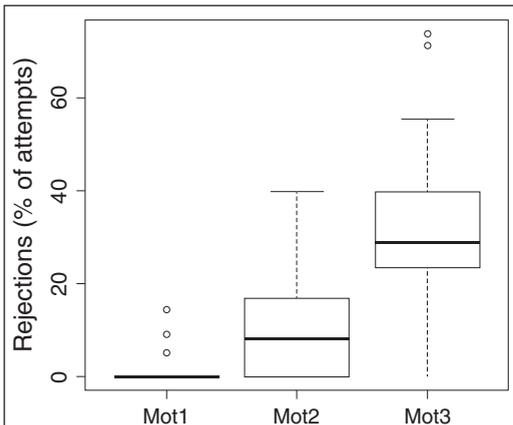

Fig.5. Percentage of attempts to establish nipple contact in which the mother rejected the infant. Total number of nursing attempts, successful or unsuccessful, by infants: Inf1 = 205, Inf2 = 251, Inf3 = 267.

The rate of infant rejection differed across the three mothers (Kruskal Wallis; X^2 = 32.394, d.f. = 2, P < 0.001). Post hoc comparisons revealed that Mot3 rejected her infant more often (32% ± 9, n = 22, 95% C.I.) than Mot1 (2% ± 2, n = 22) and Mot2 (10% ± 5, n = 22) (Fig. 5).

The proximity between the infants and their mothers differed significantly (ANOVA; F = 109.740, d.f. = 2, P < 0.001). Inf1/Mot1 were in physical contact with one another most of the time, and the amount of time they spent in different proximity categories decreased as the proximity range increased (Fig. 6). Inf2/Mot2

and Inf3/Mot3, on the other hand, were most often either in contact with one another, or more than 2 m apart. They spent very little time at distances between these two extremes.
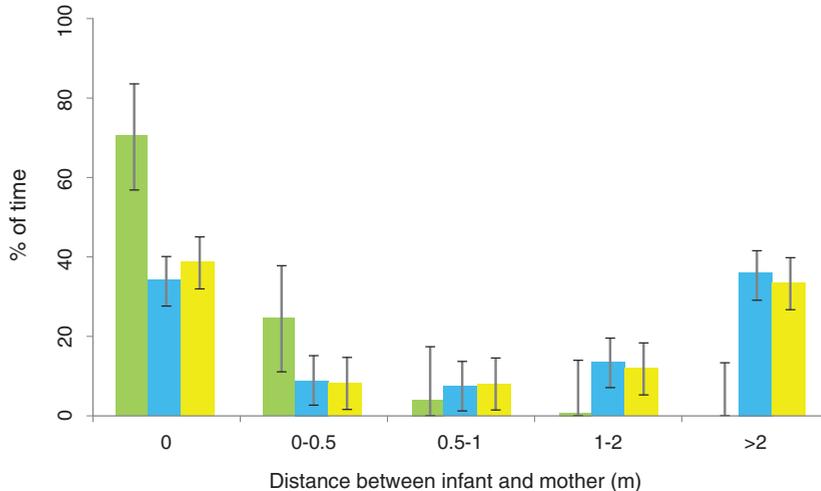

Fig.6. Proximity between mother and infant. Green = Inf1/Mot1, blue = Inf2/Mot2, yellow = Inf3/Mot3.

Discussion

In douc langurs, there appear to be several shifts in behaviour between the ages of 2 and 25 months. One of the most striking differences is in mother-offspring proximity. The older infants, Inf2 and Inf3, spent more time further away from their mothers than Inf1, and spent little time interacting with their mothers aside from nursing. They rarely, for example, held onto, climbed upon, or sat in the lap of their mothers. The youngest infant, Inf1, by contrast, rarely ventured farther than 1 m from his mother—most of his time was spent in physical contact, interspersed with brief exploratory journeys <2 m away. Brief exploratory journeys were reported for similar-aged captive red-shanked douc langurs (*Pygathrix nemaeus*) (Hick, 1972; Ruempler, 1998), which are the grey-shanked douc langurs' closest relatives (Roos et al. 2007), although not all infants make these trips so young (Brockman & Lippold, 1975). Yeong et al. (2010) observed four infant red-shanked douc langurs from their birth until an age of 18 months. The proportion of time these infants spent in physical contact with their mothers decreased dramatically during the first two to four months of life, after which the time spent in contact remained fairly constant. The proportion of time the 1-2 month old red-shanked douc langurs spent in contact with their mothers (60-90%) was similar to Inf1, and the proportions of time 18-month olds spent in contact with their mothers (20-60%) was similar to Inf2 and Inf3.

Each of the infants spent a relatively large amount of time on its mothers' nipples, but the rate of rejections by the mothers increased with infant age. This pattern of rejection presumably reflects different stages of the weaning process, which is often gradual. In the Nilgiri langur (*Trachypithecus johnii*), for example, the process begins as early as 14 weeks after birth, at which point the mother leaves her infant consistently, and alternates between accepting and rejecting her infants' attempts to nurse (Poirier, 1968). The process then continues, with the rejections increasing in frequency and severity, until the infant is over a year old. At this point, the mother regularly rejects the infant's attempts to nurse, until eventually the infant no longer seeks contact with its mother's nipple.

Maternal effects on infant development in primates act through many different factors; a mother's social partners, diet, physiology, and daily activities can all have profound and lasting effects on her offspring (Altmann, 1980; Förster & Cords, 2002; Hinde & Spencer-Booth, 1967; Mateo, 2009). In addition, there is a great deal of individual variation in growth rates, weaning age, and behavioural development, even in individuals that live in the same environment (Maestripieri, 2009; Pereira & Leigh, 2003). Infant development in primates is therefore an exceptionally complex process that can be influenced by a combination of maternal effects, social surroundings, physical environment, and individual variation.

Differences in wild and captive environments likely have a strong effect on infant development, and studies of colobines have confirmed that rates of infant independence differ in captivity and in the wild. Zhao et al. (2008) found that wild white-headed langur (*Trachypithecus leucocephalus*) infants are weaned at a younger age than captive infants. In red-shanked douc langurs, however, the opposite may occur, with captive infants undergoing accelerated maturation. In the wild, weaning begins at roughly 12-13 months of age, and continues until the infant is 2 years old (Lippold, 1995). In captivity, mothers begin to reject their infants' attempts to suckle when the infants are 4-10 months old (Yeong et al., 2010). The age at which captive infants no longer spend time on their mothers' nipple is unknown, however, because studies have not continued long enough to observe independence. Infant red-shanked douc langurs have been observed nursing through the ages of 7 months (Hick, 1972), 13 months (Brockman 1976), 17 months (Kavanagh, 1978), and 18 months (Yeong et al., 2010). The latest known age of an infant on its mother's nipple was 24 months (Ruempler, 1998), and this is likely an underestimate since the behaviour still occurred when observations stopped. In this study, the oldest infant was 25 months old, and continued to spend time on its mother's nipple at a rate that was similar to the 2-month old infant. By this advanced age, during which the individual is consuming a full diet of leaves, nipple time is no longer nutritional (and should therefore not be considered nursing), but is probably done for comfort. This study provides a snapshot of infant behaviours beyond one year of age, which is when many infant studies end. To better understand behaviours such as weaning in the grey-shanked douc langurs, however, field studies that track continuously the infants through to independence are needed. Additional captive studies are also necessary to determine how the captive environment alters infant development, particularly studies with a larger sample size.

The time course of weaning also depends on how quickly the mother gives birth again. In primates, weaning often ends with the birth of a younger sibling (Nicolson, 1987; Strier, 2000). Through extensive observations of the North Indian langur (*Semnopithecus entellus*), for example, Jay (1965) found that mothers regularly give birth to a second infant approximately two years after the first offspring was born, at which point she severs all nursing ties with the elder infant. In my study, Inf1's older brother (20 months) rarely interacted with his mother, and I never saw him attempt to establish nipple contact, even though he was 5 months younger than Inf3, who established nipple contact frequently. Also, of the four infants that Yeong et al. (2010) observed, the two that had younger siblings were weaned earlier than the two who did not. The birth of a younger sibling seems to lower the age at which an infant is weaned, and may even affect other social interactions. For example, female *Cercopithecus mitis* groomed older offspring less often when younger siblings were born (Cords, 2000).

Other behavioural differences existed among the infants, although it is difficult to ascertain whether these differences arose from social factors, which can affect many aspects of maternal behaviour and infant development (Förster & Cords 2005; Hinde & Spencer-Booth, 1967;

Maestripieri & Call 1996; Spencer-Booth, 1968) or are simply individual idiosyncrasies. Inf2 spent a significantly greater proportion of her time grooming her mother than the other two infants. Cords (2000) suggested that grooming may be more important to developing long-term relationships in female *C. mitis* than in males, and a similar sex difference could occur in douc langurs, which, like *C. mitis*, are female-philopatric (Wenting Liu et al. 2008). Such a sex difference could account for the variation in time spent grooming. Also, Inf2 spent a relatively large amount of time grabbing her mother's face and body, which could reflect the absence of other infants or juveniles with whom she could play. Captive red-shanked douc langur infants, for example, exhibit less social play when they are housed with only their parents in comparison to infants that live with other infants and juveniles (Yeong et al., 2010). Arguing against such an interpretation, however, is Inf3, who lived in a similar social environment to Inf2, yet did not "play" extensively with its mother. Further studies of infants in varying social environments could help clarify the effect of environment on behaviours such as grooming and social play.

Conservation Implications

Studies of social behaviour on captive individuals also have the potential to guide conservation management. The mother-infant relationship affects the survival abilities of infants, and to a lesser degree the mothers as well, and therefore can influence the success of reintroductions of captive individuals into the wild. We can assume that to maximize the likelihood of infant survival, the relationship in captivity should be similar to what is seen in the natural environment. This can be best ensured by imitating the douc langurs' natural social and physical environment as effectively as possible in captivity. For example, grey-shanked douc langurs currently live in groups of <20 individuals, with an average group size in the range of 8-15 (Ha Thang Long, 2004). These groups generally include multiple infants and juveniles, which provide opportunities for bonding and play. Behaviours such as the face and body grabbing exhibited by Inf2 towards her mother may result from a lack of playmates, which could affect the infant's ability to form future social bonds and therefore to integrate into a group in the wild. Also, if infant development is accelerated or delayed in captivity, the timing at which mothers and infants are reintroduced should be adjusted accordingly. If infants are reintroduced too soon, for example, they may not adjust well to the shift in setting, or may be an extra burden on their mothers, which could exacerbate the pressures of switching to a natural environment. Rosenblum and Andrews (1994) found that a mother's ability to foster normal infant development decreased when she was confronted with an unpredictable environment. Minimizing the changes from captive to natural environments could therefore be critical to maintaining a stable relationship between mothers and their infants. Creating a captive environment that imitates the natural one is especially important for species such as the grey-shanked douc langur, which is critically endangered (IUCN 2010), and may rely on successful reintroductions in the future.

If deforestation and hunting continue to reduce grey-shanked douc langur population sizes, the species' reliance on captive-breeding programs will increase. The EPRC is currently the only location to house captive grey-shanked douc langurs, which are likely just as difficult to sustain in captivity as their sister taxa, the red-shanked douc langurs, have proven to be (Hill, 1972; Hick, 1972; Ruempler, 1998). Captive behaviour studies on grey-shanked douc langurs can be used to improve their maintenance, welfare and breeding success, ensuring a stable captive population.

This is the first study on social relationships in captive grey-shanked douc langurs, however, and their relationships in the wild are equally understudied. Additional studies, both in captivity and

in the wild, will allow us to compare their behaviours in these different environments. This information can be used to increase the likelihood that captive-bred individuals will thrive in their natural environment, which could prove to be vital to their long-term conservation.

Acknowledgements

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Preliminary results on food selection of the black-shanked douc langurs (*Pygathrix nigripes*) in Southern Vietnam

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Summary

Food selection is one of the most basic aspects of a primate's ecology, particularly in their natural habitat. Several factors influence food selection of colobine monkeys, of which nutritional factors, especially protein, fibre and secondary compounds are considered key determinants. While some colobines are food selective, black-shanked douc langurs (*Pygathrix nigripes*) consume a wide range of parts of many plant species. However, the influence of phytochemistry on food choice has not yet been analysed for these doucs in their natural habitat. Based on our studies on feeding ecology of the black-shanked douc langurs in southern Vietnam we collected 15 leaf and two fruit samples of food plants and six leaf samples of non-food plants that occur in high density in the study area where the black-shanked douc langurs are the largest common folivore. Samples were analysed for protein, fibre, condensed tannins, ash, and necessary base group and trace group of minerals. The results showed that digestible fibre (cellulose and hemicellulose) was higher in food plants but there were no statistically significant differences for any other comparison. There is no evidence to suggest that the doucs were foraging strategically to maximise their protein or energy intake, most viable population after that of Khau Ca.

Những kết quả ban đầu về việc lựa chọn thức ăn của loài Chà vá chân đen (*Pygathrix nigripes*) ở Nam Việt Nam

Tóm tắt

Chọn lựa thức ăn là một trong những hướng nghiên cứu khá phổ biến trong các nghiên cứu về sinh thái dinh dưỡng của các loài linh trưởng sống trong điều kiện tự nhiên. Có nhiều yếu tố được cho là ảnh hưởng đến sự chọn lựa thức ăn như lá, hoa, quả hay hạt của các loài thuộc phân họ Voọc (Colobinae), trong đó các yếu tố dinh dưỡng như thành phần prôtêin, chất xơ hay các chất ức chế tiêu hoá được xem là yếu tố chính. Trong khi một số loài voọc có phổ thức ăn hẹp, loài chà vá chân đen được cho là loài ăn rất nhiều loại thức ăn của nhiều loài thực vật khác nhau. Cho đến nay vẫn chưa có các nghiên cứu về việc chọn lựa thức ăn của giống chà vá ở trong tự nhiên. Dựa vào các kết quả nghiên cứu về sinh thái thức ăn của chà vá chân đen trước đây, chúng tôi đã tiến hành thu mẫu 15 mẫu lá và 2 mẫu quả là thức ăn của chà vá chân đen và 6 mẫu lá của 6 loài cây có mật độ cao trong khu vực nghiên cứu nhưng không được chà vá chân đen sử dụng. Các mẫu được phân tích để xác định prôtêin, xơ, tanin, tro, các nguyên tố đa lượng và vi lượng cần thiết cho cơ thể động vật. Kết quả cho thấy, chỉ có hàm lượng xơ có thể tiêu hoá (cellulose và hemicelluloses) có thể ảnh hưởng đến sự chọn lựa thức ăn của chà vá chân đen. Chưa có chứng cứ vững chắc nào để khẳng

định chà vá chân đen có một chiến lược kiếm ăn nhằm đạt hiệu quả cao nhất về hấp thu prôtêin và năng lượng.

Introduction

Food selection is one of the most basic aspects of a primate's ecology, particularly that of colobine monkeys. Most colobines have a predominantly folivorous diet and the morphology of their masticatory and digestive systems are adapted to digest cellulose and denature toxins in leafy materials (Oates & Davies, 1994; Chivers, 1994). Colobines have sharp high molar cusps with long shearing crests, deep mandibles and narrow incisors for eating leaves (Lucas & Teaford, 1994) and the salivary glands are enlarged to enhance digestion (Oates & Davies, 1994). They also have an enlarged forestomach containing bacteria, adapted for the breakdown of cellulose, which constitutes the main part of plant material fermentation (Canton, 1998; Kay & Davies, 1994). Together with the detoxification by bacteria in the forestomach (Canton, 1998), an enlarged liver in most colobines processes the toxins which many trees produce to protect mature leaves against predation (Nadler et al., 2003). According to Yeager and Kool (2000) colobines need to select food high in essential nutrients and low in digestion-inhibiting compounds such as lignin, a structural fibre which cannot be degraded by the symbiotic gut microorganisms. Thus digestion of other cell constituents takes place over a prolonged period (Ford et al., 1979; Hungate, 1966; Marquardt et al., 1978; Osbourn, 1978). Typical Asian colobines prefer young leaves over mature ones because of the high proportion of protein and low fibre and secondary compounds in young foliage (McKey et al., 1981; Milton, 1979; Milton et al., 1980; Glander, 1982). Recent studies on a wide range of nutritional components from a wide variety of plants in the wild showed that the ratio of protein to fibre (cellulose and lignin combined) was a significant factor in food choice (for *Presbytis rubicunda*, *Presbytis melalophos*: Davies et al., 1988; *Colobus satana*: McKey et al., 1981; *Presbytis johnii*: Oates et al., 1980; *Trachypithecus delacouri*: Workman, 2010; Workman & Le Van Dung, 2009; *Nasalis larvatus*: Yeager et al., 1993).) A question that arises from these findings is whether this conclusion is true to all colobines. To answer this question we conducted a preliminary study on food selection of the black-shanked douc langur *Pygathrix nigripes*. This is a large colobine monkey (Napier & Napier, 1985) endemic to Vietnam and Cambodia (Brandon-Jones et al., 2004; Groves, 2001; Nadler et al., 2007). The species has been reported to have a diet consisting of between 40-54% of leaves depending the location (Hoang Minh Duc, 2007; Hoang Minh Duc et al., 2009; Rawson, 2009). Unlike Delacour's langur that has been reported to eat 42 plant species (Workman, 2010); the black-shanked douc langurs have been shown to consume more than 150 plant species (Hoang Minh Duc, 2007; Hoang Minh Duc et al., 2009). While nutrient intake from leaves by the doucs has never been studied quantitatively in the wild a recent quantitative study conducted on the feeding ecology of captive grey-shanked douc langurs and semi free-ranging red-shanked douc langurs concluded that these two species are highly folivorous and that they tended to avoid plant species containing high level of alkaloids, tannins, cyanogenic, and antimicrobial compounds and chose young leaves rather than mature leaves (Otto, 2005).

This paper reports a preliminary study of food selection in the wild of free-ranging black-shanked douc langurs. Specifically we aimed to examine the selection of plant species and investigate the nutrients supplied by key plants consumed by the black-shanked douc langurs.

Materials and Methods

Study Sites

We conducted this research in Nui Chua and Phuoc Binh National Parks (Fig.1). Both protected areas are located in the Greater Annamites Ecoregion which is one of the most diverse monsoon forests in Asia (Baltzer et al., 2001). These protected areas were chosen because they both have known populations of black-shanked douc langurs and allowed data to be collected in a range of altitude, climatic and forest types. Phuoc Binh (located between 11˚58' - 12˚10' N and 108˚43' - 108˚49' E) is 19,814 ha and is located in the centre of the black-shanked douc langurs range in Vietnam. It has an annual rainfall of over 2000 mm. The mean maximum and minimum temperatures are 38.8˚C and 14.2˚C respectively (Nguyen Khanh Van et al., 2000), hence this park has a wet and humid climate which allows the 3000 human inhabitants to cultivate wet rice and practice shifting cultivation in the park's buffer zone (Phuoc Binh Commune People's Committee, 2005). Nui Chua (located between 11˚39' - 11˚48' N and 109˚04' - 109˚14' E) is also known to contain a large population of black-shanked douc langurs. It covers 22,513 ha with 7,532 ha being a buffer zone in which there are 14 villages or communes, supporting more than 24,000 people (BirdLife International & Forest Inventory and Planning Institute, 2001). This park is located in the driest part of Vietnam and receives an annual average rainfall of 697 mm (Nguyen Khanh Van et al., 2000). The park has many short streams that drain steep slopes but only six that run in the dry season, hence the climate here is hot and dry. Both protected areas are described in more detail in Hoang Minh Duc et al. (2009).
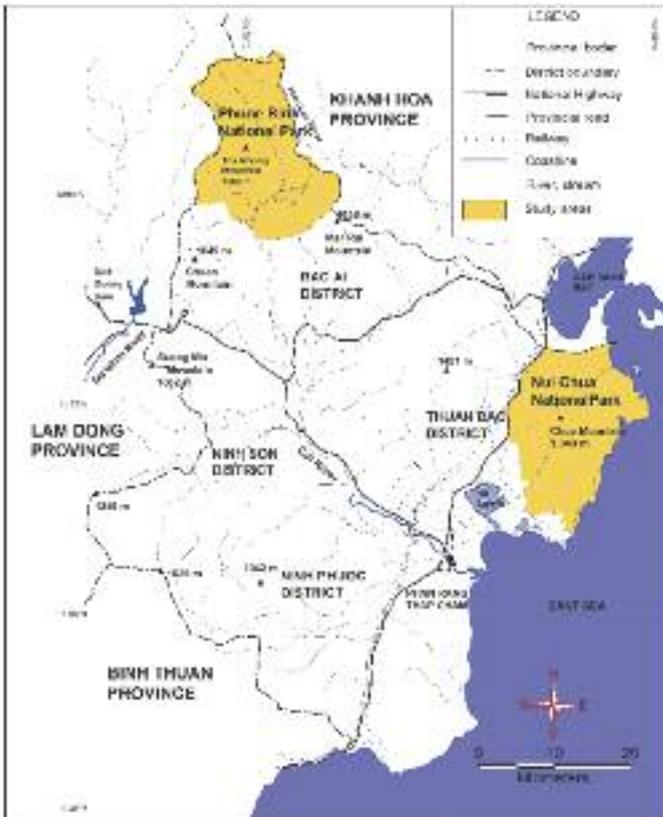

Fig.1. Location of study areas

Material

We collected seventeen food plant samples between February and August 2005 for nutritional analysis. We collected the same plant part from food plants as the doucs were observed to eat, usually from branches in the mid canopy. The sampled trees were the same species and in the same locality as the trees where the douc langur groups foraged but sometimes were not the same tree that the group fed in (following the methods of Chapman et al., 2003) due to the difficulty in collecting of leaves and or flowers. Samples were air dried in the field and then sealed in plastic bags for transport to the laboratory. Additionally, to determine if the douc langurs were preferentially foraging for specific nutrients we also collected non-food samples from plants that we did not observe the douc langurs foraging, but which were present at high density in the locality where the douc langurs were foraging.

Nutrient Analysis

Chemical analyses were undertaken in the laboratory of the Environmental Technology and Management Centre, Nong Lam University, Ho Chi Minh City by technicians employed by the laboratory. We examined crude protein, crude fat, soluble carbohydrate, fibre (total cellulose and lignin), Calcium, Zinc, Iron, Potassium, Manganese, Magnesium and tannin of each specimen (Table 1).

Table 1. Methods for Nutrient Analyses.

Parameter	Test
Crude protein	Kjeldahl test (Horwitz, 1970)
Crude fibre	Weeder analysis (Dang Dinh Boi & Phan Tat Dat,1970
Water soluble carbohydrate	Bertrand, 1913
Crude fat	Soxhlet (petroleum ether) (Carpenter, 2003)
Calcium and Magnesium	EDTA titration (Daly & Hill, 2004)
Potassium	flame emission (Daly & Hill, 2004)
Iron, Manganese, Zinc	Atomic absorption spectrometry (Miles et al., 2001)
Tannin	Lowenthal (Barua & Roberts, 1940)

Dried samples were ground in a Wiley mill to pass through a 1-mm mesh screen. Dry matter was determined by drying a portion of each sample overnight at 105°C.

The protein (nitrogen) content of the plant parts was assessed via Kjeldahl procedures (Horwitz, 1970). Crude protein was calculated by assuming a ratio of protein to nitrogen of 6.25 for leaf samples and 4.3 for fruit samples (Conklin-Bristain et al., 1999).

The structural substances present in plants are mainly composed of cellulose, hemicelluloses, pentosans, lignin, cutin, and pectin (National Research Council, 2002). The method used in this study to analyse fibre content (Weeder analysis) was a hydrolytic procedure. For logistic reasons we determined the main digestible substance as crude cellulose (cellulose and hemicelluloses combined) (van Soet, 1994), and lignin, was our measure of the indigestible fibre.

Crude cellulose (cellulose and hemicelluloses combined) was identified following Dang Dinh Boi and Phan Tat Dat (1995). This procedure is based on solubilisation of lignin and other soluble constituents. A powdered sample was solubilised in ethanol and benzene solution. After filtration and washing with water, the residue was gently boiled in distilled water for 2 hrs, then filtered and washed to solubilise water soluble substances. Lignin was isolated from the remaining liquid using

NaOH 0.25N. The residue was recovered and washed with water until alkali free. It was then dried at 600C for 16 hrs, and this yielded the crude cellulose measure.

Lignin content was determined according to the method described by Dang Dinh Boi and Phan Tat Dat (1995). The procedure is based on solubilisation of other constituents in suitable solvent and then precipitation of lignin by 72 % H_2SO_4. A powdered sample was solubilised in ethanol (110 ml) and benzene (240 ml) solution for 4 hrs in a Soxhlet apparatus. The powder was then boiled in distilled water for 1 hr. After filtration through a porous Gooch crucible, the residue was washed with boiled distilled water and then dried naturally at room temperature. The residue was solubilised in 72 % H_2SO_4 in ice water for 2 hrs. The mixture was then boiled with distilled water in a conical flask for 4 hrs then filtered and washed with hot water. The residue was dried at 105°C for 3-4 hrs, and this yielded the lignin measure.

Soluble carbohydrates in plants usually consist of the free sugars glucose, fructose and sucrose. These free sugars are often determinated by HPLC (High-Performance Liquid Chromatography). In this research, because of logistical constraints, soluble carbohydrates were assessed by the Bertrand method (Bertrand, 1913).

Two groups of mineral content were identified for the plant samples. The base group included potassium (K), magnesium (Mg), and calcium (Ca) was determined directly by comparison with the standard solution using flame emission. The trace group including iron (Fe), manganese (Mn) and zinc (Zn) was dried, weighed, ashed, and solubilised with hydrochloric acid (Miles et al., 2001) and then determined by atomic absorption spectrometry according to the procedure outlined by Daly & Hill (2004).

Secondary metabolites can be classified by their chemical structure or physical properties into three groups: terpenes, phenols (including tannin), and nitrogen-containing compounds (Taiz & Zeiger, 2000), in this research only tannin, a phenolic compound, was analysed. Tannins were assessed using the Lowenthal Permanganate Titration (Barua & Robert, 1940). This method relies on the oxidation of phenols by potassium permanganate solution in the presence of indigo carmine as a 'red-ox indicator' to show the end point. The tannin analysis was described by Barua & Robert (1940).

Data analysis

We compared means of the various nutrients in food-plant and non-food-plant groups. Based on results of frequency of feeding bouts on 60 plant species recorded during continuous scans between February and August 2005 (Hoang Minh Duc, 2007) we compared mean of nutrients and tannin between the major food plant group (frequency of feeding scores more than 2%) and the minor food plant group (frequency of feeding scores fewer than 2%).

Results

The nutrient content varied considerably in the food-plant samples (Appendix). The mean of crude protein of food plants was 12.3% (SD=3.66%) and mean of crude cellulose (cellulose and hemicelluloses) was 23.87% (SD=7.26). Within the food-plant group, fruit samples (n=2) contained less tannin than leaves (n=15) (p = 0.05 see Table 2). Mean crude protein in fruit was less than in leaves but the difference was not significant. Content of cellulose and lignin in fruit samples were similar to those in leaves samples (Table 2). Water soluble carbohydrates were in only trace quantities in the leaves of all plant samples while it was very high (28.8%) in the fruit pulp of *Canarium subulatum* (Appendix).

Table 2. Nutrient composition of leaves and fruit samples from food plants. (Note: ** p < 0.01)

Parameter	Food leaves Mean (sd) n=15	Food fruit Mean (sd) n=2	t	df	p (2-tailed)
Crude protein %	12.30 (3.66)	7.57 (5.76)	1.09	1.1	0.45
Crude cellulose %	23.87 (7.26)	21.70 (16.32)	0.19	1.1	0.88
Lignin %	22.8 (9.26)	23.52 (14.82)	-0.07	1.1	0.96
Ash %	5.51 (1.60)	5.26 (0.92)	0.35	1.9	0.77
Calcium %	0.60 (0.34)	0.36 (0.16)	1.71	2.47	0.21
Magnesium %	0.30 (0.19)	0.14 (0.09)	1.85	2.20	0.19
Potassium %	0.61 (0.25)	0.62 (0.16)	-0.03	1.69	0.99
Iron g/kg	11.04 (7.30)	18.43 (21.98)	-1.08	15	0.29
Manganese mg/kg	145.25 (131.69)	446.76 (56.70)	-5.72	2.83	0.01**
Zinc mg/kg	23.18 (21.97)	16.32 (9.19)	0.80	2.99	0.49

The mean tannin content in the leaves of the major food group (n=7 species) was significantly higher (mean ± 1 sd, 7.39% ± 5.11%) than the minor food group (4.93% ± 4.37%, n=9 species). Tannin content was lowest (1.79 %) in *Diallium cochinchinensis* and highest (18.09 %) in *Irvingia malayana* (Appendix). This latter species was observed to be consumed often during observation.

There were no significant differences in mean concentrations of the organic nutrients and crude protein nutrient composition of leaves taken from known food-plants and non-food plants (Table 3). Mean cellulose content of food-leaves was significantly higher than non-food leaves (Table 3). Mean tannin content of all food and non-food leaves was not significantly different. Minerals, mean ash, and manganese contents were similar between food and non-food leaves (all p > 0.05). Mean calcium, iron and zinc in food leaves were not significantly different to those in non-food leaves (all p>0.2, Table 3).

Table 3. Nutrient composition of food and non-food leaves (Nui Chua and Phuoc Binh combined data).

Parameter	Food leaves Mean (sd) n=15	Non-food leaves Mean (sd) n=6	t	df	p (2-tailed)
Crude protein %	12.30 (3.66)	15.36 (5.17)	-1.32	7.12	0.23
Crude cellulose %	23.87 (7.26)	16.89 (1.61)	2.30	19	0.03
Lignin %	22.80 (9.26)	24.06 (11.85)	-0.23	7.58	0.83
Ash %	5.51 (1.60)	5.39 (3.93)	0.11	19	0.92
Calcium %	0.60 (0.34)	0.86 (0.94)	-0.96	19	0.35
Magnesium %	0.30 (0.19)	0.27 (0.20)	0.36	8.98	0.73
Potassium %	0.61 (0.25)	0.59 (0.22)	0.24	10.17	0.82
Iron g/kg	11.04 (7.31)	7.89 (3.75)	1.30	17.40	0.21
Manganese mg/kg	145.25 (131.69)	133.51 (73.24)	0.26	16.46	0.80
Zinc mg/kg	23.18 (21.97)	29.29 (7.44)	-0.95	18.84	0.36

Discussion

There are two main findings from our study. First, we did not find any difference in the crude protein content of food leaves and non-food leaves, albeit in a small sample. Secondly, the black-

shanked douc langurs we studied selected leaves that were higher in cellulose than non-food leaves and we found no evidence that tannin was the key determinant of food selection in the black-shanked douc langurs.

Each primate species has a critical protein content below which it cannot maintain bodily functions (Milton, 1979; van Soest & Robertson, 1980). Early studies on phytochemistry found that a high level of protein might be one reason that certain foods were selected (Struhsaker, 1975; Oates et al., 1977). For smaller herbivorous primates such as the howler monkey (mean weight: 7.55 kg), the critical level of protein content probably ranges from 9 – 11% dry matter (Milton, 1979). Oftedal (1991) reported that primates would require only 7–11% protein on a dry matter basis for growth and maintenance, and only 14% (DM) for reproduction. With respect to colobines, protein demand might be even lower, because they have ability to reuse blood urea for protein synthesis by microbes (Kay & Davies, 1994). The mean protein content in leaves (12.3%) eaten by the black-shanked douc langurs in our study was higher than the general protein requirement for primates but lower than that of semi-free ranging *P. nemaeus* (14.4%: Otto, 2005). Some plants found in the study area contained high protein in leaves (25.4%: *Derris* sp. 2., 14.7%: *Cratoxylum cochinchinensis*; see Appendix 1) but were not eaten by the douc langurs. A similar result was found in non-food leaves of howler monkeys (Milton, 1979) suggesting that protein is perhaps a factor but not the only factor that influences these monkeys' food choices. Protein content in leaves eaten by primates ranges from 12 to16% dry matter (Glander, 1982). Oftedal (1991) argued that it is unlikely that high dietary level of protein is required by most primates. In this study, crude protein content of food-leaves did not differ to that of non-food leaves albeit in a small sample. The same result was reported for *Rhinopithecus brelichi* where crude protein of plants selected was similar to those rejected (Bleisch et al., 1998). Those authors also reported that available, rather than crude protein and total ash may be important determinants of food choice in this species. In our study, available protein was not identified. For total ash and other minerals, there was no difference between food leaves and non-food leaves. Dasilva (1992) also reported that *Colobus polykomos* on Tiwai Island, Sierra Leone did not select foods based on protein content. The same conclusion was also reported for two sportive lemur subspecies: *Lepilemur mustelinus mustelinus* and *L. m. edwardsi* (Ganzhorn, 1992).

Colobine monkeys in captivity were reported to require about 10-30% hemicelluloses of dry matter and 5-15% cellulose of dry matter (National Research Council, 2002). In our study, cellulose and hemicellulose constituted 23.87% of dry matter in food plants which was similar to the general primate requirement for cellulose and hemicelluloses. Cellulose and hemicellulose combined are the main energy constituent of the leaves, and they also maintains stable bacterial colonies in the forestomach (Kay & Davies, 1994).

Recent studies suggested that the high ratio between crude protein and crude fibre (cellulose and hemicelluloses and lignin combined) was the key determinant of food selection in primates (Davies et al., 1988; McKey et al., 1981; Oates et al., 1980; Workman, 2010; Yeager et al., 1993). In our study, we found a contradictory result that this ratio in food leaves (0.26) was significantly lower (t = -2.4, df = 7, p = 0.04) than that in non-food leaves (0.39). Since both cellulose and hemicelluloses can be digested (van Soet, 1994), we used the ratio of protein to lignin to test if the doucs prefer leaves with higher ratio of protein to lignin. However, this mean ratio was not significantly different between food leaves (0.61) and non-food leaves (0.72) (t = -1.03, df = 20, p = 0.32). The ratio of protein to fibre did not seem to be a reason certain foods were preferred in our study.

Among 15 plant species that were analysed, it is possible that each was chosen by doucs because it has a high content of a certain required nutrient. Maynard & Loosly (1969) reported that primates' food varied considerably in nutritional content and a plant or plant part that is rich in one nutrient may be poor in another. In our study no nutrient alone, except cellulose, could determine food selection in *P. nigripes*.

Plant secondary compounds may play a role in plant selection because they have a toxic effect on some herbivores or reduce the digestibility of protein (Feeny, 1976; Rhoades & Cates, 1976) and cellulose (Fischer et al., 1976, Waterman et al., 1980). In this study, we did not find a significant difference in tannin content between food leaves and non-food leaves. Moreover, we did find significantly more tannin in the plants that were consumed more often. This may be because the levels of tannin in the plants were not high enough to deter the monkeys, or that their adaptations are sufficient to deal with the negative effects of tannins. It has also been suggested that colobines might reduce the toxic effect of tannins by producing salivary proteins which have a high affinity for tannins (van Soest, 1994) and by the activity of symbiotic bacteria (Caton, 1998). An assessment based on food preferences of *P. nemaeus* (Otto, 2005) found that phenolic compounds, including tannins, were present in avoided, as well as in preferred food species and food intake was not clearly related to the presence of individual secondary plant compounds. In this study, tannins did not appear to be a determinant of food selection.

The food plants consumed by the black-shanked douc langurs do contain a number of other secondary compounds which are used in traditional Vietnamese medicine (Hoang Minh Duc, 2007). We did not measure these compounds in this study and their selection by black-shanked douc langurs awaits investigation. As for other secondary compounds, their effect on food selection of the species is still unclear. The black-shanked douc langurs consumed seeds of *Strynchnos* spp. which contains strychnine and brucine (toxic alkaloids) (de Padua et al., 1999; van Wyk & Wink, 2004), and leaves of *Derris* sp. which potentially contains toxic flavonoid compounds (Chevallier, 1996). Moreover, the secondary compound containing plants were consumed in small amounts (Hoang Minh Duc, 2007). Small amounts of these metabolites are more easily degraded and detoxified by the symbiotic bacteria in colobine's forestomach (Freeland & Janzen, 1974; Glander, 1982; McKey, 1978), preventing the host animal from damage (Freeland & Janzen, 1974; Waterman et al., 1980). A low concentration of these metabolites may act as medicine for treatment of intestinal worms, reduce fever or as bitter tonic as reported for *Strynchnos* spp. (de Padua et al., 1999; van Wyk & Wink, 2004)

Conclusion

We found no evidence to suggest that protein content or the ratio between crude protein and crude fibre influenced plant food selection of the black-shanked douc langurs, but the digestible fibre (cellulose and hemicelluloses) content may influence food selection. Our conclusions about the influence of phytochemistry on food selection must be tempered in the light of the small samples that we were able to collect and analyse.

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Preliminary observations of geophagy amongst Cambodia's Colobinae

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Key words: geophagy, soil eating, feeding ecology, red-shanked douc langur; silvered langur, Cambodia, predation

Summary

Geophagy amongst the genus *Pygathrix* and silvered langur species group have never been recorded in the wild. Here we describe several instances of primates coming to the ground in order to eat soil from salt licks in Veun Sai-Siem Pang Conservation Area, northeastern Cambodia. This camera trapped salt lick was used several times per month by both taxa and often for several hours at a time. The resource is clearly important as monkeys face predation risk in accessing it. Each species accesses the salt lick at different times of day, suggesting avoidance behavior. Soil composition has not been analysed to date, however consumption may either be an attempt to buffer the digestive system against toxins ingested through a predominantly leaf-based diet or may provide essential minerals to the diet.

Những quan sát ban đầu về tập tính ăn đất ở những loài khỉ ăn lá tại Campuchia

Tóm tắt

Tập tính ăn đất ở giống *Pygathrix* và vọc bạc chưa từng được ghi nhận ngoài tự nhiên. Trong tài liệu này chúng tôi mô tả một số quan sát về việc khỉ ăn lá xuống đất để ăn đất có khoáng muối tại khu bảo tồn Veun Sai-Siem Pang, vùng đông bắc Campuchia. Bẫy ảnh được sử dụng và ghi nhận cả hai taxa xuống đất vài lần trong tháng và vài giờ mỗi lần. Nguồn đất này rõ ràng là quan trọng đối với chúng bởi vì nguy cơ bị vật dữ ăn thịt là rất cao khi xuống đất. Mỗi loài xuống khu vực khoáng muối tại những thời gian khác nhau trong ngày chứng tỏ có sự luân phiên, tránh nhau. Tuy thành phần của đất chưa được phân tích, nhưng việc ăn đất này có thể là cách chúng chống lại độc tố có trong lá cây hoặc trong đất có chứa nguồn khoáng vi lượng hết sức cần thiết cho sự phát triển của loài.

Introduction

Due to recent taxonomic reassessments and new discoveries Cambodia's number of primate taxa has increased in recent years from nine to eleven species (Rawson, 2010). With the discovery of red-shanked douc langurs in northeastern Cambodia (Rawson & Roos, 2008) and the division of the silvered langur species group (Roos et al., 2008), Cambodia is now recognized as home to four species of leaf-eating monkey (sub-family Colobinae); the red-shanked douc langur (*Pygathrix nemaeus*), the black-shanked douc langur *(P. nigripes*) and potentially two species of silvered langurs (*Trachypithecus germaini* and *T. margarita*).

While phenotypically the one known population of red-shank douc langurs in Cambodia are widely variable, and in some instances resemble grey-shanked douc langur (*P. cinerea*) which also likely occur in northeastern Cambodia, genetic assessments suggest the population discussed here are red-shanked douc langurs (Rawson & Roos, 2008).

Colobines are known to engage relatively frequently in geophagy, defined as the deliberate consumption of soil (Krishnamani & Mahaney, 2000). Of 39 species of primate recorded as engaging in this behavior either in captivity or wild conditions, eight are colobine taxa (Krishnamani & Mahaney, 2000). A number of hypotheses have been put forward to explain the function of geophagy in primates, including: a buffer against toxins in the diet; alleviation of gastrointestinal problems; a dietary mineral supplement that it is used in times of resource scarcity; and as a cultural phenomenon (see Krishnamani & Mahaney, 2000 for a full review).

While relatively common in Colobines, and primate folivores in general, to date this phenomenon has not been recorded in either douc langurs or silvered langurs. Here we describe the first recorded instances of geophaghy in these taxa, documented with camera trap photos in Veun Sai-Siem Pang Conservation Area, northeastern Cambodia.

Materials and Methods

All data come from the Veun Sai-Siem Pang Conservation Area, located in Veun Sai and Siem Pang Districts of Ratanakiri and Steung Traeng Provinces respectively, northeastern Cambodia. The site comprises approximately 55,000 hectares of mainly semi-evergreen/evergreen forest at low elevation and is the subject of conservation partnership between the Forestry Administration, Conservation International, Poh Kao, des Tigres et des Hommes and local communities and authorities.

A significant part of this work is surveying and monitoring wildlife species found at the site. As part of this research we deployed camera traps at a number of locations across the site in order to capture photos of rare and cryptic species. We used Reconxy PC85 units, set to take bursts of three photos with one second intervals when triggered. As part of this work we captured primates utilizing salt licks, and based on this we set one camera at a single salt lick in evergreen forest that primates were known to frequent to gain further information about this behavior. The salt lick (UTM 0689051/1552440) was camera trapped for a total of 127 days between 4th January and 22nd August 2010.

We calculated encounter frequencies for primates that visited the site, with an independent encounter defined as beginning when a period of 30 minutes had expired between photos of the same taxon. A 30 minute interval was necessary as primates often disappeared for long time intervals as they accessed the salt lick, which is partially underground.

All photos were entered into a database, which included information on exact time photos were taken. We transformed times into decimals for comparison of means using Mann-Whitney U-test in SPSS Statistics version 17.0 and then transformed them back for presentation. All other time related statistics were conducted with Oriana version 3.21.

Results

We captured 1226 photos of red-shanked douc langurs on 20 days, constituting 20 encounters at the salt lick over the 127 day camera trapping period. Average encounter frequency over the period was one visit per 6.35 days. Total time spent during the 127 days of camera trapping at the
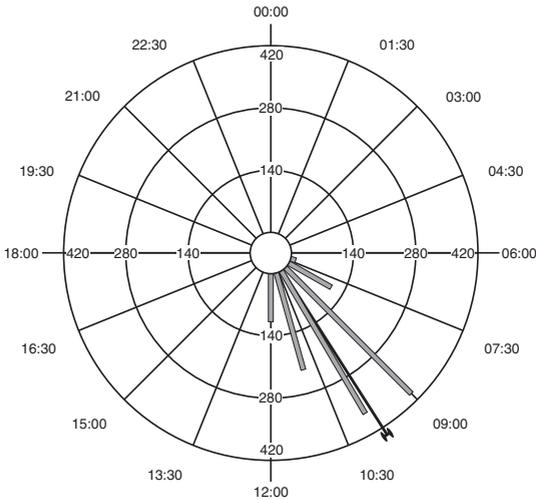
Fig.1. Frequency histogram, by hour, of camera trap photos taken of red-shanked douc langurs. Mean time of records and 95% confidence intervals shown.
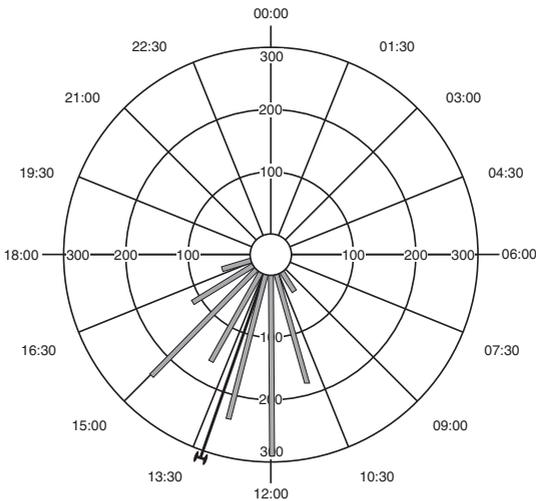

Fig.2. Frequency histogram, by hour, of camera trap photos taken of silvered langurs. Mean time of records and 95% confidence intervals shown.

salt lick for red-shanked douc langurs was 24 hours and 44 minutes with an average visit lasting 1 hour and 14 minutes (SD ± 54 mins 44 secs). Based on an analysis of the time that each photo was taken, we determined that the mean time for photos taken of douc langurs at the salt lick was 09:50 (95% CI of 09:46-09:53; Fig. 1.).

We captured 1320 photos of silvered langurs on 14 days constituting 14 encounters at the salt lick over the survey period. Average encounter frequency over the period was one visit per 9.07 days. Silvered langurs spent a total of 21 hours and 28 minutes at the salt lick, with average visit duration of 1 hour and 32 minutes (SD ± 1 hour 19 minutes). Based on an analysis of the time that each photo was taken, we determined that the mean time for visiting the salt lick was 13:18 (95% CI of 13:12-13:24; Fig. 2.).

Based on comparisons of median time for photos of both species of primate, red-shanked douc langurs and silvered langurs visited the salt lick at significantly different times of day ($U = 65839$, $p < 0.001$, $n_{douc} = 1226$, $n_{silver\ langur} = 1320$, Mann-Whitney U-test). Red-shanked douc langurs visited the salt lick predominantly in the morning and silvered langurs visited in the early afternoon. There was no significant difference between the duration of visits to the salt lick by different primate species ($U = 339.5$, $p = 0.713$, $n_{douc} = 20$, $n_{silver\ langur} = 14$, Mann-Whitney U-test).

Discussion

Geophagy or soil eating has not been recorded in wild silvered langurs or douc langurs, despite several long-term ecological studies at numerous sites (Ha Thang Long et al., 2010; Hoang Minh Duc, 2007; Kool, 1993; Lippold, 1977, 1998; Lippold et al., 2010; Mitani et al., 2010; Phaivanh Phiapalath & Pongthep Suwanwaree, 2010; Rawson, 2009). The phenomenon has however been recorded in several other Asian colobine species (see Krishnamani & Mahaney, 2000 for a review) and has been recorded in douc langurs in captivity (U. Streicher, pers. comm.).

The frequency of visits by both groups of douc langurs and silvered langurs to this salt lick to engage in geophagy suggests that this is a common behavior among these taxa at this site. The behavior has also been identified at several other camera trapped salt licks in Veun Sai-Siem Pang

Conservation Area as part of ongoing survey efforts, suggesting this is not a localized phenomenon. Limited camera trapping of salt licks in the more southerly Mondulkiri Province by WWF has however failed to record primate geophagy, despite the presence of black-shanked douc langurs and silvered langurs at the site (Gray, pers comm.).

For these primates in Veun Sai-Siem Pang Conservation Area, coming to the ground for long periods of time to consume soil may pose a significant risk of predation. Douc langurs and silvered langurs are predominantly arboreal with few published records of terrestrial behavior in douc langurs (Hoang Minh Duc, 2007; Lippold, 1998; Nadler, 2008; Rawson, 2009) and none to our knowledge of silvered langurs, although they doubtless display such behavior. Historically, at least, tiger (*Panthera tigris*) was present at the site, and other predators such as leopard *(Panthera pardus)*, clouded leopard (*Pardofelis nebulosa*), and dhole (*Cuon alpinus*) which still occur at the site may also pose a threat to primates. Leopard and tiger diets in Thailand were found to commonly comprise primates (including arboreal colobines), occurring in 10.4% and 5.0% of fecal samples, respectively (Rabinowitz, 1989). Given that the threat of predation is real, and that both douc langurs and silvered langurs are spending considerable time on the ground (Fig 3. and 4.), the benefits of geophagy must be considerable.

Red-shanked douc langurs and silvered langurs at the site use the salt lick at different times of the day. On only one occasion did both species utilize the salt lick at the same time. Douc langurs were already present at the salt lick when a group of silvered langurs arrived at 09:30. The douc langurs can be seen vocalizing as the silvered langurs arrive, and the douc langurs subsequently moved off and the silvered langurs entered the salt lick area, although the douc langurs quickly returned, led by an adult male. The two groups then continued to use the salt lick, apparently at the same time, for over one hour with no noted conflict, until the silvered langurs moved off around 10:23. It is hypothesized that in general the two species are using the salt lick at different times to avoid competition.

The function of geophagy for colobines in Veun Sai-Siem Pang Conservation Area is unknown, however, theories of soil consumption in primates fall broadly in to two categories; use for alleviation of gastro-intestinal disorders and mineral supplementation of the diet (Krishnamani & Mahaney, 2000). Neither theory can be ruled out without feeding ecology data and chemical analysis of soil samples. It is however unlikely to be related to reproduction as all age and sex classes engage in the practice. Collection and analysis of soil samples will be necessary to attempt to differentiate between hypotheses, however based on photos only, it appears that soil being consumed is kaolin clay (W. Mahaney, pers. comm.).

Salt licks are a congregation location for many mammals in addition to red-shanked douc langurs and silvered langurs, including gaur (*Bos gaurus*), sambar (*Cervus unicolor*) and muntjac (*Muntiacus muntjac*). These areas should be the target of enforcement activity based on their importance and potential level of threat. Snare removal around these areas should be a priority as these could pose a threat to these primates when moving terrestrially as well as other species.

Conclusions

Soil eating or geophagy has been recorded for red-shanked douc langurs and silvered langurs in northeast Cambodia. This is apparently a very common behavior at the site for both taxa, which spend large amounts of time on the ground consuming clayey soils. While it is not clear the role that geophagy has in these species feeding ecology, it is apparently a behavior with significant benefits

Fig.3. Red shanked douc langurs (*Pygathrix nemaeus*) accessing the salt lick. Photo: Conservation International.

Fig.4. Silvered langurs (*Trachypithecus germaini*) accessing the salt lick. Photo: Conservation International.

to these primate populations as large amounts of time engaged in terrestrial activity puts these arboreal primates at increased danger of predation.

Acknowledgments

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Community-based monitoring of southern yellow-cheeked gibbon (*Nomascus gabriellae*) in Da Te forest, Lam Dong Province, central Vietnam

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Summary

Dong Nai Conservation Landscape is situated centrally within the global range of southern yellow-cheeked gibbon and this species was identified as one of the Landscape's conservation foci. Da Te forests represent a critical point in Dong Nai Conservation Landscape in terms a of forest corridor for habitat connectivity and having a number of species of global conservation significance. Two monitoring surveys of southern yellow-cheeked gibbon in a Da Te forest were conducted in December 2007 and December 2008. The surveys estimated average density of 0.36 – 0.41 gibbon groups per km^2 of hearing area. Significant changes in average daily calling rate and cumulative number of calling groups per listening posts between 2 surveys were observed that related to newly increasing forest clearance for rubber plantation during second monitoring survey. A number of recommendations were made to mitigate forest loss and strengthen biodiversity conservation in the area.

Kết quả điều tra giám sát vượn má hung (*Nomascus gabriellae*) dựa vào cộng đồng ở rừng Đạ Tẻh, tỉnh Lâm Đồng

Tóm tắt

Vùng cảnh quan bảo tồn Đồng Nai nằm ở trung tâm vùng phân bố toàn cầu của loài vượn má vàng và loài vượn này đã được chọn là một trong số các trọng tâm bảo tồn của Vùng cảnh quan này. Rừng huyện Đạ Tẻh có vị trí rất quan trọng đối với Vùng cảnh quan bảo tồn Đồng Nai với vai trò là hành lang rừng kết nối sinh cảnh trong Vùng cảnh quan và chứa đựng quần thể của một số loài có tầm quan trọng bảo tồn toàn cầu. Hai đợt khảo sát giám sát quần thể vượn má vàng ở rừng Đạ Tẻh đã được thực hiện vào tháng 12/2007 và tháng 12/2008. Kết quả cho thấy mật độ trung bình của vượn là 0.36-0.41 nhóm/km^2 diện tích vùng nghe. Có sự thay đổi đáng kể giữa 2 lần giám sát về tần số hót trong bình ngày và số nhóm vượn hót ở các điểm nghe do ảnh hưởng của việc khai thác rừng để chuyển sang trồng cao su và các hoạt động kinh doanh khác. Một số kiến nghị đã được đưa ra nhằm giảm thiểu sự mất rừng và tăng cường bảo tồn đa dạng sinh học trong vùng.

Introduction

Southern yellow-cheeked gibbon (*Nomascus gabriellae*) is globally threatened ("Vulnerable" in IUCN Red List, 2008), nationally threatened ("Endangered" in Vietnam Red Data Book, 2007) and endemic to Vietnam, Cambodia and Thailand. In Vietnam, this species is restricted to Southern Central Vietnam, from Gia Lai Province to Dong Nai Province and Tay Ninh Province. Dong Nai Conservation Landscape is situated centrally within the global range of the species, and the species is identified as one of the landscapes 'conservation foci', components of global biodiversity that are priorities for conservation action within the landscape (Pilgrim et al., 2006).

Da Te forests (Fig. 1) are situated in a critical point of Dong Nai Conservation Landscape. Firstly, Da Te forests are contiguous with forest in Cat Tien National Park (Cat Loc sector), that provides an important buffer to the national park. More importantly, Da Te forests provide the forest habitat contiguous with forests from Di Linh highlands to the North and forests from Dong Nai Province in the South. Therefore, Da Te forest is classified as one of areas of the first conservation priority in the Landscape by Biological assessment of the Dong Nai Conservation Landscape (Pilgrim et al., 2006) and "Action plan for Biodiversity conservation of Lam Dong Province, Period 2008-2020". The latter has set an aim to develop Da Te forest as a good forest corridor for habitat connectivity in the landscape. In order to provide scientific background for relevant management of Da Te forests and also for developing Pay-for-Environment Services (PES) policy of Da Te forests, in 2007, WinRock International have developed a Communty-based

Fig.1. Location of Da Te forest.

Biodiversity Monitoring Programme for 6 key wildlife species including the southern yellow-cheeked gibbon. Two monitoring surveys were conducted in December 2007 and December 2008. This report represents the results of southern yellow-cheeked gibbon monitoring.

Materials and Methods

A listening post technique was used for monitoring southern yellow-cheeked gibbon in Da Te forest. Seven listening posts were randomly arranged in Da Te Forests (Quoc Oai and An Nhon Commune); the distance between two consecutive listening points was, at least, 3 km long to avoid overlapping count:

- Listening Post 1: Grassland 1 (forest comp.526); UTM 0773753/ 1288032; Alt. 584
- Listening Post 2: Grassland 2 (forest comp. 519B); UTM 0775498/ 1292877; Alt.630
- Listening Post 3: Đoi Tho area (forest comp. 520); UTM 0777348/ 1292296; Alt. 608
- Listening Post 4: Trang Phao grassland (forest comp. 525); UTM 0776457/ 1289821; Alt. 610
- Listening Post 5: Aircraft grassland (forest comp. 518); UTM 0769941/ 1292383; Alt. 566

- Listening Post 6: Peak 551, near Suoi Dut junction (forest comp. 536); UTM 0769016/ 1288849; Alt. 527
- Listening Post 7: Dang Ji area (forest comp. 536); UTM 0776871/ 1285315; Alt. 448

The survey team consists of 21 people (5 staff of Da Te State Forest Enterprise, 2 staff of Da Te Forest Protection Unit and 14 residents of Quoc Oai and An Nhon Communes who work as commune forestry officials or as members of contracted commune forest protection teams). The survey team received a two-day training on survey technique before the first survey and one-day repeated training before second survey.

Two surveys were conducted in December 2007 and December 2008; each survey lasted for five consecutive days that allowed catching 98.4% gibbon groups heard at each post (following estimation in Cambodia by Rawson et al., in press). During survey time, three persons are quietly posted at each listening post. Listening surveys begin early in the morning 30 minutes before sunrise (5:00 am in December 2007 and 2008) and end three hours after sunrise (8:00 am in December 2007 and 2008). Sunrise time was taken from a GPS unit. When hearing a call the following information was recorded into the survey form:

- Time of calling start of each group.
- Time of calling bout end of a calling of each group. A calling bout is defined as being a series of calls without a break of more than 5 minutes between them. If a group stops calling for more than 5 minutes it should be considered as a new bout.
- Type of calls: solo or duet. Only duet calls are used for analysis.
- Compass bearing to the calling group.
- Estimate distance from the listening spot to the calling group as "near"(< 400 m), medium (400 – 800 m) and far (>800 m).
- Weather conditions at calling start time.
- Weather conditions at sunrise time.

The number of calling groups from each post for each morning is calculated by maximum number of groups heard on the morning or mapping the calls, with groups differentiated based on the direction and timing of calling. A cumulative count of number of calling gibbon groups for five days at each post is determined by mapping callings over five days. Then, an estimated number of gibbon groups for all listening posts (including calling and not calling groups) is estimated using the formula:

$$X = \frac{\sum c_i}{p(m)}$$

x: the estimated number of gibbon groups from all listening posts,

c_i: cumulative number of calling gibbon groups heard in five days period at listening post i,

p(m): calling probability for five day survey period = 0.894 (Rawson et al., in press.),

i: number of the listening post (1…7).

The average density of gibbon groups at listening area is:

$$d = \frac{x}{\Sigma a_i} = \frac{\Sigma c_i}{p(m).\Sigma a_i}$$

d: the average density of gibbon groups in listening area;

a_i: the hearing area of post i which is estimated based on hearing cycle with radius of 1.5 km (longest distance that gibbon calls can be heard from listening post); $a_i = (1.5km)^2 \times 3.1416 = 7.0676$ km^2 (some adjustment from real terrain conditions may be necessary).

Due to low number of listening posts, total gibbon population number for whole area of Da Te forest will not be estimated and average density (d) will be used for comparison between surveys. Additional index for comparison can also average daily calling rate (R) which can be calculated using following formula:

$$Ri = \frac{\Sigma Gk}{n}$$

Ri: average daily calling rate of gibbon at listening post i;

Gk: number of recorded calling groups for each survey day at post i (k = day 1 to day 5)

n: number of survey days (n = 5).

Results and Discussion

Summary results of 2007 and 2008 gibbon monitoring surveys are presented in Table 1, Fig. 2 and 3 which include average daily calling rate for each listening post, mean value of average daily calling rate from all listening posts, cumulative number of calling groups from each listening post, total cumulative number of calling groups from all listening posts and average density of gibbon groups in hearing areas.

Table 1. Comparison of results of 2007 and 2008 monitoring surveys.

Listening post	Average daily calling rate (group/day)		Cumulative number of calling groups (group)	
	2007	**2008**	**2007**	**2008**
Post 1	1.8	0.2	3	1
Post 2	3.4	1.4	4	3
Post 3	2.4	3.0	4	5
Post 4	2.2	4.0	3	5
Post 5	1.6	0.8	2	1
Post 6	0.8	0.6	2	1
Post 7	0.0	0.0	0	0
Total			**18 groups**	**16 groups**
Mean	**1.72 groups/day**	**1.43 groups/day**		
Average density of gibbon groups in hearing areas			**0.41 groups/km^2**	**0.36 groups/km^2**
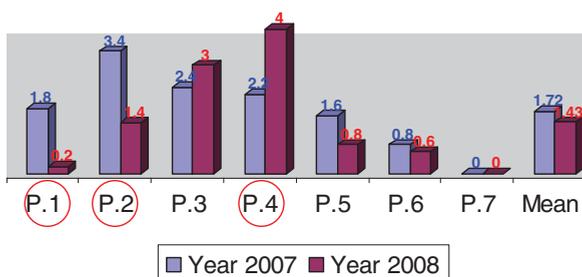
Fig.2. Change in average daily calling rates of gibbon groups in 2008 vs. 2007.
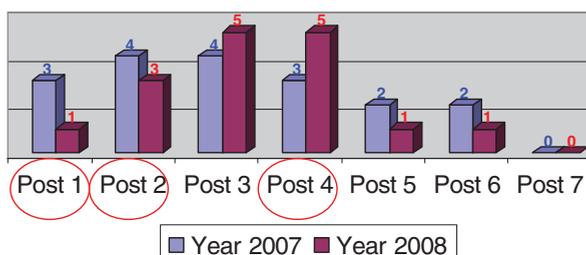
Fig.3. Change in cumulative number of calling gibbon groups.g rates of gibbon groups in 2008 vs. 2007.

Total cumulative number of calling groups is 18 for 2007 and 16 for 2008 and estimated density of gibbon groups per hearing area is 0.41 groups/km^2 for 2008 and 0.36 groups/km^2. This difference is not significant and does not mean that gibbon population in 2008 is less than those in 2007. The difference is very likely due to lower calling rates of the gibbon population during 2008 survey time than those in 2007. This no loss of gibbons is also supported by facts that no evidence of gibbon killing were recorded during period from December 2007 to December 2008 based on data from Da Te Forest Protection Unit (FPU), Da Te Forest Company (FC) and our interviewing local residents.

The monitoring results show significant change in average daily calling rate and cumulative number of calling gibbon groups. Both 2007 and 2008 surveys occur in December with very similar weather conditions, so seasonal change and weather-leading change can be ignored. This change obviously comes from different level of human impacts on forest. The forest disturbance during 2008 survey time is much higher during 2007 survey time. This relates to change in Da Te forest management system. In 2007, forest in monitoring area (Quoc Oai and An Nhon Communes) was under management of single Da Te State Forest Enterprize (SFE) and only small scale selected bamboo cutting occurred. In 2008, Da Te SFE was reformed into Da Te Forest Company and about 3,578 ha of forest in monitoring area has been allocated to 3-4 private companies for rubber tree plantation and other business purposes. Large portion of allocated forests is under clearing for growing industry trees (rubber trees, etc.). The forest clearing by these companies significantly stimulates illegal forest cutting and tree logging by local villagers. Consequently, the forests in the monitoring area are very disturbed by human encroachment, noise of timber cutting and forest destruction. The forest disturbance causes change in the home range location of gibbon groups (some gibbon groups have to move from disturbed areas to new safe areas) and depresses calling rate of the gibbon groups which results in smaller number of calling groups recorded in 2008.

The changes in average daily calling rate and cumulative number of calling groups are

significant for listening posts 1, 2 and 4. This clearly reflects the impacts of forest disturbance during monitoring survey. The reduction of average daily calling rate and cumulative number of calling groups from listening posts 1 and post 2 is due to increased forest disturbance by two private companies (Toan Xa and Dinh Thuan Companies) which conducted forest clearing during survey times, while increase of average daily calling rate and cumulative number of calling groups from listening posts 4 and possibly also from post 3 is due to movement of some gibbon groups from disturbed areas (Post 1 and 2) to these more safe areas. The reduction in post 5 and 6 is unclear, level of forest disturbance in this area was high in 2007 due to bamboo logging and remains high in 2008 due to increased human encroachment for private company establishment and illegal forest clearance and recorded calling rate is low for both years.

We could access only two studies with estimation of southern yellow-cheeked gibbon density using listening posts method. One in Cat Tien NP conducted by Technical Unit of Cat Tien NP in Cat Tien NP with technical assistance from Cambridge University and Ping Tung University (Cat Tien National Park, 2005) and another in Seima Biodiversity Conservation Area (SBCA), Mundulkiri Province, Cambodia (Rawson et al., in press).

Using a similar method of gibbon census and data analysis in SBCA (Cambodia) (Rawson et al., in press) obtained average density of southern yellow-cheeked gibbons in SBCA 0.71 ± 0.07 groups/km^2. This figure is almost twice as high as our estimated density of southern yellow-cheeked gibbon in Da Te forest area (0.36 - 0.41 groups/km^2). Explanation for this may be better forest quality and fewer disturbances in SBCA in comparison with Da Te Forest.

In Cat Loc (a section of Cat Tien National Park) which shares border with Da Te forests, Kenyon (2007) and technical staff of Cat Tien conducted gibbon census using listening post method but with some differences in listening posts arrangement and gibbon call count techniques (Cat Tien National Park, 2005). In total, 55 gibbon groups were recorded from 357 km^2 of Cat Loc sector that gives average density of about 0.15 groups/km^2. This figure is much lower than our estimated density in Da Te monitored forest area (0.36 - 0.41 groups/km^2). This difference can be explained partly by the method modification; the density was estimated for whole Cat Loc Section of Cat Tien NP not for hearing area which is much smaller than the Cat Loc Section as whole.

Due to low number of listening posts, the estimated density in this study (0.36 - 0.41 groups/km^2) does not represent the gibbon density in total for the Da Te forest. However, this density indicates that Quoc Oai and An Nhon forests can support a population of gibbons and thus, Quoc Oai and An Nhon forests represent an important habitat extension for southern yellow-cheeked gibbons which inhabit in Cat Loc Section of Cat Tien NP and other surrounding forest area.

Conclusions and Recommendations

Two monitoring surveys for southern yellow-cheeked gibbon in Da Te forest (Quoc Oai and An Nhon communes) give estimated average density of 0.36 – 0.41 gibbon groups/km^2 of hearing area. This indicates the importance of Da Te forest as habitat expansion and corridor for gibbon populations from Cat Tien NP and adjacent forests. In order to protect the gibbon population and also to secure Da Te forest as forest corridor for the Dong Nai conservation landscape following activities need to conduct:

- Da Te FPU, Da Te FC, local authorities should increase its forest protection activities (regular patrol, inspection and enforcement) to prevent illegal forest destruction by local residents and forest over logging by private companies. Forest protection and biodiversity conservation

regulations should be acknowledged to all new forest owners for strict obey. In regard to forest areas, which are planned for conversion into plantation, all processes of forest cutting, infrastructure construction should be strictly monitored to mitigate negative impacts on local environment and biodiversity.

- An educational programme should be conducted to increase awareness of workers and staff members of the new companies on importance of Da Te forests for environmental protection and biodiversity conservation, national legislation on environmental protection and biodiversity conservation.

- Allocation of some area of Da Te forests to private companies for rubber plantation and other business activities will weaken the forest corridor function of Da Te forest, however, the corridor still remains and an effort should be made to maintain this corridor as much as possible. The forest product resources and biodiversity values of remained forests should be inventored and assessed to develop sustainable use planning and relevant biodiversity conservation action.

Acknowledgements

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Population status of Francois' langur (*Trachypithecus francoisi*) at Ba Be National Park

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Key words: Francois' langur, *Trachypithecus francoisi*, Distribution, group size, threats

Summary

The Francois' Langur (*Trachypithecus francoisi*) is an endangered primate belonging to subfamily Colobinae. The species was historically widespread in seven northern provinces of Vietnam. Ba Be National Park is located in Vietnam's northern mountainous area, and is a home to several small groups of Francois' langurs. The objective of these surveys is to reveal distribution, group size and threats to the species and its habitat. Two status surveys were undertaken with a total of 21 field survey days between 13. to 26. February and 16. to 22. November 2009 at Ba Be National Park. Sightings of Francois' langurs were very rare and only two groups of two and four individuals respectively detected in Pac Ngoi. Hunting and habitat destruction are the main threats to langurs. Illegal logging poses a serious threat to the habitat, while shotguns and traps were identified as the main methods for hunting in the area. Immediate actions need to be taken to conserve the remaining small populations of Francois' langurs in Ba Be National Park.

Tình trạng quần thể Voọc đen má trắng (*Trachypithecus francoisi*) tại Vườn Quốc gia Ba Bể

Tóm tắt

Voọc đen má trắng (*Trachypithecus francoisi*) là loài Linh trưởng nguy cấp thuộc họ phụ Voọc (Colobinae). Trước đây loài này phân bố ở 7 tỉnh phía Bắc của Việt Nam. Vườn Quốc gia Ba Bể thuộc khu vực vùng núi phía Bắc Việt Nam và là nơi cư trú của một vài nhóm Voọc đen má trắng. Mục tiêu của đợt điều tra này là làm sáng tỏ phân bố, kích cỡ nhóm và các mối đe dọa đến loài và sinh cảnh của chúng. Hai cuộc điều tra được thực hiện với tổng số 21 ngày điều tra thực địa từ 13-26 tháng ba và 16 đến 22 tháng 11 năm 2009 tại Vườn Quốc gia Ba Bể. Quan sát trực tiếp Voọc đen má trắng là hiếm, chỉ có 2 nhóm khoảng 2 đến 4 cá thể được nhìn thấy tại khu vực Pắc Ngòi. Săn bắn và phá hủy sinh cảnh là các mối đe dọa chính đến loài Voọc đen má trắng và sinh cảnh của chúng. Khai thác trái phép là mối đe dọa nghiêm trọng đối với sinh cảnh của Voọc đen má trắng, trong khi đó súng và bẫy là công cụ chính được sử dụng để săn bắt Voọc đen má trắng tại đây. Các hành động cần thiết cần thực hiện ngay để bảo tồn các quần thể nhỏ Voọc đen má trắng còn lại tại khu vực điều tra.

Introduction

The Francois' Langur (*Trachypithecus francoisi*) is listed as an endangered primate species (IUCN, 2010). The species is protected in Vietnam under Decree 32/2006/ND-CP and is classified

as "Endangered" in the Red Data Book of Vietnam (Ministry of Science, Technology, and Environment (2007).

The distribution of Francois' langurs ranges from the Red River in Vietnam across the Chinese border to as far as the Daming Hills in Guangxi and Xingyi in Guizhou. It is restricted to habitats characterized by karst topography with plentiful cliffs (Groves, 2001). The species was historically widespread in seven northern provinces of Viet Nam (Lang Son, Cao Bang, Thai Nguyen, Bac Kan, Ha Giang, Tuyen Quang and Lao Cai Provinces) (Pham Nhat, 2002). Due to habitat loss and hunting for food and commercial sale, populations now only occur in four provinces (Lang Son, Ha Giang, Bac Kan and Tuyen Quang) (Pham Nhat, 2002; Nadler et al., 2003). All remaining populations are small (<50 individuals), isolated, and vulnerable to extinction (Nadler et al., 2003). The population estimates for China is about 1,400 to 1,650 individuals (IUCN, 2010), whereas the remaining population in Vietnam is estimated to be less than 300 individuals (Nadler et al., 2003). Main threats to the Langurs are hunting and habitat destruction. (Gang Hu et al., 2004; Li Youbang et al., 2007; Nadler et al., 2003).

Ba Be National Park is located in Vietnam's northern mountainous area and is home to several small groups of Francois' langurs. According to previous reports, the maximum number of individuals in a group recorded in Ba Be National Park did not exceed six individuals (Nadler et al., 2003). However, verbal reports from local communities indicated the existence of about 13 individuals in the Dau Dang karst massif (F. Potess, pers. comm., 2009). A survey on the population status of *T. francoisi* in Ba Be aims to determine population status of *T. francoisi* at Ba Be National Park and identify current threats to the species and its habitat.

Methods

Description of the survey area

Ba Be National Park is located in Ba Be District, Bac Kan Province (Fig. 1.). The national park comprises 7,608 ha of which about 85% is forested. The park is divided into three functional zones, comprising a strict protected area of 3,226 ha, a forest rehabilitation area of 4,082 ha, and administration area of 300 ha (Committee, 2001; Bui Van Dịnh, 2003). Steep limestone hills and valleys characterize the topography of the park. The elevation ranges between 150 to 1,121 m asl, with the highest peak Cang Lo. Many limestone caves are found along the steep cliffs, with Puong cave at 300 m in length being the largest one.

There are mainly three types of forest in Ba Be National Park: forest associated with limestone hills and mountains, evergreen forest, and bamboo forest. Limestone forest occupy most parts of the park and feature thick vegetation cover, while evergreen forest are distributed on low earthen hills covered with a thicker soil layer. The fauna of the Ba Be National Park is composed of 65 mammals, 214 birds, 46 reptiles and amphibians, and 87 fish species (Bui Van Dinh, 2003; Pham Nhat, 2003). Among the list, 55 species are recorded in the Vietnam Red Data Book. The occurrence of Francois' langurs has given the park greater importance as a national and international conservation area.

Data collection

The survey was separated into two missions. The first mission was implemented between 13. to 26. February 2009 at Dau Dang and Pac Ngoi and the second from 16. to 22. November 2009 at Keo Cạp, Pac Ngoi, Ta Han. The focus was on the four possible occurrence areas of the langurs:

Fig.1. Location of Ba Be National Park in northern Vietnam.

Dau Dang area (Khau Qua, Nam Dai, and Khau Cum), Keo Cạp (Tang Tang, Na Dien, and Khau Cum), Pac Ngoi (Choc Thep, Na Phoon, and Lung Quang), and Ta Han. I surveyed for 216 hours and the total area covered was 47 km^2.

Interviews

A diverse number of local people ranging from villagers, hunters, and rangers belong to national park staff were interviewed before surveys commenced. Key informants were determined by who had seen the Francois' langurs in recent times. The purpose of the interviews was to collect general information on family groups and population of the species, diet, habitat preferences and current and past distribution in the area.

Species Presence/Absence Surveys

Existing trails, transects, and reported sleeping sites were used to survey the presence/absence of family groups of Francois' langurs. The presence/absence of the species was determined from both direct and indirect evidence and utilizing the methods documented in Ross & Reeve (2003):

- Direct observation of animals;
- Indirect observation of characteristic signs (tracks, faeces, feeding signs, vocalizations, etc.);
- Observation of animals captured or killed (care should be taken to ascertain where the animals were obtained); and
- Reports from local communities.

Group size and composition

Given the small population of Francois' langurs at Ba Be National Park, opportunistic census was used to count the total number of animals in groups encountered. More effort was paid to the sleeping sites early in the morning and late in the afternoon, since this allowed observers to have good visible count of a whole group of animals, and to determine their age, sex, and composition of the group.

Sleeping site surveys

Local reports, indirect and direct evidence, as well as full-day follow were used to determine current sleeping sites of the langurs. Attempts were also made to search for caves on the middle or tops of the cliffs and brown deposits of langur excrement just below the caves (Chengming Huang et al., 2002; Qihai Zhou et al., 2007). GPS and topographic maps were used to get locations and mark sleeping sites of the species on the map.

Threats

Information on the presence of traps, snares, guns, crossbows, camps, hunting dogs, forest clearance, timber-cutting, huts, non-timber forest product collection, and livestock grazing were recorded during the surveys to assess the human impact on Francois' langurs and its habitat, as well as on wildlife as a whole.

Results

Distribution and group size of Francois' langurs

Sighting of langurs during the survey were very rare. Most information on distribution and group sizes were based on local reports (Fig. 2). Details of local people's observation of the langurs during the survey, as well as their location and group size, are shown in Table 1.

Langurs were sighted twice in the Pac Ngoi area during the surveys. A group of four individuals was observed on 22. February 2009 (0564901E/2476206N) close to the Pac Ngoi cliff. The group included one adult male, one adult female, one juvenile, and one infant. Another group of two adult–size langurs were also seen on 20. November 2009 in Pac Ngoi area (0565280E/2475689N). The observers

Fig. 2. Records of Francois' langurs (*Trachypithecus francoisi*) in Ba Be National Park.

were unable to determine the composition of the group because observation duration was too short. The actual group size of these groups is likely to be larger, since the observers might have missed some animals hidden in the dense foliage.

Based on local reports, there seems to be another small group of six to eleven animals residing in Pac Ngoi area. Old faeces of langurs were found at Na Phoon cliff (0563623E/2476201N) where according to locals from Nam Cuong Village, a group of 6 animals including 2 juveniles spent approximately 10 days in September 2009.

Table 1. Records of Francois' langurs (*Trachypithecus francoisi*) at Dau Dang, Ta Han, Na Phoon, and Pac Ngoi areas, Ba Be National Park. (Historical records in Ba Be National Park prior 1989 see Nadler et al., 2003).

No.	Date	Sites	Locations (WGS84)	Group sizes	Evidence	References
1	August 1989	Dau Dang	?	?	One hunted individual	Ratajszczak et al., 1990
2	1994	Puong cave	?	?	?	Kemp et al., 1994
3	1996	Nam Dai (outside national park)	?	4 - 5	Sighting	Hill et al.,1996
4	April 1999	Pac Ngoi	?	6	Sighting	Nong The Dien, pers. comm.. 2000 (cited in Nadler et al., 2003)
5	2004	Dau Dang	?	13	Sighting	Potess, F., pers. comm. 2009
6	2009	Dau Dang	?	4-6	Sighting	T.Nadler, pers.comm. 2009
7	Dec. 2008	Na Phoon	0563541E 2475872N	4-6	Sighting	Hoang Phuc Thanh, Nam Cuong Village, pers. comm..
8	June 2008	Na Phoon	0563541E 2475872N	11	Sighting	Nguyen Van Tam, Na Ban ranger station, pers. comm..
9	10. Jan. 2008	Dau Dang	0560543E 2482765N	1 (4)	Sighting	Dong Van Cu, Dau Dang Village, pers. comm..
10	12. Jan. 2008	Dau Dang	0560543E 2482765N	4-6	Sighting	Nong Thi Moi, Dau Dang Village, pers. comm..
11	22. Feb. 2009	Pac Ngoi-Choc Thep	0564901E 2476206N	4	Sighting	This survey
12	April 2009	Ta Han cliff	0561043E 2477336N	1	Sighting	Hoang Van Khoanh, Coc Toc Village, pers. comm..
13	August 2009	Dau Dang	0560543E 2482765N	2	Sighting	Dong Van Cu, Dau Dang Village, pers. comm..
14	Sep. 2009	Na Phoon	0563623E 2476201N	6	Sighting	Hoang Phuc Thanh, Nam Cuong Village, pers. comm..
15	20. Sep. 2009	Pac Ngoi	0565280E 2475689N	2	Sighting	This survey

No signs of langurs were observed in other surveyed areas (Dau Dang, Keo Cap, Ta Han). However, groups of two to six animals have been recently seen by local people in these areas (Table 1). In Ta Han area, one adult-size animal was seen in Ta Han Cliff (0561043E/2477336N) in April 2009. The solitary animal spent two days in this area, emitted loud vocalizations and then disappeared (Hoang Van Khoanh, pers. comm.).

Sleeping sites

No sleeping sites of the langurs were determined during the survey, though efforts were made at dawn and dusk to search for possible sleeping sites. For instance, search was carried out at caves at middle or top of the cliffs near deep brown deposits of langur excrement in Dau Dang and Pac Ngoi cliffs. Two possible sleeping caves are located at Na Phoon cliff (0563623E/2476201N and 0563541E/2475872 N), where both local people and a forest ranger have seen langurs entering

the cave last year (Tam Thanh, pers. comm.). Furthermore, old faeces were also seen on the ground below the former cave. However, it is believed the langurs may have abandoned the later sleeping cave since 2008 due to the construction of an ecotourism road to Na Phoon Cave. This observation was confirmed by locals who admitted they had not seen the langurs since the roadwork started.

Threats to the Francois' langurs

Information on the presence of human activity in the forests was also recorded during the surveys. Signs of human activity can generally be grouped into two main categories: hunting (hunters encountered, gunshots heard, dogs, and campsites for hunting) and habitat destruction (illegal logging, stacked timber, campsites for logging, fire wood and bamboo shoot collection, livestock grazing and cutting trees for grazing).

Hunting

Hunting activities appeared to remain in some parts of the survey areas. The team encountered hunters in Pac Ngoi area. Gunshots were heard several times during the first few days of each survey mission in all survey areas, and appeared to reduce during the proceeding days probably due to the presence of the surveyors and park rangers. The use of guns for hunting seemed to be more prevalent and extensive in Dau Dang, Kep Cạp and Ta Han areas than at Pac Ngoi. Old hunting campsites were observed in all survey areas.

Hunting signs were generally found in the more remote and difficult (terrain wise) areas, which hold better quality forest habitat, are more remote from established ranger stations and are less regularly visited by park rangers.

Direct evidence of killed langurs was not found during the survey. Local people reported that they once used to kill langurs to consume their meat and to make a medicine from their bones, called "cao". This product can be used for domestic family medicinal purposes, sold in locals markets and to traders. The purposes of hunting langurs may have changed from the past, as a whole animal can be sold now for VND 200.000/kg (ca. USD10/kg). It is also believed that the gall bladders of the langurs are of higher quality than those of bears for customary medicinal purposes. This may create a demand on langurs.

Habitat Destruction

Habitat destruction observed in some parts of the forest, had an impact on the forest integrity. Evidence of habitat destruction observed included trees cut for timber, stacked timber boards, campsites for logging, well used trails for transporting logs, non-timber forest product collection, livestock grazing and cutting trees for grazing fodder.

Trees cut for timber and stacked timber boards were encountered along existing trails in the forest. Much of the felling was undertaken with the use of chainsaws rather than with traditional pit sawing methods. Illegal logging often takes place at night or early morning and is very difficult to control since only a small number of park rangers are available on site. The trees cut are often large and valuable timber species such as Tong Du (*Toona sinensis*), Nghien (*Burretiodendron hsienmu*).

Livestock grazing and cutting trees for grazing are also having an impact on the habitat of the langurs and wildlife in general. This activity is far less widespread than hunting and illegal logging. Livestock grazing often takes place at abandoned cultivations and lower elevation sites in the

forest. In most cases, the tree cut for grazing is *Streblus brenieri*.

Bamboo shoot collection by local people occurs between June and September in the Keo Cạp area where there are several patches of bamboo forest. Collectors harvested fresh bamboo shoots and often dried the vast majority of product inside the forest. Dried bamboo shoot products are then transported out of the forest and sold to traders or in local markets, attracting a local value of VND 70-80.000/kg.

Firewood collection is also traditional and cultural customs of ethnic minority groups. Several times the survey team encountered groups of three to six people cutting firewood inside known langur habitat in Pac Ngoi area.

Discussion

Distribution and group size

The results indicate that the distribution of langurs is now restricted to three areas: Dau Dang, Pac Ngoi, and Ta Han. Records are extremely rare, only 15 records in 20 years (Table 1). Group size is also extremely low compared with those in reports on surveys in China. Historically, Francois' langurs were found in a number of areas within the Ba Be National Park (Nadler et al., 2003). The mean group size of Francois' langurs in China is seven (Li Youbang et al., 2007) and mean densities are 0.6 - 1.2 individuals/km^2 (Gang Hu et al., 2004). A possible explanation for these differences is the degree of pressure from threats (especially hunting) to the langurs. Encounter rates of signs of human impacts are high.

Threats to Francois' langurs

The results of the surveys show that hunting and habitat destruction are the main threats to the species. Hunting for meat and traditional medicine has been recorded in previous reports (Nguyen Xuan Dang et al., 2006; Li Youbang et al., 2007; Nadler et al., 2003). Although there was no direct evidence of hunting langurs during the survey, however according to local reports the species used to be hunted for food and traditional medicine purposes. The langurs are usually shot or trapped. Information supplied by local people indicates that shotguns have been and perhaps continue to be used to shoot langurs. Traps have not previously been reported. However, reports from local sources indicate that one individual was trapped last year in the Pac Ngoi area.

Habitat destruction is also an important factor contributing to the decline. Habitat destruction generally takes the form of logging, subsistence farming, grazing, firewood collection and non-timber forest product collection.

Conclusions

The distribution of Francois' langurs appeared to be restricted to three locations within Ba Be National Park: Dau Dang, Pac Ngoi and Ta Han areas. Sightings of langurs are very rare. Two groups of two and four animals were detected during this survey in the Pac Ngoi area. Group size seems to be lower than that in China. These surveys appear to suggest a downward trend of population numbers of Francois' langurs in Ba Be National Park

Like in other primate populations, hunting and habitat destruction are the main threats to species and its habitat. Illegal logging poses a serious threat to the habitat of Francois' langurs, and shotguns and traps are identified as the main methods used for hunting. Both activities were often observed in remote and difficult access areas where the influence and presence of park rangers is irregular due to limited number of staff.

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Primate Conservation in Indochina:
Ex-situ versus in-situ conservation – Analysis of the most viable option against primate population declines

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Summary

In primate conservation two conservation strategies are applied: *ex-situ* and *in-situ* conservation. *Ex-situ* conservation includes the caring for animals in facilities maintained by humans, whereby *in-situ* conservation focuses on protecting flora and fauna in their original habitat. While *in-situ* conservation aims at securing the functioning of whole ecosystems, ex-situ conservation normally focuses on certain species. Primate populations phase two major threats, namely hunting and habitat disturbance (commercial forestry, land encroachment through mining, agriculture, hydroelectric projects).

A small number of *ex-situ* primate conservation projects exist in Indochina (Cambodia, Laos, and Vietnam). The analysis of the centres concludes that *ex-situ* conservation is rather costly, but through these projects other significant functions are carried out such as, environmental education, an increase in awareness, research and capacity building

The rapidly decreasing primate populations make calculations of the minimum viable population size necessary and call for species recovery plans. The analysis reflects that small group sizes, the occupation of small ecological niches and long reproductive cycles are typical characteristics of the critically endangered Cat Ba langur (*Trachypithecus p. poliocephalus*), the Delacour's langur (*Trachypithecus delacouri*) and the Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) among others.

Bảo tồn thú linh trưởng ở Đông Dương:
Bảo tồn nguyên vị hay bảo tồn chuyển vị - Phân tích về các giải pháp khả thi nhất để bảo vệ các quần thể thú linh trưởng đang suy giảm

Tóm tắt

Có hai chiến lược về bảo tồn đang được sử dụng trong bảo vệ các loài linh trưởng: bảo tồn chuyển vị và bảo tồn nguyên vị. Bảo tồn chuyển vị là việc chăm sóc, nuôi dưỡng các loài động vật tại những cơ sở nuôi nhốt bởi con người, trong khi đó bảo tồn nguyên vị là việc duy trì quần thể động vật ngay tại vùng sống nguyên thủy của loài. Trong khi bảo tồn nguyên vị tập trung vào việc bảo vệ các chức năng của cả hệ sinh thái, bảo tồn chuyển vị thường tập trung vào những loài nhất định. Quần thể thú linh trưởng đối diện với hai mối đe dọa chính là việc săn bắt và tác động lên hệ sinh thái (khai thác gỗ thương mại, lở đất do khai khoáng, đất nông nghiệp và các dự án thủy điện).

Hiện có một số nhỏ các dự án bảo tồn chuyển vị cho thú linh trưởng hoạt động ở vùng Đông dương (Campuchia, Lào, và Việt Nam). Phân tích hoạt động của các trung tâm cho thấy bảo tồn chuyển vị rất tốn kém, bù lại các trung tâm này có vai trò đáng kể trong việc nâng cao nhận thức bảo tồn, giáo dục môi trường, nghiên cứu và xây dựng năng lực.

Sự suy giảm số lượng một cách nhanh chóng các quần thể thú linh trưởng trong khu vực khiến cho vai trò của các quần thể nhỏ tăng lên và đòi hỏi những kế hoạch cụ thể để phục hồi chúng. Những phân tích trên các đối tượng loài như vọc Cát bà (*Trachypithecus p. poliocephalus*), vọc mông trắng (*Trachypithecus delacouri*), và vọc mũi hếch (*Rhinopithecus avunculus*) đã cho thấy tình trạng chung là các bầy đàn có kích thước nhỏ, vùng sinh thái hẹp, nhưng chu kỳ sinh sản lại dài. Đây chính là những thử thách cần phải được xem xét cẩn trọng khi ra quyết định về chiến lược bảo tồn phù hợp cho từng loài.

Introduction

Indochina has one of the greatest concentrations of endangered primates along with West Africa, the Atlantic forests of Brazil and Madagascar (Mittermeier, 2004). In this context the strict political definition of Indochina is used which comprises the former colonial French Indochina, including Cambodia, Lao People's Democratic Republic (Laos) and Vietnam.

The region hosts seven of the 25 global most endangered primate species (Mittermeier et al., 2009).

As fruit-eaters (frugivores), leaf-eaters (folivores) and insect-eaters (insectivores); primates play a significant role in ecosystems. They act as seed dispersers, pollinators, stimulate plant growth and encourage increased branching of forest trees (Oppenheimer & Lang, 1969; Chapman, 1995; Cowlishaw & Dunbar, 2000).

The status and distribution areas of primate species in Vietnam, Cambodia and Laos were not known until about 20 years ago. Due to the decreasing primate populations in the wild and a high number of confiscated animals appearing through the wildlife trade, *ex-situ* projects were started. Currently only a few wildlife rescue centers exist in Indochina. Potential conservation strategies include captive breeding, translocation and reintroduction versus *in-situ* protection. The research thesis is based on the hypothesis: "*Ex-situ* conservation programs contribute to the survival of primate species in Indochina."

Ex-situ conservation programs herein include the activities of zoos, wildlife rescue centers and other private facilities/initiatives that hold primates outside their original habitat. A special focus is allocated to wildlife rescue centers.

Material and Methods

The research is based on data collection by the following means:
- Site visits including observations, photographs and semi-structured interviews using a questionnaire,
- Literature research, and
- Semi-structured interviews of research institutes, governmental institutions, non-governmental organizations and other conservation bodies involved in primate conservation in Indochina.

The research work analyses *ex-situ* programs according to the following set of criteria:
- Main aims and future vision of the facilities,

- Operation of captive breeding programs, availability of semi-wild facilities or release programs into the wild,
- Engagement of rescue centers in other fields such as capacity building for rangers, environmental awareness raising or research,
- Species held (including conservation status), origin of primates and numbers of individuals,
- Successful captive breeding, including number and species of offspring raised, and
- Counterpart set-up with governmental or other institutes to ensure ownership and sustainability.

Semi-structured interviews were conducted according to a questionnaire including the above mentioned criteria. Table 1 show which *ex-situ* facilities were visited.

In addition to the research on the analysis of *ex-situ* conservation programs; primate population dynamics, primate ecology in Indochina, and the socio-political and regulatory framework in Indochina were analyzed. In the context of the analysis of extinction processes and minimum viable populations the population dynamics of three Indochinese primates (Fig. 1) were conducted:

Cat Ba langur (*Trachypithecus p. poliocaphalus*),

Delacour's langur (*Trachypithecus delacouri*), and

Tonkin snub-nosed monkey (*Rhinopithecus avunculus*).

Table 1. Site visits to ex-situ conservation facilities.

Number of sites	Name of rescue centre	Country
1	Endangered Primate Rescue Center, Cuc Phuong National Park	Vietnam
2	Gibbon Rehabilitation Project, Phuket	Thailand
3	Singapore Zoo	Singapore
4	Angkor Centre for Conservation of Biodiversity	Cambodia
5	Phnom Tamao Wildlife Sanctuary	Cambodia

Fig.1. Three critically endangered primate species whose population dynamics were analyzed:
   Cat Ba langur (*Trachypithecus p. policephalus*). Photo: Tilo Nadler;
   Delacour's langur (*Trachypithecus delacouri*). Photo: Tilo Nadler;
   Tonkin snub-nosed monkey (*Rhinopithjecus avunculus*). Photo: Le Khac Quyet.

Fig.2. Red-shanked douc langur (*Pygathrix nemaeus*) at Singapore Zoo, March 2007. Photo: Marietta Sander.

Results

The field visits and semi-structured interviews at three animal rescue centres focused on the objectives of the *ex-situ* facilities, reintroduction measures, and offspring of endangered primates, visitor numbers and the costs incurred. The facilities included the Angkor Centre for Conservation of Biodiversity (Cambodia), the Endangered Primate Rescue Center (Vietnam) and Phnom Tamao Wildlife Sanctuary (Cambodia).

For all three facilities animal rescue is a crucial objective. As a result of the high international wildlife trade in the region, the core tasks of the facilities are elementary health care and quarantining after rescue. Outreach programs and awareness raising for visitors through guided nature walks, photo exhibitions in the capital and conferences are important outputs for all three centers. The Endangered Primate Rescue Center in Vietnam produces the most offspring of endangered and critically endangered primates. Due to the centre's focus on primates it hosts the largest number of endangered primates and is regarded as the most experienced. The other facilities take in a large number of species, including reptiles, amphibians, birds and mammals and are thus lacking the specialization and knowledge on specific endangered primate species.

In 2002 the IUCN compiled technical guidelines on the management of *ex-situ* populations. Herein, suggested core activities include:

- Increasing public and political awareness and understanding of important conservation issues and the significance of extinction,
- Coordinated genetic and demographic population management of threatened taxa,
- Reintroduction and support to wild populations,
- Habitat restoration and management,
- Long-term gene and bio-material banking,
- Institutional strengthening and professional capacity building,
- Appropriate benefit sharing,
- Research on biological and ecological questions relevant to *in-situ* conservation, and
- Fundraising to support all of the above.

The three *ex-situ* facilities comply with the recommendations made by the IUCN. The long term gene and bio-material banking is only conducted at the Endangered Primate Rescue Center in cooperation with German Primate Centre.

The analysis of the population dynamics of the three critically endangered primate species (Cat Ba langur, Delacour's langur, Tonkin snub-nosed monkey) reflects that they are close to becoming extinct. Cowlishaw & Dunbar (2000) illustrate species extinction traits which include dietary specialization, slow reproductive rates, large body size, small group sizes, the occupation of small ecological niches, long generation times and poor speed of demographic recovery. The three analyzed primate species all comply with these traits. Additional severe human induced threats (land encroachment, habitat disturbance, hunting) increase the pressure.

Discussion

The research hypothesis "*Ex-situ* conservation programs contribute to the survival of primate species in Indochina" can be confirmed. Site visits and the semi-structured interviews supported the core functions of the analyzed *ex-situ* conservation programs as:

1. Population management of threatened species through research and care,
2. Reintroduction and support to wild populations,
3. Increasing public and political awareness, and
4. Capacity building of national conservation experts.

Thus, the centres contribute to the survival of primate species in Indochina. However, the human induced habitat changes and resulting conservation threats put such a pressure on the primate species that the efforts of the *ex-situ* conservation facilities cannot keep up with the demand. *Ex-situ* conservation including captive breeding is costly, time intensive and requires large areas. A cost-benefit analysis as in the business world is a challenge. However, without a doubt, *ex-situ* conservation facilities fulfill a key conservation role in gathering information for their keeping, population management and reintroduction.

Also, the outreach activities are crucially important to create awareness among the population for conservation.

Conclusions

Primate conservation requires an interdisciplinary approach, political will, species-specific conservation strategies and sufficient resources. The recommended primary conservation strategy is *in-situ* protection of primates in their original habitat. Enhanced efforts are required to improve an effective protected area management system, in capacity building of staff and in protecting the *in-situ* conservation strategies. Effective *in-situ* conservation can guarantee the safeguarding of whole ecosystems and the inhabiting species. Hunting and habitat disturbance currently wipe out complete primate sub-populations in Indochina. Urgent measures need to be taken.

The findings on the minimum viable population size confirm that four primate species in Vietnam are seriously under threat. They all show the typical species extinction traits (slow reproductive rate, dietary specialization, reduced ecological flexibility, poor speed of demographic recovery). Therefore effective *in-situ* protection in protected areas is urgently required. If primates are observed in the wildlife trade it is strongly recommended to use them for captive breeding

programs.

Of the three analyzed wildlife rescue centres the Endangered Primate Rescue Centre in Vietnam positively stands out in the whole region. The centre made significant achievements in the areas of capacity building, awareness raising and primate research. Important information is now available on primate distribution areas, *in-situ* population figures, behavior, and feeding ecology and reintroduction practices.

Referring to the title of the dissertation "*Ex-situ* Conservation – A viable Solution for Primate Population Declines?" the research shows that *ex-situ* conservation is very valuable, particularly due to the additional services rendered by *ex-situ* conservation projects in awareness creation, research and capacity building. But at the current level and with current resources, existing *ex-situ* projects cannot ensure the long-term survival of the critically endangered Indochinese primates. More political commitment and national/ international funds are needed. Currently no viable *ex-situ* populations exist of three of the four most endangered Vietnamese primates. A focus on *in-situ* conservation with an effective protected area management network and an integrated interdisciplinary approach including new management schemes are thus recommended.

Nevertheless, the success achieved in the past three decades must not be underestimated. Compared to the 1980's a wealth of information is now available on the Indochinese primates, their natural distribution, ecology, behavior, taxonomic classification and phylogeny.

Acknowledgements

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Summary

The *Vietnam Primate Conservation Program* is a long-term project of Frankfurt Zoological Society in Vietnam and comprises several components to support the conservation of highly endangered primate species. The project focuses on the protection of primate habitats and wild populations with special attention paid to Delacour's langur (*Trachypithecus delacouri*), Hatinh langurs (*T. hatinhensis*) and as well as both red and grey-shanked douc langurs (*Pygathrix nemaeus, P. cinerea*).

The *Delacour's langur Conservation Project* continued in Van Long Nature Reserve as a focal point for the conservation of the "Critically Endangered" Delacour's langur. Primate surveys have mainly been focused on the monitoring of Delacour's langur populations in several areas. An ongoing decline of many subpopulations was recorded.

The *Red-shanked douc langur Conservation Project* in Son Tra Nature Reserve, Danang continued with the population study on this species. The study revealed an extremely high impact to the habitat of the langurs through intense road construction and the development of the area as a tourism spot.

The *Hatinh langur Reintroduction Project* in Phong Nha-Ke Bang National Park continued the ranger- and patrol activities, but activities to search and habituate the animals inside the semi-wild area have been extremely limited. This has now made it extremely difficult to locate the animals for the possibility to catch the animals for the planned and final release into the wild of the national park.

In January 2010, Frankfurt Zoological Society launched the *Grey-shanked douc langur Conservation Project* in the Kon Ka Kinh National Park. The capacity of the park's ranger was strengthened through training courses. The park's rangers and the FZS-project's staff patrolled about 12,000 ha of the park. 24 transects were set up in order to monitor endangered primates including the grey-shanked douc langur and the northern yellow-cheeked gibbon (*Nomascus annamensis*). About 720 school children attended lectures on wildlife conservation. Research on the population density and ecology of the grey-shanked douc langurs and northern yellow-cheeked gibbons was conducted.

The Endangered Primate Rescue Center was established in order to provide housing for confiscated endangered primates from the illegal wildlife trade. Captive breeding programs for several different taxa have been successfully employed. At the end of the year the EPRC keeps 151 primates in 16 taxa.

Based on a long-term agreement between Danang University and FZS a primate training course for students at the Danang University was organized. Thirty students attended the course.

To intensify the bonds of international cooperation and exchange experiences the "Second International Conference on Wildlife Rescue in East and Southeast Asia" was organized in Cuc Phuong National Park. This conference was part of the Wildlife Animal Rescue Network (WARN) and had more than 70 participants making up different 16 nationalities.

Báo cáo năm 2010 – Hội Động vật học Frankfurt "Chương trình Bảo tồn Linh trưởng Việt Nam" và Trung tâm Cứu hộ Linh trưởng Nguy cấp

Tóm tắt

"*Chương trình Bảo tồn Linh trưởng Việt Nam*" là một dự án dài hạn của Hội Động vật học Frankfurt tại Việt Nam và bao gồm hỗ trợ nhiều hoạt động bảo tồn đối với một số loài thú linh trưởng có nguy cơ đe dọa bị tuyệt chủng cao. Dự án đã tập chung tới việc bảo vệ môi trường sống cho linh trưởng và bảo tồn các quần thể còn trong tự nhiên của một số loài như voọc mông trắng, voọc Hà Tĩnh, chà vá chân đỏ và chà vá chân xám.

Dự án -*Bảo tồn loài voọc mông trắng*- tại Khu bảo tồn thiên nhiên đất ngập nước Vân Long, tỉnh Ninh Bình vẫn được hoạt động tích cực nhằm bảo tồn loài voọc mông trắng, một trong số những loài thú linh trưởng đặc hữu và nguy cấp cao. Các hoạt động ngoại nghiệp về linh trưởng hầu như được tập chung điều tra theo dõi các quần thể voọc mông trắng còn trong tự nhiên. Các số liệu cho thấy sự tiêu giảm và có nhiều tác động tiêu cực đến nhiều tiểu quần thể của loài này.

Dự án -*Bảo tồn loài chà vá chân đỏ*- tại Khu bảo tồn thiên nhiên Sơn Trà, thành phố Đà Nẵng vẫn được tiếp tục nghiên cứu các tiểu quần thể của loài này trên Bán đảo Sơn Trà. Sơ bộ nghiên cứu cho thấy sự tác động tiêu cực rất mạnh tới quần thể loài này tại đây là do các hoạt động xây dựng đường giao thông và gia tăng các khu du lịch mới.

Dự án -*Bảo tồn loài voọc Hà Tĩnh*- tại Vườn Quốc gia Phong Nha-Kẻ Bàng, tỉnh Quảng Bình vẫn được tiếp tục thực hiện với các hoạt động hỗ trợ lực lượng kiểm lâm thông qua công tác tuần tra rừng. Nhưng công tác theo dõi động vật được thả trong Khu bán hoang dã đã không được thực hiện liên tục, do vậy số liệu về hoạt động của chúng rất bị hạn chế. Đây cũng là một trong những khó khăn của Dự án bởi không xác định được vị trí trú ngụ của động vật để có thể tiến hành các bước tiếp theo nhằm bắt lại động vật cho việc thả động vật vào trong tự nhiên như đã dự kiến.

Tháng 1 năm 2010, Hội Động vật học Frankfurt đã chính thức triển khai Dự án -*Bảo tồn loài chà vá chân xám*- tại Vườn Quốc gia Kon Ka Kinh, tỉnh Gia Lai. Nâng cao năng lực hoạt động cho cán bộ kiểm lâm đã được trải nghiệm qua các cuộc tập huấn. Cán bộ kiểm lâm của Vườn và cán bộ sinh học của dự án FZS đã hợp tác chặt chẽ trong công tác tuần rừng trên diện tích 12,000 ha. Thông qua công tác tuần rừng đã lập được 24 tuyến điều tra theo dõi linh trưởng của loài chà vá chân xám và vượn má hung. Nghiên cứu về mật độ bầy đàn và tập tính sinh thái của loài chà vá chân xám vẫn liên tục thu thập dữ liệu từ những năm trước. Về loài vượn má hung, sơ bộ điều tra đã ước tính có 40 đàn với khoảng 139 cá thể của loài này tại đây. Tất cả các số liệu điều tra và dữ liệu nghiên cứu linh trưởng này sẽ có giá trị khoa học quan trọng nhằm hỗ trợ cho công tác bảo vệ và bảo tồn tại Vườn Quốc gia Kon Ka Kinh. Có 720 lượt học sinh tham gia các buổi sinh hoạt bảo tồn thiên nhiên, tìm hiểu về động thực vật hoang dã được cán bộ kiểm lâm và cán bộ dự án phối hợp cùng các nhà trường trong địa bàn thực hiện.

Trung tâm Cứu hộ Linh trưởng Nguy cấp vẫn hoạt động với nhiệm vụ cứu hộ, phục hồi sức khỏe và chăm giữ thú linh trưởng sau khi được tịch thu từ những vụ săn bắt và buôn bán động vật hoang dã trái phép. Chương trình cho sinh sản một số loài và phân loài (taxa) thú linh trưởng nguy cấp cao vẫn được thực hiện thành công. Số lượng động vật đến cuối năm là 151 cá thể của 16 taxa.

Trên cơ sở hợp tác khoa học lâu dài giữa Đại học Đà Nẵng và FZS đã tổ chức thành công khóa tập huấn ngắn hạn trong mùa hè cho 30 sinh viên về kỹ năng ngoại nghiệp đối với linh trưởng.

Nhằm tăng cường quan hệ hợp tác quốc tế trong khu vực, trao đổi kinh nghiệm cứu hộ và bảo tồn động vật hoang dã, -Hội thảo lần thứ II về Cứu hộ Động vật hoang dã trong khu vực Đông và Đông Nam Á- đã được tổ chức thành công tại Vườn Quốc gia Cúc Phương trong tháng 11/2010. Đã có hơn 70 đại biểu đến từ 16 quốc gia tham dự Hội thảo.

Introduction

The *Vietnam Primate Conservation Program* is a long-term project of Frankfurt Zoological Society in Vietnam and comprises several components to support the conservation of highly endangered primate species. The project focuses on the protection of primate habitats and wild populations, which include the support of forest protection activities and education for communities surrounding such areas. In the past the intensified management of ranger activities to combat poaching and illegal wildlife trade led to an increase in the confiscation of endangered primate taxa. The Endangered Primate Rescue Center provides housing for such animals and has also started with breeding programs for further reintroduction projects.

Endangered Primate Rescue Center

Staff

The Vietnamese staff of 20 workers continued for the care of the animals. The workers are responsible for animal husbandry duties (i.e. feeding, cleaning, and cage maintenance) as well as the general up-keep of the center from landscaping to building maintenance.

The foreign head animal keeper Denny Lohse from Zoo Leipzig began his work in September 2009 and continued until August 2010. He was then replaced by Elke Schwierz who is also an animal keeper from Zoo Leipzig. Elke has worked for a total of five years at the EPRC.

Two volunteers from the German "DED-weltwaerts-Program", Jonas Hagemann and Bastian Boge also worked from September 2009 until August 2010. In continuation of this program the volunteers Felix Schlieszus and Jonas Elsner began their work at the center in September 2010.

Jeremy Phan a volunteer from US worked from January to June in order to gain experience as an animal keeper. The four month period served as an internship to complete his Bachelor's Degree in Zoology at Michigan State University.

Nguyen Thi Thu Hien continued her work as Vietnamese manager of the EPRC.

Primates housed at the EPRC

At the end of 2010 the EPRC housed 151 primates, including 122 langurs, 18 gibbons, and 11 lorises. In cooperation with Forest Protection Authorities in several provinces 7 douc langurs and 1 loris were confiscated (Fig. 1). During the year 13 langurs were born and 20 died this number

Fig.1. Confiscated red-shanked douc langur baby (*Pygathrix nemaeus*) in the care of an animal keeper at the Endangered Primate Rescue Center. Photo: Elke Schwierz.

includes 5 of the confiscated langurs. Despite intensive care and veterinary treatment the animals died due to severe injuries or due to the poor status of their health conditions.

Education

Mai Quang Tuan and Do Dang Khoa, working as animals keepers at the EPRC continued with their part time study at the Forest University Hanoi to receive diplomas as forest engineers.

Cooperation with Authorities to combat poaching and illegal wildlife trade

The Endangered Primate Rescue Center continued the cooperation with several provincial Forest Protection Authorities for the confiscation of primates (Fig. 2). A cooperation and exchange

Fig.2. Confiscated red-shanked douc langur (*Pygathrix nemaeus*) in a provincial Forest Protection Department prior to its transfer to the Endangered Primate Rescue Center. Photo: Tilo Nadler.

of information also exists with the Vietnamese Organization ENV (Education for Nature Vietnam) which is closely involved in the fight against illegal wildlife trade.

Delacour's langur Conservation Project in Van Long Nature Reserve

Van Long Nature Reserve continued as a focal point for the conservation of the "Critically Endangered" Delacour's langur. Several meetings with the province administration were organized in an effort to extend the area into the neighboring Hoa Binh Province. (Fig. 3, 4 and 5). For this reason the number of guards was also increased once more and is now at 27. As a very important step towards the protection of the area outside the future protected area of the nature reserve members of the community police and one hamlet leader were included as guards in the staff of the nature reserve. Hamlet leader and community police are powerful authorities in the communes in the control of gun use and forest encroachment.

FZS continues to pay salaries for the community guards who serve as the most important unit for the protection of the nature reserve. All guards also received new sets of uniforms.

Quarterly meetings with guards, commune leaders and commune police were organized to report protection activities, impact to the nature reserve, illegal activities and for the planning of protection activities (Fig. 6).

Fig.3. Event in the neighbouring Hoa Binh Province to Van Long Nature Reserve for the extension of the reserve. Photo: Tilo Nadler.

Fig.4. Discussion with authorities from communes of the proposed extension area for the nature reserve. Photo: Nguyen Thi Thu Hien.

Fig.5. The representative of the Forest Protection Authority of Yen Thuy District, Hoa Binh Province, Nguyen Manh Hong, and the representative of Frankfurt Zoological Society in Vietnam, Tilo Nadler, after a fruitful discussion about the extension of the nature reserve. Photo: Nguyen Thi Thu Hien.

Fig.6. Guards of Van Long Nature Reserve in preparation for a patrol. Photo: Tilo Nadler.

A gun control activity was organized by the Management Board of the nature reserve and the police in Dong Tam Commune. Guns were not confiscated, they are personal property, but the owners made commitments not to use the guns for hunting. The use of guns for hunting will be prosecuted by law.

Fig.7. The Vice Director of Van Long Nature Reserve, Mai Van Quyen, receives the certificate of the registration into the "Vietnamese Guinness Book of Records" for the protection of the worlds largest Delacour's langur population. Photo: Tilo Nadler.

Fig.8. The certificate of the "Vietnamese Guinness Book of Records" for the protection of the worlds largest Delacour's langur population. Photo: Tilo Nadler.

In December, Van Long Nature Reserve was rewarded with entry into the "Vietnamese Guinness Book of Records" for the protection of the highest existing Delacour's langur population (Fig. 7 and 8).

Red-shanked douc langur Conservation Project in Son Tra Nature Reserve

The PhD student Larry Ulibarri continued his study on the red-shanked douc langur population in Son Tra Nature Reserve on Son Tra Peninsula. The study revealed an extremely high impact to the habitat of the langurs through intense road construction and the development of the area as a tourism spot, including the construction of several resorts. The road contraction causes an increase in fragmentation of the douc langur population.

Hatinh langurs Reintroduction Project in Phong Nha-Ke Bang National Park

The ranger and patrol activities continued with the evaluation of the GPS coordinates collected during ranger patrols, especially for the designated release area for the Hatinh langur group currently at the semi-wild area.

Unfortunately activities to search and habituate the animals inside the semi-wild area have been extremely limited with the result that no information about the activities of these animals is available. The possibility to use the transmission of the radio collars carried by the animals at the groups inside the semi-wild area to identify sleeping places of the group failed. The radio transmission from radio collars has stopped after three years of the animals being released inside the large (20 ha) semi-wild area. This has now made it extremely difficult to locate the animals and sleeping places which could allow for the possibility to catch the animals for the planned and final release into the wilds of the national park. In the current situation it would take a great deal of time and expense to locate the animals and secure them for release.

Grey-shanked douc langur Conservation Project in Kon Ka Kinh National Park, Gia Lai Province (by Ha Thang Long)

In January 2010, Frankfurt Zoological Society launched a project in the Kon Ka Kinh National Park to save the grey-shanked douc langurs in the Central Highlands. The activity of the project has achieved several remarks:

The capacity of the park's ranger was strengthened through training courses. 20 rangers (of about 40 in total) from 7 stations and as well as from the head-quarter office were trained on biodiversity knowledge and the methodology of biodiversity monitoring. Skills for using orientation equipments such as GPS, maps, and compass for patrols were improved. Rangers also had a better understanding of biodiversity and forest protection laws.

The park's rangers and the FZS-project's staff patrolled about 12,000 ha of the park. 24 transects (about 84 km) were set up in order to monitor endangered primates including the grey-shanked douc langur and the northern yellow-cheeked gibbon.

The attitude of local people toward wildlife protection in Kon Ka Kinh National Park was improved. Since October 2010 the education team of the park conducted visits to the schools. About 720 school children attended lectures on wildlife conservation and the conservation the grey-shanked douc langurs. About 300 posters of the douc langurs and their conservation status were distributed.

Research on the population density and ecology of the grey-shanked douc langurs and northern yellow-cheeked gibbons was conducted. A population of about 40 gibbon groups with 139 individuals is estimated to live in the park. A small population of grey-shanked douc langurs with about 45 individuals was found outside the park border.

Primate Surveys and Research

Primate surveys have mainly been focused on the monitoring of Delacour's langur populations. These efforts are extremely important for the reintroduction and possible translocations. The biologist Nguyen Van Linh continued primate surveys in Van Long Nature Reserve and other areas where the Delacour's langurs occur. He and the biologist Nguyen Ai Tam were working as assistants for a genetic population study in Van Long Nature Reserve. The population study was carried out by the German

biologist Anja Ebenau from March to July. To examine the population genetic structure of Delacour's langur within VLNR, fecal samples were collected for genetic analysis.

Since September, the biologist Nguyen Hong Chung was employed and was also involved in surveys for Delacour's langurs.

In March, a brief Delacour's langur survey was carried out in Cuc Phuong National Park. Only a few individuals could be recorded and this information confirmed the decrease of the population.

An outstanding research result, documented from researchers at the German Primate Center and the EPRC has been the discovery and scientific description of a new gibbon species – the northern yellow-cheeked gibbon *Nomascus annamensis*.

Education an PR activities

In August a one-week primate training course by Danang University was organized based on a long-term contract between Danang University and FZS. Thirty students attended the course which provided information about conservation strategies and methods, primate systematics, identification of Vietnamese primates, primate research and research techniques, wildlife trade, and wildlife protection laws of Vietnam.

The course also included two days field training and observation of primates in Son Tra Nature Reserve. FZS project leaders Tilo Nadler, Ha Thang Long and the PhD student Larry Ulibarri, who carried out the douc langur research in Son Tra Nature Reserve, served as lecturers of the course (Fig. 9 and 10).

In May a BBC-Team visited the EPRC to make a report about the keeping of rare and highly endangered primates (Fig. 11).

Vietnam TV visited two time the EPRC to report about the conservation work.

Fig.9. Participants at the primate training course at Danang University. Photo: Tilo Nadler.

Fig.10. Ceritificate for the students attending the primate training course. Photo: Tilo Nadler.

Fig.11. In May, a BBC Team visited the EPRC to report about Vietnams rare and endangered primates. Photo: Tilo Nadler.

"Second International Conference on Wildlife Rescue in East and Southeast Asia"

On November 9th through the 13th the "Second International Conference on Wildlife Rescue in East and Southeast Asia" of the Wildlife Animal Rescue Network (WARN) took place in Cuc Phuong National Park and was organized by the Endangered Primate Rescue Center / Frankfurt Zoological Society in cooperation with the Forest Protection Authority of Vietnam and Cuc Phuong National Park.

This second conference should strengthen the newly established cooperation between rescue centres in the region and provide a platform to exchange information and experiences in rescue centre management and animal rescue issues.

More than 70 participants of 16 nationalities attended the conference (Fig. 12). During the three day conference 24 presentations were made about general and species-specific issues on wildlife rescue activities. Visits to the three rescue centres at Cuc Phuong National Park – for primates, turtles, small carnivores and pangolins – and a visit to Van Long Nature Reserve with observations made of the Delacour's langurs in the wild rounded out the program (Fig. 13).

Fig.12. Participants of the "Second International Conference on Wildlife Rescue in East and Southeast Asia" of the Wildlife Animal Rescue Network (WARN), on November 9th to the 13th at Cuc Phuong National Park. Photo: Luong Van Hien.

Fig.13. Participants of the "Second International Conference on Wildlife Rescue in East and Southeast Asia"
visit the Endangered Primate Rescue Center. Photo: Tilo Nadler.

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Leipzig Zoo, Germany

International Primate Protection League and Arcus Foundation, USA

Zoological Society of Philadelphia, USA

Zoological Society for the Conservation of Species and Populations, Germany

German Primate Centre

We are grateful for this support which makes our work possible towards the conservation of primate species which are on the brink of extinction.

Publications, reports and presentations resulting from the FZS "Vietnam Primate Conservation Program" and the Endangered Primate Rescue Center

The fourth issue of the *Vietnamese Journal of Primatology* came out in 2010 with the description of a new gibbon species as its highlight. The Journal can be ordered at the EPRC but is also available on the website of the IUCN/Primate Specialist group and on the website of Frankfurt Zoological Society.

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Appendix

Register of primates at the EPRC 2010 - (up to date 31.12. 2010)

(*species or subspecies only held in EPRC anywhere in the world)

No.	Date of arrival	Sex	Date born or estimated	Sire	Dam	Source	Current status
Delacour's langur *Trachypithecus delacouri* (*)							
1-01	Jan.93	M	1990	wild	wild	confiscated	EPRC
1-02	Jan.93	M	1990	wild	wild	confiscated	EPRC
1-04	17.5.94	M	1993	wild	wild	confiscated	EPRC
1-06	28.7.96	F	28.7.96	1-01	1-03	born EPRC	EPRC
1-07	21.2.98	M	21.2.98	1-01	1-03	born EPRC	EPRC
1-09	3.4.01	F	3.4.01	1-01	1-03	born EPRC	EPRC
1-10	4.6.01	M	4.6.01	1-02	1-05	born EPRC	EPRC
1-12	7.12.02	M	7.12.02	1-01	1-03	born EPRC	EPRC
1-13	9.7.03	F	9.7.03	1-02	1-06	born EPRC	EPRC
1-15	14.7.04	M	14.7.04	1-01	1-03	born EPRC	EPRC
1-16	1.6.05	M	1.6.05	1-04	1-08	born EPRC	EPRC
1-17	27.10.05	F	27.10.05	1-02	1-06	born EPRC	EPRC
1-18	19.4.07	M	19.4.07	1-04	1-08	born EPRC	EPRC
1-19	8.1.08	M	8.1.08	1-02	1-06	born EPRC	EPRC
1-20	30.1.08	M	30.1.08	1-10	1-09	born EPRC	EPRC
1-21	29.7.08	F	29.7.08	1-07	1-13	born EPRC	EPRC
1-23	20.5.10	F	20.5.10	1-02	1-06	born EPRC	EPRC
1-24	1.11.10	?	1.11.10	1-02	1-17	born EPRC	†2.11.10
Hatinh langur *Trachypithecus laotum hatinhensis* (*)							
2-01	11.5.93	M	1990	wild	wild	confiscated	EPRC
2-03	13.1.94	F	1993	wild	2-02	confiscated	EPRC
2-05	9.4.94	F	1994	wild	2-04	confiscated	EPRC
2-09	14.1.96	F	ad.	wild	wild	confiscated	EPRC
2-10	6.2.96	M	6.2.96	2-01	2-08	born EPRC	EPRC
2-11	27.4.96	F	27.4.96	2-01	2-04	born EPRC	EPRC
2-12	27.11.96	M	1995	wild	wild	from private	EPRC
2-13	28.3.97	M	28.3.97	2-01	2-09	born EPRC	EPRC
2-14	22.5.97	F	22.5.97	2-01	2-08	born EPRC	EPRC
2-15	15.10.97	M	1995	wild	wild	from tourists	†1.9.10
2-17	11.12.97	F	1994	wild	wild	from tourists	EPRC
2-20	11.3.98	F	1995	wild	wild	from tourists	EPRC
2-22	24.2.99	M	24.2.99	2-01	2-08	born EPRC	EPRC
2-23	9.4.99	M	9.4.99	2-01	2-09	born EPRC	EPRC
2-24	25.3.00	M	25.3.00	2-15	2-17	born EPRC	EPRC
2-26	20.11.00	M	20.11.00	2-15	2-11	born EPRC	EPRC
2-27	7.1.01	F	7.1.01	2-15	2-20	born EPRC	EPRC
2-32	4.4.02	F	4.4.02	2-15	2-17	born EPRC	EPRC
2-36	14.11.03	F	14.11.03	2-12	2-05	born EPRC	EPRC
2-41	28.11.04	M	28.11.04	2-01	2-09	born EPRC	EPRC
2-46	1.8.05	F	ca. 2004	wild	wild	confiscated	EPRC
2-47	27.11.05	M	27.11.05	2-12	2-05	born EPRC	EPRC
2-48	14.2.06	F	14.2.06	2-15	2-11	born EPRC	EPRC
2-49	29.6.06	F	29.6.06	14-01	2-14	born EPRC	EPRC
2-50	28.9.06	M	28.9.06	2-12	2-03?	born EPRC	EPRC
2-51	20.10.06	M	20.10.06	2-10	2-27	born EPRC	EPRC
2-52	31.10.06	F	31.10.06	2-10	2-32	born EPRC	EPRC

2-53	10.12.06	M	10.12.06	2-15	2-20	born EPRC	EPRC
2-54	30.3.07	M	30.3.07	2-15	2-17	born EPRC	EPRC
2-56	??.9.07	M	??.9.07	2-12	2-36	born EPRC	EPRC
2-57	18.3.08	F	18.3.08	14-01	2-14	born EPRC	EPRC
2-58	19.5.08	F	19.5.08	2-15	2-11	born EPRC	EPRC
2-59	29.12.08	?	29.12.08	2-12	2-03	born EPRC	EPRC
2-60	1.4.09	M	1.4.09	2-10	2-27	born EPRC	EPRC
2-62	???	M	??.5.09	2-12	2-05	born EPRC	EPRC
2-63	4.6.09	F	4.6.09	2-15	2-20	born EPRC	EPRC
2-64	20.1.10	M	20.1.10	14-01	2-14	born EPRC	EPRC
2-65	21.1.10	M	21.1.10	2-01	2-09	born EPRC	†22.1.10
2-66	1.5.10	F	1.5.10	2-10	2-32	born EPRC	†6.5.10
2-67	8.5.10	F	8.5.10	2-47	2-46	born EPRC	†9.5.10
2-68	28.10.10	M	28.10.10	2-15	2-20	born EPRC	EPRC
2-69	27.11.10	M	27.11.10	2-01	2-09	born EPRC	EPRC

Black langur *Trachypithecus laotum hatinhensis* morph "ebenus" (*)

14-01	12.1.98	M	1996	wild	wild	from tourists	EPRC

Laos langur *Trachypithecus laotum laotum* (*)

3-01	26.9.95	M	1995	wild	wild	confiscated	EPRC

Grey langur *Trachypithecus crepusculus*

4-04	22.1.97	F	1996	wild	wild	from private	EPRC
4-05	14.4.00	F	1999	wild	wild	confiscated	EPRC
4-07	24.1.02	F	24.1.02	4-06	4-04	born EPRC	EPRC

Cat Ba langur (Golden-headed langur) *Trachypithecus p. poliocephalus* (*)

15-01	8.11.98	F	1998	wild	wild	Cat Ba NP	EPRC
15-04	2.6.03	M	2.6.03	15-02	15-01	born EPRC	EPRC
15-06	1.3.10	M	1.3.10	15-04	15-01	born EPRC	EPRC

Francois' langur *Trachypithecus francoisi*

17-01	8.1.02	F	1997	wild	wild	Na Hang NR	EPRC
17-02	30.9.05	M	2003	wild	wild	confiscated	EPRC

Red-shanked douc langur x Hatinh langur *P. nemaeus* x *T. laotum hatinhensis* (*)

18-01	14.10.03	F	14.10.03	6-9/12?	2-03	born EPRC	EPRC

Red-shanked douc langur *Pygathrix nemaeus*

6-02	17.3.96	F	1992	wild	wild	confiscated	EPRC
6-05	8.5.97	M	ad.	wild	wild	confiscated	EPRC
6-06	24.5.97	M	1994	wild	wild	from tourists	EPRC
6-09	10.7.97	M	1997	wild	wild	confiscated	EPRC
6-12	28.11.97	M	1997	wild	wild	from tourists	EPRC
6-14	12.1.98	M	1996	wild	wild	from tourists	EPRC
6-16	2.4.98	M	1994	wild	wild	from tourists	EPRC
6-21	30.12.98	F	30.12.98	6-05	6-02	born EPRC	EPRC
6-28	19.8.00	M	1996	wild	wild	confiscated	EPRC
6-29	25.4.01	M	25.4.01	6-05	6-13	born EPRC	EPRC
6-30	6.6.01	F	6.6.01	6-06	6-02	born EPRC	†24.1.10
6-31	21.4.02	F	21.4.02	6-06	6-02	born EPRC	EPRC
6-32	24.2.03	F	24.2.03	6-06	6-02	born EPRC	EPRC
6-37	25.8.04	M	25.8.04	6-06	6-02	born EPRC	EPRC

6-38	13.12.04	F	ad.	wild	wild	confiscated	EPRC
6-39	13.4.05	M	ad.	wild	wild	confiscated	EPRC
6-41	9.5.05	F	9.5.05	6-12	6-21	born EPRC	EPRC
6-42	11.6.05	M	April 05	wild	wild	confiscated	EPRC
6-46	17.8.06	F	2001	wild	wild	confiscated	EPRC
6-53	17.10.07	F	2003	wild	wild	confiscated	EPRC
6-55	2.2.08	F	2.2.08	6-28	6-46	born EPRC	EPRC
6-56	6.2.08	F	6.2.08	6-08	6-30	born EPRC	EPRC
6-58	27.3.08	F	27.3.08	6-16	6-32	born EPRC	EPRC
6-60	20.7.08	M	2007	wild	wild	confiscated	EPRC
6-61	14.3.09	M	14.3.09	6-12	6-21	born EPRC	EPRC
6-62	17.3.09	M	17.3.09	6-28	6-31	born EPRC	EPRC
6-63	9.6.09	M	2002	wild	wild	confiscated	EPRC
6-64	31.3.10	F	ad.	wild	wild	confiscated	EPRC
6-65	7.4.10	M	ad.	wild	wild	confiscated	†8.4.10
6-66	21.4.10	M	ad.	wild	wild	confiscated	†5.8.10
6-67	2.5.10	M	2.5.10	6-16	6-38	born EPRC	EPRC
6-68	9.7.10	M	ad.	wild	wild	confiscated	†25.12.10
6-69	19.8.10	M	19.8.10	6-28	6-46	born EPRC	EPRC
6-70	21.8.10	M	21.8.10	6-16	6-38	born EPRC	EPRC
6-71	4.9.10	F	June 10	wild	wild	confiscated	†4.10.10

Grey-shanked douc langur *Pygathrix cinerea* (*)

7-01	31.8.95	M	1992	wild	wild	confiscated	†27.12.10
7-04	4.8.97	M	1994	wild	wild	confiscated	EPRC
7-09	13.2.01	M	ca.1996	wild	wild	confiscated	EPRC
7-11	15.12.01	F	ca. 1997	wild	wild	confiscated	EPRC
7-13	12.7.02	F	ad.	wild	wild	confiscated	EPRC
7-14	18.8.02	M	1998	wild	wild	confiscated	EPRC
7-16	11.12.02	M	ad.	wild	wild	confiscated	EPRC
7-19	13.3.03	M	subad.(1998)	wild	wild	confiscated	EPRC
7-24	15.1.04	F	15.1.04	7-04	7-13	born EPRC	EPRC
7-25	9.11.04	M	2000	wild	wild	confiscated	EPRC
7-28	6.6.05	F	6.6.05	7-01	7-11	born EPRC	EPRC
7-29	14.8.05	F	ca. 2005	wild	wild	confiscated	EPRC
7-30	9.11.05	F	ad.	wild	wild	confiscated	EPRC
7-31	5.3.06	M	5.3.06	7-04	7-13	born EPRC	†25.9.10
7-34	19.10.06	F	2000	wild	wild	confiscated	EPRC
7-35	3.11.06	F	ad.	wild	wild	confiscated	EPRC
7-37	24.12.06	M	2003	wild	wild	confiscated	EPRC
7-39	17.3.07	M	2003	wild	wild	confiscated	EPRC
7-40	10.10.07	M	10.10.07	7-09	7-34	born EPRC	EPRC
7-43	5.5.08	M	5.5.08	7-01	7-11	born EPRC	†8.9.10
7-45	5.12.08	F	2007	wild	wild	confiscated	EPRC
7-46	4.3.09	M	2005	wild	wild	confiscated	EPRC
7-47	11.3.09	M	2004	wild	wild	confiscated	EPRC
7-48	8.3.09	F	8.3.09	7-19	7-30	born EPRC	EPRC
7-49	8.4.09	M	2009	wild	wild	confiscated	EPRC
7-52	25.2.10	M	2005	wild	wild	confiscated	EPRC
7-53	31.3.10	M	ad.	wild	wild	confiscated	EPRC
7-54	8.4.10	M	ad.	wild	wild	confiscated	†17.4.10
7-55	6.5.10	M	6.5.10	7-09	7-34	born EPRC	EPRC

Black-shanked douc langur *Pygathrix nigripes*

13-05	15.3.01	M	1996	wild	wild	confiscated	EPRC

Northern White-cheeked gibbon *Nomascus leucogenys*

8-01	30.9.94	F	1993	wild	wild	from foreigner	EPRC
8-03	28.5.02	M	1999	wild	wild	confiscated	EPRC
8-08	19.11.04	F	2001	wild	wild	confiscated	EPRC

Southern white-cheeked gibbon *Nomascus siki*

9-02	18.9.93	F	1993	wild	wild	from foreigner	EPRC
9-05	10.11.94	M	1992	wild	wild	from foreigner	EPRC
9-06	24.2.95	F	1993	wild	wild	from tourists	EPRC
9-07	30.10.96	M	1996	wild	wild	from tourists	†16.5.10
9-08	1.12.98	F	1998	wild	wild	from tourists	EPRC
9-09	23.6.99	M	23.6.99	9-05	9-02	born EPRC	EPRC
9-11	25.7.02	F	25.7.02	9-03	9-06	born EPRC	EPRC
9-12	17.12.02	M	17.12.02	9-05	9-02	born EPRC	EPRC
9-13	21.11.06	F	21.11.06	9-05	9-02	born EPRC	EPRC
9-15	15.7.09	M	15.7.09	9-05	9-02	born EPRC	EPRC

Northern Yellow-cheeked gibbon *Nomascus annamensis (*)*

20-01=10-02	6.2.97	F	1994	wild	wild	confiscated	EPRC
20-02=10-09	9.7.10	M	2005	wild	wild	confiscated	EPRC

Southern Yellow-cheeked gibbon *Nomascus gabriellae*

10-02 = now identified as *N. annamensis* 20-01

10-04	3.6.01	F	1997	wild	wild	confiscated	EPRC
10-05	11.6.04	F	2001	wild	wild	confiscated	EPRC
10-06	21.5.04	F	2003	wild	wild	confiscated	†12.4.10
10-07	7.10.06	F	2005	wild	wild	confiscated	EPRC
10-08	7.10.06	F	2005	wild	wild	confiscated	EPRC

10-09 = now identified as *N. annamensis* 20-02

Slow loris *Nycticebus bengalensis*

11-09	20.11.07	F	ad.	wild	wild	confiscated	EPRC
11-10	22.6.08	F	ad.	wild	wild	confiscated	EPRC
11-11	9.5.09	F	ad.	wild	wild	confiscated	EPRC

Pygmy loris *Nycticebus pygmaeus*

12-36	22.2.01	F	22.2.01	12-09	12-04	born EPRC	EPRC
12-70	24.2.06	F	ad.	wild	wild	confiscated	†15.9.10
12-72	24.2.06	F	ad.	wild	wild	confiscated	EPRC
12-88	17.5.07	F	ad.	wild	wild	confiscated	EPRC
12-97	12.4.08	F	ad.	wild	wild	confiscated	EPRC
12-99	7.1.09	F	ad.	wild	wild	confiscated	†19.12.10
12-100	14.3.09	F	ad.	wild	wild	confiscated	†29.8.10
12-101	Febr.09	M	2/2009	wild	12-98	born EPRC	†23.2.10
12-102	Febr.09	F	2/2009	wild	12-99	born EPRC	EPRC
12-103	Febr.09	M	2/2009	wild	12-99	born EPRC	EPRC
12-110	6.8.09	M	2008	wild	wild	from private	EPRC
12-112	29.4.10	M	ad.	wild	wild	confiscated	EPRC

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Books and Monographs
Groves CP (2001): Primate Taxonomy. Washington DC.

Edited books and book chapters
Groves CP 2004: Taxonomy and Biogeography of Primates in Vietnam and Neighbouring Regions. In: Nadler T, Streicher U. & Ha Thang Long (eds.): Conservation of Primates in Vietnam; pp. 15-22. Frankfurt Zoological Society, Hanoi.

Dissertations
Otto C (2005): Food intake, nutrient intake, and food selection in captive and semi-free Douc langurs. PhD dissertation, University Cologne.

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