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Cover: Black-shanked douc langur, male (*Pygathrix nigripes*). Photo: T. Nadler

Announcement

Symposium
- Conservation of Primates in Indochina -

November 26-30, 2008
Cuc Phuong National Park, Vietnam

The Frankfurt Zoological Society and Conservation International are pleased to announce a symposium "Conservation of Primates in Indochina" to be held November 26-30, 2008 in Cuc Phuong National Park, Vietnam.

Five years ago, Vietnamese and foreign scientists gathered in Cuc Phuong National Park to discuss current research on, knowledge of, and conservation issues facing the primates of Vietnam. Conservation of Primates in Vietnam, an edited volume of the symposium's lectures, was published in 2004.

Subsequent years have seen a flourish and expansion of studies on the genetics, behavior, ecology, taxonomy, and conservation of Vietnam's primates. In addition, primatological studies from neighbouring countries have deepened our knowledge about other closely related and closely situated Asian primates, particularly the leaf monkeys of southern China. Vietnam has four endemic primate species yet several of the primates have distributions that, while restricted in range, extend across political boundaries. Vietnamese primates share habitat types and conservation threats with primates of eastern Laos, Cambodia, and southern China. Consequently, it is most fitting that this year's symposium prominently features the broader region of Indochina.

This is a serendipitous year in which to meet, as 2008 marks the 15th anniversary of the Endangered Primate Rescue Center in Cuc Phuong National Park and the 150th anniversary of the Frankfurt Zoological Society.

Please submit abstracts and questions to:

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The fee for participants - US$100 (for foreigners); 800,000 VND (for Vietnamese) includes:

- Accommodation in Cuc Phuong National Park (4 nights)
- Meals during the symposium (4 breakfast, 3 lunch, 4 dinner)
- Transportation Hanoi-Cuc Phuong National Park-Hanoi (by bus)
- Visit Van Long Nature Reserve (transportation by bus and boat)

The production of the Vietnamese Journal of Primatology is generously sponsored by German Primate Centre.

Thông báo

Hội thảo chuyên đề
- Bảo tồn Linh trưởng ở Đông Dương -

26-30 tháng 11 năm 2008
Vườn Quốc gia Cúc Phương, Việt Nam

Hội Động vật học Frankfurt (FZS) và Tổ chức Bảo tồn Quốc tế (CI) xin thông báo về Hội thảo chuyên đề - Bảo tồn Linh trưởng Đông Dương - dự kiến tổ chức từ ngày 26-30 tháng 11 năm 2008 tại Vườn Quốc gia Cúc Phương, tỉnh Ninh Bình, Việt Nam.

Năm năm trước tại Vườn Quốc gia Cúc Phương, các nhà khoa học trong và ngoài nước đã tham dự Hội thảo chuyên đề - Bảo tồn Linh trưởng ở Việt Nam - để cùng thảo luận và trao đổi nhiều thông tin khoa học, kinh nghiệm bảo tồn linh trưởng ở Việt Nam trong thập niên 90 và đầu những năm 2000 của thập kỷ 21th. Ấn phẩm - Bảo tồn Linh trưởng ở Việt Nam - đã được phát hành vào đầu năm 2004 bao gồm các bài tham luận khoa học trong Hội thảo chuyên đề này.

Trong những năm tiếp theo đã có thêm nhiều nghiên cứu về linh trưởng được mở rộng và thực hiện với nhiều đề tài như gien di truyền, tập tính sinh thái, phân loại và nhiều hoạt động bảo tồn linh trưởng ở Việt Nam. Bên cạnh đó cũng có nhiều nghiên cứu về thú linh trưởng ở các nước láng giềng và luôn có sự hợp tác khoa học bởi mối quan hệ mật thiết giữa các loài thú linh trưởng của Châu Á và đặc biệt là các loài khỉ ăn lá ở khu vực Nam Trung Quốc. Việt Nam có bốn loài linh trưởng đặc hữu, hầu hết các loài linh trưởng có vùng phân bố ở khu vực rừng bảo vệ nghiêm ngặt, vùng mở rộng hay trong ranh giới đường biên các quốc gia. Nhiều loài linh trưởng của Việt Nam cùng chia sẻ môi trường sống và chịu nhiều áp lực đến bảo tồn với nhiều loài linh trưởng của các nước bạn Lào, Cămpuchia và Nam Trung Quốc. Do vậy, từ Hội thảo chuyên đề năm nay sẽ được mở rộng cho khu vực Đông Dương.

Năm 2008 này cũng là một năm đặc biệt để kỷ niệm 15 năm thành lập Trung tâm Cứu hộ Linh trưởng Nguy cấp (EPRC) ở Vườn Quốc gia Cúc Phương và 150 năm thành lập Hội Động vật học Frankfurt (FSZ).

Xin liên hệ và đăng ký tham luận với:

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- VND 800.000 (đối với người Việt Nam)

bao gồm:

- Phòng nghỉ ở Cúc Phương (4 đêm)
- Ăn trong thời gian hội thảo (4 ăn sáng, 3 ăn trưa, 4 ăn tối)
- Vận chuyển Hà Nội - Cúc Phương (xe buýt)
- Thăm Khu bảo tồn đất ngập nước Vân Long (xe buýt và thuyền nan)

Xuất bản ấn phẩm Chuyên đề Linh trưởng Việt Nam được tài trợ bởi Trung tâm Nghiên cứu Linh trưởng, CHLB Đức.

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Radio tracking of Hatinh langurs (*Trachypithecus laotum hatinhensis*) at a semi-wild enclosure in Phong Nha - Ke Bang National Park, Central Vietnam

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Key words: primate reintroduction, radio tracking, monitoring

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Summary

In 2005 the Frankfurt Zoological Society in cooperation with the Cologne Zoo launched a reintroduction program for two endangered primate species, the Hatinh langur (*Trachypithecus laotum hatinhensis*) and red-shanked douc langur (*Pygathrix nemaeus*) in the Phong Nha – Ke Bang National Park in central Vietnam. As a first step, a semi-wild enclosure was built and in early September 2007 two groups of Hatinh langurs from the Endangered Primate Rescue Center were transferred. All individuals were equipped with radio collars and continuously monitored over a period of nine weeks. The langurs settled-in well and rapidly explored the whole enclosure. Original group composition was not maintained, but the langurs finally merged into one group. While during the first half of the study, observation conditions were limited as animals were hiding under dense liana covers, later on the langurs gradually exposed themselves in more open areas, allowing extended data sampling periods. Follow-up studies are recommended.

Theo dõi loài vọoc Hà Tĩnh (*Trachypithecus laotum hatinhensis*) bằng tín hiệu radio tại khu nuôi nhốt bán hoang dã, vườn quốc gia Phong Nha - Kẻ Bàng, Việt Nam

Tóm tắt

Năm 2005, Hội Động vật Frankfurt, Vườn thú Cologne kết hợp Vườn Quốc gia Phong Nha - Kẻ Bàng khởi động chương trình tái thả hai loài linh trưởng có nguy cơ bị tuyệt chủng về môi trường tự nhiên tại Vườn Quốc gia Phong Nha - Kẻ Bàng, miền Trung Việt Nam. Hai loài được thả gồm vọoc Hà Tĩnh (*Trachypithecus laotum hatinhensis*) và loài vọoc chà vá chân nâu (*Pygathrix nemaeus*). Tháng 9 năm 2007, hai đàn nhỏ loài vọoc Hà Tĩnh từ Trung tâm Cứu hộ Linh trưởng Nguy cấp Cúc Phương đã được thả về khu vực bán hoang dã. Tất cả các cá thể đều được gắn vòng thu sóng radio và được theo dõi liên tục 9 tuần sau khi thả. Kết quả cho thấy, các cá thể hòa nhập tốt với môi trường sống. Cấu trúc của hai đàn nhỏ không còn, thay vào đó các cá thể nhập thành một đàn lớn. Trong suốt nửa thời gian đầu của việc theo dõi, động vật thường lẩn trốn trong lùm cây nên khó quan sát. Sau một thời gian làm quen với môi trường mới, động vật đã dần dần xuất hiện ở những chỗ trống thuận tiện hơn cho việc thu thập số liệu. Nghiên cứu tiếp theo cần được tiến hành.

Introduction

In 2005, as part of the Vietnam Primate Conservation Programme of the Frankfurt Zoological Society (FZS), FZS in cooperation with Cologne Zoo initiated a primate reintroduction program in the Phong Nha - Ke Bang National Park (PNKB) in central Vietnam (Nadler & Streicher, 2003; Vogt et al., 2006; Vogt & Forster, in press). In the long run, the project strives to enforce and link currently isolated populations of two endangered primate species, the Hatinh langur (*Trachypithecus laotum hatinhensis*) and red-shanked douc langur (*Pygathrix nemaeus*) (IUCN 2006). Hatinh and red-shanked douc langurs are endemic to Laos and Vietnam. Both species are mainly threatened by poaching.

High hunting pressure, especially in the past, led to a severe decline of their natural populations (Nadler et al., 2003). The distribution of Hatinh langurs is limited to a small stretch south of 17°59’N and the only confirmed occurrence in Vietnam is located in two provinces of central Vietnam. Today, the total population in Vietnam is estimated to consist of between 520 to 670 individuals (Nadler et al., 2003). However these animals appear not to be a closed population. The complete eradication of Hatinh langurs in various areas has led to a high degree of fragmentation, and subpopulations might be separated from each other by large areas. The distribution of red-shanked douc langurs ranges from about 18°40’N latitude in Laos and 19°30’N in Vietnam southwards to about 14°33’N. In Vietnam the occurrence of this species has been confirmed in seven areas, but there are currently no estimates of the remaining population sizes. Over the last few years a rapid decline of the species has been observed and the remaining population appears to be small and highly fragmented (Nadler et al., 2003).

The PNKB National Park (17°22’-17°35’N; 105°58’-106°23’E), located along the border to Laos in Quang Binh Province, comprises about 90.000 ha and is one of the last remaining areas of retreat for both Hatinh langurs and red-shanked douc langurs. The animals for reintroduction will come from the Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park, northern Vietnam. As an initial step of the program, a semi-wild enclosure was built at the border of PNKB, where the langurs will be maintained, protected and monitored for an appropriate period of time. A single forested limestone hill of 18 ha in size (Fig. 1), containing different vegetation types as well as some small streams, rocks and limestone cliffs was selected as an appropriate area and was surrounded by an electrical fence.

Fig.1. The semi-wild area at Phong Nha – Ke Bang National Park.

In early September 2007, the first primates were transferred to the semi-wild enclosure. Two groups of Hatinh langurs, comprising four individuals each, all equipped with radio transmitters, were brought to the site. At that time a long-term, continuous monitoring program was started for general supervision and protection of the langurs, as well as to collect data on their behavioural ecology.

Objectives

The present report deals with the results of the initial monitoring period from September to November 2007. The purpose of the study was to document the release and adjustment of the langurs to the enclosure, providing particular information on the following topics:

1. Spatial use of habitat, i.e. movement, ranging and frequency use patterns
2. Group structure, i.e. will original groups remain stable in size and composition
3. General observation conditions and notes on behavioural ecology, as related to further studies

Material and Methods

The study animals

Both Hatinh langur groups, comprising one male and three females each, had been established at the EPRC several weeks prior to the transfer (Table 1, Fig. 2). They were kept in adjacent cages, and therefore all animals were familiar with each other. Each group contained one adult female, the other members were still sub-adult. All animals could be individually recognised by characteristic morphological features such as the shape and colour of the sideburns, face, tail, and body, as well as identified by distinct radio transmitter frequencies.

Table 1. Composition of transferred Hatinh langur groups.

Group	Name	Sex	Date of birth
A	Paul	M	03.12.2003
	Lila	F	08.04.2005
	Thom	F	06.02.2002
	Sonja	F	23.08.2004
B	Russ	M	21.11.2003
	Catherine	F	13.08.2000
	Tute	F	03.07.2005
	Wully	F	13.04.2003

The following radio tracking equipment was used to locate the langurs: HOLOHIL MI-2Transmitters (weight 40,2 g, battery life 150 weeks), a TELONICS Receiver TR-4 164/168MHz, assembled to a TELONICS Antenna RA-14K 164/168MHz (+/-35 angle).

Direct observation was carried out by means of two types of binoculars, a NIKON SPORTER 10x36 and a SWAROVSKI HABICHT 10x42. The distance to detected animals was measured with a BUSHNELL YARDAGE PRO Rangefinder (14 - 850m).

Fig. 2. Group B. From left to right: Tute, Catherine, Wully, Russ.

Methods

For easier orientation in the field, some preparatory work was carried out prior to the transfer.

The fence pillars encircling the enclosure (total girth 1700m, 781 pillars) were marked with red numbers. Following photographs, maps of the hill from four different views were drawn by COREL DRAW 8 (Fig. 3) and used as data sheets for recording movement patterns.

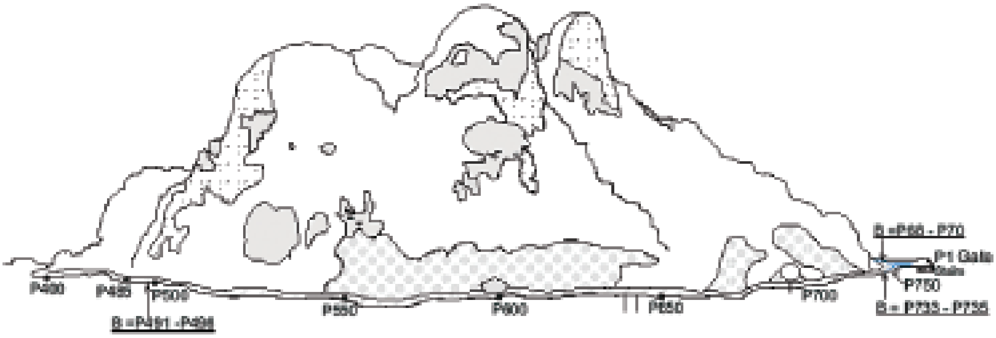

Fig. 3. Map of the semi-wild area, showing the front view, between pillar No. 400 to 1.

The Hatinh langurs were brought to the semi-wild area on September 4, 2007, kept in the enclosureís cages for two days and finally released to the hill on September 7, 2007. An intense study of their adjustment phase was started, mainly conducted by J. Riedel, a student from the University of Bonn, as well as the project managers B. Forster and M. Vogt. The study stretched over a total time period of 64 days, from September 7-November 9, 2007). Data recording took place on 49 days, totaling 232 survey hours, 129 in the morning, and 103 in the afternoon (Table 2). On the remaining 15 days monitoring was not possible, mostly due to bad weather conditions.

Table 2. Time schedule of study period.

Week	Date	Observ. days	Observ. hours	Observ. h morning	Observ. h afternoon
1	07.-14.09.	8	78,5	42,0	36,5
2	15.-21.09.	7	31,5	21,0	10,5
3	22.-28.09.	6	27,3	18,3	9,0
4	29.-05.10.	4	13,3	9,5	3,8
5	06.-12.10.	6	24,8	13,3	11,5
6	13.-19.10.	2	8,0	2,5	5,5
7	20.-26.10.	5	17,7	9,2	8,5
8	27.-02.11.	5	8,0	3,8	4,3
9	03.-09.11.	6	22,8	9,8	13,0
TOTAL		**49**	**231,7**	**129,2**	**102,5**

During the first week, the langurs were tracked and observed from sunrise to sunset. In order to determine the animals positions at the hill in regular intervals, telemetric records were taken every 30 minutes from different positions. Time, pillar number, individual signal records, the intensity of signals (1 = low, 2 = normal to high), as well as the direction of the signal (frontal = in line of direct extension of the recording pillar; from left or from right) were noted. At any time animals were visible, their perpendicular distance to the fence was measured and the location was marked in the map. In addition, behavioural data were collected by ad-libitum-sampling (Altmann, 1974).

After seven days of observation, recording time was reduced to two to three hours in the morning and afternoon, respectively. The first aim of each monitoring day was to locate all eight individuals, to make sure that all langurs were still at the site and in good condition. To do this, telemetric records were taken at five fixed points around the enclosure (Pillars No. 1, 150, 300, 450 and 600). In this way, the complete area could be tracked. After the first contact, we tried to detect the langurs by binoculars and observe them as long as possible. If animals were not visible, radio records were taken in regular intervals to note travel activities. If the langurs were split up in several subgroups and spread over different locations, the observer either stayed with the biggest group or changed position from time to time, depending on subgroup size and observation conditions. Telemetric and behavioural data sampling took place as described above.

Data analysis

In total 2.992 individual signal records (ISR) were received: 2.232 frontal, 275 from left and 485 from right. To describe the langursí spatial use, the semi-wild enclosure was divided into six sectors (Table 3), following topographical features, which determined tracking and observation conditions. Only frontal records were used for calculation.

Sightings correspond to the visible detection of one or more individuals. To analyse the use frequencies of the sectors, not all sightings could be regarded, in order to avoid multiple counts of the same individuals at the same area in a definite time span. Therefore, the maximum number of all visible individuals (MVI) at a certain place per continuous observation period was calculated. For example, if the langurs stayed in sector 3 from 8 to 10 am, and at 8:00 3 individuals were seen and at 9:00 5 animals, the latter number was taken into account as the maximum number of sighted individuals for sector 3 during the morning session. The total number of all MVI for the whole study period was 177.

Table 3. Division of sectors according to pillar number.

Sector No.	1	2	3	4	5	6
Pillar No.	65-209	210-319	320-444	445-569	570-699	700-64

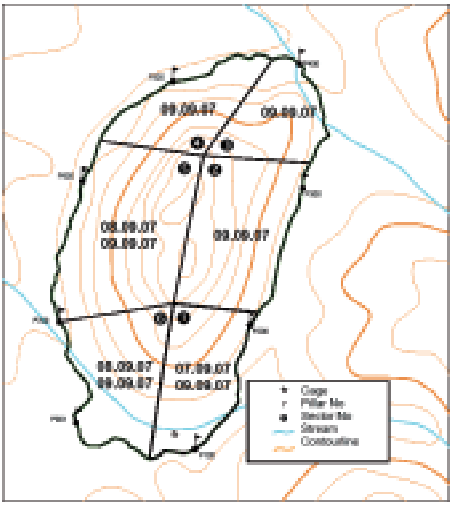

Fig. 4. Composition of transferred Hatinh langur groups.

Results and discussion

Spatial use of semi-wild enclosure

Figure 4 shows the use of the semi-wild area by the langurs during the first three days after release. The cage is located in sector 1, on the level of pillar No. 80.

On September 7 at 9 am, the two cage compartments were opened. Both groups rapidly left the cage, but stayed and explored the area in its close vicinity for the rest of the day. The following morning (September 8, 2007), the langurs were recorded around pillar No. 19 in sector 6, moving further to the southern part of sector 5, being finally located around pillar No. 676 in the evening. On the next day telemetric records indicated that the langurs had split up into several subgroups (see below). The main troop was found in sector 5, moving to the top of the hill and subsequently entering sectors 4, 3 and 2, respectively. One female, Wully, was seen on the top of the southern peak (sector 2). In addition, single individuals could be located in sectors 6 and 1. So within only three days, the langurs already had explored the whole enclosure.

As the study progressed, certain areas were used with different frequencies. Figure 5 shows the distribution of all frontal records (ISR, n = 2.232) as well as of all maximum sightings (MVI, n = 177) over the six sectors. With a total of 658 ISR (29.5%) and 51 MVI (28,8%) sector 4 was the most used part of the enclosure. Next, according to the telemetric records, the langurs spent approximately equal amounts of time in the three areas 6, 5 and 2 (23,5%; 21,1% and 20,9%), while sightings indicate a bit different order (25,4% for sector 2; 18,6% for sector 3, followed by 13,6% for sector 1). The lowest frequency was obtained for sector 3 (1,7% of ISR and MVI).

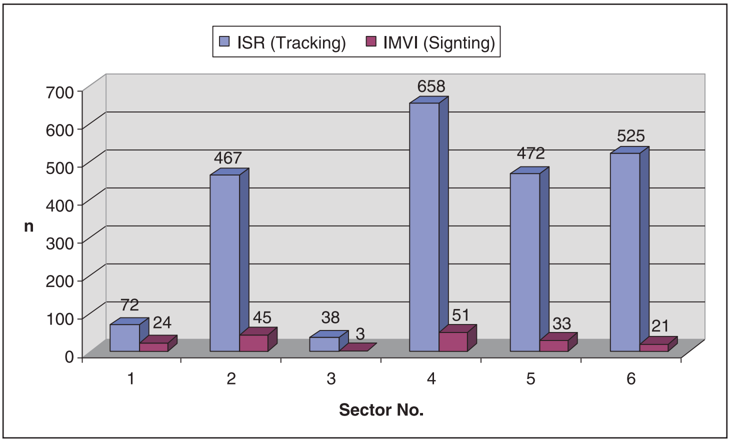

Fig. 5. Distribution of telemetric and visible records over sectors.

While during the first three weeks the Hatinh langurs often stayed in the upper parts of the enclosure between the peaks, they subsequently used the lower areas more frequently. This can be determined from Fig. 6, presenting the average daily distance to the fence on days with visible records. Especially beginning in the middle of October until the conclusion of the study, the langurs were mainly encountered just 40 - 60 m away from the fence. During the first weeks, the animals might have felt safer on the top of the hill, away from the enclosure's border and human observers. As they became more familiar with their new home, they might have started to feel more comfortable in the lower parts of the enclosure. Moreover, the lower levels of the enclosure are densely covered with an invasive climber species, which turned out to become a preferred food source for the langurs (see below). Therefore the increased use

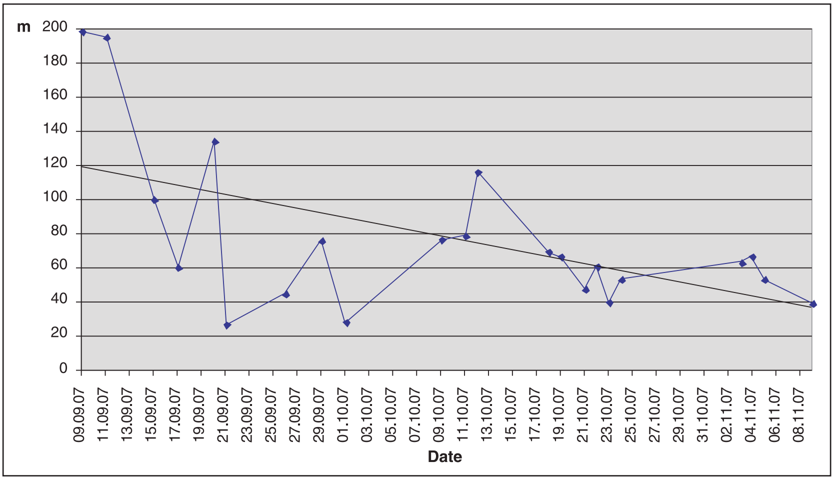

Fig. 6. Average daily distance of sighted langurs to the fence (in m).

of these parts might be linked to their feeding ecology. In general, the use of a habitat over time and space is related to essential ecological factors such as food availability, shelter, and sleeping places (Clutton-Brock, 1977; Dunbar, 1988; Fleagle, 1999). However, these considerations already go far beyond the scope of the present study and must be left open for further research.

Group structure

On the release day, all langurs gathered around the cage. Besides exploring the close surroundings, they were mainly engaged in social contact with each other. On the second day, they already had split up into several subgroups, which is how they remained for the next weeks. According to radio tracking data, most of the time there was one main group containing 4 to 5 langurs, the remaining animals hanging around alone or in pairs. Although the latter often were females, no stable pattern could be recognised in the composition of all the different clusters. Constantly new groups formed and dissolved, showing new combinations from one day to the next across the whole enclosure.

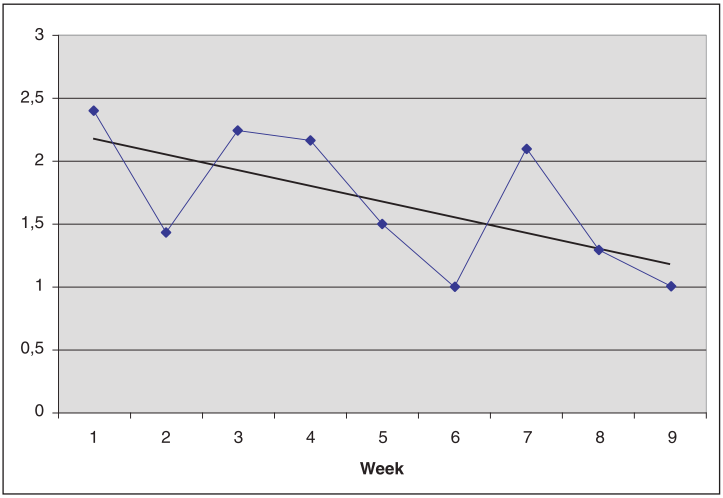

Fig. 7. Average daily number of subgroups over study period (week 1: without release day).

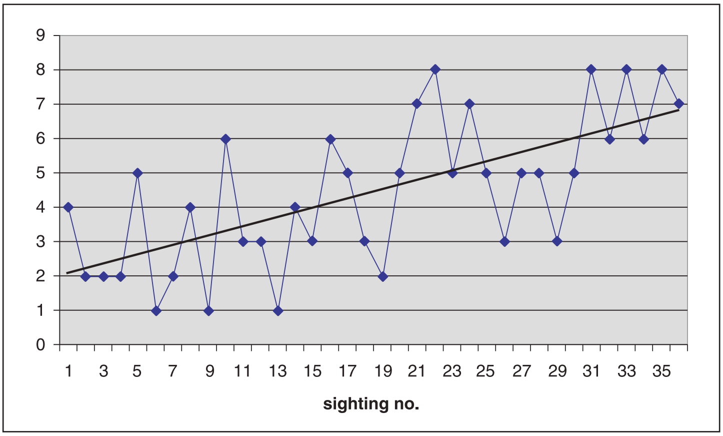

Fig. 8. Number of individuals counted per sighting (in chronological order).

Over time, the Hatinh langurs gradually came together (Fig. 7). The average daily number of subgroups constantly decreased over time, and by the end of the study period, all eight individuals merged into one group. This is also indicated by the increasing number of individuals per sighting, as shown in Fig. 8. On October 19, 2007 (sighting No. 22 in Fig. 8), all eight langurs were observed together for the first time.

The original composition of both groups was not maintained, but animals finally formed one group, comprising two males and six females. Langurs usually are organised in one-male-multi-female groups (Yeager & Kool, 2000). In the present case, most langurs were still quite young, familiar with each other and brought to a new, unknown and spacious environment, which likely caused them to stay together. Throughout the study period, no severe conflicts of males competition for females were observed. Besides their familiarity, this lack of conflict might be due to their young age, and changes are expected when they reach sexual maturity.

Observation conditions and notes on behavioural ecology

Hatinh langurs could be seen on 26 days (including the release day), which corresponds to 53,1% of all monitoring days (n=49). Time spans of visible records ranged from less than one minute to two hours. Continuous observation (defined as periods with regular sightings) for more than 30 minutes was possible on just 14 days. Especially during the first half of the study phase, it was quite hard to spot any langurs and animals often could be detected only by radio tracking.

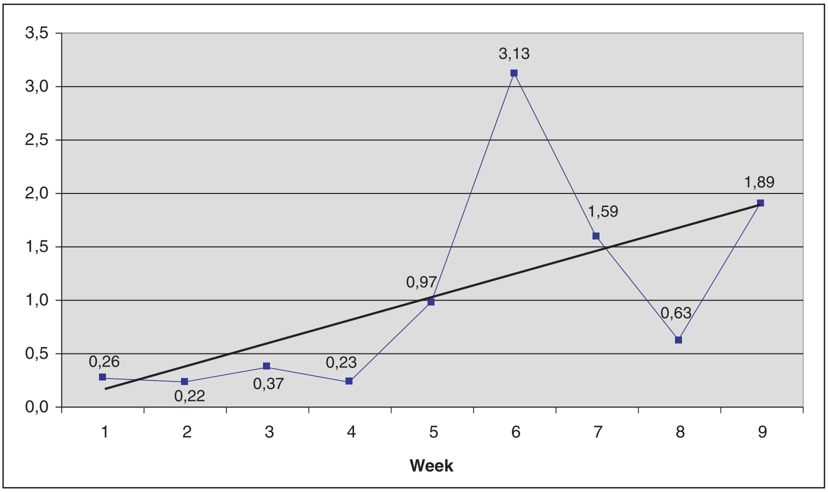

Fig. 9. Average number of sighted langurs per observation hour in the course of the study period (week 1: without release day).

Figure 9 shows the average number of sighted langurs per observation hour over the course of the study. During the first four weeks, an average of five observation hours were necessary to see one individual. Starting in week five (early October), the number of langurs visible per observation time increased. This is not only due to the progressive enlargement of group size (see above), but also to better observation conditions. Figure 10 shows the percentage of observation time during which animals could be observed for all sighting days (release day excluded) in chronological order, independent of group size. In the further course of the study, longer observation periods were achieved. On the one hand, this was related to the fact that around the same time, the Hatinh langurs started to use the

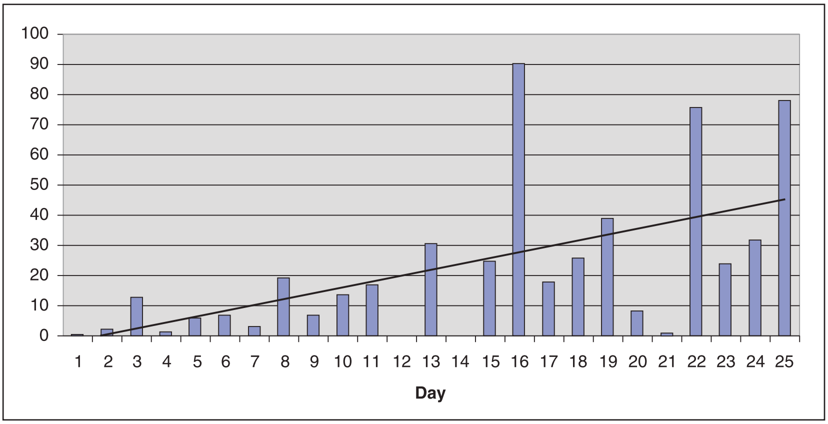

Fig. 10. Amount of time langurs were visible (as % of observation time) on days with sightings (days arranged in chronological order).

lower parts of the enclosure more frequently and therefore could be more easily detected and monitored. During the first weeks after the release the animals often were hiding under dense liana covers, thwarting study. There were often days without any sightings, but acoustic and visual clues (such as shaking tree branches and sounds of jumping between trees/scrub) indicated the presence of the langurs to the observer. As weeks passed, the animals increasingly exposed themselves on the outer / upper parts of vegetation substrates as well as in more open areas such as tree gaps, and therefore extended data sampling periods were possible.

Although observation conditions were limited, some data on their behavioural ecology could be collected. First, all langurs seemed to be in good health throughout the study period. No major injuries were detected and everytime langurs were seen they appeared to be in good physical condition.

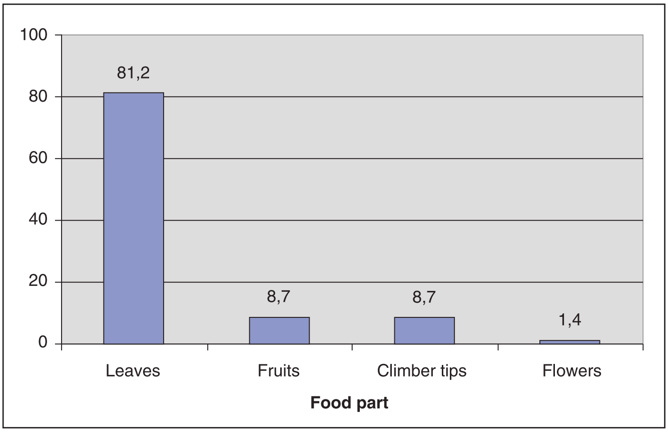

Fig. 11. Food parts eaten by the langurs.

In total 121 data points feeding records were obtained, but the food source or part was only identified in 46 cases. Out of the remaining 75 data points, 28% of the time a climber plant was eaten (leaves 9 = 42.9%; tips 6 = 28.6%; rest unknown). Regarding all identified feeding records for food parts (n=69 data points Fig. 11), the langurs mostly ate leaves (81.2%), followed by fruits (8.7%), climber tips (8.7%) and flowers (1.4%).

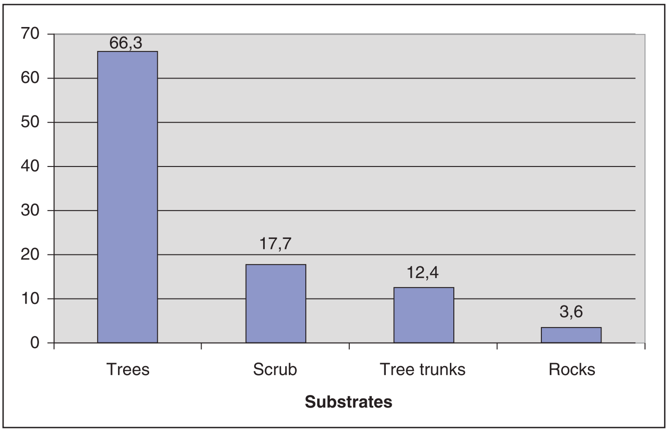

Fig. 12. Substrates used by the langurs.

During 169 sightings, the substrate used by the langurs could be identified (Fig. 12). Most of the time, they were seen inside trees (112 times = 66,3%), 30 times (17,7%) on scrubs, 21 times (12,4%) on fallen dead tree trunks and 6 times (3,6%) on rocks. In the course of this study it could not be determined where the Hatinh langurs spent the night, due to reduced visibility at dusk. As species belong to of the "limestone langurs" (Nadler & Streicher, 2004), free-ranging Hatinh langurs are adapted to limestone cliffs, which they often use as sleeping sites. The released langurs from the EPRC however have been born in captivity and therefore are not familiar with these natural conditions. So it would be interesting if they show a different behaviour than wild Hatinh langurs. This and other important aspects of their biology should be evaluated in future studies.

Acknowledgements

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Tail postures of four quadrupedal leaf monkeys (*Pygathrix nemaeus*, *P. cinerea*, *Trachypithecus delacouri* and *T. hatinhensis*) at the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam

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Key words: primate locomotion, kinematics, arboreality, limestone langurs, douc monkeys

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Summary

The kinematics of colobine locomotion, particularly the folivorous primates of Vietnam, has to date received little attention. Recent work at the Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park is the first to examine kinematics during arboreal quadrupedal locomotion in these endangered leaf monkeys, revealing marked differences in tail postures among four similarly sized species. During symmetrical walking, doucs (*Pygathrix nemaeus* and *P. cinerea*) typically allow the tail to hang down, falling along the substrate behind them during travel. Delacour's langurs (*Trachypithecus delacouri*) exhibit greater variability, often arching the tail higher in concave-downwards posture. Hatinh langurs (*Trachypithecus hatinhensis*) exhibit the greatest variability in tail posture, often raising the tail in a concave-upwards arc with the tip reaching over the thorax. These patterns are evident in our kinematic data, and may reflect different adaptations to rapid locomotion over varied substrates in the wild. Whereas doucs are typically observed in arboreal settings and utilize forelimb suspensory movements when traveling rapidly, wild Delacour's and Hatinh langurs include steep limestone karst formations to varying extents in their substrate repertoires, and travel rapidly using quadrupedal running and bounding. Further exploration of postural and locomotor adaptations in these taxa is pivotal to their conservation and captive management.

Hình dáng đuôi của bốn loài khỉ ăn lá (*Pygathrix nemaeus*, *P. cinerea*, *Trachypithecus delacouri*, và *T. hatinhensis*) tại Trung tâm Cứu hộ Linh trưởng Nguy cấp, Vườn Quốc gia Cúc Phương, Việt Nam.

Tóm tắt

Động học khi di chuyển ở các loài khỉ ăn lá (colobine) còn ít được nghiên cứu, đặc biệt với các loài khỉ ăn lá ở Việt Nam. Ở đây chúng tôi nghiên cứu vấn đề này trên đối tượng là các loài khỉ ăn lá có nguy cơ tuyệt chủng cao được cứu hộ tại Vườn Quốc gia Cúc Phương. Kết quả cho thấy có sự khác biệt rõ rệt về hình dáng đuôi khi di chuyển của bốn loài có kích thước tương đương. Ở các loài vọoc chà vá (*Pygathrix nemaeus* và *P. cinerea*) khi di chuyển bằng bốn chi, đuôi thường thõng xuống dưới bề mặt giá đỡ. Còn ở loài vọoc mông trắng (*Trachypithecus delacouri*) đuôi thể hiện nhiều hình dáng khác nhau khi di chuyển, đuôi thường nâng cao, uốn cong xuống, mút đuôi chúi xuống. Đối với loài vọoc Hà Tĩnh (*Trachypithecus hatinhensis*) đuôi thể hiện đa dạng nhất về hình dáng khi di chuyển, thông thường đuôi nâng cao, uốn cong lên và mút đuôi kéo dài ngang ngực. Các cách biểu hiện trên có liên quan đến động học trong di chuyển của mỗi loài, và chúng có thể phản ánh sự thích nghi của mỗi loài trên các loại giá đỡ khác nhau ngoài tự nhiên. Những quan sát cho thấy ở các loài vọoc chà vá thích nghi với đời sống trên cây thường sử dụng kiểu di chuyển treo người bằng hai chi trước để di chuyển nhanh. Trong khi đó các loài vọoc thích nghi với đời sống trên núi đá vôi, nơi có sự đa dạng hơn về các loại giá đỡ như vọoc mông trắng và vọoc Hà Tĩnh lại chạy bằng bốn chi hoặc nhảy. Cần có những nghiên cứu sâu hơn về hình dáng, sự thích nghi khi di chuyển ở các loài trên nhằm phục vụ việc bảo tồn và nuôi dưỡng chúng.

Introduction

The primates of Vietnam engage in broad range of locomotor behaviors, ranging from the high-velocity ricochetal brachiation of the crested gibbons to the deliberate movements of the slow and pygmy lorises. Although the locomotor behaviors of some of these forms are relatively well-understood based on detailed laboratory research (e.g. Demes et al., 1990; Bertram, 2004), notably absent are kinematic studies of colobines, in general, and Vietnam's leaf monkeys in particular. This is likely due to the fact that many of these animals are critically endangered, having remote and restricted ranges in places where collecting locomotor and positional data can present a number of challenges. Moreover, Vietnam's colobines are found in only a handful of captive settings, restricting their availability for laboratory kinematic studies. In an effort to understand variation in kinematics and positional behavior among this fascinating radiation of primates, we have examined differences in tail posture and kinematics during quadrupedal locomotion among captive leaf monkeys housed at the Endangered Primate Rescue Center in Cuc Phuong National Park, Vietnam.

The leaf monkey species included in this study fall within in two distinct groups of Southeast Asian colobines; the odd-nose doucs, represented here by the red-shanked douc (*Pygathrix nemaeus*) and the grey-shanked douc (*P. cinerea*), and the langurs, represented in this study by the Hatinh langur (*Trachypithecus hatinhensis*) and Delacour's langur (*T. delacouri*). All four species are characterized as having long, sweeping tails that exceed the length of their pre-sacral vertebral column (Dao Van Tien, 1985; Nadler, 1997; Nadler et al., 2003). The doucs exhibit a tail that is nearly equal in length to their total head and body length, ending in a tuft of longer hairs. The Hatinh langurs exhibit a similarly formed tail that exceeds total head and body length, and lacks a tuft at the terminal end. Delacour's langurs exhibit an average tail length similar to that found in Hatinh langurs, but are unique among Southeast Asian langurs in possessing a tail that is larger in diameter at its proximal end and more thickly furred (Nadler et al., 2003).

Taking into account the various roles for which different primates employ their tails, and the variation in social behavior, positional repertoire, and tail form across primates, the posture and movements of the tail are of interest (e.g., Garber & Rehg, 1999; Lawler & Stamps, 2002; Schmitt et al., 2005; Larson & Stern, 2006). This structure features in, among other behaviors, alarm calls and displays, it plays an important role in maintaining balance and change of direction during locomotion (Rollinson & Martin, 1981; Dunbar & Badam, 2000; Anapol et al., 2005; Larson and Stern, 2006). Early investigation of the role of the non-prehensile tail in Old World primates determined that longer tails were associated with a higher degree or arboreality, while more terrestrial species exhibited shorter tails (Rollinson & Martin, 1981). However, subsequent investigations have revealed that the relationships among tail length, substrate use, and positional behavior in primates is more complex than first assumed (Dunbar & Badam, 2000; Anapol et al., 2005; Larson & Stern, 2006). Here, we seek to document whether habitual tail postures and kinematics vary among leaf monkey taxa, and whether there is anything that distinguishes those that prefer the arboreal environment (i.e. *Pygathrix*), from those that incorporate acrobatic locomotion on limestone karst cliffs (i.e. *Trachypithecus*).

Differences in tail and body posture among the leaf monkeys of Vietnam were first observed by Nadler & Ha Thang Long (2000). This contribution complements previous descriptions of the positional behavior of Delacour's langurs (*Trachypithecus delacouri*) and Hatinh langurs (*Trachypithecus hatinhensis*) (Byron & Covert, 2004; Workman & Covert, 2005), and provides the first kinematic examination of tail posture for any of the leaf monkey species considered herein.

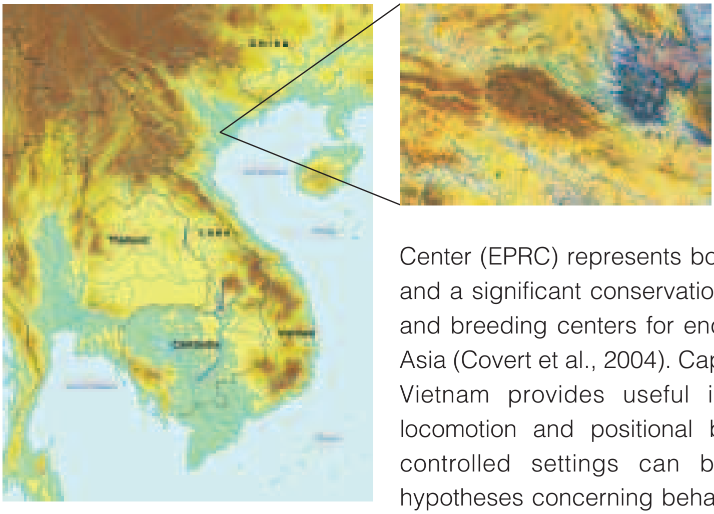


Fig. 1. Location of study site, Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park, Vietnam.

Location

Established in 1962, Cuc Phuong National Park represents the first nationally protected area in Vietnam (Fig. 1). Within the park, the Endangered Primate Rescue Center (EPRC) represents both an important research facility and a significant conservation site as one of the only rescue and breeding centers for endangered primates in Southeast Asia (Covert et al., 2004). Captive research on the primates of Vietnam provides useful information regarding primate locomotion and positional behavior, as data gathered in controlled settings can be used to develop testable hypotheses concerning behaviors in the wild. The integration of captive and wild data is essential for understanding the ecological tolerances and requirements of these fragile, endangered animals.

Study sample

As noted previously, the species considered herein include two genera of leaf monkey species ranging in size from 8-12 kg. Hatinh langurs (*Trachypithecus hatinhensis*), Delacour's langurs (*T. delacouri*), red-shanked doucs, and grey-shanked doucs (*Pygathrix nemaeus* and *P. cinerea* respectively). Delacour's langurs are among the most critically endangered primate species in the world, with an estimated total number of less than 300 individuals remaining in the wild. Endemic to limestone mountainous habitats in the Ninh Binh, Ha Nam, Hoa Binh and Thanh Hoa provinces of northern Vietnam, these langurs are restricted to a narrow geographic distribution ranging between 20-21 degrees North and 105-106 degrees East (Nadler et al., 2003). Delacour's langurs are adept arboreal quadrupeds that also engage in locomotion on steep karst cliff faces, exhibiting dramatic, sweeping motions of their long tails, along with a broad range of locomotor behaviors as they negotiate these challenging near-vertical substrates (Fig. 2).

Fig. 2. Delacour's langurs in Van Long Nature Reserve. Photo: T. Nadler.

Like their congeners, the endangered Hatinh langurs have been referred to as "limestone langurs" (Groves, 2004). This taxon is restricted to karst habitats in the western Quang Binh Province of Vietnam and the eastern Khammouane Province of Laos, representing a severe range contraction in recent times (Nadler et al., 2003). A close relative of the Laotian langur (*Trachypithecus laotum*), there are thought to be less than 700 Hatinh langurs alive today, with hunting and habitat loss continuing to exert pressure on the remaining populations. Little is known of their habits in the wild, although they also reportedly use caves as sleeping sites, and at least at the EPRC, they appear to be more active than *T. delacouri* (T. Nadler, pers. obs.).

Grey-shanked doucs are slightly larger than the Hatinh and Delacour's langurs, while the red-shanked doucs are similarly sized (Nadler et al., 2003). Both douc species appear to move more slowly than either the Hatinh or Delacour's langurs. In addition to hunting pressure, doucs are susceptible to habitat loss, with many populations isolated in small and unprotected forest fragments. Red-shanked doucs inhabit forested areas with some limestone karsts in north-central Vietnam, whereas grey-shanked doucs are primarily found in central Vietnam, although *P. nemaeus* and *P. cinerea* are thought to exist in sympatry in some areas along the boundaries of the species' ranges (Nadler et al., 2003). A third species, the black-shanked douc (*P. nigripes*) is not well represented in captivity at the EPRC and hence not considered in this study. Notably, none of the *Pygathrix* species have been reported to use limestone karst cliffs with high frequency during locomotion, however the black-shanked douc has been reported to use granite outcroppings found within its habitat relatively often (Ha Thang Long & Nadler, 2007). Interestingly the tail length of black-shanked doucs exceeds that of red- and grey-shanked doucs by about 100mm on average, hence this taxon may provide an interesting perspective on tail posture in a future study. Along with Hatinh langurs, red- and black-shanked doucs are listed as Endangered in the IUCN Red List of Threatened Species (Southeast Asian Mammal Databank, 2006). Moreover, Delacour's langurs and grey-shanked doucs are among the world's 25 most critically endangered primate species (Mittermeier et al., 2007). The need for more information on the habits and ecological constraints of these animals sets a context for a detailed examination of their locomotor biology.

Methods

Kinematic data collection

Several adult male and female Hatinh (n=7), Delacour's (n=5) langurs, red-shanked (n=6), and grey-shanked (n=4) doucs were filmed in the EPRC, Cuc Phuong National Park, Vietnam between July, 2005 and May, 2006. Housed in semi-naturalistic enclosures, subjects were filmed walking along horizontal supports approximately 5cm in diameter. Following methods described in Stevens

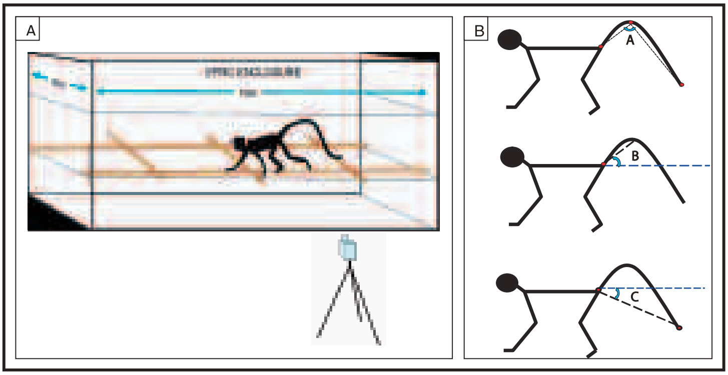


Fig. 3. A) Kinematic data collection setup using semi-naturalistic enclosures at the Endangered Primate Rescue Center. B) Angles measured in this study.

(2003) and Stevens et al. (2006), cameras were positioned in lateral view at a distance of greater than 5m to reduce effects of parallax (Fig. 3A). Frame rates were optimized to catch rapid movements by splitting 30Hz fields to achieve 60Hz, and shutter speeds were set to reduce motion blur. For each species, 15 strides without visible changes in speed or direction were analyzed. Video clips were imported into Peak Motus and kinematic points along the back, limbs and tail were digitized at forelimb and hind limb touchdown, midsupport and lift off events. Differences in tail posture were quantified using three different measures (Fig. 3B): Tail inflection angle (Angle A) is defined by three kinematic points: the base of the tail, the inflection point of the greatest tail curvature, and the tip of the tail. This angle describes the relative convexity/concavity of the tail posture in lateral view. Angle B is defined by the tail's inflection point, the base of the tail, and an imaginary x-axis (horizontal line) drawn through the tail base marker. This angle describes the relative projection of the midpoint of the tail above or below the tail base. The final angle, Angle C, is defined by the tail tip, the tail base, and an imaginary x-axis through the tail base marker. Angle C describes the degree to which the tail tip projects above or below the tail base.

Kinematic statistical analyses

Because kinematic data can not be expected to follow a normal distribution, data were rank-transformed prior to analysis of variance. Rank transformation permits analysis of variance without loss of power in datasets that are not normally distributed (Conover & Iman, 1981). Kinematic differences correlated with velocity may simply reflect differences in locomotor speed, hence speed-correlated variables were also examined using analysis of covariance with locomotor velocity as the covariate.

Behavioral data collection

Kinematic analyses were supplemented with behavioral data on posture and locomotor mode. Instantaneous focal animal sampling (Altmann, 1974) and bout sampling were used simultaneously to record positional behavior and posture on the substrate (Table 1). Instantaneous samples were recorded at 25 second intervals for several hours on male and female adult Hatinh langurs (n=3), Delacour's langurs (n=3), grey-shanked doucs (n=2) and red-shanked doucs (n=3), yielding over 4500 observations in all. One benefit of observing these species at the EPRC is that the enclosures are relatively uniform in size and construction, making it possible to rule out variation in structural environment as a possible causative factor when interpreting similarities or differences in behavioral patterns between the four leaf monkey species. Nonetheless, due to low numbers of individuals and observations, data reported herein are preliminary in nature and are interpreted with caution.

Several tail postures were identified that could be quickly assessed and recorded by the behavioral observer. This lends ecological relevance to the study of kinematic "hows" by providing frequency data on "how often" a given tail posture was assumed. Tail postures were sorted into five major categories: tail hanging down (Fig. 4A), tail on branch (Fig. 4B), tail back (Fig. 4C), tail back with a concave-upwards arch (Fig. 4D), tail back with a concave-downwards arch (Fig. 4E). Tail posture frequencies in each category were compared across species and in relation to locomotor mode.

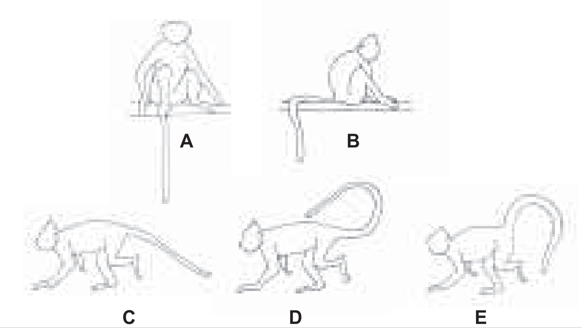
Results and Discussion

Tail kinematics

Because the leaf monkey species included in this study do not differ greatly in body size or available habitat type, our null hypothesis was that they would not exhibit significant differences in tail posture or locomotor/ positional behavior. Interestingly, kinematic analysis revealed that tail postures differ considerably among species. In all taxa, the tail inflection angle (Angle A) remained relatively constant throughout the stride cycle (Fig. 5A). Both douc species, along with the Delacour's langurs, exhibited a concave downward arch of the tail as reflected by positive tail inflection angles (Fig. 5A), whereas Hatinh langurs exhibited a concave-upward tail inflection (Fig. 5A). Tails were significantly more arched in Hatinh langurs at all kinematic events. For all of the leaf monkeys, a strong correlation was observed between tail inflection and speed (p<.005 for all species at all kinematic events; Table 2), with tails drooping and trailing along the support at lower speeds and rising into a higher arch at more rapid speeds. Doucs

Table 1. Ethograms of postures and locomotor modes recorded in this study.

Postures	
QS	Quadrupedal stand
SP	Sit perpen di cular to substrate
SPL	Sit parallel to substrate
PV	Prone ventral
PD	Prone dorsal
BP	Bipedal stand
S	Suspension (any suspensory posture)
SG	Sit on ground
Motions	
QW	Quadrupedal walk
QF	Fast quadrupedalism
QB	Quadrupedal bound
LU	Leap up (can be hands or feet first)
LD	Leap down (can be feet or feet first)
CU	Climb up
CD	Climb down (can be hands or head first)
AS	Arm swing (any suspensory motion)
BPW	Bipedal walk
PU	Pull up (can be one handed or two handed)
DROP	Drop down from substrate



A. Tail Hang Down (THD/TD) **B.** Tail On Branch (TOB/DB) **C.** Tail Back (TO/TB) **D.** Tail Back Concave Up (TA) **E.** Tail Back Concave Down (TQ)

Fig. 4. Tail postures recorded in this study.

Table 2. Significance levels for comparisons among langur species in this study.

	person	speaman	anova	ancova
Fore TD	0.000	0.000	0.000	0.001
Fore MS	0.010	0.010	0.000	0.001
Fore TO	0.000	0.000	0.000	0.000
HInd TD	0.001	0.001	0.000	0.005
HInd MS	0.000	0.000	0.000	0.000
HInd TO	0.000	0.000	0.000	0.000

never attained the locomotor velocities of the *Trachypithecus* species, but even so, at higher speeds their tails exhibited a slight inferiorly-concave arch. The highest speeds were observed in *T. delacouri* (p<.01 for all events), which may have contributed to their more acute mean tail inflection angle relative to doucs.

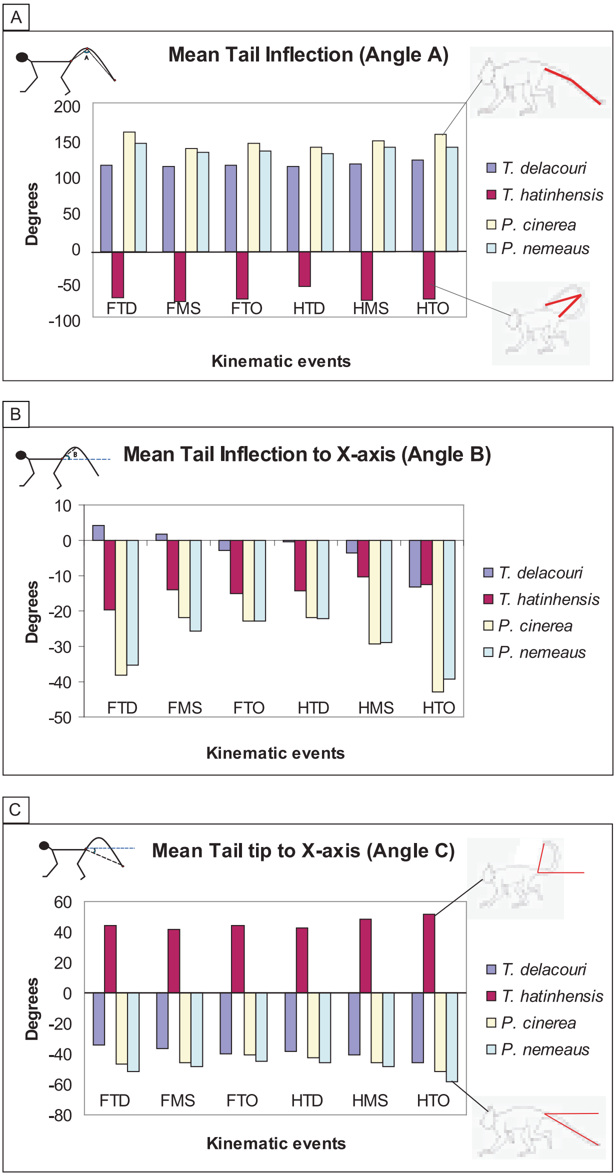


Fig. 5. Tail kinematics and forelimb and hind limb events. FTD=forelimb touch down; FMS=forelimb midsupport; FTO=forelimb lift off; HTD=hind limb touch down; HMS=hind limb midsupport; HTO=hind limb lift off. N=15 for each species.
A) Mean tail inflection angle (Angle A) at forelimb and hind limb events. Note concave upward posture in *T. hatinhensis*—comparisons between all other species and *T. hatinhensis* exceed significance levels of p<0.05.
B) Mean mid-tail angle (Angle B), made by inflection point, the base of tail and an imaginary horizontal line through the base of the tail. Tails are typically less arched in *Pygathrix* than in *Trachypithecus*.
C) Mean tail tip angle (Angle C), defined as the angle between the tip of the tail, the base of the tail, and an imaginary horizontal line through the tail base. Note that tail tip generally is carried above the level of the substrate in *T. hatinhensis*, in contrast with below-substrate tail tip typically exhibited by the other species in the study.

Further analyses revealed that Delacour's langurs tend to carry the body of the tail slightly higher than do Hatinh langurs or doucs, as revealed by higher values for Angle B (Fig. 5B). Interestingly, the tail inflection to x-axis angle (Angle B) suggests more change in tail position during the stride cycle than did the tail inflection angle, yet no correlation was observed between Angle B and travel velocity. Perhaps not surprisingly, the tail tip to x-axis angle (Angle C) was a good discriminator of the Hatinh langurs as they tend to arch their tails concave-upwards, particularly at higher speeds, such that the tail tip is in a much different position than is observed in the Delacour's langurs and the doucs (Fig. 5C). As Hatinh langurs exploit similar habitat types as the other leaf monkeys, there is not a clear explanation for their unique tail posture at this time, but we are pursuing more detailed studies of axial postcranial morphology and will soon have the opportunity observe this species in its natural habitat.

Positional behavior

Patterns in tail kinematics may reflect differences in postural and locomotor preferences between the leaf monkey species. For example, Delacour's langurs prefer to sit the majority of the time, whereas Hatinh langurs tend to use prone positions and spend more time on the ground. Moreover, Hatinh langurs exhibit higher frequencies of quadrupedal walking than do Delacour's langurs (Fig. 6), whereas the latter leap and bound more frequently (p< 01). In contrast, doucs spend a significant portion of time in forelimb suspensory postures (48-60%) and less time walking and leaping than do either of the *Trachypithecus* species.

With respect to tail posture, the most notable difference among the four species is that Delacour's and Hatinh langurs exhibit a great deal more variability in tail postures than do the doucs, frequently using tail postures that involve holding the tail up and/or arched over the body (TA), or back away from the body (TQ) (Fig. 7A-B). Only Delacour's langurs used the TQ tail position, whereas only Hatinh

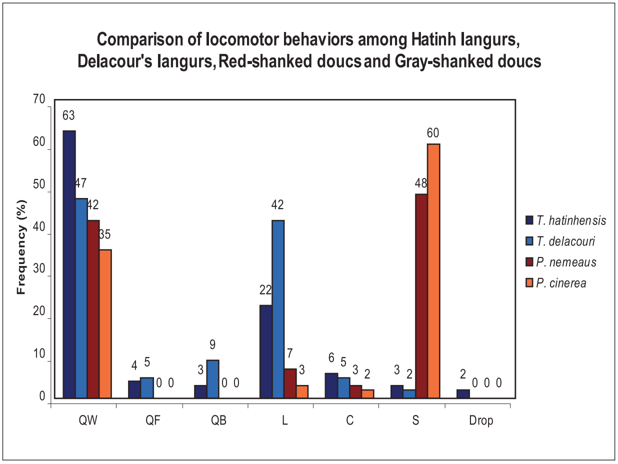


Fig. 6. and postural behavior by category in the four leaf monkeys.

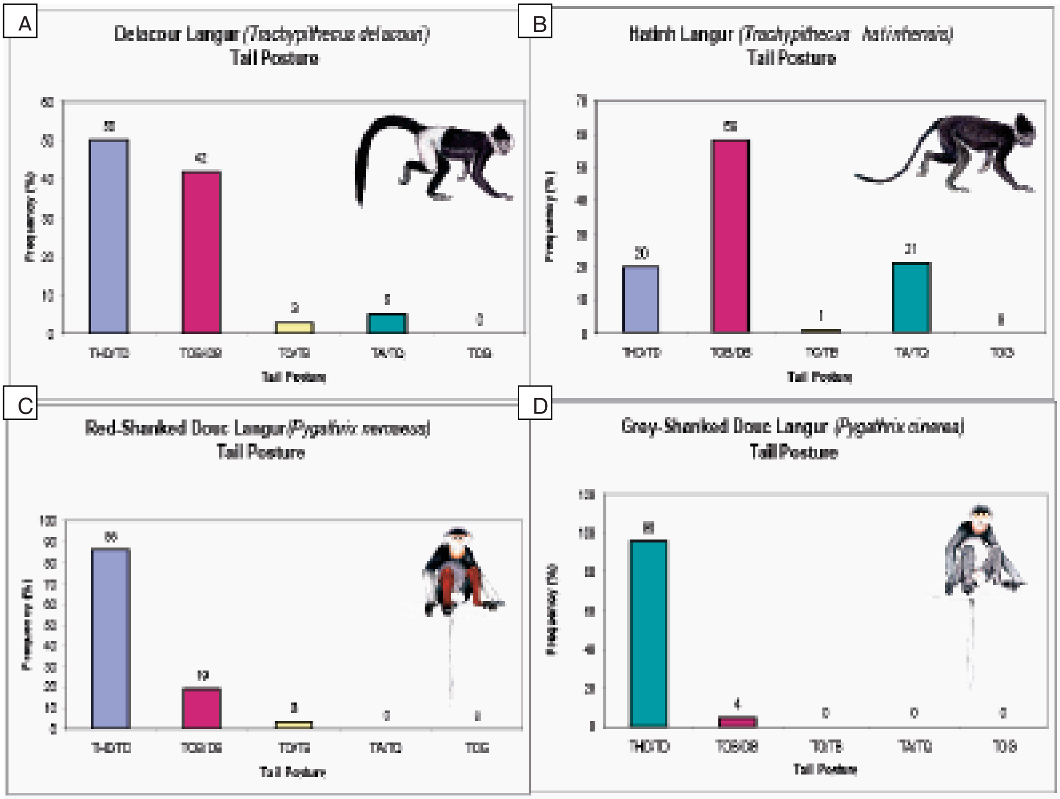


Fig. 7. Percent time spent exhibiting different tail postures by the four leaf monkeys. A) *Trachypithecus delacouri*, B) *T. hatinhensis*, C) *Pygathrix nemaeus* , and D) *P. cinerea*.

langurs were observed using the TA tail posture The most frequent tail postures for both *Trachypithecus* species allowed the tail to hang freely in a pendulous position (THD/TD), or draped over a substrate (TOB/DB) (Fig. 7A-B). Delacour's langurs used the TQ tail position 100% of the time when they were walking fast, or bounding (Fig. 8A).

During slower quadrupedalism they tended to let the tail hang down (50%) or rest on a substrate (27%). TQ tail position was used only 14% of the time during walking. Hatinh langurs used the TA tail position almost exclusively during quadrupedal locomotion (87% for walk, 100% for fast quadrupedalism, and 100% for bounding). This suggests that tail posture may vary as a function of locomotor mode and/or velocity. The only tail postures observed for the red-shanked and grey-shanked doucs were postures that allowed the tail to hang freely in a pendulous position (THD/TD), drape on substrate (TOB/DB), or that held the tail up off of the substrate, but straight out in back of the body (TO/TB) (Fig. 7C-D). Both the red-shanked and grey shanked doucs used THD/TD most frequently during quadrupedal walking (84% and 95%, respectively) (e.g. Fig. 8B). Interestingly, neither the red-shanked nor the grey-shanked doucs were observed using faster modes of quadrupedalism or bounding. This may relate to the use of more suspensory modes for faster travel in *Pygathrix* species.

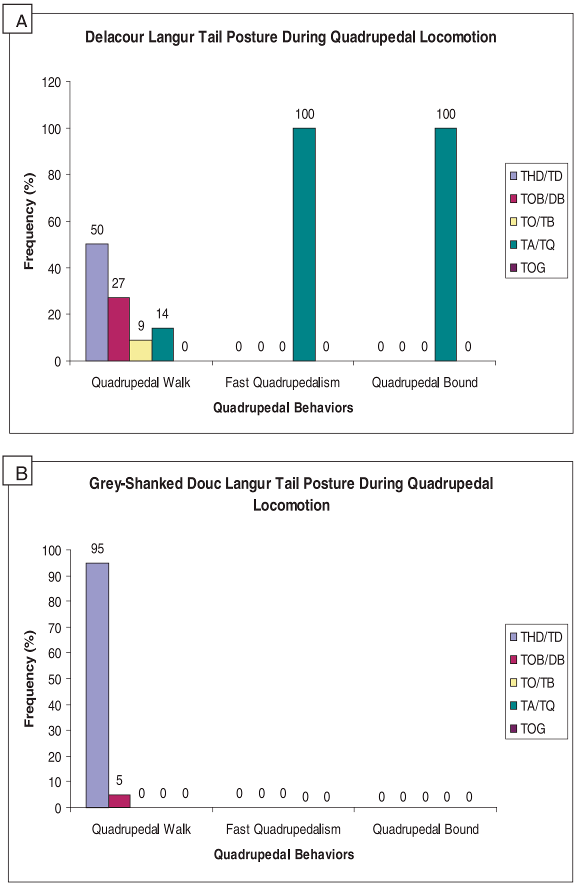


Fig. 8. Tail posture during quadrupedal locomotion in A) *Trachypithecus delacouri*, and B) *Pygathrix cinerea*. Note that whereas the Delacour's langur engages in fast quadrupedal walking and bounding in addition to slow walking, quadrupedal locomotion in grey-shanked doucs is typically slow. Patterns are similar in each of their congeners.

Discussion and conclusions

Recent work in Cuc Phuong National Park is the first to document detailed locomotor kinematics among the colobines of Vietnam. This study reveals marked differences in tail postures among four leaf monkey species explored herein. During symmetrical walking, doucs (*Pygathrix nemaeus* and *P. cinerea*) typically allow the tail to hang down, falling along the substrate behind them during travel. Delacour's langurs (*Trachypithecus delacouri*) exhibit greater variability, frequently arching the tail higher in concave-downwards posture. Hatinh langurs (*Trachypithecus hatinhensis*) exhibit the most variability in tail carriage, often raising the tail into a concave-upwards arc with the tip reaching over the thorax. These patterns may reflect phylogeny and/or different locomotor adaptations used in the wild. Genetic findings detail a deep split in the "limestone langurs" (e.g.

Roos et al., 2001) with a northern group including *T. delacouri*, *T. francoisi*, *T. poliocephalus*, and *T. leucocephalus*, and a southern group including *T. hatinhensis*, *T. laotum*, and in some analyses *T. ebenus*. Anecdotally, tail posture with each of these genetic/geographic groups of *Trachypithecus* is very similar, with the northern species exhibiting concave-downward tail carriage and the southern species exhibiting concave-upward postures.

From the standpoint of differing locomotor adaptations, doucs differ from *Trachypithecus* species in exhibiting relatively little overall movement of the tail during quadrupedal locomotion. These species typically use quadrupedal behaviors to move slowly, transitioning to suspensory locomotion to travel at higher speeds. In contrast, Delacour's and Hatinh langurs exhibit a range of locomotor speeds during quadrupedalism, favoring fast quadrupedalism and bounding over arm-swinging behaviors (Wright et al., in press) and may incorporate sweeping motions of their tails to assist with balance when negotiating steep limestone karst formations in the wild. Further exploration of habitat use and locomotor adaptation of leaf monkeys is needed to test these ideas, and better knowledge of their natural habitats is pivotal for conservation and captive management of these critically endangered forms.

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Mandibular morphology as it relates to ingestive and digestive folivory in *Trachypithecus* and *Pygathrix*

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Key words: mandibular morphology, digestive strategies, *Trachypithecus*, *Pygathrix*, Southeast Asian colobines

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Summary

Recent studies have identified differences in patterns of food selection, ingestive behavior, dental morphology, and gut physiology among the three major genera of leaf monkey found within Vietnam; *Pygathrix*, *Rhinopithecus*, and *Trachypithecus*. Building on this previous work, Wright et al. (2008) compared chewing rates between *Trachypithecus* and *Pygathrix* when masticating leaves of comparable toughness. *Trachypithecus* was found to chew leaves faster and to have significantly larger lower molars. These findings were argued to support the hypothesis that *Trachypithecus* species rely more on ingestive behaviors for the processing of leaves, whereas *Pygathrix* species, with slower chewing rates, smaller molars, and the presence of a “gastric mill” (i.e. presaccus of the stomach) rely more on their digestive tract for the processing of leaves. This study augments the findings of Wright et al. (2008) by comparing four mandibular variables (width and depth of the mandibular symphysis and the mandibular corpus) between *Trachypithecus* and *Pygathrix*. These variables are indicative of the ability of the mandible to withstand high or repetitive biting or chewing forces. Measurements were taken on skeletal specimens housed at the Endangered Primate Rescue Center, Cuc Phuong, Vietnam and at the National Museum of Natural History, Washington, D.C., USA. The two genera were found to be comparable in absolute and size adjusted symphyseal depth, symphyseal width, and corpus width. However, *Trachypithecus* significantly exceeded *Pygathrix* in corpus depth. These findings, when placed in the context of previous work, further support the hypothesis of ingestive folivory for *Trachypithecus* and digestive folivory for *Pygathrix*. Additional comparisons of molar occlusal morphology are proposed to supplement these findings as are studies of activity, food chemistry, and metabolism in these genera to assess the relative energetic efficiency of their respective ingestive and digestive dietary strategies.

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Đặc điểm hình thái hàm dưới trong mối quan hệ với việc lấy thức ăn và tiêu hóa thực vật ở giống *Trachypithecus* và *Pygathrix*

Tóm tắt

Những nghiên cứu gần đây đã chỉ ra sự khác biệt trong cách thức lựa chọn thức ăn, tập tính nhai nuốt, hình thái răng, và hoạt động của dạ dày giữa ba giống khỉ ăn lá ở Việt Nam gồm: *Pygathrix*, *Rhinopithecus*, và *Trachypithecus*. Kế thừa những nghiên cứu trên, Wright và cộng sự (2008) so sánh tần xuất nhai giữa hai giống *Trachypithecus* và *Pygathrix* khi chúng ăn lá. Kết quả cho thấy ở giống *Trachypithecus* việc nhai lá diễn ra nhanh hơn, và hàm dưới có cấu tạo lớn hơn hàm dưới ở giống *Pygathrix*. Khám phá này ủng hộ giả thuyết: giống *Trachypithecus* tiêu hóa lá phụ thuộc nhiều vào tập tính nhai nuốt, còn giống *Pygathrix* với tần xuất nhai chậm hơn, hàm dưới nhỏ hơn việc tiêu hóa lá phụ thuộc nhiều hơn vào hệ thống chuyển hóa và hấp thụ bên trong.

Nghiên cứu này nhằm cung cấp thêm dẫn chứng cho khám phá của Wright và cộng sự. Nghiên cứu đã so sánh bốn đặc trưng (gồm độ rộng, độ sâu của hàm dưới, sự kết nối của hàm dưới, và thân hàm dưới) ở các loài thuộc hai giống *Trachypithecus* và *Pygathrix*. Các thông số này được chọn vì chúng thể hiện khả năng chịu lực tạo ra bởi việc cắn và nhai liên tục của hàm dưới. Mẫu vật được đo có tại Trung tâm Cứu hộ Linh trưởng Nguy cấp, Cúc Phương, Việt Nam và Bảo tàng Lịch sử Tự nhiên Hoa Kỳ, Mỹ. Một vài thông số được so sánh tuyệt đối như: độ rộng, độ sâu của điểm kết nối hàm, và độ rộng của thân hàm. Tuy nhiên, độ sâu của thân hàm ở giống *Trachypithecus* vượt hẳn giống *Pygathrix*. Kết quả một lần nữa ủng hộ giả thuyết về tiêu hóa lá phụ thuộc việc nhai nuốt ở giống *Trachypithecus*, và tiêu hóa phụ thuộc vào sự chuyển hóa ở dạ dày của giống *Pygathrix*.

So sánh về hình thái bề mặt của răng hàm đang được tiến hành để củng cố kết quả trên. Ngoài ra cần có thêm những nghiên cứu về hoạt động, hóa học dinh dưỡng, và quá trình trao đổi chất ở hai giống trên để chứng minh cho giả thuyết.

Introduction

Ten species of leaf eating primate representing three genera are found within Vietnam's borders (Nadler & Streicher, 2004). Dietary studies of these taxa are few, but diverse, having focused on critical aspects of their ecology (Kirkpatrick, 1998), anatomy (Jablonski, 1998; Wright, 2008), digestive physiology (Caton, 1998; 1999) and ingestive behavior (Wright et al., 2008). These studies hint at different patterns of food selection, ingestive behavior, dental morphology, and gut physiology among the three major genera found within Vietnam; *Pygathrix*, *Rhinopithecus*, and *Trachypithecus* (Wright et al., 2008), revealing a level of dietary and morphological variability among these primates that had previously gone unrecognized. The present study adds to the current literature by comparing the ability of the mandibles of *Trachypithecus* and *Pygathrix* to withstand high or repetitive chewing forces.

How to eat a leaf

Leaves demand both mechanical and chemical mechanisms for the extraction of water and nutrients (Cheng et al., 1980; Lucas et al., 1995; Dominy et al., 2001). To gain required nutrients from leaves they must be exposed to microbes that ultimately convert structural and non-structural carbohydrates into volatile fatty acids (Cheng et al., 1980; Van Soest, 1994, Waterman & Kool, 1994). Three different steps can be modified to shape this process. First, leaves may be maintained in the gut for extended periods, thus extending the time of exposure to digestive microbes. Secondly, some part of the gut may be enlarged increasing the volume of microbes and digesta that may interact. Finally, leaves may be broken into smaller pieces to increase the surface area on which microbes may act (Lucas et al., in prep). Reptiles exhibit adaptations permitting the first two methods (Pafilis et al., 2007 [gut retention]; O'Grady et al., 2005 [gut morphology]). Birds also exhibit variation in gut retention times (Fukui, 2003) and morphology (Grajal et al., 1989; Battley & Theunis, 2005), and some exhibit adaptations of the bill and hyoid bone for food processing (Korzoun et al., 2003). Mammals and primates, like birds, exhibit gut and oral adaptations, particularly dental adaptation, for the ingestion and digestion of foliage (Lucas, 2004). But this begs the question: Do folivorous mammals and primates emphasize any of these methods to the exclusion of others, and does emphasis on any one method differ among closely related taxa?

Holding dietary toughness constant, Wright et al. (2008) found that captive *Pygathrix cinerea* and *P. nemaeus* at the Endangered Primate Rescue Center (EPRC), Cuc Phuong National Park, Vietnam, chew more slowly and have relatively smaller molar teeth than *Trachypithecus hatinhensis*, and *T. delacouri*. A slower chewing rate and smaller teeth suggests that larger leaf particles, which also have less surface area for microbial digestive action, are swallowed by *Pygathrix*. With this the case, one may expect that additional comminution of swallowed leaf matter will take place in the stomach or intestines, and indeed, this appears to be the case. Caton (1998,1999) recognizes two distinct gut types among the Colobinae, one group exhibiting a presaccus of the stomach (*Procolobus*, *Rhinopithecus*, *Pygathrix*, and *Nasalis*) and the other lacking a presaccus (*Colobus*, *Semnopitheucs*, *Trachypithecus*, and *Presbytis*). The presaccus, which is densely muscled and has a stratified squamous epithelial lining, was hypothesized by Caton (1998) to be a "gastric mill," which breaks digestiva into smaller pieces, much like a component of the gizzard found in birds, reptiles, some fish, and some other animals. Given these findings, Wright et al. (2008) defined *Pygathrix* species as digestive folivores; exhibiting slower chewing rates, smaller teeth, and a gastric mill, and *Trachypithecus* species as ingestive folivores; exhibiting faster chewing rates, larger teeth, and a gastrocolic digestive system, with a stomach that lacks a presaccus and a colon similar to that of cercopithecines and apes (Caton, 1999).

In the present study, we compare the ability of *Pygathrix* and *Trachypithecus* mandibles to withstand chewing forces. Given the more ingestive strategy of *Trachypithecus*, and knowledge gleaned from in vivo studies of strain in the primate mandible, we predicted that members of this genus would exhibit deeper and wider mandibular corpora and wider (anteroposteriorly) and taller mandibular symphyses.

Material and Methods

Measurements

Because the goal of this study was to evaluate the ability of the mandibles of *Pygathrix* and *Trachypithecus* species to withstand masticatory loads we compared absolute mandibular corpus width (CW), mandibular corpus depth (CD), symphyseal width (SW), and symphyseal depth (SD), in turn we used total mandibular length from the posterior edge of the mandibular ramus to infradentale (MLI) to calculate shape variables for each of the absolute measures (e.g. CW/MLI x 100). Figure 1 illustrates all of the mandibular measures used in this study. These shape variables are based on those used by Taylor (2006) in a comparison of African ape mandibular morphology. In this way we could compare biomechanically relavant and size adjusted corpus depth shape

(CDS), corpus width shape (CWS), symphyseal width shape (SWS), and symphyseal depth shape (SDS) between the two genera. Using MLI to calculate the shape variables estimates the load arm during incision and to a lesser degree during mastication (Bouvier, 1986a; b; Taylor, 2006). We further set the corporal and symphyseal measurements relative to cheek tooth row length from the posterior ramus edge to the mesial edge of P3 (MLP3) to evaluate the contribution of the anterior dentition to the total mandibular load arm and to use a more appropriate estimate of the load arm during mastication (Fig. 1). A wide mandibular corpus efficiently counteracts high strains due to torsional bending of the corpus along its long axis during mastication and incision, while a deeper corpus counteracts strains due to inferiorly oriented bending of the corpus in the sagittal plane when chewing or incising. A wide symphysis counteracts lateral bending ("wishboning") of the corpora, while a deep symphysis is an efficient means of countering torsional stresses on the corpora (Hylander, 1979a; b; 1984; 1985; Taylor, 2006).

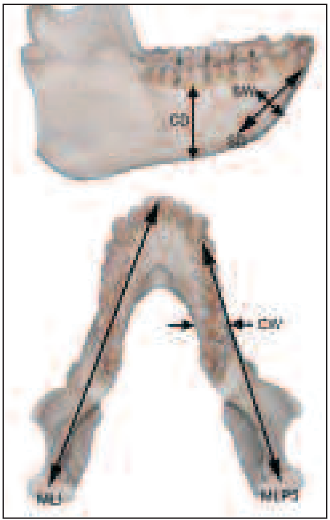

Fig. 1. Measurements superimposed on the jaw of a female specimen of *P. cinerea* (EPRC). CD = corpus depth; SW = symphyseal width (measured at the widest point on the symphysis perpendicular to SD); SD = symphyseal depth (measured from infradentale to gnathion); MLI = Mandibular length to infradentale (referred to as maximum mandibular length in the text, measured from a point on the distal edge of the mandibular ramus in a line parallel to the occlusal plane to infradentale. The posterior position approximates the center of the mandibular condyle.); MLP3 = mandibular length from the same distal position as MLI to the mesial edge of P_3); CW = corpus width (measured at the widest point below M_2). Photo B. Wright.

Statistical analysis

All specimens used in this study were measured at the Endangered Primate Rescue Center or at the National Museum of Natural History, USA (Table 1). Nine adult pecimens of *P. cinerea* and seven adult specimens of *P. nemaeus* composed the *Pygathrix* sample. One adult specimen of *T. delacouri*, two adult specimens of *T. hatinhensis* and one adult specimen of *T. cristatus* composed the *Trachypithecus* sample (see Table 1 for number of individuals per sex). The relatively small sample sizes and the possibility of taxonomic misdiagnosis for some of the *P. nemaeus* individuals, lead us to pool specimens into their representative genera for analysis. Although these samples are small, they are taken from possibly the largest and best curated sample of *P. cinerea* (EPRC) and some of the most endangered primates in the world (e.g. *T. delacouri*).

Table. 1. Species, number of individuals per sex, and collection for measured specimens.

Species	Sex	Collection
P. nemaeus	5 ♂; 2 ♀	NMNH & EPRC*
P. cinerea	4 ♂; 5 ♀	EPRC
T. hatinhensis	1 ♂; 1 ♀	EPRC
T. delecouri	1 ♂	EPRC
T. cristatus	1 ♂	EPRC

EPRC = Endangered Primate Rescue Center.
NMNH = National Museum of Natural History, USA.
* One adult female from EPRC.

In an initial evaluation of histograms and point density plots for each of the variables, only corpus depth shape was found to be distributed normally or slightly playkurtically. Given the primarily leptokurtic nature of the data for each genus, we log (LN) transformed the shape ratios prior to analysis (Jungers et al., 1995). We tested if *Trachypithecus* significantly exceeded *Pygathrix* in all four mandibular shape variables using one-tailed, two-sample Student's t-tests, in Systat 11.0. A Bonferroni adjustment was applied during analysis in order to avoid Type I errors.

Results

Our prediction at the outset of analysis was that the ingestive folivores (*Trachypithecus*) would exceed the digestive folivores (*Pygathrix*) in all shape variables. The genera overlapped in symphyseal width shape (Fig. 2), symphyseal depth shape (Fig. 3), and in corpus width shape (Fig. 4) when the shape variables were calculated using MLI (Table 2). However, *Trachypithecus* species were found to have significantly deeper mandibular corpora when the shape variables were calculated using MLI ($p < 0.01$; Table 2; Fig. 5). Since a larger absolute jaw size could also increase the ability of the jaw to resist comparable biting and masticatory forces we compared

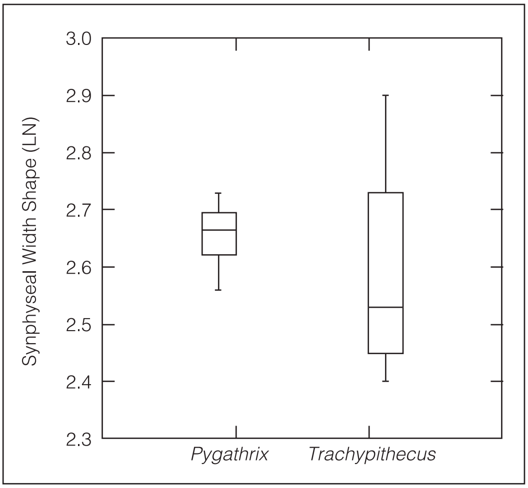

Fig. 2. Box and whisker plot comparing log normal SWS between the two primate genera (Student's *t*, p > 0.05). Horizontal center line = median, length of box = range within which the central 50% of the values fall, hinges = first and third quartiles.

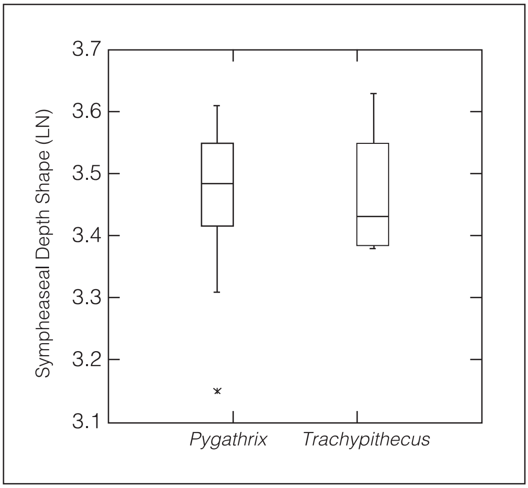

Fig. 3. Box and whisker plot comparing log normal SDS between the two primate genera (Student's *t*, p > 0.05). The statistical findings were the same with and without the outlier (asterisk). Horizontal center line = median, length of box = range within which the central 50% of the values fall, hinges = first and third quartiles.

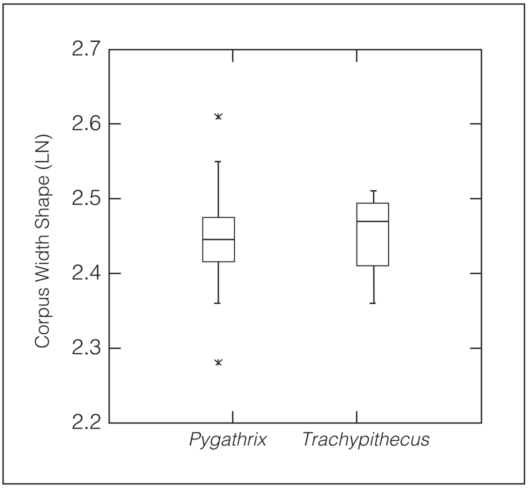

Fig. 4. Box and whisker plot comparing log normal CWS between the two primate genera (Student's *t*, p > 0.05). The statistical findings were the same with and without the outliers (asterisks). Horizontal center line = median, length of box = range within which the central 50% of the values fall, hinges = first and third quartiles.

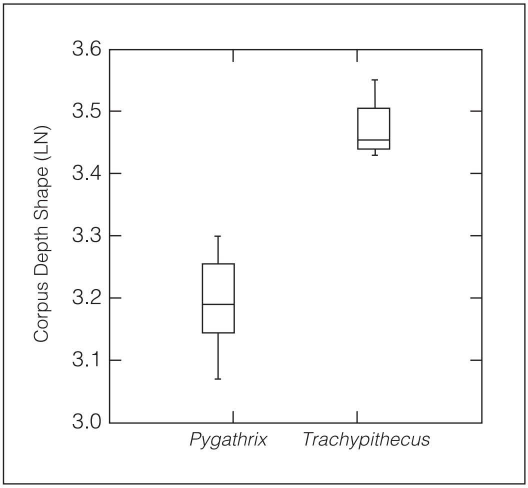

Fig. 5. Box and whisker plot comparing log normal CDS between the two primate genera (Student's *t*, p < 0.01). Horizontal center line = median, length of box = range within which the central 50% of the values fall, hinges = first and third quartiles.

absolute measurements of the corpus and symphysis. These findings were the same as those for the size adjusted shape variables with *Trachypithecus* significantly exceeding *Pygathrix* in corpus depth (p = 0.01; Table 2), but being of a comparable shape for the other three variables of interest (Table 2).

Table. 2. Results of Student's *t*-test on log normal adjusted absolute and shape variables for both genera. Mean values are presented for both genera.

Variable	*Pygathrix*		*Trachypithecus*	*t*-test	p
SW	2.30	>	2.15	1.53	0.12
SD	3.11	>	3.02	1.03	1.00
CW	2.08	>	2.01	1.39	0.73
CD	2.84	**<**	3.03	-3.67	**0.007**
SW/MLI	2.66	>	2.59	1.21	0.97
SD/MLI	3.47	=	3.47	-0.04	1.00
CW/MLI	2.44	=	2.45	-0.23	1.00
CD/MLI	3.20	**<**	3.47	-7.80	**0.0001**
SW/MLP3	2.80	>	2.67	2.23	0.16
SD/MLP3	3.61	>	3.55	0.35	1.00
CW/MLP3	2.59	>	2.53	1.31	0.83
CD/MLP3	3.34	**<**	3.55	-5.29	**0.0001**

SW = symphyseal width; SD = symphyseal depth; CW = corpus width; CD = corpus depth; MLI = mandibular length to infradentable (maximum length); MLP3 = mandibular length to mesial edge of p_3 (cheek tooth row length).

Bold type indicates significant difference.

In order to evaluate the relative mechanical advantage when biting with the cheek teeth or chewing, we compared shape variables calculated using MLP3 to those caluclated using MLI. The average values for both symphyseal shape variables and for CWS when using cheek tooth row length are relatively higher for *Pygathrix* than when the shape variables are calculated using MLI (Table 2). This suggests that *Pygathrix* may have relatively greater mechanical advantage when chewing than when incising. However, the overall pattern remained the same when using MLP3 for calculating the shape variables: *Trachypithecus* significantly exceeded *Pygathrix* in MDS (p < 0.01; Table 2). The two genera were comparable in the other three shape variables of interest (SDS, SWS, and CWS).

Discussion

This study builds on previous findings concerning the dietary strategies of *Trachypithecus* and *Pygathrix* and provides additional evidence in support of the hypothesis that *Trachypithecus* uses its mouth more intensively for the comminution of food while *Pygathrix* uses specializations of its stomach for comminution and digestion. The *Trachypithecus* species in this study were found to be comparable to *Pygathrix* in three out of four absolute measures of the mandible and in six out of eight biomechanically relevant shape variables. However, *Trachypithecus* species were found to significantly exceed *Pygathrix* species in CD and in CDS. This increased mandibular depth can be qualitatively identified when the mandibles of members of each genus are compared visually (Fig. 6). This same pattern was found when the shape variables were calculated using MLP3 as opposed to MLI. While the increase for *Pygathrix* in average values of SD, SW, and CW when using MLP3 suggests that the anterior dentition compose a slightly greater proportion of total mandibular length in this genus, it cannot be statistically argued to represent a difference of great functional value. Hence, it may be hypothesized that, relative to *Pygathrix*, *Trachypithecus* has evolved greater CD and CDS in response to increased strains in a sagittal plane during incising, cheek tooth biting, or mastication.

It remains to be seen if there are particular ways in which *Trachypithecus* processes foods with

Fig. 6. Lateral views of the mandibles of a male *T. hatinhensis* specimen (left) and a male *P. cinerea* specimen (right) (EPRC). Images have been scaled by eye to have approximately the same ramus height. Note the deeper corpus and more inferoposteriorly flared ramus of *T. hatinhensis*, which is similar to that of New World *Alouatta* spp., and the relatively more prognathic anterior dentition of *P. cinerea*. See text for further discussion. Photos B. Wright.

its cheek teeth, or in the way it recruits its masticatory muscles when chewing, that could account for these higher strains. Given preliminary data on the length of chewing bouts and chewing rates (Wright et al., 2008) we hypothesize that this increase in corpus depth is due in part to *Trachypithecus*' significantly higher chewing rate relative to the rate found for *Pygathrix*. It is notable that, qualitatively, the shape of the *Trachypithecus* jaw is more like that of the New World howling monkey (*Alouatta* spp.) than is the jaw of *Pygathrix* (Fig. 6, legend). Howling monkeys have been defined as behavioral folivores due to their reliance on low activity levels to compensate for a heavily folivorous diet (Milton et al., 1979). As with *Trachypithecus* spp., with which it shares a convergent mandibular shape, *Alouatta* spp. may also rely on oral ingestive behaviors (e.g. increased chewing rates or increased chewing bout lengths) to compensate for a relatively unfolivore-like gut.

Relative to *Pygathrix*, *Trachypithecus* species have been found to have a significantly larger M_2, higher chewing rates (Wright et al., 2008) and, as found in this study, deeper mandibular corpora. However, they lack the presaccus, or "gastric mill" (Caton, 1998) found in *Pygathrix* species. We are presently augmenting these findings with additional craniometric data and detailed analysis of tooth crown morphology. We also plan to combine these findings with data on the metabolic rate of these species, chemical analysis of their foods, their activity pattern, and their locomotor repertoire in order to identify if the ingestive strategy *Trachypithecus* or the digestive strategy of *Pygathrix* is more energetically efficient.

Conclusions

1) *Trachypithecus* and *Pygathrix* are comparable in mandibular symphyseal width and depth and in mandibular corpus width.
2) However, *Trachypithecus* has a significantly deeper mandibular corpus than *Pygathrix*.
3) Deeper mandibular corpora suggest an evolved response to increased and inferiorly directed strains in a sagittal plane during either incision or during mastication.
4) These findings support the hypothesis proposed by Wright et al. (2008) that *Trachypithecus* is an ingestive folivore whereas *Pygathrix*, with its "gastric mill" is a digestive folivore.
5) Research investigating differences between these genera in molar occlusal morphology and in metabolic efficiency are ongoing.

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The placenta of the Colobinae

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Key words: Colobinae, langurs, placenta, bilobed, hemochorial

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Summary

Leaf-eating monkeys have a hemomonochorial placenta that is usually composed of two lobes and these are connected by large fetal vessels. In general, the placenta is similar to that of the rhesus monkey (*Macaca mulatta*) and, like that species, occasional placentas possess only a single lobe. This paper describes the structure, weights and cord lengths of all colobine monkeys examined by the author to date and it provides an overview of the placentation of langurs in general.

Nghiên cứu về nhau thai của nhóm khỉ ăn lá

Tóm tắt

Ở nhóm khỉ ăn lá (leaf-eating monkeys) nhau thai thường được tạo bởi hai thùy, và hai thùy này nối với nhau bởi những mạch máu lớn từ bào thai. Nhìn chung, cấu tạo nhau thai của nhóm này giống ở nhóm khỉ vàng (*Macaca mulatta*). Và cũng như ở khỉ vàng, thỉnh thoảng nhau thai chỉ có một thùy. Trong nghiên cứu này tác giả mô tả cấu tạo, cân nặng cũng như chiều dài nhau thai của các loài thuộc nhóm khỉ ăn lá. Qua đó cung cấp thông tin toàn diện về cấu tạo nhau thai của các loài khỉ ăn lá.

Introduction

Colobine monkeys have a placentation that is similar to that of other members of the Cercopithecidae. The placentas are usually bilobed, generally their cord insertion site is on the larger of the two lobes, and they have a hemomonochorial relationship to the maternal organism. In addition, there are no atrophied villi in the free membranes, presumably because of the paucity of a true decidua capsularis as well as their superficial endometrial implantation. This paper describes all known placental publications of the Colobinae and provides a summary of the placental weights and other physical characteristics of placentas examined by the author.

Materials and Methods

The placentas presented here come from the births of all Colobinae at the Zoological Society of San Diego and the Endangered Primate Research Center at Cuc Phuong National Park, Vietnam.

The following species were studied:

Delacour's langur	*Trachypithecus delacouri*
Francois' langur	*Trachypithecus francoisi*
Hatinh langur	*Trachypithecus laotum hatinhensis*

Red-shanked douc langur	*Pygathrix nemaeus*
Grey-shanked douc langur	*Pygathrix cinerea*
Cat Ba langur	*Trachypithecus p. poliocephalus*
African Colobus monkey	*Colobus angolensis*
Hanuman langur	*Presbytis (Semnopithecus) entellus*

Some of these placentas were received fresh; others had been fixed in 10% formalin solution before examination. Although a bilobed placenta is typical of these species, as will be seen in Table 1, three placentas had only a single lobe despite being apparently complete specimens and, in addition, there were 3 of 20 Hanuman langurs (*Presbytis* (*Semnopithecus*) *entellus*) with single lobe. This has also been the case in some rhesus monkey placentas; they are described as having two disks in only about ~80% of term gestations (Myers, 1972; Chez et al., 1972). The real reason for this discrepancy is unknown at this time, but Chez et al. (1972) considered this to represent a hereditary phenomenon. In the closely related baboon, however, only a single placental disk is found. Similarly, in Hanuman langurs 3 of 20 placentas examined by me had only a single lobe (Benirschke, 2007). It is likely that some factors at early implantation are responsible for the discrepancy but no specific suggestions of what they are have been made.

Results

Langurs have a typical hemo-monochorial type of placenta. That is to say, only one trophoblastic layer separates the villous structures from the maternal intervillous blood. The villous structure is very similar to that found in the human placenta and it is shown next. Three to four fetal capillaries nearly fill the tertiary villi that are covered with a continuous layer of syncytiotrophoblast. This trophoblast with its microvillous surface is bathed by the maternal blood. The syncytium derives from the cytotrophoblast, but that is difficult to discern by light microscopy and becomes discontinuous as a layer with advancing gestation. In electronmicroscopic studies, however, it can always be identified and the cytotrophoblast can also be seen in very young langur placental light microscopic preparations (e.g. Burton, 1980). There are also a few connective tissue cells in the terminal villi while they and the fetal villous macrophages (“Hofbauer cells”) are more common in secondary villi. The syncytium has a contiguous cytoplasm in which its diploid nuclei are dispersed. There is continuous growth of the syncytiotrophoblast during maturation and thus the formation of syncytial “knots” increases with age. The syncytial knots may detach and get swept away in the intervillous blood stream.

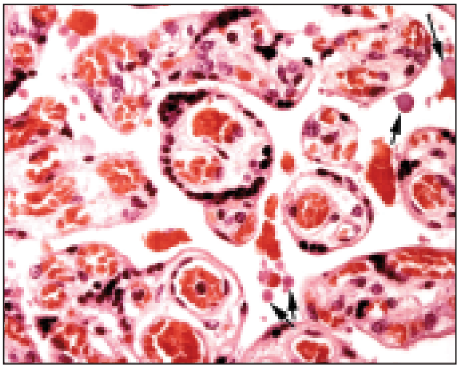

Fig. 1. Villi of mature langur placenta with syncytiotrophoblastic vesicles indicated at arrows.

Fig. 2. Higher magnification of terminal mature villi and syncytial “knot”.

They end up in the maternal lung and there they disintegrate because they do not have the capacity to reproduce. Additionally, numerous ‘cytoplasmic vesicles’ are found in the intervillous space that arise from the syncytial cytoplasm. These vesicles contain various enzymes and hormones (Fujikura & Mukai, 2006). The syncytiotrophoblast has a microvillous surface and is responsible for the nutrient and gaseous traffic across the placenta and for hormone production. It is essentially indistinguishable from human trophoblast when studied fine-structurally (Wynn et al. 1971).

The typical feto-maternal ‘barrier’ thus consists of fetal endothelium, and syncytiotrophoblast. The tertiary villi shown in Fig.2 branch from larger stem villi which have their connective tissue connection to the chorionic membrane (Fig. 3).

The mature placenta also contains numerous foci of calcification. They occur both, in fibrinoid and in the more sclerotic villi. Such a degenerated ‘intermediate’ villus with calcification is shown next (Fig. 4).

The variability of the length of the umbilical cord shown in Table 1 is surely the result of inadequate removal at birth or because of incomplete submission to the pathologist. In general, the umbilical cord must be about 25-30 cm long; it possesses two arteries and one vein (Fig. 5). No ducts are present in most umbilical cords and the surface is covered with a single layer of squamous amnionic epithelium. There are few twists of the cord, but when they are present, the twisting is found to occur in both directions.

Many langur placentas have small regions of complete infarction, yellow areas that have become necrotic; most of these are located at the margins. As is true of human placentas with infarcts, these areas merely atrophy and do not become ‘organized’ by replacement with a scar; and they also do not elicit an inflammatory reaction. More importantly, the mechanism that leads to these frequent infarcts in cercopithecine placentas is unclear. Unlike the findings in human gestations, in which maternal decidual vascular occlusions or ‘atherosis’ (in preeclampsia) may be held responsible for the infarcts, such changes have not been demonstrated in langurs.

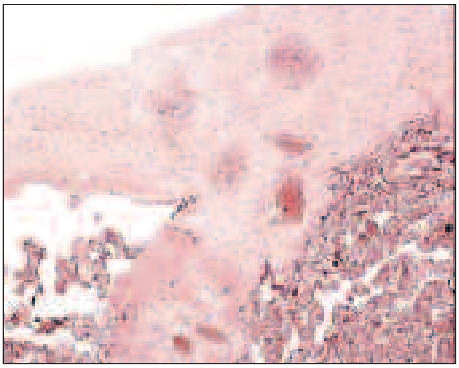

Fig. 3. Fetal surface of placenta with main stem villus arising from the chorionic membrane.

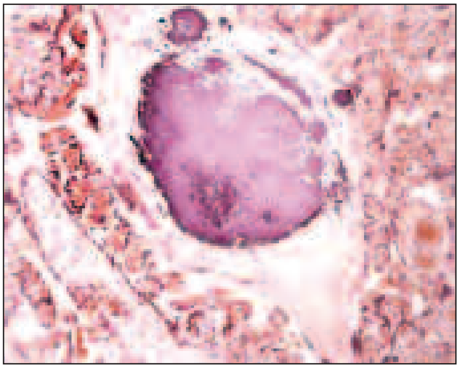

Fig. 4. Calcification (blue) within a degenerated villus.

Fig. 5. Cross section of the umbilical cord. The two umbilical arteries are at the top, the vein is below.

Table 1. The weights of placentas, lengths of umbilical cords and fetal weights (when available) are listed sequentially.

SPECIES	WEIGHT [g]	CORD [cm]	LOBES	FETAL WEIGHT [g]
Delacour's langur	145 g	26 cm	2	
	82 g	21 cm	2	
Hatinh langur	150 g	13 cm	1	
	87 g	?	2	
Francois' langur	141 g	20 cm	2	
	119 g	16 cm	2	
	70 g	8 cm	2	
Grey-shanked douc langur	118 g	5 cm	2	
Red-shanked douc langur	68 g	14 cm	2 stillborn (see Resnik et al., 1978)	465 g
	145 g	19.7cm	2	
	95 g	33 cm	2	
	150 g	31.5cm	2	
	100 g	0	2	367 g
	72 g	18 cm	1	
	130 g	23 cm	2	510 g
	133 g	30 cm	2	577 g
	100 g	24 cm	2	475 g
	87.5 g	31 cm	2	304 g
	68.4 g	32 cm	2 stillborn	475 g
	195 g	21 cm	2 stillborn	
	110 g	24 cm	2	
Cat Ba langur	65 g	16 cm	2	
African Colobus monkey	120 g	21 cm	2	
	135 g	?	2	
	105 g	10 cm	2	
	129 g	3.5cm	2	750 g
	112 g	23 cm	2	272 g
	31 g	15 cm	1	
Hanuman langur	20 placentas are shown in tabular form by Benirschke (2007).			

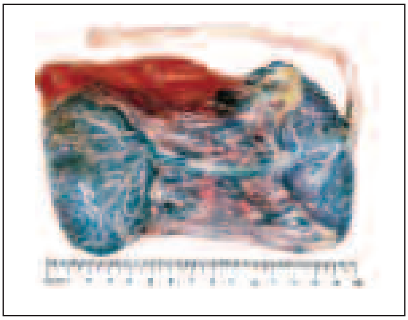

Fig. 6. Delacour's langur placenta with two lobes, cord insertion on larger lobe and connecting vessels between the two lobes. Photo: T. Nadler.

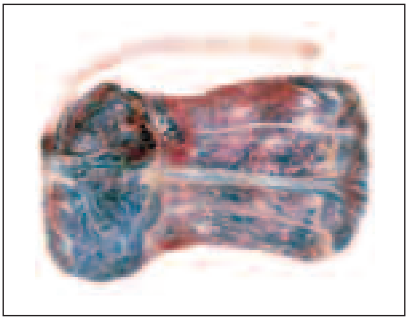

Fig. 7. Maternal surface of the same placenta as in Fig. 6. Photo: T. Nadler.

The membranes are composed of an inner layer of amnionic epithelium that sits on a thin layer of avascular connective tissue. While the amnion is compressed against the chorionic membrane, it can always be detached since it is not physically fused with the chorion. In the free membranes between the two lobes and those making up the gestational sac there are the large blood vessels that connect the two placental lobes (Fig. 6,7). A thin layer of trophoblast and some small quantity of decidua 'capsularis' form the outer surface of the free membranes (Fig. 8). In contrast to human placentas, however, the free membranes contain no atrophic villi. While they are characteristically found in the decidua capsularis of human placental membranes, they are absent in langurs, marmosets, rhesus monkey and most other primates studied in sufficient detail.

Fig. 8. Free membranes with amnion above, followed by a potential space, the chorion, trophoblast and decidua capsularis.

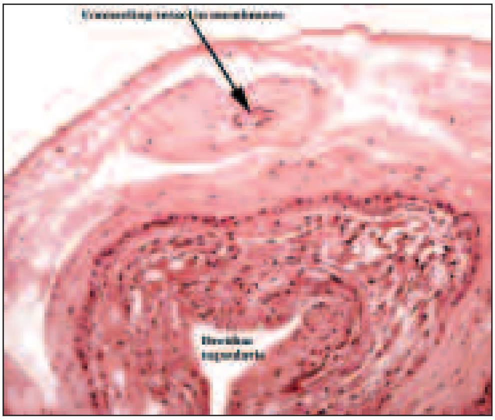

Fig. 9. Maternal surface of the placenta with the shell of extravillous trophoblast and peripheral decidua basalis. One of the large connecting blood vessels is carried in the chorion.

Another feature that distinguishes the langur placenta from that of human gestations is the nature of the maternal floor. The langurs have a relatively smooth outer surface on their disks. Peripheral to the villous tissue is a nearly smooth shell of extravillous trophoblast, cells that represent the invasive, implanting elements, and then follows a layer of less modified decidua basalis (compared to that seen in human term placentas) in which the placenta separates by shearing. It does not appear to be infiltrated by trophoblast very deeply (Fig. 9).

Discussion

The findings of different species of leaf-eating monkey's placentas show great similarities in their macroscopic and histological appearance. While most are bilobed, a smaller number has no secondary lobe despite being associated with the development of a normal offspring. Thus, these placentas resemble closely those of the better studied placentas of rhesus monkeys and baboon (Ramsey, 1982; Mossman, 1987), including the occasional unilobar structure. In addition, it is striking that none of these species possesses the numerous atrophic villi on the free membranes that are so characteristic of human placentation. Thus, there must be a sufficient difference in the early implantational relationship between trophoblast and endometrium. Unfortunately, the only early langur implantation sites described are restricted to two young implantations in dusky leaf monkeys (*Trachypithecus obscurus*) by Burton (1980). He found a number of significant differences from early human implantations. First, he suggested that there was little typical 'decidual' change in the endometrium. Remarkably though, the detachment of placentas from the uterus at term suggests that some decidualization must exist at the base. Also, some maternal endometrial tissue is found on the membranes and that tissue as well as that on the placental base is similar to endometrial stroma. It may not show the histological increase of glycogen content and the compaction of endometrial stromal cells may be different, but it must serve similar function. But then, the formation of the decidua is very complex, as was so well delineated in Kliman's review (2000); differences surely exist amongst primates. Burton (1980) also described the invasion of maternal blood vessels by extravillous trophoblast that is similar to human placentation without, however, causing the typical vascular modification of spiral arterioles to takes place in human implantation.

Most importantly though, Burton (1980) depicts the very large and superficial nature of the implanting trophoblast. In his second specimen, typical villous formation is shown, while there is apparently no evidence of the development of a secondary disk at those stages. As far as the pattern of fetal blood vessels is concerned, the majority show the 'magistral' type, as was true of baboon placentas (Houston & Hendrickx, 1968). Regrettably, no studies were conducted to ascertain the possible absence of the transverse umbilical arterial anastomosis ('Hyrtl anastomosis'). This is a definite need for future investigations.

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Gibbon hand preference studies at the Endangered Primate Rescue Center, Vietnam

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Key words: Gibbons, locomotion, hand preference

Summary

The project involved a total of 150 hours of continuous focal animal sampling for 16 crested gibbons, genus *Nomascus*, at the Endangered Primate Rescue Center of Cuc Phuong National Park, in Ninh Binh Province, Vietnam. The results of this preliminary observation into the effect of brachiation level (Enclosure Level Drop) on hand preference during gibbon locomotion revealed that seven of nine adults showed greater hand index (HI) scores in the Enclosure Level Drop Leading Limb measure in comparison to two individuals (1 adult, 1 sub-adult) that had higher HI scores in Uni-level Leading Limb. Significant population-level effects were found for brachiation level and age class which demonstrates the importance for researchers of primate hand preference to conduct observations across multiple variables.

Nghiên cứu về sở trường hoạt động tay của vượn tại Trung tâm Cứu hộ Linh trưởng Nguy cấp, Việt Nam

Tóm tắt

Nghiên cứu này đã thực hiện trong 150 tiếng liên tục tập trung vào 16 cá thể vượn thuộc giống *Nomascus* được nuôi giữ tại Trung tâm Cứu hộ Linh trưởng Nguy cấp ở Vườn Quốc gia Cúc Phương, tỉnh Ninh Bình, Việt Nam.

Những kết quả quan sát bước đầu đã đưa được ra tính hiệu quả của các tầng giá đỡ (Chuồng có Tầng Giá đỡ) cho sở trường vận động tay của vượn. Vận động của bảy trong số chín cá thể trưởng thành cho thấy sự giá trị của tay trong quá trình vận động (HI) chính ở trong Chuồng có Tầng Giá đỡ cho các chi được so sánh với hai cá thể (1 trưởng thành, 1 bán trưởng thành) thì thấy (HI) chính ở trong Chuồng có Tầng Giá đỡ cho các chi là cao hơn. Mật độ - quần thể có ý nghĩa tác động tới tính hiệu quả của các tầng giá đỡ và độ tuổi đã chứng minh được tầm quan trọng việc các chuyên gia linh trưởng nghiên cứu về sở trường vận động tay của vượn nhằm quan sát được nhiều biến đổi và thu thập được nhiều số liệu trong quá trình vận động của động vật.

Introduction

Studies of hand preference (and laterality), based upon whether hand/limb choice differs from a 50-50 proportion, allow researchers to observe various aspects of primate behavior including locomotion, vocalization, and feeding in order to learn more about brain and body function, adaptations, age and development, and sex (McGrew & Marchant, 1997; Hopkins, 1999; Hopkins et al., 2002; Hopkins & Cantero, 2003). Gibbons are important subjects for hand preference (and laterality) studies because of their vocalizations, morning calls related to territory and pair bonds, and their specialized locomotor form of arm swinging called ‘brachiation’ (Geissman, 2000; Redmond & Lamperez, 2004). Environmental adaptation for suspensory locomotion behaviors, such as brachiation, i.e. suspensory swings beneath tree branches, involves specialized body and muscle activity suitable for suspension in an arboreal environment (Fleagle, 1988; Larson, 1993; Bertram, 1999; Byron & Covert, 2004; Thorpe & Crompton, 2006). Previous studies of gibbon hand preference during brachiation have focused primarily on leading limb across the same horizontal axis without explicitly accounting for brachiation level (Stafford et al., 1990; McGrew & Marchant, 1997; Redmond & Lamperez, 2004). In the wild, gibbons have been observed to travel at heights of up to 30m in the emergent forest canopy (Cannon & Leighton, 1994). From a functional perspective, gibbons need strong and skilled hands and arms for maneuvers like dropping to lower branches or levels in their enclosure, and generally avoiding falls and energy loss (Bertram, 1999; Chang et al., 2000; Usherwood et al., 2003). Skill is likely to be expressed through performance differences and the presence of hand preference likely reflects differences in coordination (Hopkins et al., 2002). Despite the variability that gibbons encounter in their natural habitat and the fact that gibbons do not always move across a single horizontal plane in their environment, effect of descent or ascent during locomotion has not been considered in previous studies of gibbon hand preference.

Methods

Subjects were 16 individuals, males and females, ages 2 to 14, including northern white-cheeked gibbons (*Nomascus leucogenys leucogenys*), southern white-cheeked gibbons (*Nomascus leucogenys siki*) and yellow-cheeked gibbons (*Nomascus gabriellae*) at the Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park, Ninh Binh Province, Vietnam. Animals were housed in 9 x 5.5 x 3.5 meter enclosures composed of chain-linked fencing with levels of bamboo poles dispersed throughout the enclosure at horizontal intervals of 80 cm separated by vertical distances of approximately 1.5 m.

A total of 150 hours of continuous focal animal sampling for 16 individuals was completed in March and April of 2007. Each individual was observed for a total of approximately 9 hours and 20 minutes. The total number of observations was 6,905 Data points. The researcher recorded leading limb during locomotion in ‘Uni-level Leading Limb’ and ‘Enclosure Level Drop Leading Limb.’ In order to insure independence of data-points only leading limb was considered, a minimum of 2 full arm swings was needed, and a 3-second pause (or depending on the substrate, a three-step walk) marked the beginning and ending of a brachiation event (Redmond & Lamperez, 2004). ‘Enclosure Level Drop’ involved swinging from on top of to underneath a support level so that the individual swings under the support level on which it had been sitting or standing (based on Hunt et al., 1996). Hand index (Hopkins et al., 2002) was calculated separately for Uni-level, Enclosure Level Drop and Total Combined Leading Limb behaviors by subtracting the total number of left-hand responses from the total number of right-hand responses for the behavior, the result of which was divided by the total number of responses in the behavior: [(R – L)/(R + L)]. In this study, hand index, one-tailed binomial tests, standard two-sample t-tests, and ANOVA were used to determine if statistically relevant results were present across brachiation level, sex and age class.

Results

Leading Limb and Hand Preference

In this study, ABS 20 HI was considered the cut-off point for marginal significance in order to demonstrate a proportional increase of hand bias across Uni-level Leading Limb and Enclosure Level Drop Leading Limb. For among-population analyses, 9 of 16 individuals exhibited a minimum of ABS 20 HI scores in either Uni-level Leading Limb or Enclosure Level Drop Leading Limb which was not a significant proportion based on a two-tailed binomial test. However, for among-population analyses of adult individuals, a significant proportion (8 of 9) had the minimum ABS 20 HI in either Uni-level Leading Limb or Enclosure Level Drop Leading Limb based on a two-tailed binomial test, p = .05. For within-population results, the analysis of variance model revealed a significant correlation between individual subject and hand index scores, F(1, 839) = 4.87, p < .001.

Fig. 1. In Enclosure Drop Leading Limb, a gibbon leaps or drops from a bamboo support or platform and falls downward to grab a support at the lower enclosure level, followed by a minimum of two full arm swings. Note: Uni-level Leading Limb, i.e. two full arm swings across the same horizontal axis does not include the drop from above level. (Fig. 1. modified from Usherwood et al., 2003)

Brachiation Level and Hand Preference

An analysis of variance model revealed a significant correlation between brachiation level and hand index scores, *F*(1, 958) = 2.02, *p* < .01 and a standard two-sample t-test also revealed a significant correlation between brachiation level and hand index, *t*(856) = -2.99, *p* < .01. A minimum of a 60% hand preference bias was revealed for only 2 individuals (1 adult, 1 sub-adult) in Uni-level Leading Limb compared with 7 of 9 adults in Enclosure Level Drop Leading Limb. ANOVA revealed that a significant two-way interaction was found between adult age class and brachiation level, *F*(1, 534) = 7.29, *p* = .01. Daisy, with a dependent infant, had the highest HI score and the fewest total number of Enclosure Level Drop Leading Limb behaviors: 25. For the overall population, the mean number of Enclosure Level Drop Leading Limb behaviors per individual was 117 with a mean number of Uni-level Leading Limb behaviors per individual of 314.

Sex and Hand Preference

All four adult males and three of five adult females showed a minimum ABS score of a 0.20 HI in Enclosure Level Drop Leading Limb. A one-way ANOVA test revealed a significant correlation between sex and hand index scores, *F*(1, 958) = 4.88, *p* < .05. However, a significant correlation was not revealed for HI scores between adult age class and sex. The fact that there were only five males in the sample and four of five were adult males that had an ABS HI score of at least .20 HI may have contributed to ANOVA results for the influence of sex.

Age Class and Hand Preference

A standard two-sample *t*-test revealed a significant correlation between age class and hand index scores, t(958) = -2.97, *p* < .01. Additionally, ANOVA revealed a significant two-way interaction between age class and Enclosure Level Drop Leading Limb Hand Index, *F*(1, 476) = 6.02, *p* < .05. Table 1 demonstrates that the mean adult ABS HI score for Enclosure Level Drop Leading Limb was ABS 29 HI compared with ABS 03 HI for sub-adults. No significant correlation was found between age class and Uni-level Leading Limb Hand Index.

Table 1. Hand Index (HI) Scores and Percentages

HI scores [(R – L)/(R + L)] and percentages for Combined Leading Limb SUM-HI (1), Uni-level Leading Limb HI (2) and Enclosure Level Drop Leading Limb HI (3) across sex, age, and age class. Note: Percentages and preferred Left or Right direction are shown in parentheses; HI scores of ABS .20 HI and 60% percentage or above in bold italic.

Animal	Sex	Age	Age Class	1	2	3
Gorbi	M	14	ADULT	-0.02 (51% L)	-0.05 (53% L)	***0.39 (69% R)***
Daisy	F	13	ADULT	0.18 (59% R)	0.15 (57% R	***0.60 (80% R)***
Lilly	F	13	ADULT	-0.15 (58% L)	***-0.20 (60% L)***	-0.07 (53% L)
Gabi	F	12	ADULT	0.01 (51% R)	0.04 (52% R)	***-0.20 (60% L)***
Rudi	M	10	ADULT	***0.20 (60% R)***	0.17 (59% R)	***0.26 (63% R)***
Ina	F	9	ADULT	0.00 (50-50%)	0.15 (57% R)	-0.13 (56% L)
Simba	F	8	ADULT	0.04 (52% R)	-0.13 (56% L)	***0.45 (73% R)***
Hugo	M	7	ADULT	-0.19 (59% L)	-0.16 (58% L)	***-0.24 (62% L)***
C. Cuong	M	7	ADULT	0.00 (50-50%)	-0.10 (55% L)	***0.30 (65% R)***
Victoria	F	5	SUB-ADULT	-0.02 (51% L)	-0.02 (51% L)	-0.02 (51% L)
Xuan	F	5	SUB-ADULT	***-0.20 (60% L)***	***-0.26 (63% L)***	-0.11 (55% L)
Quynh	F	4	SUB-ADULT	0.00 (50-50%)	0.02 (51% R)	-0.05 (52% L)
Rafi	M	4	SUB-ADULT	0.06 (53% R)	0.06 (53% R)	0.03 (51% R)
Moppi	F	3	SUB-ADULT	-0.06 (53% L)	-0.10 (55% L)	0.05 (52% R)
Platsch	F	2	SUB-ADULT	0.00 (50-50%)	-0.05 (53% L)	0.16 (58% R)
Muckel	F	2	SUB-ADULT	-0.02 (51% L)	0.01 (50-50%)	-0.08 (54% L)

Discussion

Due to the variability that gibbons encounter during brachiation in their natural environment, quantifications of leading limb across multiple brachiation levels are likely to be more accurate measures of gibbon hand preference. In this study, a significant correlation was revealed between age class and brachiation level with a majority of adults demonstrating increased HI scores in Enclosure Level Drop Leading Limb while no sub-adults revealed a marginally significant increase in hand index for the Enclosure Level Drop behavior. The significant correlation found between age class, brachiation level and hand index scores suggests that growth and development contribute to gibbon laterality during descending brachiation.

The Enclosure Level Drop Leading Limb behavior in this study involved a downward drop of a maximum of one meter. Gibbons in the wild have been observed to swing or drop distances of ten meters during brachiation (Nadler, pers. comm.). The proportionally increased HI scores for the majority of adult individuals for enclosure level drops of no more than one meter suggests that there could be even greater hand/arm preference rates for gibbons when dropping farther distances during brachiation as there is more skill and risk involved.

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Female crested gibbons (genus *Nomascus*) sing male song

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Key words: crested gibbons, male song, population survey, song behaviour

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Summary

Crested gibbons (genus *Nomascus*) belong to a group of gibbons that is still relatively unexplored. Their vocal behaviour, in particular, raises a number of important questions. One question is whether the loud calls, also known as "songs", are species- and/or sex-specific. Past studies have suggested that the song repertoire of crested gibbons is sex-specific, particularly in adult individuals (Geissmann 2002). In this study (Geissmann 2002), only a few male individuals were reported to imitate the song phrase of a female. This report was presented as evidence that, on some occasions, female crested gibbons can sing male song phrases. In the present study, it was found that six of sixteen yellow-cheeked (*Nomascus gabriellae*) and white-cheeked crested gibbons (*Nomascus leucogenys*), housed in zoos of the Zoological Park Organization, Thailand and of the Endangered Primate Rescue Center, Vietnam, produced male song phrases. Six females were found to be able to produce at least the multi-modulated call phrases as thought to be exclusively belonging to the male song repertoire, and one female white-cheeked crested gibbon even produced several types of male song phrases. The structure of the first element of these multi-modulated call phrases was examined and found to be similar between males and females, but the females scored were found to have a larger frequency range than the males. Most females also produced first elements of longer duration than the males. These results lead us to believe that crested gibbons generally may have the ability to produce song phrases of either sex. However, it is unknown what exact factors may contribute to males or females producing elements of song by the opposite sex. Further study is needed. Resolving this question may have an impact on the current methods used in population surveys of crested gibbons.

Con cái các loài vượn (giống *Nomascus*) bắt chước kiểu hót của con đực

Tóm tắt

Các loài vượn thuộc giống *Nomascus* hiện còn ít được nghiên cứu. Những nghiên cứu về giọng hót của chúng đặt ra nhiều câu hỏi cần được làm sáng tỏ. Một trong số câu hỏi đó là, liệu tiếng kêu lớn tạo thành "bài hát" của chúng có phải là biểu hiện đặc trưng của loài hoặc của riêng một giới. Những nghiên cứu trước đây cho rằng các "bài hát" ở các loài vượn mang đặc trưng cho mỗi giới tính, đặc biệt là những cá thể trưởng thành (Geissmann, 2002). Trong nghiên cứu của mình (Geissmann, 2002), tác giả cũng ghi nhận những trường hợp một số cá thể đực bắt chước một phần trong "bài hát" của con cái.

Nghiên cứu của chúng tôi ghi nhận một vài trường hợp con vượn cái có thể hót những kiểu hót của con đực. Sáu trong số mười sáu cá thể nuôi nhốt tại Vườn thú Zoological Park Organisation, Thái Lan và Trung tâm Cứu hộ Linh trưởng Nguy cấp, Cúc Phương, Việt Nam, thuộc hai loài vượn đen má hung (*Nomascus gabriellae*) và vượn đen má trắng (*Nomascus leucogenys*) đã hót kiểu hót của con đực. Cả sáu cá thể trên đều hót được ít nhất một kiểu hót của con đực cùng loài, đặc biệt một cá thể còn hót được một vài kiểu hót khác nhau của con đực cùng loài với nó.

Cấu trúc của đoạn đầu trong nhiều kiểu hót của con đực đã được kiểm tra, kết quả cho thấy con đực và con cái hót giống nhau, tuy nhiên tần số tạo ra bởi con cái lớn hơn và các con cái hót đoạn này đều kéo dài hơn so vơi con đực. Kết quả trên cho phép suy luận rằng các loài vượn nói trên có thể tạo ra kiểu hót giống nhau ở cả hai giới. Tuy nhiên nhân tố dẫn đến hiện tượng này chưa được tìm thấy và cần có thêm những nghiên cứu sâu hơn. Việc tìm ra câu trả lời sẽ có ảnh hưởng rất quan trọng đến phương pháp đánh giá quần thể đang được sử dụng ngoài thực địa.

Introduction

Although studied for the last few decades, song behaviour remains one of the least researched aspects of gibbon behaviour. It has been suggested that gibbon songs of all species may be species and sex-specific (Marshall & Marshall, 1976). However, these studies largely have concerned species of the *Hylobates* genus, but relatively little is known about crested gibbons (*Nomascus* sp). Crested gibbons are the gibbons of Cambodia, China, Laos and Vietnam and this genus contains several endangered species and it is these species in which we are interested. Several studies on crested gibbons have focused on the physical features and organization of their song (Deputte & Goustard, 1978; Deputte, 1982; Goustard, 1984; Haimoff, 1984; Schilling, 1984). They have found that crested gibbons use different song elements in their songs. Geissmann (2002) argued that the song phrase used in adult crested gibbons represents the highest degree of specificity in their song repertoire. However, some studies have found that male adult crested gibbons occasionally produce some female-like great call notes, whereas none of the papers have reported similar issues in the song of female crested gibbons (Schilling 1984; Geissmann, 2002). Importantly, the song of crested gibbons has been used in various census surveys to estimate their population size (Jiang Xue Long et al,. 2006; Park, 2007; Ruppell, 2007). If an individual, particularly a female, produces song phrases of the opposite sex this may result in miscalculations and incorrect sexing in population surveys of crested gibbons. We report here the singing behaviour of several adult female crested gibbons in which the question of sex specificity is raised and it is shown that females may produce male songs.

Materials

The study was conducted in the five Thai zoos under the auspices of the Zoological Park Organization of Thailand (ZPO) and the Endangered Primate Rescue Center (EPRC), Vietnam during several visits between August 2006 and January 2008. Sixteen female crested gibbons, including four yellow-cheeked crested (*Nomascus gabriellae*) and 12 white-cheeked crested gibbons (*Nomascus leucogenys*), in different housing conditions, were observed and their songs were recorded (Table 1). Only four individuals were paired with a male, the other 12 females were housed singly or with another female. None of the female adult individuals was born in captivity. Most of the individuals have been housed in their respective enclosures for over ten years, except two that arrived in the EPRC in 1998, and thus have spent slightly under ten years in their present housing.

Table 1. Location and Housing information of each observed female crested gibbons.

Individual	Species	Location	Housing condition	Producing male song	Recording
1	*N. gabriellae*	Chiangmai	1	Yes	No
2	*N. leucogenys*	KKOZ	1	No	No
3	*N. gabriellae*	EPRC	2	No	No
4	*N. gabriellae*	Dusit	2	Yes	Yes
5	*N. leucogenys*	Dusit	2	No	No
6	*N. gabriellae*	Songkla	3	No	No
7	*N. leucogenys*	Songkla	3	No	No
8	*N. leucogenys*	Chiangmai	3	Yes	Yes
9	*N. leucogenys*	Chiangmai	3	No	No
10	*N. leucogenys*	EPRC	3	Yes	Yes
11	*N. leucogenys*	EPRC	3	Yes	Yes
12	*N. leucogenys*	EPRC	3	Yes	No
13	*N. leucogenys*	Dusit	4	No	No
14	*N. leucogenys*	KKOZ	4	No	No
15	*N. leucogenys*	Korat	4	No	No
16	*N. leucogenys*	EPRC	4	No	No

1. The individual is singly housed, and no individuals of the opposite sex are housed in the same zoo.
2. The individual is singly housed, but conspecifics of the opposite sex are housed within auditory distance.
3. The individual is housed in the same or in an adjacentcage with another individual of the same sex.
4. The individual is housed as part of a pair or a family group.

Methods

The vocal recordings were taken between 05.00am and 11.00am on each day of observation because gibbons vocalise mostly at dawn or a few hours after dawn (Geissmann, 2002), and each individual was observed for five random days during the period of visit between August 2006 and January 2008. A directional microphone (Sennheisser ME-66) and a digital recorder (Marantz PMD-670) were used to record the songs. Some of the recordings are incomplete due to human factors (e.g.: one individual in Thailand and two individuals in Vietnam stopped producing male song phrases whenever the keeper or the observer appeared). All recorded songs were sampled digitally into the computer at a 16- bit quantization and 44.1 kHz sampling rate. Vocalizations were analysed by sound software (Raven 1.3, Connell University).

Sixty-four multi-modulated call phrases were collected from four adult females. As all individual recordings of females contained multi-modulated call phrases, the physical features of multi-modulated call phrases were analysed. Several studies (Deputte, 1982; Goustard, 1984; Schilling, 1984) have described the structure of multi-modulated call phrases, and these were shown to contain high variability in the number of elements and modulations. The first element of the multi-modulated call phrases was analysed, thus overcoming the variability problem of other elements in multi-modulated call phrases. In order to compare the differences in structure of multi-modulated call phrases in males and females, the first element of multi-modulated call phrases produced by an unpaired (M-1) and by a paired (M-2) adult male white-cheeked crested gibbon (*Nomascus leucogenys*) was also analysed. Four parameters (start frequency, lower most frequency, frequency range and duration) were measured (Fig 1).

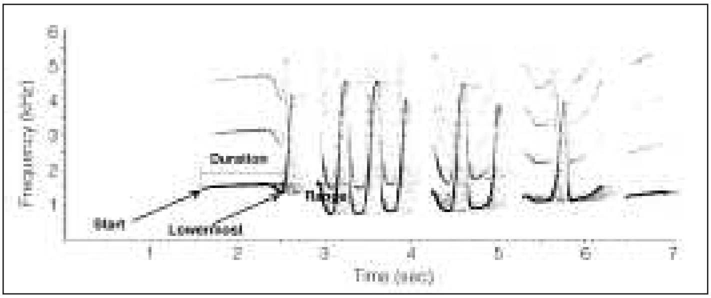

Fig. 1. The sonogram is an example of male multi-modulated call phrases. This graph shows the four parameters that were selected to measure the structure of the first element of multi-modulated call phrases.

Results

Six of the 16 females produced male song phrases and all individuals were visually identified by the observer. In the case of four of the six we were able to record them producing a male song phrase (Fig. 2). As the housing conditions in Table 1 show, none of the females were housed together with males. The six individuals that produced male song phrases were housed singly or with another female, and two of them were housed in zoos without any male crested gibbons in the same zoo.

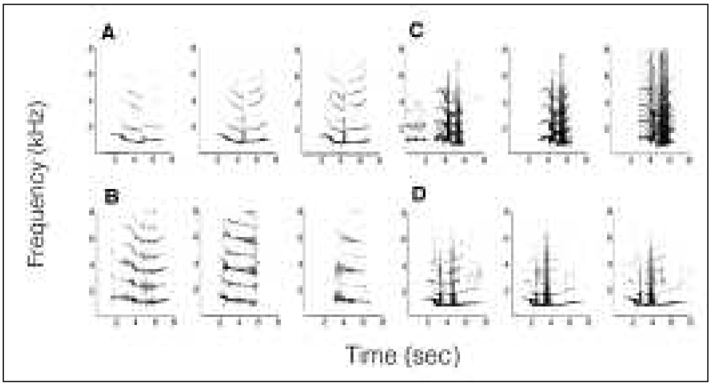

Fig. 2. The sonograms of multi-modulated call phrases produced by four adult females. The graph shows that the female produced fewer elements in each multi-modulated call phrase.

Three females produced male song consistently at least over one month's period. All females produced at least multi-modulated call phrases. One female individual even produced boom and staccato call phrases (Fig 3). Multi-modulated call phrases produced by females have less variety compared to males. The number of elements of each multi-modulated call phrase produced by females usually consisted of two elements, whereas the males' multi-modulated call phrases contained up to six elements.

Two individuals produced multi-modulated call phrases along with their own (female) great call sequence, whereas two other individuals were recorded when they only produced multi-modulated call phrases on their own.

The first element of multi-modulated call phrases was analysed (Table 2). Results showed that the multi-modulated call phrases produced by females have some structural similarity with adult male individuals. The duration of the first elements produced by females is generally longer than that produced by males. The frequency range in females is also larger than in males. Moreover, the

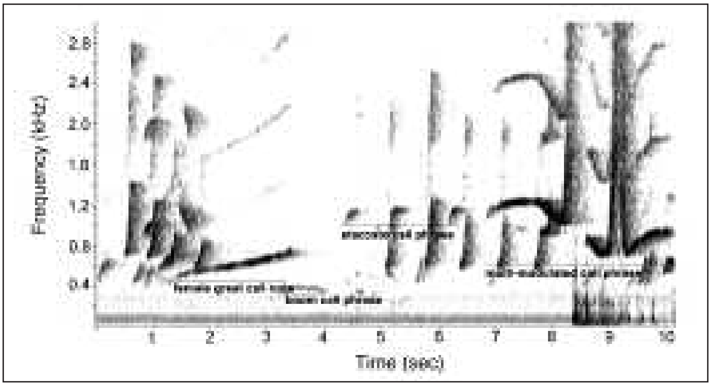

Fig. 3. Song element types produced by a female white-cheeked crested gibbon. During the recording, lar gibbons (*Hylobates lar*) also sang in the background. The female great call note was produced by another female crested gibbon housed in the adjunct cage. The two female white-cheeked crested gibbons produced great call phrases five seconds later in the same song sequences.

results showed that the call structure in the first element of multi-modulated call phrases may be individually different.

Table 2. Call parameters of the first element in multi-modulated call phrases. The first column shows the available number of notes collected from the individual. M-1 and M2 (bottom) are adult males (*Nomasucs leucogenys*).

Individual (N)	Start Frequency (Hz)	Lowermost Frequency (Hz)	Frequency Range (Hz)	Duration (s)
10 (27)	1384.53 ± 10.69	881.93 ± 11.85	502.60 ± 18.51	3.28 ± 0.12
3 (27)	1347.31 ± 7.37	803.78 ± 4.84	543.50 ± 10.61	1.88 ± 0.09
8 (7)	1189.92 ± 10.79	961.76 ± 12.03	228.16 ± 15.92	1.36 ± 0.15
4 (3)	1435.17 ± 34.26	1100.37 ± 8.37	334.70 ± 32.69	4.17 ± 0.55
M-1 (32)	1412.40 ± 7.98	1300.83 ± 5.53	111.57 ± 7.52	1.23 ± 0.06
M-2 (33)	1258.06 ± 11.20	1110.01 ± 15.24	148.05 ± 10.30	1.41 ± 0.03

Discussion

Crested gibbons are known to produce sex-specific song phrases in their duet song (Haimoff, 1984; Schilling, 1984) and it was considered unlikely that crested gibbons produce song phrases of the opposite sex. It has been noted in some studies that, in some rare cases, juvenile or adult males may produce female great call-like phrases synchronized with female great call phrases (Goustard, 1984; Schilling, 1984; Geissmann et al., 2000; Chen pers. observ.). The findings of this study provide some evidence of the ability of crested gibbons to use song elements of the opposite sex. Coupled with early findings in juvenile and adult male crested gibbons (Goustard, 1984; Schilling, 1984; Geissmann, 2002), it may now be argued that crested gibbons have the ability to produce the elements of song of either sex but they do so rather regularly in captivity, and in the absence of individuals of the opposite sex housed in the same zoo.

The ability to produce song elements of the song of the opposite sex may be partially inherent (a vocal template) and then refined and developed into sex specific song via social learning. Several studies conducted in hybrid individuals *(Hylobates* sp.) in captivity and in the wild have shown that the hybrid individuals produced intermediate song patterns between their parents

(Brockelman & Schilling, 1984; Geissmann, 1984). These studies suggest that genetic components may play a role in the development of gibbon song, and Geissmann (2002) has addressed this in his review of gibbon song. In this study, two female individuals produced male song phrases housed in a context where no male conspecifics were present in the same zoo. It could be argued that, as all gibbons studied here were wild-born and brought to the zoo from the wild as adolescents or adults, they would have heard and seen male female interactions of other adults in the wild, including, most notably, their song, giving them ample opportunity to learn song phrases of either sex. However, one of the female individuals was known to have been sent to the zoo while still an infant. This particular female would have had few or no opportunities to practise or learn male song phrases from other individuals. Therefore, social learning may not be the major influence affecting the ability of female crested gibbons to produce song elements of the opposite sex.

Such issues of singing song phrases of the opposite sex have also been found in other gibbon species (Geissmann, 1993). However, none of the studies have reported that female crested gibbons produce the male-like song phrases, except for one case that reported this to be so in a newly paired female pileated gibbon (*Hylobates pileatus*) that produced a male call sequence (Geissmann, 1983). In this study, all female crested gibbons that produced male song phrases were not housed with nor were they in the vicinity of male conspecifics. In contrast, those that were housed with male conspecifics were not once found to produce male song phrases. Further investigation still needs to be conducted to clarify how both genetic components and social learning affect the song behaviour in crested gibbons.

The finding of this singing behaviour may have some impact on current surveys of crested gibbons, as mentioned before. The duet song in crested gibbons was thought to present the highest degree of sex-specific behaviour among gibbon species (Geissmann, 2002). This study, however, showed that, in some cases, the duet song may be partly produced by a single female. It is not clear what exact circumstances, if any, make female gibbons produce the male song phrases. All these cases were found in captive environments. There is no direct evidence to show that such cases occur in the wild, yet one should not rule out this possibility. It may be very difficult to confirm the sex of an individual caller unless visual contact can be maintained at the time of vocalization. If this is the case in the wild, such behaviour may lead to biases in survey results as the current census population surveys mainly apply the method of spot listening (Brockelman & Ali, 1987; Brockelman & Srikosamatara, 1993). Therefore, one would need to be careful in deriving the sex of an individual from listening to its call.

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Chromosomal and molecular studies of a hybrid between red-shanked douc langur (*Pygathrix nemaeus*) and Hatinh langur (*Trachypithecus laotum hatinhensis*)

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Key words: Hybrid, *Pygathrix nemaeus*, *Trachypithecus laotum hatinhensis*, Colobinae, chromosomes, FISH, DNA

Summary

Four male douc langurs (*Pygathrix nemaeus*) and a group of Hatinh langurs (*Trachypithecus laotum hatinhensis*) were released to a five hectare semi-wild enclosure at the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam. After one month, the douc langurs approached the Hatinh langur group and copulation of a douc langur male and a Hatinh langur female was observed. After a gestation period of 205-207 days, a hybrid was born.

The coloration of the newborn hybrid more closely resembled a douc langur, but changed over a period of about four months to complete black. Some features of the newborn's appearance resembled douc langurs, such as the capped-like form of the hairs on the head, the long and grey whiskers and the tassel on the tail.

Chromosomal and molecular genetic investigations have been carried out. Chromosome preparations were made from peripheral blood lymphocytes. Fluorescence *in situ* hybridization (FISH) revealed reciprocal translocations and/or inversions that unequivocally distinguish the karyotypes of *Trachypithecus* and *Pygathrix*. DNA sequences were generated from a region of the maternal inherited mitochondrial genome and two autosomal loci.

The hybrid status of the female offspring could be most clearly defined as *Pygathrix nemeaus* (pat) x *Trachypithecus laotum hatinhensis* (mat) with a 44,XX karyotype.

Nghiên cứu về nhiễm sắc thể và phân tử của con lai giữa loài vọoc chà vá chân nâu (*Pygathrix nemaeus*) và loài vọoc Hà Tĩnh (*Trachypithecus laotum hatinhensis*)

Tóm tắt

Bốn cá thể đực của loài vọoc chà vá chân nâu (*Pygathrix nemaeus*) và một đàn vọoc Hà Tĩnh (*Trachypithecus laotum hatinhensis*) được thả chung trong khu bán hoang dã rộng 5 hecta tại Trung tâm Cứu hộ Linh trưởng Nguy cấp, Vườn Quốc gia Cúc Phương. Một tháng sau khi thả, các cá thể chà vá tiếp cận đàn vọoc Hà Tĩnh, giao phối giữa cá thể đực chà vá và cá thể cái vọoc Hà Tĩnh đã được quan sát. Quá trình mang thai từ 205-207 ngày, một cá thể con lai đã được sinh.

Màu sắc lông của con lai ban đầu giống với màu sắc lông của loài chà vá nhiều hơn, tuy nhiên sau 4 tháng màu lông chuyển toàn bộ sang sắc đen. Một số đặc điểm giống với loài chà vá vẫn hiện hữu như: hình dáng kiểu lông trên đầu, đuôi dài và có màu kem xám, có túm lông đuôi.

Nghiên cứu về hệ thống nhiễm sắc thể được phân tích dựa trên tế bào máu Lympho ngoại biên. Chiếu hình quang cho thấy có hiện tượng trao đổi đoạn và/hoặc nghịch đoạn đã xảy ra một cách rõ rệt trên những nhiễm sắc thể nhuộm thuộc *Trachypithecus* và *Pygathrix*. Trình tự ADN (DNA) đã được tổng hợp tại một vùng của gien ti thể di truyền theo dòng mẹ và hai nhiễm sắc thể thường.

Tình trạng lai của cá thể cái được diễn đạt như sau: *Pygathrix nemaeus* (cha) x *Trachypithecus laotum hatinhensis* (mẹ) với 44 NST, XX.

Introduction

On March 19, 2002 four male red-shanked douc langurs (*Pygathrix nemaeus*) were released to a five hectare electric fenced semi-wild enclosure at the Endangered Primate Rescue Center, Vietnam. The semi-wild enclosure comprises a limestone hill with typical primary limestone forest and vegetation.

The douc langurs had been confiscated from the illegal animal trade and the age of the animals was estimated to between five and six years based on comparisons to a number of red-shanked douc langurs born and raised at the EPRC.

In January 2003 a group of Hatinh langurs (1,3) (*Trachypithecus laotum hatinhensis*) was released to the same area. All these animals were also confiscated and the ages estimated to (male: 8 years, females: 3, 7 and 10 years).

The Hatinh langur group and the douc langur “bachelor-group” formed separate stable social units, similar to groups in the wild. Each species moved and foraged as a single group in the enclosure. After one month, the douc langurs approached the Hatinh langur group, and the male Hatinh langur began keeping his distance from the group. Grooming between douc langur males and Hatinh langur females was occasionally observed and increased. On March 22, 2003 copulation of a douc langur male and a Hatinh langur female was observed. Two days later all four douc langurs were isolated in a cage inside the semi-wild area and translocated back to a cage at the Endangered Primate Rescue Center, outside the semi-wild area.

A hybrid from the species - a female – was born on October 14, 2003. The gestation period for the hybrid was at least 205-207 days. Urine analyses from douc langurs indicate the species’ gestation period is 210 days (Lippold, 1981), though a shorter length of 165-190 days has been suggested (Benirschke, http:medicine.uscd.edu/cpa). The gestation period of Hatinh langurs is estimated to about 180 days (Nadler, pers. obs.) and is most probably similar to the closely related Francois’ langur (*Trachypithecus francoisi*) which is 184+15 days (Mei Qui Nian & Lai Mao Qing, 1998).

Description of the *Pygathrix nemaeus* x *Trachypithecus laotum hatinhensis* hybrid

The coloration of the newborn hybrid more closely resembled a douc langur, but changed over a period of about four months to completely black (Fig. 1). Newborn Hatinh langurs have a bright golden-yellow fur. Only the tail is dark chocolate-brown. The face of a young Hatinh langur is light

Fig. 1. Hybrid red-shanked douc langur (*Pygathrix nemaeus*) x Hatinh langur (*Trachypithecus laotum hatinhensis*) four months old. Photo: T. Nadler.

Fig. 2. Hatinh langur (*Trachypithecus laotum hatinhensis*) one month old. Photo: T. Nadler.

flesh-colored (Fig. 2). The coloration of newborn douc langurs is more similar to adults: most of the body is grey, with a darker, blackish line on the back along the spine and reddish-grey legs. The face of a young douc langur is variable, mostly slate grey with yellow patches, often with yellow eye rings but can be also totally slate grey (Fig. 3 and 4).

Fig. 3,4. Young douc langurs (Pygathrix nemaeus) with different face coloration. Photo: T. Nadler.

The fur color of the hybrid changed to completely black after four months, a time similar to that of Hatinh langurs. During this time the cheeks changed to light grey, not to white like in Hatinh langurs. The length of the hair on the cheeks was longer but didn't form the typical whiskers of the douc langurs. The fur on the head formed the typical cap of the doucs and not the crest of the Hatinh langurs (Fig. 1, 5). Nostril form is also intermediate between the features of the parental species (Fig. 6). The tail length of 78 cm is intermediate between douc and Hatinh langurs (*Pygathrix* 55,6 cm; 52-58 cm, n=6 adult females [EPRC]; *Trachypithecus* 85,3 cm; 78-90 cm; n=7 adult females [EPRC]), but the tassel on the end more closely resembles that of doucs (Fig. 7). The brachial index and the crural index (fore and hind limbs, respectively) correspond to

Fig. 5. Adult hybrid (four years old). Photo: T. Nadler.

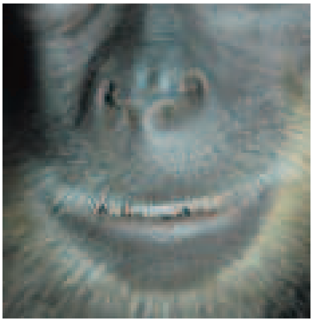

Fig. 6. The form of the nostril is intermediate between the features of the parental species. Photo: T. Nadler.

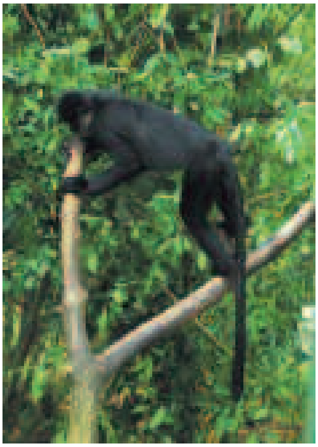

Fig. 7. Adult hybrid (four years old). Photo: T. Nadler.

Trachypithecus but the proportions between the fore limbs and hind limbs (humerofemoral and intermembral indices, respectively) are intermediate (Table 1).

Table 1. Long bone proportions.

	Pygathrix nemaeus	*Trachypithecus laotum hatinhensis*	Hybrid (female)
Brachial Index (Radius x 100/Humerus)	**106.6** (100-109.5) n=4 ad. females (Nadler, unpubl.) **97-107** (Groves, 1970) **104** (100-107) n=4 (sex?) (Napier & Napier, 1967)	**100.6** (100-103) n=5 ad. females (Nadler, unpubl.)	**100.0**
Crural Index (Tibia x 100/Femur)	**87.0** (82-90.5) n=5 ad. females (Nadler, unpubl.) **88** (Napier & Napier, 1967)	**92.9** (88.6-97.5) n=5 ad. females (Nadler, unpubl.)	**93.8**
Humerofemoral Index (Humerus x 100/Femur)	**88.1** (84-92.9) n=5 ad. females (Nadler, unpubl.)	**79.5** (72.7-85.0) n=5 ad. females (Nadler, unpubl.)	**83.3**
Intermembral Index ([Humerus+Radius] x 100/ [Femur+Tibia])	**97.1** (92.3-101.3) n=5 ad. females (Nadler, unpubl.) **93** (92-94) n=3 (sex ?) (Napier & Napier, 1967) **92-96** (Groves, 1970)	**82.2** (77.1-87.0) n=5 ad. females (Nadler, unpubl.)	**86.0**

Material and Methods

Chromosome preparations

Chromosome preparations were made from peripheral blood lymphocytes according to standard methods, with minor modifications (Schempp & Meer 1983). During the last 7 – 8 hours before harvesting, bromodeoxyuridine (BrdU) was added to the culture. As a result, thymidine was incorporated into early-replicating and BrdU into late-replicating chromosomal segments, allowing the presentation of RBA-banding pattern on metaphase chromosomes (ISCN 2005). Slides carrying interphase cells and metaphase spreads were dehydrated in a series of concentrations of ice-cold ethanol then air-dried and stored at –80°C. Before using for in situ hybridization, the slides were dehydrated again and then air dried.

Fluorescence in situ hybridization (FISH)

Prior to FISH, the slides were treated with RNase followed by pepsin digestion as described (Ried et al. 1992). FISH followed the method described by Schempp et al. (1995). Chromosome in situ suppression (CISS) was applied to human whole-chromosome painting (WCP) libraries (Jauch et al. 1992). After FISH the slides were counterstained with DAPI (0.14 µg/ml) and mounted in Vectashield (Vector Laboratories). Preparations were evaluated using a Zeiss Axiophot epifluorescence microscope equipped with single-bandpass filters for excitation of red, green, and blue (Chroma Technologies, Brattleboro, VT). During exposures, only excitation filters were changed allowing for pixel-shift-free image recording. Images of high magnification and resolution were obtained using a black-and-white CCD camera (Photometrics Kodak KAF 1400; Kodak, Tucson, AZ) connected to the Axiophot. Camera control. Digital image acquisition involved the use of an Apple Macintosh Quadra 950 computer.

Molecular genetics

To further confirm the hybrid status of the study specimen, DNA sequences were generated from a region of the maternal inherited mitochondrial genome and two autosomal loci. Since the study specimen is a female, Y-chromsomal loci were not studied. DNA was extracted from blood with the Qiagen mini-kit following recommendations of the supplier. Loci were amplified via PCR using standard PCR conditions. For the amplification of the hypervariable region I of the mitochondrial D-loop, the oligonucleotide primers 2068 and 2270 were used. The two autosomal loci (transition protein 2 and transthyretin, intron 1) were amplified with the oligonucleotide primer pairs 5'-GCA GGT GTA CAA AAC CAA G-3'/5'-GTC TCA TTA GTT GGA TTT CC-3' and 5'-GGC CCT ACG GTG AGT GTT-3'/5'-ACT TTG ACC ATC AGA GGA CA-3', respectively. PCR reactions were identical for all amplifications and included a predenaturation step at 94°C for 2 min., followed by 40 cycles consisting of denaturation at 94°C for 1 min., annealing at 58°C for 1 min., and elongation at 72°C for 1 min. At the end, a final elongation step at 72°C for 5 min. was added. PCR products were run on agarose gels and excised from the gel. After purification with the Qiagen gel extraction kit, PCR products were sequenced on an ABI3100 capillary sequencer. To evaluate the hybrid status of the study specimen, the generated sequences were compared with those obtained from pure *Pygathrix nemaeus* and *Trachypithecus laotum hatinhensis* individuals.

Results and Discussion

Asian leaf-eating monkeys (Colobinae) have rather conserved karyotypes. With the exception of the proboscis monkey (*Nasalis larvatus*; 2n = 48), all species of *Trachypithecus* and *Pygathrix* investigated have the diploid chromosome number of 2n = 44 (Bigoni et al. 1997a;1997b; 2003; 2004; Nie et al. 1998; Wienberg 2005). Fluorescence in situ hybridization (FISH) using human whole-chromosome paints (WCP) revealed reciprocal translocations and/or inversions that unequivocally distinguish the karyotypes of *Trachypithecus* and *Pygathrix*.

First, a reciprocal translocation homologous to human 6/16 appears to be a distinguishing characteristic of the genus *Trachypithecus* (Bigoni et al. 1997a; 1997b; Nie et al. 1998), while this translocation is absent in *Pygathrix* (Bigoni et al. 2004). Applying human WCP 6 and WCP 16 we could demonstrate this rearrangement in our female *Trachypithecus laotum hatinhensis* (TLA) individual, mother of our putative hybrid offspring: both chromosome pairs 15 and 18 exhibit the rearranged painting pattern of human 6/16 (Fig. 8a). However, in our putative female hybrid offspring this reciprocal painting pattern appeared only in the haploid state marking TLA chromosome 15 and 18, while one larger submetacentric and one small metacentric were painted in total with WCP 6 and WCP 16, respectively (Fig. 8b). Indeed, the larger submetacentric chromosome painted by WCP 6 is characteristic for chromosome 2, and the small metacentric chromosome painted by WCP 16 is characteristic for chromosome 19 of *Pygathrix nemaeus* (Bigoni et al. 2004).

Furthermore, all Asian colobines studied so far share a reciprocal translocation of homologues of human chromosomes 1 and 19 resulting in chromosomes 4 and 5 of *Trachypithecus* (Nie et al. 1998), and in chromosomes 8 and 10 of *Pygathrix* (Bigoni et al. 2004). In *Pygathrix* the primitive ancestral translocation pattern is found, whereas a derived and more complex alternating painting pattern is seen on chromosome 5 of *Trachypithecus*. This derived complex pattern that can be explained by pericentric inversion should have occurred in *Trachypithecus* after the divergence from *Pygathrix* (Bigoni et al. 2003; 2004). Using human WCP 1 and WCP 19 as painting probes the reciprocal translocation pattern on chromosomes 4 and 5 becomes clearly visible in our female *Trachypithecus laotum hatinhensis* (TLA) individual (Fig. 8c). In addition the alternating 1/19 pattern on both chromosomes 5 of TLA (Fig. 8c) confirms the pericentric inversion specific for *Trachypithecus*. In our putative hybrid offspring the derived alternating 1/19 painting pattern specific for *Trachypithecus* appears only on one chromosome, TLA 5 (Fig. 8d), while the primitive ancestral 1/19 painting pattern specific for *Pygathrix* is seen on the other chromosome, PNE 10 (Fig. 8d).

The molecular genetic study provides further evidence for the hybrid status of the study specimen and confirms the findings obtained from the chromosomal investigations. Since the mitochondrial genome is solely maternally inherited, the hybrid should carry only the mitochondrial genome of the mother. In fact, the sequence of the hypervariable region I of the D-loop of the putative hybrid is closely related or even identical with those generated from several *Pygathrix nemaeus* individuals, but differs greatly from *Trachypithecus laotum hatinhensis* haplotypes. In contrast to the mitochondrial genome, autosomal loci are inherited by both parents, so that in F1 hybrids alleles of both parents should be traceable. For this hybrid, we obtained sequences from two autosomal loci, which in fact carry alleles of both *Pygathrix nemaeus* and *Trachypithecus laotum hatinhensis*, so that a hybrid status of the study specimen is confirmed.

In conclusion, the cytogenetic and chromosomal painting as well as the molecular genetic studies shed light on the hybrid status of this female offspring in question. Although no material from the paternal *Pygathrix nemaeus* individual was available for our cytogenetic studies, the hybrid status of the female offspring could be most clearly defined as *Pygathrix nemeaus* (pat) x *Trachypithecus laotum hatinhensis* (mat) with a 44,XX karyotype.

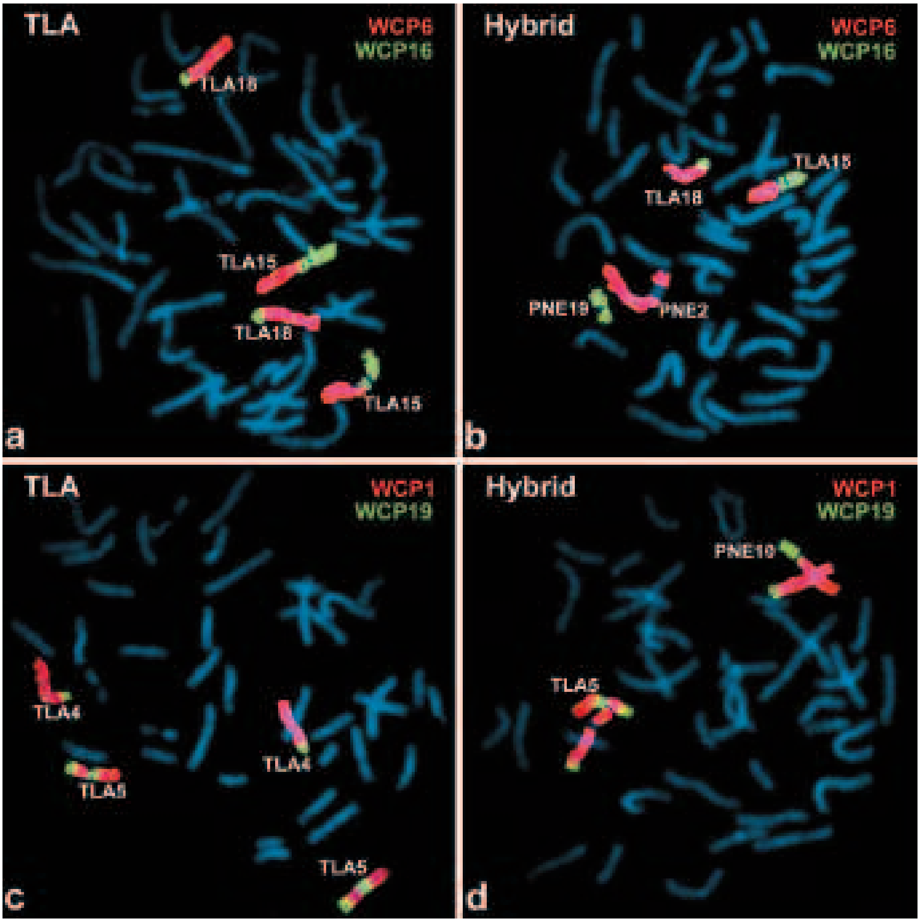

Fig. 8. FISH with human painting probes (WCP) to metaphase spreads of female *Trachypithecus laotum hatinhensis* (TLA) and the female hybrid offspring (Hybrid).

a. Rearranged pattern of human 6/16 on both chromosome pairs 15 and 18 of TLA.

b. The hybrid offspring shows rearranged 6/16 pattern only on one TLA chromosome 16 and 18, respectively, derived from the mother. The larger submetacentric chromosome painted in toto by WCP 6 characterizes chromosome 2 of *Pygathrix nemaeus* (PNE2), and the small metacentric one painted by WCP 16 characterizes chromosome 19 of *Pygathrix nemaeus* (PNE 19), derived from the father of the hybrid.

c. Both chromosomes TLA5 indicate the derived alternating 1/19 painting pattern specific for *Trachypithecus*.

d. The hybrid offspring shows only one chromosome TLA5, derived from the mother. PNE10 shows the primitive ancestral 1/19 painting pattern specific for the paternal *Pygathrix nemaeus*.

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Identification of the species, origin and sex of smuggled douc langur (*Pygathrix* sp.) remains

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Key words: *Pygathrix*, species identification, illegal trade, mtDNA, PCR-based sex-typing

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Summary

Information of illegal wildlife trade routes provides trustworthy evidence of the smuggling activities of endangered wildlife. Therefore, identifying the species and origin of confiscated samples provide important data. In this study, mitochondrial control region sequences, compared to the Indochinese Primate Genetics Project database at the German Primate Center, identified 28 dried samples smuggled to China as black-shanked douc langurs (*Pygathrix nigripes*). Among them, 20 samples most likely originated from Khanh Hoa Province in Vietnam. Another three came from Binh Phuoc Province in Vietnam or Cambodian Mondulkiri Province, whereas one sample came from Dak Lak or Lam Dong Provinces, Vietnam. The origin of four further samples was not clearly traceable. Based on sex-typing PCRs, 17 samples are from females and 11 from males.

Những nhận dạng về loài, nguồn gốc và giới tính từ những mẫu vật buôn lậu là bộ phận vọoc chà vá (*Pygathrix* sp.)

Tóm tắt

Thông tin về tình trạng mua bán động vật hoang dã bất hợp pháp đã cung cấp nhiều bằng chứng xác thực cho quá trình điều tra hoạt động buôn lậu động vật hoang dã gây tổn hại đến giới tự nhiên. Vì vậy, thông qua những nghiên cứu về chủng loại và nguồn gốc từ các mẫu vật được tịch thu, chúng tôi đã thu được những số liệu quan trọng. Nghiên cứu này sử dụng phương pháp giám định gien di truyền dị thể (mtDNA D- loop), đối chiếu với số liệu gien di truyền của Dự án nghiên cứu về gien di truyền thú linh trưởng Đông Dương tại Trung tâm Nghiên cứu Linh trưởng, CHLB Đức, nhận dạng chính xác 28 mẫu vật buôn lậu đến Trung Quốc là bộ phận của loài voọc chà vá chân đen (*Pygathrix nigripes*). 20 mẫu trong số đó có xuất xứ ở tỉnh Khánh Hòa (Việt Nam), 3 mẫu khác có thể ở tỉnh Bình Phước (Việt Nam) hoặc tỉnh Mondulkiri thuộc nước láng giềng Cămpuchia. Ngoài ra, một mẫu vật có thể ở tỉnh Đắc Lắc hoặc Lâm Đồng (Việt Nam). Bốn mẫu vật còn lại còn chưa được xác định rõ nguồn gốc. Dựa vào phương pháp giám định giới tính đặc biệt (PCRs), 17 mẫu đã được xác định là của giống cái và 11 mẫu còn lại là của giống đực.

Introduction

Illegal wildlife trade has become increasingly serious in recent years (Yang Guang et al., 1999; Traffic, 2006). Accordingly, populations of a large number of species around the world have been reduced (Robert, 2000; Karlsson & Holmlund, 2007), resulting in many species now being endangered or even close to extinction (Zhou Zhihua & Jiang Zhigang, 2004).

Governments and NGOs dealing with wildlife conservation pay more and more attention to the illegal wildlife trade and conduct measurements to efficiently control it (Martin, 1997; Wright & Kumar, 1997; Wang & Li, 1998). These illegal trades particularly occur in less developed countries with an abundance of natural wildlife resources. Illegal trade often exists in border regions between two countries with one having high abundance of wildlife resources and the other, a high consumption demand (Li Yiming et al., 2000). For example, local forestry policemen in China frequently confiscate smuggled wildlife coming from the neighbouring countries Vietnam, Lao or Myanmar.

To control illegal trans-border activities, knowledge about trade routes and networks is required, which then can be used to establish efficient strategies to reduce or even stop them. Therefore, the species identity of confiscated specimens or their remains as well as their origin must be correctly identified. However, policemen are not well trained to identify species, and it is mainly specimen fragments, which are difficult to identify morphologically, that are traded. To overcome this problem, DNA analyses represent a useful alternative. Mitochondrial sequence data are especially easy to generate from low-quality DNAs extracted from tissue, feces, hairs, etc. Moreover, a large number of mitochondrial sequence data are available in various databases, and comparisons with such databases allow a rapid identification of specimens and/or their origin. Accordingly, genetic methods are already widely used to study the species identity of e.g. freshwater fishes, white abalones, tigers and primates (Yang Guyang et al., 2002; Wan Qiuhong & Fang Shengguo, 2003; Gruenthal & Burton, 2005; Liu Hui & Wu Xiaobing, 2006; Kyle & Wilson, 2007), or to trace the original place of cetaceans (Baker & Palumbi, 1994; Phipps et al., 1998).

In the present study, we sequenced the hypervariable region I of the mitochondrial control regions of confiscated douc langur remains and compared obtained data with sequence information deposited at the Indochinese Primate Conservation Genetics Project database. We traced the origin of study specimens and determined whether they are males or females. With this study, we highlight the power of molecular techniques in elucidating the species identity of confiscated primate remains, their geographic origin and sex.

Materials and Methods

Samples

The 28 dried skeleton samples including muscle fragments were smuggled from Vietnam to China and confiscated by Chinese customs in Guangxi Province in 2005. We collected some samples (hairs, bones, muscle) from each body and stored them at 4°C before further processing. A preliminary species identification of the specimens based on photographs was conducted by T. Nadler and U. Streicher (both Endangered Primate Rescue Center, Vietnam).

Laboratory methods

Genomic DNA from dried muscle samples was extracted with a standard proteinase K/SDS/phenol/chloroform method (Sambrook & Russel, 2001). To identify the species and the origin of samples, a ~450 bp long fragment of the hypervariable region I (HVI) of the mitochondrial control region was amplified via PCR using the oligonucleotide primers 5'-ATTGATTTCACGGAGGATGGT-3' and 5'-AACTGGCATTCTATTTAAACTAC-3'. PCR based sex-typing was conducted by amplifying fragments of the amelogenin X (AMELX) and the Y-linked sex-determining region (SRY) gene. The primate-specific primers AMEL-F1 and AMEL-R1 amplify a ~200bp fragment, while SRY-F1 and SRY-R1 amplify a ~165bp fragment (Fiore, 2005). Each PCR reaction contained at least 25ng DNA template, 4μL 10x reaction buffer, each 0.4 μmol/L forward and reverse primer, 0.25 mmol/L mixed dNTPs, 0.75 mg/ml BSA, 2.5 unit of Taq DNA polymerase, and sterile distilled water add to 40μL. Amplifications were performed in an ABI Gene Amp PCR System 9700. Cycling conditions include an initial denaturation at 94° for 2min, 40 cycles each with 94° for 1 min, 60° for 1 min and 72° for 1 min, and a final extension at 72° for 5 min.

All reactions were run with negative controls. PCR products were checked by agarose gel electrophoresis. Mitochondrial products were further purified and sequenced by BGI Life Tech Co Ltd (Beijing, China). Sequences were checked and edited using MEGA3 (Kumar et al., 2004) and aligned with Clustalx1.83 (Thompson et al., 1997).

Statistical analysis

The species identity and local origin of specimens was traced by comparing respective sequences with orthologous sequences from all douc langur species and various localities deposited at the database of the Indochinese Primate Conservation Genetics Project. Pairwise differences between confiscated specimens and those deposited in the database were estimated in MEGA. Phylogenetic relationships based on the neighbor-joining algorithm and uncorrected distances were estimated in PAUP* v4.0b10 (Swofford, 2002).

Results and Discussion

All 28 confiscated langur remains were morphologically identified as douc langurs. However, due to the pure condition of samples and the lack of detailed information, no further identification of specimens concerning species, sex or origin was possible. Hence, various genetic methods were applied. We successfully extracted DNA from all 28 individuals and amplified the HVI of the mitochondrial control region. Pairwise differences between all three douc langur species range from 6.6 to 13.7%. The herein analyzed samples show lowest similarities to *Pygathrix nigripes* (0.0-6.4%), whereas differences to *P. nemaeus* and *P. cinerea* range from 10.9 to 13.7%. Accordingly, all confiscated specimens can be identified as *P. nigripes*. Among all tested *P. nigripes* individuals, the confiscated specimens are most similar to samples from Khanh Hoa (individuals W1, W2, W3, W4, W5, W7, W8, W9, W10, W11, W12, W14, W15, W16, W17, W19, W21, W22, W23, W25; 0.5-2.0%), Binh Phuoc-Mondulkiri (individuals W6, W24, W27; 1.0-2.3%) and Dak Lak-Lam Dong (individual W18; 1.0-1.5%). Individuals W13, W20, W26 and W28 show lowest differences to samples from Dak Lak, Lam Dong, Dong Nai, Binh Phuoc and Mondulkiri (3.3-5.5%), but due to the relative large differences, the exact origin is not traceable. Based on phylogenetic tree reconstructions, all confiscated animals cluster together with *P. nigripes*, and are clearly separated from *P. nemaeus* and *P. cinerea* (Fig. 1).

The close affiliation of the confiscated specimens to *P. nigripes*, as indicated by the relative low pairwise differences, is also supported by the obtained tree topology (Fig. 1) and clearly confirms that the confiscated remains are from black-shanked douc langurs. Further tree reconstructions including *P. nigripes* samples from various locations provide a more detailed view on the

Fig. 1. Phylogenetic affiliation of confiscated specimens (labeled with "W" and number) in relationship to each one representative of the three known douc langur species. The tree was outgroup-rooted with *Rhinopithecus avunculus*.

geographic distribution of black-shanked douc langur haplotypes (Fig. 2).

Samples W1, W2, W3, W4, W5, W7, W8, W9, W10, W11, W12, W14, W15, W16, W17, W19, W21, W22, W23 and W25 from the confiscated pool cluster together with samples from Khanh Hoa, whereas samples W6, W24 and W27 form a clade together with individuals from Binh Phuoc and Mondulkiri. Sample W18 clusters with samples from Dak Lak and Lam Dong. The four samples W13, W20, W26 and W28 are either distantly related to animals from Dong Nai or branched off first in a clade consisting of animals from Dak Lak, Lam Dong, Dong Nai, Binh Phuoc and Mondulkiri. Accordingly, their exact origin is not traceable. Based on pairwise differences and phylogenetic tree reconstructions and due to the female philopatric nature of douc langurs, samples W1, W2, W3, W4, W5, W7, W8, W9, W10, W11, W12, W14, W15, W16, W17, W19, W21, W22, W23 and W25 most likely originated from animals from Khanh Hoa, whereas samples W18 and W6, W24 and W27 are derived from individuals from Dak Lak-Lam Dong and Binh Phuoc-Mondulkiri, respectively (Fig. 3).

Sex typing was performed by amplifying a region of the Y chromosomal SRY gene. To exclude PCR artifacts, a control PCR amplifying a fragment of the X chromosomal AMELX gene was selected. In all 28 samples, a successful amplification of the AMELX product was generated, indicating the reliability of the data (Fig. 4).

In 17 samples, the SRY product is absent, indicating that these individuals are females, whereas in the other eleven animals a SRY product is present, indicating that these animals are males.

Douc langurs comprise three species, which are distributed in Vietnam, Laos and Cambodia (Roos & Nadler, 2001; Roos et al., 2007). All of them are endangered to different degrees (Nadler et al., 2003), mainly due to hunting and habitat loss throughout their range. Black-shanked douc langurs are found in southern Vietnam and south-east Cambodia, only east of the Mekong. The species occurs from Dak Lak Province in the north southwards to Dong Nai Province. There is no actual population estimate for Vietnam (Nadler et al., 2007), but hunting is the major threat to the species. It seems that species of this genus can adapt to relatively heavily disturbed forests (Nadler et al., 2003), which increases also the contact between langurs and humans. Accordingly, douc langurs can easily be discovered and hunted. The species is believed to have undergone a decline of more than 50% in the last three generations (35 years, based on a generation length of 10-12 years) due to forest loss and hunting (Southeast Asia Mammal Data Bank, 2006). It is protected in Vietnam on the highest level under the wildlife protection law (Government of Vietnam, 2006), and listed in the Red Data Book of Vietnam (Ministry of Science and Technology & Vietnamese Academy of Science and Techology, 2007) as "Endangered". The IUCN Red List lists the species also as "Endangered" (EN) under criteria "A2cd" and CITES in appendix 1.

Besides a high demand for douc langurs as food or medicine or even as pet monkeys in Vietnam, Lao or Cambodia, the consumption of wildlife including douc langurs in neighbouring countries is steadily increasing, especially in China. The presented case of confiscated douc langurs in Guangxi Province, China is only one example therefore. Due to ongoing wildlife trade including douc langurs, methods are required to follow such trades by identifying species, their origin and sex. Mitochondrial DNA has become a powerful tool in forensic identification because of the high copy number per cell and the lack of recombination (Balitzki-Korte et al., 2005). The present study successfully used mitochondrial control region sequences for the identification of confiscated douc langur samples. This also shows that mitochondrial DNA sequencing is a quick, reliable and simple approach for species and original identification.

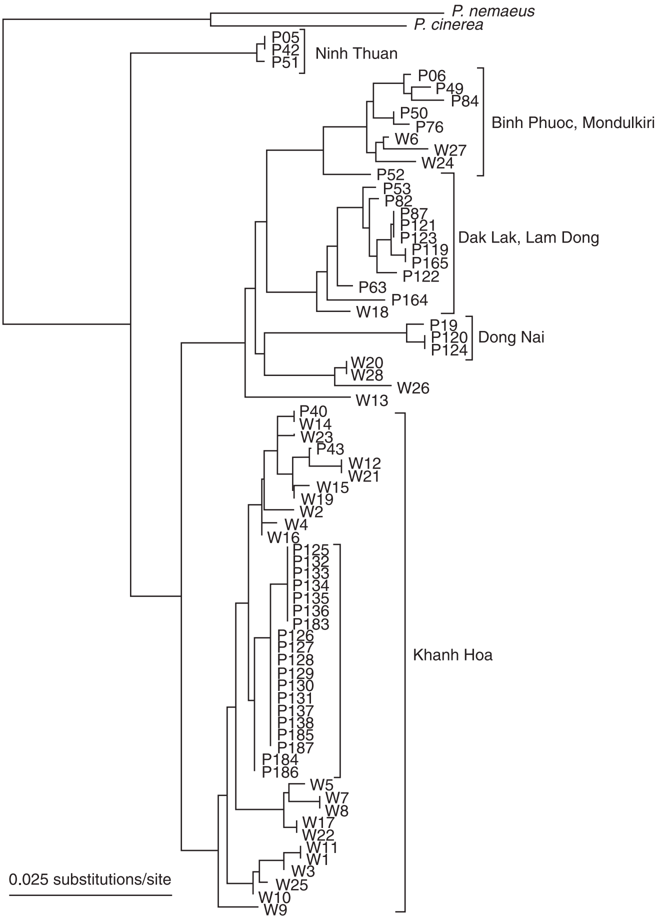

Fig. 2. Phylogenetic affiliation of confiscated specimens (labeled with "W" and number) in relationship to different black-shanked douc langurs from various provinces. The tree was outgroup-rooted with *Pygathrix nemaeus* and *Pygatyhrix cinerea*.

Based on the confirmed origin of most of the confiscated samples, smuggling routes can be traced. Looking at the map of Vietnam and neighbouring countries (Fig. 3), it becomes obvious that the original places of the confiscated samples are

Fig. 3. Map of Vietnam and neighbouring countries. Circles indicate the putative origin of confiscated douc langurs.

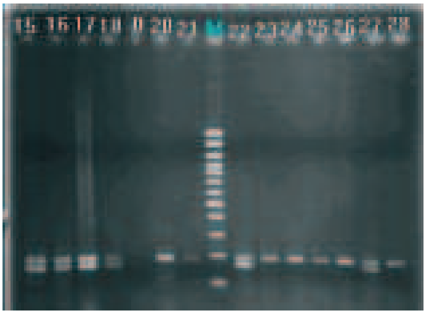

Fig. 4. Agarose gel showing examples for the results of the sex-typing PCRs. Numbers indicate different individuals and "0" the control. Individuals with one and two bands represent females and males, respectively.

all well connected by a good transport infrastructure to trading centers such as Ho Chi Minh City or directly to the South China Sea. Accordingly, an efficient and large-scale wildlife trade is possible.

Our results suggest that more attention has to be paid by national and international organizations to prevent wildlife trade across borders. In Vietnam, the province is the most critical implementation unit for strategies, polices and plans of the government, and the conservation in provincial scales is the most efficient level for magnifying the impact of conservation activities (Long et al., 2004). Hence, customs and law enforcement agencies in provinces need to uphold national laws (Polet et al., 2004).

Cross-border cooperation is urgently required to prevent wildlife trade, but also to improve the management of protected areas. Especially sharing experiences and information would be helpful to strengthen conservation efforts and to establish concerted actions against the illegal wildlife trade (Bleisch & Zhang Yingyi, 2004).

Acknowledgements

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Color variation in black-shanked douc langurs (*Pygathrix nigripes*), and some behavioural observations

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Key words: *Pygathrix nigripes*, coloration, behaviour

Summary

Black-shanked douc langurs on Hon Heo Peninsula, Khanh Hoa Province, Vietnam show remarkable color variations. Individuals with different white extensions on the forearms and partly red coloration on the lower legs have been observed. This is a typical feature of red-shanked douc langurs (*Pygathrix nemaeus*).

Molecular genetic investigations have shown that douc langurs from Hon Heo Pensinsula cluster together with other black-shanked douc langurs from southern Vietnam. Moreover, no genetic signs of red-shanked douc langurs were observed in the population, so that the Hon Heo population indeed represents black-shanked douc langurs.

Observations show that black-shanked douc langurs spend about 20% of the daily time budget on the ground, mostly on large granite blocks. These observations contradict the assumption of the entirely arboreal way of life of the species. Water consumption of doucs on the ground has been observed.

Sự đa dạng màu sắc lông ở loài chà vá chân đen (*Pygathrix nigripes*), và một số quan sát tập tính

Tóm tắt

Quần thể vọoc chà vá chân đen trên bán đảo Hòn Hèo, tỉnh Khánh Hòa, Việt Nam có sự thay đổi đáng kể về màu sắc lông. Nhiều cá thể có mảng lông trắng rộng ở chi trước và một phần lông màu đỏ ở cẳng dưới của chi sau đã được quan sát. Những đặc điểm về màu sắc lông nêu trên là đặc trưng của loài chà vá chân nâu (*Pygathrix nemaeus*)

Phân tích di truyền học phân tử cho thấy quần thể vọoc chà vá ở bán đảo Hòn Hèo cùng nhóm với các quần thể chà vá chân đen miền Nam, Việt Nam. Hơn nữa, không có dấu hiệu nào về mặt di truyền học của chà vá chân nâu trong quần thể này, có thể khẳng định quần thể chà vá ở Hòn Hèo là loài chà vá chân đen. Quan sát quần thể chà vá chân đen ở đây còn cho thấy 20% tổng thời gian hoạt động trong ngày động vật di chuyển dưới đất, thường là các tảng đá lớn. Kết quả trên ngược với giả định về cách sống hoàn toàn trên cây của loài này. Những hành vi động vật uống nước trên nền đất cũng đã được quan sát.

Introduction

Ongoing studies have recently been initiated on the black-shanked douc langur (*Pygathrix nigripes*), yet, compared to other Indochinese primates, the species is poorly studied. Such a dearth of knowledge about *Pygathrix nigripes* has contributed to its provisional systematic placement as a subspecies within the red-shanked douc langur *Pygathrix nemaeus* (Chaplin & Jablonski, 1998; Lippold, 1998) and its incorrect assignment of "already extinct" (Warhol & Benirschke, 1986).

The coloration of the black-shanked douc langur was initially described based on a few museum specimens and it is this description that has appeared in several publications (Jablonski, 1995; Lippold, 1998; Napier, 1985; Napier & Napier, 1997). With the intensified field work on primates in Vietnam and with the information from poached, confiscated and kept animals there is more detailed knowledge about this species which provides new insights.

Material

In May 2007 a survey was carried out on Hon Heo Peninsula, Khanh Hoa Province, south Vietnam (Ha Thang Long & Nadler, 2007) to gather an overview about a recently discovered population of black-shanked douc langurs (S. Lamarche, pers. comm.). During several additional visits to Hon Heo Peninsula douc langurs were observed with binoculars and documented with photographs and video.

Fecal samples of 16 individuals were collected and used for molecular genetic analyses.

Results

Coloration

Detailed observations of the douc langurs were made on several occasions. A maximum of 32 different individuals was observed belonging to three groups. Twelve animals were identified as having coloration that didn't match the typical "standard description" of the black-shanked douc langur body coloration: dark grey forearms, and black lower legs (Groves, 1970; Chaplin & Jablonski, 1998; Fig. 1).

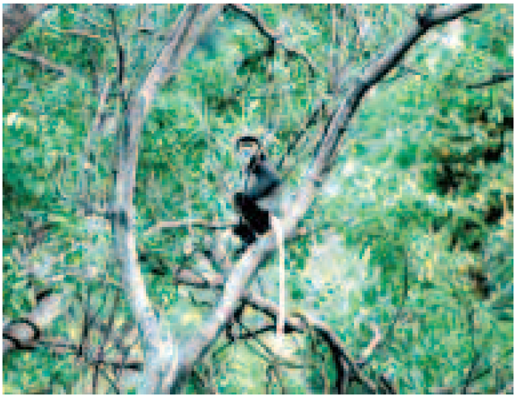
Fig. 1. A group of black-shanked douc langurs in Nui Chua National Park with typical "standard coloration". Photo: Le Khac Quyet.

Two typical features of different coloration could be recognized:

1. White coloration of varying length on the forearms. White coloration can extend from small patches, like a wristwatch, to a complete white forearm (Fig. 4-6).
2. Red coloration on the lower leg. The red can be differ from a red shine, resulted in a number off red colored hairs to dark red coloration to at least the half lower leg (Fig. 7).

Both features are typical coloration for the red-shanked douc langur (*Pygathrix nemaeus*) (Groves, 1970, Chaplin & Jablonski, 1998).

Pygathrix nigripes populations in Cambodia (B. Rawson, pers. comm.), and the Vietnamese National Parks Nam Cat Tien (G. Polet, pers. comm.; Nadler, pers. obs.) and Nui Chua (Hoang Minh



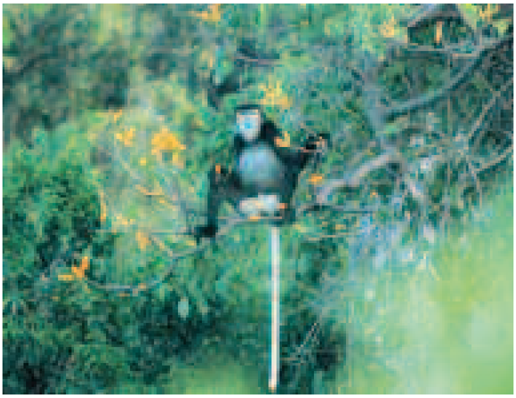


Fig. 2, 3. Black-shanked douc langur with typical "standard coloration"; Hon Heo Peninsula, Khanh Hoa Province. Photo: T. Nadler.

Fig. 4. Black-shanked douc male with small white patch on the wrist and black lower legs; Hon Heo Peninsula, Khanh Hoa Province. Photo: T. Nadler.

Fig. 5. Black-shanked douc male (middle) with more extended white patch on the wrist and black lower legs; Hon Heo Peninsula, Khanh Hoa Province. Photo: T. Nadler.

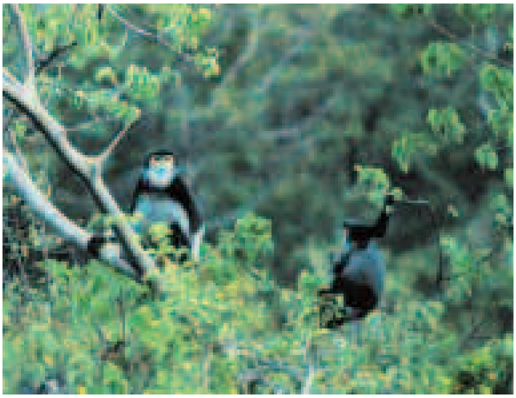


Fig. 6. Black-shanked douc male with complete white fore arms and black lower legs; Hon Heo Peninsula, Khanh Hoa Province. Photo: T. Nadler.

Fig. 7. Black-shanked douc male with complete white fore arms and dark red on lower legs; Hon Heo Peninsula, Khanh Hoa Province. Photo: T. Nadler.

Duc, pers. comm.) show the typical black-shanked coloration. Also poached animals from neighbouring Khanh Hoa Province (only 30 to 40 km from the Hon Heo Peninsula) show no different coloration (Fig. 8).

Fig. 8. Poached black-shanked douc langurs from Khanh Hoa Province. Photo: FPD.

Molecular genetics

From all 16 fecal samples collected, the hypervariable region I of the mitochondrial D-loop was successfully amplified and sequenced. Based on phylogenetic tree reconstructions, all obtained sequences cluster significantly together with black-shanked douc langurs and not with red- or grey-shanked douc langurs. Haplotypes from the latter two were not observed in the population (Roos, unpubl.). Although only maternally inherited markers were analyzed, it seems highly unlikely that paternal–inherited markers would provide a different relationship, which might be explained by hybridization. However, to definitively exclude hybridization further markers should be analyzed.

Behavioural observations

Contrary to the assumption that doucs are entirely arboreal (Lippold, 1998; Napier & Napier, 1997) the black-shanked douc langurs on Hon Heo Peninsula spend about two hours per day (20% of the daily time budget) on the ground, seeming to prefer granite blocks for resting and social contact, like grooming and mating (Fig. 9-13).

One reason to move to the ground is the need to drink water. A group of douc langurs was observed drinking extensively from a water source every morning (Fig. 14-15). Little is known about the water consumption of colobines and it is often overlooked that water is an essential nutrient (Committee of Animal Nutrition, 2003). Douc langurs have a relatively high water demand (Ruempler, 1998). This high demand exists not only for animals fed compressed food or pellets under captive conditions (Lippold, 1977). Observations on doucs at the EPRC, kept in cages and

Fig. 9, 10. Black-shanked douc langurs resting on a granite block. Photo: T. Nadler.

Fig. 11, 12, 13. Female offered mating, laying down for mating, and mating. The male has a white wrist patch. Photo: T. Nadler.

on a semi-wild area, show that doucs also drink extensive water when natural food is available (Nadler, unpubl.). In the wild doucs probably also use arboreal cisterns (such as depressions at junctures of tree limbs and trunks) as has been documented for mantled howlers (*Alouatta palliata*) (Glander, 1978), but doucs often form relatively large groups and therefore arboreal water sources are likely insufficient, necessitating animals to move to the ground to use terrestrial water sources.

Fig. 14. Black-shanked douc langur group moved to a water hole on the top of a granite block. One male with an extended white patch on the wrist. Photo: T. Nadler.

Fig. 15. Black-shanked douc langurs drinking from a water hole on the top of a granite block. Photo: T. Nadler.

The reason that field investigators previously had not seen douc langurs on the ground and drinking directly from water sources (Lippold, 1977; 1998) can be attributed to unhabituated animals exercising caution in the presence of observers.

Acknowledgements

Special thanks goes to Sylvio Lamarche who brought attention to the occurrence of the black-shanked douc langur population on Hon Heo Peninsula, for his commitment to conservation, his great support during all visits and his friendship.

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Five years of the "Indochinese Primate Conservation Genetics Project"

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Key words: genetics, Indochinese primates, systematics

Summary

After a five year period of the "Indochinese Primate Conservation Genetics Project" some important results have widened the knowledge of some primate taxa and provide also background information for conservation measures. Highlighted results are: (1) Two species of silvered langurs (*Trachypithecus germaini* and *T. margarita*) are confirmed for the Indochinese bioregion. (2) The Indochinese grey langur (*T. crepusculus*) is present in Pu Long Nature Reserve. Although no specimen was observed since the 1960s, genetic analyses of fecal material collected in the Nature Reserve confirmed its occurrence there. (3) *Nomascus hainanus* represents a distinct species. The genus *Nomascus* comprises now the five species *N. nasutus*, *N. hainanus*, *N. concolor*, *N. leucogenys* and *N. gabriella*e. (4) *Nomascus siki* represents a subspecies of *N. leucogenys*. However, the exact distribution zones of *N. gabriellae*, *N. l. siki* and *N. l. leucogenys* are not clarified yet, and maybe another subspecies is present in the region. (5) The sympatry of *Pygathrix cinerea* and *P. nemaeus* is confirmed for Chu Mom Ray National Park. Hybridization among them is highly likely, but not genetically confirmed yet.

Sample collection is requested for the continuation of the work.

Năm năm thực hiện chương trình “Nghiên cứu bảo tồn gien của các loài linh trưởng Đông Dương”

Tóm tắt

Sau 5 năm thực hiện chương trình “Nghiên cứu bảo tồn gien của các loài linh trưởng Đông Dương” một số kết quả quan trọng đã được phát hiện. Đặc biệt là những kiến thức mới về phân loại học và ý nghĩa của nó đối với các chương trình bảo tồn loài. Kết quả như sau: (1) Có hai loài vọoc bạc gồm *Trachypithecus germaini* và *T. margarita* được khẳng định tại vùng Đông Dương. (2) Loài vọoc xám Đông Dương (*T. crepusculus*) hiện diện ở khu bảo tồn thiên nhiên Pu Luông, Việt Nam. Mặc dù không có mẫu vật nào được quan sát từ những năm 1960, nhưng phân tích di truyền học dựa trên mẫu phân thu thập ngoài tự nhiên đã khẳng định sự hiện diện của chúng. (3) Loài vượn *N. hainanus* là một loài riêng biệt. Giống *Nomascus* gồm có năm loài khác nhau: *N. nasutus*, *N. hainanus*, *N. concolor*, *N. leucogenys* và *N. gabriella*e. (4) Vượn *N. siki* là một loài phụ của *N. leucogenys*. Tuy nhiên vùng phân bố của ba loài và phân loài *N. gabriellae*, *N. l. siki*, và *N. l. leucogenys* chưa rõ. Có thể tồn tại một loài phụ khác trong vùng phân bố trên. (5) Vùng phân bố trùng nhau của hai loài *Pygathrix cinerea* và *P. nemaeus* được khẳng định tại Vườn Quốc gia Chư Mom Rây. Con lai của hai loài này nhiều khả năng

tồn tại nhưng chưa được kiểm chứng về thông tin di truyền.

Để chương trình được tiếp tục nhiều mẫu vật cần được thu thập thêm trong thời gian tới.

Introduction

November 2008 will mark the 5th anniversary of the "Indochinese Primate Conservation Genetics Project." The idea for such a project came up at the symposium "Conservation of Primates in Vietnam" at Cuc Phuong National Park in November 2003. The project aimed to improve exchange of information between scientists involved in primate science and conservation in the Indochinese bioregion in order to straighten conservation efforts as well as studies in different fields of biological research.

In fact, in the last five years, knowledge on several biological aspects of Indochinese primates was deepened, and the distribution of taxa was improved in many ways as such as by publishing a new journal, the *Vietnamese Journal of Primatology*. Although comprehensive collaborations with many organisations in Vietnam, Cambodia, Laos and China have existed for several years, further cooperations with e.g. Fanjingshan National Nature Reserve and the University of Bejing, Guizhou Provice, China were established.

In recent years, trans-boundary actions became increasingly important. With the exception of *Nomascus hainanus*, *Trachypithecus delacouri*, *T. poliocephalus poliocephalu*s, *T. poliocephalus leucocephalus* and the four *Rhinopithecus* species, all other primate species are distributed in at least two countries. Of special conservation relevance are *Nomascus nasutus*, *T. laotum hatinhensis* and *Pygathrix cinerea*, which occur in restricted areas in trans-boundary regions of Vietnam-China, Vietnam-Laos and Vietnam-Laos-Cambodia, respectively. Accordingly, collaborations between governments and other conservation organisations are urgently required to improve conservation efforts across borders.

Results

Besides research on behaviour, socioecology and distribution conducted in the field or on captive populations, genetic studies based on non-invasively collected material provide further insights into the biology of species. Accordingly, based on the collection of different material types by various scientists and field workers of the consortium, comprehensive genetic analysis were conducted, which have led to important results. Highlighted results include:

1. Two species of silvered langurs (*Trachypithecus germaini* and *T. margarita*) are confirmed for the Indochinese bioregion (Groves, 2007; Nadler et al., 2005; Roos et al., 2007)
2. The Indochinese grey langur (*Trachypithecus crepusculus*) is present in Pu Long Nature Reserve. Although no specimen was observed since the 1960s, genetic analyses of fecal material collected in the Nature Reserve confirm its occurrence there (Nadler et al., 2004).
3. *Nomascus hainanus* represents a distinct species. The genus *Nomascus* comprises now the five species *N. nasutus*, *N. hainanus*, *N. concolor*, *N. leucogenys* and *N. gabriellae* (Roos, 2004; Roos et al., 2007).
4. *Nomascus siki* represents a subspecies of *N. leucogenys*. However, the exact distribution zones of *N. gabriellae*, *N. l. siki* and *N. l. leucogenys* are not clarified yet, and maybe another subspecies is present in the region (Roos, 2004; Roos et al., 2007).
5. The sympatry of *Pygathrix cinerea* and *P. nemaeus* is confirmed for Chu Mom Ray National Park. Hybridization among them is highly likely, but not genetically confirmed yet (Roos, unpubl.).

The population genetic studies focus on gibbons, douc langurs and "limestone langurs". Using different genetic marker systems, we have established a rough distribution map of genetic haplotypes for these three primate lineages. Preliminary data provide insights into taxonomy, recent and historic dispersal events, the influence of climate zones, rivers and mountains on the distribution, and gene flow between populations.

Genetic studies revealed new information on taxonomy, evolution, dispersal mechanisms, behaviour and ecology of Indochinese primates. Accordingly, these and other data deepen our knowledge about the biology of these species, which in practise help to protect primate populations and species. For example genetic methods allow the geographic origin of confiscated primates to be traced with a relative high accuracy. Moreover, population sizes can easily be estimated and the occurrence of a species in an area can be confirmed without seeing any specimen. Accordingly, genetic analyses are useful tools to reduce wildlife trade and to improve survey work. Furthermore, basic information for establishing migration corridors between protected areas and for reintroduction or resettlement programs can be provided.

Call for contribution and cooperation

Although many issues were settled in recent years, others are still unresolved and further investigations are needed. The utility of a reference collection to identify confiscated animals depends on the amount of existing sample material. Therefore, further samples from many individuals and locations all over the distribution areas are required. I would like to thank all the field workers who provided samples in last years and encourage them to further collection of samples to expand the database. In principal, all types of material are useful. Detailed information (species, sex, location, coordinates) for all collected samples should be provided. Sample materials should be handled and preserved in the following way:

1. Hairs: pluck out the hairs, don’t cut them, so that root cells are still present. Preserve hairs dried in small plastic or paper bags.
2. Dry tissue/skin (also smoked material): cut off a piece and preserve it dry in a small plastic or paper bag.
3. Fresh tissue/skin: cut off a piece and preserve it in a tube with 70-90% ethanol.
4. Fecal sample: put sample (about 1cm^3) with a stick into a tube with 70-90% ethanol.

The shipment of samples, especially across the border, should be in accordance with national and international laws and regulations. For some species samples CITES permits are required. Information and support can be requested at the Endangered Primate Center at Cuc Phuong National Park, Vietnam (s.a. www.primatecenter.org).

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Frankfurt Zoological Society: "Vietnam Primate Conservation Program" and Endangered Primate Rescue Center, Vietnam – Report 2007

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Key words: primates, Vietnam, Endangered Primate Rescue Center

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Summary

Frankfurt Zoological Society (FZS) started the "Vietnam Primate Conservation Program" in 1991. Included in this program are the management of the Endangered Primate Rescue Center, primate protection, primate habitat protection, primate research, and education.

At the end of 2007 the EPRC housed 141 individuals of 17 taxa. Nine individuals of 5 taxa were born during the year and 16 individuals died; nine of those that died were pygmy lorises. Eight captive bred Hatinh langurs were transferred in September to Phong Nha-Ke Bang National Park for the reintroduction project.

Sixteen confiscated animals arrived at the center (1 slow loris, 11 pygmy lorises and 4 douc langurs). A big loss for the breeding program at the EPRC has been the disappearance of two female white-cheeked gibbons, most probably shot on the semi-wild area, close to the EPRC and reception area of the national park.

The care of all the EPRC animals has been continuously managed by a staff of 20 Vietnamese workers under the supervision of an experienced animal keeper from Zoos in Berlin and Leipzig, Germany. Five biologists and one project assistant complete the staff of the EPRC.

Scientific research was carried out in cooperation with several Universities from the USA, the Zoological Museum Dresden, Germany, and with the German Primate Center.

Students from German Universities have been working for several months to continue the work on the Primate Data Base.

One survey was carried out on Hon Heo Peninsula, Khanh Hoa Province, to assess the status of a black-shanked douc langur population which was discovered in 2006.

The support of protection activities at Van Long Nature Reserve continued. Salaries, equipment, and uniforms were provided for 20 guards. A PhD student from Duke University, USA started an ecological study of the Delacour's langurs with support from FZS.

A three-week primate training course was organized by Conservation International, Hanoi National University, and FZS for students and staff of protected areas and Forest Protection Departments.

A one-week primate training course was organized by Da Nang University and FZS, and carried out for students at Da Nang University.

The project assistant of the "Vietnam Primate Conservation Program", and one of the foreign head keepers of the center participated in a three-week training course "Conservation of Endangered Species" at the Durrell International Training Conservation Center at Jersey Zoo, UK.

Several TV reports from German and Vietnamese TV stations were made and broadcasted.

The guidebook “Protected Animals of Vietnam” was completed. The guidebook should be a tool for the rangers of the Forest Protection Departments and other law enforcement authorities.

Posters, postcards, and T-shirts were printed as information and education material.

Hội động vật học Frankfurt: “Chương trình Bảo tồn Linh trưởng Việt Nam” Trung tâm Cứu hộ Linh trưởng Nguy cấp, Việt Nam – Báo cáo 2007

Tóm tắt

Hội động vật học Frankfurt (FZS) đã triển khai “Chương trình Bảo tồn Linh trưởng Việt Nam” từ năm 1991. Nội dung của Chương trình bao gồm các hoạt động quản lý và duy trì Trung tâm Cứu hộ Linh trưởng Nguy cấp, bảo vệ thú linh trưởng, bảo vệ môi trường sống của thú linh trưởng, nghiên cứu về thú linh trưởng, tuyên truyền giáo dục và nâng cao nhận thức bảo tồn thú linh trưởng.

Cuối năm 2007, Trung tâm Cứu hộ Linh trưởng Nguy cấp đã nuôi giữ 141 cá thể của 17 loại khác nhau. Chín cá thể của 5 loài và phân loài được sinh ra trong năm và 16 cá thể bị tử vong, loài cu li nhỏ chiếm số lượng lớn nhất là 9 cá thể. Tám cá thể được sinh ra và nuôi dưỡng trong điều kiện nuôi nhốt tại Trung tâm đã được đưa tới Vườn Quốc gia Phong Nha- Kẻ Bàng cho Chương trình tái thả thú linh trưởng vào tự nhiên. 16 cá thể được tiếp nhận về Trung tâm bao gồm (1 cu li lớn, 11 cu li nhỏ và 4 voọc chà vá). Một tổn thất lớn đối với chương trình cho sinh sản thú linh trưởng của Trung tâm là sự mất tích của hai cá thể vượn má trắng, dường như chúng bị bắn trong khu bán hoang dã thứ 2 nằm ở giữa khu vực của Tung tâm và Ban du lịch của Vườn.

Công tác chăm sóc động vật luôn được duy trì với 20 nhân viên Việt Nam dưới sự giám sát có kinh nghiệm của chuyên gia nuôi thú của Vườn thú Berlin và Vườn thú Leizig, CHLB Đức. Năm cán bộ sinh học và một trợ lý dự án nữa là toàn thể nhân viên của dự án.

Nghiên cứu thú linh trưởng được hợp tác với một số Trường Đại học của Hoa Kỳ, Bảo tàng động vật Dresden và Trung tâm Nghiên cứu Linh trưởng của Đức.

Sinh viên của một số Trường Đại học cuar Đức đã làm tình nguyện cho Chương trình Dữ liệu Linh trưởng điện tử.

Một cuộc khảo sát ngắn đã được thực hiện tại khu vực núi Hòn Hèo, huyện Ninh Hoà, tỉnh Khánh Hoà nhằm đánh giá sơ bộ về hiện trạng của một quần thể của loài voọc chà vá chân đen mới được phát hiện trong năm 2006.

Hoạt động tăng cường và hỗ trợ cho công tác bảo vệ ở Khu bảo tồn đất ngập nước Vân Long vẫn được tiếp tục thực hiện thông qua việc hỗ trợ phụ cấp, bảo hộ và thiết bị nhỏ cho 20 nhân viên bảo vệ của Khu bảo tồn. Một nghiên cứu cấp Tiến sĩ về tập tính sinh thái voọc mông trắng tại Vân Long của mootj sinh viên Trường Đại học Duke, Hoa Kỳ đã được thực hiện với sự hỗ trợ của FZS.

Tập huấn đào tạo ngắn trong 3 tuần về bảo tồn linh trưởng được thực hiện lần thứ hai cho sinh viên, nhân viên các khu bảo tồn và lực lượng Kiểm lâm với sự hợp tác tổ chức của Tổ chức Bảo tồn Quốc tế (CI), Trường Đại học Khoa học và Tự nhiên Quốc gia Hà Nội và Hội động vật học Frankfurt.

Tập huấn đào tạo ngắn trong 1 tuần về kỹ năng nghiên cứu ngoại nghiệp thú linh trưởng cho sinh viên Trường Đại học Đà Nẵng với sự hợp tác tổ chức của Trường Đại học Đà Nẵng và Hội động vật học Frankfurt.

Trợ lý dự án của “Chương trình Bảo tồn Linh trưởng Việt Nam” và một chuyên gia nuôi thú đã tham gia khoá học ngắn trong 3 tuần về “Bảo tồn những loài nguy cấp” tại Trung tâm Đào tạo Bảo tồn

Quốc tế - Durrell tại đảo Jersey, Anh quốc.

Nhiều lượt đoàn làm phim trong và ngoài nước đã đến quay phim và đưa tin về hoạt động của dự án trên các kênh truyền hình quốc gia và quốc tế.

Cuốn sách "Cẩm nang về các loài động vật được bảo vệ ở Việt Nam" đã được hoàn tất. Cuốn sách này sẽ là một dụng cụ hữu hiệu hỗ trợ cho lực lượng Kiểm lâm và các cơ quan chức năng bảo vệ luật pháp khác.

Tranh áp phích, bưu thiếp, áo phông được in để đưa thông tin giáo dục bảo tồn thú linh trưởng.

Introduction

The Endangered Primate Rescue Center is part of the "Vietnam Primate Conservation Program" of Frankfurt Zoological Society (FZS). Similar to the Center's "EPRC-Newsletter" published in previous years, the report should give an overview about the work and activities carried out as part of the program and expand on the previous report from 2004 to 2006 (Nadler, 2007).

Endangered Primate Rescue Center

Animals of the EPRC

At the end of 2007 the EPRC housed 141 individuals of 17 taxa, including two hybrids (s. Appendix). Nine individuals of 5 taxa were born during the year and 16 individuals died; nine of those that died were pygmy lorises. Eight captive born Hatinh langurs were transferred to Phong Nha-Ke Bang National Park for the reintroduction project. A single slow loris, which had been kept for more than six years at the EPRC, was released to the national park.

Sixteen confiscated animals arrived at the center (1 slow loris, 11 pygmy lorises and 4 douc langurs). There are probably two reasons for the decrease of langur confiscations: a dramatic decline of the wild populations, and a new law which permits the selling of confiscated wildlife. Ranger stations and Forest Protection Departments can now earn additional income by selling the confiscated wildlife – sometimes back to the wildlife trader – whereas informing the rescue center and handing over the animals yields a relatively low reward or no monetary compensation (more details see also Nadler, 2007b).

A big loss for the breeding program at the EPRC has been the disappearance of two female white-cheeked gibbons, listed as "Critically Endangered" species from one of the semi-wild areas. The mail gibbon, paired with these two females, was found with an injured shoulder, after a two day intensive search.. X-ray investigation showed some bullets in his shoulder. It is likely that the two female gibbons were also shot in the semi-wild area, which is inside the national park and very close to the reception and hotel area of the park.

Construction activities

After 12 years the asbestos-concrete roof on a wooden construction of the station house was replaced by a steel-roof construction with metal sheets. This allowed for the construction of an additional storage room.

Staff at the EPRC

The care for the animals has been continuously managed by a staff of 20 Vietnamese workers under the supervision of Elke Schwierz, from 2003 until July 2007. Falk Wicker, an experienced animal keeper from Zoo Leipzig, Germany started his work in April 2007 and continued the work as head keeper after Elke Schwierz finished her work at the center.

Despite repeated efforts to employ a second Vietnamese project assistant, only Nguyen Thi Thu Hien, has been available to manage the manifold work.

Four Vietnamese biologists have been working at Kon Ka Kinh National Park, Gia Lai Province under the leadership of Ha Thang Long for the research on grey-shanked douc langurs and to gather data for his PhD study at Cambridge University, UK.

Under supervision of the EPRC and in cooperation with the German Primate Centre the biologist Van Ngoc Thinh continued his field studies on gibbons for his PhD.

Scientific work

The scientific work at the center continues, mostly in cooperation with foreign institutions but also with support from volunteers.

Prof. Dian Brockman, University of North Carolina at Charlotte, USA started a study about “Biomarkers of Agent Orange Exposure in Nonhuman-Primates”.in December 2006 and continued with laboratory work in 2007.

Prof. Robin Bernstein, George Washington University, USA studied “Stress in red-shanked douc langurs” based on hormone analyses of samples collected by staff at the EPRC over a period of about two months.

Prof. Kristin Wright, Kansas City University of Medicine & Biosciences, USA studied morphological structures and locomotion of douc langurs at the EPRC.

The mammal curator of the Zoological Museum Dresden, Germany (Museum für Tierkunde) Dr. Clara Stefen conducted a study about skull morphology of douc langurs at the EPRC.

Nguyen Xuan Dang, biologist of the Institute for Ecology and Biological Rescources, Hanoi carried out a one-year-study on the feeding ecology of white-cheeked gibbons in the semi-wild enclosure (January to December).

Megan Barker, student at the University of California at Santa Cruz, USA made a study about hand preferences on gibbons at the EPRC.

Michael Chen, PhD student at the University of New England, Armidale, Australia continued his study about gibbon vocalization and vocalization development at the EPRC.

The study on plant phenology and feeding of Delacour’s langurs on the semi-wild area at the EPRC - originally a part of the biological study of the Center’s biologist Le Van Dung in middle 2006 - was continued by the staff of the center to complete a full year of data collection.

The close cooperation with the German Primate Center especially for molecular genetic research on Indochinese primate species continued.

Three German students, Tobias Schneider from University Bochum, Benjamin Stisser and Lea Roos from University Landau worked in total for 5 months on the Primate Data Base Project at the EPRC. The Primate Data Base should develop to a data information base about Vietnamese primates and provide information about the distribution and biology of primate species which can be used as a tool for conservation activities, land use planning and research.

Surveys

In May one survey was carried out in cooperation with the Forest Protection Department of Ninh Hoa District, Khanh Hoa Province to provide the first information about the status of black-shanked douc langurs on Hon Heo Peninsula, Khanh Hoa Province, a population which was discovered in 2006. The survey also assessed the value of the area for this species and provided basic information for the possibility of the establishment of a protected area (Ha Thang Long & Nadler, 2007).

Habitat protection at Van Long Nature Reserve

The support of protection activities of the nature reserve continued. FZS continued the salary payments for 20 guards, and provided the guards with equipment and uniforms.

Meetings with guards and communes have been organized in cooperation with the management board of the nature reserve.

Catherine Workman, PhD student at Duke University, USA started her study on the ecology on Delacour’s langurs in Van Long Nature Reserve in cooperation with and supported by the EPRC, and in cooperation with the EPRC’s biologist Le Van Dung. Le Van Dung is also in charge of the contact with, and the training of the guards, and the cooperation with the communes around the nature reserve.

Reintroduction project

A 20ha electric fenced semi-wild area was completed early 2007 in Phong Nha-Ke Bang National Park, Central Vietnam. In September 2007 the first 8 captive bred Hatinh langurs were transferred from the EPRC to Central Vietnam for release. The priority for a release to the national park is the elimination of poaching. Surveys were carried out and planning is ongoing to locate a safe reintroduction area.

Education

In July/August a three-week primate training course was carried out for students and staff of protected areas and Forest Protection Departments. The course was organized by Conservation International, Hanoi National University, and FZS. The one-week practical part of the course took place in Cuc Phuong National Park and the Endangered Primate Rescue Center.

In August a one week primate training course was organized by Da Nang University and FZS, and carried out for students at Da Nang University. The course comprised both theoretical and practical components. The practical part was organized on Son Tra Nature Reserve in cooperation with the Da Nang Forest Protection Department.

During the year the guidebook “Protected Animals of Vietnam” was completed. The guidebook should be a tool for the rangers of the Forest Protection Departments and other law enforcement authorities in Vietnam to identify protected animal species, to provide information for temporary care, and to place confiscated animals.

For the fourth straight year, the 6 grade students at the United Nations International School, Hanoi visited the EPRC for one week to get information about nature conservation in general and primate conservation in particular.

Posters, postcards, and T-shirts were printed as informational and educational materials and were sold at the EPRC.

In July/August the project assistant of the “Vietnam Primate Conservation Program” Nguyen Thi Thu Hien, and the foreign head keeper of the center Elke Schwierz participated in a three-week training course “Conservation of Endangered Species” at the Durrell International Training Conservation Center at Jersey Zoo, UK.

Publications, reports, and presentations about the “Vietnam Primate Conservation Program” and the Endangered Primate Rescue Center

Ha Thang Long (2007): Distribution, population and conservation status of the grey-shanked douc (Pygathrix cinerea) in Gia Lai Province, Central Highlands of Vietnam. Vietnamese J. of Primatol. 1, 55-60.

Ha Thang Long & **Nadler T** (2007): Rapid assessment of the status of black-shanked doucs (Pygathrix nigripes) on Hon Heo Peninsula, Khanh Hoa Province. Report to Forest Inventory and Planning Institute Hanoi and Frankfurt Zoological Society.

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Nadler T (2007b): Endangered Primate Rescue Center, Vietnam – Report 2004 to 2006. Vietnamese J. of Primatol. 1, 89-104.

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Streicher U (2007): Morphological data of pygmy lorises (Nycticebus pygmaeus). Vietnamese J. of Primatol. 1, 67-74.

Wright BW, **Le Khac Quyet**, **Prodhan R**, **Covert HH** & **Nadler T** (2007): Does craniofacial variation among Rhinopithecus species follow an altitudinal cline? Poster presentation.

Wright KA, **Ruff CB**, **Stevens NJ**, **Covert HH** & **Nadler T** (2007): Long bone articular and diaphyseal structure in douc langurs: evidence of suspensory adaptations Am. J. Phys. Anthropol. Suppl. 44, 253.

Wright KA, **Stevens NJ**, **Covert HH** & **Nadler T** (2006): Hanging Around: Comparisons of suspensory behaviors among doucs (Pygathrix) and gibbons (Nomascus) at the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam. Int J. Primatol. Vol. 27, Supplement 1, 322.

TV reports

- German TV made a report about the transfer of Hatinh langurs from the EPRC to the release site in Phong Nha-Ke Bang National Park.
- German TV made a report about the FZS activities on Hon Heo Peninsula, Khanh Hoa Province to preserve the black-shanked douc langur population
- Vietnam TV (VTV2) made a report about the reintroduction project for Hatinh langurs in Phong Nha-Ke Bang National Park
- Vietnam TV (VTV2) made two reports about the work of the EPRC
- Ninh Binh TV made one report about the work of the EPRC

Appendix

Register of primates by the EPRC 2007 - (up to date 31. 12. 2007)
(* species or subspecies held only at the EPRC and nowhere else 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
1-03	17.5.94	F	ad.	wild	wild	confiscated	EPRC
1-04	17.5.94	M	1993	wild	1-03?	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
1-08	16.8.99	F	16.8.99	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
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	EPRC
2-17	11.12.97	F	1994	wild	wild	from tourists	EPRC
2-20	11.3.98	F	1995	wild	wild	from tourists	EPRC
2-21	11.3.98	M	11.3.98	2-01	2-04	born EPRC	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
2-25	13.8.00	F	13.8.00	2-12	2-05	born EPRC	2.9.07 to Phong Nha
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
2-30	4.2.02	F	4.2.02	2-01	2-04	born EPRC	2.9.07 to Phong Nha
2-32	4.4.02	F	4.4.02	2-15	2-17	born EPRC	EPRC
2-35	13.4.03	F	13.4.03	2-01	2-09	born EPRC	2.9.07 to Phong Nha
2-36	14.11.03	F	14.11.03	2-12	2-05	born EPRC	EPRC
2-37	19.11.03	M	19.11.03	2-15	2-11	born EPRC	2.9.07 to Phong Nha
2-38	3.12.03	M	3.12.03	2-15	2-20	born EPRC	2.9.07 to Phong Nha
2-40	23.8.04	F	23.8.04	14-1	2-14	born EPRC	2.9.07 to Phong Nha

2-41	28.11.04	M	28.11.04	2-01	2-09	born EPRC	EPRC
2-42	8.4.05	F	8.4.05	2-12	2-03	born EPRC	2.9.07 to Phong Nha
2-45	3.7.05	F	3.7.05	2-15	2-17	born EPRC	2.9.07 to Phong Nha
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	14-1	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
2-55	17.5.07	M	17.5.07	2-01	2-09	born EPRC	EPRC
2-56	??.9.07	M	??.9.07	2-12	2-36	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	confiscated	EPRC
15-04	2.6.03	M	2.6.03	15-02	15-01	born EPRC	EPRC

Francois' langur *Trachypithecus francoisi*

17-01	8.1.02	F	1997	wild	wild	confiscated	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
6-13	6.1.98	F	ad.	wild	wild	confiscated	†19.3.07
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
6-26	6.5.00	M	6.5.00	6-05	6-17	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	EPRC
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
6-34	26.3.04	F	2001	wild	wild	confiscated	EPRC
6-35	17.4.04	W	17.4.04	6-05	6-13	born EPRC	EPRC
6-36	28.6.04	M	ad.	wild	wild	confiscated	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
6-45	31.5.06	F	Jan. 06	wild	wild	confiscated	†13.2.07
6-46	17.8.06	F	2001	wild	wild	confiscated	EPRC
6-47	17.8.06	F	ad.	wild	wild	confiscated	†14.1.07
6-48	21.11.06	F	21.11.06	6-06	6-02	born EPRC	†14.2.07
6-49	23.3.07	M	23.3.07	6-16	6-38	born EPRC	†6.7.07
6-50	29.4.07	M	29.4.07	6-12	6-34	born EPRC	EPRC
6-51	9.5.07	F	9.5.07	6-12	6-21	born EPRC	†30.5.07
6-52	14.9.07	M	2003	wild	wild	confiscated	EPRC
6-53	17.10.07	F	2003	wild	wild	confiscated	EPRC

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

7-01	31.8.95	M	1992	wild	wild	confiscated	EPRC
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	EPRC
7-34	19.10.06	F	2000	wild	wild	confiscated	EPRC
7-35	3.11.06	F	ad.	wild	wild	confiscated	†11.2.07
7-37	24.12.06	M	2003	wild	wild	confiscated	EPRC
7-38	5.3.07	M	2007	wild	wild	confiscated	†6.10.07
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

Black-shanked douc langur *Pygathrix nigripes*

13-05	15.3.01	M	1996	wild	wild	confiscated	EPRC

Red-shanked douc langur x Black-shanked douc langur *Pygathrix nemaeus* x *P. nigripes* (*)

16-01	1.1.00	M	1.1.00	6-06	13-02	born EPRC	EPRC

White-cheeked gibbon *Nomascus leucogenys leucogenys*

8-01	30.9.94	F	1993	wild	wild	from foreigner	EPRC

8-02	30.9.94	F	1994	wild	wild	from foreigner	EPRC
8-03	28.5.02	M	1999	wild	wild	confiscated	EPRC
8-04	28.5.02	F	2001	wild	wild	confiscated	†18.8.07]poached or
8-05	28.5.02	F	2001	wild	wild	confiscated	†18.8.07]stolen
8-08	19.11.04	F	2001	wild	wild	confiscated	EPRC

Southern white-cheeked gibbon *Nomascus leucogenys 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	EPRC
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
9-10	10.3.00	M	1999	wild	wild	confiscated	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
9-14	30.12.07	M	30.12.07	9-07	9-06	born EPRC	EPRC

Yellow-cheeked crested gibbon *Nomascus gabriellae*

10-01	26.2.95	F	1994	wild	wild	from tourists	EPRC
10-02	6.2.97	F	1994	wild	wild	confiscated	EPRC
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	EPRC
10-07	7.10.06	F	2005	wild	wild	confiscated	EPRC
10-08	7.10.06	F	2005	wild	wild	confiscated	EPRC

Slow loris *Nycticebus bengalensis*

11-05	5.4.01	M	ad.	wild	wild	confiscated	16.11.07 released
11-09	20.11.07	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
12-48	12.12.03	M	ad.	wild	wild	Uni Hanoi	†8.8.07
12-60	13.6.05	M	Febr.05	wild	12-59	confiscated	†13.9.07
12-62	27.7.05	M	ca. 2004	wild	wild	confiscated	†21.8.07
12-66	24.2.06	F	ad.	wild	wild	confiscated	†30.11.07
12-67	24.2.06	M	ad.	wild	wild	confiscated	EPRC
12-68	24.2.06	M	ad.	wild	wild	confiscated	EPRC
12-69	24.2.06	F	2/2005	wild	wild	confiscated	EPRC
12-70	24.2.06	F	ad.	wild	wild	confiscated	EPRC
12-72	24.2.06	F	ad.	wild	wild	confiscated	EPRC
12-78	15.3.06	F	ad.	wild	wild	confiscated	EPRC
12-83	7.10.06	M	ad.	wild	wild	confiscated	EPRC
12-84	3.11.06	M	ad.	wild	wild	confiscated	EPRC
12-85	7.2.07	M	2006	wild	wild	confiscated	†5.7.07
12-86	17.5.07	F	ad.	wild	wild	confiscated	EPRC
12-87	17.5.07	M	ad.	wild	wild	confiscated	EPRC
12-88	17.5.07	F	ad.	wild	wild	confiscated	EPRC

12-89	17.5.07	F	ad.	wild	wild	confiscated	EPRC
12-90	17.5.07	M	ad.	wild	wild	confiscated	EPRC
12-91	7.6.07	M	2007	wild	wild	confiscated	†27.6.07
12-92	7.6.07	F	2007	wild	wild	confiscated	†27.6.07
12-93	19.6.07	F	2006	wild	wild	confiscated	†30.9.07
12-94	28.10.07	M	ad.	wild	wild	confiscated	EPRC
12-95	10.11.07	F	ad.	wild	wild	confiscated	†15.11.07

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Books and Monographs

Groves CP (2001): Primate Taxonomy. Smithsonian Institution Press, Washington DC.

Edited books and book chapters

Groves CP 2004: Taxonomy and Biogeography of Primates in Vietnam and Neighbouring Regions. In: Nadler, Streicher, Ha Thang Long (eds.): Conservation of Primates in Vietnam; p 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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