VIETNAMESE Journal of
Primatology
CHUYÊN ĐỀ LINH TRƯỞNG HỌC VIỆT NAM
ISSN 1859-1434

VOLUME 2 - ISSUE 2
OCTOBER 2013

Vietnamese Journal of Primatology

Vietnamese Journal of Primatology (ISSN 1859-1434) is a peer-reviewed open access journal published yearly by the Endangered Primate Rescue Center. The subscription price outside Vietnam is $40.00 including shipment (for one copy). The journal can be ordered from the Endangered Primate Rescue Center, Cuc Phuong National Park, Ninh Binh Province, Vietnam or by mail: <eprc.info@gmail.com>. All subscriptions will be sent by air. Payments should be made by transfer to: Indovina Bank, 88 Hai Ba Trung, Hanoi, SWIFT CODE: IABBVNVX; Account: Frankfurt Zoological Society; Account no.: 2023828.

DPZ Deutsches Primatenzentrum
Leibniz-Institut für Primatenforschung

The **Vietnamese Journal of Primatology** is produced with support from German Primate Centre, Göttingen.

Cover: Pygmy loris (*Nycticebus pygmaeus*). Photo: T. Nadler.

Vietnamese Journal of Primatology (2013) vol.2(2), 1-12

Twenty years Endangered Primate Rescue Center, Vietnam – Retrospect and Outlook - Report 2012

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Key words: Primates, Vietnam, Endangered Primate Rescue Centre

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Summary

As the first rescue center in the whole of Indochina, the Endangered Primate Rescue Center over the years has developed into a recognized leading institution both on national and international level. After twenty years of existence the Endangered Primate Rescue Centre has grown, and now it is a good opportunity to review the development and to summarize the work of the EPRC.

The year 2012 started with a new protectorate for the Endangered Primate Rescue Center. In the past a part of Frankfurt Zoological Society's projects in Vietnam with beginning of the year Leipzig Zoo, Germany became the partner and bearer of the EPRC. The EPRC continues the "Vietnam Primate Conservation Program" for a number of highly endangered primate species.

During the last twenty years the number of cages at the EPRC has grown to now 50 enclosures with a total surface of 3200 m2, which house 150 primates which are either 'Critically Endangered' or 'Endangered' species. More than 180 primates of 9 species are born at the center, several for the first time in captivity. The reintroduction of captive bred individuals is the final goal of the center and started with the reintroduction of lorises, followed by Hatinh langurs and Delacour's langurs.

The EPRC is dedicated to educating the public about primates, it is able to do this through: guided visitor tours, TV-reports, publications, training courses for students, rangers, and caretakers from other animal holding facilities in Vietnam.

The scientific work conducted at the EPRC, is often carried out in close cooperation with national and foreign institutions and is represented in over 130 publications, which include scientific papers, books and presentations at international conferences and events.

Hai mươi năm – Nhìn lại quá trình phát triển của Trung tâm Cứu Hộ Linh trưởng Nguy cấp, Việt Nam – Báo cáo 2012

Tóm tắt

Sau hai mươi năm thành lập và tồn tại của Trung tâm Cứu hộ Linh trưởng Nguy cấp, đây chính là thời điểm để nhìn lại sự phát triển và tổng kết các hoạt động mà EPRC đã thực hiện trong thời gian qua. Là một trung tâm cứu hộ động vật hoang dã được thành lập đầu tiên trong khu vực Đông Dương, Trung tâm đã phát triển qua thời gian và được sự công nhận và hoang nghênh cả trong nước và quốc tế.

Năm 2012 là một mốc đánh dấu thay đổi đối tác và nhà tài trợ cho Trung tâm Cứu hộ Linh trưởng Nguy cấp. Trước đây, được Hội Động vật học Frankfurt, CHLB Đức tài trợ thì nay Trung tâm được Vườn thú Leipzig. CHLB Đức tiếp quản. Trung tâm Cứu hộ Linh trưởng Nguy cấp Việt Nam vẫn tiếp tục tiến hành nhiều hoạt động của dự án "Chương trình Bảo tồn Linh trưởng Việt Nam" nhằm quan tâm tới những loài thú linh trưởng nguy cấp cao.

Trong 20 năm hoạt động và phát triển, Trung tâm đã cho xây dựng 50 chuồng nuôi có tổng diện tích mặt chuồng là 3.200 m^2, đang cứu hộ và nuôi dưỡng hơn 150 cá thể của 15 loài linh trưởng "Cực kỳ Nguy cấp" và "Nguy cấp". Với 180 cá thể của 9 loài đã được sinh sản tại Trung tâm, trong số đó có một số loài linh trưởng lần đầu tiên được sinh sản thành công trong điều kiện nuôi nhốt trên toàn thế giới. Chương trình tái hòa nhập linh trưởng quý hiếm được sinh sản trong nuôi nhốt trở về tự nhiên là mục tiêu cuối cùng của Trung tâm với một số thử nghiệm đã thực hiện với loài Cu li nhỏ, voọc Hà Tĩnh và voọc mông trắng.

Trung tâm Cứu hộ Linh trưởng Nguy cấp đã đóng góp cho việc phổ cập kiến thức về linh trưởng và nâng cao nhận thức bảo tồn linh trưởng trong cộng đồng thông qua công tác của nhân viên chăn nuôi thú, hướng dẫn viên du lịch, nhiều phóng sự và tin bài trên các phương tiện thông tin đại chúng. Nhiều ấn phẩm khoa học được công bố cả trong nước và quốc tế, đào tạo và tập huấn cho sinh viên, lực lượng kiểm lâm về nghiên cứu ngoại nghiệp, cứu hộ và chăm sóc linh trưởng.

Công tác nghiên cứu khoa học của Trung tâm rất chuyên sâu và luôn có sự hợp tác khoa học với các viện nghiên cứu, các trường đại học và các tổ chức bảo tồn trong và ngoài nước. Đến nay, Trung tâm Cứu hộ Linh trưởng Nguy cấp đã công bố được hơn 130 ấn phẩm bao gồm các bài báo khoa học, tạp chí, sách và tham luận khoa học tại nhiều Hội nghị khoa học Quốc gia và Quốc tế.

Introduction

After twenty years of existence the Endangered Primate Rescue Center has grown, and now is a good opportunity to review the development and to summarize the work of the EPRC. The EPRC first started as an experiment to rescue some single individuals of highly endangered primate species. But before long, the center developed over the years into a recognized leading institution both on national and international level, which is highly praised through visits of numerous politicians and renowned scientists (Fig. 1 and 2). Since its establishment in 1993 – as the first rescue center in Indochina – the number of primate species and individuals has grown over the years and the center now keeps a high number of 'Critically Endangered' and 'Endangered'

Fig.1. The former President of Vietnam Tran Duc Luong and his wife on their third visit at the Endangered Primate Rescue Center. Photo: Luong Van Hien.

Fig.2. The former President of Vietnam, and successor of Tran Duc Luong, Nguyen Minh Triet, and the Chairman of Ninh Binh Province Bui Van Trang visit the Endangered Primate Rescue Center. Photo: Luong Van Hien.

primates in captivity (Fig. 3).

The year 2012 represented a new start for EPRC as a new partner and sponsor emerged. In the past the center was run as a project of Frankfurt Zoological Society. With an intensified focus from Frankfurt Zoological Society on the protection and conservation of large wilderness areas with a high biodiversity, the function and profile of a rescue facility doesn't correspond anymore with the strategic profile of the organisation.

Fig.3. The EPRC was recognized in the "Vietnamese Guinness Book of Records" as the facility with the highest number of 'Critically Endangered' and 'Endangered' primates.

Leipzig Zoo in Germany made the generous offer to act as organizational and financial bearer for the Endangered Primate Rescue Centre. The funding and support offered from Leipzig Zoo, which itself is a leading European zoo in animal husbandry and care is a profitable advantage for the management and future development of the center. This connection opens up the chance for future improvement at the center, through innovative techniques regarding captive breeding, nutrition, enrichment, husbandry, veterinary care and reintroduction.

The center acts as refuge for confiscated primates of endangered species. The possibility for housing of the endangered primates at the EPRC supports the activities of forest protection authorities in combating the illegal wildlife trade. With the increasing number of animals at the center, the establishment of captive breeding programs for highly endangered primate species was applicable as a source for future reintroduction. Beyond this the center provides an unique possibility for research and studies on highly endangered primates under captive conditions and in the field.

The EPRC continues the *Vietnam Primate Conservation Program* for a number of highly endangered primate species.

Frankfurt Zoological Society continues the conservation work in Vietnam, focusing on the conservation of the 'Critically Endangered' grey-shanked douc langur and protection of its habitat, in particular Kon Ka Kinh National Park...

Endangered Primate Rescue Center

Facilities at the EPRC

The facilities at the EPRC have grown along with the center; temporary cages built in 1993 were replaced by two 275 m^2 double cage units which were constructed in a new area of the EPRC (Fig. 4). Over the years the number of cages has grown to 50 with a total surface of 3.200 m^2 (Fig. 5). In addition to the enclosures three houses were built. These houses are equipped with electrical heating systems to provide suitable conditions for individuals from southern Vietnam, confiscated during the winter season and for groups with young animals. In 1997 a separate quarantine complex was built with four indoor and four outdoor cages, a surgery and a preparation room (Fig. 6). In 1998 the first 2 ha electric fenced semi-wild area with primary forest was completed which enables the center to keep langur groups under natural conditions without provisioning. A second 5 ha semi-wild area was built in 2001 (Fig. 7). The total area of the center now comprises 1.5 ha with cage constructions and the two semi-wild areas.

Fig.4. In 1995 the first two cages and a station house were constructed in the new area of the EPRC at Cuc Phuong National Park. Photo: Tilo Nadler.

Fig.5. Currently the EPRC has 50 cages in the shadow of large native trees which were planted at the beginning of the cage construction in this area. Photo: Tilo Nadler.

Fig.6. In 1997 a separate quarantine complex was finished with indoor and outdoor cages and a surgery. Photo: Jens Gerlach.

Fig.7. In 2001 a second and 5 ha large semi-wild area with primary forest was completed. Photo: Tilo Nadler.

Animals at the EPRC

In 1993 the EPRC started off with the housing of 8 primates, four langurs, two gibbons and two lorises. The number increased during twenty years to 153 individuals in 2012 (Fig. 8). The increase was a result from high numbers of incoming individuals and animals born at the EPRC, following the established captive breeding programs at the center. In total 249 primates have arrived at the center since it was established, on average 12.45 individuals per month. Most of these individuals were confiscated from Forest Protection Departments in cooperation with the EPRC (Fig. 9). Few individuals, especially lorises, were donated from their owners, who were illegally keeping them. Roughly 30% of the confiscated primates didn't survive, despite intensive veterinarian care. This is often due to the state the animals are in when they arrive at the center, as many show physiologically as well as physical signs of distress. Some arrive badly injured through snare traps or have other physical problems including heavy digestion disorder.

The center is very successfully with breeding programs. In 1996 it saw the first animals born at the center, and this number has grown to 187 from 9 different species. In average 11 individuals a year are born at the EPRC (Fig. 10).

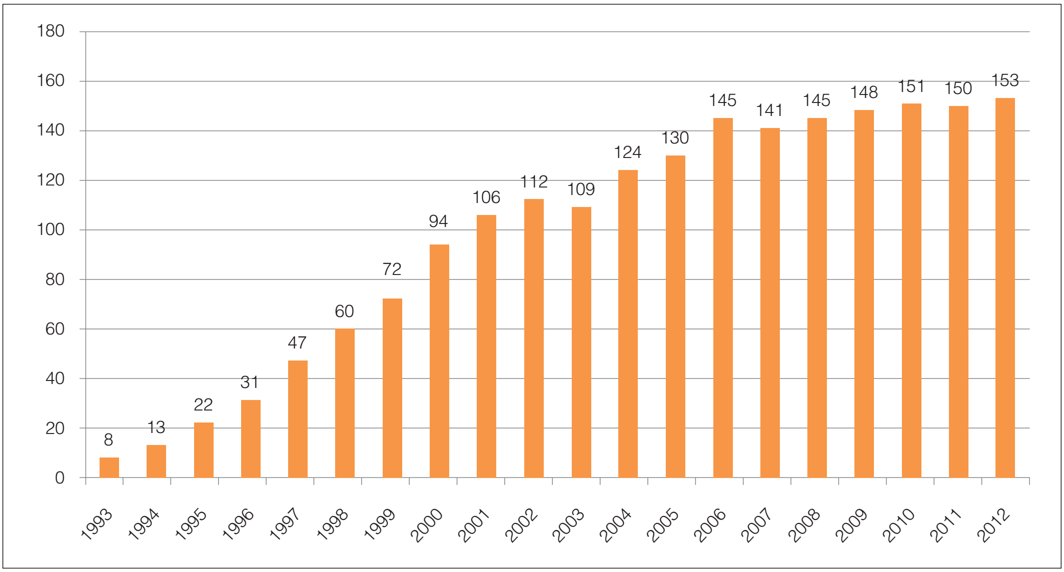

Fig.8. Number of primates housed at the EPRC.

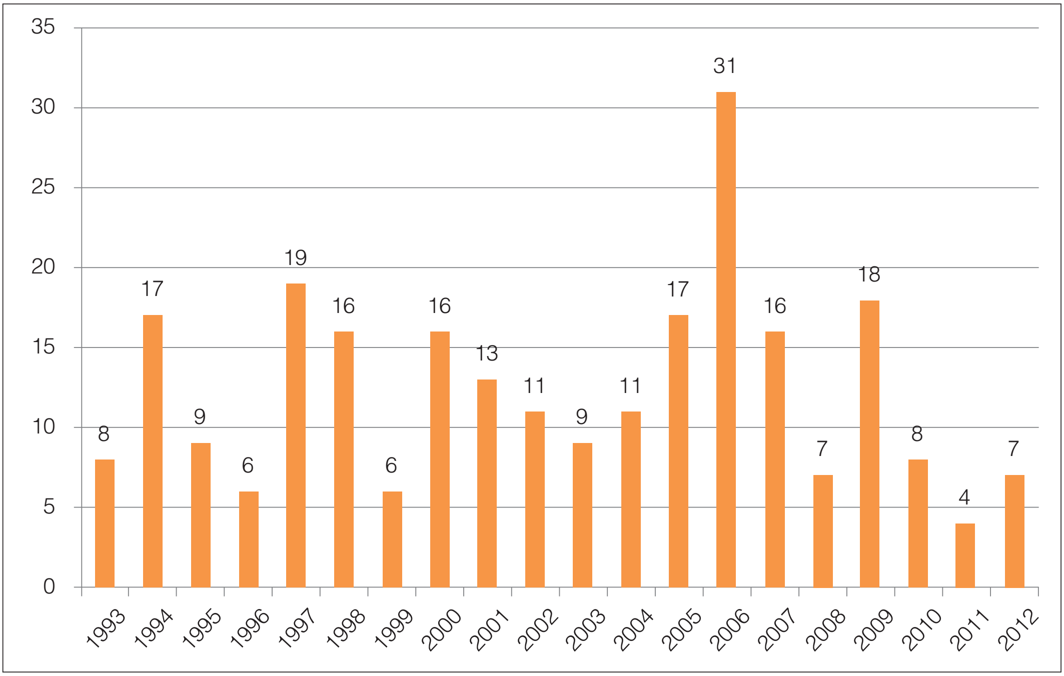

Fig.9. Primates arrived at the EPRC.

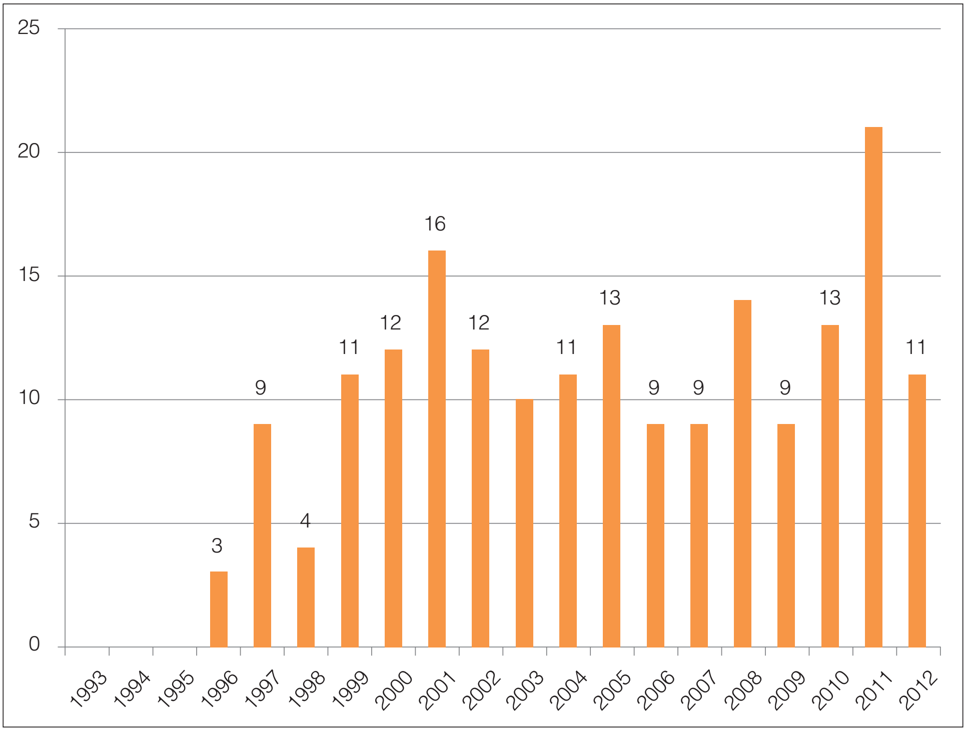

Fig.10. Primates born at the EPRC.

The highlight of the EPRC is the successful keeping of sensitive leaf-eating primate species and the establishment of successful breeding programs with these species. Currently the EPRC houses 15 species, four listed as ‘Critically Endangered’ (IUCN-List of Threatened Species) and three of them belong to the “25 World’s Most Endangered Primates” (Mittermeier et al., 2012). Seven species listed as ‘Endangered’ and three as ‘Vulnerable’. The newly discovered northern yellow-cheeked gibbon is not evaluated by IUCN but also most probably has the status ‘Endangered’.

Three ‘Critically Endangered’ species, - the Delacour’s langur, the Cat Ba langur, and the grey-shanked douc langur – which are kept only at the EPRC bred for the first time in captivity.

With 5 founder individuals of the Delacour’s langur, all confiscated from the illegal wildlife trade, 22 individuals were born at the center, four already in the F2-generation.

Three Cat Ba langurs, the world’s rarest langur species, were born at the center and the four individuals at the EPRC makes more than 5% of the total population with only about 60 individuals in the wild.

For the grey-shanked douc langur - a species discovered in 1997 at the EPRC based on confiscated primates – a captive breeding program was initiated and in 2002 the first individual was born. Now in total 17 individuals are born at the EPRC.

For the ‘Endangered’ Hatinh langur and red-shanked douc langur breeding programs with confiscated individuals started in 1996 and 1998 respectively. From 11 founder individuals of Hatinh langurs 65 individuals, and from 12 red-shanked douc langurs 34 individuals have been born at the center. A number of Hatinh and red-shanked douc langurs are already born in the F2-generation.

The ‘Endangered’ southern white-cheeked gibbon have successfully bred 8 individuals.

The center has developed a successful nutrition and feeding regime for confiscated juvenile leaf-eating langurs. Ten red-shanked douc langur babies were hand reared from the first day of life. They are the offspring from a female with missing milk glands. As a consequence she was unable to rear their babies. And more than 10 individuals arrived at a very young age of only two to three month. Nearly all of these hand reared babies have grown up.

A special advantage of the center is the possibility to integrate the juveniles in a "kindergarten" to enable contact with other young orphaned individuals, even of different species. This contact seems to be an important behavioral impact for the development of the juveniles and several hand reared female langurs have bred already without problems (Fig. 11 and 12).

Fig.11. The EPRC developed successful nutrition and feeding concepts for babies of leaf-eating primates. Photo: Tilo Nadler.

Fig.12. A number of sensitive leaf-eating langurs were successfully hand reared at the EPRC. Photo: Tilo Nadler.

All new arrivals undergo a quarantine time of about six weeks and a health check including blood screening for diseases. The genetics of all these individuals are determined and their DNA samples stored at the Gene Bank at the German Primate Centre.

Staff

Parallel to the increasing number of animals also is the number of animal keepers which has increased considerably since 1993 (Fig. 13). The Vietnamese workers are recruited from villages close to the national park (Fig. 14). This job creation enables local workers to gain a stable income in an area which is primarily inhabited by a Muong minority where job possibilities are limited. The income from the work they complete at the center enables considerable support for a number of families within the village. The staff is working under supervision of three Vietnamese head keepers, with more than 10 years of work experience at the EPRC. In addition an experienced and trained foreign animal keeper will be present. In January and February Denny Lohse, animal keeper from Leipzig Zoo filled this position. In July started Maria Bischoff in this position, who is also animal keeper from Zoo Leipzig and continued the work to early 2013.

Nguyen Thi Thu Hien oversees the work at the center as a Vietnamese manager and also works as a secretary for the director of the EPRC, helping to translate and working as a contact person for the administration of the park and the ministry.

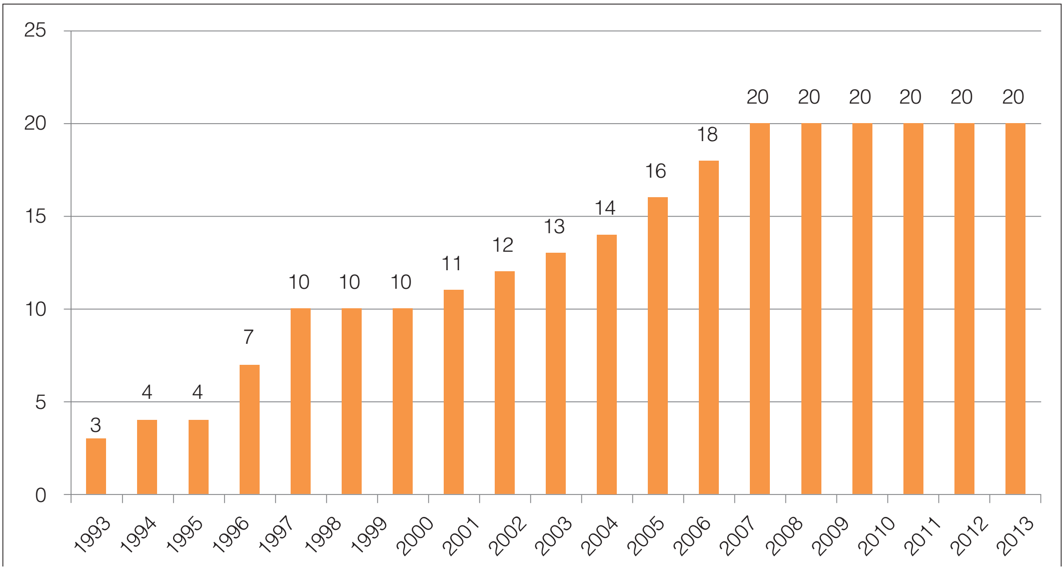

Fig.13. Number of Vietnamese animal keepers at the EPRC.

Fig.14. Animal keepers at the EPRC.

Animal food

For the members of the three primate families at the center (Lorisidae: lorises; Cercopithecidae: langurs and douc langurs; Hylobatidae: gibbons) different food is prepared depending on individual requirements. Lorises are fed with an array of different fruits and additional insects like grasshoppers, stick insects and occasionally meal worms. The gibbon food consists mainly of vegetables and some fruits purchased at the local markets. The langurs are fed exclusively with leaves, collected two times daily in the surrounding of the national park. The daily food composition comprises leaves from 8 to 12 different plant species, mostly trees. The offered plant species changes seasonally, depending from the availability of young shoots. The necessary daily amount of leaves for roughly 100 langurs is more than 300 kg (Fig. 15).

Fig.15. The preparation of more than 300 kg leaves for the leaf-eating primates requires a high amount of working time. Photo: Tilo Nadler.

Reintroduction

The final goal of the captive breeding programs of highly endangered primate species is the reintroduction of captive born individuals to strengthen decreased wild populations, or to establish new populations were the species originally existed but has been eradicated. For confiscated individuals who are not integrated in captive breeding programs, release recommendations and techniques should be developed to support their survival in the wild after a release.

In 2000 the first release study of pygmy lorises was carried out. Pygmy lorises are hunted and also confiscated in remarkably high numbers. Ten pygmy lorises were released with radio collars, tracked and the data analyzed.

In 2005 begun the construction of a 20 ha semi-wild area in Phong Nha-Ke Bang National Park as a cooperation project between Frankfurt Zoological Society and Cologne Zoo, as first step for the reintroduction of Hatinh langurs. In 2007 8 captive bred Hatinh langurs were transferred to this facility in preparation for the release. Through mismanagement of the reintroduction project the release of the animals was never completed and the animals still exist in this large facility and have now developed to a small population.

After more than 10 years close cooperation with Van Long Nature Reserve, successful improvements have been seen in the protection through a community based protection unit. Education programs in the surrounding communes, field surveys and a genetic population study in the area, which has allowed the area to be assessed as a safe and suitable reintroduction locality for the ‘Critically Endangered” Delacour’s langur (Ebenau, 2011; Ebenau et al. 2011). In 2011 three captive bred individuals were equipped with GPS radio collars, released into the nature reserve and tracked for about one year (Nadler, 2012). In November 2012 two further individuals with GSP radio collars were released and tracked. The data will be analyzed in PhD and master theses.

Education and capacity building

The EPRC is open for the public, but only on guided tours. More than 10.000 visitors per year visit the center and get information about the primates of Vietnam, the problems about their conservation and the critical situation of wildlife in the country.

A number of lectures and training courses were carried out at the EPRC, for guides of the national park, for animal keepers from other animal keeping facilities in Vietnam, for rangers, students, and school children. The center produced a number of posters, leaflets, cartoons and story books for children, and toys and games, occasionally in cooperation with other organizations.

Special events are school programs for children at Hanoi’s International School and Hanoi’s UN-International School (UNIS) at the EPRC. In each program about 80 children visit Cuc Phuong

National Park and the EPRC to get information about Vietnamese primates and to carry out "primate studies" at the EPRC.

Television reports and PR

Several Vietnamese TV-stations produced reports about the work of the EPRC with screen time ranging from 30 to 45 minutes. Additionally short reports and information about the center are broadcasted in average four times per year.

In total about 20 reports (20 to 45 min.) were also produced from foreign TV-stations and companies, based in Germany, UK, Japan, China, Singapore, Australia, US, Finland, France and Holland.

All reports focus on the critical situation of wildlife in Vietnam, and in particular on primates.

Since 1993 and the beginning of the project, 260 papers, articles and books were published referring to the primate work. About 50% of the publications and reports contains popular information in newspapers and magazines, especially highlighting the importance of raising awareness and attention about protection and conservation of Vietnamese primates in the public, nationally and internationally.

Scientific research

The EPRC offers with a number of endemic and highly endangered primates an unique research base. Running programs are linked with national and foreign universities and institutions. Both Vietnamese and foreign students and scientists study at the EPRC. More than 130 scientific papers have been published and contribute to the knowledge we have of Vietnamese primates, including information about biology, systematics, distribution and status.

Special highlights are the contribution of the EPRC to the discovering of two new primate species, the grey-shanked douc langur (Nadler, 1997) and the northern yellow-cheeked gibbon (Van Ngoc Thinh et al., 2010). An important contribution to the conservation of Vietnamese primates is also the clarification of the taxonomy. A remarkable number of systematic changes are based on studies at the center and the collection of DNA samples of the housed individuals (Nadler et al., 2005; Roos et al., 2007; 2008, Hoang Minh Duc et al., 2012).

More than 20 Diploma, master and PhD theses were written based on studies at the EPRC or in connection with field projects under supervision of the EPRC (references Appendix 1).

Since 2000 results of research and activities of the EPRC were presented on all biannual congresses of the International Primatological Society.

Future planning

The EPRC continue to stabilize small captive populations of highly endangered species as source for future reintroduction projects. A special challenge outside the EPRC, is securing adequate habitats for reintroductions of these individuals. Reintroduction is only justifiable if a safe habitat is available. To provide a safe habitat in Vietnam is unfortunately a long and difficult road and requires close cooperation with rangers, strict law enforcement and especially a very close cooperation with the communes in selected areas. The involvement of the local communes in such projects is essential and is one of the key points for a successful reintroduction project.

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Vietnamese Journal of Primatology (2013) vol. 2(2), 13-26

An updated taxonomy of primates in Vietnam, Laos, Cambodia and China

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Key words: *Nycticebus*, *Macaca*, *Trachypithecus*, *Semnopithecus*, *Rhinopithecus*, *Pygathrix*, *Hoolock*, *Hylobates*, *Nomascus*, Vietnam, Laos, Cambodia, China

Summary

The present publication summarises and updates information on the taxonomy of primates in Vietnam, Laos, Cambodia and China based on a new multi-author synthesis. For each species is included taxonomic authorities, species' type localities, subspecies, current distribution and conservation status. In total, the primate fauna in these countries comprises 41 species and 47 taxa. A breakdown of species is given by country, endemic species per country and the IUCN Red List conservation status category for each species.

Cập nhật về phân loại học của các loài thú linh trưởng ở Việt Nam, Lào, Campuchia và Trung Quốc

Tóm tắt

Tài liệu này sẽ cung cấp những thông tin cập nhật và ngắn gọn về tình trạng phân loại học của các loài thú linh trưởng ở Việt Nam, Lào, Campuchia và Trung Quốc dựa trên sự tổng hợp của nhiều tác giả. Đối với mỗi loài, chúng tôi sẽ bao gồm tác giả phân loại, loại sinh cảnh, phân loài phụ và thực trạng phân bố, bảo tồn. Về tổng thể, có 41 loài và 47 phân loài thú linh trưởng ở những nước trên. Các loài được sắp xếp theo mỗi nước, loài đặc hữu và tình trạng bảo tồn theo chuẩn IUCN.

Introduction

To conserve the primate fauna of Asia, there has long been a need for an accessible, updated checklist of recognized taxa. Volume 3 (Primates) of *The Handbook of the Mammals of the World*, edited by Russell A. Mittermeier, Anthony B. Rylands and Don E. Wilson, was published in April 2013 (Mittermeier et al., 2013). The book integrates new information on primates, including data on morphology, behaviour, acoustics, and genetics. For each primate family, there is an introductory section with reviews of systematics, morphology, habitat, general habits, communication, food and feeding, breeding, movements, home range and social organization, relationship with humans, and conservation status. They are followed by species accounts with more detailed information, along with illustrations by Stephen D. Nash, and range maps of each.

According to *The Handbook*, the order Primates comprises 16 families, 78 genera, 480 species and 682 taxa. For Vietnam, Laos, Cambodia and China, this multi-author compilation recognizes three families, nine genera, 41 species and 47 taxa. With 25 confirmed species, the highest number of species is found in Vietnam, followed by China with 22–25 species and Laos with 20–22 species. Cambodia, with only 13 species, has the lowest number (Table 1). Concerning endemic species, China has the highest number with 5–6 species, followed by Vietnam with three, and Laos with one species; Cambodia has no endemic species (Table 2). Of the 40 primate species that have been assessed (*Nomascus annamensis* has not yet been evaluated), 10 (25%) are Critically Endangered, 19 (47.5%) are Endangered, 6 (15%) are Vulnerable and 2 (5%) are Near Threatened. Only 3 species (7.5%) are Least Concern (Table 3).

Table. 1. Primate species by country.

Country	**Species**
China (22–25 species)	*Nycticebus bengalensis, (N. pygmaeus), Macaca leonina, M. assamensis, M. thibetana, (M. munzala), M. arctoides, M. mulatta, Trachypithecus shortridgei, T. phayrei, T. crepusculus, T. francoisi, T. leucocephalus, Semnopithecus schistaceus, Rhinopithecus bieti, R. brelichi, R. roxellana, R. strykeri, (Hoolock hoolock), H. leuconedys, Hylobates lar, Nomascus hainanus, N. nasutus, N. concolor, N. leucogenys*
Cambodia (13 species)	*Nycticebus bengalensis, N. pygmaeus, Macaca leonina, M. arctoides, M. fascicularis, Trachypithecus germaini, T. margarita, Pygathrix cinerea, P. nemaeus, P. nigripes, Hylobates pileatus, Nomascus annamensis, N. gabriellae*
Laos (20–22 species)	*Nycticebus bengalensis, N. pygmaeus, Macaca leonina, M. assamensis, M. arctoides, M. fascicularis, M. mulatta, Trachypithecus germaini, T. margarita, T. crepusculus, T. laotum, T. hatinhensis, T. ebenus, (Pygathrix cinerea), P. nemaeus, (P. nigripes), Hylobates pileatus, H. lar, Nomascus concolor, N. leucogenys, N. siki, N. annamensis*
Vietnam (25 species)	*Nycticebus bengalensis, N. pygmaeus, Macaca leonina, M. assamensis, M. arctoides, M. fascicularis, M. mulatta, Trachypithecus germaini, T. margarita, T. crepusculus, T. francoisi, T. poliocephalus, T. delacouri, T. hatinhensis, T. ebenus, Rhinopithecus avunculus, Pygathrix cinerea, P. nemaeus, P. nigripes, Nomascus nasutus, N. concolor, N. leucogenys, N. siki, N. annamensis, N. gabriellae*

Note: Species records in brackets are those the presence of which is unconfirmed.

Table. 2. Endemic primate species by country.

Country	Species
China (5–6 species)	*(Macaca thibetana), Trachypithecus leucocephalus, Rhinopithecus bieti, R. brelichi, R. roxellana, Nomascus hainanus*
Vietnam (3 species)	*Trachypithecus poliocephalus, T. delacouri, Rhinopithecus avunculus*
Laos (1 species)	*Trachypithecus laotum*
Cambodia (0 species)	-

Note: Macaca thibetana might also occur in NE India and thus might not be endemic to China.

Table. 3. IUCN Red List of primates by category.

Threatened Status	Species
Critically Endangered (10 species)	*Trachypithecus poliocephalus, T. leucocephalus, T. delacouri, Rhinopithecus avunculus, R. strykeri, Pygathrix cinerea, Nomascus hainanus, N. nasutus, N. concolor, N. leucogenys*
Endangered (19 species)	*Macaca munzala, Trachypithecus shortridgei, T. germaini, T. margarita, T. phayrei, T. crepusculus, T. francoisi, T. hatinhensis, T. ebenus, Rhinopithecus bieti, R. brelichi, R. roxellana, Pygathrix nemaeus, P. nigripes, Hoolock hoolock, Hylobates pileatus, H. lar, Nomascus siki, N. gabriellae*
Vulnerable (6 species)	*Nycticebus bengalensis, N. pygmaeus, Macaca leonina, M. arctoides, Trachypithecus laotum, Hoolock leuconedys*
Near Threatened (2 species)	*Macaca assamensis, M. thibetana*
Least Concern (3 species)	*Macaca fascicularis, M. mulatta, Semnopithecus schistaceus*
Not Evaluated (1 species)	*Nomascus annamensis*

Following the publication of *The Handbook*, there remained a need to make this information widely available and easy to access. In the following an overview is given of primates in Vietnam, Laos, Cambodia and China, including taxonomic authorities, type localities, subspecies, current distribution and conservation status. Whilst formal punctuation is retained for taxonomic purposes (scientific names and author initials), we have omitted this for general use (notably compass directions) for ease of reading.

Taxonomy

Family Lorisidae

Genus *Nycticebus*

Bengal Slow Loris *Nycticebus bengalensis* (Lacépède, 1800)

Type locality: India, Bengal.

Subspecies: None.

Distribution: E Bangladesh, NE India (Arunachal Pradesh, Assam, Meghalaya, Tripura, Nagaland, Manipur and Mizoram states), S China (S Yunnan Province from c.25°N in Yunnan and the Pearl River in the E, and possibly S Guangxi Autonomous Region), Myanmar (including the Mergui Archipelago), N and C Vietnam, Laos, Cambodia, Thailand, and possibly N of peninsular Malaysia.

Conservation status: Vulnerable.

Pygmy Slow Loris *Nycticebus pygmaeus* Bonhote, 1907

Type locality: Nha Trang, Annam, C Vietnam.

Subspecies: None.

Distribution: Laos, Vietnam and E Cambodia (E of the Mekong River). The precise W limit of the distribution is uncertain, but it appears to be absent (or at least very scarce) in the extreme W of the Mekong plain; records from S China (SE Yunnan Province) are uncertain and may be based merely on released captives brought in from elsewhere.

Conservation status: Vulnerable.

Family Cercopithecidae

Genus *Macaca*

Northern Pig-tailed Macaque *Macaca leonina* (Blyth, 1863)

Type locality: "Mountainous and rocky situation", Arakan District, SE Burma (Myanmar).

Subspecies: None.

Distribution: NE India (S of the Brahmaputra River in the states of Assam, E Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura), extending SE through E Bangladesh and Myanmar (including the Mergui Archipelago), SW China (SW Yunnan Province), Thailand, Laos, Vietnam and Cambodia; S to Surat Thani-Krabi depression in peninsula Thailand (8–9°N). Lack of records in C and NE Myanmar between 20° and 25°N suggests that this may be a natural gap in the distribution of the species.

Conservation status: Vulnerable.

Assamese Macaque *Macaca assamensis* (McClelland, 1839)

Type locality: India, Assam.

Subspecies: 2 subspecies are recognized:

Eastern Assamese Macaque, *M. a. assamensis* (McClelland, 1839); Western Assamese Macaque, *M. a. pelops* (Hodgson, 1840).

Distribution:

M. a. assamensis: SE Asia, 200–2750 m, E of the great bend of the Brahmaputra River, in SW

China (SE Xizang and SW Guangxi Autonomous Regions, SW Yunnan and Guizhou Provinces), NE India (E Arunachal Pradesh, E Assam, Nagaland, Meghalaya, Manipur, Mizoram and Tripura states), S and E through N and E Myanmar, N and W Thailand, Laos and N Vietnam.

M. a. pelops: Himalayas up to 3100 m, from C Nepal (W limit Tipling, 83°36'E) E through NE India (N West Bengal, Sikkim and W Assam states), and Bhutan (E limit Manas River, 90°58'E), with a widely disjunct record, of what may be a geographic relict, in coastal SW Bangladesh (Sundarbans).

Conservation status: Near Threatened (both subspecies).

Tibetan Macaque *Macaca thibetana* (Milne-Edwards, 1870)

Type locality: China, near Moupin (Baoxing), Sichuan.

Subspecies: None.

Distribution: EC China (25–33°N, 102°30'–119°30'E) in E Xizang (Tibet), Sichuan, S Gansu, S Shaanxi, Hubei, Anhui, Zhejiang, N Yunnan, Guizhou, Jiangxi, Fujian, N Guangxi and N Guangdong Provinces; W limit in the Yangtze Gorge in W and NW Sichuan and S limit at 23°48'N, c.110°E in Guangxi. The species may range into NE India (Arunachal Pradesh, Assam and Meghalaya states), although some reports, at least, appear to be based on misidentifications.

Conservation status: Near Threatened.

Arunachal Macaque *Macaca munzala* Sinha et al., 2005

Type locality: Zemithang (27°42'N, 91°43'E, 2180 m), Tawang District, Arunachal Pradesh, India.

Subspecies: None.

Distribution: NE India (W Arunachal Pradesh State), in Tawang and West Kameng Districts at elevations of 2000–3000 m; it possibly extends into Bhutan and Xizang (Tibet) in S China. There are reports of its occurrence in Mouling National Park in the Upper Siang District of C Arunachal Pradesh, but they have yet to be confirmed.

Conservation status: Endangered.

Stump-tailed Macaque *Macaca arctoides* (I. Geoffroy Saint-Hilaire, 1831)

Type locality: Cochinchine, a region in southern Vietnam; based on the mounted skin of an adult male collected by P. M. Diard in June 1822; exact place of collection unknown.

Subspecies: None.

Distribution: S and SE Asia, NE India S and E of the Brahmaputra River (Assam, Arunachal Pradesh, Meghalaya, Nagaland, Manipur, Mizoram and Tripura states), SW China S of 25°N (Yunnan, Guizhou, Guangxi and Guangdong Provinces), N Myanmar, Bangladesh, Thailand, Laos, Cambodia, Vietnam and N Peninsula Malaysia where it could be a recent colonizer dispersed from S Thailand as recently as 1959; it may be now extinct in Bangladesh where it was last recorded in 1982 and 1989. Known records are, at present, concentrated along N and S margins of its distribution, and it is rare or absent in C Indochinese peninsula, where deciduous forests predominate.

Conservation status: Vulnerable.

Long-tailed Macaque *Macaca fascicularis* (Raffles, 1821)

Type locality: Indonesia, Sumatra (Bengkulu).

Subspecies: 10 subspecies are recognized:

Common Long-tailed Macaque, *M. f. fascicularis* (Raffles, 1821);

Dark-crowned Long-tailed Macaque, *M. f. atriceps* Kloss, 1919;

Burmese Long-tailed Macaque, *M. f. aureus* É. Geoffroy Saint-Hilaire, 1831; Con Song Long-tailed Macaque, *M. f. condorensis* Kloss, 1926;

Simeulue Long-tailed Macaque, *M. f. fuscus* (G. S. Miller, 1903);

Kemujan Long-tailed Macaque, *M. f. karimondjawae* Sody, 1949;

Lasia Long-tailed Macaque, *M. f. lasiae* (Lyon, 1916);

Philippine Long-tailed Macaque, *M. f. philippinensis* (I. Geoffroy Saint-Hilaire, 1843);

Maratua Long-tailed Macaque, *M. f. tua* Kellogg, 1944;

Nicobar Long-tailed Macaque, *M. f. umbrosus* (G. S. Miller, 1902).

Distribution:

M. f. fascicularis: S Laos, S Vietnam, Cambodia, E and S Thailand (and offshore islands), S to the Malay Peninsula, Borneo, Sumatra, Java, Bali and most but not all offshore islands, also extending into the Sulu Archipelago and Zamboanga Peninsula of W Mindanao in the Philippines; probably artificially introduced in the Nusa Penida-Timor Islands chain;

M. f. atriceps: Ko Khram Island (Khram Yai), off SE coast of Thailand; *M. f. aureus*: SW Bangladesh (Teknaaf Peninsula), S Myanmar (including the Mergui Archipelago), WC Thailand (S to c.10°N) and Laos;

M. f. condorensis: SE Vietnam (Con Son and Hon Ba Islands in the East Sea);

M. f. fuscus: Simeulue Island, off NW Sumatra;

M. f. karimondjawae: Karimunjawa Island and presumably nearby Kemujan Island, N of Java;

M. f. lasiae: Lasia Island, off NW Sumatra;

M. f. philippinensis: Philippine Archipelago N of c.10°N;

M. f. tua: Maratua Island, off E Borneo;

M. f. umbrosus: Nicobar Islands (Katchall, Little Nicobar and Great Nicobar islands).

Conservation status: Least Concern;

M. f. fascicularis Least Concern;

M. f. condorensis, *M. f. umbrosus* Vulnerable; *M. f. philippinensis* Near Threatened;

M. f. atriceps, *M. f. aureus*, *M. f. fuscus*, *M. f. karimondjawae*, *M. f. lasiae*, *M. f. tua* Data Deficient.

Rhesus Macaque *Macaca mulatta* (Zimmermann, 1780)

Type locality: India. Based solely on Tawny Monkey, a menagerie animal (presumably observed in London), not preserved, identified by Pennant in 1771. Restricted by R. Pocock in 1932 to "Nepal Tarai" (Terai), the narrow plain that extends along the southern border of Nepal.

Subspecies: Due to missing data provisionally none.

Distribution: S and SE Asia from c. 36° N (in Afghanistan, Pakistan, India and China) S to c.15° N (in India, Thailand, Laos and Vietnam), also in Nepal, Bhutan, Bangladesh, Myanmar and Hainan Island. An isolated population at 40°24'N in NE China was extirpated in 1987.

Conservation status: Least Concern.

Genus *Trachypithecus*

Shortridge's Langur *Trachypithecus shortridgei* (Wroughton, 1915)

Type locality: Burma, Homalin, upper Chindwin.

Subspecies: None.

Distribution: NE Myanmar, E of the Chindwin River (Kachin State N to Myitkyina District) and SW China (Dulong River Valley in Gongshan County, NW Yunnan Province).

Conservation status: Endangered.

Germain's Langur *Trachypithecus germaini* (Milne-Edwards, 1876)

Type locality: Cochin China and Cambodia.

Subspecies: None.

Distribution: Mainland SE Asia in S Myanmar, S Thailand, S Laos, Cambodia (W of Mekong River), and the S tip of Vietnam; Mekong River as E limit not yet confirmed.

Conservation status: Endangered, but assessment included T. margarita.

Annamese Langur *Trachypithecus margarita* (Elliot, 1909)

Type locality: Vietnam, Langbian.

Subspecies: None.

Distribution: S Laos (N limit is 16°23'N), SC Vietnam (N limit is 14°30'N), and E Cambodia (Ratanakiri and Mondulkiri Provinces); most likely the W limit is the Mekong River, but further studies are needed to confirm this. Records in Vietnam N to 16°37'N are questionable.

Conservation status: Endangered (but as a synonym of *T. germaini*).

Phayre's Langur *Trachypithecus phayrei* (Blyth, 1847)

Type locality: Burma, Arakan.

Subspecies: 2 subspecies are recognized;

Phayre's Langur: *T. p. phayrei* (Blyth, 1847);

Shan States Langur: *T. p. shanicus* (Wroughton, 1917).

Distribution:

T. p. phayrei: E Bangladesh, NE India (Assam, Mizoram and Tripura states), and W Myanmar (SE through Arakan to Pegu);

T. p. shanicus: SW China (Yingjiang-Namting River and Tunchong-Homushu Pass districts in W Yunnan Province), and N and E Myanmar (Shan States and neighbouring dry zone of N Myanmar).

Conservation status: Endangered (both subspecies).

Indochinese Gray Langur *Trachypithecus crepusculus* (Elliot, 1909)

Type locality: Burma (Myanmar), Mt Muleiyit, 1500 m.

Subspecies: None.

Distribution: SW China (E of Salween River and S of Xishuangbanna, S Yunnan Province), S Myanmar, N Thailand (S to Raheng, and W to the coast of the Bay of Bengal), N and C Laos, and N Vietnam, the W limit is most likely the Salween River; S of the distribution of Phayre's Langur (T. phayrei).

Conservation status: Endangered (as T. phayrei crepuscula).

François' Langur *Trachypithecus francoisi* (Pousarges, 1898)

Type locality: Longzhou, China.

Subspecies: None.

Distribution: SC China (Chongqing, Guizhou and Guangxi Provinces) and N Vietnam (Ha Giang, Cao Bang, Tuyen Quang, Bac Kan and Thai Nguyen Provinces).

Conservation status: Endangered.

Cat Ba Langur *Trachypithecus poliocephalus* (Trouessart, 1911)

Type locality: The type locality is supposed to be the village Cai Kien (21°19'N, 107°44'E) or the Cai Kinh limestone massif (21°45'N, 107°30'E).

Subspecies: None.

Distribution: Cat Ba Island, Ha Long Bay, off N Vietnam.

Conservation status: Critically Endangered (as T. poliocephalus poliocephalus).

White-headed Langur *Trachypithecus leucocephalus* Tan, 1957

Type locality: Fusui County, Guangxi, China.

Subspecies: None.

Distribution: S China, restricted to Fusui, Chongzuo Ningming and Longzhou Counties, SW Guangxi Autonomous Region; it is bordered on NW and N by the Zuojiang River, in the SW and S by the Mingjiang River, and to the SE by the Shiwan Mts.

Conservation status: Critically Endangered (as T. poliocephalus leucocephalus).

Delacour's Langur *Trachypithecus delacouri* (Osgood, 1932)

Type locality: Hoi Xuan, Vietnam.

Subspecies: None.

Distribution: NC Vietnam (Hoa Binh, Ha Nam, Ninh Binh and Thanh Hoa Provinces).

Conservation status: Critically Endangered.

Laos Langur *Trachypithecus laotum* (Thomas, 1921)

Type locality: Ban Na Sao, SW French Laos, on the French side of the Mekong, in latitude about 17°30'N.

Subspecies: None.

Distribution: WC Laos, from S Bolikhamsai Province, probably with the Nam Kading River as N barrier of the distribution, S to C Khammouane Province, at c.17°40'N; there are also reports from the corridor to Nakai-Nam-Theun National Protected Area, and from Khamkeut District, close to Lak Sao (18°13'N, 104°47'E).

Conservation status: Vulnerable.

Hatinh Langur *Trachypithecus hatinhensis* (Dao Van Tien, 1970)

Type locality: Xom Cuc (17°56' N, 105°47' E), Ha Tinh Province (now Tuyen Hoa District, Quang Binh Province), Vietnam.

Subspecies: None.

Distribution: NC Vietnam (Quang Binh and Quang Tri Provinces), and EC Laos (Khammouane Province). The records in other Vietnamese provinces (Nghe An, Thu Thien Hue, Gia Lai, Phu Yen) are errors.

Conservation status: Endangered.

Black Langur *Trachypithecus ebenus* (Brandon-Jones, 1995)

Type locality: “Indo China”: probably either Lai Chau or Fan Si Pan mountain chain (c.22°30'N, 103°50'E), Vietnam.

Subspecies: None.

Distribution: EC Laos, in S Khammouane Province, close to the border with Vietnam and in scattered limestone blocks to the S into N Savannakhet Province to c.16°50' N, the species is also recorded in C Vietnam, Quang Binh Province (17°59'N, 105°40'E).

Conservation status: Endangered (as synonym of *T. hatinhensis*).

Genus *Semnopithecus*

Nepal Sacred Langur *Semnopithecus schistaceus* Hodgson, 1840

Type locality: Nepal.

Subspecies: None.

Distribution: NW Pakistan, N India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, and NW Bengal states, and Sikkim), S China (Tibetan regions of Bo Qu, Ji Long Zang Bu and Chumbi Valleys in Xizang Autonomous Region), Nepal, and W Bhutan (E to Sankosh River); its presence in E Afghanistan is uncertain.

Conservation status: Least Concern.

Genus *Rhinopithecus*

Tonkin Snub-nosed Monkey *Rhinopithecus avunculus* Dollman, 1912

Type locality: Yen Bay, Songkoi River, Vietnam.

Subspecies: None.

Distribution: NW Vietnam, known only from small forest patches in Ha Giang, Tuyen Quang, Bac Kan, and Thai Nguyen Provinces.

Conservation status: Critically Endangered.

Yunnan Snub-nosed Monkey *Rhinopithecus bieti* Milne-Edwards, 1897

Type locality: China, Yunnan, left bank of upper Mekong, Kiape, a day’s journey South of Atuntze (28°25'N, 98°55'E).

Subspecies: None.

Distribution: SW China, in SE Xizang Autonomous Region (Tibet) and NW Yunnan Province (fragmented populations in the Yun Ling Mts), W of the Yangtze River and E of the Mekong River.

Conservation status: Endangered.

Guizhou Snub-nosed Monkey *Rhinopithecus brelichi* Thomas, 1903

Type locality: Van Gin Shan (Fanjingshan), Guizhou Province, China.

Subspecies: None.

Distribution: SC China, Guizhou Province, Fanjingshan in the Wuling Mts.

Conservation status: Endangered.

Golden Snub-nosed Monkey *Rhinopithecus roxellana* (Milne-Edwards, 1870)

Type locality: China, Sichuan, near Moupin (Baoxing) (30°26'N, 102°50'E).

Subspecies: 3 subspecies are recognized;

Moupin Golden Snub-nosed Monkey: *R. r. roxellana* (Milne-Edwards, 1870);

Hubei Golden Snub-nosed Monkey: *R. r. hubeiensis* Wang et al., 1998;

Qinling Golden Snub-nosed Monkey: *R. r. qinlingensis* Wang et al., 1998.

Distribution:

R. r. roxellana: WC China (S Gansu, S Shaanxi, W Sichuan Provinces);

R. r. hubeiensis: WC China (Shennongjia in W Hubei Province and in NW Sichuan Province);

R. r. qinlingensis: WC China (Qinling Mts, S Shaanxi Province).

Conservation status: Endangered (all three subspecies).

Stryker's Snub-nosed Monkey *Rhinopithecus strykeri* Geissmann et al., 2011

Type locality: 26.43101°N, 98.38894°E (2815 m), Maw River area, NE Kachin State, NE Myanmar.

Subspecies: None.

Distribution: NE Myanmar (Salween–N'mai Hka divide in NE Kachin State, only around the Maw River, as far E as the mountains above the village of Chichitago, 26.31°–26.51°N and 98.34°–98.61°E) and S China (Gaoligongshan Nature Reserve, Yunnan Province).

Conservation status: Critically Endangered.

Genus *Pygathrix*

Gray-shanked Douc *Pygathrix cinerea* Nadler, 1997

Type locality: Vietnam, Gia Lai Province, Play Ku.

Subspecies: None.

Distribution: C Vietnam between 16°N and 13°38'N (Quang Nam, Kon Tum, Quang Ngai, Gia Lai, and Binh Dinh provinces), and a small part of NE Cambodia. Probably also S Laos.

Conservation status: Critically Endangered.

Red-shanked Douc *Pygathrix nemaeus* (Linnaeus, 1771)

Type locality: Vietnam, "Cochin-China".

Subspecies: None.

Distribution: EC and SE Laos, N and C Vietnam (but very fragmented), and a small area in NE Cambodia (Voensei, Ratanakiri Province).

Conservation status: Endangered.

Black-shanked Douc *Pygathrix nigripes* (Milne-Edwards, 1871)

Type locality: Vietnam, Saigon (Ho Chi Minh City).

Subspecies: None.

Distribution: E Cambodia (E of the Mekong River and S of the Srepok River) and SW Vietnam from c.14°N to S Cat Tien National Park. The presence of this species in S Laos is suspected but not confirmed.

Conservation status: Endangered.

Family Hylobatidae

Genus *Hoolock*

Western Hoolock Gibbon *Hoolock hoolock* (Harlan, 1834)

Type locality: India, Garo Hills, Assam.

Subspecies: None.

Distribution: Bangladesh and NE India (states of Assam, Arunachal Pradesh, Nagaland, Meghalaya, Manipur, Mizoram and Tripura) between the Brahmaputra and Salween rivers, and to the S of the Brahmaputra and E of the Dibang Rivers, extending into NW Myanmar, W of the Chindwin River. The identity of a population in the Medog Nature Reserve in SE Tibet, across the border from Arunachal Pradesh, is unknown.

Conservation status: Endangered.

Eastern Hoolock Gibbon *Hoolock leuconedys* (Groves, 1967)

Type locality: Burma (Myanmar), Sumprabum, 1200 m.

Subspecies: None.

Distribution: NE India, marginally in the states of Arunachal Pradesh (Lohit and Lower Dibang Valley Districts) and Assam (Tinsukia District), E Myanmar (E of the Chindwin River), and SW China in W Yunnan Province.

Conservation status: Vulnerable.

Genus *Hylobates*

Pileated Gibbon *Hylobates pileatus* Gray, 1861

Type locality: Cambodia.

Subspecies: None.

Distribution: SE Thailand (E and S of the Mun and Takhong rivers, W limit may have formerly been the Bang Pakong River), SW Laos (W of the Mekong River), and N and W Cambodia (W of the Mekong River); may have formerly occurred in S Vietnam (W of the Mekong River).

Conservation status: Endangered.

Lar Gibbon *Hylobates lar* (Linnaeus, 1771)

Type locality: None. Malaysia, Malacca (restricted by C. Kloss in 1929).

Subspecies: 5 subspecies are recognized;

Malaysian Lar Gibbon, *H. l. lar* (Linnaeus, 1771);

Carpenter's Lar Gibbon, *H. l. carpenteri* Groves, 1968;

Central Lar Gibbon, *H. l. entelloides* I. Geoffroy Saint-Hilaire, 1842;

Sumatran Lar Gibbon, *H. l. vestitus* G. S. Miller, 1942;

Yunnan Lar Gibbon, *H. l. yunnanensis* Ma & Wang, 1986.

Distribution:

H. l. lar: Malay Peninsula, from 9°N to the Mudah River and S of the Perak River;

H. l. carpenteri, E Myanmar, NW Laos, and NW Thailand, from Chieng Dao at 19°22'N to c.16°N;

H. l. entelloides, S Myanmar and SW Thailand, from c.15°N to 10°N;

H. l. vestitus, N Sumatra, NW of Lake Toba and the Singkil River;

H. l. yunnanensis, SW China (SW Yunnan Province), originally between the Salween and Mekong Rivers in the counties of Cangyuan, Menglian and Ximeng, but probably now extinct.

Conservation status: *H. l. lar*, *H. l. vestitus*, *H. l. carpenteri* Endangered; *H. l. entelloides* Vulnerable; *H. l. yunnanensis* Data Deficient.

Genus *Nomascus*

Hainan Crested Gibbon *Nomascus hainanus* (Thomas, 1892)

Type locality: China, Hainan.

Subspecies: None.

Distribution: China, Hainan Island. Formerly widespread, but today restricted to Bawangling Nature Reserve in the W of the island.

Conservation status: Critically Endangered.

Eastern Black Crested Gibbon *Nomascus nasutus* (Künckel d'Herculais, 1884)

Type locality: Vietnam, Along (Halong Bay).

Subspecies: None.

Distribution: Formerly S China to N Vietnam as far S and W as the Red River, but now restricted to a small area along the Sino-Vietnamese border in Trung Khanh District (Cao Bang Province, NE Vietnam) and Jingxi County (Guangxi Autonomous Region, SW China).

Conservation status: Critically Endangered.

Western Black Crested Gibbon *Nomascus concolor* (Harlan, 1826)

Type locality: Vietnam, Tonkin.

Subspecies: 2 subspecies are recognized;

Tonkin Black Crested Gibbon, *N. c. concolor* (Harlan, 1826);

Laotian Black Crested Gibbon, *N. c. lu* (Delacour, 1951).

Distribution:

N. c. concolor: SW China (C & SW Yunnan Province) including a small population W of the Mekong River near the Myanmar border, and N Vietnam (Lao Cai, Son La, and Yen Bai

Provinces) between the Black and Red Rivers from c.20°N to 23°45'N;

N. c. lu: NW Laos (Bokeo and Luang Namtha Provinces) in an isolated population E of the Mekong River at c.20°17'N–20°25'N.

Conservation status: Critically Endangered (both subspecies).

Northern White-cheeked Crested Gibbon *Nomascus leucogenys* (Ogilby, 1841)

Type locality: Siam (restricted by J. Fooden in 1987 to Laos, Muang Khi).

Subspecies: None.

Distribution: S China (extreme S Yunnan Province), N Laos, and NW Vietnam between the Mekong and Black Rivers, to the S possibly limited by the Khading River in Laos and the Rao Nay River in Ha Tinh Province in Vietnam.

Conservation status: Critically Endangered.

Southern White-cheeked Crested Gibbon *Nomascus siki* (Delacour, 1951)

Type locality: Vietnam, Thua Luu.

Subspecies: None.

Distribution: C Laos and C Vietnam, restricted by the Rao Nay River in Vietnam and the Khading River in Laos in the N, the Mekong River in the W, the Vietnamese coastal agricultural areas in the E, and around the Thach Han River (16°40'–16°50'N) in Quang Tri Province, Vietnam, and the Savannakhet Province in Laos, to the S.

Conservation status: Endangered.

Northern Yellow-cheeked Crested Gibbon *Nomascus annamensis* Van Ngoc Thinh et al., 2010

Type locality: Vietnam, Ja Boc, Sa Thay District, Kon Tum Province (c.14°25'N, 107°35'E, Chu Mom Ray National Park).

Subspecies: None.

Distribution: S Laos (E of Mekong River, up to approximately the Banhiang River, 16°00'–16°03'N, in Savannakhet Province), C Vietnam (from the Thach Han River, 16°40'–16°50'N, in Quang Tri Province in the N to approximately the Ba River, 13°00'–13°10'N, in Gia Lai and Phu Yen Provinces in the S), and NE Cambodia (Ratanakiri and Stung Treng Provinces, E of the Mekong River and N of the Srepok River, 13°30'N).

Conservation status: Not Evaluated.

Southern Yellow-cheeked Crested Gibbon *Nomascus gabriellae* (Thomas, 1909)

Type locality: Vietnam, Langbian, 460 m.

Subspecies: None.

Distribution: S Vietnam (from Ba River, 13°00'–13°10'N, in Gia Lai and Phu Yen Provinces in the N to the Nui Ong Nature Reserve, 11°01'N, in Binh Thuan Province in the S) and SE Cambodia (E of the Mekong River and S of the Srepok River).

Conservation status: Endangered.

Acknowledgements

The following are the authors who contributed to *The Handbook of the Mammals of the World*, Volume 3, providing the family overviews and species accounts for the primates in Vietnam, Laos, Cambodia and China.

Family LORISIDAE: K. Anna I. Nekaris;

Family CERCOPITHECIDAE: Dietmar Zinner, Gisela Fickenscher and Christian Roos.

Species accounts: Martina V. Anandam, Ardith A. Eudey, Colin P. Groves, Sanjay Molur, Tilo Nadler, Matthew C. Richardson, Christian Roos and Lori K. Sheeran.

Family HYLOBATIDAE: David J. Chivers.

Species accounts: Martina V. Anandam, Sanjay Molur, Colin P. Groves, Benjamin M. Rawson, Matthew C. Richardson and Christian Roos.

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Vietnamese Journal of Primatology (2013) vol. 2(2), 27-35

A survey to evaluate public opinion about the reintroduction of the 'Critically Endangered' Delacour's langur (*Trachypithecus delacouri*) in Van Long Nature Reserve, Ninh Binh Province, Vietnam

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Key words: Delacour's langur, *Trachypithecus delacouri*, survey, conservation awareness, Van Long Nature Reserve.

Summary

In November 2012 two individuals of the critically endangered Delacour's langur (*Trachypithecus delacouri*) were released into Van Long Nature Reserve, northern Vietnam. In parallel to the monitoring we carried out a survey in order to evaluate the public opinion and conservation awareness around the reintroduction project. From 22nd January 2013 to 2nd February 2013 we randomly interviewed 50 local residents in and around the reserve, not taking into account any personal characteristics as gender, age or profession.

Respondents were mostly unaware about the fact that the Delacour's langur is an endemic species and about half of all respondents could not estimate the approximate amount of individuals existing in Van Long Nature Reserve and in total for Vietnam. However, it is remarkable that local residents are characterized by a high level of conservation awareness. Almost half of all respondents were informed about the reintroduction and 48 out of 50 respondents knew about the conservation work of Frankfurt Zoological Society (FZS) in cooperation with the Management Board of Van Long Nature Reserve and the Forest Protection Department of Ninh Binh Province. All respondents were speaking against poaching. They confirmed that primates are not hunted in Van Long Nature Reserve for a very long time and need to be protected in the long run, further improvements were recommended.

All in all, the outcomes of the survey confirm that the area is highly suitable for further reintroductions due to elevated awareness of the local residents and therefore, conservation measures are well on their way in Van Long Nature Reserve.

Kết quả khảo sát ý kiến của cộng đồng về việc tái hòa nhập loài linh trưởng cực kỳ nguy cấp, voọc Mông Trắng (*Trachypithecus delacouri*) tại khu bảo tồn thiên nhiên Vân Long, Tỉnh Ninh Bình, Việt Nam

Tóm tắt

Hai cá thể của loài linh trưởng cực kỳ nguy cấp, voọc Mông Trắng được tái thả vào khu bảo tồn thiên nhiên Vân Long, tháng 11 năm 2012. Song song với chương trình giám sát chúng tôi thực hiện một khảo sát đánh giá ý kiến của cộng đồng về dự án tái thả. Từ ngày 22 tháng 1 đến 2 tháng 2 năm 2013, chúng tôi đã phỏng vấn ngẫu nhiên 50 người dân địa phương trong và xung quanh khu bảo tồn. Khi phỏng vấn chúng tôi không quan tâm đến yếu tố cá nhân như giới tính, độ tuổi và nghề nghiệp. Hầu hết những phản hồi của người dân đều không biết loài Voọc Mông Trắng là loài đặc hữu và hơn một nửa không thể ước lượng được số lượng cá thể của loài linh trưởng này ở khu BTTN Vân Long và ở Việt Nam. Tuy nhiên, người địa phương lại có sự nhận thức rất tốt về việc bảo tồn loài linh trưởng này. Gần một nửa số người được hỏi biết về chương trình tái thả voọc, và 48 trên 50 người được hỏi biết về chương trình hợp tác giữa hội động vật học Frankfurt (FZS) và khu bảo tồn thiên nhiên Vân Long, và chi cục kiểm lâm Ninh Bình. Hầu hết những người được hỏi đều phản đối việc sắn bắn. Họ cũng khẳng định các loài linh trưởng không bị săn bắn trong khu bảo tồn thiên nhiên Vân Long thời gian vừa qua. Họ cũng đề xuất việc bảo vệ cần được cải thiện tốt hơn và loài voọc Mông Trắng cần được bảo vệ lâu dài. Kết quả của khảo sát khẳng định khu vực tái thả rất thích hợp cho các hoạt động tiếp theo của dự án, bởi vì ý thức của cộng đồng địa phương đã được nâng cao. Các giải pháp bảo tồn đang thực sự có hiệu quả tại khu bảo tồn thiên nhiên Vân Long.

Introduction

The Delacour´s langur (*Trachypithecus delacouri*) is defined as “Critically Endangered” (IUCN-Red List of Threatened Species, 2012) and also listed as one of “The 25 Most Endangered Primates in the World” (Mittermeier et al., 2012) and thus facing an extremely high risk of becoming extinct in the 21st century. As an endemic species to Vietnam, the Delacour´s langurs’ occurrence is restricted to the northern area between 20°-21° N and 105°-106° E closely related to limestone mountain ranges (Nadler et al., 2003; 2010). The species is threatened by hunting pressure, habitat destruction and fragmentation due to a small population size (Nadler, 2012).

Within the framework of the “Vietnam Primate Conservation Program”, started in 1993 by the Frankfurt Zoological Society (FZS), the occurrence of the langur was confirmed for Van Long Nature Reserve (VLNR), Ninh Binh Province. The reserve was established in 2001 and since then, FZS supports the Management Board of the reserve in close collaboration, e.g. providing funds for salaries and equipment and building ranger stations (Nadler, 2011). Those efforts resulted in recognizing the Delacour´s langur as a flagship species and, due to a strict protection program, the population in the reserve increases steadily. The population in VLNR comprised roughly 100 individuals in 2011 (Ebenau et al., 2011; Nadler, 2010) and increased to 110-120 individuals in 2013 (Nadler, pers. comm.) constituting about 50 % of the world’s total population (Mai Dinh Yen et al., 2010; Nadler, 2010).

Beneath the captive breeding program of the Endangered Primate Rescue Center (EPRC), it is further recommended in the Biodiversity Action Plan for Vietnam (Government of the Socialist Republic of Vietnam & Global Environment Facility Project, 2004) to reintroduce individuals from the breeding program into suitable habitats in order to stabilize the wild population. Out of all areas with Delacour´s langurs’ natural occurrence, VLNR was identified to provide the highest chances for a long-term existence (Nadler, 2004; 2012; Workman, 2010). In August 2011, three captive born individuals were introduced in the reserve for the first time ever, followed by an introduction of two individuals in November 2012.

Background for the survey

Following the Guidelines for Reintroduction of Non-human Primates (Baker, 2002), it is necessary to carry out public relations activities within a reinforcement project, especially conservation education and awareness and to assess their impact subsequently. Accordingly, local communities need to be integrated into conservation projects and the local residents have to be informed. An evaluation of attitudes to the project is necessary to contribute to the success of the reintroduction projects.

With regard to this recommendation, a survey was carried out in order to get an overview of local people's knowledge and acceptance of primates in VLNR. With the first release of two individuals in August 2011 and the second in November 2012, a survey of this kind was performed for the very first time in VLNR, and also most probably the first time in Vietnam at all. The survey was performed in course of a diploma thesis with the main focus on the radio collar monitoring of the two released Delacour's langurs in 2012.

Material & Methods

Human impact in Van Long Nature Reserve

VLNR is the largest wetland reserve in the northern Red River Delta area, surrounded by human-dominated, intensively cultivated landscape. 1.700 people reside inside the core zone of the reserve, mostly living from farming. The total human population of the seven communes surrounding VLNR is 47,949 people (Mai Dinh Yen et al., 2010). Economically, the region is not developed and the people are poor. However, the tourism sector has developed quickly during the last decade. Before any efforts were made to protect the area, people regularly entered the reserve collecting firewood as well as hunting big mammals (Workman, 2010). Local use and management of the wetland and the limestone forest have been ongoing for centuries, but nowadays hunting and collection of timber is banned (Nguyen Thi Dao, 2008).

Performing the survey

Over a period of 12 days, from 22nd January 2013 to 2nd February 2013, we randomly interviewed 50 local people around and inside of Van Long Nature Reserve, e.g. on the road, working on rice fields, in corner shops or at their home. We did not take into account any personal characteristics as gender or age, although we avoided questioning children and teenagers. Most days we combined the survey with the monitoring of the two released individuals and therefore interviewed people early in the morning or in the afternoon.

The interviews were conducted in Vietnamese by the Vietnamese biologist Nguyen Hong Chung (Fig. 1). The questionnaires were filled in by him during the interviews and were later translated into English. The question style varied between open-ended questions (respondent formulates his own answer) and closed-ended questions (yes/no questions and questions with grouping where answers are predetermined). A system of categories was developed to sort the answers to open-ended questions, where multiple answers were possible.

Fig.1. Biologist Nguyen Hong Chung (right) interviewing a local man while tending cattle. Photo: Sarah Elser.

Results and Discussion

All 50 respondents knew about the existence of the Delacour's langur in Van Long Nature Reserve (Fig. 2), whereas most of them, namely 47 people, have actually seen them with their own eyes (Fig. 3). With regard to macaques, 43 people knew about their existence, but only 34 ever saw one. The fact that only respondents who saw a loris knew that they exist in that area is striking and indicates a low level of awareness for this nocturnal primate (Fig. 2 and 3).

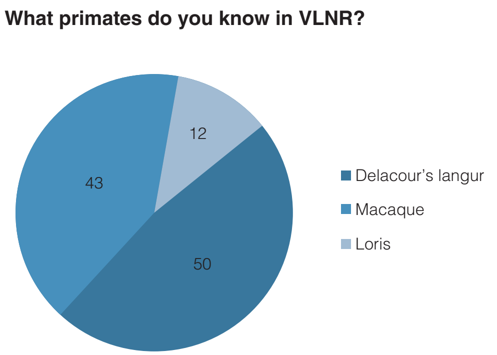

Fig.2. (Question 1) Amount of respondents who know named primates in VLNR. 50 people questioned/multiple replies possible.

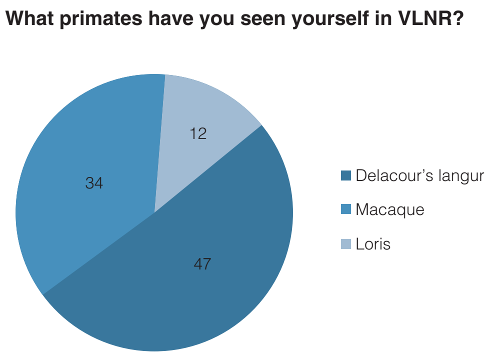

Fig.3. (Question 2) Amount of respondents who have seen named primates in VLNR. 50 people questioned/multiple replies possible.

Although we showed the respondents pictures of different primate species, they were unable to distinguish and identify different macaque and loris species from each other. In many cases the respondents were also troubled to identify a Delacour's langur if a picture showed an individual in a juvenile stage with cream-colored trousers and not a characteristic white coloration like in the adult stage. To distinguish a representative of the pygmy loris (*Nycticebus pygmaeus*) from a representative of the larger northern slow loris (*Nycticebus bengalensis*) requires expert-knowledge and experience as well as good binoculars. This also applies for the different species of the genus *Macaca*, especially in the dense vegetation existing in VLNR.

Question 3 and 4 aimed to find out if people know other places of Delacour's langurs' occurrence than VLNR and if they are aware of the fact that it is an endemic species to northern Vietnam.

With one exception all respondents referred to Van Long (Fig. 4). Six people named Dong Tam, Hoa Binh, an area immediately adjacent to VLNR which is planned to become its extended area. Another six people were aware of a relic population in Cuc Phuong

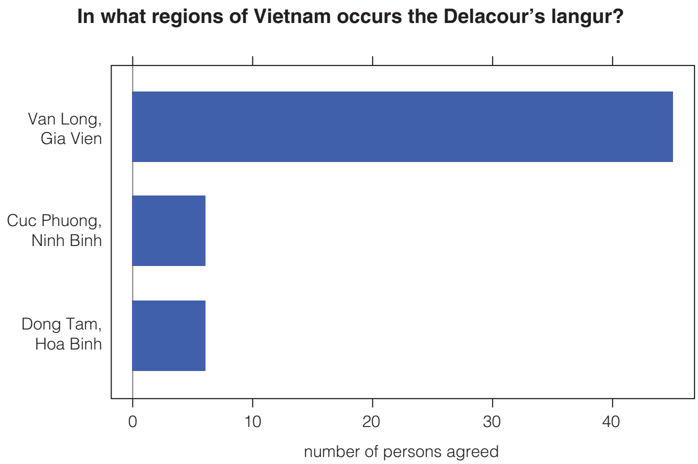

Fig.4. (Question 3) Regions where the Delacour's langur occurs according to local residents. 50 people questioned/multiple replies possible.

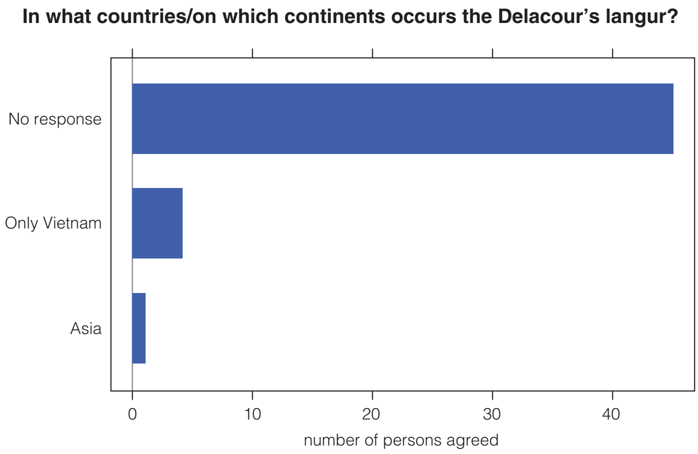

Fig.5. (Question 4) Countries/continents where the Delacour's langur occurs according to local residents. 50 people questioned/ multiple replies possible.

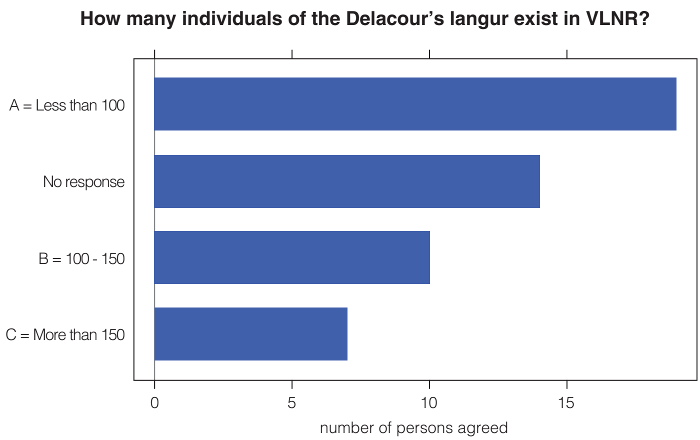

Fig.6. (Question 5) Amount of individuals of the Delacour's langur existing in VLNR according to local residents. 50 people questioned.

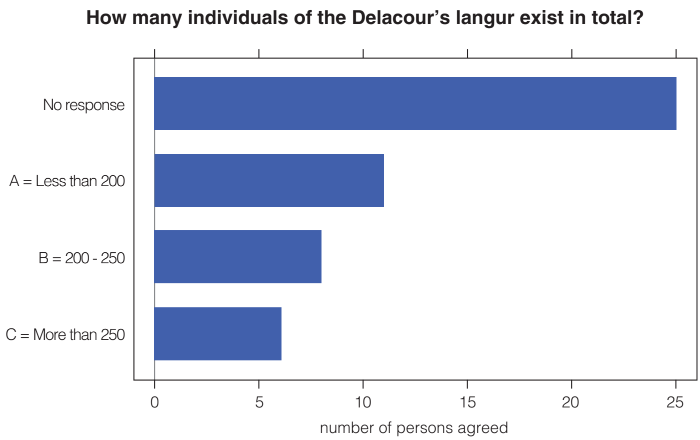

Fig.7. (Question 6) Amount of individuals of the Delacour's langur existing in total according to local residents. 50 people questioned.

National Park, 35 km west from VLNR, the location of the Endangered Primate Rescue Center.

Only four people were aware that the Delacour's langur is an endemic species to Vietnam; 45 people were unable or unwilling to respond (Fig. 5).

The results of question 5 (Fig. 6) about an estimate of the population size of the Delacour's langur are striking: Altogether 29 people selected option A and option B, declaring that less than 150 individuals live in VLNR (matching exactly the current population size of 110-120 individuals in total VLNR in 2013) (Nadler, pers. comm.). Still, 14 people refused to settle on a given option.

In contrast to the previous question, a lot more respondents were unwilling to settle for an option, namely 25 people, 50 % of all respondents (Fig. 7). Nevertheless, 19 respondents (option A and B) were right with the assumption that no more than 250 individuals of the Delacour's langur exist in total. Generally, it was quite difficult to elicit a response from the local people to questions 5 and 6. Without offering categories to them, nearly everybody refused to suggest a number, probably out of concern to give a wrong answer. After interviewing 15 people without receiving an answer, we decided to offer categories to the last 35 people. Due to a lack of time we were unable to start the survey all over again.

Question 7 and 8 refer to the success rate of former and current public relations around the Delacour's langur and the awareness of two reintroductions of overall five individuals of the Delacour's langur in 2011 and 2012. All in all, 48 people named either "rangers" or "guards" or both together as people that make efforts to protect the langurs (Fig. 8). The expression "rangers" refers to employees of the Forest Protection Department of Ninh Binh Province, whereas "guards" are employees of the Management Board of Van Long Nature Reserve sponsored by FZS. Although this is a fundamental difference, we cannot inevitably presume that all respondents are aware of that fact. Five people named "rangers" as well as "commune" whereby, strictly speaking, both expressions refer presumably to the Forest Protection Department of the province. Nine respondents referred to Tilo Nadler, director of the EPRC, or Frankfurt Zoological Society. All in all, 104 entries were made by 50 respondents, indicating a high level of conservation awareness.

Question 8 (Fig. 9) revealed that 23 out of 50 respondents were aware of the reintroduction program of the Endangered Primate Rescue Center. Six respondents even stated the amount of released individuals and the month and year of the particular release whereas 22 respondents were unaware of the performed introductions.

Being one of the most relevant questions for us, we asked 50 respondents if primates are still hunted in Van Long Nature Reserve. All respondents negated the question immediately and emphatically. Nine people stated that there was no hunting in VLNR in a long time ranging from 3 up to 15 years ago. Four times it was stated that small animals and three times that birds are still being hunted with traps in VLNR. Two respondents indicated that hunters with guns are still seen on the weekends. From our own experience we can confirm that

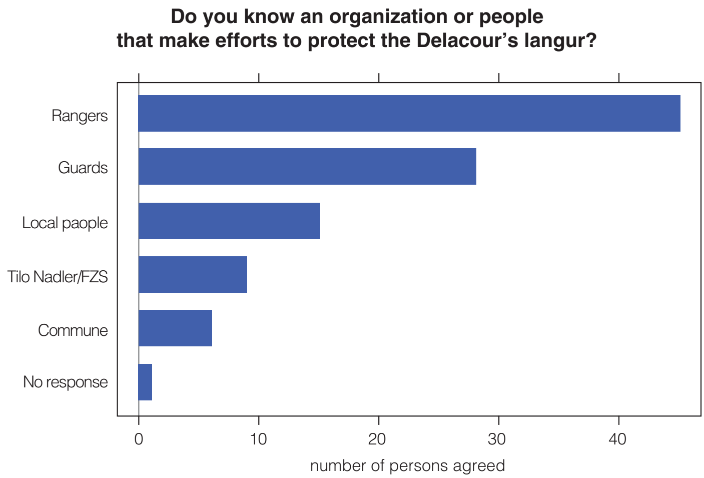

Fig.8. (Question 7) Organizations or people known by local residents. 50 people questioned/ multiple replies possible.

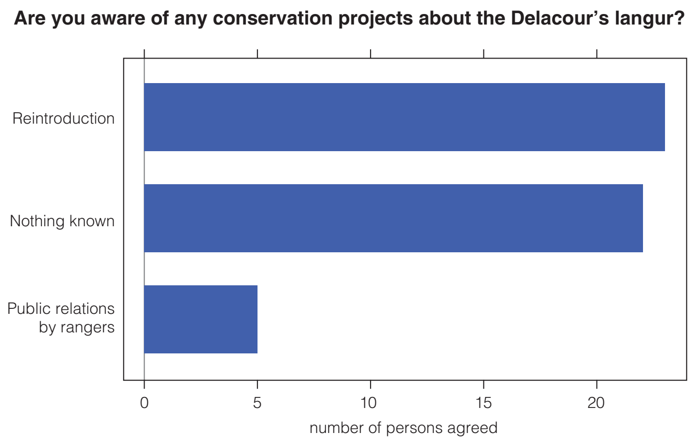

Fig.9. (Question 8) Awareness of local residents about conservation projects. 50 people questioned/ multiple replies possible.

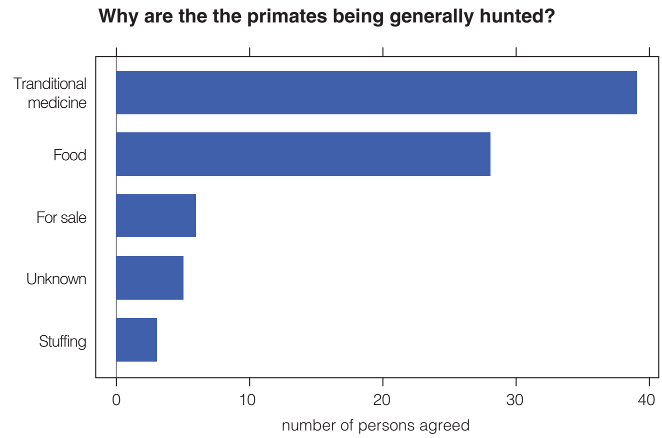

Fig.10. (Question 10) Why primates are being hunted generally according to local residents. 50 people questioned/ multiple replies possible.

bird hunting, although forbidden, is still a common practice in VLNR.

That "Traditional medicine" is the biggest reason why primates are being generally hunted stated by 39 respondents (Fig. 10). "Food" is considered to be the second most common cause for primate hunting.

Some people described how the animals are processed for traditional medicine: the flesh is eaten, bones are boiled for several days and afterwards the brew which has originated is consumed. The traditional belief is that health is improved and life is prolonged.

On the contrary, one respondent explained that eating primate flesh might bring bad luck and one declared that primates look similar to humans and for this reason are eaten reluctantly. All in all, 50 respondents gave 81 answers.

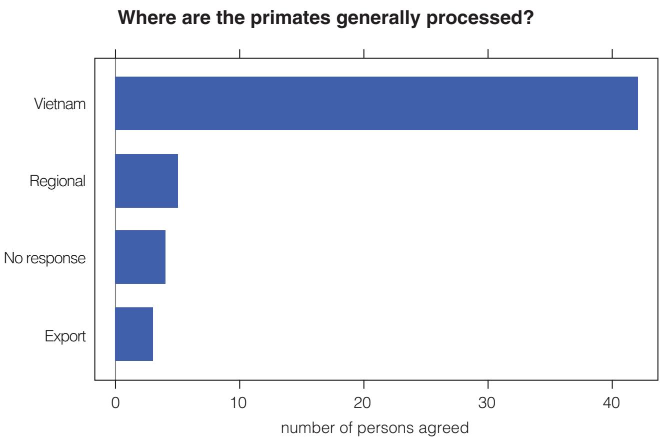

Fig.11. (Question 11) Where primates are generally processed according to local residents. 50 people questioned/ multiple replies possible.

Question 11 (Fig. 11) aimed to gain information about the place of primate processing. 42 respondents stated "Vietnam", indicating that also transport of hunted primates takes place within the country. However, it can be assumed that "Vietnam" is a generic term and that primates are mostly processed where they are hunted, accordingly "regional". Only 5 respondents stated that the processing takes place regionally for self-use, although other reports prove that primates are usually processed and eaten on the spot (Tilo Nadler, pers. comm.).

As a final question, we wanted to determine peoples' opinion whether primates should be protected or should be used for food and medicine ("Do you think it is better to protect primates or is it better to make use of them?"). All 50 respondents were convinced that primate protection is important and preferable towards hunting. Different aspects of respondents' own comments regarding that question are summarized (Fig. 12). 23 out of 50 respondents expressed themselves in addition to the initial question.

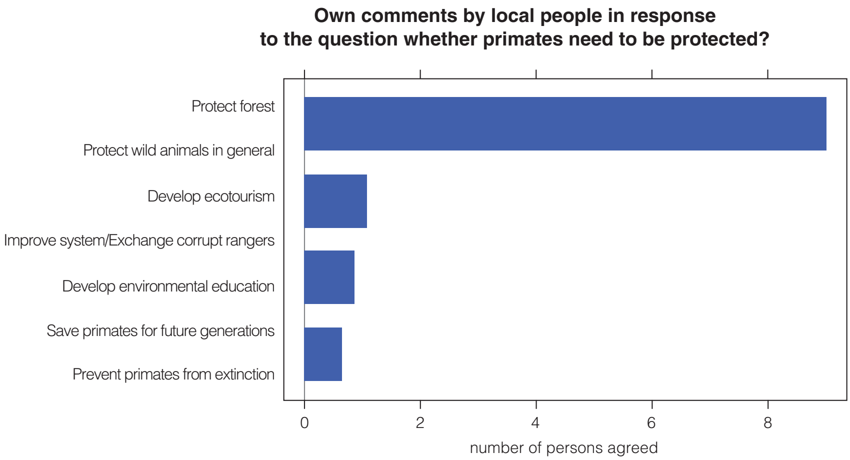

Fig.12. 23 local residents expressed their opinion to the question if primates need to be protected.

Conclusions

The results of this survey give a first overview of local people's knowledge and acceptance of primates and especially the critically endangered Delacour's langur in VLNR. Since the establishment of the reserve in 2001, a good piece of work has been done with regard to species conservation. For example, illustrated charts in the reserve, television reports as well as public relation efforts of FZS in cooperation with the Management Board of the nature reserve made the conservation of the Delacour's langur known to a broad public. It is strongly recommended to carry out such surveys simultaneous to reintroduction projects. Local residents need to be incorporated further in order to ensure successful conservation work.

Acknowledgements

We would like to thank the local authorities of Ninh Binh Province for permission to conduct the survey. Furthermore, special thanks go to the Management Board of Van Long Nature Reserve including all the guards for their kindness and close cooperation. We are very grateful to Tilo Nadler and Nguyen Thi Thu Hien from the EPRC for their great support, organization and guidance during the entire study period. And many thanks to on reviewer of the paper for comments and corrections.

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Vietnamese Journal of Primatology (2013) vol. 2(2), 37-47

The status of Delacour's langur (*Trachypithecus delacouri*) in the planned extension area of Van Long Nature Reserve

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Key words: Delacour's langur, *Trachypithecus delacouri*, interviews, subpopulation size, threats, Dong Tam Commune

Summary

Vietnam has five primate species on the list of "The world's 25 most endangered primates 2012–2014. One of them, the Delacour's langur (*Trachypithecus delacouri*), occurs only in a limited area of northern Vietnam and is critically endangered. The size of its population is estimated to be 200 individuals grouped in 10 subpopulations. The biggest viable subpopulation occurs in Van Long Nature Reserve. However, the details of the number of groups and individuals in the adjacent area outside the reserve and in the planned extension area, in Dong Tam Commune, are unknown. The aim of our study was to estimate the subpopulation status in the adjacent area of the nature reserve, and to determine the number of groups, individuals and recognition of possible threats. For this purpose we combined the Local Ecological Knowledge (LEK), specifically interviews and species presence/absence records, which were carried out simultaneously August to November 2012. According to local people, this area holds six groups of Delacour's langur with the average and maximum number of 34 and 51 individuals, respectively. If confirmed, this would be one of the biggest known subpopulations of this species outside Van Long Nature Reserve. We observed three groups of Delacour's langurs in two sightings. Moreover, we heard langur vocalization and observed langur faeces. This subpopulation is facing threats from hunting, decreased forest cover, growing settlements and limestone quarrying. However, the forest in Dong Tam Commune is an important strategic area for Delacour's langur conservation, and therefore needs to be included as an extension of Van Long Nature Reserve.

Tình trạng của loài Voọc Mông Trắng (*Trachypithecus delacouri*) ở khu vực dự kiến mở rộng của khu bảo tồn thiên nhiên Vân Long

Tóm tắt

Việt Nam có 5 loài linh trưởng trong danh sách "25 loài linh trưởng nguy cấp nhất thế giới năm 2012-2014". Một trong số đó là loài Voọc Mông Trắng (Trachypithecus delacouri). Loài linh trưởng đang nguy cấp này có vùng phân bố hẹp ở phía bắc Việt Nam. Hiện còn khoảng 200 cá thể sống trong 10 tiểu quần thể khác nhau. Quần thể lớn nhất và có khả năng tồn tại lâu dài nhất thuộc khu BTTN Vân Long. Tuy nhiên, số lượng bầy đàn và cá thể ở khu vực giáp ranh với khu bảo tồn, xã Đồng Tâm hiện vẫn chưa có thông tin. Mục tiêu của nghiên cứu này là ước lượng số lượng bầy đàn, cá thể ở vùng tiếp giáp với khu bảo tồn, cũng như xác định các mối đe dọa đến loài. Từ tháng 8 đến tháng 12, năm 2012. Chúng tôi đã thực hiện phương pháp phỏng vấn người dân địa phương để xác định sự có mặt của loài. Theo người dân địa phương, khu vực Đồng Tâm hiện có khoảng 6 đàn Voọc Mông Trắng với khoảng 34-51 cá thể. Nếu thông tin này được khẳng định thì quần thể voọc này có số lượng lớn nhất trong những khu vực bên ngoài Vân Long. Kết quả thực địa cho thấy, có 3 đàn đã được quan sát trong khu vực này. Chúng tôi còn nghe tiếng kêu của loài Voọc này và nhìn thấy phân của chúng. Tiểu quần thể này đang bị tác động bởi việc săn bắn, suy giảm diện tích rừng, các khu định cư tăng lên và khai thác đá vôi. Diện tích rừng ở xã Đồng Tâm là quan trọng cho công tác bảo tồn loài Voọc Mông Trắng, do đó khu vực này cần được đưa vào trong vùng mở rộng của khu BTTN Vân Long.

Introduction

With 5 of its species on the world's 25 most endangered primates list, Vietnam has a disproportionate number of imperiled primates (Mittermeier et al., 2012). The Delacour's langur *(Trachypithecus delacouri)* has been on this list since the first edition in 2000 and is considered as "Critically Endangered" (IUCN Red-List of Threatened Species), and protected in Vietnam on the highest level (Government of Vietnam, 2006). The distribution of Delacour's langur is restricted to limestone mountain ranges of northern Vietnam, located between 20°-21°N and 105°-106°E in four provinces: Ninh Binh, Ha Nam, Hoa Binh and Thanh Hoa (Nadler et al., 2003). After renewed discovery of this species in Cuc Phuong National Park in 1987 (Ratajszczak et al., 1990), an intensive program of surveys by Frankfurt Zoological Society started in 1993 to determine total species population size and distribution. Currently, about 200 individuals are known to exist in 10 isolated subpopulations (Nadler, 2010, Nadler, pers. comm.), down from a high of 300 individuals (Nadler, 1996). Population decline is due to several factors, but the most serious threat seems to be poaching (Nadler et al., 2003). Delacour's langurs occurs in five protected areas: Cuc Phuong National Park, Pu Luong Nature Reserve, Hoa Lu Cultural and Historical Site, Huong Son Cultural and Historical Site and Van Long Nature Reserve. It is believed that only Van Long Nature Reserve contains a viable subpopulation with the chance of long-term survival (Nadler, 2004; 2010). After establishment of Van Long Nature Reserve in 2001, as a result of collaboration between Frankfurt Zoological Society and the Management Board of the nature reserve, significant conservation results have been achieved and the subpopulation of Delacour's langur has doubled during the last decade (Nadler, 2010). Presumably, the total subpopulation size of VLNR is now around 100 individuals (Workman, 2010; Ebenau et al., 2011).

Conservation activities require assessing species abundance and distribution, yet the efficacy of survey and census work varies based on field site conditions and species. Due to lack of habituation and the rugged karst habitat, Delacour's langur are extremely difficult to spot and even more difficult to follow. Using Local Ecological Knowledge (LEK) can therefore be useful (Brook & McLachlan, 2008), while also being mindful that for long-term monitoring, abundance and distribution data need to be confirmed and quantified (Jones et al., 2008; Anadon et al., 2009). LEK has recently been employed in determining the status for various Indochinese primates, for example pygmy loris (*Nycticebus pygmaeus*) (Starr et al., 2011), Francois' langur (*Trachypithecus francoisi*) (Dong Thanh Hai, 2011), macaques (Hamada et al., 2010), as well as in previous surveys of Delacour's langur (Nadler, 1996).

The occurrence of Delacour's langur in Dong Tam Commune, Hoa Binh Province was previously reported (Ebenau et al., 2011) as well known in local communities. This area consists of suitable and, in some parts, intact habitat for langurs, and the inclusion of this area into Van Long Nature

Reserve and the upgrade to a national park is being discussed. Therefore, in this study we aimed to estimate the subpopulation status of Delacour's langur in the extension area, linking two methods: interviews (LEK) and species presence/absence records in order to gather comprehensive information on abundance and distribution of Delacour's langurs in Dong Tam Commune, as well as determine the threats for them and their habitat.

Materials and Methods

Study site

Dong Tam commune is located in Lac Thuy District, Hoa Binh Province, between 20°24' - 20°31' N and 105°46' - 105°52' E. The total area covers 4926.6 ha, including 2154.22 ha of unprotected forest, 268.96 ha of farmlands, 719.89 ha of utilitarian area and 1771.73 ha of other land use types. This commune is settled by 5679 people in 18 villages. Eight villages with 2509 people (44% of total commune's population) are located in close proximity to the forest. Sixty-five percent of people are involved in agriculture activities and 35% in industry. The area is characterized by a seasonal climate with distinguishable hotter, wetter summers and colder, drier winters. The elevation ranges between 27 m and 392.5 m asl. The landscape is similar to that observed in Van Long Nature Reserve with mostly evergreen mixed with some deciduous forest on karst limestone mountainous terrain (Fig. 1). There are no studies conducted on vegetation cover and wildlife in the Dong Tam area.

Fig.1. The landscape of Dai Dong in Dong Tam Commune. Photo: Filip Wojciechowski.

Data collection

In order to obtain relatively comprehensive information on the Delacour's langurs status in the Dong Tam area, the research was divided into three parts, carried out simultaneously between 1st August and November 2012: interviews with local people, presence/absence recording, and threat estimation.

Interviews with local people

We used a structured questionnaire, which can simplify comparisons between respondents and allows quantification (Huntington, 2000). During 10 randomly days we interviewed 100 people from 8 villages with the focus close to the forested area (Dai Dong, Suoi Tep, Dong Moi, Dong Tien, Dong De, Dong Noi, Dong Hai, Doc Yeng). Respondents had been chosen randomly, with the aim to avoid any bias in terms of livelihood, gender or age. However, to strengthen reliability of answers, children and teenagers were not interviewed.

Species presence/absence records

Based on interview answers, we chose areas where we expected occurrence of Delacour's langurs. Some areas where langurs might occur are visible from the village. To better observe langurs, however, we surveyed the habitat over 9 days to find the best observation spots in the hilly habitat. We established 13 observation points (Fig. 2) and spent 52 survey days totaling approximately 350 hours and covering an area of 23 km^2. An observation day lasted from 5.30-6.30 am until 17.00-18.00 pm, depending on the weather conditions and visibility. Surveys were undertaken regardless of weather conditions. We used 10X42 Nikula binoculars. We determined presence/absence from direct observations of animals as well as from indirect observations, mostly feces and vocalization (Ross & Reeve, 2011). We recorded the GPS coordinates of direct and indirect observations using a Garmin G60 ™ device.

Fig.2. The observation points in Dong Tam Commune.

Threats

We interviewed local people and local authorities about hunting and habitat changes in the area and we recorded observations of either throughout the survey.

Data analysis

Questionnaire answers were recorded in Excel and all location data were put in Google Earth. The weighed-average for group size was calculated in each place. When we had a range of numbers, we used the lowest number to avoid overestimation. Additionally, highly unreliable answers were excluded, and ultimately, 98 questionnaires were analysed. Based on locations and the average groups' size of the langurs, as well as known barriers, the home range of the groups was roughly delineated in Google Earth.

Results

Results of interviews

We interviewed farmers (83% of people questioned), guards of Van Long Nature Reserve (4%), construction workers (3%), miners of the local quarry (3%) and others like policemen, students etc. (7%). Local knowledge about Delacour's langur is high in this area, as 94% of the respondents could recognize the species and correctly described its features. More than the half of the interviewed people (59%) had stated that they can recognize different age classes of individuals, mostly infants. However, only 12 infants had been seen in the area this year. 66% of respondents had seen langurs, 42 had made a direct observation, 13 had both seen and heard them and 11 had only heard the animals. Observation frequency of langurs differed among local people from 1 to more than 10, but the latter was hearing rather than sighting the individuals. In total, langurs had been seen 144 times in 16 places and had been heard 87 times in 13 places within the study area (Table 1). Combining all information together, including location, group sizes and natural barriers, the number of groups in the area is 6 and the group size ranged from 2 to 12 (Table 1, Fig. 5). The total subpopulation average is 34 individuals; however the maximal number can reach 51. According to local people, there is no best time to spot langurs, as respondents had seen and heard them almost evenly in the morning and afternoon (Fig. 3). Most of the people had stated that they had seen or heard them between 6 and 9 am and between 3 and 5 pm. Additionally, local people said they usually saw langurs when the weather changed, i.e. rainy morning and sunny afternoon. Villagers saw langurs more often on the middle of the mountain slope, and much less on the top and foothills (Fig. 4). Only seven respondents had seen langurs entering or exiting a cave.

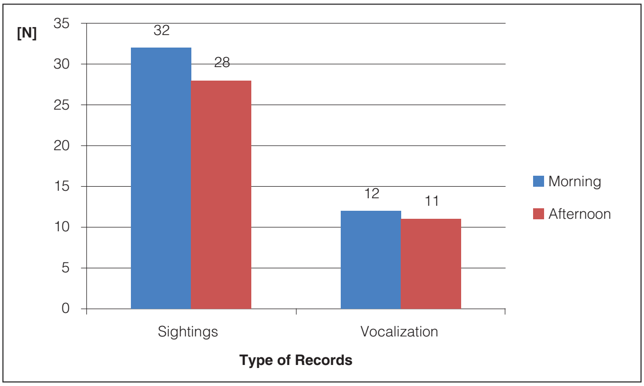

Fig.3. Comparison of sightings and hearings of Delcaour's langur (*Trachypithecus delacouri*) noticed by local people during mornings and afternoons (n=83).

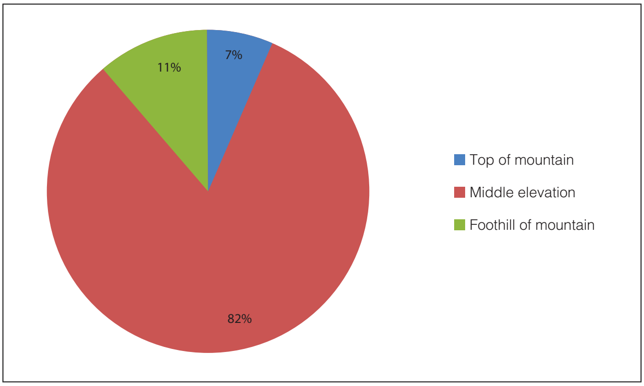

Fig.4. Comparison of number of sightings of Delacours' langurs (*Trachypithecus delacouri*) on different elevation by local people (n=62).

Fig.5. The presumable distributions of groups of Delacour's langur (*Trachypithecus delacouri*) in Dong Tam Commune. The blue colour - places where langurs had been seen and heard, the yellow colour – places where they had been only heard, the pink colour – places where they had been seen by research team, the green colour – spots with feces recorded.

Table. 1. Records of occurrence of Delacour's langur (*Trachypithecus delacouri*) at Dong Tam Commune, based on local people statements.

No.	Name of the place	Number of records		Group size in the spots		Overall group size	
		Sightings	Hearings	Max.	Min.	Max.	Min.
1	Trai Hui	21	4	10	5	10	6
2	Dong Moi	26	4	10	7		
3	Khi Cave	10	16	7	2		
4	x	4	-	10	-		
5	Gieng Valley	2	5	10	-		
6	Dai Dong	5	>10	3	-		
7	Quang Valley	10	>10	8	6	8	6
8	Quan Le 1*	4	-	15	8	18	9
9	Quan Le 2*	6	-	10	-		
10	Quan Le 3*	19	-	18	12		
11	Quan Le 4*	1	1	5	-		
12	Quan Le 5*	7	>10	3	2		
13	Mountain 2	5	3	2	-	2	2
14	Mountain 4000	-	2	-	-		
15	Nha Vit Valley	14	>10	7	6	7	5
16	Chen Valley	3	>10	2	1		
17	Ngo Valley	7	2	6	6	6	6
Total number		144	87	126	55	51	34

Species presence/absence records

We only observed langurs on two occasion's. Additionally we found indirect evidence of langurs on three accounts. We heard callings on one occasion and two times faeces were found. One adult male was seen mid afternoon probably with two other individuals out of view in dense vegetation. There was another sighting of three individuals; adult male and female, and an unidentified individual early in the morning. Finally, we found old faeces in a sleeping cave, and again old faeces on a rock.

Threats to Delacour's langur

Strong anthropogenic pressure exists on the langur subpopulation in the area of Dong Tam Commune. However, human impact is not evenly distributed between all places where langurs had been observed. The main threats which affect the langurs are hunting, destruction of the forest cover (livestock grazing, fuel wood collection, logging), growing settlements in the area with increasing infrastructure and limestone quarrying.

Hunting

The direct threat for the species in the area is hunting. Twice we encountered hunters, one we saw with a gun, and another one carried bird traps. Apart from direct sightings we found possible hunter campsites in the area. Local people reported a high presence of hunters in the area, mostly for bird trapping and snake collection but also occasionally with guns. Local authorities of Dong Tam Commune reported no cases of receiving or encountering killed or illegally caught Delacours' langurs in recent years.

Activities affecting the forest cover

The human impact on vegetation is high in Dong Tam Commune. Livestock grazing is widespread in one part of the area which can have an effect on the forest cover. The grazing by goats is prevalent. Almost always during observations in these areas, we saw one or more groups of goats, usually with one person following them. Four times we encountered collectors of different plants. The most commonly collected plants are Song đá (*Calamus rudentum*), Mây Nếp (*Calamus tetradactylus*), Bình Vôi (*Stephania glabra* (Roxb.) Miers), Bời Lời Nhớt (*Litsea glutinosa* (Lour.) C.B. Rob.) and Huyết Giác (*Dracaena cambodiana* Pierre ex Gagnep) partly for medical purposes. Logging was observed in two main areas, one close to a point of langur observation.

Growing settlements

The human population growth in the Dong Tam Commune is almost 1% per year (local authorities, pers. comm.). Additionally there is a resettlement program planned to relocate 120 families from a threatened terrain of the Da River in Mai Chau District in 2013 (Fig. 6). The disturbance from construction was very high, caused by using heavy equipment vehicles and high numbers of workers.

Limestone quarrying

Limestone quarrying for construction and the opening of trails for tracks destroy larger areas. The extraction work creates a high disturbance (Fig. 7). An intensifying of the quarrying is planned. The quarrying area is close to the home range of a langur group.

Fig.6. The construction of a new estate in front of Quan Le Mountain for the translocation of 120 families. Photo: Filip Wojciechowski.

Fig.7. The effect of limestone quarrying. Photo: Filip Wojciechowski.

Discussion

Limitations of methodology

Delacour's langurs are difficult to study in the wild because of the extremely rough and inaccessible karst (Workman, 2010; Ebenau et al., 2011). Point transect sampling are recommended from other studies in similar habitats (Haus et al., 2009; 2010). With the limited time and staff capacity we could not afford transect sampling. This method would be also of low efficiency through the very difficult terrain, low visibility and subpopulation density. Interviews with local people are a cost-effective method for assessing poorly populations (Hines et al., 2005; Meijaard et al., 2011). However the reliability of our data could be affected by recall ability, causing either underestimation or overestimation of individuals within the groups, mixing up the period of sightings or places where they noted the presence of Delacour's langurs.

Distribution and group size

Inferred subpopulation size of Delacour's langur in Dong Tam Commune is 34 individuals in 6 groups, constituting a considerable part of the world's population of this species. This is a significantly larger number than the 14 individuals mentioned in previous studies in Dong Tam Commune (Ebenau et al., 2011). We recorded infants in 4 groups, so these are probably viable groups and will increase in size. The number of individuals in groups of the study area is consistent with Delacour's langur in a normal population density, claimed as 9 (Nadler et al., 2003). The daily movements of Delacour's langur was estimated as 666.3 m per day in VLNR (Nguyen Vinh Thanh & Le Vu Koi, 2006) and this distance is similar to other groups of colobines, which move on average between 500 to 600 meters per day, (Struhsaker & Leland, 1987). Based on these facts and our sightings it can be assumed that the the langurs have relatively large home ranges in this area.

One langur with radio collar was seen in the area. It was one of the three released individuals in VLNR in 2011. It potentially shows the suitability of this forest for new reintroduced langurs.

Threats to the Delacour's langur

It is not clear whether langur poaching still exists in the area and how the population is affected. The forest cover is highly affected by human activities, especially by goat grazing. Goats can damage vegetation as well as compete for food with langurs (Nadler et al., 2003). The scale of logging and collection of forest products seems to be less destructive to the habitat. The langurs in the area of Quan Le Village are highly affected by growing settlements and limestone quarrying.

Patrols by rangers and community guards in the area are insufficient and rare.

Conclusions

The subpopulation of Delacour's langur in Dong Tam Commune holds 6 groups comprising of 34 to 51 individuals. This is roughly 20% of the entire population of the. species. The involvement of this area as an extension of Van Long Nature Reserve would be a considerable support for the conservation for one of the world's rarest primates.

Current conservation activities should focus on:

- education activities for local people about the importance of the area and the conservation of the Delacour's langur,
- the establishment of a monitoring program of this subpopulation by local people,

- strengthening the efforts of rangers and guards, increasing patrol activities and intensifying the law enforcement.

Acknowledgments

The study could not be possible without significant contributions from a number of people. We would like to especially express our gratitude to Tilo Nadler and Nguyen Thi Thu Hien from Endangered Primate Rescue Center, Cuc Phuong National Park for supporting and facilitating this research. Our thanks go also to the local authorities of Hoa Binh Province for their permission to conduct the fieldwork in Dong Tam Commune as well as their positive attitude and invaluable help at the course of the research period. We are also grateful to guards of Van Long Nature Reserve, especially Bui Van Toi, Dinh Van Hong, Phạm Van Hoan, Tran Ngọc Canh, Tran Tu Anh for their strong engagement, assistance and optimism in the field. Moreover, we would like to thank Mrs. Hoa and Mr. Tuan from Dai Dong for their kindness and help during observations and additionally we are grateful to all people who we interviewed for sharing their time and knowledge. Finally our thanks and gratitude goes to Dr. Eliza Głowska from Adam Mickiewicz University for her comments and to a reviewer which helped to improve this paper.

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Vietnamese Journal of Primatology (2013) vol. 2(2), 49-64

Agent Orange exposure in black-shanked douc langurs (*Pygathrix nigripes*) at Nam Cat Tien National Park, Vietnam

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Key words: dioxin, fecal enzyme immunoassay, douc langurs, Nam Cat Tien National Park

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Summary

Vietnam is home to some of the world's most critically endangered primates, and while hunting and habitat loss/fragmentation continue to threaten many taxa, few data exist on the impact of environmental herbicides, such as dioxins (Agent Orange, TCDD) and related compounds (DRC), on the health of Vietnamese wildlife. We previously demonstrated the utility of a novel enzyme immunoassay (EIA) procedure to quantify faecal dioxin (fTCDD) concentrations in douc langurs (*Pygathrix*) housed at the Endangered Primate Rescue Center (EPRC), Cuc Phuong National Park, Vietnam and found significant fTCDD-age effects, juveniles exhibiting levels of fTCDD substantially above those observed in adults. In this study we field-tested the utility of fTCDD EIAs for quantifying dioxin in wild black-shanked douc langurs (*Pygathrix nigripes*) residing in the Nam Cat Tien National Park (NCTNP), and confirmed fTCDD-age effects in a larger sample of juvenile *Pygathrix* at EPRC. EIA analyses were based on faecal samples obtained from 22 *P. nigripes* at NCTNP, 6 *P. cinerea* and 3 *P. nemaeus* housed at EPRC. NCTNP *P. nigripes* and EPRC *Pygathrix* exhibited similar equivalent fTCDD concentrations, averaging 8.62 pg/g +/- 5.6 SD and 11.1 pg/g +/- 7.4, respectively. Age continued to be a strong predictor of fTCDD levels in individuals originating from south of the 17th Parallel, but in divergent ways: EPRC juveniles exhibited nearly 3-fold higher fTCCD levels than those observed in adults, whereas CTNP adults exhibited nearly 2-fold higher dioxin concentrations than those observed in juveniles. The significance of these results resides in the development of a novel method to quantify dioxin levels in wild *P. nigripes* populations which has broad utility for assessing dioxin exposure in other *Pygathrix* populations inhabiting "dioxin hotspots" in southern Vietnam.

Nghiên cứu phơi nhiễm chất phát quang da cam ở quần thể voọc chà vá chân đen (*Pygathrix nigripes*) Vườn quốc gia Nam Cát Tiên, Việt Nam

Tóm tắt

Việt Nam là ngôi nhà của nhiều loài linh trưởng quý hiếm và đang đứng trước nguy cơ tiệt chủng. Trong khi những mối đe dọa chính như việc săn bắn, môi trường sống bị chia cắt và phá hủy được nói đến nhiều thì những số liệu về ảnh hưởng của chất phát quang, diệt cỏ chẳng hạn như dioxin (Agent Orange, TCDD) và các hợp chất liên quan (DRC) lên sức khỏe của động vật hoang dã lại ít được báo cáo. Trước đây chúng tôi đã chứng minh được việc hiệu quả của việc sử dụng enzyme miễn dịch (EIA) trong việc đánh giá sự nhiễm chất dioxin (fTCDD) tập trung ở các cá thể chà vá (*Pygathrix*) được nuôi nhốt tại Trung tâm cứu hộ thú linh trưởng (EPRC) Vườn quốc gia Việt Nam. Kết quả khi đó cho thấy việc nhiễm chất fTCDD ở các cá thể bán trưởng thành luôn cao hơn những cá thể trưởng thành. Trong nghiên cứu này, chúng tôi sử dụng enzym EIAs để đánh giá mức nhiễm fTCDD ở quần thể chà vá chân đen (*Pygathrix nigripes*) tại Vườn quốc gia Nam Cát Tiên và tái khẳng định ảnh hưởng của độ tuổi đến mức nhiễm fTCDD tại trung tâm cứu hộ linh trưởng EPRC. Chất EIA được phân tích dựa trên việc thu thập các mẫu phân từ 22 cá thể chà vá chân đen tại VQG Nam Cát Tiên, 6 cá thể chà vá chân xám và 3 cá thể chà vá chân nâu tại EPRC. Kết quả, những cá mức nhiễm fTCDD của những cá thể chà vá chân đen tại Nam Cát Tiên và chà vá tại EPRC là tương đối như nhau, trung bình 8.62 pg/g+/- 5.6 SD và 11.1 pg/g +/- 7.4 SD. Độ tuổi có ảnh hưởng rõ rệt đến mức nhiễm fTCDD ở những cá thể có nguồn gốc ở phía nam vĩ tuyến 17 với hai khuynh hướng khác nhau. Tại trung tâm cứu hộ, những cá thể bán trưởng thành mức nhiễm fTCDD cao gấp ba lần con trưởng thành. Tại Nam Cát Tiên, mức nhiễm ở con trưởng thành lại cao gấp hai lần con bán trưởng thành. Kết quả trên cho thấy việc sử dụng phương pháp đánh giá mức nhiễm dioxin trong quần thể chà vá chân đen *P. nigripes* là hiệu quả và mở ra hướng nghiên cứu rộng hơn trên những quần thể khác ở miền Nam Việt Nam, nơi mà chất dioxin được sử dụng nhiều.

Introduction

Vietnam is home to some of the world's most critically endangered primates, including the Delacour's langur (*Trachypithecus delacouri*, <250 individuals) and Cat Ba langur (*Trachypithecus poliocephalus*, ca. 60 individuals) (Mittermeier et al., 2012). Encompassed within the Indo-Burma Biodiversity Hotspot region, Vietnam is one of the top 25 hotspots identified for urgent conservation action and is also among the nine leading hotspots in terms of endemics (Myers et al., 2000). The principle threat to biodiversity in this region continues to be habitat loss/degradation via the use of chemical defoliants [TCDD], logging, and clearing of land for agriculture, but when coupled with subsistence/trophy hunting for body parts, these combined threats can have a devastating impact on primate populations (Nadler & Streicher, 2004).

To date, there are few data on the impact of environmental herbicides, such as dioxins (i.e., Agent Orange, TCDD) and dioxin-related compounds (DRC), on the health of Vietnamese wildlife. Considered the most toxic of the organohalgen compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a lipophilic chemical that bioaccumulates in the fat of vertebrates through the food chain (Fries, 1995) and persists in the environment for decades, thereby making it extremely hazardous to both wildlife and humans who are subject to long-term exposure. Anthropogenic sources of DRCs include by-products from the manufacture of chlorinated phenols used in pesticides in the 1930's, incineration, forest fires (Field & Sierra-Alvarez, 2008) and chemicals (i.e. TCDD), aerial transport of dioxins in the former being deposited in soils, plants, and water through combustion, industrialization, and e-waste recycling processes (Tanabe & Tu Binh Minh, 2010). While it is well known that dioxins are subject to photodegradation, it has been only recently demonstrated that TCDD can be degraded through the actions of aerobic bacteria from the genera *Sphingomonas*, *Pseudomonas* and *Burkholderia* (reviewed in Field & Sierra-Alvarez, 2008). Chemicals are the primary vehicle for the introduction of dioxin into the Vietnamese environment (Hatfield & 10-80 Committee, 2006), Agent Orange having been used extensively to defoliate forests and destroy crops during the Vietnam War. The United States government sprayed more than 45 million liters of TCDD on the forests and villages of Vietnam between 1965 and 1971, U.S. military CORPS Tactical Zones I and III receiving the vast majority of the herbicide (Stellman et al., 2003 in Brockman et al., 2009). Humans residing in “dioxin hotspots” such as Bien Hoa City (Dong Nai Province), have been shown to exhibit markedly elevated serum dioxin levels (Schecter et al., 2003), but until recently, few data have been available on the extent of dioxin exposure in Vietnamese primates (Brockman et al., 2009; Vu Ngoc Thanh et al., 2010).

In previous research (Brockman et al., 2009), we demonstrated the utility of a novel enzyme immunoassay (EIA) procedure to quantify faecal dioxin (fTCDD) concentrations in *Pygathrix* housed at the Endangered Primate Rescue Center (EPRC), Cuc Phuong National Park (Ninh Binh Province), Vietnam and found significant fTCDD-age effects, juveniles exhibiting substantially elevated fTCDD levels above those observed in adults, suggesting that immature individuals *may* be at risk of functional developmental effects. We identified two adult individuals, a female *P. nemaeus* (6-02) and a male *P. cinerea* (7-25), which appeared to exhibit developmental consequences of possible TCDD exposure (Brockman et al., 2009), but we have found no definitive clinical links between TCDD exposure and reproduction or frequency of stillbirths in the *Pygathrix* population at EPRC.

The objectives of this research were 1) to field-test the utility of fTCDD EIA procedures to quantify dioxin in the faeces of a wild population of endangered black-shanked douc langurs (*Pygathrix nigripes*) (Fig. 1), residing in the Nam Cat Tien National Park, an area located in CORPS Tactical Zone III, which is known to have been extensively sprayed with TCDD during the Vietnam War (see below), and 2) to confirm fTCDD-age effects in a larger sample of red-shanked douc langurs, (*P. nemaeus*) (Fig. 2), and grey-shanked douc langurs (*P. cinerea*), residing at EPRC (Fig. 3). Based upon the results of our previous studies of fTCDD-age effects in *Pygathrix* housed at the EPRC (Brockman et al., 2009), we predicted that fTCDD concentrations in juvenile *P. nigripes* at NCTNP would be significantly elevated above those observed in adults.

Fig.1. Black-shanked douc langur (*Pygathrix nigripes*) at Nan Cat Tien National Park. Photo: Jonathan A. O’Brien.

Fig.2. Red-shanked douc langur (*Pygathrix nemaeus*) at the Endangered Primate Rescue Center. Photo: Tilo Nadler.

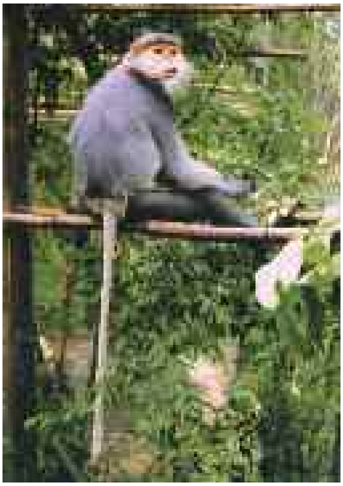

Fig.3. Grey-shanked douc langur (*Pygathrix cinerea*) at the Endangered Primate Rescue. Photo: Tilo Nadler.

Material and Methods

Study site

Cat Tien National Park is located ~160 km northwest of Ho Chi Minh City and covers an area of approximately 740 km^2 situated within the three Provinces of Dong Nai, Lam Dong, and Binh Phuoc,

Fig.4. Map of Cat Tien National Park Complex, indicating locations of Tay Cat Tien and Nam Cat Tien.

11o21' – 11o48'N, 107o 10' – 107o 34'E. The CTNP complex is divided into two large sections, Cat Loc in the north and Cat Tien in the south, the latter being further subdivided into Tay Cat Tien and Nam Cat Tien (Fig. 4) which is home to nine species of primates (Polet et al., 2004), including *P. nigripes*. Militarily strategic districts located in the Provinces of Dong Nai and Binh Phuoc were targeted by the U.S. military for extensive aerial spraying with Agent Orange during the Vietnam War, including Bien Hoa (837,755 L), Long Khanh (6,017,499 L), Phuoc Long (7,570,823,568 L) and Binh Long (148,762 L). 367,090 L of Agent Orange was sprayed on the entire Province of Lam Dong (calculated from HERPS Tape, 2000). CTNP is highly seasonal, a five-month dry season from December to April alternating with a seven-month wet season from May to December, when rainfall averages 2175-2975 mm annually (Vidya et al., 2007). Habitats within the NP are diverse and include not only one of the last remaining lowland evergreen/semi-evergreen forests in Vietnam, but also large expanses of deciduous and secondary forests, dominated by bamboo forests, grassland, and wetlands bordering the Dong Nai River (Polet et al., 2004). This research was conducted at NCTNP where a substantial population of black-shanked douc langurs is known to reside, i.e. 18 groups, ~109 animals, three of which have been reported to contain habituated adults and immatures which are regularly encountered and easily observed (Polet et al., 2004).

Subjects

Subjects in this study included individuals from three species of douc langurs: *P. nigripes* (black-shanked douc langurs, 14 adults, 8 juveniles) residing at NCTNP, and *P. nemaeus* (red-shanked douc langurs, 2 adults, 1 juvenile) and *P. cinerea* (grey-shanked douc langurs, 4 adults, 2 juveniles) residing at EPRC, the latter having been donated from tourists or confiscated from known capture locations south of the 17th Parallel (demilitarized zone, DMZ). FTCDD results from 17 subjects from our previous study conducted at EPRC in 2006 (Brockman et al., 2009) were included in our analyses here in order to increase sample sizes of individuals in the adult and immature age classes. As reviewed previously in Brockman et al. (2009) with the exception of Hong Minh Duc's studies (2007) of *P. nigripes* at Nui Chua and Phuoc Binh National Parks (Ninh Thuan Province), quantitative data on the social structure, day range, mating behaviour and behavioural ecology of douc langurs is extremely limited, and data on life history parameters are virtually nonexistent, the likely consequence of the scarcity of habituated populations of douc langurs available for long-term demographic study. Recent studies of ontogenetic cranial development and dental eruption patterns in captive *Pygathrix* at EPRC suggest, however, that the adult age-class is attained at 5-6 years of age (Stephen & Nadler, 2012). Previous studies by Ruempler (1998 in Nadler et al., 2003) indicate that male and female douc langurs become sexually mature at ~5-8 years and ~5-7 years, respectively. Age estimates for wild *P. nigripes* in this study were based on observations of body size differences, adults being approximately twice the size (or more) than juveniles. Sexes of the wild subjects could not be reliably determined. EPRC subjects ranged in age from 1 to 14 years, age estimates having been assigned by EPRC staff during quarantine based upon patterns of dental eruption and tooth wear (Nadler, unpubl. data). I received permission to conduct research at CTNP and EPRC from Mr. Tranh Van Thanh, Park Director, Nam Cat Tien National Park and from Tilo Nadler (TN), Director EPRC (June 2004), respectively. This research was approved by the University of North Carolina-Charlotte Institutional Animal Care and Use Committees (UNCC protocol no. 09-404).

Data collection

Nam Cat Tien National Park

A total of 102.5 hours were spent in NCTNP surveying the population of ~100 resident largely unhabituated black-shanked douc langurs (Fig. 1) located ~.5-1.5 km from the Ranger Station. Four different groups were subsequently located (Table 1). Visual contact occurred most frequently at dawn and terminated at noon when groups moved into inaccessible regions of the park dominated by thick bamboo forests. During the total 2.78 hrs of animal contact, groups were in view for 5-20 min during which 22 (~10 g) faecal samples were collected immediately after voiding (or soon thereafter) from adult and immature individuals (Fig. 5 and 6; Table 1) and placed in 2 x 3 inch Ziploc bags which were labelled as to individual, date and time of collection (Brockman et al., 2009). At the end of each daily field session, the faecal samples were vacuum sealed in individual zip-loc bags and then repackaged again in quart-sized Zip-loc bags (labeled adult and immature) until they could be transported to an ultra-cold freezer at the Institute of Tropical Biology in Ho Chi Minh City and later shipped frozen to CAPE Technologies, Inc. (South Portland, MA) where they were stored at -20°C until they were extracted and enzyme immunoassayed by Robert Harrison, Director (CAPE Technologies, Inc.). Vietnamese and U.S. permits were obtained to ship the faecal samples to CAPE Technology laboratory, South Portland, ME where EIA procedures were employed to quantify fTCDD concentrations as described below.

Fig.5. Adult faecal sample from black-shanked douc langur (*Pygathrix nigripes*) at Nan Cat Tien National Park. Photo Diane K. Brockman

Fig.6. Juvenile faecal sample from Black-shanked douc langur (*Pygathrix nigripes*) at Nan Cat Tien National Park. Photo Diane K. Brockman

Table. 1. Faecal samples collected from wild *Pygathrix nigripes* at Nam Cat Tien National Park (n=22 samples).

Sample ID	Group	Adult/Immature	g sample/tube	pg/gm TCDD	Notes
B1-1	Gang of 5	Adult	9.7	5.8	conclusive; negative spike[1]
B1-2	Gang of 5	Adult	6.2	2.8	EPA Method 4025m (soil)
B1-3	Gang of 5	Immature	7.8	7.0	
B2-1	Gang of 11	Adult	6.6	16.5	Mean TCDD of 2 runs (8.3, 24.0)
B2-2	Gang of 11	Adult	8.1	4.4	
B2-3 & 10-3	Gang of 11	Immature pooled sample	7.1	1.9	EPA Method 4025m (soil)
B3-1	Gang of 35	Adult	9.2	7.8	
B3-2	Gang of 35	Adult	7.8	0.0	Inconclusive; negative spike[1]
B4-1	Gang of 35	Adult	5.4	10.3	EPA Method 4025m (soil)
B4-2	Gang of 35	Adult	6.6	15.2	
B4-3	Gang of 35	Adult	5.5	8.7	
B4-4	Gang of 35	Adult	4.0	19.9	
B5-1/2	Yellow Trail Group	Immature pooled sample	5.1	2.9	EPA Method 4025m (soil)
B5:2/18-2	Yellow Trail Group	Adult	9.8	20.0	
B5:2/19-1	Yellow Trail Group	Adult	6.6	6.0	EPA Method 4025m (soil)
B6-1/3/4	Gang of 5	Immature pooled sample	6.3	5.0	Mean of 2 unspiked/dupes
B6-2	Gang of 5	Adult	4.0	8.2	EPA Method 4025m (soil)
B7-1/2/3/4	Gang of 35	Immature pooled sample	7.9	18.2	Inconclusive; negative spike[1]
B8-1/2/3/4	Gang of 35	Immature pooled sample	6.7	11.1	Mean of 2 unspiked/dupes
B9-1/2/3/4	Gang of 35	Immature pooled sample	5.9	5.4	
B11-5/6/7/8	Gang of 35	Immature pooled sample	4.9	5.9	EPA Method 4025m (soil)
B11-9	Gang of 35	Adult	9.7	4.4	EPA Method 4025m (soil)
Adult mean TCDD				**10.35 +/- 6.1 SD**	**1.8-fold higher**
Immature mean TCDD				**5.64 +/- 3.1 SD**	**p=0.08**

[1]: Not included in analysis of results.

Cuc Phuong National Park

Tilo Nadler, Director of EPRC, identified three juvenile and six adult douc langurs from southern Vietnam which had come into the population since my previous research there in December 2006 and were thus candidates for faecal collections (Table 2). Single (10 g) faecal samples were collected from the nine subjects and placed in individually labelled 2 x 3 inch Ziploc bags and placed in EPRC's ultra-cold freezer until they could be shipped to the U.S. for analysis. Vietnamese and U.S. permits were obtained to ship the fecal samples to the CAPE Technology laboratory, South Portland, ME where EIA procedures were used to quantify fTCDD levels as described previously (Brockman et al., 2009).

Table. 2. Faecal samples collected from gray-shanked (*P. cinerea*) and red-shanked (*P. nemaeus*) douc langurs at the Endangered Primate Rescue Center, Cuc Phuong National Park (n=9 samples).

Animal ID	Species	Sex	Birth Date	Capture Location	Age	g sample/tube	pg/gm TCDD	Notes
7-49	*P. cinerea*	Male	2009	Quang Nam	~ 1 yrs	2.84	27.0	
7-45	*P. cinerea*	Female	2007	A Lao	~ 3 yrs	4.45	11.8	
6-60	*P. nemaeus*	Female	2007	?	~ 3 yrs	5.07	3.9	See Note[1]
7-46	*P. cinerea*	Male	2005	Ba To	~ 5 yrs	4.41	9.8	
7-52	*P. cinerea*	Male	2005	?	~ 5 yrs	8.25	6.6	
7-47	*P. cinerea*	Male	2004	An Lao	~ 6 yrs	6.10	6.1	
7-39	*P. cinerea*	Male	2003	An Lao	~ 7 yrs	5.25	11.0	
6-53	*P. nemaeus*	Female	2003	Binh Dinh	~ 7 yrs	6.19	7.7	
6-63	*P. nemaeus*	Male	2002	Hue	~ 8 yrs	4.43	6.6	
Adult mean TCDD							**7.0 +/- 2.6 SD**	**p=0.07**
Immature mean TCDD							**19.4 +/- 10.7 SD**	**2.8-fold higher**

[1]: Not included in analysis of results; could not confirm origin south of the 17th Parallel.

Faecal dioxin extraction and EIA

Nam Cat Tien NP Phase I Faecal Dioxin Extraction and EIA

Dr. Robert H. Harrison (Director, CAPE Technologies) received 22 frozen *P. nigripes* faecal samples in April 2010 which were immediately placed in his laboratory's ultra-cold freezer at -20^{o} C. Fourteen faecal samples were subsequently hydrolyzed and extracted prior to being enzyme immunoassayed using EPA Method 4025m which had been previously validated for *Pygathrix* (Brockman et al., 2009). EIAs of the remaining 8 faecal samples were postponed until issues relating to poor spike recoveries and incomplete hydrolysis (n=3 samples) could be resolved (see Phase II below).

Processed sample appearance and method suitability

Sample hydrolysates appeared grossly different from those reported in Brockman et al. (2009) and different than any other sample matrix previously analyzed at CAPE Technologies using this protocol. After hydrolysis and extraction the centrifuged samples were well separated (Fig. 7) and the extracts appeared "normal", but the hydrolysates did not. "Normal" hydrolysates are entirely liquid and have a distinct purple color. These hydrolysates were dark brown to black with a very slight purplish cast and contained a large amount of solid residue, including a significant amount of material adhering to the glass above the hydrolysate, something not seen with other samples.

Fig.7. 125 ml hydrolysis/extraction bottles after hydrolysis, extraction, and centrifugation. The acid hydrolysate is dark material on bottom and the solvent extract of that hydrolysate is the clear liquid on top. This liquid was removed by suctioning and processed through the cleanup described in the materials and method section.

After the extracts were removed for cleanup and analysis, the hydrolysates were examined further. Several hydrolysates were treated with additional conc. HCl, approximately doubling to tripling the ratio of acid to sample. After shaking, solvent extraction, centrifugation, and extract removal, these hydrolysates remained largely insoluble. Heating of the hydrolysate to roughly 50°C had no visible effect on the hydrolysate.

Other hydrolysates were treated by filtering the solids (which often had a weight approaching that of the original sample), washing with water, then treatment with 6 N NaOH. The remaining processing was otherwise identical to the repeat acid treatments, including heat treatment. After treatment, these samples appeared nearly identical to the acid treated samples.

The possibility of effect of sample size on the process was also considered. Sample sizes were variable, but within a fairly narrow range (mean ± SD = 7.2 ± 1.6 g). The maximum sample size analyzed in the current group (9.8 g) was smaller than the largest sample analyzed previously (11.3 g; Brockman et al., 2009). Additionally, the protocol was also modified slightly from Brockman et al. (2009) to include a larger amount of conc. HCl (60 ml here vs. 50 previously). Since the resulting hydrolysates appeared uniform across the range of sample sizes, neither acid:sample ratio nor sample size appeared to explain these divergent outcomes.

There is no currently obvious explanation for this difference between EPRC samples (Brockman et al., 2009) and the current CTNP samples other than perhaps species-specific diet differences and/or gut microflora composition.

QA data

Standard curve data (and their QA parameters), used as the basis for concentration calculations, were within recent norms. Method blanks, unspiked and spiked, gave results within normal ranges with one exception (flagged as possible invalid due to protocol deviation). Three sets of intra-batch duplicates gave % cv values of 19, 5, and 17. However, sample spike recoveries ranged from 160% to -62%, with no obvious pattern.

Only two original samples were large enough to split into 4 subsamples. One of these, B1-3, had all four subsamples (two unspiked and two spiked) analyzed in different sub-batches of Run A. The calculated pg/g TEQ values ranged from 2.1 to 7.6, all below the unspiked method blank for Run A. The spike recovery values for the two unspiked/spiked pairs were 10 and -62%. The other large sample, B2-1, was split between Run A and Run B. Spike recoveries were 57% and 76%, both within acceptable ranges. Unspiked sample values, uncorrected for spike recovery, were 8.3 and 24.0 pg/g. While there is no obvious explanation for this discrepancy, it should be noted that this sample was different than all the other samples in one potentially important respect. The amount of color in the extracts was significantly greater than all the other samples (Fig. 8); for repeat processing of original sample hydrolysates- see legend for complete explanation).

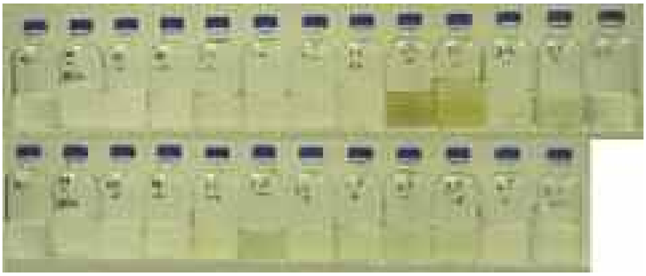

Fig.8. Solvent extracts removed from repeat acid hydrolysis treatments of original hydrolysates. Variations in volume primarily reflect the effect of different amounts of solid residue in the original hydrolysate. Extract color and variations thereof were parallel to what was seen for the original extracts (no photos taken at that point). Note color difference in pairs 2-1, 3-2, and 7-P (triplicates).

While color by itself does not imply sample preparation issues, outlier samples often pose challenges which are difficult to assess and overcome with limited sample quantities such as in this study. The only way to guarantee sample homogeneity during preparation is by complete liquefaction during acid hydrolysis. Since this was not observed for any samples, it is reasonable to assume that none of the samples were adequately homogenized after splitting.

Because of the unusual nature of the hydrolysates noted above and the variable spike recovery values, the repeat treatments noted in the Method Suitability section above included some spikes, of both previously spiked and previously unspiked samples. The resulting data showed a wide range of spike recovery values similar to that seen with the original samples.

Interpretation of data for unspiked unknown samples

Quality assurance data for some samples were acceptable, yet it remains difficult to justify specific conclusions about any individual sample concentrations. Since all samples presented the same difference in physical appearance after hydrolysis, it is reasonable to assume they all showed whatever differences exist in sample chemistry between these samples and the previous EPRC samples. It is possible that dietary components such as foliar lignin vary greatly due to both species and geography. Such a difference might explain both poor spike recoveries and incomplete hydrolysis.

Regardless, significant solid residue in the acid hydrolysate indicated that the entire sample was not available to the solvent for extraction. In contrast, a fully liquid acid hydrolysate can interact completely with the extraction solvent and thus the entire sample is available for extraction and subsequent analysis.

Revised sample preparation protocol

The above data and observations lead to the possibility of an alternative sample preparation protocol being superior (see Phase II below). The justification for the current protocol was that vegetation samples previously analyzed at CAPE Technologies gave adequate results in terms of appearance and QA data. Hence, faecal samples from CTNP folivores should have been amenable to the same sample preparation protocol. Such a protocol allows the complete removal of even intracellular and membrane integrated dioxin, which may not be removable with a simple solvent extraction. This was borne out in the earlier work reported in Brockman et al. (2009), to the maximum degree possible within the structure of the QA samples analyzed.

In this case however, it was more reasonable to use a solvent extraction coupled with vigorous mechanical agitation, such as used for soils analyzed by Method 4025m. Samples are agitated vigorously in a glass vial containing sample, solvent, sodium sulfate for drying, stainless steel BBs for gross scale pulverization, and sand for fine scale pulverization. The resulting pellet after extraction and centrifugation is generally extremely fine-grained (like silt) and entirely homogeneous. However, since this method is not routinely used by CAPE Technologies for biological samples, validation for fecal samples was highly desirable (see below).

Nam Cat Tien Phase II faecal dioxin extraction and EIA

In Phase II, the remaining eight (of 22) NCTNP faecal samples were extracted using an acetone:hexane soil method of extraction and subsequently enzyme immunoassayed to quantify dioxin concentrations.

Soil method extraction and EIA

All glassware to be in contact with method blanks or samples was first rinsed with toluene, and then air dried before use. Samples were weighed using an identical tare bag to determine net weights. Sodium sulfate was added directly to samples in their original Ziploc bags, the bags were then closed and the sample was kneaded by hand until the mixture was uniform and free flowing. Pooled samples were combined and mixed further, and the samples were then split by weight (as appropriate), and transferred to 125 ml glass bottles for extraction. Sand (10 g) and BBs (n=5) were added to each bottle. Spikes of 100 pg 2378-TCDD (10 µl of 10 ppb stock ppb in toluene) were added to selected samples for quality assurance and the bottles were shaken until no clumps were visible.

Acetone:hexane (50 ml of 1:1) was added to each bottle and all were shaken vigorously for 10 hours. Bottles were centrifuged for 20 min and the supernatants were poured off into 60 ml vials and weighed. The sample pellets were washed with 30 ml hexane, hand shaken, and spun and the supernatants recovered and weighed as before. A second wash was performed in the same manner using 20 ml hexane. These washes were added to the evaporation vials, 1 ml of tetradecane was added to each vial, and the pooled supernatants were evaporated under a stream of clean compressed air at approx. 45° to 65°C. After evaporation of the acetone and hexane, the tetradecane residue was diluted with 5 ml hexane. Fine acid silica (FAS) was added to each hexane sample and swirled until the supernatant was clear and colorless.

Carbon columns (CAPE Technologies) were attached to 15 mm acid silica columns (CAPE Technologies) and 8 ml hexane was added to each column. Interstitial air was removed by vacuum depressurization, followed by pressurization to purge residual air from the carbon column. Samples were added to the acid silica columns, including both supernatant hexane and acid silica from sample pretreatment. Columns were pressurized to push the sample into the columns, followed by a hexane wash. Hexane (3 x 10 ml per column) was added and pushed through the coupled column system. When air penetrated the lower portion of the acid silica columns, flow was stopped and the carbon columns were moved to clean 15 mm reservoirs. Carbon columns were washed in the forward direction with 6 ml 1:1 hexane:toluene, which was discarded. Carbon columns were reversed on the same reservoir and eluted with 12 ml toluene, which was captured in 16x125 mm tubes. Keeper solution (62.5 µl/tube) was added and the toluene was evaporated under a stream of clean compressed air at ≤85°C. Evaporation tubes were centrifuged 15 min to concentrate the keeper residue at the bottom. Methanol was added to reconstitute the samples, which were added to the immunoassay tubes and incubated 16 hrs. The EIA was finished according to the kit insert (IN-DF1).

EPRC fecal dioxin extraction and EIA

Nine (10 g) faecal samples were collected from wild-born douc langurs arriving at EPRC since December 2006 and subsequently extracted and enzyme immunoassayed as described previously (Brockman et al., 2009). The procedure is briefly described as follows: solvents utilized were HPLC grade (Fisher Scientific), except for toluene, which was residue analysis grade (Burdick & Jackson). Acids were ACS grade (Fisher). Analytical standard grade 2,3,7,8-TCDD was obtained from Ultra Scientific. All cleanup columns and immunoassay kit materials were manufactured by CAPE Technologies. All glassware was rinsed with toluene and dried before use.

Faecal samples were collected in 2 x 3 inch Ziploc bags, shipped frozen, then stored at -20°C until analysis. All subsequent procedures were performed at 20-25°C unless noted otherwise. Ziploc bags were weighed to 1 mg before thawing and then weighed again after the sample was removed to determine the weight of the sample delivered to the cleanup procedure.

Samples were removed from their bags by dispersing the sample in conc. HCl and pouring bottle for hydrolysis. Sample bags were rinsed with additional conc. HCl to remove as much residue as possible. Total volume of HCl used was 50 ml per sample. The entire sample and rinsate were poured into a 250 ml borosilicate glass bottle with a Teflon lined cap. Solvent (50 ml 3:1 hexane:dichloromethane) was added and the 2378-TCDD spike, if any, was added at this point (spike levels were 10 pg/g). Bottles were capped and rotated end over end at 30 rpm for 12-15 hrs. Bottles were centrifuged 15 min @ 1000 x g and the supernatant solvent was removed and passed through a column of 5 g NaHCO3. The treated solvent was then oxidized by mixing with acid silica (activated chromatographic silica with conc. H2SO4 adsorbed) until the solvent was clear.

The dioxin in the oxidized supernatant was captured for analysis using the CAPE Technologies coupled column cleanup system (ref AN-008). The oxidized supernatant was passed through a column of the same acid silica as used previously, then directly onto a column of activated carbon. Hexane washes of the acid silica oxidation bottle (2 x 50 ml) were added sequentially to the acid silica column to maximize sample recovery. After washing, the carbon column containing the captured dioxin was removed and attached to a clean empty reservoir. The column was washed in the forward direction with 6 ml of 1:1 hexane:toluene, then eluted in the reverse direction with 12 ml toluene (ref TN-005) and captured in a 16 x 125 mm glass tube. A keeper solution of methanol containing 20% polyethylene glycol and 100 ppm of Triton X-100 was added and the toluene was evaporated at 70-80°C under a stream of filtered dry compressed air. The samples were reconstituted by centrifuging the evaporation tubes 5 min @ 1000 x g, then replacing the evaporated methanol. Dioxin levels in the prepared samples were then analyzed by immunoassay according to the kit insert (ref INDF1).

Standards in keeper and samples prepared as described above were added to sample diluent in tubes coated with antidioxin antibody. After incubation for 14-17 hours, the sample was removed and the tube washed 4 times with 1 ml of distilled water plus 0.01% Tween 20 detergent. Enzyme conjugate was added and tubes were incubated 15 min., and then washed with distilled water. Enzyme substrate was added and color was allowed to develop for 30 min. Stop solution containing 1 N HCl was added and the optical density (OD) of each tube was read at 450 nm using a portable differential photometer (Artel).

Statistical Tests and Assessments of fTCDD-Population and-Age Interactions

Statistical analyses were performed using SIGMAPLOT 12 (Systat Software, Inc. Point Richmond, CA). Univariate statistical tests (Student's t-test, Mann-Whitney U test) were used to examine variation in fTCDD levels in NCTNP and EPRC douc langurs. The effect of age on fTCDD concentrations in adult and juvenile douc langurs was tested using multivariate (multiple linear regression) and univariate analyses. Variables which departed from normality were adjusted by removal of outliers (samples more than two standard deviations from the mean) or were log transformed. Results are reported as means ± SD with significance set at $P < 0.05$.

Results

Sample preparation in phase I vs phase II

Results of faecal extraction/EIA procedures of 3 of 22 Phase I faecal samples were eliminated from further analysis due to negative spike recoveries and incomplete hydrolysis. Employing the soil method of faecal extraction in Phase II analyses completely eliminated the problematic issues encountered in the Phase I extraction procedures.

FTCDD concentrations in *Pygathrix* from South of the 17^{th} Parallel

Results from a reanalysis of FTCDD concentrations obtained from the 17 *Pygathrix* in 2006 (Brockman et al., 2009) pooled with those from this study, showed that *Pygathrix* originating from south of the 17^{th} Parallel exhibit fairly low fTCDD levels, averaging 8.11 pg/g fTCDD ± 5.8 SD (n=44). Assessments of the effect of age on fTCDD levels showed that dioxin concentrations in juveniles were ~69% higher than those observed in adults (juvenile: 10.73 pg/g fTCDD ± 8.2, n=11; adult: 7.39 pg/g fTCDD ± 4.7 SD, n=33, P=0.31, Table 3).

Table. 3. Faecal TCDD concentrations in *Pygathrix*.

Location/ species	Total Mean fTCDD +/- SD (pg/g wet weight)	P Value	Adult Mean fTCDD +/- SD (pg/g wet weight)	Juvenile Mean fTCDD +/- SD (pg/g wet weight)	P Value
Pygathrix: south of the DMZ	8.11 +/- 5.8 (range: 1.9 – 27.0) (n=44)		7.39 +/- 4.7 (range: 2.8 – 20.0) (n=33)	10.73 +/- 8.2 (range: 1.9 – 27.0) (n=11)	0.31
NCTNP: *P. nigripes*	8.62 +/- 5.6 (range: 1.9 – 20.0) (n=19)		10.35 +/- 6.1 (range: 1.9 – 20.0) (n=12)	5.60 +/- 3.0 (range: 1.9 – 11.1) (n=7)	0.07
EPRC: *Pygathrix*	7.94 +/- 6.1 (range: 3.0 – 27.0) (n=25)	0.58	5.7 +/- 2.5 (range: 3.0 – 11.0) (n=21)	19.7 +/- 6.3 (range: 11.8 – 27.0) (n=4)	0.001

FTCDD and the effect of age in *P. nigripes* at NCTNP

At the population level, *P. nigripes* exhibited low total fTCC levels, averaging 8.62 pg/g fTCDD ± 5.6 SD, n=19. Results of Student's t-tests showed that adult *P. nigripes* exhibited nearly 2-fold higher fTCDD levels than those observed in juveniles (adult: 10.35 pg/g ± 6.1 SD, n=12; juvenile: 5.64 pg/g ± 3.1 SD, n=7) though not significantly so (P=0.07, Table 3, Fig. 9).

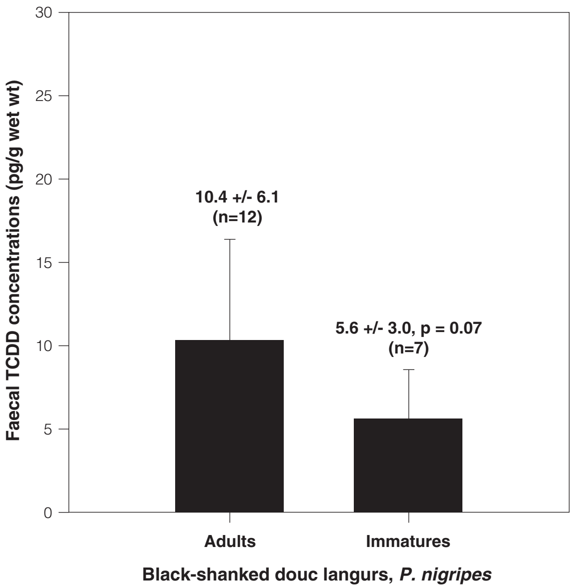

Fig.9. Age related variation in fTCDD concentrations in black-shanked douc langurs (*Pygathrix nigripes*) at Nam Cat Tien National Park.

FTCDD concentrations and the effect of age in *P. nemaeus* and *P. cinerea* at EPRC

The small sample size of *P. nemaeus* originating from south of the 17th Parallel (n=2) precluded an examination of species differences in fTCDD levels; previous studies demonstrate, however, no effect of species on fTCDD concentrations (Brockman et al., 2009). At the population level, EPRC douc langurs in this study exhibited low total fTCDD concentrations, averaging 11.1 pg/g TCDD ± 7.4 SD, n=9. Results from Mann-Whitney U tests indicate that juvenile douc langurs exhibited nearly 3-fold higher fTCDD levels than those observed in adults (juvenile: 19.4 pg/g fTCDD ± 10.7 SD, n=2; adult: 7.0 pg/g fTCDD ± 2.6 SD, n=7) though not significantly so (P=0.07, Table 2). Results from pooling the fTCDD values obtained previously from the 17 *Pygathrix* at EPRC (Brockman et al., 2009) with those obtained here indicate that total mean fTCDD concentrations in this EPRC population were somewhat lower than the subpopulation above, averaging 7.94 pg/g fTCDD ± 6.1 (n=25), being broadly equivalent to those obtained from *P. nigripes* at NCTNP (i.e. 8.62 pg/g fTCDD, Table 3). Pearson Product Moment Correlation tests showed a significant negative correlation between fTCDD levels and age (r = - 0.72, P = < 0.0001, n = 25). Mann-Whitney Rank U tests indicated that fTCCD levels in immatures were significantly higher than those in adults south of the 17th parallel, juveniles exhibiting nearly four-fold higher fTCDD levels that those observed in adults (juvenile: 19.7 pg/g fTCDD, ± 6.3 SD, n=4; adult: 5.7 pg/g fTCDD ± 2.5 SD, n=21, P=0.001, Table 3, Fig. 10).

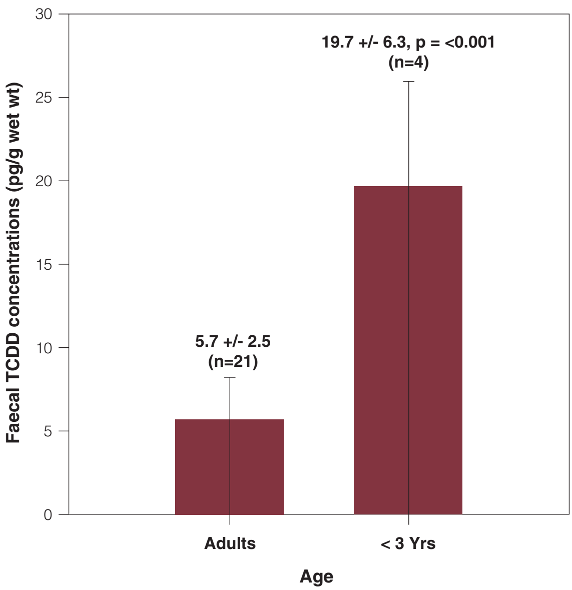

Fig.10. Age-related variations in fTDD concentrations in douc langurs (*Pygathrix*) at the Endangered Primate Rescue Center.

Discussion

The results of this study demonstrate that the extraction procedures employed in our previous study (Brockman et al., 2009) were ineffective in extracting TCDD from faeces obtained from wild *P. nigripes*. However, by employing a modified soil method (EPA Method 4025m) of fTCDD extraction, we were able to obtain a fully liquid acid hydrolysate which could interact completely with the extraction solvent, thereby enabling the entire *P. nigripes* faecal sample to be available for extraction and subsequent analysis. This study further demonstrates that our previously validated novel EIA procedures reliably quantified relative fTCDD concentrations in wild black-shanked douc langurs residing at NCTNP, an area that was exposed to high levels of dioxin and dioxin-like chemicals during the Vietnam War. Although total fTCDD concentrations in this population were fairly low (mean: 8.6 pg/g fTCDD), they were broadly equivalent to those observed in wild-caught red-shanked and grey-shanked douc langurs residing at EPRC (~7.94 pg/g fTCDD, Table 3), though not as high as might be expected for a site exposed to high levels of dioxin. As noted previously (Brockman et al., 2009), these low fTCDD levels likely reflect background levels of environmental dioxin and/or exposure pathways associated with a folivorous diet and arboreal lifestyle, the latter limiting dermal exposure to dioxin in soils.

Potential sources of dioxin in *P. nigripes* include ingestion of contaminated plants and soils at NCTNP as this species has been known to feed on the ground, particularly in the dry season, and occasionally consume mud at small pools in the forest (Hoang Minh Duc, 2007). Black-shanked douc langurs at Hon Heo (Khanh Hoa Province) have been observed spending as much as ~20% of their daily time budget on the ground where they can access terrestrial water sources (Nadler, 2008), but no data are available on how much time (if any) *P. nigripes* spend resting/moving on the ground at NCTNP. As noted previously (Brockman et al., 2009), one of the known pathways of dioxin/dioxin-like compounds into vertebrates includes ingestion of contaminated soil by animals (Fries, 1995). Previous studies have shown that soil-to-plant transport of organohalgen compounds such as dioxin occur principally via volatization from the soil surface/disturbed soils and subsequent atmospheric deposition on plants (Fries, 1995), and are thus a likely source of exposure in this population of *P. nigripes* at NCTNP.

Concordant with our previous findings (Brockman et al., 2009), age was a reliable predictor of fTCDD concentrations in *Pygathrix* originating from south of the 17th parallel, fTCDD levels among juveniles being moderately elevated above those observed in adults (Table 3). Age was also a strong predictor of fTCDD concentrations in douc langur populations residing at NCTNP and EPRC, but in divergent ways. FTCDD levels in juvenile *P. cinerea* at EPRC were nearly 3-fold higher than those observed in adults, whereas dioxin concentrations in adult *P. nigripes* at NCTNP were nearly 2-fold higher than those observed in juveniles for reasons that are unclear. Overall, fTCDD concentrations in *Pygathrix* at EPRC were found to decline significantly with age (Fig. 11).The higher concentrations of fTCDD observed in the two juvenile *P. cinerea* at EPRC may derive from having originated from areas which were heavily sprayed with TCDD during the Vietnam War. Confiscation location data from EPRC quarantine records indicate that male 7-49 (1 year) and female 7-45 (3 years) originated from Quang Nam and An Lao District, Binh Dinh Provence, respectively (Nadler, unpubl. data), these regions having been intensively spayed with dioxin during the Vietnam War. Between September 1965 and June 1970, 289 and 194 US military missions were flown into Binh Dinh and Quang Nam Provinces, spraying 2,094,510 l and 1832953 l of Agent Orange in those provinces, respectively (calculated from HERPS Tape, 2000). The levels of likely residual TCDD concentrations remaining in soils in these provinces are unknown.

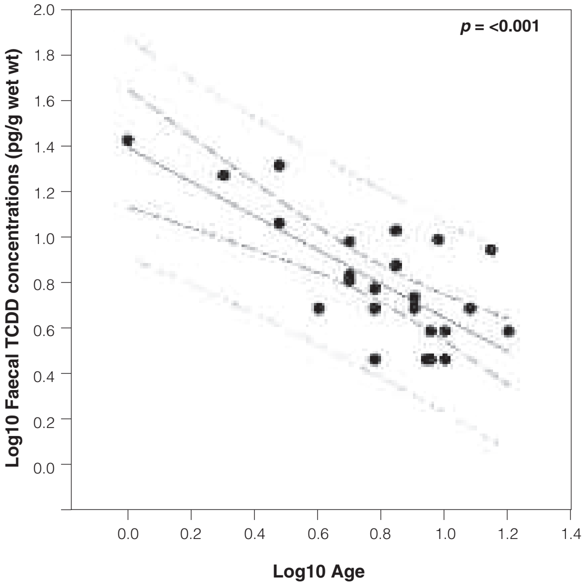

Fig.11. FTCDD concentrations decline with age in douc langurs at the Endangered Primate Rescue Center.

The divergent fTCDD-age results obtained from *P. nigripes* at NCTNP are difficult to explain, but they may be related to the potentially contaminated herbivorous diet consumed by obligate folivores. As noted previously (Brockman et al., 2009), dioxin has the capacity to persist in soils to a depth of greater than 10 cm for decades (Hatfield & 10-80 Committee, 1998), and has been estimated to be available for plant uptake for as long as 100 years (Paustenbach et al., 1992). Residual TCDD in soils at NCTNP (if any) can therefore function as a TCDD reservoir, having the capacity to remobilize and transport TCDD as dust which is deposited on the leaves and buds of *Afzelia xylocarpa* known to be consumed by adult *P. nigripes* at NCTNP.

Alternatively, divergent age-related variations in TCDD concentrations observed in NCTNP *P. nigripes* and *Pygathrix* at EPRC may be a consequence of species-specific and age-related variations in microbial communities that inhabit the gastrointestinal tract (Ochman et al., 2010). The array of microorganisms that coexist peacefully with their hosts is collectively referred to as microbiota or microflora, those inhabiting the gut being composed of strict anaerobes, over 50 bacterial phyla of which have been described to date (reviewed in Sekirov et al., 2010). The composition of microfloral communities is known to be host specific, changing continually throughout an individual's lifetime and susceptible to external (i.e. diet, geography) and internal (i.e. disease state, stress) modifications. Studies in humans show that infants are inoculated with maternal microbes at birth as they transverse the birth canal and that during the first year of life these communities are relatively simple, but then diversifies and stabilizes after weaning, resembling the microbiota communities of young adults/adults. Microbial communities maintain homeostasis of the intestinal mucosa and are crucially important for the development of systemic immune systems and for modulating nutritional and metabolic responses (Sekirov et al., 2010). Recent studies of fecal community composition in mammals show that phylogeny and diet influence bacterial diversity, bacterial phyla being observed to be partitioned among hosts according to diet, herbivore microbiota containing the most phyla, followed by omnivores and carnivores (Ley et al., 2008). An investigation of microbial communities in great apes (chimpanzees, gorillas) and humans reveals clear species-specific differences in microbial community structure (Yildirim et al., 2010), and while gut microbial communities of *Pygathrix* are unknown, one might reasonably posit that the divergent fTCDD-age effects observed between the NCTNP and EPRC populations of douc langurs may be related to species-specific differences in microbial communities and the likely influence of geography and diet on the microbiota in these respective populations. While it has been previously demonstrated that aerobic bacteria effectively degrade 2,3,7,8-TCDD in sediments (Field & Sierra-Alvarez, 2008), a similar role of anaerobic/gut microflora for dioxin degradation in vertebrates, including primates, has yet to be demonstrated.

Conclusion

The results of this research show that by employing a modified soil method (EPA Method 4025m) of fTCDD extraction in conjunction with a novel EIA procedure, we could reliably quantify fTCDD concentrations in wild *P. nigripes* at NCTNP. We also confirmed fTCDD-age effects in a larger sample of *P. nemaeus* and *P. cinerea* residing at EPRC and showed that juveniles exhibited substantially elevated fTCDD levels above those observed in adults whereas fTCDD concentrations in adult *P. nigripes* were substantially elevated above those observed in juveniles for reasons that are unclear, but may be related to diet and species-specific variation in gut microflora.

Acknowledgements

We are grateful to Tranh Van Thanh, Park Director, and Nguyen Van Thanh, Manager of Science and Technical Development, for granting DKB permission to conduct research at NCTNP. We are especially grateful to Bert Covert for his unflagging encouragement and support of this research and to Tilo Nadler, Director, and his staff for their continued enthusiastic support of our research at EPRC. We are also indebted to an anonymous reviewer who provided comments that substantially improved the manuscript. Funding was provided by the Schwartz Family Foundation Trust and by a Senior Faculty Grant from the College of Liberal Arts and Science, University of North Carolina Charlotte.

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Vietnamese Journal of Primatology (2013) vol. 2(2), 65-73

Seed dispersal by rhesus macaques (*Macaca mulatta*) in Son Tra Nature Reserve, central Vietnam: A preliminary report

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Key words: macaque, *Macaca mulatta*, defecation, seed dispersal

Summary

We studied the characteristics of seeds within feces, an important aspect of endozoochorous seed dispersal, in wild rhesus macaques (*Macaca mulatta*) inhabiting Son Tra Nature Reserve, central Vietnam. Fecal samples (n = 110) were collected from July to December 2011 (except November), and rate of seed occurrence, species/genus composition, rate of intact seeds, number of plant species, and seeds per single fecal sample were examined. We recorded 16 plant species including nine families from macaque fecal samples. The mean cubic diameter of seeds ranged from 0.9 to 17.1 mm. Seeds were found within the fecal samples during every month except December. The rate of seed appearance and the number of plant species within fecal samples were stable over the study period, but the number of intact seeds observed within fecal samples was higher in July than in other months. All seeds were intact except those of one of the *Garcinia* sp., which had a mean diameter of 10.5 mm. The rhesus macaques in Son Tra Nature Reserve thus seem to act as potential endozoochorous seed dispersers for seeds with mean cubic diameter <10 mm. We compared the characteristics of the defecated seeds in our study with those collected at other sites of studies among macaques to examine regional variations in the role of endozoochorous seed dispersers. We found that the rhesus macaques in Son Tra Nature Reserve are more likely to disperse relatively large seeds (5–10 mm^3) and to disperse smaller numbers of plant species within single feces than do macaques at other study sites. These differences are likely due to differences among study sites in both vegetation and the dietary habits of the macaques. Our results suggest that the characteristics of the seeds dispersed by macaques are not universal, but rather vary in accordance with habitat properties.

Nghiên cứu ban đầu về sự phát tán hạt bởi loài khỉ vàng (*Macaca mulatta*) tại khu bảo tồn thiên nhiên Sơn Trà, miền Trung Việt Nam

Tóm tắt

Chúng tôi nghiên cứu về đặc điểm của các hạt thực vật còn lưu trong phân, một yếu tố quan trọng khi nghiên cứu về sự phát tán hạt nhờ động vật, ở loài khỉ vàng tại khu bảo tồn thiên nhiên Sơn Trà, miền Trung Việt Nam. Chúng tôi đã thu 110 mẫu phân từ tháng 7 đến tháng 12 năm 2011 (trừ tháng 11). Chúng tôi phân tích tỷ lệ hạt xuất hiện, thành phần loài và giống, tỷ lệ hạt còn nguyên. Và phân tích số lượng loài thực vật, số hạt trong mỗi mẫu phân. Kết quả ghi nhận 16 loài thực vật thuộc 9 họ trong những mẫu phân của loài khỉ vàng. Đường kính trung bình của hạt thay đổi từ 0.9 đến 17.1 mm. Hạt được tìm thấy trong tất cả các mẫu phân trong các tháng ngoại trừ tháng 12. Tỷ lệ hạt xuất hiện và số lượng các loài thực vật trong những mẫu phân là ổn định trong suốt thời gian nghiên cứu, nhưng số lượng hạt còn nguyên được phát hiện trong tháng 7 cao hơn các tháng còn lại.Tất cả các hạt còn nguyên đều có đường kính trung bình khoảng 10.5 mm, ngoại trừ đối với loài *Garcinia* sp. Có thể nói loài khỉ vàng tại khu BTTN Sơn Trà có vai trò phát tán hạt có đường kính nhỏ hơn 10 mm. Chúng tôi cũng đã so sánh đặc điểm của các hạt trong nghiên cứu này với các địa điểm khác trong cùng loài nhằm đánh giá sự biến thiên.Chúng tôi nhận thấy rằng loài khỉ vàng ở khu BTTN Sơn Trà phát tán các hạt tương đối lớn (5-10 mm^3) nhưng lại ít hơn về số lượng loài thực vật trong mỗi mẫu phân so với những khu vực nghiên cứu khác. Sự khác nhau đó bắt nguồn từ sự khác nhau về thảm thực vật và thói quen ăn của loài khỉ vàng tại những điểm nghiên cứu khác nhau. Kết quả của chúng tôi cho thấy những đặc điểm phát tán hạt bởi khỉ vàng không đồng nhất, mà ngược lại rất riêng tùy thuộc vào đặc điểm của sinh cảnh sống.

Introduction

Macaques (Macaca spp.) constitute one of most widely distributed primate clades in the world (Thierry, 2011). The diets of macaques include at least some fruits in both temperate (rhesus macaques *Macaca mulatta*: Goldstein & Richard, 1989; Japanese macaques *M. fuscata*: Tsuji et al., 2006; Barbary macaques *M. sylvanus*: Ménard, 1985; Tibetan macaques *M. thibetana*: Zhao et al., 1991) and tropical/sub-tropical regions (long-tailed macaques *M. fascicularis*: Lucas & Corlett, 1998; rhesus macaques: Corlett, 2011; southern pig-tailed macaques *M. nemestrina*: Caldecott, 1986; Taiwanese macaques *M. cyclopis*: Su & Lee 2001; crested macaques *M. nigra*: O'Brien & Kinnard, 1997). Compared with their sympatric avian seed dispersers, macaques consume larger amounts of fruit at once (Terakawa et al., 2008), have larger gape sizes (Noma & Yumoto, 1997; Kitamura et al., 2002), and exhibit longer retention times in the gut: 5–224 min for birds (Murphy et al., 1993; Kitamura, 2007) versus 37–54 h for macaques (Lambert, 2002; Otani, 2004; Tsuji et al., 2010). All these characteristics contribute to greater/wider seed-dispersal potential of macaques compared to birds. Indeed, macaques sometimes disperse seeds as far as several hundred meters (Terakawa et al., 2008; Albert, 2011; Liu et al., 2012). Moreover, the sites at which seeds are dispersed by macaques are known to be characterized by enhanced germination and/or growth of several plant species (Tsujino et al., 2009; McConkey & Brockelman, 2011; but see Nakashima et al., 2010). Finally, the passage of seeds through the digestive tract has a neutral or positive effect on seed germination and viability for several species (Otani & Shibata, 2001; Albert, 2011). Thus, macaques are likely to strongly affect the spatial and genetic structure of plant populations and thereby contribute to the dynamics of their habitat's plant community via seed dispersal.

Macaques have two dispersal methods: spitting and defecation. In general, macaques spit out large seeds, whereas they ingest smaller seeds without destroying them (Corlett & Lucas, 1990; Yumoto et al., 1998; Kitamura et al., 2002). To evaluate the role of macaques as endozoochorous seed dispersers, it is essential to examine which plant species and/or how many seeds are present in their feces (Otani, 2003). For example, Japanese macaques disperse plant seeds in both warm and cool temperate forests in the Japanese archipelago through defecation and spitting (Yumoto et al., 1998; Otani, 2003; Tsuji, 2011; Tsuji et al., 2011). However, little quantitative data on the seed-dispersal characteristics of macaques in tropical regions exist in terms of the number of plants whose seeds are dispersed, the rate of seed appearance, the size of defecated seeds, the number of seeds per feces, the number of plant species per feces, and the rate of intact seeds. We present a preliminary report on the characteristics of seeds dispersed through defecation. We analyzed fecal samples from wild rhesus macaques in Son Tra Nature Reserve (STNR), Danang City, central Vietnam and then compared the results with those from Japanese macaques inhabiting the temperate region to examine the unique characteristics of the macaques living in subtropical regions.

Materials and methods

Son Tra Nature Reserve (16°07'N, 108°18'E) is located on Son Tra Peninsula, which is ca. 10 km northeast from the central area of Danang City. Danang City lies in the typical tropical monsoon zone and has average annual temperature and average annual rainfall of ca. 26°C and ca. 2505 mm, respectively (Linh et al., 2012). There are two main seasons annually: a period with lower precipitation (January–July) and a per-humid period, sometimes with excessive precipitation (August–December) (Nguyen Thi Hien et al. 2000; Linh et al., 2012;) (Fig. 1). The majority of the nature reserve is covered by secondary forests, composed of Dipterocarpaceae, Moraceae, and Theaceae in lowland areas (<300 m asl), and by tropical rain forests, composed of Fagaceae, Dipterocarpaceae, Anacardiaceae, Moraceae, and Sapindaceae, at higher altitudes (300–650 m asl) (Lippold 1977; Birdlife International Vietnam Programme & Forest Inventory and Planning Institute, 2001).

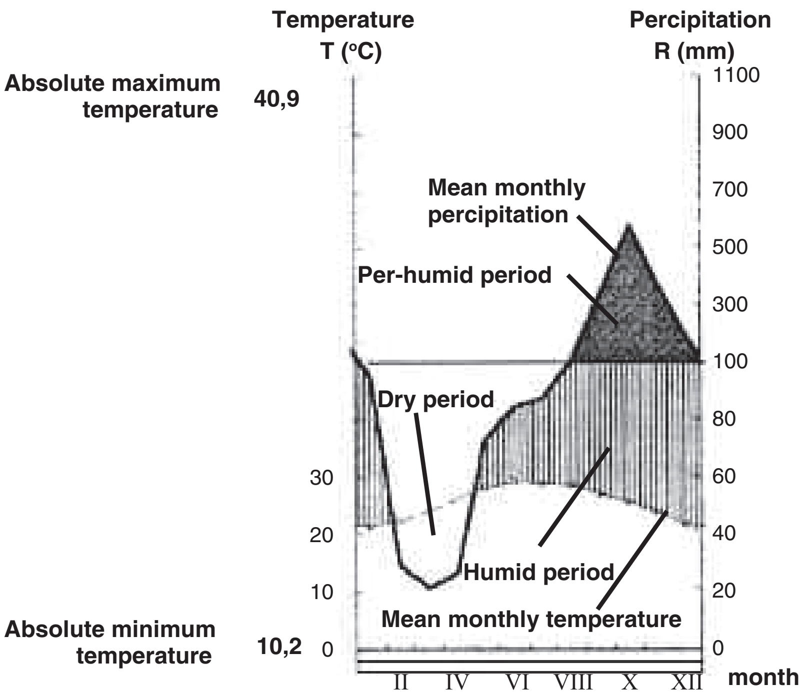

Fig.1. Climate diagram for Danang.

With the exception of November, when heavy rain prevented us from going into the forest, we conducted field surveys between July and December 2011. We collected the feces of rhesus macaques (range: 18–29) by cycling (less than 10 km/h) surveys along the paved road within the STNR on clear days (3–5 surveys per month). We discriminated between the feces of primates and those of sympatric canids (small Indian civet *Viverricula indica* and small Asian mongoose *Herpestes javanicus*) on the basis of shape and size. We also assumed that collected fecal samples were from rhesus macaques and not from other species of macaques or douc langurs because we observed only rhesus macaques on or near the paved road during the study (Tsuji et al., 2012).

Collected fecal samples were taken to Hue University, central Vietnam, where they were washed through a 0.5-mm mesh sieve and seeds remaining in the sieve were collected. We then identified and counted the seeds. Due to lack of information about seeds of Vietnamese plants, seed identification was based on characteristics of similar species in other South-East Asian countries (Thailand and Malaysia); due to this limitation, the majority of plants could be identified only to the genus level, and not the species level. We measured the longest axis (a_1), second-longest axis (a_2), and third-longest axis (a_3) of randomly selected seeds of each plant species/genus to the nearest 0.05 mm using a Vernier caliper (Tsuji et al., 2011). When large numbers of seeds were obtained for a given species, we measured 10 randomly selected seeds, whereas we measured all seeds along each dimension when we could not obtain sufficient numbers. The mean cubic diameter (MCD) of seeds was calculated from averaged values (Tsuji et al., 2011).

We tested monthly changes in the seed-appearance ratio with the *chi*-square test for independence. We tested the numbers of seeds and plant species per single fecal sample with the Kruskal–Wallis test. For the latter analysis, *post hoc* Steel–Dwass tests were conducted. All statistical analyses were performed using the statistical software R. 2.15.0 (R Developmental Core Team, 2012). For these analyses, significance (α) was set at 0.05.

Results

A total of 110 fecal samples were collected during the study period. In total, 58.2% (64/110) of feces contained the seeds of 16 plant species (Table 1). Rates of seed appearance were 72.2% (13/18) in July, 37.9% (11/29) in August, 42.3% (11/26) in September, 52.4% (11/21) in October, and 0% (0/16) in December, and the monthly change in the appearance-ratio of seeds was marginally significant (*chi*-square test, χ^2 = 9.45, *df* = 4, *P* = 0.051). The numbers of plant species (including unidentified species) detected from July to October were six, eight, six, and three, respectively. The composition of the plants seeds detected differed between July and the three other months (rainy season): smaller seeds, such as *Ficus* sp. (MCD: 0.9 mm), were detected in fecal samples collected in July, but large seeds, with the exception of those of *Microcos tomentosa*, were detected in samples collected in the dry season. On the other hand, the plant compositions reflected in the seeds collected during the other months within the rainy season were relatively similar to one another and contained large seeds, such as *Baccaurea* sp and *Annonaceae* species (Table 1). The mean ± SD number of seeds in a single fecal sample was 61.1 ± 204.2 (*n* = 64). The numbers of seeds in individual fecal samples collected between July and October were 155.9 ± 319.4, 6.3 ± 6.4, 4.8 ± 3.8, and 3.4 ± 2.7, respectively (Table 1), and the profiles changed significantly by month (Kruskal–Wallis, *df* = 3, χ^2 = 15.5, *P* < 0.001). Furthermore, the Steel–Dwass post hoc tests indicated that the mean seed number in July was significantly greater than those in the other three months (*P* < 0.05). The mean ± *SD* number of plant species whose seeds were detected in a single fecal sample was 1.3 ± 0.6 (n = 64). The numbers of seeds in single fecal samples collected in each month from July to October were 155.9 ± 319.4, 6.3 ± 6.4, 4.8 ± 3.8, and 3.4 ± 2.7, respectively (Table 1); these figures reflect significant changes according to month (Kruskal–Wallis, df = 3, ♩ 2 = 12.3, P = 0.007). Furthermore, the Steel–Dwass *post hoc* test indicated that the mean seed number in August was significantly lower than that in July ($P < 0.05$). Seed size (in MCD) ranged from 0.8 mm (unidentified plant) to 17.1 mm (*Gnetum* sp.) with a mean ± *SD* of 6.7 ± 4.4 mm (n = 16). Evidence of seed destruction was obtained only for *Garcinia* sp.—one cracked seed of this relatively large-seeded species was found in fecal samples.

Discussion

We found a clear seasonal pattern of seed dispersal by rhesus macaques in Son Tra Nature Reserve; the rate of seed appearance and number of seeds in single fecal samples in July were greater than were those in the other months, and this was attributable primarily to the greater number of *Ficus* seeds in July. Seasonality in dispersal has also been found in study sites in a cool temperate region (Otani, 2003; Hayashi, 1992; Tsuji et al., 2011); for example, on Kinkazan Island in northern Japan, the rate of seed appearance and number of seeds in single fecal samples were greater in summer (corresponding to plant growth/fruiting season) and fall (fruiting season) and lower in winter (dormant season) (Tsuji et al., 2011). In Bonbori in western Tokyo, on the other hand, the corresponding values were greater in spring (dormant/germinating season) and summer (Tsuji, 2011). However, collection of a sufficient number of samples at Son Tra Nature Reserve between January and June may change the present results. For example, on Yakushima Island, in a warm temperate region, a greater number of *Ficus* seeds were found during a longer period (7–8 months per year), and the degree of seasonality of seed dispersal was unclear (Yumoto et al., 1998). To confirm seasonal dispersal patterns in STNR, it will be necessary to collect long-term data.

The annual mean MCD of seeds detected from single fecal samples collected at STNR (6.7 mm, range: 0.8–17.1) was greater than those collected from macaques at other study sites: Yakushima Island (2.5 mm, range: 1.1–6.4 mm) (Otani and Shibata 2000); Shimokita Peninsula (2.7 mm, range: 1.2–4.1 mm) (Otani, 2003); Kashima (2.3 mm, range: 1.2–4.1 mm) (Otani, 2003); Bonbori (4.2 mm, range: 1.6–11.8 mm) (Tsuji, 2011); Kinkazan Island (3.0 mm, range: 0.8–10.8 mm) (Tsuji et al., 2011); and Bukit Timah, Singapore (4.3 mm, range: 0.2–13.4 mm) (Lucas & Corlett, 1998) (Table 2). Furthermore, the maximum MCD of seeds collected at Son Tra Nature Reserve (17.1 mm) was also greater than those collected from macaques at other study sites (Table 2). These observations suggest that the macaques at STNR may disperse seeds with a wider range of sizes than do macaques in other populations.

In Son Tra Nature Reserve, only one seed from *Garcinia* sp., which is relatively large (MCD = 10.5 mm (Table 1)), was destroyed by the rhesus macaques. It has been reported that seeds of *Garcinia* sp. are among the larger species of seeds swallowed by northern pig-tailed macaques (Albert, 2011) and long-tailed macaques (Lucas & Corlett, 1998). Tsuji et al. (2011) showed that the rate of intact seeds was negatively correlated with the MCD of the seeds in Japanese macaques. These findings suggest that macaques at Son Tra Nature Reserve act as seed dispersers for relatively small seeds (MCD <10 mm) and as seed destroyers for large seeds that are accidentally swallowed (MCD ≥10 mm). The ease with which a given species is peeled may affect the possibility that its seeds will be swallowed. We will test the generalizability of this hypothesized relationship in future studies with more fecal samples from Son Tra Nature Reserve and/or other study sites.

Table. 1. Monthly changes in mean+ SD number of seeds obtained from fecal samples mof the rhesus macaques (*Macaca mulatta*) at Son Tra Nature Reserve, Central Vietnam in 2011.

Family (genus, species)	MCD (mm) (mean ± *SD*)		Number of seeds / feces (mean ± *SD*) July *n* = 18	August *n* = 29	September *n* = 26	October *n* = 21	December *n* = 16
Moraceae (*Ficus* sp.)	0.85 ± 0.09	(*n* = 10)	416.2 ± 497.3 (5)	—	—	—	—
Tiliaceae (*Microcos tomentosa*)	10.56 ± 1.08	(*n* = 10)	1.0 (1)	4.0 (1)	1.0 (1)	—	—
Euphorbiaceae (*Baccaurea* sp.)	8.44 ± 1.11	(*n* = 10)	—	23.0 (1)	2.6 ± 2.3 (7)	3.4 ± 2.6 (10)	—
Annonaceae	7.36 ± 0.22	(*n* = 10)	—	5.7 ± 4.5 (3)	9.0 ± 8.5 (2)	2.0 (1)	—
Sapotaceae	5.87 ± 0.59	(*n* = 10)	—	2.0 (1)	6.0 (1)	—	—
Guttiferae (*Garcinia* sp.)	10.53 ± 1.80	(*n* = 8)	—	2.0[a] (1)	—	—	—
Gnetaceae (*Gnetum* sp.)	17.05 ± 1.04	(*n* = 10)	—	—	7.0 (1)	—	—
Sapindaceae (*Lephisanthes* sp.)	8.17 ± 0.76	(*n* = 10)	—	1.0 (1)	—	—	—
Annonaceae	7.19 ± 0.07	(*n* = 2)	—	—	1.0 (1)	—	—
Unidentified 1	0.79 ± 0.08	(*n* = 10)	66.8 ± 82.5 (5)	—	—	—	—
Unidentified 2	1.18 ± 0.18	(*n* = 10)	41.5 ± 23.3 (2)	—	—	—	—
Unidentified 3	1.00 ± 0.07	(*n* = 10)	16.8 ± 16.2 (9)	—	—	—	—
Unidentified 4	8.90 ± 0.44	(*n* = 10)	1.0 (1)	—	—	—	—
Unidentified 5	4.72 ± 0.23	(*n* = 7)	—	7.0 (1)	—	—	—
Unidentified 6	9.20 ± 0.35	(*n* = 10)	—	6.5 ± 3.5 (2)	—	—	—
Unidentified 7	5.12 ± 0.48	(*n* = 10)	—	—	—	1.0 (1)	—
Total number of seeds			155.9 ± 319.4 (13)	6.3 ± 6.4 (11)	4.8 ± 3.8 (11)	3.4 ± 2.7 (11)	—
# Species detected / feces			1.8 ± 0.9 (13)	1.0 ± 0.0 (11)	1.2 ± 0.4 (11)	1.1 ± 0.3 (11)	—

Species are arranged in decreasing order of the mean seed number. *n* means number of collected fecal samples. MCD: cubic diameter.
Figures in parenthesis mean number of fecal samples containing seeds of given species.
[a] One seed was destroyed

Table. 2. Regional variations in characteristics of seeds detected from feces of wild macaques (*Macaca fuscata*, *M. leonine*, *M. fascicularis*, *M. mulatta*).

Study site	Yakushima	Bonbori	Kashima	Kinkazan	Shimokita	Khao Yai	Bukit Timah	Son Tra
Climate zone	WT	WT	CT	CT	CT	TR	TR	TR
Sampling period	1995-2004	2007-2008	2000-2001	2000-2009	2000-2001	2009-2010	1986-1987	2011
Dispersal agent	*Macaca fuscata*	*M. fuscata*	*M. fuscata*	*M. fuscata*	*M. fuscata*	*M. leonina*	*M. fascicularis*	*M. mulatta*
# Fecal samples	132	52	107	1294	75	335	76	110
# Feces containing seeds (%)	131 (99.2)	44 (84.6)	99 (92.5)	747 (57.7)	59 (78.7)	298 (89.0)	-	64 (58.2)
# Identified plant species	33	20	18	35	12	93	24	16
MCD (Mean± *SD*) (mm)	2.5± 1.7 (*n* = 12)	4.2± 2.6 (*n* = 20)	2.3± 1.1 (*n* = 15)	3.0± 2.0 (*n* = 35)	2.7± 1.2 (*n* = 11)	-	4.3± 4.4 (*n* = 24)	6.7± 4.4 (*n* = 16)
Range	1.1–6.4	1.6-11.8	1.2-4.1	0.8-10.8	1.2-4.1	< 20[a]	0.2-13.4	0.8-17.1
# Species/feces (Mean± *SD*)	2.8± 1.4 (*n* = 131)	1.8± 0.9 (*n* = 44)	2.4± 1.5 (*n* = 99)	2.0± 0.9 (*n* = 747)	1.9± 0.8 (*n* = 59)	1.9 ± 1.4 (*n* = 298)	-	1.3± 0.6 (*n* = 64)
References	Otani (2005)	Tsuji (2011)	Otani (2003)	Tsuji et al. (2011)	Otani (2003)	Albert (2011)	Lucas and Corlett (1998)	This study

CT: cool temperate zone, WT: warm temperate zone, TR: tropical zone, MCD: cubic diameter of seeds.
[a] Shown in length

The ratio of seed appearance at Son Tra Nature Reserve during our study was 58%, which was lower than the corresponding values for macaques at other study sites: Yakushima Island, 99% (Otani & Shibata, 2000); Shimokita Peninsula, 79% (Otani, 2003); Kashima, 93% (Otani, 2003); and Khao Yai, 89% (Albert, 2011). However, the ratio on Kinkazan Island, 58% (Tsuji et al., 2011), was similar to that in the present study (Table 2). Furthermore, the number of species detected in single fecal samples collected at Son Tra Nature Reserve was lower than that in those collected from single samples from macaques at other study sites (Table 2). Although we cannot eliminate the possibility that these lower values were due to the small amount of data in the dry season, when larger numbers of smaller seeds (such as *Ficus* spp.) were swallowed, our results suggest regional variations in dispersal variables. Seeds in the tropical region are larger than are those in the temperate region (Moles et al., 2007). As the macaques tend to spit out large seeds (≥10 mm in seed length) without swallowing (Corlett & Lucas, 1990; Yumoto et al., 1998; Albert, 2011), regional variations in plant composition due to climate may cause regional variations in seed dispersal by macaques. To test this possibility, further studies are required with other macaque populations/species, especially those inhabiting the tropical region.

Conclusions

Similar to macaques in the temperate region, macaques in the subtropical seasonal forests of central Vietnam (and perhaps South-East Asia) disperse seeds of various plant species. The results indicated the following: 1) a low ratio of seed appearance in feces; 2) the large size of dispersed seeds; and 3) the small number of seed species per single feces. Some of these observations seemed to reflect the environmental conditions (e.g., vegetation) and environment-related foraging behavior of the macaques. The findings of the present study suggest that the role of macaques as endozoochorous seed dispersers may show regional variation.

Acknowledgments

We would like to thank Mr. Le Van Nhi, Tran Van, Mr. Ly Thi Kiem, and Mr. Ho Ngoc Luong for their kind support during the field survey at Son Tra NR. We are also grateful to Mr. Le Van An for his facilities at Hue University. We would also like to thank Mr. Le Thai Hung for plant identification. Many thanks go also to a reviewer for comments and corrections.

This study was funded by AS-HOPE and ITP-HOPE of the Japan Society for the Promotion of Science to Y. Tsuji (2010-2011) and by Grants-in-Aid from the Department of Academy and Technology of Japan (Japan Society for the Promotion of Science, nos. 23780160 and 20255006).

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Vietnamese Journal of Primatology (2013) vol. 2(2), 75-82

Vocalization of red- and grey-shanked douc langurs (*Pygathrix nemaeus* and *P. cinerea*)

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Key words: Vocalization, red-shanked douc langur, grey-shanked douc langur, *Pygathrix nemaeus*, *Pygathrix cinerea*

Summary

The vocal repertoires of red- and grey-shanked douc langurs (*Pygathrix nemaeus* and *P. cinerea*) have never been described before. Information derived from the recordings of the captive groups of the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam, was used for a first quantitative description of these species' calls based on the calculation of pairwise dissimilarity indexes and cluster analysis. Distinctive features were found across the 8 vocal types identified for each species. Some of the clusters are feasibly representing call variants belonging to an acoustic continuum, but others clearly represent distinct types. The potential changes occurring due to sex- and age-dependent characteristics were not investigated in the present study.

Nghiên cứu về âm thanh học của hai loài chà vá chân nâu và chà vá chân xám (*Pygathrix nemaeus* và *P. cinerea*)

Tóm tắt

Nghiên cứu về âm thanh học của loài chà vá chân nâu và chà vá chân xám chưa từng được thực hiện trước đây. Những dữ liệu về tiếng kêu thu thập từ những đàn chà vá nuôi nhốt tại Trung tâm cứu hộ thú linh trưởng, Vườn quốc gia Cúc Phương, Việt Nam đã được phân tích một cách định lượng căn cứ vào các chỉ số không đồng nhất từng cặp và theo nhóm. Những đặc điểm khác biệt đã được tìm thấy ở 8 loại tiếng kêu khác nhau ở mỗi loài. Một số nhóm âm thanh thể hiện sự biến thiên đa dạng của tiếng kêu trong cùng một dải âm vực, tuy nhiên một số khác lại thể hiện sự khác biệt rõ ràng. Những thay đổi về âm thanh do sự khác nhau về giới tính và độ tuổi chưa được tìm hiểu kỹ trong nghiên cứu này.

Introduction

The study of primate vocal behaviour can offer important insights into the communication processes within a species, especially when seen in the light of the ecological and social information available (Maretti et al., 2010). Knowledge of the vocal repertoire can also be crucial to understand the biology of a species and set the appropriate policies for its conservation (Laiolo, 2010).

Previous studies have shown a strong influence of genetics on primate vocal repertoires (Hammerschmidt & Fischer< 2008) frequently leading to species-specific vocal types (Gamba & Giacoma, 2005) or showing shared types differing across species by more subtle acoustic properties basically due to morpho-anatomic variability (Gamba et al., 2012a). Sex-, age- and individual-specific features of primate calls may also derive from morpho-anatomical or physiological differences (Gamba et al., 2012b).

In this study we investigated the vocal repertoires of the red- and grey-shanked douc langur (*Pygathrix nemaeus* and *P. cinerea*). We identified the vocal types emitted by captive individuals using Affinity Propagation (hereafter AP) clustering. The resulting clusters were then confirmed as discrete vocal categories using multivariate analysis.

The information obtained is feasibly not sufficient to describe the complete vocal repertoire but largely increase knowledge of the species vocal communication. The description of the vocal types can be also valuable to recognize these monkeys during acoustic surveys in their natural habitats.

Material and Methods

The animals recorded in this study were housed at the Endangered Primate Rescue Center, Cuc Phuong National Park, Vietnam. This institution is part of the ‘Vietnam Primate Conservation Programme’ of the Frankfurt Zoological Society. For each species we had a different number of study groups and individuals. We recorded 10 individuals of *Pygathrix cinerea* hosted in 4 different enclosures, 15 *P. nemaeus* kept in 3 different enclosures (Table 1).

Table. 1. Composition and sex ratio of the study groups.

Species	N_{males}	$N_{females}$	$N_{youngsters}$	Total
Pygathrix cinerea	3	4	3	10
Pygathrix nemaeus	4	6	5	15

Recordings

We recorded the animals between March 4^{th} and March 23^{rd}, 2010. Recordings were collected using solid-state audio recorders Sound Devices 702 and Marantz PMD 671, equipped with Sennheiser ME66 (with windshield Sennheiser MZW66) and G.R.A.S. 40 BE microphones. During all sessions we used the *ad libitum* sampling method (Altmann, 1974). Vocal signals were recorded at a sampling rate of 96000 Hz (16 bit depth). All vocalizations were spontaneously emitted and nothing was done to elicit the emission of vocal signals, no vocal or visual playbacks were used. All subjects were treated according to the guidelines for animal behaviour studies (Anonymous, 2002).

Acoustic analyses

The recording sessions were split into shorter files using SoundForge 7.0 (Madison Media Software Inc., Sony Corporation of America, U.S.A.) and Adobe Soundtrack 2 (Apple Inc., U.S.A.). Once a vocalization was isolated from the recording session file, it has been saved in a new file and a silence of 0.5 sec was inserted at the beginning and the end. All sounds were normalized using the function “scale to peak” in the software Praat (Boersma & Weenink, 2006). The files prepared in this way were then treated in two different ways: (a) they were submitted to a similarity measuring procedure or (b) they were analysed using a semi-automatic extraction of the acoustic properties.

The generation of a pairwise similarity index (a) across the species-specific vocalizations was performed using an implementation of the dynamic time warping. The dynamic time warping is a procedure of spectrogram alignment increasingly used in bioacoustics to compare sounds belonging to large datasets. At the basis of this procedure, there is a method commonly used in speech science relying on the calculation of cepstrum coefficients (Davis & Mermelstein, 1980). These coefficients provide a representation of the energy distributed at the various frequencies in the sound spectrum and, even if the computation of cepstral coefficients is usually performed to match the sensitivity of the human ear, they have been shown to be useful in the study of animal calls (Trawicki et al., 2005; Clemins & Johnson, 2006; Ranjard & Ross, 2008; Tao et al., 2008; Brown & Smaragdis, 2009; Ranjard et al., 2010). We used a method currently implemented in a free package called DTWAVE (University of Auckland). A sequence of cepstrum coefficients was computed for each call by means of a mel filterbank (Ranjard et al., 2010) using the Hidden Markov Model Toolkit (Young, 1994). When they were submitted to the Hidden Markov Model Toolkit we used a target rate of 50000 ns and a window size of 100000 ns. Once all the cepstral coefficients were aligned and rescaled, the software constructed an average vector sequence. Then the dynamic time warping calculated the pairwise distances between all the calls in the dataset until only the sequence representing an average of all call sequences remained (see Ranjard & Ross, 2008).

The measurement of the acoustic properties of each vocalization was performed in Praat in the perspective of the source filter theory (Fant, 1960; Gamba & Giacoma, 2007; Taylor & Reby, 2010). To detect source features (MeF0, MiF0, MaF0, enstF0, sumvar, rangeF0, F0stdev, F0start, F0end), Fast Fourier transforms were generated for each call (frequency range: 0-12000 Hz; maximum: 100dB/Hz; dynamic range: 60 dB; pre-emphasis: 6.0 dB/Oct; dynamic compression: 0.0). The fundamental frequency and its variation were measured by using the autocorrelation method ["Sound: To pitch (ac)…"]. We adjusted the analysis parameters according to the range of variation in each vocalization (Gamba & Giacoma, 2005; Gamba et al., 2012a; 2012b).

Slightly different settings were used for those calls showing very low pitch. The files containing these calls were resampled to 22050 Hz. For these calls we have also used a 'stop Hann band' filter in Praat for frequency between 0 and 100 Hz (smoothing factor: 100 Hz) and were then analysed with specific settings [(advanced pitch settings) max number of candidates: 35; voice threshold: 0.15; octave-jump cost: 0.01; voiced/unvoiced cost: 0.15]. All measurements were taken during a step-by-step monitored process, where the operator could interrupt the analysis and modify the analysis parameters. A Praat script was used to automate file opening and editing and file saving of the measurements.

Quantitative and statistical analyses

The matrix of dissimilarity indexes generated from DTWAVE was submitted to a clustering procedure called AP (Frey & Dueck, 2007) using the *apcluster* package in R (Hornik, 2013). The algorithm used a squared negative Euclidean distance to measure dissimilarity and identify clusters. The number of clusters and the number of vocalizations used as exemplars were not user-defined. The result of the application of AP to measure vocal signal similarity were diagonally symmetric matrices, one for each species, in which each matrix element quantified the pairwise agreement between two of the calls. We extracted two parameters from this analysis: the number of the clusters and the filename of the calls assigned to each cluster. Then, the information concerning each vocalization and the cluster to which it was assigned was transferred into a table and used as 'grouping variable' in IBM SPSS Statistics 20 for Mac (IBM Corporation, Armonk, New

York, USA) for a stepwise Discriminant analysis (hereafter DFA). It is to be noticed that neither the parameters used during the dynamic time warping procedure nor the information derived from the clustering process were directly related with the acoustic measurements used in the Discriminant process. We have reported the average percentage of correctly classified instances (CCI) to the cluster and the cross-validated percentage of correctly classified instances (c.-v.) for each DFA.

Results

Description of the vocal repertoire

High quality calls entered the procedure for the calculation of the pairwise distances using DTWAVE. We set a procedure for each species.

The vocal emissions of *Pygathrix cinerea*.

We aimed to understand whether groups of the recorded calls could be identified using the dissimilarity matrix generated in DTWAVE. The AP analysis yielded 8 clusters (number of samples = 179; number of iterations = 191; input preference = -3.811533; sum of similarities = -32.84342; sum of preferences = -34.3038; net similarity = -67.14722). The cluster membership was then used as grouping variable in the DFA. For *P. cinerea* we found a significant model and high rate of correct classification [N = 179, Wilks' Lambda = 0.027, $F_{14,340}$ = 139.785, $p < 0.001$, CCI = 91.1% (c.-v. 89.4%)]. The most important parameters in this Discriminant model were duration (0.992) and F0start (0.078).

The spectrograms of the vocal types identified during the clustering process and then validated using the DFA as discrete categories (Fig. 1, Table 2).

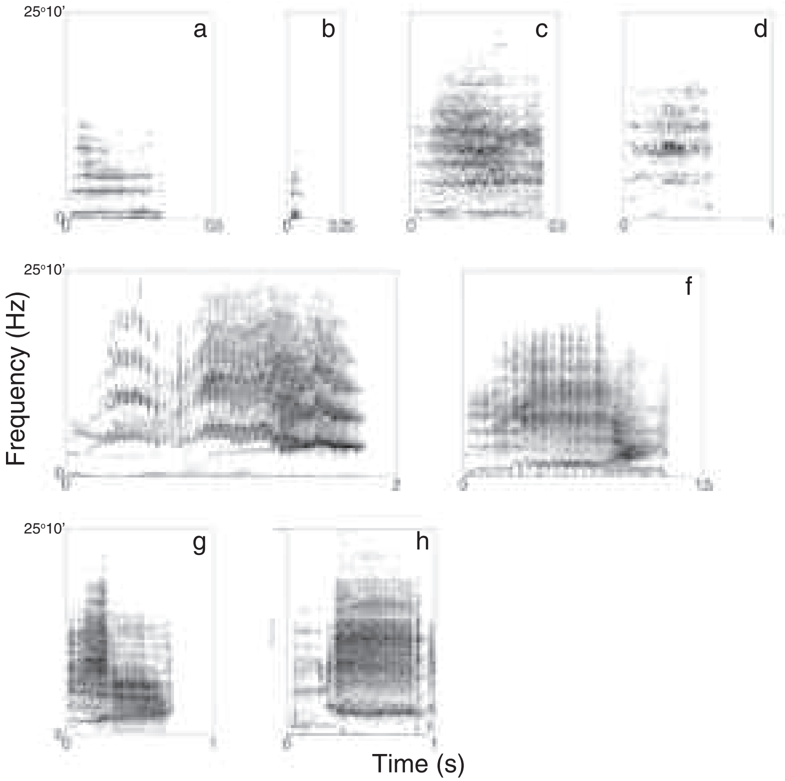

Fig.1. The spectrograms of the vocal types we recorded for *Pygathrix cinerea*: (a) Wroo, (b) Up, (c) Tzii, (d) Trill, (e) Friii, (f) Trill-wroo, (g) Phre, (h) Uo-traa. All spectrograms were generated in Praat with the following parameters: window length: 0.025 sec, time range as shown (0.25-2.00 s); frequency range: 0-24000 Hz; maximum: 50 dB/Hz; dynamic range: 35-55 dB; pre-emphasis: 6.0 dB/Oct; dynamic compression: 0.0.

Table. 2. Description of the *Pygathrix cinerea* vocal types. The spectrogram of a call resulting near to the vocal type centroid during the multivariate analysis is shown in Fig. 1.

Wroo	Medium-range low-pitched guttural call. The sonograms show a clear formants structure (Fig. 1a).
Up	Low-pitched short harsh vocalization (Fig. 1b).
Tzii	High-pitched vocalization showing frequency modulation. Compared to the Trill the harmonic structure is less visible due to the remarkable chaotic pattern (Fig. 1c).
Trill	Short high-pitched vocalization with frequency modulation (Fig. 1d).
Friii	Long and harsh high-pitched vocalization with remarkable frequency modulation (Fig. 1e).
Trill-wroo	High-pitched vocalization showing with frequency modulation followed by a short low-frequency emission (with a wroo-like structure, Fig. 1f).
Phre	Similar to *trill-wroo* but shorter and with different fundamental frequency values in the final portion (Fig. 1g).
Uo-traa	Vocalization with a high-pitched modulated part preceded and followed by short low-pitched units (with visible harmonics, Fig. 1h).

The vocal emissions of *Pygathrix nemaeus*.

The AP analysis of the pairwise distances generated for the calls of *Pygathrix nemaeus* yielded 8 clusters (number of samples = 264; number of iterations = 143; input preference = -4.804802;

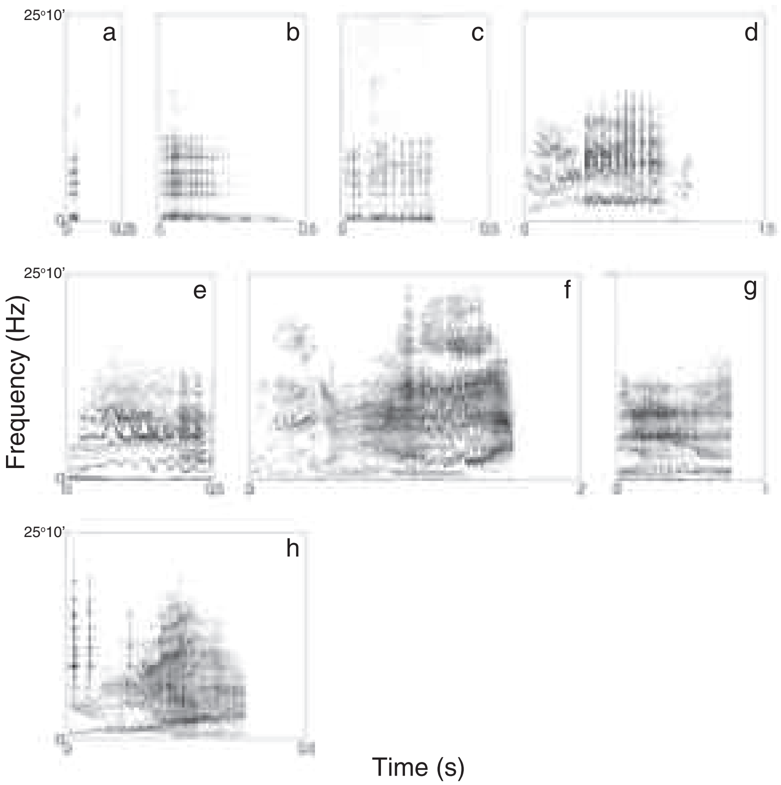

Fig.2. The spectrograms of the vocal types we recorded for *Pygathrix nemaeus*: (a) Up, (b) Wroo, (c) Slow Wroo, (d) Friii, (e) Trill, (f) Ruae-Ruae, (g) Uo-traa, (h) Trill-wroo. All spectrograms were generated in Praat with the following parameters: window length: 0.025 sec, time range as shown (0.25-2.00 s); frequency range: 0-24000 Hz; maximum: 50 dB/Hz; dynamic range: 35-55 dB; pre-emphasis: 6.0 dB/Oct; dynamic compression: 0.0.

sum of similarities = -65.32299; sum of preferences = -43.24321; net similarity = -108.56628), which were then submitted to DFA. The classification process showed a consistent part of the set correctly assigned to the previously identified clusters (CCI = 84.8%, c.-v. 83.0%) and a significant Discriminant model (N = 264, Wilks' Lambda = 0.036, $F_{14,510}$ = 172.351, p < 0.001). The six acoustic measures participating primarily to the Discriminant model were duration (0.937), enstF0 (0.118), sumvar (0.062), rangeF0 (0.149), F0stdev (0.143), and F0start (0.018). The spectrograms of the call types identified using AP and classified in the DFA (Fig. 2, Table 3).

Table. 3. Description of the *Pygathrix nemaeus* vocal types. The spectrogram of a call resulting near to the vocal type centroid during the multivariate analysis is shown in Fig. 2.

Up	Low-pitched and short harsh vocalization (Fig. 2a).
Wroo	Low-pitched guttural calls with clear formants structure (Fig. 2b).
Slow Wroo	Low-pitched guttural calls with clear formants structure. They are usually given at low intensity (Fig. 2c).
Friii	Long and harsh high-pitched vocalization with remarkable frequency modulation (Fig. 2d).
Trill	Short high-pitched vocalization with frequency modulation (Fig. 2e).
Ruae-ruae	Long and harsh high-pitched vocalization with frequency modulation. Units with low pitch occur during the emission (Fig. 2f).
Uo-traa	Long and harsh high-pitched vocalization, with frequency modulation preceded and followed by a low-pitched unit (Fig. 2g).
Trill-wroo	Long duration high-pitched vocalization with frequency modulation follow by short low-frequency emission (with a wroo-like structure, Fig. 2h).

Discussion

The recorded calls of *P. cinerea* and *P. nemaeus* showed an acoustic variability that allowed identifying 8 different vocal types for each species, all of which are distinguishable by visual inspection of the spectrograms and by ear. As we mentioned before, taking into account that the dataset has not been checked for sex-, age- or individual-specific characteristics, the vocal types identified in the present study can potentially represent individually given variants of the same call, although in most cases spectrographic inspection seems actually to support the discreteness of most vocal types.

Comparison between *Pygathrix cinerea* and *P. nemaeus*

We can notice that calls with similar acoustic structure are present in the repertoire of both species. Six clusters for each species are showing calls with similar acoustic structure: the *up*, the *wroo*, the *frii*, the *trill*, the *trill-wroo*, and the *uo-traa*. The *ups* are very short low-pitched vocalizations with visible formants. They sound like a burp. The *wroos* are low-pitched calls showing longer duration when compared to the *ups*.

A common acoustic unit across many of the call types is that they include a *trill* unit. The *trills* are high-pitched calls showing remarkable frequency modulation. In addition to the *trills*, we found other three vocal types showing a *trill*-like unit. The *friiis* are similar to *trills*, but compared to these, they are longer in duration and with stronger frequency modulation. The other two vocal types, the *trill-wroo* and the *uo-traa* sound harsh. They are both high-pitched and showed remarkable frequency modulation. Moreover is important to highlight that all the vocal types showing a *trill*-like structure often occur with subharmonics and other nonlinear phenomena.

Comparison with other species

Qualitatively speaking, we screened the published spectrograms of taxa phylogenetically close to the genus *Pygathrix*, as the species belonging to the genera: *Nasalis* (Messeri & Trombi, 2000; Srivathsan & Meier, 2011), *Presbytis* (Wich et al., 2008; Meyer et al., 2012), *Simias* (Schneider et al., 2007) and *Trachypithecus* (Krishnamurthy, 1991).

Messeri & Trombi (2000) recognized six vocal types in *Nasalis larvatus* using acoustic measurement partially overlapping those used in this study. Thus the repertoire size of *N. larvatus* seems reasonably similar to those found for *Pygathrix cinerea* and *P. nemaeus* in the present study.

Previous studies often concentrated on specific aspects of communication signals but only provided information on context-specific utterances In as contact calls (Rawson, 2009) or male loud calls of other species (Wich & Nunn, 2002). From these investigations we should expect various loud call types showing their main energies concentrated in the spectral window with the lowest background noise (e.g. low-pitched calls) and having a whistle-like structure with scarce frequency modulation (Schneider et al., 2007). This is not what we have found in the present study, where most types showed remarkable frequency modulation and a relatively high pitch. It is to be noticed that very high-pitched calls were recently found in many primate species and our findings support and even larger presence of these vocalizations in the repertoire of colobines. In fact, Riondato (*unpublished data*) also found high-pitched frequency modulated vocalizations in *Rhinopithecus brelichi*. The adaptive significance of vocalizations with such a high fundamental frequency is still unclear but their acoustic structure could actually mediate communication between conspecifics in a noisy and densely forested environment (Snowdon & Hodun, 1981).

It would be of great relevance to perform further recordings and analyses and more fieldwork on the study species, including eco-ethological observations, could provide a valuable framework for generalisation and functional interpretation of the results shown in this paper.

Acknowledgements

We are grateful to Truong Quang Bich, Director of Cuc Phuong National Park and Mr. Do Van Lap, Chief of the Department of Scientific and International Collaborations for granting permission to conduct research at the Endangered Primate Rescue Center. We are especially grateful to Tilo Nadler, Director of the EPRC and Nguyen Thi Thu Hien, Manager of the EPRC for their continued support of our research.

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Pygmy lorises (*Nycticebus pygmaeus*) without sublingua

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Key words: Pygmy loris, *Nycticebus pygmaeus*, sublingua.

Summary

Since establishment of the Endangered Primate Rescue Center (EPRC) in 1993 the center received a total of 89 pygmy lorises (*Nycticebus pygmaeus*) and 9 northern slow lorises (*Nycticebus bengalensis*). The animals are mostly confiscated from Forest Protection Departments in cooperation with the EPRC or through activities of the organization Education for Nature Vietnam (ENV). Some animals also donated from private persons after they realize that it is illegal to keep the lorises, or they are donated from tourists which bought the animals from hunters, traders or in an illegal market with the intention to rescue the animals but unaware that buying protecting animals is an illegal and criminal act.

On arrival at the EPRC all animals undergo a health check and are quarantined for a six week period. During these routine health checks, we accidentally discovered that two pygmy lorises did not have a sublingua, which is a special morphological feature of some mammals, including lorises. We have only just started to look systematically for this feature and can to date not determine how many of the pygmy lorises kept at the EPRC do lack a sublinga and it what the ecological implications of the lack of this feature are.

Về những các thể cu li nhỏ (*Nycticebus pygmaeus*) không có lưỡi thứ cấp

Tóm tắt

Từ khi xây dựng trung tâm cứu hộ thú linh trưởng (EPRC) năm 1993, trung tâm đã tiếp nhận 89 cá thể cu li nhỏ (*Nycticebus pygmaeus*) và 9 cá thể cu li lớn (*Nycticebus bengalensis*). Những cá thể này phần lớn được tịch thu và chuyển giao từ các chi cục kiểm lâm, kết quả hợp tác cùng với trung tâm cứu hộ EPRC và tổ chức giáo dục thiên nhiên Việt Nam (ENV). Một số cá thể khác được tự nguyện giao nộp từ những cá nhân đã nuôi loài cu li làm vật cảnh, hoặc do khách du lịch mua lại từ thợ săn, người mua bán động vật hoang dã với mục đích cứu hộ. Những người giao nộp không nhận thức được việc mua bán các động vật hoang dã là vi phạm pháp luật. Sau khi đến trung tâm cứu hộ, các cá thể này được kiểm tra sức khỏe và cách ly kiểm dịch trong vòng 6 tuần. Trong quá trình kiểm tra sức khỏe, chúng tôi đã tình cờ phát hiện hai cá thể loài cu li nhỏ không có lưỡi thứ cấp. Lưỡi thứ cấp là một đặc điểm hình thái đặc trưng của một vài loài thú bao gồm cả loài cu li. Chúng tôi đã bắt đầu việc quan sát một cách có hệ thống về đặc điểm này, tuy nhiên hiện nay vẫn chưa xác định được bao nhiêu cá thể cu li ở EPRC không có lưỡi thứ cấp và ý nghĩa của đặc điểm này với sinh thái học.

Introduction

Some prosimians possess below the tongue a special structure, the sublingua, or “under-tongue” (Fig. 1). A similar structure can be also found in several other groups of primitive mammals, like marsupials, treeshrews and colugos (Ankel-Simons, 2000).

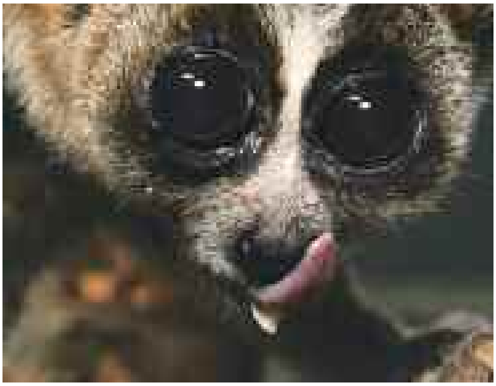

Fig.1. Pygmy loris shows the sublingua, the “under-tongue”. Photo: Duke Lemur Center.

Lorises lower incisors and canines form the “toothcomb”, which lorises use to gouge holes into trees to lick the excreted gum (Streicher 2009; Streicher et al. 2013). The toothcomb is also assumed to play a role in grooming. However the very narrow position of the teeth in the toothcomb makes it prone to accumulation of food remains or hair

The sublingua, the “under-tongue” of the pygmy lorises and northern slow lorises is a muscular structure below the tongue (Hershkovitz, 1977, Jones, 1918), about 10 mm long and 5 mm wide on the base (Fig. 2, 3 and 4). The front of the sublingua ends in mostly 13 keratinized serrations - “denticles” (Osman Hill, 1953) (Fig. 5). These denticles correspond with the narrow gaps between the teeth in the tooth comb and the sublingua is assumed to serve to remove remains of bark or food items from the toothcomb (Jones, 1918; Osman Hill, 1953, Sonntag 1921).

Fig.2. The sublingua, or "under-tongue", is a secondary tongue located below the primary tongue. Photo: Tilo Nadler.

Fig.3. The front edge of the sublingua is lined with keratinized serrations. Photo: Tilo Nadler.

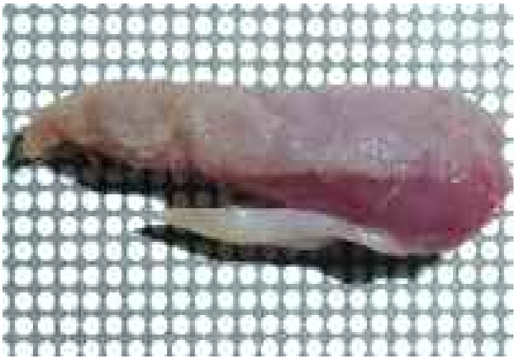

Fig.4. The sublingual in pygmy lorises is about 10 mm long and 5 mm wide. Photo: Tilo Nadler.

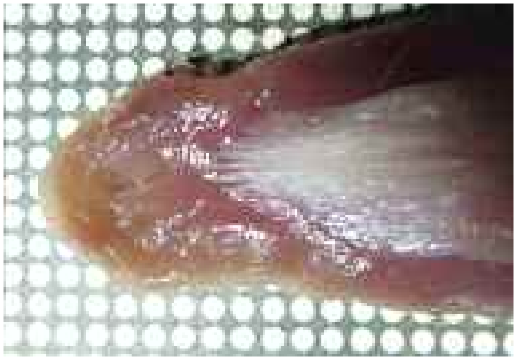

Fig.5. There are an uneven number of serrations, mostly 13, on the tip of the sublingua. Photo: Tilo Nadler.

Material

Since 1993 the Endangered Primate Rescue Center received 89 pygmy lorises (*Nycticebus pygmaeus*) and 9 northern slow lorises (*N. bengalensis*). Two northern slow lorises and 31 pygmy lorises were born at the center, all other animals are victims of poaching and illegal wildlife trade and were confiscated country wide.

On arrival at the EPRC all animals undergo a comprehensive health check before they are quarantined for a six week period. During these routine health checks we accidentally found that two pygmy lorises were missing a sublingua (Fig. 6). We have only just started to look systematically for this feature and can to date not determine how many of the pygmy lorises kept in the past at the EPRC do lack a sublingua and it what the ecological implications of the lack of this feature are.

The northern slow lorises, which we examined, all show the same morphological feature (Fig. 7).

Fig.6. Pygmy loris with missing sublingua. Photo: Tilo Nadler.

Fig.7. Northern slow loris with sublingua. Photo: Tilo Nadler.

Discussion

The sublingua is assumed to be important to clean food remains of the toothcomb and thus maintain dental health. However the two individuals found at the EPRC without sublingua were adult and in good condition. They arrived at the EPRC as adult individuals without any signs of malnutrition or insufficient care of the fur. The question arises, if the sublingua has functions other than only the cleaning of the tooth comb, as animals appear to be able to clean their teeth and maintain dental health as well when the sublingua is missing,

The discovery that the sublingual is missing was rather accidental. During the routine health check only the teeth are checked. We have only just started to look systematically for this feature and can to date not determine how many of the pygmy lorises lack the sublingua.

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VIETNAMESE Journal of Primatology
CHUYÊN ĐỀ LINH TRƯỞNG HỌC VIỆT NAM

VOLUME 2 - ISSUE 2

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ISSN 1859-1434