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Avahi meridionalis ramanantsoavani
a new woolly lemur from SE Madagascar

Cover photo: *Avahi meridionalis ramanantsoavani* from the Reserve of Manombo (Photograph by N. Andriaholinirina).

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COMMON ACOUSTIC FEATURES IN THE VOCAL EXPRESSION OF EMOTIONS IN MONKEYS AND MAN

Jürgens U and Hammerschmidt K

Introduction

Non-verbal emotional vocal utterances of humans, such as laughing, crying, moaning or jubilating, as well as the emotional intonations superimposed on the verbal component during affective speech have been found to show transcultural similarities, suggesting that the vocal expression of emotion is to some extent genetically determined (SCHERER et al., 2001). A similar conclusion can be drawn with respect to monkey calls: Kaspar-Hauser experiments in the squirrel monkey have shown that all call types of the species-specific vocal repertoire can be produced by animals which never had an opportunity to hear these calls from conspecifics (WINTER et al., 1973; HAMMERSCHMIDT et al., 2001). From this the question arises of whether there are common acoustic features used by humans as well as monkeys in the expression of specific emotional states. Such features would indicate common phylogenetic roots of human and non-human primate emotional vocal behaviour; and such common phylogenetic roots would justify the use of monkeys as animal models in studies investigating the central nervous control of emotional vocal behavior in humans. In the study to be reported about here, acoustic analyses of squirrel monkey calls and human emotional intonations were carried out with the aim to find out in which way aversive emotional states differ from non-aversive ones in their vocal expression.

Human emotional intonations

In the case of the human emotional intonations, we asked 23 students of dramatic art to pronounce one and the same word, the name "Anna", with six different intonations. Three of them expressed aversive emotional states; these were rage, despair and disgust. Three expressed hedonic emotional states; these were joyful surprise, voluptuous enjoyment and affection. All speech samples were analysed acoustically, using a program developed by one of the authors (K.H.). By this program, we determined the values of 94 acoustic parameters. As many of them were highly correlated, we reduced the number to 14 by the help of a correlation analysis. These 14 are shown in Table 1. They include the amplitude, duration, proportion of non-harmonic time segments in relation to harmonic ones (noise), mean and maximum fundamental frequency (F0 mean, F0 max), mean and maximum peak frequency (PF mean, PF max), that is, the frequency of highest energy in the power spectrum, amplitude ratio of the lowest and second lowest dominant frequency bands (DFB ratio), mean harmonic-to-noise ratio (HNR mean), mean frequency range (Range mean), that is, the difference between highest and lowest frequency; DFA2 is the center frequency of the power spectrum, that is, the frequency at which 50 % of the energy of the power spectrum is reached, if the frequency amplitudes are summed up from lower to higher frequencies; DFB1 local mod is a measure of the variability of the lowest dominant frequency band, which in harmonic segments is identical with the funda-

mental frequency; Pf amp max is the frequency of the amplitude maximum of the peak frequency; and PF max loc indicates the relative position of the maximum of the peak frequency with respect to vocalization onset.

Table 1: List of acoustic parameters tested in human emotional intonations. Arrows in the column "Aversion" indicate that the corresponding parameter has a significantly higher value in the aversive intonations as a group compared with the hedonic intonations as a group. Arrows in the column "Amplitude" indicate a positive correlation between amplitude and acoustic parameters listed in column 1.

Parameter	**Aversion**	**Amplitude**
Amplitude	↑	
Duration		
Noise	↑	
F0 mean		↑
F0 max		↑
PF mean	↑	↑
PF max	↑	↑
DFB ratio		↑
HNR mean		
Range mean	↑	↑
DFA2 mean	↑	↑
DFB1 local mod		↑
PF amp max		↑
PF max loc	↑	

If all aversive emotional intonations as a group are compared with the hedonic emotional intonations as a group, it turns out that 7 out of the 14 parameters have a significantly higher value for the aversive intonations. These are the amplitude, amount of noisy segments, mean and maximum peak frequency, frequency range, center frequency of the power spectrum and the time from vocalization onset to the maximum of the peak frequency (Table 1).

Not all of the aversion parameters are independent of each other. If the amplitude is related to the remaining parameters, it turns out that 4 of the 6 aversion parameters show a correlation with amplitude. These are PF mean, PF max, Range mean and DFA2 mean. This rises the question of whether such parameters as peak frequency or frequency range have an explanatory value beyond that of amplitude. We, therefore, made in addition a pairwise comparison of aversive and hedonic intonations showing the same amplitude.

Two pairs with virtually identical amplitude of aversive and hedonic intonation were found. These are "voluptuous enjoyment" against "disgust" and "affection"

against "disgust". The results show that 4 of the 6 aversion parameters have a significantly higher value for the aversive intonation in both pairs. The other 2 parameters also have higher values in the aversive intonations, but reach significance in only one of the two pairs. The numbers in Table 2 give the percentage by which the value of the respective parameter in the aversive intonation surmounts that in the hedonic one. From these numbers, it is evident that Pf max contributes most to the characterization of aversive intonations. Smaller, but nevertheless significant contributions in both pairs come from the mean peak frequency (PF mean), frequency range (Range mean) and center frequency of the power spectrum (DFA 2 mean).

Table 2: Comparisons between single human emotional intonations with same amplitude, but differing valence. The numbers indicate by which percentage the corresponding acoustic parameter is higher in the aversive intonation than in the hedonic one. ns: difference between intonations not significant.

Parameter	**Voluptuous. - Disgust**	**Affection - Disgust**	**Mean**
Noise	75 ns	139	107.0
PF mean	97	101	99.0
PF max	179	85	132.0
Range mean	67	74	70.5
DFA2 mean	33	46	39.5
PF max loc	118	3 ns	60.5

Squirrel monkey calls

For comparison with the human emotional intonations, we analysed the vocalizations of 25 squirrel monkeys (*Saimiri sciureus*). Vocalizations were elicited by electrical stimulation of various limbic brain structures. Six call types belonging to three different call classes were analysed (Table 3). Cackle and twitter belong to one call class, shriek and caw to a second and growl and purr to a third. Determination of the aversive or hedonic character of the emotional state accompanying these calls was made in a self-stimulation test (Jürgens, 1979). In this test, the animals received the opportunity to switch on and off the vocalization-eliciting brain stimulation themselves. The time the animals spent self-stimulating provided us with a measure of the aversive or hedonic quality of the emotional state accompanying a specific call type. In this test, it turned out that twitter is accompanied by a hedonic emotional state, while cackle is accompanied by a moderately aversive state; caw also expresses a moderately aversive state, while shriek expresses a highly aversive state; purr expresses a neutral state, growl a moderately aversive state (Table 3).

If the calls are analyzed acoustically in the same way as the human emotional intonations, and pairwise comparisons are made between two calls of the same class, but differing in degree of aversion, we find that the only parameter showing a significantly higher value for the more aversive call in all three comparisons is PF max,

that is, exactly that parameter which also best characterized aversion in the human emotional intonations. The other parameters having been shown to play a role in the expression of human aversive states, namely mean peak frequency, mean frequency range and noise, also show in the monkey significantly higher values for the more aversive calls in two of the three pairs (Table 3).

Table 3: Comparisons of squirrel monkey calls belonging to the same call class, but differing in valence. + hedonic, ± neutral, - slightly aversive, -- highly aversive. Arrows pointing upward (downward) indicate that the corresponding parameter has a significantly higher (lower) value for the more aversive one of the two calls being compared. The table is based on the results reported in FICHTEL et al. (2001).

Parameter	**Cackle (-) vs. Twitter (+)**	**Shriek (- -) vs. Caw (-)**	**Growl (-) vs. Purr (±)**
PF max [Hz]	↑	↑	↑
PF mean [Hz]		↑	↑
Range mean [Hz]		↑	↑
Noise [%]	↑	↑	
DFB1 max [Hz]	↓	↑	
DFA1 mean [Hz]		↑	↑
DFA2 mean [Hz]		↑	↑

Conclusions

We conclude from this that monkey and man differentiate aversive from non-aversive emotional states vocally in a very similar way. This suggests that the vocal expression of emotional states in humans and monkeys is homologous in the strict sense. As that branch of the phylogenetic tree leading to the squirrel monkey already separated from that branch leading to modern humans about 45 million years ago, we may conclude that the phylogenetic roots of human emotional vocal behavior reach back at least 45 million years.

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MOLECULAR PHYLOGENY AND TAXONOMIC REVISION OF THE EASTERN WOOLLY LEMURS (*AVAHI LANIGER*)

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Key words: *Avahi*, Strepsirrhini, taxonomy, mtDNA, cytogenetics, new species

Abstract

The western and northern populations of woolly lemurs (*Avahi*) have been divided into three distinct species (*A. cleesei*, *A. occidentalis* and *A. unicolor*), whereas the eastern populations are still considered to represent a single species (*A. laniger*), despite the wider distribution of woolly lemurs in this region. To analyze the diversity within the eastern population and among the eastern and western populations, we compared cytogenetic data and mitochondrial DNA (mtDNA) sequences from woolly lemurs from 14 sites in the east of Madagascar and from three sites in the west, representing three of the four recognized species. Cytogenetic and mtDNA data are in agreement and confirm the distinctiveness of *A. laniger* and *A. occidentalis*. Within *A. laniger* the molecular data revealed large genetic distances among local populations. On the basis of these new data we propose to split *A. laniger* into three species: (1) north of the Mongoro/Onive Rivers, (2) south of the Mongoro/Onive Rivers at least as far south as Mahasoarivo, and (3) from the south-east (Manombo, Sainte Luce). Within the south-eastern species (3) two clearly separated subspecies can be distinguished, one from the region of Manombo and the other from the region of Sainte Luce. The northern species (1) shows considerable intraspecies genetic distances and may consist of several populations distinguishable as subspecies. However, our sampling has not as yet resolved the pattern of these taxa. Additionally, based on our mtDNA analysis, the separate specific status of *A. cleesei* is questionable.

Introduction

Woolly lemurs (genus *Avahi*) are small nocturnal primates living in Madagascar. Traditionally, the genus has been considered monotypic (*A. laniger*), with two subspecies: *A. l. occidentalis* in northern, northwestern and western forests, and *A. l. laniger* in the eastern forests (HILL, 1953; PETTER et al., 1977). However, based on cytogenetic studies by RUMPLER et al. (1990) the two subspecies were elevated to full species: *A. laniger* and *A. occidentalis*. Their specific rank was later confirmed by molecular studies performed on samples from Ampijoroa for *A. occidentalis* and Ranomafana for *A. laniger* (RAZAFINDRAIBE et al., 1997, 2000; PASTORINI et al., 2003). Recently, within *A. occidentalis* two new species were identified on morphological characteristics: *A. unicolor* and *A. cleesei* (THALMANN and GEISSMANN 2000, 2005).

By using molecular biology techniques, especially mitochondrial DNA sequencing, new species and distribution refinements have recently been reported for several nocturnal lemurs, among them cheirogaleids (RASOLOARISON et al., 2000; YODER et al., 2000; KAPPELER et al., 2005; LOUIS et al., 2006a) and lepilemurs

(ANDRIAHOLINIRINA et al., 2006; LOUIS et al., 2006b; RABARIVOLA et al., 2006).

Given the extensive distribution of *A. laniger* - from Vohimara (Vohemar) in the north to Tolagnaro (Fort Dauphin) in the south - and the fact that thus range extends over several potential geographic barriers such as the Mangoro/Onive Rivers, it is of great interest to examine possible chromosomal and genetic variance within the range of *A. laniger*. We therefore did a systematic chromosomal and mitochondrial DNA study on *A. laniger* at a variety of geographic locations, with a focus on populations living in the eastern forest on either side of geographic barriers that constitute the species boundaries of other taxa such as *Lepilemur*, *Microcebus* or *Propithecus* (PETTER et al., 1977; ANDRIAHOLINIRINA et al., 2005; LOUIS et al., 2006a).

Materials and Methods

Fieldwork

Samples from 55 individuals were collected during several field surveys (2002-2006) in different parts of the eastern forests from Antsahaporetiny (48°42'E, 16°56'S) in the north to Sainte Luce in the south (47°11'E, 24°47'S). In the west samples were obtained from individuals caught in the Ampijoroa and Bemaraha reserves (Table 1; Fig. 1). Animals were captured in the wild using blowpipe projection. Skin biopsies were taken under general anaesthesia with a 2mg/kg injection of ketamine solution (Ketalar, Parke-Davis). A part of each sample was directly frozen in liquid nitrogen, while the other part was preserved with a cryoprotector (DMSO 10 %), with the aim of growing fibroblast cultures. Standard morphometric measurements were collected, including body mass, head body length, ear length, tail length and hind foot length. After recovery from the anesthesia, animals were released in their respective capture areas.

Table 1: Origin and number of samples.

species	areas of capture	coordinates	# samples	# haplotypes
Avahi occidentalis	Ampijoroa	46°49'E-16°18'S	1	1
	P.N. Ankarafantsika	47°00'E-16°00'S	4	3*
Avahi cleesei	Bemaraha	44°42'E-18°51'S	4	1
Avahi laniger 1	Ambodifamelona / Antsahaporetiny	48°41'E-16°56'S	3	2
	Fatita / Ivongo	48°55'E-17°11'S	2	2
	Ambolo	48°39'E-17°14'S	1	1
	Zahamena	48°50'E-17°38'S	2	2
	Anjozorobe	47°53'E-18°20'S	3	3
	Andasibe	48°25'E-18°56'S	1	1
	Maromizaha	48°27'E-19°03'S	8	5*
	Vatateza	47°48'E-19°42'S	3	3

species	areas of capture	coordinates	# samples	# haplotypes
Avahi laniger 2	Andalameloka / Sangalampona	47°51'E-19°48'S	2	1
	Mahasoarivo	47°26'E-21°16'S	4	4
	Vatoalatsaka	47°48'E-19°50'S	1	1*
	Manara	47°48'E-19°55'S	5	5
Avahi laniger 3	Manombo	47°41'E-23°01'S	6	1
	Sainte Luce	47°11'E-24°47'S	5	4
total			55	40

(1) = north of Mongoro/Onive Rivers; (2) = south of Mongoro/Onive Rivers at least as far south as Mahasoarivo; (3) = south-east
* one haplotype from the respective sampling site is identical to another sequence of a different site (P.N. Ankarafantsika to Ampijoroa; Maromizaha to Vatateza; Vatoalatsaka to Andalameloka / Sangalampona)

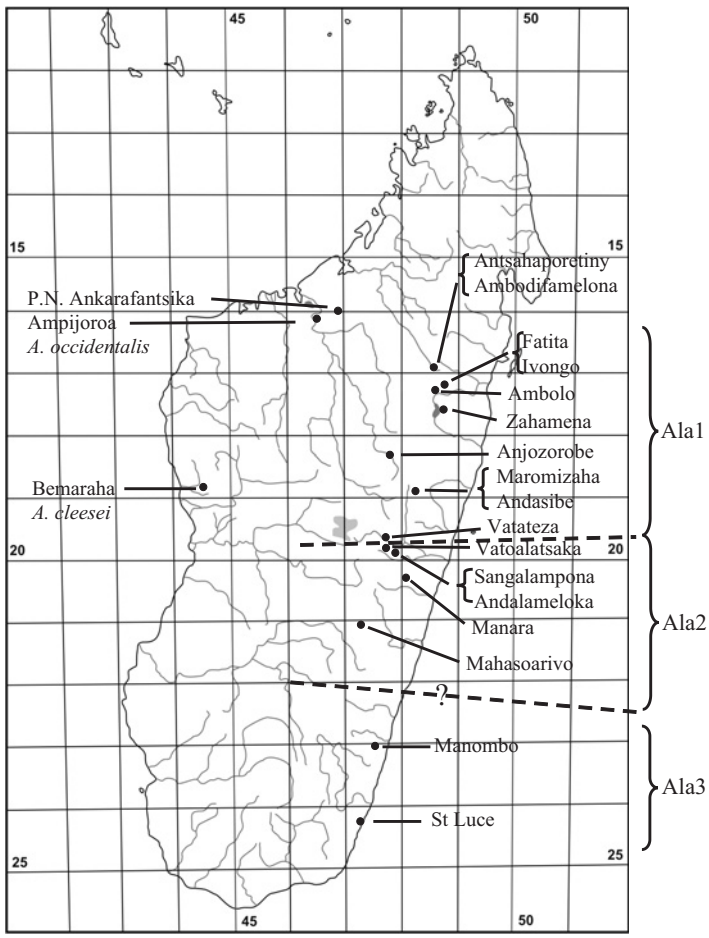

Fig. 1: Sampling sites of woolly lemurs, *Avahi* spp. (geographic coordinates s. Tab. 1).
Ala1 = *Avahi laniger* 1, north of the Mangoro/ Onive Rivers;
Ala2 = *A. laniger* 2, south of Mangoro/Onive Rivers to Mahasoarivo;
Ala3 = *A. laniger* 3, populations of the south-east.
(----- = taxon border;
? = exact taxon border not known)

Cytogenetics

Cytogenetic analyses using R-banded and C-banded chromosomes were performed, following classical methods (DUTRILLAUX and COUTURIER, 1981), for at least one specimen per location on both sides of the Betsiboka River on the western coast, and Mangoro/Onive Rivers in the east. Karyotypes were established on fibroblast cultures.

Molecular genetics

DNA from the biopsies was extracted using the QIAamp DNA Mini Kit according to the manufacturer's procedures, and was stored at -20° C before further processing.

The complete mitochondrial cytochrome b gene (1,140bp) was amplified via PCR using the oligonucleotide primers CYT-AVA-L (AHC223): 5'-TGACTAATGATATG AAAAACCATCG-3' and CYT-AVA-H (AHC226): 5'-GGTTGATGCTTCTTCCTT GAG-3'. Wax-mediated hot-start PCRs were carried out for 40 cycles, each with a denaturation step at 94° C for 60 s, annealing at 60° C for 60 s, and extension at 72° C for 90 s, followed by a final extension step at 72° C for 5 min. Aliquots of the PCR amplifications were checked by agarose gel electrophoresis. Subsequently, PCR products were cleaned using the Qiagen PCR Purification Kit and sequenced on an ABI 3100-Avant sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems), primers as indicated above, and the internal primers AHE267 5'-CAGGGTTTGATGAGATGCCTA-3' and AVAF2 5'-CCGATTCTTCGCATTTC ACT-3'. Sequences were easily aligned by eye due to the lack of insertions or deletions, and were checked for their potential for correct transcription in order to eliminate data set contamination with pseudogenes.

We omitted identical sequences from the same location so that the final alignment comprised 41 sequences including 40 woolly lemurs as well as one *Propithecus verreauxi coronatus* (AY441734, ROOS et al., 2004), which was used as outgroup for phylogenetic reconstructions. Sequences were submitted to GenBank and are available under the accession numbers EF103291-EF103330. The origins of the individuals analysed are shown in Figure 1.

Uncorrected pairwise distances within and between species and major populations were calculated with MEGA 3.1 (KUMAR et al., 2004).

Phylogenetic trees were constructed using the maximum-parsimony (MP), neighbour-joining (NJ) and maximum-likelihood (ML) algorithms, as implemented in PAUP 4.0b10 (SWOFFORD, 2002) or TREEPUZZLE 5.0 (STRIMMER and VON HAESELER, 1996). For MP analyses, all characters were treated as unordered and equally weighted throughout. A heuristic search was performed with the maximum number of trees set to 100. NJ and ML trees were constructed using standard models as well as the TrN + I (=0.5681) + G (=1.1745) model of sequence evolution which was selected as the best-fitting model with MODELTEST 3.06 (POSADA and CRANDALL, 1998). Relative support of internal nodes was performed by bootstrap analyses with 1,000 replications (MP, NJ), or by quartet puzzling support values on the basis of 10,000 puzzling steps (ML).

Results

Cytogenetics

No differences in R-banding were detected among the woolly lemur individuals from the eastern forest, all of which were characterized by the classical karyotype of *A. laniger* (Fig. 2). The R-banded karyotype of *A. cleesei* is identical to that of *A. occidentalis* (Fig. 3). The karyotype of *A. occidentalis* differed from that of *A. laniger* by three chromosomal rearrangements, one inversion and two Robertsonian translocations (Fig. 4). The cytogenetic data confirm previous results (RUMPLER et al., 1990).

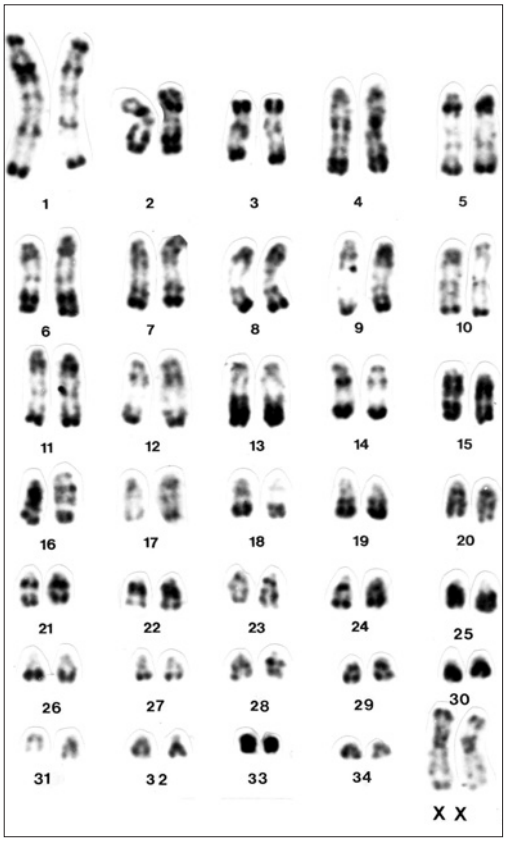

Fig. 2: Karyotype of *A. laniger* female established after R-banding.

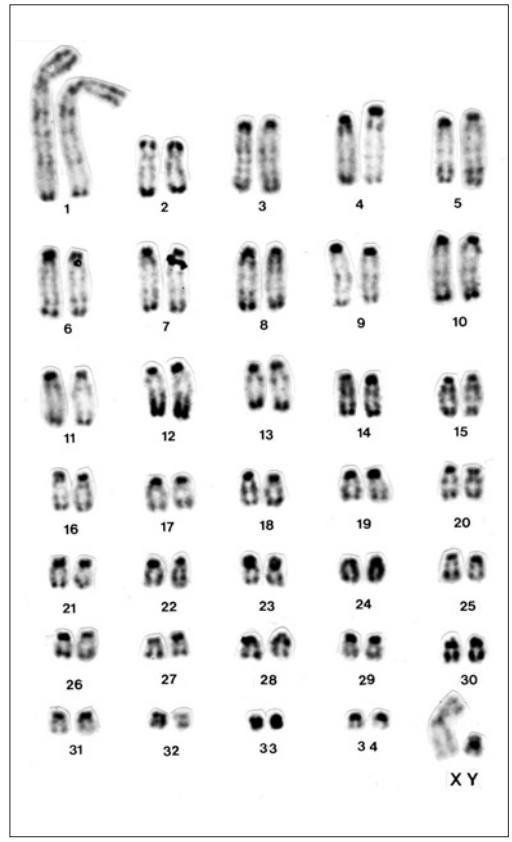

Fig. 3: Karyotype of *A. occidentalis* male established after R-banding.

Molecular genetics

Complete cytochrome b gene sequences (1,140 bp) were obtained from 55 animals representing two of the three published western species (*A. occidentalis* and *A. cleesei*) as well as populations covering a large portion of the *A. laniger* range. Among the 55 sequences we detected 37 haplotypes. In three cases identical haplotypes have been found at two sampling sites each. We added these three sequences to the

alignment so that the final number of sequences in our analysis is 40 (Tab. 1). Because of the absence of stop codons and indels, it seemed to us justifiable to regard the sequences as representing the functional mitochondrial cytochrome b gene rather than a nuclear pseudogene (ZHANG and HEWITT, 1996). Average uncorrected pairwise distances within the genus range from 2.68 to 9.50 %, with overlapping intra- and inter-specific distances (intra-specific: 0.00-4.82 %, inter-specific: 2.54-10.53 %; Table 2).

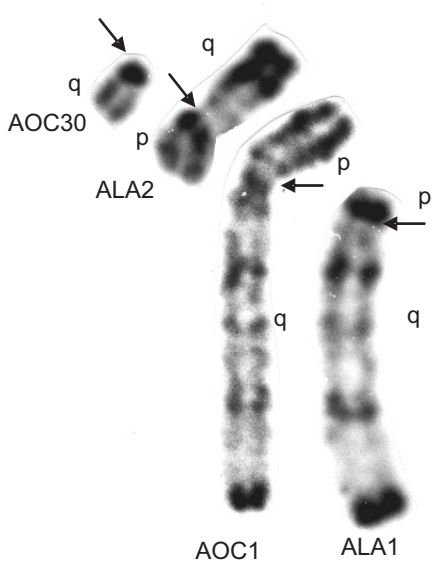

Fig. 4: Chromosomal rearrangements between *Avahi occidentalis* (AOC) and *A. laniger* (ALA). The three chromosomal rearrangements are depicted. One inversion and two Robertsonian translocations separate *Avahi occidentalis* (AOC) from *A. laniger* (ALA). ALA1 and AOC1 differ by one inversion and one translocation. AOC1q corresponds to ALA1p+q (they differ by one inversion); ALA2p corresponds to the acrocentric chromosome AOC30; AOC1p corresponds to ALA2q. (Arrow = centromeric position; p = short arm; q = long arm).

Table 2: Genetic distances among three *Avahi* species. Uncorrected pairwise distances are given below the diagonal with range above. Mean distances within each species are given in bold on the diagonal. All estimates are expressed as percentages.

	A. occidentalis	***A. cleesei***	***A. laniger***
A. occidentalis	**1.07**	2.54-2.81	8.77-10.53
A. cleesei	2.68	**-**	8.42-9.65
A. laniger	9.50	9.06	**2.61**

A division of *A. laniger* populations into three phylogeographical groups (*A. laniger* 1 from north of the Mangoro/Onive Rivers, *A. laniger* 2 from south of the Mangoro/Onive Rivers, and *A. laniger* 3 from the south-east [Manombo and Sainte Luce]) results in a reduction of the average intra-taxon distances within *A. laniger* from 2.61 % (Table 2) to a maximum average of 1.15 % (*A. laniger* 1; Table 3), whereas the inter-taxon distances among the three *A. laniger* taxa are on average 3.10 to 3.75 % (overall range 2.65-4.83 %). Furthermore, a comparatively large pairwise distance (mean 1.89 %; range 1.75-2.11%) was found between the two populations of *A. laniger* 3 from Manombo and Sainte Luce, respectively.

Table 3: Genetic distances among phylogeographical taxa of *Avahi*. Uncorrected pairwise distances are given below the diagonal with range above. Mean distances within each taxon are given in bold on the diagonal. All estimates are expressed as percentages.

	A. occidentalis	***A. cleesei***	***A. laniger* 1**	***A. laniger* 2**	***A. laniger* 3**
A. occidentalis	**1.07**	2.54-2.81	8.77-10.09	8.86-10.18	9.30-10.53
A. cleesei	2.68	**-**	8.42-9.21	9.04-9.65	9.12-9.47
***A. laniger* 1**	9.47	8.86	**1.15**	3.07-4.83	3.25-4.65
***A. laniger* 2**	9.43	9.27	3.74	**0.67**	2.72-3.68
***A. laniger* 3**	9.79	9.30	3.75	3.10	**0.90**

Phylogenetic trees reconstructed on the basis of various algorithms produced identical tree topologies, for the most part with significantly supported branching patterns (Fig. 5). Two distinct major haplotype lineages were detected, representing the western and eastern populations. This major split is confirmed by cytogenetic evidence. The western clade further splits into two subgroups representing the two species *A. occidentalis* and *A. cleesei*, respectively. The eastern lineage separates into two subgroups: a northern lineage, containing all *A. laniger* populations from north of the Mangoro/Onive Rivers (*A. laniger* 1), and a southern one. The latter further divides into two subgroups with geographically non-overlapping ranges. The first of these comprises all populations from the Mangoro/Onive Rivers as far south as Mahasoarivo (*A. laniger* 2), and the second consist of the populations of Manombo and Sainte Luce (*A. laniger* 3). Both populations of the *A. laniger* 3 lineage can be clearly recognized as distinct clades. In contrast, no clear subdivision is obvious in the *A. laniger* 1 lineage, although populations between Anjozorobe and the Mangoro/Onive Rivers constitute a clade that is recognisably separate from the populations north of Zahamena.

Discussion

Among nocturnal mammals pelage coloration is rarely an ideal characteristic for distinguishing among taxa. Nonetheless, *A. occidentalis* clearly differs in its facial mask from *A. laniger*, and to a lesser degree from *A. cleesei* and *A. unicolor*. However, among the eastern populations currently assigned to *A. laniger*, the populations of Sainte Luce and Manombo, and the two subgroups living north and south of the Mangoro/Onive Rivers, only slight external differences were detected.

The cytogenetic data clearly distinguish *A. occidentalis* from *A. laniger*, and the mitochondrial sequences also strongly support separate specific status for these forms. This conclusion is in agreement with studies on restriction genomic DNA banding pattern (RAZAFINDRAIBE et al., 1997).

Among the western woolly lemurs, *A. cleesei* and *A. occidentalis* form two distinct clades (Fig. 5). However, cytogenetically no differences are detectable and the pairwise distances are relative low (2.68 %) between the two taxa compared to other closely related lemur species, so that the specific status of *A. cleesei* has to be evaluated by further studies.

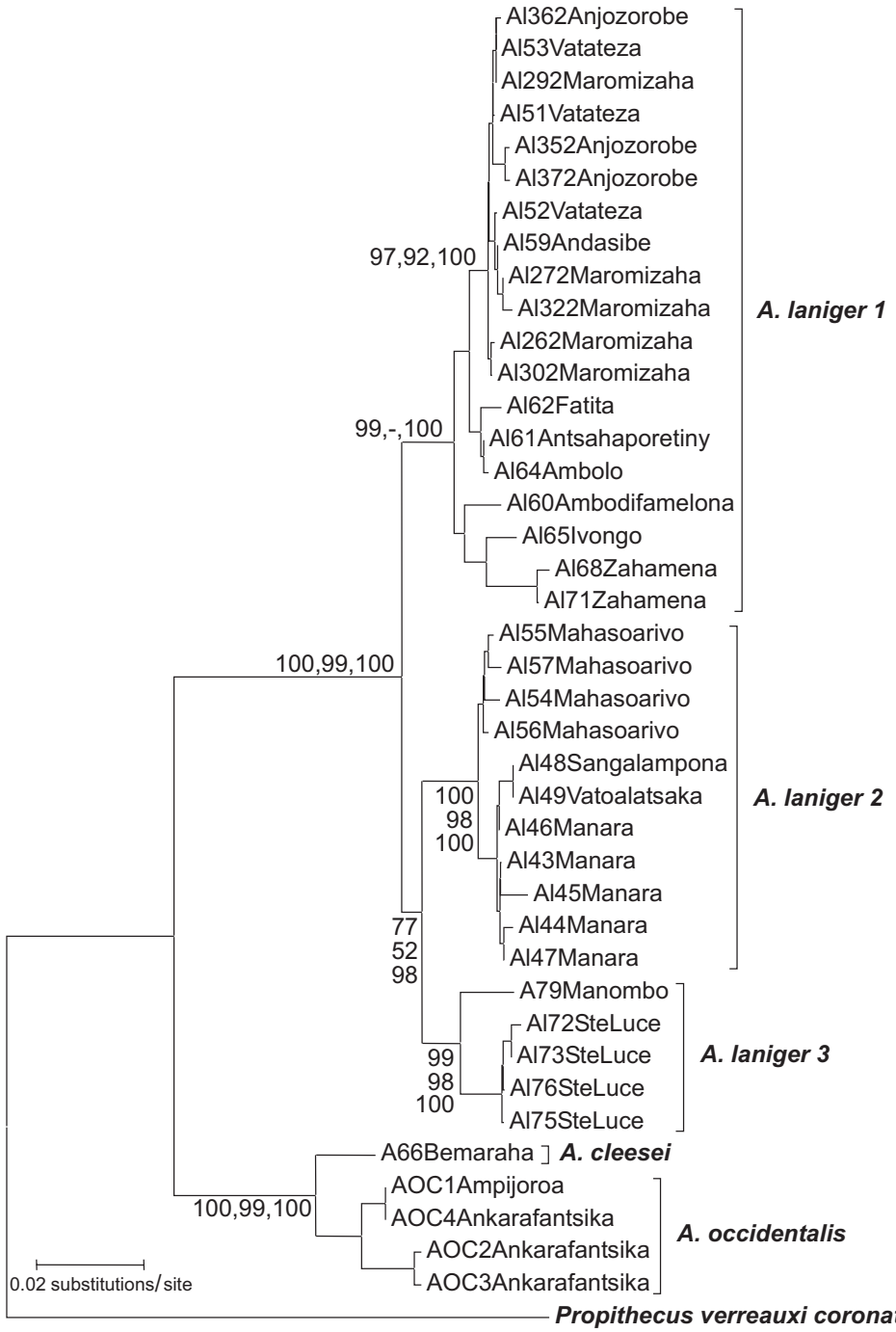

Fig. 5: Phylogenetic relationships as obtained from complete mitochondrial cytochrome b sequence data. Branch lengths are based on the NJ tree with numbers on branches indicating internal support (first: NJ, second: MP, third: ML).

Among the eastern woolly lemurs, all populations studied present the same karyotype. However, the molecular data reveal substantial variety, and point to the conclusion that all *A. laniger* populations that are separated by high genetic distances (Table 3), and that are distributed in distinct local clades (Fig. 5), should be formally recognised as separate taxa. These should probably be recognised at the specific level, except that individuals from Sainte Luce and Manombo are separated by lower genetic distances, which may warrant separation only at the subspecies level. To provide some perspective, the pairwise distances between the different *A. laniger* groups are in the same range (3.10-3.75 %) as those between the species of *Lepilemur dorsalis* and *L. ankaranensis* (ANDRIAHOLINIRINA et al., 2006), and *Mirza coquereli* and *M. zaza* (KAPPELER et al., 2005).

The type locality of *A. laniger* is unknown. The species, named by Gmelin in 1788, is based on an illustration published by Sonnerat in 1782. Given what is known of Sonnerat's itineraries, it is virtually certain that the individual illustrated came from the northeastern quadrant of Madagascar, plausibly from around Maroantsetra. If multiple woolly lemur species are recognised in eastern Madagascar, it is thus the populations living to the south of the Mangoro/Onive Rivers (*A. laniger* 2), at Manombo, and in the area of Sainte Luce (*A. laniger* 3), that require new names. Below we describe the woolly lemurs from these three areas as three new taxa.

Avahi peyrierasi sp. nov. (Fig. 6)

Type Series: DNA from eight specimens stored at the University Louis Pasteur Strasbourg, France, and from three specimens stored at the Gene Bank of Primates, German Primate Centre, Germany.

Type locality: Mahasoarivo (Ranomafana approx. 47°26'E, 21°16'S), Province Fianarantsoa, Madagascar.

Description: Dorsal fur is grey-brown, while ventrum is grey or white. The tail is red-brown. Outside thighs are grey-brown and insides are white. Small white

Fig. 6: *Avahi peyrierasi* from the forest of Ranomafana (Photograph by Ph. Barazer).

bands are visible along the inferior part of the legs and in some animals along the upper part also. A white border of fur completely encircles the face in some individuals, and white beards and cheeks are also present. *A. peyrierasi* has a mean body mass of 1050 g for females and 991 g for males, and a mean head-body length for females is 289 mm and for males 279 mm (Table 4).

Diagnosis: In the mitochondrial cytochrome b gene, *A. peyrierasi* differs from the other woolly lemurs of the eastern forest (*A. laniger*, *A. meridionalis meridionalis* and *A. m. ramanantsoavani*) by average genetic distances of 3.79, 3.87 and 3.95 %, respectively.

Comparison and remarks: *A. peyrierasi* is smaller than *A. laniger* (Table 4).

Etymology: The species name *peyrierasi* is proposed in honour of André Peyrieras, a French naturalist who worked in the Park of Tsimbazaza and studied a broad range of Malagasy species from dipters to lemurs. He was strongly involved in the discovery of *Hapalemur aureus* (MEIER et al., 1987).

Distribution: *A. peyrierasi* is currently known from south of the Mangoro/Onive Rivers in the forests of Manara, Vatoalatsaka, Sangalampona, Mahasoarivo and Ranomafana.

Avahi meridionalis sp. nov. (Fig. 7)

Type Series: Tissue and DNA from five specimens stored at the University Louis Pasteur Strasbourg, France, and from one specimen stored at the Gene Bank of Primates, German Primate Centre, Germany.

Type locality: Sainte Luce (approx. 47°11'E, 24°47'S), Province Toliara, Madagascar.

Description: Dorsal fur is grey-brown toning down to light grey distally, while ventrum is grey. The tail is red-brown and darkens distally. *A. meridionalis* has a mean weight of 1200 g (females) and 1100 g (males) and the mean head-body length is 270 mm and 250 mm for females and males, respectively.

Diagnosis: In the mitochondrial cytochrome b gene, *A. meridionalis* differs from *A. laniger* and *A. peyrierasi* in 3.75 % and 3.10 %, respectively. The two populations of *A. meridionalis* from Sainte Luce and Manombo differ in 1.89 %.

Comparison and remarks: *A. meridionalis* is slightly smaller than *A. laniger* and *A. peyrierasi* (Table 4).

Fig. 7: *Avahi meridionalis meridionalis* from Sainte Luce (Photograph by I. Norscia).

Etymology: *A. meridionalis* is named *meridionalis* as, like *Hapalemur meridionalis*, it occupies the most southern part of the woolly lemur range in eastern Madagascar.

Distribution: The species is restricted to the reserve of Andohahela and the area of Sainte Luce. Further studies are required to determine the exact distribution range and especially the limits with its sister species *A. peyrierasi* and the Manombo population, which is described below as distinct subspecies of the nominate form of *A. meridionalis*.

Avahi meridionalis ramanantsoavani ssp. nov. (Fig. 8)

Type Series: Tissue and DNA from eight specimens stored at the University Louis Pasteur Strasbourg, France, and from three specimens stored at the Gene Bank of Primates, German Primate Centre, Germany.

Type locality: Reserve of Manombo (approx. 47°41'E, 23°01'S), Province Finarantsoa, Madagascar.

Description: Dorsal fur is grey-brown, while ventrum is grey. The tail is red-brown. The facial mask slightly differs from that of *A. laniger* as the fur of some animals is lighter while the outline from some others is more pronounced. The ventral fur is grey and overtakes laterally from a white band on posterior legs. The mean body mass is 1019 g for females and 897 g for males and the mean head-body length of 269 mm for females and 257 mm for males (Table 4).

Diagnosis: In the mitochondrial cytochrome b gene, *A. meridionalis ramanantsoavani* differs from the nominate form by 1.89 %.

Comparison and remarks: *A. meridionalis ramanantsoavani* is smaller than *A. laniger* and *A. peyrierasi* (Table 4).

Etymology: *A. m. ramanantsoavani* is named in honour of Georges Ramanantsoavana, the first Director des Eaux et Forêts de Madagascar, who strongly supported the studies on lemur taxonomy and conservation in the 1960-1970s.

Distribution: The species is restricted to the type locality of the Manombo reserve. Further field studies are required to determine the exact distribution range and especially the limits with its two related taxa, *A. peyrierasi* and *A. m. meridionalis*.

Fig. 8: *Avahi meridionalis ramanantsoavani* from the Reserve of Manombo (Photograph by N. Andriaholinirina).

Table 4: Morphometric measurements of *Avahi*.

taxon	sex (N)	body mass (g)	head-body length (mm)	ear length (mm)	lower hind leg length (mm)	hind foot length (mm)	tail length (mm)
A. occidentalis	female (n=2)	938.5 (877-1000)	270 (270)	20 (20)	122.5 (120-125)	62.5 (60-65)	360 (360)
	male (n=2)	750 (750)	255 (230-280)	21 (20-22)	112.5 (110-115)	60 (60)	275 (250-300)
A. cleesei	female (n=3)	1058.3 (675-1250)	278.3 (230-305)	25.8 (25.0-27.5)	128.3 (120-135)	65 (60-70)	346.6 (330-360)
	male (n=4)	1000 (750 1250)	262.5 (200-315)	30 (30)	127.5 (125-130)	67.5 (65-75)	326.2 (315-350)
A. laniger	female (n=7)	1410.7 (1000-2000)	305.7 (260-350)	25 (20-30)	151.4 (140-170)	78.8 (70-95	362.8 (320-390)
N Mangoro/ Onive Rivers	male (n=2)	1375 (1250-1500)	320 (300-340)	26 (25-27)	142.5 (135-150)	92.5 (80-105)	327.5 (320-330)
A. peyrierasi	female (n=5)	1050 (950-1125)	289 (260-310)	20 (15-25)	146 (130-160)	71 (60-75)	344 (320-370)
S Mangoro/ Onive Rivers	male (n=6)	991.6 (850-1100)	279.1 (255-280)	22.1 (20-25)	136.6 (120-150)	68.3 (60-75)	329.1 (320-350)
A. meridionalis meridionalis	female (n=3)	1200 (950-1400)	270 (230-290)	26 (24-27)		77 (61-95)	318.3 (300-330)
Sainte Luce	male (n=2)	1100 (1100)	250 (250)	25.5 (25-26)		87.5 (75-100)	312.5 (300-325)
A. meridionalis ramanant-soavani	female (n=5)	1019 (900-1255)	269 (240-310)	22 (20-30)	140 (135-145)	69.4 (65-77)	364 (330-400)
Manombo	male (n=3)	896.6 (875-925)	256.6 240-(270)	18.3 (13-22)	135 (130-140)	72.6 (70-75)	370 (360-380)

Conclusion

The genus *Avahi* is very stable cytogenetically, so that only two different karyotypes are found, separating populations from the eastern and western forests of Madagascar. However, the molecular data reported here revealed significant inter-population diversity. As inferred from mitochondrial sequencing, three previously undescribed taxa exist in the eastern forests of Madagascar and the same data set doubts the separate specific status of one western population that was previously recognized as such only on the basis of morphological characteristics. Further morphological studies, and more specimens from each locality, are expected to provide new data corroborating the independent taxonomic status of the three new forms named here, and may additionally indicate the existence of yet more. Although our new descriptions are mainly based on mitochondrial sequence variation, we point out that there is clearly an urgent need for further field and laboratory research to clarify the complete diversity of woolly lemurs. The three new forms represent at

least three "significant evolutionary units" which should be protected to preserve the complete biodiversity of Madagascar.

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CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF THE NEWLY DESCRIBED SPORTIVE LEMUR *LEPILEMUR JAMESI* (LOUIS et al., 2006)

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Key words: *Lepilemur*, Strepsirrhini, cytogenetics, mtDNA, taxonomy

Abstract

A comparative cytogenetic and molecular study was performed on the newly described sportive lemur species *Lepilemur jamesi*. *L. jamesi* has a diploid chromosome number of 2N=26 and differs in six (*L. mustelinus*) to 23 chromosomal rearrangements from all other karyotyped sportive lemur species. In the mitochondrial cytochrome b gene, *L. jamesi* shows distances of 9.7-15.5 % to the other studied species. Although its closest neighbour to the north is *L. microdon*, in phylogenetic tree reconstructions *L. jamesi* clusters together with *L. mustelinus*. In combination with the relative high chromosomal similarity between *L. mustelinus* and *L. jamesi*, this indicates an uncommon biogeographic pattern leading to the current distribution of these sportive lemur species. Based on our cytogenetic and molecular data, the distinct species status of *L. jamesi* is confirmed.

Introduction

The sportive lemurs are small nocturnal primates endemic to Madagascar living in almost all forested areas of the island. Because pelage coloration shows larger inter-individual than interspecific variation, their classification remained controversial until a comprehensive cytogenetic approach was performed (PETIT, 1933; PETTER and PETTER-ROUSSEAUX, 1960). On the basis of cytogenetic characteristics it was possible to distinguish six species: *Lepilemur dorsalis*, *L. edwardsi*, *L. leucopus*, *L. mustelinus*, *L. ruficaudatus* and *L. septentrionalis* (PETTER et al., 1977; RUMPLER and ALBIGNAC, 1978). Recently, the karyotype of *L. microdon* was established (ANDRIAHOLINIRINA et al., 2005; ZARAMODY et al., 2005). Based on karyotypes differences *L. septentrionalis* was split into two separate species *L. septentrionalis* and *L. ankaranensis* (GROVES, 2001; RUMPLER et al., 2001; RAVAOARIMANANA et al., 2004) and *L. mittermeieri* was discovered and karyotyped (RABARIVOLA et al., 2006) so that there are now nine cytogenetically recognized species.

Thus cytogenetics allowed us to distinguish in the eastern forests *L. mustelinus* living north of the Mangoro River up to the area of Sambava from *L. microdon* south of the river. In order to determine more precisely the southern limit of *L. microdon* we performed a field study in the area of Manombo (Fig. 1) where we caught one specimen. The cytogenetic analysis was unsuccessful, however a complete cytochrome b gene sequence was generated. The specific status of the Manombo sportive lemurs was proposed by LOUIS et al. (2006) on the basis of other molecular markers than cytochrome b, and the taxon was described as a new species *Lepilemur jamesi* (LOUIS et al., 2006) (Fig. 2).

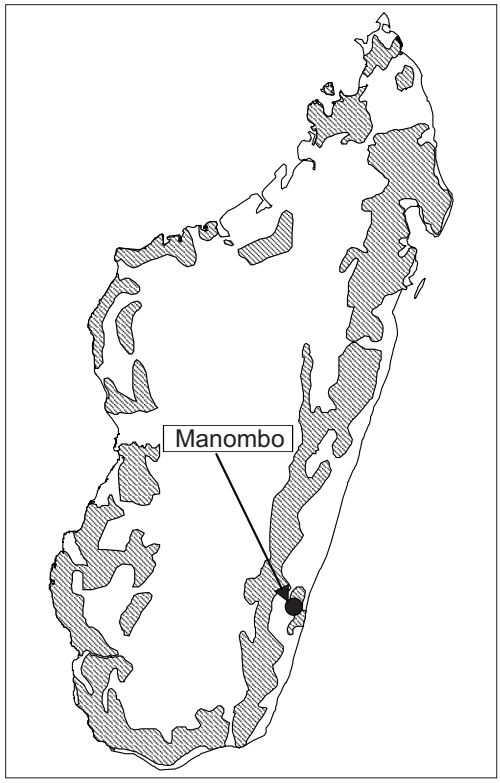

Fig. 1: Sketch of the distribution of *Lepilemur* and the geographic position of the Manombo Reserve, the origin of the *L. jamesi* specimens.

Fig. 2: *Lepilemur jamesi* in the Mamombo Reserve (Photograph by N.A.).

In a recent second attempt, we were able to sample additional individuals in Manombo for chromosomal analyses. The aim of the study was to describe the karyotype of the sportive lemurs from Mamombo, and to clarify whether their karyotype supports their specific status and their phylogenetic relationships inferred from mitochondrial DNA sequences.

Materials and methods

Fieldwork

Four animals, three females and one male, were caught in the reserve of Manombo (Fig. 1) by NA using a blowpipe projection of darts containing a 2mg/kg ketamine solution (Ketalar Parke-Davis). Skin biopsies and 0.5 ml blood samples were taken under general anaesthesia. The blood samples were taken in heparinized tubes for lymphoblast short term cultures in the Institute Pasteur of Madagascar. A part of each skin biopsy was directly frozen in liquid nitrogen while the other part was preserved with a cryoprotector (DMSO 10 %) with the aim of growing

fibroblast cultures. The frozen samples were sent to Europe for further molecular investigations. After recovery from anaesthesia, the animals were immediately released in their capture area.

Cytogenetics

Karyotypes were established on fibroblast cultures. R-and C-banding techniques were applied on the chromosomes following classical methods (DUTRILLAUX and COUTURIER, 1981).

Molecular genetics

DNA was extracted from the skin biopsy with the QIAamp DNA minikit as recommended by the supplier. The complete mitochondrial cytochrome b gene (1,140 bp) was amplified via PCR using oligonucleotide primers and PCR conditions as described in ANDRIAHOLINIRINA et al. (2006). An aliquot of the PCR amplification was checked by agarose gel electrophoresis, and subsequently excised from the gel. After purification with the Qiagen Gel Extraction kit, the PCR product was sequenced on an automated ABI-3100 Avant capillary sequencer with the BigDye cycle sequencing kit (Applied Biosystems). Each sample was sequenced from both directions.

For phylogenetic analyses, the data set was expanded with available orthologous sequences from 12 other sportive lemur species (*L. ankaranensis*, *L. aeeclis*, *L. dorsalis*, *L. edwardsi*, *L. leucopus*, *L. microdon*, *L. mittermeieri*, *L. mustelinus*, *L. randrianasoli*, *L. ruficaudatus*, *L. sahamalazensis*, *L. septentrionalis*), which are deposited in GenBank. As outgroup, *Phaner furcifer* was selected. Uncorrected pairwise distances were calculated with PAUP 4.0b10 (SWOFFORD, 2002). Phylogenetic tree reconstructions using maximum parsimony (MP), neighbor-joining (NJ) and maximum-likelihood (ML) algorithms were performed with PAUP or TREEPUZZLE 5.0 (STRIMMER and VON HAESELER, 1996). For MP analyses, all characters were treated as unordered and equally weighted throughout. NJ and ML trees were constructed with the TVM + I (=0.5319) + G (=2.9942) model of sequence evolution as it was selected as best-fitting model with MODELTEST 3.06 (POSADA and CRANDALL, 1998). Relative support of internal branches was performed by bootstrap analyses with 1,000 replications (MP, NJ), or by the quartet puzzling support values on the basis of 10,000 puzzling steps (ML).

Results

Cytogenetics

We applied the conventional banding techniques RHG and CBG on the metaphases (ISCN, 1978). The karyograms of the *L. jamesi* individuals showed a diploid chromosome number of 2N=26 (Fig. 3). Among the autosomes, we detected seven pairs of metacentric and submetacentric chromosomes and five pairs of acrocentric chromosomes. The smallest metacentric pair showed satellites. The X chromosome was metacentric and the Y was a small acrocentric one (Fig. 3). The C-banding technique revealed heterochromatic pericentromeric regions on all chromosomes (Fig. 4). *L. jamesi* differs from other sportive lemur species in six to 23 chromosomal rearrangements (Table 1). The smallest number of six rearrangements was found be-

tween *L. jamesi* and *L. mustelinus*, i.e. four Robertsonian translocations and two pericentric inversions (Fig. 5).

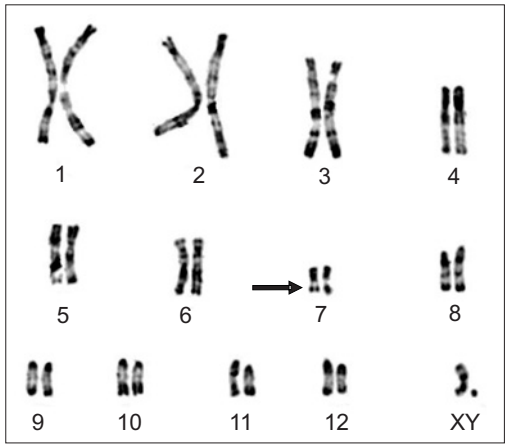

Fig. 3: R-banded karyotype of *Lepilemur jamesi*.Arrow indicates satellites.

Fig 4: C-banded karyotype of *Lepilemur jamesi*.

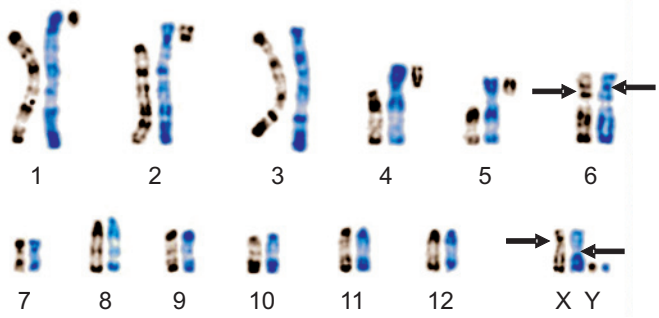

Fig. 5: Comparison of the R-banded chromosomes of *Lepilemur mustelinus* (left, in black) and *Lepilemur jamesi* (right, in blue). The two karyotypes differ by four Robertsonian translocations and two inversions. Arrows indicate the centromeric positions.

Table 1: Number of chromosomal rearrangements among *L. jamesi* and the other karyotyped sportive lemur species.

	Lra	**Lru**	**Lae**	**Led**	**Lmu**	**Ldo**	**Lsa**	**Lmt**	**Lse**	**Lan**	**Lle**	**Lmi**
Lja	21	21	21	23	6	19	19	20	18	19	20	22

Lra=*L. randrianasoli*; Lru=*L. ruficaudatus*; Lae=*L. aeeclis*; Led=*L. edwardsi*; Lmu=*L. mustelinus*; Ldo=*L. dorsalis*; Lsa=*L. sahamalazensis*; Lmt=*L. mittermeieri*; Lse=*L. septentrionalis*; Lan=*L. ankaranensis*; Lle=*L. leucopus*; Lmi=*L. microdon;*Lja=*L. jamesi*.

Molecular genetics

The complete mitochondrial cytochrome b gene sequence (1,140bp) was generated from one *L. jamesi* specimen and compared with orthologous sequence data ob-

tained from 12 other sportive lemur species (ANDRIAHOLINIRINA et al., 2006; RABARIVOLA et al., 2006). *L. jamesi* differs from all these 12 species in 9.7-15.5%. The smallest distance was detected between *L. jamesi* and *L. mustelinus* and not between *L. jamesi* and *L. microdon* (Table 2), although the latter species is *L. jamesi*'s closest neighbour to the north. Likewise, in phylogenetic tree reconstructions, *L. jamesi* clusters with *L. mustelinus* and not with *L. microdon* (Fig. 6). This relationship is significantly supported by all algorithms applied.

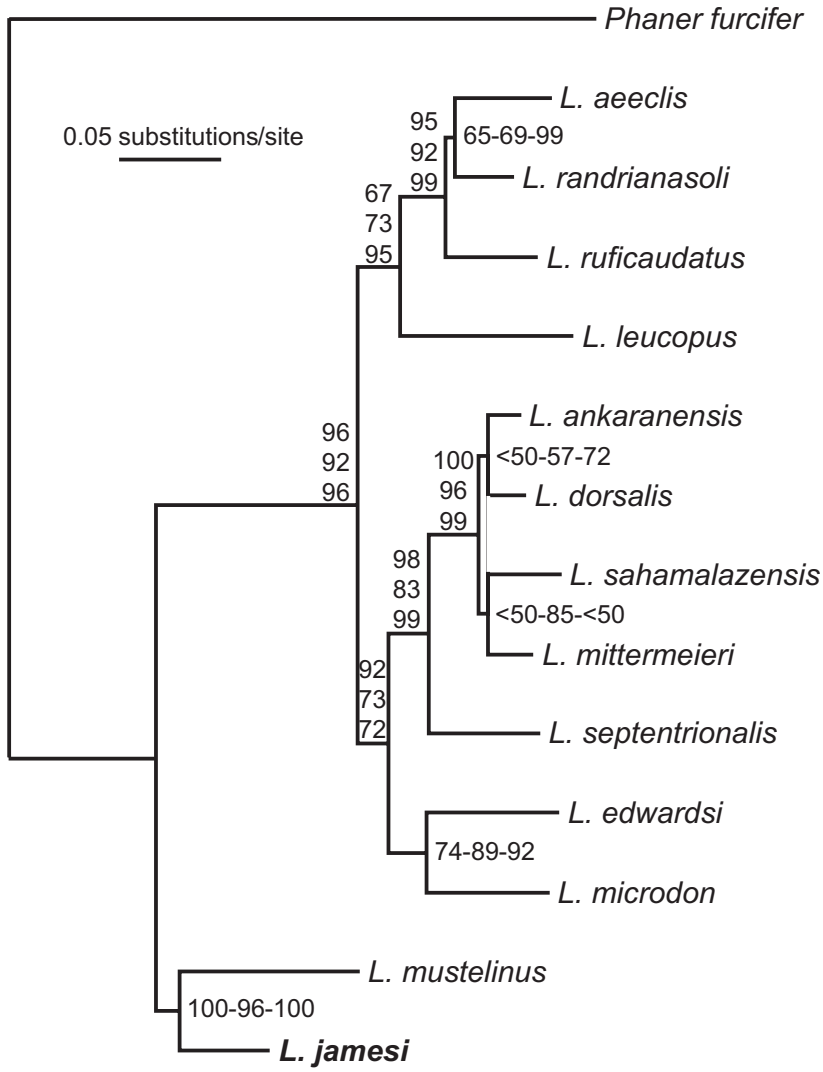

Fig. 6: Phylogenetic relationships among 13 sportive lemur species as obtained from complete mitochondrial cytochrome b sequence data. Branch lengths are based on the NJ tree with numbers on branches indicating internal support (first: MP, second: NJ, third: ML).

Table 2: Uncorrected pairwise genetic distances (in %) among analyzed sportive lemur species. For abbreviations see Table 1.

	Lra	**Lru**	**Lae**	**Led**	**Lmu**	**Ldo**	**Lsa**	**Lmt**	**Lse**	**Lan**	**Lle**	**Lmi**	**Lja**
Lra	-												
Lru	6.4	-											
Lae	6.3	7.6	-										
Led	11.7	12.8	12.5	-									
Lmu	15.4	14.8	15.5	16.2	-								
Ldo	11.6	11.7	11.0	10.7	16.1	-							
Lsa	11.8	11.8	12.9	12.2	16.8	5.5	-						
Lmt	11.0	11.4	11.5	10.4	15.5	4.9	5.0	-					
Lse	11.0	11.7	11.8	10.6	16.0	7.5	9.5	8.3	-				
Lan	11.1	11.1	11.1	10.9	15.5	3.2	4.7	4.3	7.6	-			
Lle	10.3	10.7	11.3	12.5	15.6	12.6	13.2	12.0	13.2	12.7	-		
Lmi	11.7	12.5	12.7	9.2	16.1	10.4	11.3	9.8	10.0	10.4	11.4	-	
Lja	14.3	13.0	14.5	15.3	9.7	13.9	14.7	13.5	14.4	13.7	15.5	15.1	-

Discussion

L. jamesi differs from the other karyotyped sportive lemur species by six to 23 chromosomal rearrangements (Table 1). This large number of chromosomal rearrangements needs some commentaries on its taxonomic classification. It is likely that these rearrangements result in a male sterility of possible hybrids because of disturbance of the meiotic synapses during spermatogenesis (RUMPLER and DUTRILLAUX, 1990; ISHAK et al., 1992). Indeed, it is well known that even minor chromosomal rearrangements which have no effect per se in a homogenous population become selective when they occur in hybrids of genetic distant populations (DJELATI et al., 1997). Also in the molecular data set, large genetic differences were detected between *L. jamesi* and the other studied sportive lemur species. The lowest differences between *L. jamesi* and any other species (9.7 %) were observed to *L. mustelinus* (Table 2). This difference is about three times higher than the lowest difference between chromomsomally different taxa (*L. ankaranensis and L. dorsali*s), indicating an ancient split between *L. jamesi* and *L. mustelinus*. In summary, both cytogenetic and molecular data support the classification of *L. jamesi* as distinct species, and indicate that *L. mustelinus* constitutes its sister taxon. Moreover, the data reveal that both species represent the first split within the genus.

Conclusion

The karyotype of *Lepilemur jamesi* differs from that of all the other karyotyped sportive lemur species, supporting its distinct species status as proposed by LOUIS et al. (2006). Both cytogenetic and molecular data show that the closest relative of *L. jamesi* is *L. mustelinus*. Although both occur along the eastern forests of Madagascar, their distribution is not contiguous, but they are separated by the range of *L. microdon*, which is closely related to the north-western *L. edwardsi*. These findings

show how limited our knowledge about Madagascar's fauna and its evolution still is and calls for further investigations to explain and understand the diversity and biogeography of sportive and other lemurs on the island.

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THE "SIBERUT CONSERVATION PROJECT": FIELD RESEARCH EMBEDDED IN A CONSERVATION PROGRAM

Ziegler T, Abegg C and Hodges K

Introduction

Located about 130 km off the west coast of Sumatra, the Mentawai islands are noted for their unusual species richness and high degree of endemism. Among the 19 endemic mammals, the archipelago currently harbours at least five primate species unique to this region: two species of macaques (*Macaca siberu*, *Macaca pagensis*), two species of langurs (*Presbytis potenziani*, *Simias concolor*) and one lesser ape, the Kloss` gibbon (*Hylobates klossii*).

Despite the uniqueness of Mentawai islands' ecosystems and the importance of natural resources for local people, logging activities have already destroyed large areas on the southern islands of Sipora, North and South Pagai. Only Siberut, the northernmost and biggest island of the archipelago, has remained relatively untouched, with about 60 % of its surface still covered with primary rainforest. However, even Siberut´s forest habitat is now seriously threatened by large scale clearance for agriculture and by illegal and legal logging activities. The latest concession was awarded for a 50,000 ha area in northern Siberut late last year although final approval to commence logging is still pending.

In 2002, scientists from the German Primate Centre (DPZ), Göttingen (D), in cooperation with the Agricultural University of Bogor (Indonesia), established the "Siberut Conservation Project" (SCP), with the aim of combining field research with the implementation of community-based conservation measures. The project is based on a 4000 ha area within the Peleonan forest one of the last remaining, relatively undisturbed and accessible primary rainforests on Siberut (Fig. 1). In addition to conducting basic primatological research and the establishment of biological database required for the development of long-term conservation measures for the region, project activities include a community education programme, improvement of river-based transportation to facilitate marketing of local agro-forestry products and the establishment of controlled eco-tourism.

The SCP field station is situated 7 km upstream from the north coast village of Politcioman in a largely undisturbed primary forest habitat currently protected on the basis of contracts between the SCP, local clans and Indonesian officials. The field station, inaugurated in 2003, comprises six traditionally constructed wooden buildings for accommodation and basic office work. It is situated in the centre of a radial transect system, composed of 13 main transects of 1.5-2 km length, connected by inter-transects, all of which are GPS mapped and systematically labelled. Being the only operational field base on the island, the SCP station provides a valuable opportunity for primatologists and scientists from other disciplines to study Siberuts unique and relatively unknown fauna and flora.

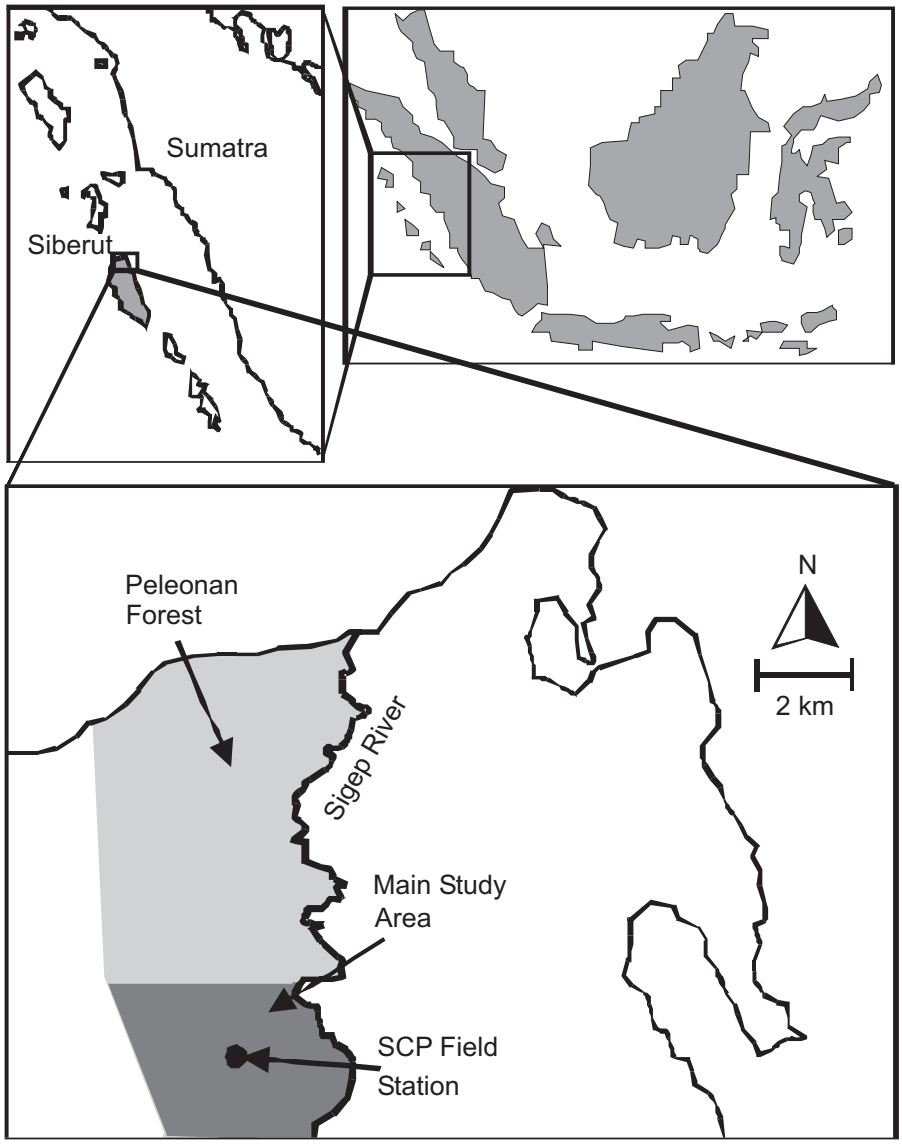

Fig. 1: Location of Siberut Island off Sumatras West coast, the Siberut Conservation Project's field station and the main study area within the Peleonan forests.

Research

SCP-research objectives currently focus on the phylogeny, taxonomy and socio-ecology of the four primate species found on Siberut Island. Habituation is well underway and several groups, especially of *Presbytis potenziani* and *Simias concolor* can be regularly followed and studied from a close distance. In addition to supporting numerous DPZ-initiated projects, the SCP also includes ongoing collaborations

with other institutions from within Germany, as well as from Europe, North America and Australia.

Ongoing and recently completed studies

Molecular phylogeny and taxonomy

One of the first projects to be completed was the clarification of the molecular phylogeny and taxonomy of the Mentawai macaques (ROOS et al., 2003). Based on mitochondrial DNA isolated from faecal samples, the study showed, somewhat surprisingly that the macaques from Siberut island are more closely related to *M. nemestrina* from Sumatra than to the macaques on the neighbouring Mentawai island of Sipora, North and South Pagai. In doing so, the data indicated an paraphyletic origin of the Mentawai macaques and provided the first molecular genetic support for a separation of the Mentawai macaques at a full species level (i.e. *M. siberu* and *M. pagensis*) (Fig. 2, 3).

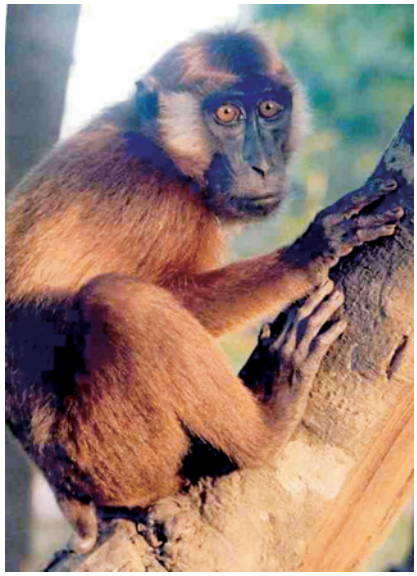

Fig. 2: *Macaca siberu*

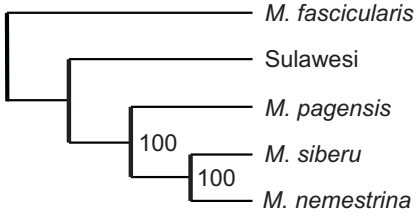

Fig. 3: Molecular-phylogenetic relationships among Mentawai macaques.

In a follow up study, based on a much broader geographic sampling we have examined the phylogenetic relationships of all twelve members of the so called *silenus*-species group and been able to shed new light on the evolutionary history of this most diverse lineage within the genus *Macaca (*ZIEGLER et al., submitted). Based on our findings, we have proposed possible scenarios in one of which peripheral SE Asian islands like Mentawai and Sulawesi served as ecological refuges for progenitors of the *silenus*-group macaque species during relatively cold and dry glacial periods. According to this hypothesis these relatively small macaque populations, surviving in the periphery of Sundaland, may have served as ancestral stocks for the oldest macaque radiation in Asia.

Primate vocalizations

In a recently completed project (Diploma thesis), the structure and usage of the vocalizations of all four sympatric primate species of Siberut (*Macaca siberu*, *Presbytis potenziani*, *Simias concolor*, *Hylobates klossii*) have been examined by Christina Schneider (WG Cognitive Ethology, DPZ). The specific aims of the study were to characterize the sound profile of the habitat the species live in, to analyze the acoustic structure of the long-distance calls produced by these species, and to document the variation of usage over the day. The results are important in helping us to ascertain to what extent species overlap in

terms of the spectral and temporal characteristics of their vocalizations and whether and how the general acoustic characteristics of the habitat influence the structure of the calls (Fig. 4, 5).

Fig. 4: Juvenile Kloss`s Gibbon.

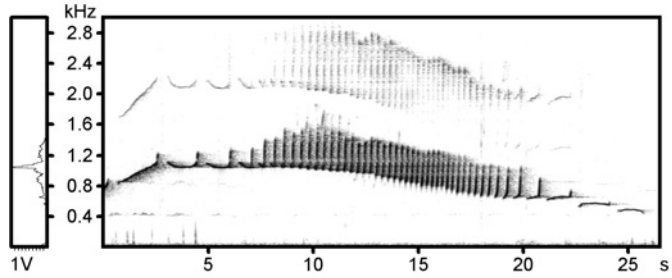

Fig. 5: Typical spectogram of a male Kloss`s Gibbon.

Primate census

In order to assess the conservation status of the four endemic primates within the Peleonan forest, a census of the 11 km² SCP core study area was recently carried out. Using a line transect approach, 104 km of survey effort was accumulated and a total of 391 primate observations made. The results (WALTERT et al., 2006, submitted) indicate a relatively high overall primate biomass density, although the Pig-tailed snub-nosed langur (*Simias concolor*) was clearly the most abundant species present. The data also confirm the conservation importance of the Peleonan forest, which contains a significant proportion of the global populations of all four species.

In absence of detailed topographical maps of the SCP study area necessary to implement and coordinate research and management activities in the field, GPS based mapping of the transect system and the adjacent area within the Peleonan forest was recently initiated. The mapping forms part of an ongoing cooperation between the DPZ and the Centre for Nature Conservation (CNC), University of Göttingen with the combined aims of:

(a) producing accurate and reliable topographical maps for use by researchers in the field and in SCP publications,
(b) establishing a geographic database management system for the project, and
(c) making recommendations for future mapping requirements in the area.

Ecology of langurs

A collaborative study between the DPZ and the CNC, University of Göttingen together with two Indonesian counterparts IPB Bogor and Gadjah Mada University, Yogjakarta, has been initiated to examine niche differentiation of the two sympatric

colobines (*Simias concolor* and *Presbytis potenziani*) in the Peleonan forest (Fig. 6). The study, which represents the PhD project of Susilo Hadi from Yogjakarta, aims to address the following questions: i) how is niche differentiation between the two sympatric colobines characterized with regard to space (preference of habitat type, canopy use, home range size, and daily travel distances) and time (daily patterns of activity and time budgets)? ii) what are the differences in diet composition and food preferences of the two species? iii) how does niche differentiation of the Mentawaian colobines compare to that between other sympatric colobines? iv) to what extent do home ranges overlap and interspecific associations and interspecific interactions occur? v) what are the implications of the findings for species specific conservation strategies.

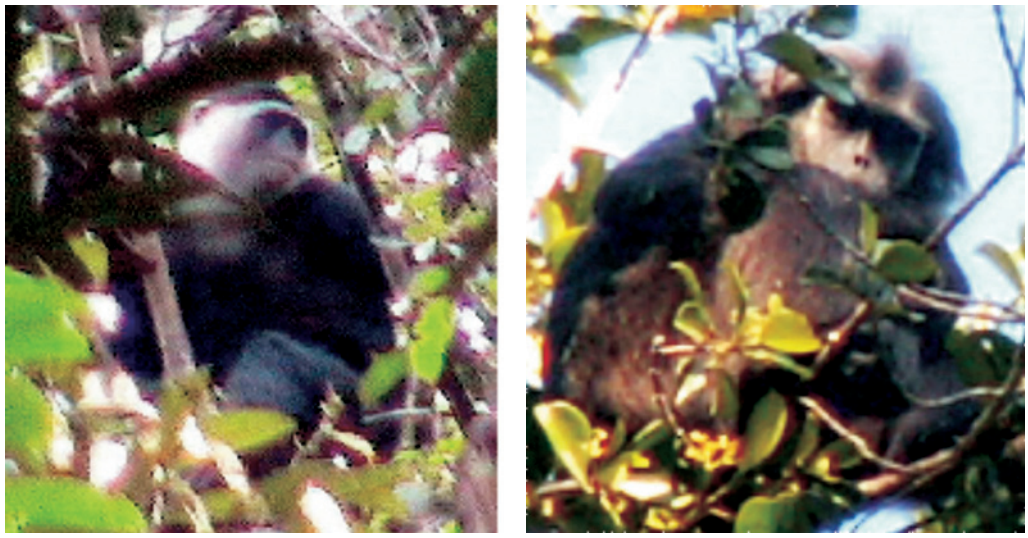

Fig. 6: Sympatric langurs, *Presbytis potenziani* (left) and *Simias concolor* (right), endemic to the Mentawai islands.

Fig. 7: Data on diet resources and food preferences are essential for studies on the "socioecological model" and niche differentiation of the Mentawaian Colobines.

Future studies

Mating system of *Presbytis potenziani*

According to the literature, *Presbytis potenziani*, is the only old world monkey with a monogamous mating system. Since available information on the biology of this Colobine species however is limited and somewhat inconsistent (TILSON and TENAZA, 1976; FUENTES, 1996) a thorough reappraisal of this issue is needed. Due to the long period of isolation and exceptional ecological circumstances found on the Mentawai archipelago (unique type of forest, minimized predation pressure) the Mentawai langurs provide a useful opportunity to test the "socioecological model" (WRANGHAM, 1980; van SCHAIK, 1989, 1996; STERCK, 1999) and to examine the relative influence of ecology and phylogeny on the development of primate social organization, social structure and mating systems (i.e. the social system). A study with these objectives is planned to begin by the end of this year (Fig. 7).

Intergroup aggression in *Simias concolor*

After a successful three month pilot study on site in 2005, another PhD project will be initiated in cooperation with the University of Stony Brook, New York. This study focusses on intergroup aggression and long-distance communication in the Pig-tailed langur (*Simias concolor*) and seeks to test hypotheses about the function of long calls and intergroup aggression. Collection of behavioural and ecological data on three focal groups of habituated animals will begin in October to examine, i) calling rates, frequencies, temporal patterns and durations, ii) social and ecological contexts of calling, iii) intergroup encounter rates, frequencies and durations, and iv) social and ecological contexts of intergroup aggression. The information gained will aid in the design of longer termed research goals.

The Siberut macaques (*M. siberu*) will soon be subject of the first socio-ecological study in this species. Systematic data collection on social behaviour and ecology will start in late 2006, when habituation of these semi-terrestrial primates is sufficiently advanced. Preliminary behavioural observations (ABEGG and THIERRY, 2002) have shown striking similarities to Sulawesi macaques and *M silenus*, characterised by a less despotic hierarchy and more relaxed social relationships within groups compared to *M. nemestrina*.

The SCP also offers research opportunities for scientists from other biological disciplines. One non-primatological study, initiated in March of this year, investigates the effects of habitat disturbance on forest understorey bird assemblages of N Siberut. This study is designed to form the basis for a diploma thesis in cooperation with the the CNC, University of Göttingen.

Apart from scientific studies on site, SCP scientists are also involved in organizing seminars and lectures on Primatology, Behavioural Ecology and Conservation Biology, as well as in conducting workshops in methods of field research and laboratory endocrine analysis at the Agricultural University Bogor (IPB), West Java.

Conservation

In parallel to scientific research, and in line with its initial objectives of promoting sustainable resource management for the region, the SCP has also set up a comprehensive programme of community based conservation. The main components of this are:

Improved river transportation and marketing of agroforestry products

In absence of a road system outside the main villages on the east coast, rivers are the only means of transportation internally on Siberut. To improve transportation and facilitate the marketing of agro-forestry products in the more populated areas of Siberut and particularly on Sumatra, the SCP provides small engines and fuel to operate the traditional dug-out canoes. Transport of local people (workers and pupils) between villages and agro-forestry fields as well as cargo is also supported by the SCP speedboat. A collaborative project with the University of Agriculture/INA, Paris Grignon, France has been investigating ways of improving the economic potential of marketing agro-forestry products in the Politcioman region (Fig. 8).

Fig. 8: Traditional river transportation needs to be improved in order to effectively market agro-forestry products from Siberut.

Education

In addition to provision of basic equipment for the school in the projects partner-village of Politcioman the SCP provides an educational programme in the form of lessons in English and on the value and sustainable use of natural resources. Financed by donations from the French NGO Planète Urgence, with which it co-operates closely, the SCP is currently constructing an education and community centre, which will be used to promote the unique biodiversity of Siberut and to increase awareness of the value of forest resources and the need to protect them. The education centre will also provide information for eco-tourists, on the natural heritage of the island and cultural traditions of the locals. The marketing of handicraft products will broaden the range of measures to generate cash income for the locals (Fig. 9).

Fig. 9: Regular school classes in English and on sustainable use of forest resources are held in the SCP partner-village of Politcioman.

Controlled eco-tourism

Another long term project within the conservation program of the SCP is the development of controlled eco-tourism in northern Siberut. Ecotourism has considerable potential for generating sizable amounts of cash income for the local community and to provide a viable alternative to selling land for logging. In this context the SCP has recently completed stage 1 of the construction of a wooden lodge at the edge of the coastal forest in order to provide initial tourist accommodation. When operational this will provide the basis for a small eco-tourism program, involving visits to two local villages, the field station within primary forest habitat as well as the opportunity to explore Northern Siberuts exceptional marine and coastal ecosystems. A significant portion of the SCP investment into eco-tourism in northern Siberut is provided as a loan to the people of the Salamanang Clan, which is closely cooperating with SCP. These locals will be trained to provide the services needed for eco-tourism, to enable them to take over the eco-tourism management and generate income independently.

As eco-tourism has high economic potential and nature related tourism is incompatible with forest destruction through commercial logging, eco-tourism initiatives can be considered as an effective conservation measure for the region. Furthermore they support SCP initiatives to raise awareness of the value of natural resources not only among the eco-tourists, but also within the local communities of northern Siberut itself.

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Further information on the Siberut Conservation Programme: **www.siberutisland.org**, E-mail contacts: **christopheabegg@yahoo.com**, **tziegl@dpz.eu**, **khodges@gwdg.de**

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NONHUMAN PRIMATES AS LABORATORY ANIMALS

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Introduction

Nonhuman primates belong to the highest developed animals with outstanding abilities in the field of sensory and cognitive physiology. Due to their close evolutionary relationship to humans they are used as laboratory animals and serve as subjects for scientific studies in numerous fields. Scientists include biologists, veterinarians, physicians, ethologists, ecologists and many others who carry on research about, with and for primates. Due to ethical and legal reasons the use of nonhuman primates in biomedical research has to be limited only to problems for which no alternative animal model exists (SCC, 2002).

Taxonomy and biology of primates

Phenotypically primates are not marked by one unique characteristic feature but may be defined by the combination of different characteristics. These typical features belong among others five-radial ends of limbs, fingers which may be opposed, eyes at the front of the head, a strongly furrowed CNS with occipital lobules and a pair of breast-related teats. More important than the morphological criteria are functional similarities which lead to the use of nonhuman primates as animal models in a number of important areas.

Primates do not represent a homogenous phylogenetic group and are today classified into two suborders: prosimians with a rhinarium (Strepsirrhini) and monkeys without a rhinarium (Haplorrhini). The difference between the two classifications is the formerly prosimian family of tarsiers (Tarsiidae). Tarsier may represent the bridge between the prosimians and the true monkeys (GROVES, 2001, 2005). Apart from the family of tarsiers New World monkeys (Platyrrhini) and Old World monkeys (Catarrhini) belong to the Haplorrhini.

New World monkeys evolved in South America, their incidence is limited to Central and South America. Due to changing concepts New World monkeys are classified into five families: Callitrichidae (e.g. marmosets, tamarins), Cebidae (e.g. capuchin monkeys, squirrel monkeys), Aotidae (night monkeys), Atelidae (spider monkeys, howler monkeys, woolly monkeys) and Pithecidae (titi monkeys, sakis and uakaris). Apart from the night monkeys New World monkeys are diurnal animals living in trees, they are characterized by a broad rhinarium and long tails. Males and females show hardly any sex dimorphism, a sexual swelling, often observed in Old World monkeys, is missing.

Two groups can be distinguished within the **Old World monkeys:** One is the group of tailed Old World monkeys (Cercopithecidae), the other one is the group of Hominoidea with the family of Hylobatidae (gibbons) and the family of Hominidae (man and great apes). The Cercopithecidae comprise numerous different species living in a wide range of habitats. Species include macaques, baboons, colobines, guenons and mangabeys. Old World monkeys predominantly live on the ground, have narrow noses and distinct ischial callosities. They lack prehensile tails and

have opposable thumbs. Apart from the barbary macaques in Gibraltar their natural habitats are Asia and Africa. Due to a more or less distinctive sexual dimorphism male and female animals may be distinguished easily, during the receptive phases the females show a sexual swelling.

The apes play a particular role within the order of primates. The family of Hylobatidae or lesser apes (gibbons), great apes with the families of orangutans (*Pongo* spp.), gorillas (*Gorilla* spp.) and chimpanzees (*Pan* spp.), and man (*Homo sapiens)* taxonomically form the superfamily of Hominoidea (ROWE, 1996). Contrary to other Old World monkeys apes (chimpanzees, orang-utans, gorillas) they are restricted to the tropical forests of Africa and Asia (ROWE, 1996).

Nonhuman primate species in biomedical research

Apart from prosimians the following nonhuman primate species are commonly used in biomedical research. Old World monkeys: macaques (*Macaca* sp.), baboons (*Papio* sp.), vervets (*Chlorocebus aethiops*) and mangabeys (*Cercocebus atys*); New World monkeys: squirrel monkeys (*Saimiri sciureus, Saimiri boliviensis*), night monkeys (*Aotus trivirgatus*), marmosets (*Callithrix jacchus*) and tamarins (*Saguinus oedipus*, *Saguinus fuscicollis*) (FORTMAN et al., 2002; WOLFE-COOTE, 2005). Since 2004 apes respectively chimpanzees are no more used in biomedical research in Europe. The last colony of laboratory chimpanzees in Rijswijk was closed down and the animals were transferred to sanctuaries. At the moment there are only a few research areas in which it is mandatory to use them in particular in hepatitis C research and vaccine development. The question remains whether Europe has to provide facilities with chimpanzees to face future challenges of unknown risks like new infectious diseases that might occur and where chimpanzees are the only available model.

The **common marmoset** (*Callithrix jacchus*) is the mainly used species within the group of New World monkeys. Common marmosets may reach a body weight of up to 450 g, their heart frequency varies between 215-265 beats/minute at a body temperature of 36,8-38,6° C (Tab. 1). Due to their blood volume of approximately 30 ml the extraction of blood samples in experimental series is limited. As a rule the animals reach sexual maturity at the age of 18 (female animals) to 24 months (male animals). After a gestation of 144 days usually twins are born. Their social system consists of complex family groups, obviously only one adult pair within a group seems to be reproductive. Monogamous family groups are the rule in captivity in order to avoid attacks of adult members of the same species (FORTMAN et al., 2002; WOLFE-COOTE, 2005).

In biomedical research the representatives of macaques (*Macaca (M.)* sp.) play an outstanding role. The two most important species are **rhesus monkeys** (*Macaca mulatta*) and **cynomolgus monkeys** (*Macaca fascicularis*). The body weight of rhesus monkeys varies from 6 to 11 kg (male), resp. 4 to 9 kg (female). The heart frequency is 98-122/min, the breath frequency 35-50/min, the normal body temperature is between 37-39° C. Depending on the body weight the animals have a blood volume of approx. 500 ml. The animals, which have a life span of more than 30 years, reach sexual maturity at the age of about 3,5 (female) or 4,5 (male) years. After a gestation of 165 to 178 days usually one infant is born each year. The animals live in

large, socially complex groups with several males, the social structure is considerably influenced by female hierarchies (Tab. 1). Depending on spatial circumstances breeding groups with single males are usual in captivity, too.

Table 1: Biological Data of Rhesus Monkeys and Marmosets.

	Rhesus monkey (*Macaca mulatta*)	**Marmoset (*Callithrix jacchus*)**
Classification	Old World or narrow-nosed monkeys (Catarrhini)	New World or broad-nosed monkeys (Platyrrhini)
Natural habitat	Indian subcontinent, southern China	Southeast Brazilian coastal rain-forest
Body weight	m: 6-11 kg; w: 4-9 kg	300-450 g
Life span	30 years	12 years
Sexual maturity	m: 4,5 years; w: 3,5 years	m: 2 years; w: 1,5 years
Heart frequency	98-122/min	215-265/min
Body temperature	37-39° C	36,8-38,6° C
Reproductive data	seasonal estrus cycle of 28 days; 1 offspring after gestation of 165-178 days; birth interval 12-24 months	estrus cycle of 28 days; 2 and more offsprings after gestation of 144 days; birth interval 154 days
Social structure	multimale groups with matrilineal hierarchy	complex family group with one adult reproductive couple
Nutrition	predominantly frugivorous; 400-600 g/day	frugivorous, insectivorous, high proportion of tree exsudates (gums) 20 g/day

In addition to macaques baboons (e.g. *Papio anubis*, olive baboon), guenons (e.g. *Chlorocebus aethiops*, African green monkey), mangabeys (e.g. *Cercocebus torquatus atys*, sooty mangabey) are more or less frequently used laboratory primates.

Actual situation in Germany

According to the annual reports on animal protection of the German Federal Government in which all data of animal experiments are listed, the number of used primates (lemurs not included) varied between 1.000 and 2.000 animals in the past ten years in Germany with a slight increase in the last years (Fig. 1). In 2006, 1585 Old World monkeys (OWM) and 421 New World monkeys (NWM) were used in Germany. In Europe, each year more than 10.000 animals are used in different experiments. Great Britain, France, the Netherlands and Germany together contribute the principal share with approximately 9.000 animals per annum. Especially rhesus and cynomolgus monkeys often have to be imported from breeding colonies in Asia and Mauritius. Subsequently they have to be put in quarantine in a costly procedure

as larger breeding capacities are missing in Europe, respectively are still under construction.

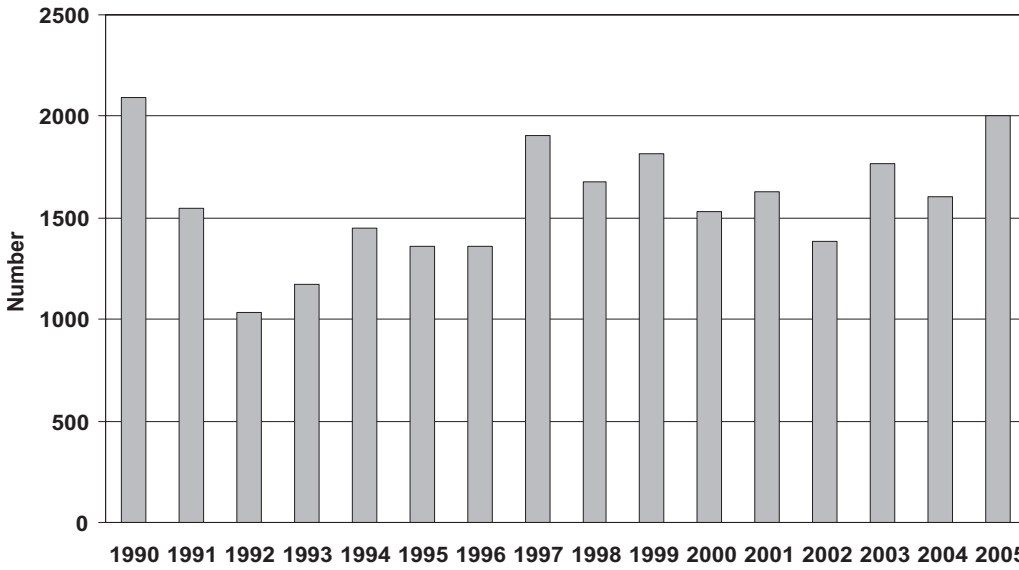

Fig. 1: Nonhuman primates as laboratory animals in Germany 1990-2005 (without prosimians).

Biomedical Research with Laboratory Nonhuman Primates

Numerous studies of anatomy and physiology, growth and development, ethology and sociobiology, and diseases of nonhuman primates have shown the similarities between humans and nonhuman primates. The major use of laboratory nonhuman primates is in toxicology procedures including those for pharmaceutical safety and regulatory procedures. In Germany around 70 % (1299 OWM, 122 NWM) of the total number (2006) are needed in this field (BMELV, 2006).

Since the Thalidomid-tragedy (Contergan®) it was realized that nonhuman primates are valuable for the preclinical risk assessment, particularly as model for the toxicology of development and reproduction (HENDRICKX and BINKERD, 1990), as Thalidomid is causing the same malformations in macaques as in men. However, as a matter of principle pharmaceutical safety tests also have to be critically evaluated. This was shown in Great Britain where severe side effects occurred in human volunteers in a phase-I-clinical study although only mild signs were observed in preclinically tested cynomolgus monkeys. The test using a monoclonal antibody (TGN1412) against CD 28 showed that the involved primate species has to be taken into consideration, too. From this experiences some authors consider chimpanzees as the most relevant animal model in the testing of humanized antibodies (VANDEBERG et al. 2006), although considerable ethical reflections have to be taken into account when chimpanzees are used (GAGNEUX et al., 2005).

Apart from toxicology primates are used in diverse fields of biological or biomedical basic research. Most of the experiments are performed in **neurosciences**, research of infectious diseases, reproductive biology and medicine, in primatology of free ranging primate populations and in veterinary medicine.

It is an undisputed fact that the cognitive abilities of this animal group with the highest developed physiological senses are the closest to men. This does not only affect morphological conditions but particularly neurophysiology which plays an important role in cognitive features as hearing, seeing, attentiveness or vocalization. Such morphofunctional investigations are applied in the corresponding pathophysiological studies and animal models in neuroclinical research. At present nonhuman primates are used in important diseases like e.g. Morbus Parkinson, Morbus Alzheimer, multiple sclerosis and dementia (BROK et al., 2001; SZABO 2005).

The basis for the use of nonhuman primates as close to human model is the genetic similarity of these animals with men. In general 99 % of the primate genome is identical to that of men. For instance the 3´-untranslated mRNA region of the dopamine transporter gene (DAT) in humans presents tandem repeats with a variable length. Similar to human, the rhesus monkey DAT gene contains a series of tandem repeats in the 3´-untranslated region while the rat DAT gene does not contain analogous repeat sequences in this region (MILLER and MADRAS, 2002, 2005). The dopamine transporter gene plays a central role in numerous processes in the CNS and is involved in diverse pathophysiological procedures. This comprises e.g. the ADH (attention deficit hyperactivity) syndrome of children or the problems of drug addiction. For this reason the rhesus monkey is used as corresponding animal model for this kind of questions (MILLER and MADRAS, 2005).

Apart from neurosciences nonhuman primates play an outstanding role in **infection research** (KAUP, 2002). Nonhuman primates either suffer spontaneously from the same infectious diseases as humans or they can be infected experimentally. The resulting clinical symptoms and courses of disease may be identical or similar. This applies for numerous agents, but the main focus is on virus infections and their exploration. In the past this was already shown in the development and production of polio vaccine (SABIN, 1985; FURESZ, 2006) or in the development of a vaccine against hepatitis B (TABOR, 2000; WIELAND and CHISARI, 2005). In both infectious diseases nonhuman primates played an outstanding role. At present chimpanzees, the only animal species which can be infected successfully with the human hepatitis C virus, are still used in the USA for the development of vaccine and therapies against hepatitis C (WIELAND and CHISARI, 2005; VANDEBERG et al., 2005, BARTH et al., 2006).

Old World monkeys, especially macaques, are of particular importance in HIV-/SIV-research. The simian immune deficiency virus (SIV) occurs spontaneously in numerous African primate species. In contrast to the human disease this virus infection does not cause an AIDS-like syndrome although the corresponding number of CD4-cells may decrease in the diverse animal species, too. Certain SIV-strains were successfully adapted to Asian macaque species where they cause an AIDS-like syndrome. The resulting opportunistic infectious diseases and the actual course of the immune deficiency are comparable to those of humans (among many others KAUP et al., 1998; SESTAK, 2005). The macaque SIV/HIV model is the golden standard of experimental AIDS research. The advantages of this model are obvious. On the one

hand a well characterized laboratory animal is used which is infected with defined viruses or virus components. In addition to SIV-variants transgene viruses (SHIV) are used. These viruses offer special possibilities for pathogenetic investigations and the development of vaccines (BATTEN et al., 2006). Moreover a known infectious dose and the possibility of follow up studies with regular tissue and blood sampling are optimal conditions of this close to human animal model. Results from pathogenic investigations have been directly included into clinical research leading to the fact that the life expectancy of human AIDS-patients increased considerably due to the therapies which are now possible. Scientists work flat out at this animal model to develop a safe and long lasting HIV-vaccine.

It has to be mentioned that the natural SIV_{cpz}-infection of the chimpanzee is assumed to be the starting point of the human HIV1-epidemic (GAO et al., 1999). Based on molecular biological and epidemiological investigations it is assumed that there were possibilities of a human infection during the processing of meet from hunted chimpanzees.

Another actual highly topical field in which nonhuman primates are used in infectious disease research is the improvement of the existing pox vaccines. Against the background of the bioterrorist threat the question arose once again whether corresponding protective measures for the prevention of pox infections are necessary. In this context the monkeypox model offers the possibility to develop both strategies for antiviral treatment and efficient new vaccines (STITTELAAR et al., 2006).

The list of viral diseases could be continued but in bacterial diseases as e.g. tuberculosis, lepra, lyme disease and in *Helicobacter pylori*-infections nonhuman primates are at present used as experimental animals in order to develop corresponding new therapeutic strategies or vaccines. Among the parasitic diseases nonhuman primates play an outstanding part especially as to malaria and Chagas Disease. Both parasitic diseases occur spontaneously in primates, therefore they present an ideal animal model.

Apart from the fields of neurosciences and infectious diseases nonhuman primates are used in reproduction biology as the corresponding menstrual cycle with hormonal steering is very close to that of women. In this context it is important to mention that the birth control pill was developed with the participation of nonhuman primates. Animal experiments in which primates are used are also realized in further, very diverse fields of biomedical research. Up to date approaches in the fields of stem cell research, gene therapy or xenotransplantation have to be mentioned here, too (SCHUURMAN and SMITH, 2005; HORN et al., 2006; ROOD et al., 2006).

In the discussions about the abolition of animal experiments using primates it is often forgotten that veterinary medical research is carried out, too, in order to investigate particular syndromes. One example is the Marmoset Wasting Syndrome, here it is possible to get insights into the course of the disease by corresponding investigations and to develop particular therapy concepts (GORE et al., 2001; ZÖLLER, 2006). Animal research saves animal lives. Similar considerations also apply to basic research within the scope of primate biology. Interventions and treatments for scientific investigations are carried out in the wild in nonhuman primates in order to obtain new findings as to evolution, etiology or genetics of this highly interesting animal group.

In summary it may be said that the use of nonhuman primates in scientific research is of particular importance in the following fields:

- Evaluation, assessment and production of vaccines;
- Basic and applied research as to pathogenesis and therapy of human diseases, especially in the field of neurosciences and infections;
- Toxicology and safety tests of critical pharmaceutical substances prior to clinical use in men;
- Basic research for the biological characterization of the diverse primate species;
- Veterinary medical research for the characterization and pathogenesis of defined diseases of nonhuman primates.

In all animal experiments the scientist faces the ethical responsibility to weigh up the damage caused to the animal in the experiments and the benefits for man. This particularly applies for nonhuman primates who are very closely related to man, due to their physiology it has to be assumed that there is a high degree of accordance regarding sensitivity to pain and suffering. However, the ethical responsibility also has to be applied to the people who suffer from chronic or at present incurable diseases. This is the actual ethical dilemma in the responsible handling of animal experiments with and for primates. In this context the involved scientists, veterinarians and animal care takers have to develop the settings for the keeping of infrahuman primates in captivity according to their requirements and they have to offer their expertise to scientists of all provenances in interventions in experimental procedures (POOLE, 1999; McCANN et al., 2006). Here the veterinarian is a responsible coordinating point in the complex interactions and the area of conflict between the requirements of animal protection and those of science, between research for the benefit of man and the integrity of the animals' life.

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