

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

Zaramody A, Fausser J-L, Roos C, Zinner D, Andriaholinirina N, Rabarivola C, Norscia I, Tattersall I and Rumppler Y

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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 this 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 anaesthesia, animals were released in their respective capture areas.

Table 1: Origin and number of samples.

species	areas of capture	coordinates	# samples	# haplotypes
<i>Avahi occidentalis</i>	Ampijoroa	46°49'E-16°18'S	1	1
	P.N. Ankarafantsika	47°00'E-16°00'S	4	3*
<i>Avahi cleesei</i>	Bemaraha	44°42'E-18°51'S	4	1
<i>Avahi laniger</i> 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
<i>Avahi laniger</i> 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
<i>Avahi laniger</i> 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)

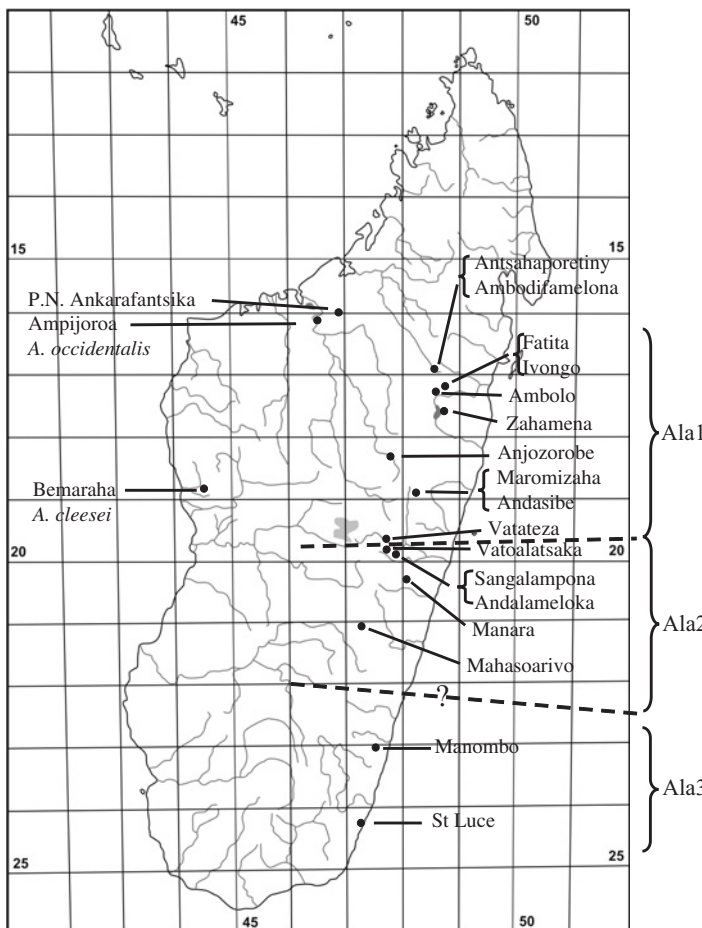


Fig. 1: Sampling sites of woolly lemurs, *Avahi* spp. (geographic coordinates s. Tab. 1).  
 Ala1 = *Avahi laniger* 1, north of the Mangoro/Onive Rivers;  
 Ala2 = *A. laniger* 2, south of Mangoro/Onive Rivers to Mahasoarivo;  
 Ala3 = *A. laniger* 3, populations of the south-east.  
 (----- = taxon 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'-CCGATTCTTCGCATTTT 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 HAESLER, 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).

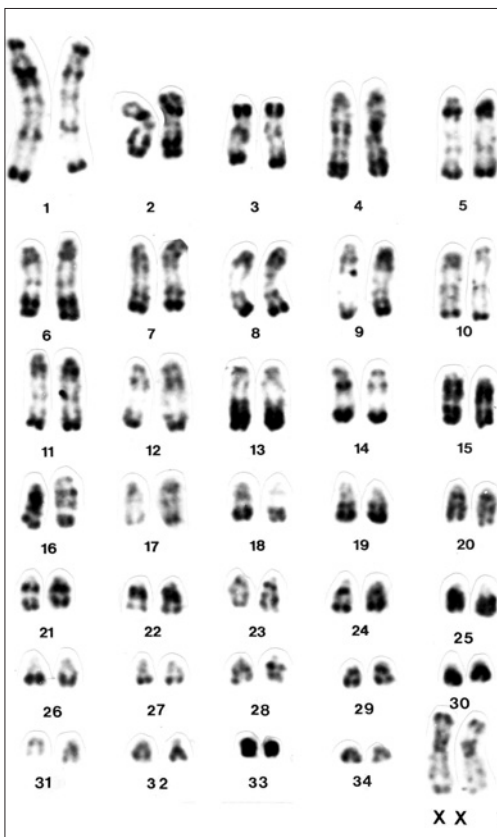


Fig. 2: Karyotype of *A. laniger* female established after R-banding.

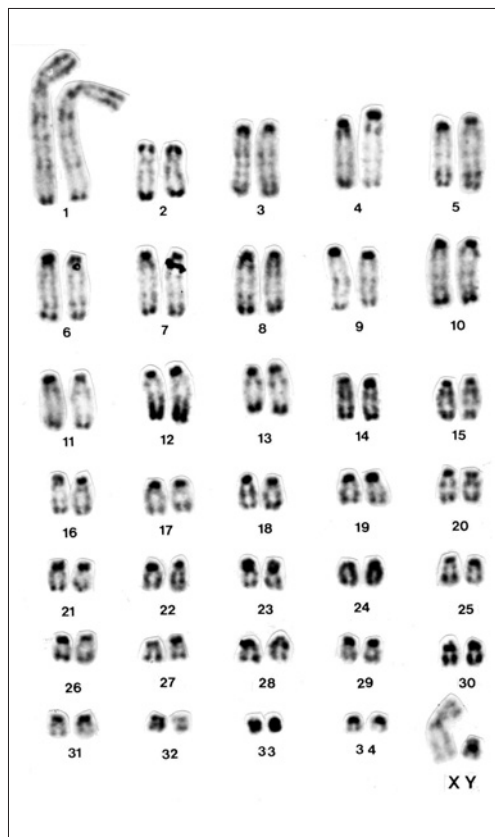


Fig. 3: Karyotype of *A. occidentalis* male established after R-banding.

### 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).

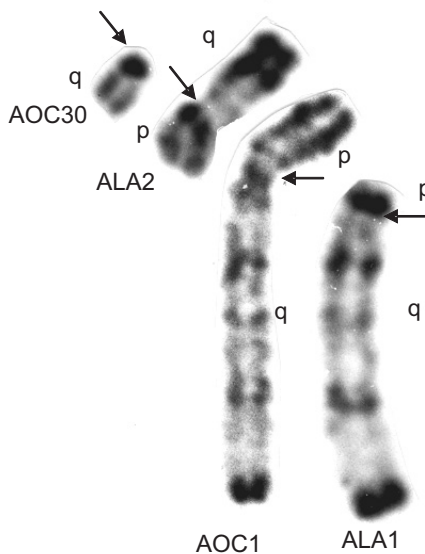


Fig. 4: Chromosomal rearrangements between *Avahi occidentalis* (AOC) and *A. laniger* (ALA). The three chromosomal rearrangements are depicted. One inversion and two Robertsonian translocations separate *Avahi occidentalis* (AOC) from *A. laniger* (ALA). ALA1 and AOC1 differ by one inversion and one 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.

	<i>A. occidentalis</i>	<i>A. cleesei</i>	<i>A. laniger</i>
<i>A. occidentalis</i>	<b>1.07</b>	2.54-2.81	8.77-10.53
<i>A. cleesei</i>	2.68	-	8.42-9.65
<i>A. laniger</i>	9.50	9.06	<b>2.61</b>

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.

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

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.

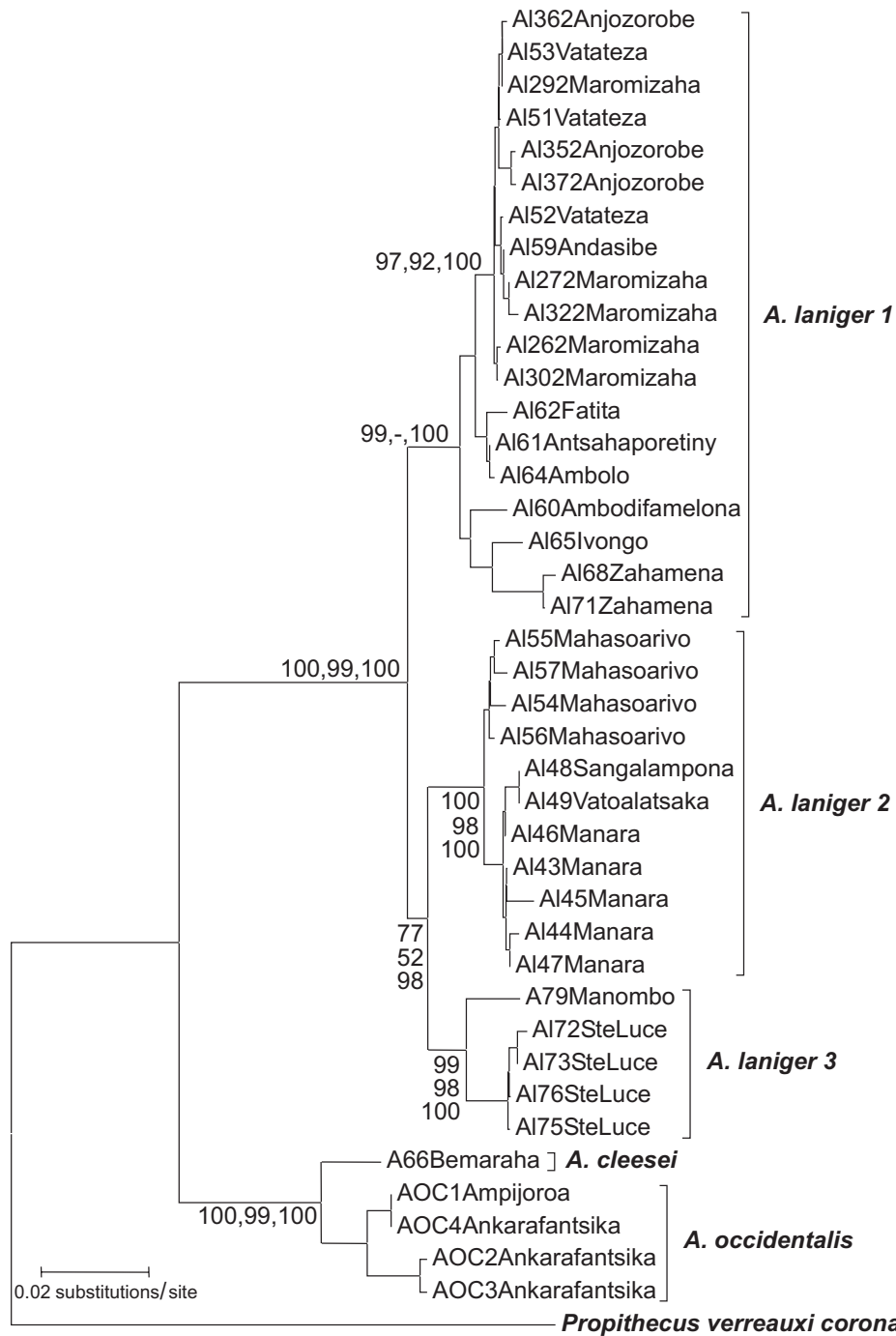


Fig. 5: Phylogenetic relationships as obtained from complete mitochondrial cytochrome b sequence data. Branch lengths are based on the NJ tree with numbers on branches indicating internal support (first: NJ, second: MP, third: ML).



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 *Haplemur 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 Fianarantsoa, 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)
<i>A. occidentalis</i>	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)
<i>A. cleesei</i>	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)
<i>A. laniger</i>	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	1375 (1250-1500)	320 (300-340)	26 (25-27)	142.5 (135-150)	92.5 (80-105)	327.5 (320-330)
<i>A. peyrierasi</i>	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	991.6 (850-1100)	279.1 (255-280)	22.1 (20-25)	136.6 (120-150)	68.3 (60-75)	329.1 (320-350)
<i>A. meridionalis meridionalis</i>	female (n=3)	1200 (950-1400)	270 (230-290)	26 (24-27)		77 (61-95)	318.3 (300-330)
	Sainte Luce	1100 (1100)	250 (250)	25.5 (25-26)		87.5 (75-100)	312.5 (300-325)
<i>A. meridionalis ramanant-soavani</i>	female (n=5)	1019 (900-1255)	269 (240-310)	22 (20-30)	140 (135-145)	69.4 (65-77)	364 (330-400)
	Manombo	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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*Authors' addresses*

Alphonse Zaramody, Université de Mahajanga, Faculté des Sciences, Dépt. de Biologie Animale, B.P. 652, Mahajanga 401, Madagascar, [zaramody@univ-mahajanga.mg](mailto:zaramody@univ-mahajanga.mg)

Jean-Luc Fausser, Institut d'Embryologie, Université Louis Pasteur, Faculté de Médecine-EA3428, 11 rue Humann, 67085 Strasbourg, France, [Jean-luc.Fausser@medecine.u-strasbg.fr](mailto:Jean-luc.Fausser@medecine.u-strasbg.fr)

Christian Roos, Primate Genetics and Gene Bank of Primates, Deutsches Primatenzentrum, Kellnerweg 4, 37077 Göttingen, Germany, [croos@dpz.eu](mailto:croos@dpz.eu)

Dietmar Zinner, Cognitive Ethology, Deutsches Primatenzentrum, Kellnerweg 4, 37077 Göttingen, Germany, [dzinner@gwdg.de](mailto:dzinner@gwdg.de)

Nicole Andriaholinirina, Faculté des Sciences Dépt. d'Anthropologie Biologique, Antananarivo, Madagascar, [nicole-ludes@netcourrier.com](mailto:nicole-ludes@netcourrier.com)

Clément Rabarivola. Université de Mahajanga, Faculté des Sciences, Dépt. de Biologie Animale, B.P. 652, Mahajanga 401, Madagascar, [cjrabary@hotmail.com](mailto:cjrabary@hotmail.com)

Ivan Norscia, Museo di Storia Naturale e del Territorio, Università di Pisa, Italy, [norscia@lunet.it](mailto:norscia@lunet.it)

Ian Tattersall, Division of Anthropology, American Museum of Natural History New York, New York 10024, USA, [iant@amnh.org](mailto:iant@amnh.org)

Yves Rumpler, Institut d'Embryologie, Université Louis Pasteur, Faculté de Médecine-EA3428, 11 rue Humann, 67085 Strasbourg, France, [marguerite.lavaux@embryo-ulp.u-strasbg.fr](mailto:marguerite.lavaux@embryo-ulp.u-strasbg.fr) (Corresponding author)