THE "KAPANY LEMURS PROJECT": MORPHOMETRIC AND GENETIC STUDY OF THE TWO KNOWN SUBSPECIES OF *EULEMUR MACACO* AND A POPULATION OF INTERMEDIATE MORPHOLOGY, IN THE SAHAMALAZA-ILES RADAMA NATIONAL PARK, NORTH WEST OF MADAGASCAR.

CONTENTS

- FIELDWORK AND TEAM 1
- ECOLOGICAL DATA 2
- MORPHOLOGICAL DATA 2
- GENETIC DATA 3
- TAXONOMIC REVISION 3
- CONCLUSION 4
- BIBLIOGRAPHY 4
- APPENDIX 6
- THANKS 31
- PRESENTATION OF THE BUDGET 33

In 2003, lemurs of intermediate morphology between *Eulemur macaco macaco* (Linnaeus, 1766) and *Eulemur macaco flavifrons* (Gray 1867 in Koenders *et al.*, 1985), have been observed by AEECL members in the Sahamalaza-Iles Radama national park. It raised the question of their taxonomic status. The main hypotheses of the result were:

- a new subspecies of *Eulemur macaco* (less probable),
- a hybrid population of *E. m. macaco* x *E. m. flavifrons* (Rabarivola *et al.*, 1991; Wyner *et al.*, 2002; Pastorini *et al.*, 2009),
- a phenotypic cline of one of the two known subspecies (Meyers *et al.*, 1989).

Individuals who belong to this population are designated by the acronym IML (Intermediate Morphology Lemur).

❖ Fieldwork and team

The fieldwork took place in the Sahamalaza-Iles Radama National Park, from April 25th 2010 to July 22nd 2010, under the direction of Dr Christoph Schwitzer. On addition to the main team (Mr Nosy, guide; Mlle Fredine Stelat Masindrazana, cooker; Mlle Odette Razanamahafaly, primatology student and myself), two catching team helped us with the project: team 1 with Dr Borome Ramaromilanto and the team 2 dispatched by the « Madagascar Biodiversity Biogeography Project » (leading by Dr Edward Louis) composed by M. Jean-Claude Rakotoniaina, M. Gerard Nalanirina and M. Jean Razafidraibe.

For catches, we used two anaesthetic products: ketamine and a mix of tiletamine and zolazepam (Telazol®, Ford Dodge). Ketamine induced a lighter sleep and a longer awakening than Telazol®. Ketamine also induced a hyperthermia which was hard to manage, whereas Telazol® led to hypothermia, easier to manage (with warm transmission, placing animals close to yourself). Consequently, for fieldwork, Telazol® showed some advantages.

❖ Ecological data

Some ecological features were noted (Appendix 1 and 2). We found bigger and more numerous forest in the north of the Bevoey village. There were only a few fragments between Bevoey and Maromandia, which could be the result of an important deforestation pressure.

More particularly, the repartition area of IML was determinated (Appendix 3, 4 and 5).

Our study was in line with the studies of many authors (Koenders *et al.*, 1985; Meyers *et al.*, 1989; Groves, 2001; Mittermeier *et al.*, 1994, 2006 and 2008; Schwitzer *et al.*, 2005 and 2006) as regards to the northern boundary of *E. m. flavifrons* depicted by the Andranomalaza River.

According to villagers of Bevoey, lemurs belonging to *Eulemur macaco flavifrons* would be present in the north of the Andranomalaza River, in the forest AMPRI. But, despite many visits, we were not able to observe any lemurs in this forest. A recent hunting could explain it. The introduction of blue-eyed black lemurs by humans would be possible (as pet). This potential population of *E. m. flavifrons* could have mated with black lemurs and resulted in a morphological evolution.

For black lemurs, authors disagreed over the southern boundary: Koenders *et al.* (1985), Meyers *et al.* (1989), Schwitzer *et al.* (2005 and 2006) and Mittermeier *et al.* (2006) thought it was the Andranomalaza river whereas Groves (2001) and Mittermeier *et al.* (1994 and 2008) defined it as the Sambirano river.

Our study will end the debate: if IML are linked to the *E. m. macaco*, the southern boundary will be represented by the Andranomalaza River; otherwise the southern boundary of this subspecies will be considered as the Sambirano River.

❖ Morphological data

The catches of 27 intermediate lemurs and 13 *E. m. flavifrons* allowed a morphometric characterization. 23 biometric criterions (Baden *et al.*, 2008; Craul *et al.*, 2007 and Olivieri *et al.*, 2007; Appendix 6, 7 and 8), and some phenotypic aspects (from pictures) were compared to those of subspecies of *E. macaco*. These data created a first morphometric database. In fact, in literature, we found few information in Mittermeier *et al.* (1994 and 2006)'s study. These authors indicated that *E. m. macaco* weighted 2.0-2.9kg (IML: ♀: 2.03kg, ♂: 1.98kg), had a body length of 39-45cm (IML: 39.6cm) and a tail's length of 51-65cm (IML: ♀: 53.61cm, ♂: 50.89cm). Mittermeier *et al*. (2006) described almost the same measures for *E. m. flavifrons.* We were not able to distinct IML from one of the two subspecies of *E. macaco*, based on biometric data.

We calculated appendicular index (Napier and Napier, 1967 in Jungers, 1985; Appendix 9). These calculations did not allow any distinction either.

Pictures of IML allowed us to describe these animals and to compare them to data from literature (Appendix from 10 to 17). IML had yellow to reddish eyes, ear tufts and beard. Males were totally black. Females were identified by a reddish-brown fur, excepted white ear tufts, beard and belly, and a black face and muzzle. Furthermore, IML females showed a white forehead, sometimes separated by a black or brown interocular line and their arch of eyebrow varied from white to back, including brown colour. We noted, among some females, a difference of colour separating the tail in two parts (light and deep).

Our description was in line with *Eulemur macaco macaco* description in literature (Groves, 2001; Mittermeier *et al.*, 1994, 2006 and 2008 and Rabarivola *et al.*, 1991). But Groves (2001) noted a whitish fur for female whereas we observed deep or light fur. This author spoke about a black interocular line but no colour variation (brown or absence of it). Mittermeier *et al.* (2006) noted a possible separation in deep and light colour on the tail as we did, but they did not observe interocular line. Meyers *et al.* (1989), Rabarivola *et al.* (1991) and Goodman and

Schütz (2000) described some hybrid phenotypic form, in the east of our study area. According to the comparison of pictures from their study (of *ex situ* hybrids) with our animals, IML did not match the description of hybrids forms.

Finally, IML description was more similar to *E. m. macaco* than to *E. m. flavifrons.*

❖ Genetic data

Dr JL Fausser and Dr D Montagnon (Strasbourg's University) analysed our skin biopsies (27 from IML and 13 from *E. m. flavifrons*). The variability of the mitochondrial D-loop (which was appropriate to distinct subspecies, as genetic distances in *Eulemur* genus are very few, Ventura *et al.,* 2001) was analyzed and compared to a genetic database of 98 sequences of the two subspecies of *E. macaco* from Strasbourg's University. Absolute and Tamura-Nei genetic distances were calculated (Tamura and Nei, 1993) and used to build cladograms.

The 27 IML caught were characterized by only 4 haplotypes including a haplotype grouping 23 animals together and another one grouping 2 individuals together. This first data showed a small genetic diversity in IML.

The average genetic distance separating IML to *E. m. macaco* was 10±0.67 bp (base pair); IML to *E. m. flavifrons* was 23±0.16 bp. Average genetic distances separating individuals among the same subspecies were 10±1.28 bp in *E. m. macaco* and 9±0.16 bp in *E. m. flavifrons.* The average genetic distance separating the two known subspecies of *E. macaco* was 25±0.17 bp. According to a Mann-Whitney/Wilcoxon test, IML were genetically closer to *E. m. macaco* than to *E. m. flavifrons.*

Analyzing cytochrome B (Dr D. Montagnon, personal communication), genetic distance between *E. m. macaco* and *E. m. flavifrons* were 2.63-5.78%, was similar to the result of Pastorini *et al.* (2002): 2.83-3.25%.

Building the cladograms showed that 25 of IML (93%) were grouped in the same clade together (Appendix 18 and 19). The two IML left differed from the others only by 6bp. All IML were grouped in the *E. m. macaco* subspecies together. According to Wyner *et al.* (2002), our sample size was big enough to conclude as regards to the taxonomic status of IML.

Finally, we concluded that IML were a **phenotypic cline of *Eulemur macaco macaco*** and that they showed a **very weak genetic diversity**.

❖ Taxonomic revision

In 2002, the « IUCN/Conservation Breeding Specialist Group » (CBSG, reference group working on lemurs) accepted to rise *E. albocollaris, E. collaris* and *E. sanfordi* to species status (based on specific karyotype) but to keep subspecies's status for *E. fulvus fulvus, E. f. rufus, E. f. mayottensis et E. f. albifrons* (Mittermeier *et al.*, 2006). Groves (2001) on the other hand proposed to rise all subspecies of *E. fulvus* to species status, based on phenotypic and cranio-dental criterions. Tattersall (2007) noted that this rising up would be a problem because *E. macaco* would be the only species divided in two subspecies in the entire *Eulemur* genus. Mittermeier *et al.* (2008) approved Groves (2001) based on Pastorini *et al.* (2009)'s study: genetic distance separating *E. fulvus* subspecies (29-90bp) were in the same order of magnitude than those of *E. m. macaco* et *E. m. flavifrons* (68-72bp). But Pastorini *et al.* (2009) explained this genetic proximity by an introgression of gene and refused a taxonomic revision.

Genetic distances were not a perfect tool to use in order to determinate taxonomic status. As underlined by Dr D Montagnon (personal communication, study on cytochrome b on different species of lemurs), there is a bigger genetic distant in the same species (*Avahi laniger*: 0.00088 à 0.12634) than between two distinct species (*P. d. edwardsi* et *P. v. coronatus*: 0.09550 à 0.10117).

To end the debate, in the line of Pastorini *et al.* (2002; important genetic proximity), Groves (2001; similar morphology), Rumpler (2004; same karyotype and production of viable and fertile hybrid) and based on our morphometric study, we suggest to **keep subspecies status for *Eulemur macaco macaco* and *Eulemur macaco flavifrons***.

❖ Conclusion

Finally, we were able to determinate that **IML were phenotypic/morphological cline/variant of *Eulemur macaco macaco***.

Our results, as regards to the repartition area, showed that the range of the *E. m. macaco* extends as far as the Andranomalaza river (in line with Koenders *et al.*, 1985 ; Meyers *et al.*, 1989 ; Schwitzer *et al.*, 2005, 2006 ; Mittermeier *et al.*, 2006) unlike the Sambirano river (Groves, 2001 et Mittermeier *et al.*, 1994 et 2008).

The absence of discovery of hybrids in our study's area, which seemed to be a hybridization area (Meyers *et al.*, 1989; Rabarivola *et al.*, 1991; Schwitzer *et al.*, 2006; Mittermeier *et al.*, 2008), could be a sign of a decrease of genetic mixing. Add to the low genetic diversity observed in this population, these assessments imply urgent decisions for its protection, especially in reducing the habitat fragmentation.

❖ Bibliography

BADEN AL, BRENNEMAN RA, LOUIS EEJ (2008) Morphometric of Wild Black-and-White Ruffed Lemurs [*Varecia variegata*; Kerr, 1792]. *Am J Primatol*, **70**(10), 913-926.

CRAUL M, ZIMMERMANN E, RASOLOHARIJAONA S, RANDRIANAMBININA B, RADESPIEL U (2007) Unexpected species diversity of Malagasy primates (*Lepilemur* spp.) in the same biogeographical zone: a morphological and molecular approach with the description of two new species. *BMC Evol Biol*, **7**, 83-98.

GOODMAN S, SCHUTZ H (2000) The lemurs of the northeastern slopes of the Réserve spéciale de Manongarivo. *Lemur news*, **5**, 30-33.

GROVES C (2001) *Primate taxonomy*. Smithsonian Institution press, Washington and London, 350p.

JUNGERS WL (1985) Body size and scaling of limb proportions in Primates. *In:* Size and scaling in primate biology. New York and London, Plenum press, 345-381.

KOENDERS L, RUMPLER Y, RATSIRARSON J, PEYRIERAS A (1985) *Lemur macaco flavifrons* (Gray, 1867): a rediscovered subspecies of primates. *Folia Primatol*, **44**, 210-215.

MEYERS D. M, RABARIVOLA C, RUMPLER Y (1989): Distribution and conservation of Sclater's lemur: implications of a morphological cline. *Primate conservation* **10**, 77-81.

MITTERMEIER RA, GANZHORM JU, KONSTANT WR, GLANDER K, TATTERSALL I, GROVES CP *et al.* (2008): Lemur diversity in Madagascar. *Int J Primatol*, **29**, 1607-1656.

MITTERMEIER RA, KONSTANT WR, HAWKINS F, *et al.* (2006) Lemurs of Madagascar. *Conservation international, tropical field guides series.*2nd ed. Washington DC, Conservation International, 520p.

MITTERMEIER RA, TATTERSALL I, KONSTANT WR *et al.* (1994) Lemurs of Madagascar. *Conservation international, tropical field guides series*. Washington DC, Conservation International, 355p.

OLIVIERI G, ZIMMERMANN E, RANDRIANAMBININA B, RASOLOHARIJAONA S, RAKOTONDRAYONY D, GUSCHANSKI K *et al.* (2007) The ever-increasing diversity in mouse lemurs: Three new species in north and northwestern Madagascar. *Mol Phylogenet Evol*, **43**(1), 309-327.

PASTORINI J, ZARAMODY A, CURTIS D, NIEVERGELT CM, MUNDY NI (2009) Genetic analysis of hybridization and introgression between wild mongoose and brown lemurs. *BMC Evol Biol*. **9**, 32-45.

PASTORINI J, FORSTNER MR, MARTIN RD (2002) Phylogenetic relationships among Lemuridae (Primates): evidence from mtDNA. *J Hum Evol*, **43**(4), 463-478.

RABARIVOLA C, MEYERS D, RUMPLER Y (1991) Distribution and morphological characters of intermediate forms between the black lemur (*Eulemur macaco macaco*) and the sclater's lemur (*E. m. flavifrons*). *Primates* **32**(2), 269-273.

RUMPLER Y (2004) Complementary approaches of cytogenetics and molecular biology to the taxonomy and study of speciation processes in lemurs. *Evolutionary Anthropology*, **13**, 67-78.

SCHWITZER C, SCHWITZER N, RANDRIATAHINA GH, RABARIVOLA C, KAUMANNS W (2005) Inventory of the *Eulemur macaco flavifrons* population in the Sahamalaza protected area, northwestern Madagascar, with notes on an unusual color variant of *E. macaco*, *Primate Report Special Issue* **72**-1 : 39-40.

SCHWITZER C, SCHWITZER N, RANDRIATAHINAG H, RABARIVOLA C, KAUSMANNS W (2006): "Programme Sahamalaza": New perspectives fort he *in situ* and *ex situ* study and conservation of the blue-eyed black lemur (*Eulemur macaco flavifrons*) in a fragmented habitat. *In:* SCHWITZER C, BRANDT S, MAMILIJAONA O, *et al*.: *Proceedings of the German-Malagasy research cooperation in life and earth sciences* (135-149), Berlin: Concept Verlag.

TATTERSALL I (2007) Madagascar's lemurs: cryptic diversity or taxonomic inflation? *Evol Anthrop,* **16**, 12-23.

VENTURA M, BONIOTTO M, CARDONE MF, FULIZIO L, ARCHIDIACONO N, ROCCHI M *et al.* (2001) Characterization of a highly repeated DNA sequence family in five species of the genus *Eulemur*. *Gene*, **275**(2), 305-310.

WYNER Y, JOHNSON SE, STUMPF RM, DESALLE R (2002) Genetic assessment of a white-collared x red-fronted lemur hybrid zone at Andringitra, Madagascar. *Am J Primatol*, **57**(2), 51-66.

❖ Appendix

Appendix 1 : Classification of forest with presentation of GPS coordinates, counting of animals and place of realisation of catches. 7
Appendix 2 : Ecological description of forest. 9
Appendix 3: localisation of forest between Ankitsika and Bevoey. 11
Appendix 4: Localisation of forest between Bevoey and Maromandia. 12
Appendix 5: Localisation of forest between Maromandia and Ambodimanga. 13
Appendix 6: Presentation and definition of the 23 morphometric criterions. 14
cm: centimetreAppendix 7: Average of morphometric measures (in cm) of the 27 IML, by sex and age. 15
Appendix 7: Average of morphometric measures (in cm) of the 27 IML, by sex and age. 16
Appendix 8: Average of morphometric measures (in cm) of the 13 *E. m. flavifrons*, by sex and age. 18
Appendix 9: Average of appendicular index (modified by Baden *et al.*, 2008) of adult female and male IML and *E. m. flavifrons* caught during our study. 20
Appendix 10: Lines and colour of IML adult and no-adult females and males (personal pictures). 21
Appendix 11: Lines and colour of *Eulemur macaco flavifrons* adult and no-adult females and males (personal pictures). 22
Appendix 12: Lines and colour of forehead of IML adult and no-adult females (personal pictures). 23
Appendix 13: Lines and colour of forehead of *Eulemur macaco flavifrons* adult and no-adult females (personal pictures). 24
Appendix 14: Lines and colour of back of *E. m. flavifrons* adult and no-adult females (personal pictures). 25
Appendix 15: Lines and colour of back of IML adult and no-adult females (personal pictures). 26
Appendix 16: Lines and colour of abdomen of *E. m. flavifrons* adult and no-adult females (personal pictures). 27
Appendix 17: Lines and colour of abdomen of IML adult and no-adult females (personal pictures). 28
Appendix 18: Phylogenesis of lemurs of our study and of the database of Strasbourg's University in ML cladogram. 29
Appendix 19: Phylogenesis of lemurs of our study and of the database of Strasbourg's University in NJ cladogram. 30

Appendix 1 : Classification of forest with presentation of GPS coordinates, counting of animals and place of realisation of catches.

Forest name	Code	Altitude (en m)	GPS coordinates (degree-minute decimal)	Presence of lemurs	Counting					Realisation of catches
					Total	F ad	M ad	F nad	M nad	
Ambalavato	BALATO	107	S14°07.407' E048°04.106'	No	-					No
Ambatomadosobe	MADOBE	81	S14°06.917' E048°03.281'	Observed	2F 2M	1	1	1	1	No
Ambatomadosohely	DOSOHE	72	S14°07.391' E048°03.290'	Vocalisations	-					No
Ambodimadrirofo	DRIRO	0	S14°06.355' E048°02.747'	No	-					No
Ambodivanio-Ankaramihely	AMBOKARA	17	S14°06.481' E048°02;855'	No	-					No
Ambodivanio-Bevoey	AMBEVO	56	S14°10.496' E048°04.584'	Observed	2F 2M	1	1	-	-	Yes
Amparikely	AMPRI	50	S14°11.122' E048°03.991'	No	*E. m. flavifrons* supposing					No
Analabetsigny	ALABET	161	S14°05.782' E048°04.121'	Observed	3F 3M 2F 3M 4 à 6 individuals					Yes
Analafady-Ambodimanga	AFADY	4	S14°14.302' E048°02.252'	Observed	3F 3M 3F 4M 1M observed					Yes
Analafaly	FALY	33	S14°07.610' E048°03.047'	Observed	12	4	1	2	5	Yes
Analalavahely	LALAVA	78	S14°14.174' E048°03.000'	Observed	2F observed					No
Analamisakana	SAKANA	3	S14°05.373' E048°02.184'	No	-					No
Analamora	MORA	112	S14°05.673' E048°03.884'	No	-					No
Andebinirakoto	RAKOTO	65	S14°06.180' E048°03.232'	No	-					No

Presence of *E.flavifrons*

- : no data

F: female M: male ad: adult nad: no adult

Appendix 1(following-up): Classification of forest with presentation of GPS coordinates, counting of animals and place of realisation of catches.

Forest name	Code	Altitude (en m)	GPS coordinates (degree-minute decimal)	Presence of lemurs	Counting					Realisation of catches
					Total	F ad	M ad	F nad	M nad	
Andengilava	ANGIL	72	S14°12.088' E048°04.100'	No	-					No
Andranomiditra	MIDITRA	141	S14°07.670' E048°04.614'	No	-					No
Andilatany	LATANY	57	S14°06.307' E048°03;017'	No	-					No
Andohaniankaramihely 1	ANDO 1	82	S14°05.979' E048°03.810'	Observed	1F 3M					Yes
Andohaniankaramihely 2	ANDO 2	171	S14°05.927' E048°04.131'	Observed	6 à 7 individuals					Yes
Andohaniankaramihely 3	ANDO 3	78	S14°06.035' E048°03.493'	No	-					No
Andohaniankaramihely 4	ANDO 4	91	S14°05.863' E048°03.717'	No	-					No
Andohaniankaramihely 5	ANDO 5	150	S14°06.000' E048°04.041'	No	-					No
Andoloambo	ANLOBO	77	S14°11.680' E048°04;453'	No	-					No
Ankaramihely	ANKARA	20	S14°06.540' E048°03.062'	Observed	3F 3M					Yes
Ankiririka	KIRIR	88	S14°11.725' E048°05.487'	No	-					No
Ankitsika	ANKI	44	S14°05.063' E048°03.861'	Observed	4 à 6 individuals					No
Antandrarafa	DRAFA	74	S14°09.431' E048°04.012'	No	-					No
Beazatambo	BEAZA	143	S14°07.517' E048°04.377'	No	-					No
Beteimbengny	BETEI	144	S14°06.149' E048°03.857'	No	-					No
Kapany	KAPY	110	S14°06.856' E048°03.719'	Observed	-					No
Mangrove face à AFADY	Mang-AFADY	16	S14°14.392' E048°01.905'	Observed	1M observed					Yes

Presence of *E.flavifrons* - : no data

F: female M: male ad: adult nad: no adult

The counting of lemurs was very difficult because of the shyness of the animals. So our counting is probably an under estimation.

Appendix 2 : Ecological description of forest.

Code	Status	Qualitative density	Average height of tree	Ecological comment	Presence of deforestation
BALATO	II	low dense	7-10m	-	Coupe bois
MADOBE	I, II	high dense à dense	4-12m	Primary status forest in a inaccessible mudslide	Stubble-burning, village, zebu
DOSOHE	I, II	high dense à low dense	6-15m	-	Paddy field, stubble-burning, houses, zebu
DRIRO	I	dense	10m	Mangrove	Wood cut
AMBOKARA	II	low dense	5-10m	-	Paddy field
AMBEVO	II	high dense	15m	On the side of the patch Maromandia-Bevoey	Wood cut
AMPRI	I	dense	15-20m	High narrow forest on the side of a cliff, supposing presence of *E. flavifrons*	Paddy field, stubble-burning, hunting
ALABET	I	high dense	15-20m	Fady (=traditional forbidden)	Deforestation on the periphery
AFADY	I	dense	10-15m	Fady, few tree (5m) linked two principals parts (a)	Village, paddy field
FALY	I	high dense	15-20m	-	Deforestation on the periphery
LALAVA	II	dense	5-10m	Very difficult access (cliff, cutting plants)	Wood cut
SAKANA	II	dense	5-10m	Presence of lemurs in March (mango)	Stubble-burning, wood cut
MORA	II	low dense	-	-	Village
RAKOTO	II	low dense	7-10m	Plantation of bananas	Plantation of bananas, stubble-burning

Presence of *E. flavifrons* observed

I: primary status

II: secondary status

I, II: Forests with a primary status in its centre with a secondary status periphery, their surfaces are similar.

II, I central: forest with a primary status in its centre of a very few surface with a secondary status periphery of a very bigger surface

(a): Groups of Mang-AFADY crossed paddy field to go in AFADY.

Same code of forest than in Appendix 1

Appendix 2 (following-up): Ecological description of forest.

Code	Status	Qualitative density	Average height of tree	Ecological comment	Presence of deforestation
ANGIL	II	dense	5m	Coffee cultivated land	Village, coffee cultivated land
MIDITRA	II	low dense	12m	-	Deforestation of periphery
LATANY	II	dense	-	Interdiction linked to interdiction of Kapany	-
ANDO 1	II	low dense	10-12m	-	Stubble-burning
ANDO 2	II	low dense	10m	-	Deforestation of periphery
ANDO 3	II	dense	5m	-	Wood cut
ANDO 4	II	low dense	5m	-	Wood cut
ANDO 5	II	dense	7-12m	-	Deforestation of periphery
ANLOBO	II	dense	7-10m	Coffee cultivated land	Coffee cultivated land, village, wood cut
ANKARA	II	low dense	3-10m	Coffee cultivated land	Coffee cultivated land, stubble-burning, village
KIRIR	II, I central	low dense	2-3m à 10-12m	On the side of national road N6	Stubble-burning
ANKI	I	high dense	15-20m	-	Stubble-burning , hunting, wood cut
DRAFA	II, I central	high dense	5-10m à 15-20m	On the side of patch Bevoey-Maromandia	Wood cut
BEAZA	II	dense	10m	-	Wood cut
BETEI	II	low dense	5-7m	-	Stubble-burning
KAPY	-	-	-	Interdiction of Kapany	-
Mang-AFADY	II	dense	5-10m	Mangrove (a)	Wood cut
				Presence of *E. flavifrons* observed	

I: primary status

II: secondary status

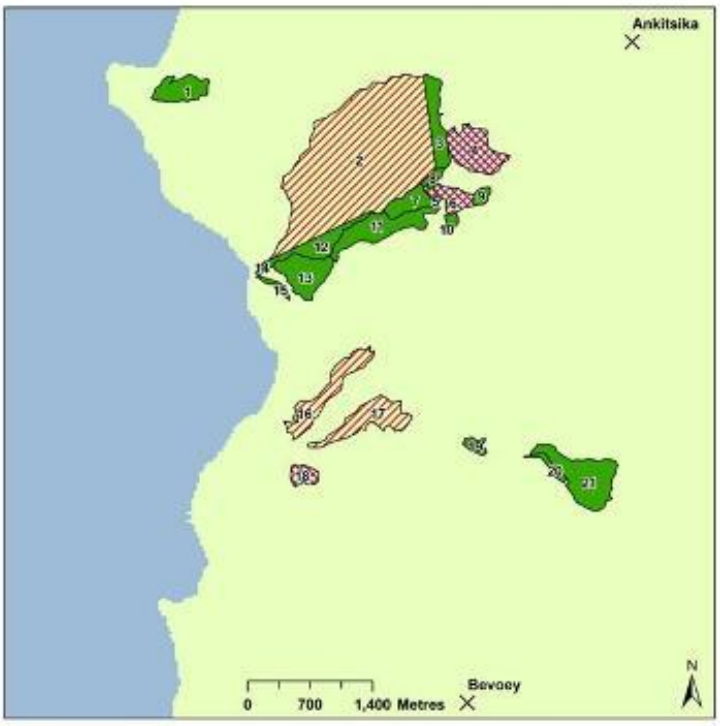
I, II: Forests with a primary status in its centre with a secondary status periphery, their surfaces are similar.

II, I central: forest with a primary status in its centre of a very few surface with a secondary status periphery of a very bigger surface

(a): Groups of Mang-AFADY crossed paddy field to go in AFADY.

Same code of forest than in Appendix 1

Appendix 3: localisation of forest between Ankitsika and Bevoey.

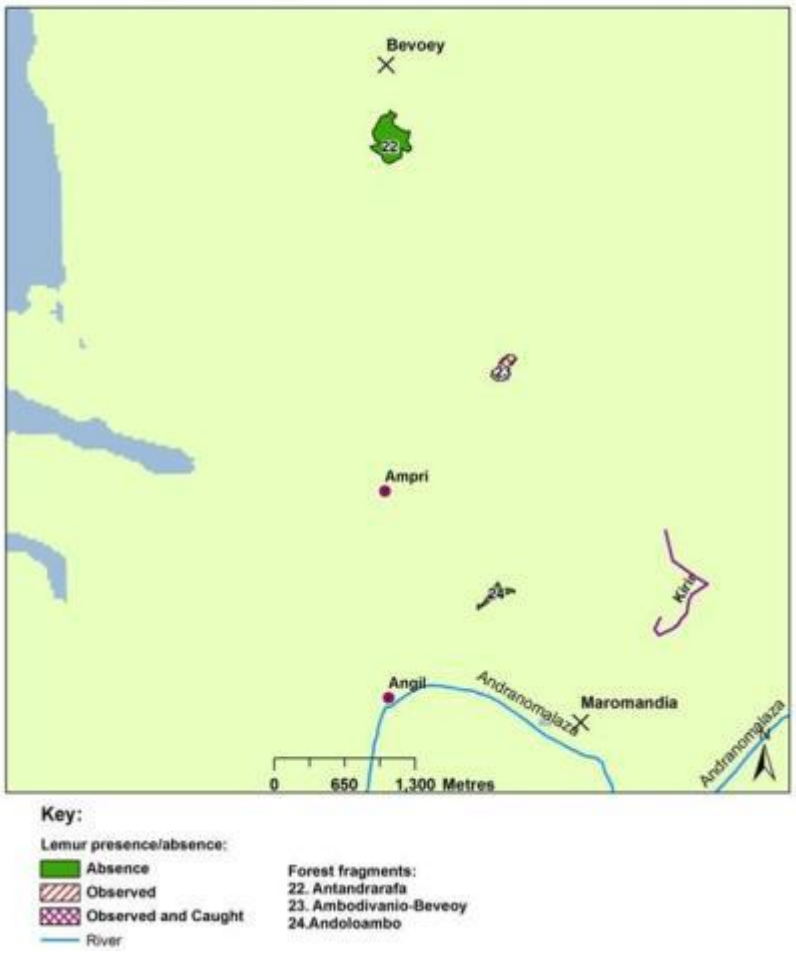


Key:

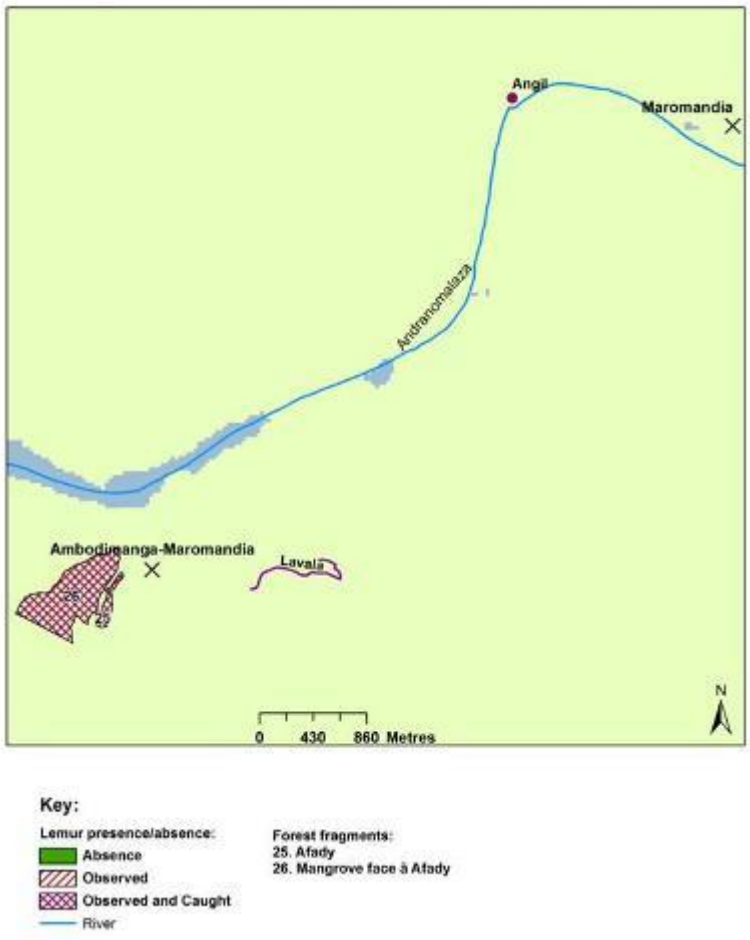
Lemur presence/absence:
- Absence
- Observed
- Observed and Caught

Forest fragments:
1. Analamisakana
2. Kapany
3. Analamora
4. Analabetsigny
5. Andohaniankaramihely 1
6. Andohaniankaramihely 2
7. Andohaniankaramihely 3
8. Andohaniankaramihely 4
9. Andohaniankaramihely 5
10. Beteimbegny
11. Andebinirakoto
12. Andilantany
13. Ankaramehely
14. Ambodimadrirofo
15. Ambodivanio_Ankaramihely
16. Ambatomadosobe
17. Ambatomadosohely
18. Analataly
19. Ballato
20. Beaza
21. Miditra

Appendix 4: Localisation of forest between Bevoey and Maromandia.



Appendix 5: Localisation of forest between Maromandia and Ambodimanga.



The evaluation of the repartition area in being linked to the observation of lemurs, our estimation could be probably an under estimation too. These data could be improve using transmitter collar and a longer study to consider the potential move of animals depending on different fructification.

Appendix 6: Presentation and definition of the 23 morphometric criterions.

Morphometric criterion	Measure unity	Abbreviation	Definition
Weight	Kg	Weight	Body weight
Interobital distance	cm	Interobit dist	Measure between medial angles of eyes.
Ear length	cm	ear lg	Measure vertically of the height of the auricle.
Ear tuft length	cm	Tuft lg	Specific to the IML, subtraction of ear length to the measure from the basis of auricle to the extremity of ear tufts
Beard length	cm	Beard lg	Specific to the IML, measure vertically from the temporo-mandibular joint to the extremity of the beard.
Canine height	cm	Canine ht	Measure buccally on the midline of the canine from the maxillary gumline to the tip of the canine crown.
Second premolar height	cm	2nd PM ht	Measure buccally on the midline of the canine from the mandibular gumline to the tip of the second premolar crown.
Muzzle length	cm	Muzzle lg	Measure from the glabella to the distal extremity of the muzzle.
Headcrown length	cm	Headcr lg	Measure from the glabella to the midpoint of the superior nuchal line.
Head length	cm	Head lg	Measure from the distal extremity of the muzzle to the midpoint of the superior nuchal line.
Body length	cm	Body lg	Measure dorsally from the midpoint of the nuchal line to the base of the tail at the junction with the perianal region.
Tail length	cm	Tail lg	Measure dorsally from the base of the tail to the distal tip of the last caudal vertebra with the tail extended straight out behind animal.

Kg: kilogramme cm: centimetre

Appendix 6 (following-up): Presentation and definition of the 23 morphometric criterions.

Morphometric criterion	Measure unity	Abbreviation	Definition
Brachium length	cm	Brach lg	Measure laterally from the proximal tip of the greater tuberosity to the distal tip of the lateral humeral epicondyle.
Antebrachium length	cm	Antbrac lg	Measure laterally from the oleocran process to the tip of the ulnar styloid process.
Hand length	cm	Hand lg	Measure palmarly at the midline from the radio-carpal joint to the distal tip of the longest digit, excluding the nail.
Pollex length	cm	Pollex lg	Measure palmarly from the first metacarpal-phalangeal joint to the distal tip of the thumb, excluding the nail.
Third digit of hand length	cm	3rd digit lg	Measure palmarly from the third metacarpal-phalangeal joint to the distal tip of the third digit, excluding the nail.
Waistline length	cm	Waist lg	Measure of the length of the abdomen thought the last lumbar vertebra.
Thigh length	cm	Thigh lg	Measure laterally with the knee at the 90° angle from the tip of the greater trochanter to the most distal point of the lateral femoral condyle.
Leg length	cm	Leg lg	Measure laterally from the proximal edge of the lateral tibial condyle to the lateral fibular malleous.
Foot length	cm	Foot lg	Measure plantarly from the proximal tip of the heel to the distal tip of the longest digit, excluding the nail.
Hallux length	cm	Hallux lg	Measure plantarly, with the hallux abducted at a 90° angle to the other digits, from the proximal tip of the first metatarsal-phalangeal joint to the tip of the toe, excluding the nail.
Third digit of foot length	cm	3rd dig foot lg	Measure plantarly from the third metatarsal-phalangeal joint to the tip of the longest digit, excluding the nail.

cm: centimetre

Appendix 7: Average of morphometric measures (in cm) of the 27 IML, by sex and age.

12 F ad	Weight	Interobit dist	Ear lg	Tuft lg	Beard lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg
Average ±standard error	2,03 ±0,08	2,49 ±0,05	3,32 ±0,10	3,99 ±0,24	3,98 ±0,14	0,87 ±0,02	0,45 ±0,02	3,88 ±0,11	22,91 ±0,42	10,07 ±0,07	30,96 ±0,57	53,61 ±1,20	9,64 ±0,18

2 F nad	Weight	Interobit dist	Ear lg	Tuft lg	Beard lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg
Average ±standard error	1,35 ±0,15	2,23 ±0,13	3,15 ±0,05	3,85 ±1,15	3,55 ±0,15	-	0,35 ±0,05	3,85 ±0,2	20,65 ±0,55	-	26,20 ±1,6	49,30 ±2,2	7,55 ±1,35

9 M ad	Weight	Interobit dist	Ear lg	Tuft lg	Beard lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg
Average ±standard error	1,98 ±0,09	2,59 ±0,08	3,49 ±0,06	4,02 ±0,29	4,72 ±0,35	0,92 ±0,06	0,44 ±0,02	3,81 ±0,07	22,61 ±0,37	-	27,74 ±0,70	50,89 ±1,47	9,43 ±0,17

4 M nad	Weight	Interobit dist	Ear lg	Tuft lg	Beard lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg
Average ±standard error	1,56 ±0,03	2,60 ±0,05	3,38 ±0,15	4,11 ±0,65	3,79 ±0,15	0,89 ±0,03	0,45 ±0,02	3,64 ±0,13	22,10 ±1,01	-	28,55 ±0,89	49,75 ±1,70	9,20 ±0,15

M: Male F: Female ad: adult nad: no-adult

Number before abbreviation of the sex and age category : number of individuals catching in this category.

- : no data

Lg: length, Dist: distance, interorbit: interorbital, Ht: height, 2^{e} PM: second premolar.

Appendix 7 (following-up): Average of morphometric measures (in cm) of the 27 IML, by sex and age.

12 F ad	Antbrac lg	Hand lg	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	10,90 ±0,17	6,98 ±0,11	2,57 ±0,07	4,25 ±0,17	22,06 ±0,77	13,53 ±0,28	14,75 ±0,20	10,36 ±0,18	3,32 ±0,08	4,13 ±0,22

2 F nad	Antbrac lg	Hand lg	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	9,23 ±1,07	6,40 ±0,80	2,35 ±0,10	3,73 ±0,73	15,75 ±3,55	11,40 ±0,90	13,40 ±0,50	9,63 ±0,82	3,10 ±0,65	3,63 ±1,03

9 M ad	Antbrac lg	Hand lg	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	10,76 ±0,22	7,19 ±0,12	2,63 ±0,06	4,31 ±0,09	20,54 ±0,54	13,37 ±0,20	14,52 ±0,18	10,09 ±0,10	3,41 ±0,07	4,36 ±0,09

4 M nad	Antbrac lg	Hand lg	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	10,39 ±0,19	6,84 ±0,17	2,36 ±0,06	4,08 ±0,13	19,15 ±0,56	12,86 ±0,29	13,95 ±0,31	9,50 ±0,22	3,31 ±0,09	4,23 ±0,16

M: Male F: Female ad: adult nad: no-adult

Number before abbreviation of the sex and age category: number of individuals catching in this category.

- : no data

Lg: length, Dist: distance, interorbit: interorbital, Ht: height, 2^{e} PM: second premolar.

Appendix 8: Average of morphometric measures (in cm) of the 13 *E. m. flavifrons*, by sex and age.

3 F ad	Weight	Interobit dist	Ear lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg	Antbrac lg	Hand lg
Average ±standard error	2,04 ±0,09	2,48 ±0,02	3,18 ±0,12	1,03 ±0,03	0,47 ±0,02	3,60 ±0,09	21,67 ±0,52	10,03 ±0,23	33,53 ±0,98	52,57 ±1,22	9,70 ±0,23	11,23 ±0,17	7,30 ±0,13

1 F nad	Weight	Interobit dist	Ear lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg	Antbrac lg	Hand lg
only one individual caught													

7 M ad	Weight	Interobit dist	Ear lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg	Antbrac lg	Hand lg
Average ±standard error	1,84 ±0,06	2,62 ±0,04	3,07 ±0,07	1,16 ±0,06	0,47 ±0,03	3,51 ±0,12	22,07 ±0,41	10,24 ±0,10	30,70 ±0,73	49,06 ±1,75	9,69 ±0,10	10,97 ±0,13	7,09 ±0,08

2 M nad	Weight	Interobit dist	Ear lg	Canine ht	2nd PM ht	Muzzle lg	Headcr lg	Head lg	Body lg	Tail lg	Brach lg	Antbrac lg	Hand lg
Average ±standard error	1,45 ±0,07	2,38 ±0,02	3,05 ±0,15	0,75 ±0,15	0,38 ±0,03	3,38 ±0,23	18,98 ±0,42	9,75 ±0,05	29,00 ±0,20	48,25 ±0,65	9,28 ±0,13	10,25 ±0,05	6,68 ±0,12

M: Male F: Female ad: adult nad: no-adult

Number before abbreviation of the sex and age category: number of individuals catching in this category.

- : no data

Lg: length, Dist: distance, interorbit: interorbital, Ht: height, 2^{e} PM: second premolar.

Appendix 8 (following-up): Average of morphometric measures (in cm) of the 13 *E. m. flavifrons*, by sex and age.

3 F ad	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	2,58 ±0,09	4,32 ±0,10	22,88 ±0,16	13,83 ±0,19	15,07 ±0,09	10,27 ±0,02	3,53 ±0,06	4,27 ±0,12

1 F nad	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
only one individual caught								

7 M ad	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	2,66 ±0,04	4,29 ±0,08	20,09 ±0,64	13,70 ±0,15	14,51 ±0,15	10,04 ±0,21	3,52 ±0,06	4,35 ±0,07

2 M nad	Pollex lg	3rd digit lg	Waist lg	Thigh lg	Leg lg	Foot lg	Hallux lg	3rd dig foot lg
Average ±standard error	2,60 ±0,30	3,98 ±0,02	18,25 ±0,35	13,05 ±0,25	13,95 ±0,15	9,85 ±0,15	3,33 ±0,03	4,00 ±0,10

M: Male F: Female ad: adultt nad: no-adult

Number before abbreviation of the sex and age category: number of individuals catching in this category.

- : no data

Lg: length, Dist: distance, interorbit: interorbital, Ht: height, 2^{e} PM: second premolar.

Appendix 9: Average of appendicular index (modified by Baden *et al.*, 2008) of adult female and male IML and *E. m. flavifrons* caught during our study.

	Species	IML		*Eulemur macaco flavifrons*	
	Sex	Female	Male	Female	Male
Intermembral index	N	12	9	3	7
	Average	72,72±0,83	72,41±0,81	72,43±1,26	73,23±0,40
Humero-femoral index	N	12	9	3	7
	Average	71,44±1,22	70,59±0,85	70,16±2,18	70,70±0,27
Brachial index	N	12	9	3	7
	Average	113,31±1,75	114,19±2,20	115,86±1,40	113,28±0,82
Crural index	N	12	9	3	7
	Average	109,37±1,82	108,74±1,67	108,96±1,87	105,910,81

IML: Intermediate Morphology Lemurs

Intermembral index: $\frac{\text{brachial length} + \text{antebrachium length}}{\text{thigh length} + \text{leg length}} \times 100$

Humero-femoral index: $\frac{\text{brachial length}}{\text{thigh length}} \times 100$

Brachial index: $\frac{\text{antebrachium length}}{\text{brachium length}} \times 100$

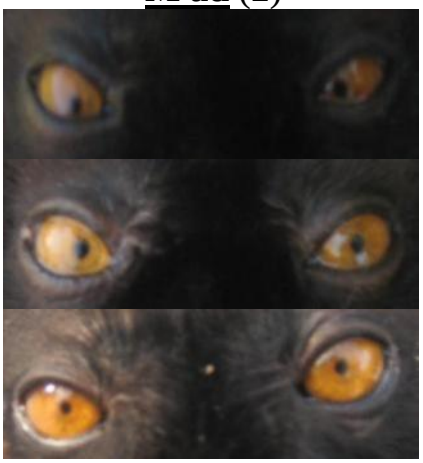
Crural index: $\frac{\text{leg length}}{\text{thigh length}} \times 100$

N: number of individual in the category

Appendix 10: Lines and colour of IML adult and no-adult females and males (personal pictures).

F ad (1)

M ad (2)



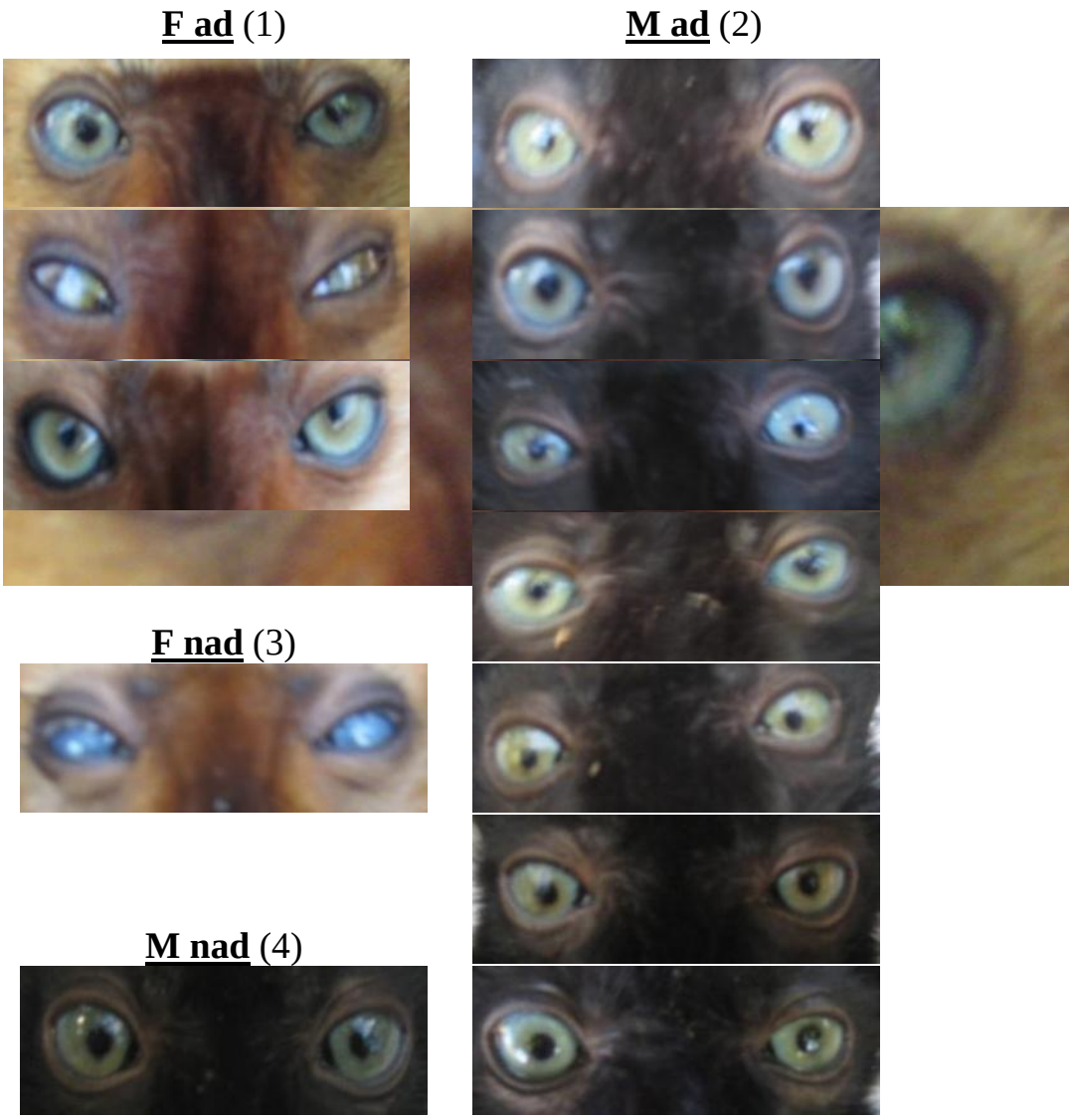
F nad (3)

M nad (4)

Key, number and place of catching (up to down)

(1): F ad, adult females: 8 FALY, 14 FALY, 17 FALY, 19 AMBEVO, 23 ALABET, 26 ANKARA and 27 ANKARA
(2): M ad, adult males: 15 FALY, 20 AMBEVO and 22 ALABET
(3): F nad, no-adult females: 10 FALY and 24 ANKARA
(4): M nad, no-adult males: 11 FALY, 13 FALY, 16 FALY, 18 FALY, 21 ALABET and 25 ANKARA

Appendix 11: Lines and colour of *Eulemur macaco flavifrons* adult and no-adult females and males (personal pictures).



Key, number and place of catching (up to down):

Place of catching: AFADY, excepted individual 40 in Mang-AFADY

(1): F ad, adult females: 34, 36, and 39

(2): M ad, adult males: 28, 30, 32, 33, 37, 38, and 40

(3): F nad, female no-adult: 31

(4): M nad, no-adult male: 29

Appendix 12: Lines and colour of forehead of IML adult and no-adult females (personal pictures).

F ad (1)

F ad (1bis)

F nad (2)

M ad (3)

Key, number and place of catching (up to down):

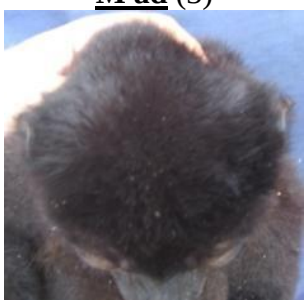
(1): F ad, adult females: 8 FALY, 12 FALY, 14 FALY et 17 FALY.
(1bis): F ad, adult females: 19 AMBEVO, 23 ALABET, 26 ANKARA et 27 ANKARA.
(2): F nad, no-adult female: 10 FALY. (3): M ad, adult male (as an example): 15 FALY

Appendix 13: Lines and colour of forehead of *Eulemur macaco flavifrons* adult and no-adult females (personal pictures).

F ad (1)

F nad (2)

M ad (3)



Key, number and place of catching (up to down):
Place of catching : AFADY
(1): F ad, adult females: 34, 36 et 39
(2): F nad, no-adult female: 31 (3): M ad, adult male (as an example): 28

Appendix 14: Lines and colour of back of *E. m. flavifrons* adult and no-adult females (personal pictures).

<u>F ad</u> (1)

F nad (2)

<u>M ad</u> (3)

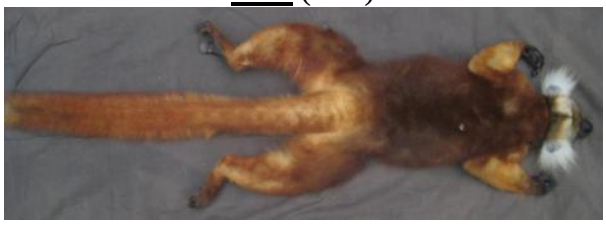
<u>Key, number and place of catching</u> (up to down):
Place of catching: AFADY

(1): F ad, adult females: 34, 36 and 39
(2): F nad, no-adult female: 31
(3): M ad, adult male (as an example): 28

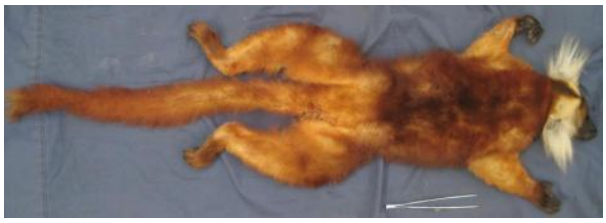
Appendix 15: Lines and colour of back of IML adult and no-adult females (personal pictures).

F ad (1)

F ad (1bis)



F nad (2)



M ad (3)

Key, number and place of catching (up to down):

(1): F ad, adult females: 8 FALY, 12 FALY, 14 FALY, 17 FALY, 19 AMBEVO, 23 ALABET and 26 ANKARA
(1bis): F ad, adult females: 27 ANKARA.
(2): F nad, no-adult females: 10 FALY and 24 ANKARA (3): M ad, adult male (as an example):15 FALY.

Appendix 16: Lines and colour of abdomen of *E. m. flavifrons* adult and no-adult females (personal pictures).

F ad (1)

F nad (2)

M ad (3)

Key, number and place of catching (up to down):
Place of catching: AFADY
(1): F ad, adult females: 34, 36 and 39 (2): F nad, no-adult female: 31
(3): M ad, adult male (as an example): 28

Appendix 17: Lines and colour of abdomen of IML adult and no-adult females (personal pictures).

F ad (1)

F ad (1bis)

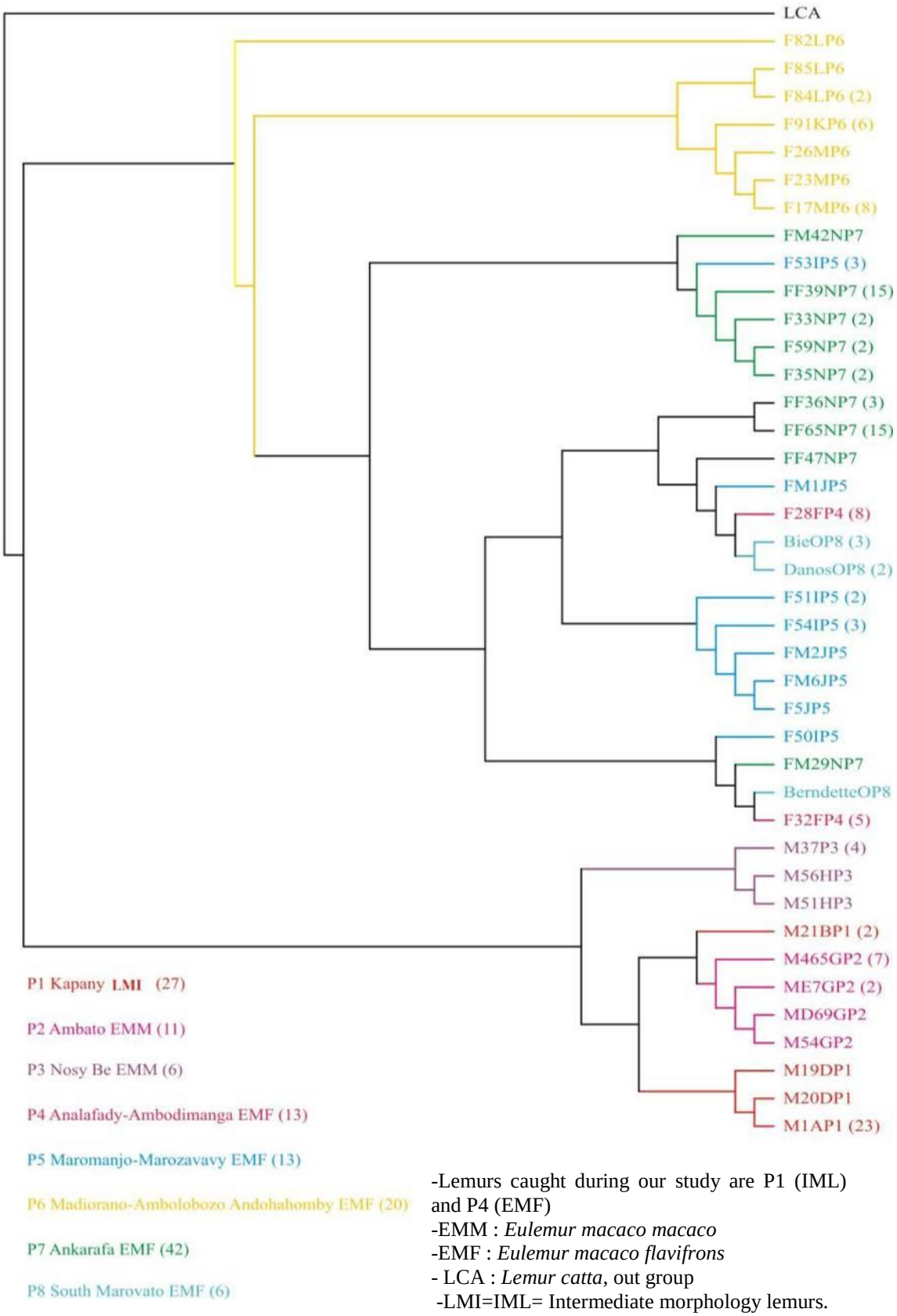
F nad (2)

M ad (3)

Key, number and place of catching (up to down):

(1): F ad, adult females: 8 FALY, 12 FALY, 14 FALY, 17 FALY, 19 AMBEVO and 23 ALABET
(1bis): F ad, adult females: 27 ANKARA
(2): F nad, no-adult females: 10 FALY and 24 ANKARA (3): M ad, adult male (as an example): 15 FALY

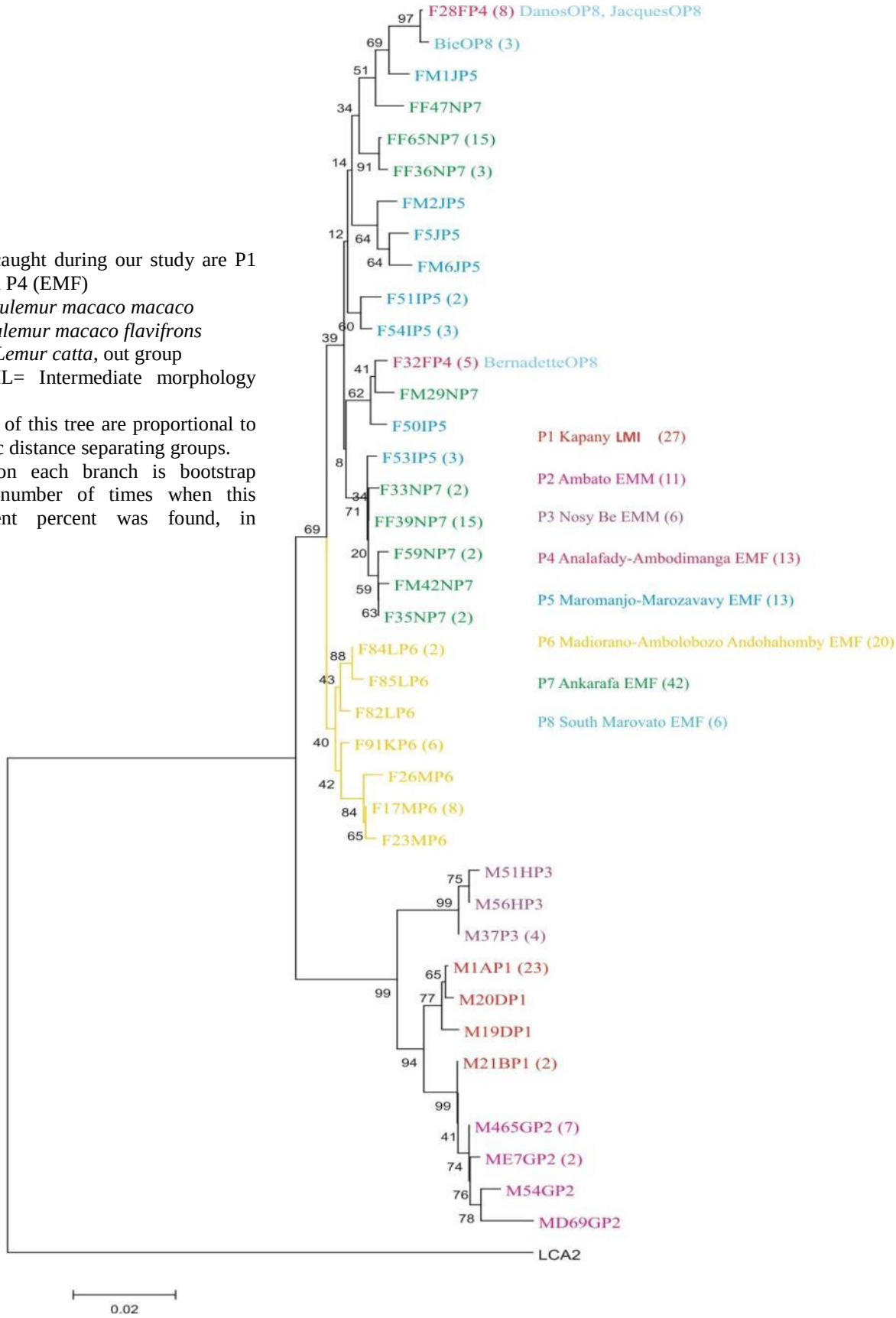
Appendix 18: Phylogenesis of lemurs of our study and of the database of Strasbourg's University in ML cladogram.



-Lemurs caught during our study are P1 (IML) and P4 (EMF)
-EMM : *Eulemur macaco macaco*
-EMF : *Eulemur macaco flavifrons*
- LCA : *Lemur catta*, out group
-LMI=IML= Intermediate morphology lemurs.
-Branches of this tree are not proportional to the genetic distance separating groups.

Appendix 19: Phylogenesis of lemurs of our study and of the database of Strasbourg's University in NJ cladogram.

-Lemurs caught during our study are P1 (IML) and P4 (EMF)
-EMM : *Eulemur macaco macaco*
-EMF : *Eulemur macaco flavifrons*
- LCA2 : *Lemur catta*, out group
-LMI=IML= Intermediate morphology lemurs.
-Branches of this tree are proportional to the genetic distance separating groups.
Number on each branch is bootstrap value = number of times when this arrangement percent was found, in percent.



❖ **Thanks**

Thanks to Dr Christoph Schwitzer to entrust me with this study.

Thanks to Rufford Small Grant, to Bristol and Mulhouse zoological parks, to AEECL to finance my expedition.

Thanks to the Minister of environment and forest of Madagascar to allow me to realize this study in the Sahamalaza-Iles Radama national park.

Thanks to the team of « Madagascar Biodiversity Biogeography Project », M. Jean-Claude Rakotoniaina, M. Gérard Nalanirina and M. Jean Razafidraibe, to be efficient and their good mood.

Thanks to Mss Fredine Stelat Masindrazana and M. Nosy to their good work and their kindness.

Thanks to Dr Guy Randriatahina and to M. Urbain Randrianarivelo to help me to put in place the expedition in the Sahamalaza-Iles Radama national park.

Thanks to Mss Odette Razanamahafaly for her translations.

Thanks to Dr Jean-Luc Fausser and Dr Daniel Montagnon to realize the genetic analyses.

Thanks to Andrew Arnell to realize the repartition area map.

❖ Presentation of the budget

Category	Description	Anticipated expenditures in euro	Actual expenditures in euro
Trip	Flight to Madagascar, trip in Madagascar, trip to Strasbourg and Bristol.	1200	2040,6
Accommodation and livehood	Subsistence (3month)	600	768,7
	Accommodation out of camp and miscellaneous (battery loading, journal…).	160	221,4
Logistic	Search licence (MICET)	300	260,0
	Rights of entry in National Park (x3)	300	43,0
	Salary of guides and cooker	3000	1202,6
	Visa	50	100,0
	Prevention and remedial treatment against malaria	150	215,7
	Other treatment	280	36,0
	Bank charges	100	50,0
Malagasy student	Trip, subsistence, salary	1350	366,7
Material	Local cell phone and communications	20	71,3
	GPS tracer (AEECL loan and interface cable)	200	104,0
	Morphometric material (weighing machine, etc.)	30	46,9
	Camcorder	130	own device
	Sampling conservation	50	50,0
	Blowpipe and consumable to catch	200	543,3
	Camp material (x2)	500	756,1
	Genetic analysis	600	600,0
Total		**9220**	**7476,1**