





**Preliminary Report** 

# **GEOGRAPHICAL VARIATION IN VOCALIZATION, BEHAVIOUR,**

# **MORPHOLOGY AND GENETICS OF MOUSE LEMURS IN NORTHWESTERN**

# MADAGASCAR

HASINIAINA Irene Alida Frankline

PhD Program "Veterinary Research and Animal Biology"

Tierärztliche Hochschule Hannover, Germany.

#### I – INTRODUCTION

Madagascar is, due to its early isolation from the African continent and an undisturbed evolution, one of the most biological diverse places on earth (Mittermeier *et al* 2010). The biggest threat for the Malagasy biodiversity in general is the rapid reduction of habitats through deforestation. In particular, the northern and north-western dry forests were recently diminished at a rapid pace by fires and illegal logging (Sommer *et al* 2002), threatening the survival of forest-adapted animal species, such as lemurs. The knowledge about the lemur species diversity and distribution as well as about the behaviour and ecology of lemurs is urgently needed and the first step for any conservation and management program, since only what is known can be protected. In parallel the sensibilization of the local population for forest management is needed.

Acoustic signaling systems are discussed to play a major role in animal speciation and adaptation as well as in non-invasive species identification (Wilkens et al., 2013). Empirical research on the geographical variation of acoustic signaling systems and the significance for adaptation and evolution in lemurs is currently in its infancy. The aim of my project funded by the Small Rufford fund is therefore to study the geographical variation of acoustic signaling behavior and its implication for taxonomy and conservation biology using the radiation of the smallest bodied lemurs of Madagascar, the mouse lemurs. The species diversity of mouse lemur in northwestern and western Madagascar is based solely on phylogenetic methods (Olivieri *et al.* 2007, Rasoloarison *et al.* 2010), for most species data on their biology are lacking. The genetically described species are, however, endangered due to their very limited range and the high fragmentation of their habitats (Radespiel 2016). Previous bioacoustic research on mouse lemurs described them as highly vocal, exhibiting a complex vocal repertoire with calls in the sonic and ultrasonic range (Zimmermann 2016). Specific call types are used in the context of startle (e.g., grunt), disturbance or predation (e.g., short whistle), aggression/submission (e.g., tsaks), and in the group-reunion and mating contexts (e.g. Trill).

My project will enhance our current knowledge on the conservation biology of six endangered mouse lemur species (from which the biology of five is totally unknown) and will contribute to unravel the role of bioacoustics for primate evolution, taxonomy and conservation biology. I will combine standardized morphological, bioacoustic and ethological approaches in the field along a transect in northwestern and northern Madagascar with phylogenetic and molecular genetic approaches in the laboratory and with our current knowledge of the bioacoustics of mouse lemurs. By using such an integrative approach, I can also get insight into the use of vocalizations as a non-invasive tool for species identification in mouse lemurs.

#### **General Remarks**

In 2015, I visited three of the six planned study sites according to my proposal: Ankarafantsika National Park (ANP), Marosely forest (MF) and Bombetoka forest (BF) to perform my study. To conduct the study, it was necessary to organize research permits from the Ministry of the Forestry and Environment and the Madagascar National Park (MNP). After getting the research permits, we had to contact the responsible of the Park for the case of ANP and the Head of the village for MF and BF. I have established the following research team to perform the study: Rina Evasoa Mamy (PhD candidate, University of Medicine and Veterinary Hannover, working on social dominance in lemurs) and two Master students as research assistant: Mahatoly Ursulla Laura and Andriamendrikaja Angelo Stephan from the University of Mahajanga. Our first study site ANP, where we were trained to conduct the study by our supervisors Prof. Dr. Elke Zimmermann and Prof. Dr. Solofonirina Rasoloharijaona, is accessible by car by a paved national road and we don't have any problem with the transportation, accommodations, electricity, water since our camp was inside the park and we could use the established infrastructure. We had access to electricity 24h/24 h, so we could transfer data each day in the computer. .Our second study site MF is inaccessible by car and paved road. Thus, it was necessary to take a taxi bus from Mahajanga to Port Bergé, and to stay first in Port Bergé because we needed to negotiate our stay in MF with the Responsible of the Management of Forest in Marosely. After he agreed with our stay there, we had to take another taxi bus to the Village of Marosely. From there our research team with all the materials for camping and research had to walk because the car cannot reach the study site. Thus, we organized a team of villagers with oxcarts for our luggage and we are walking for about two hours to reach the forest. We established our camp in the middle of the forest without any infrastructure and consequently there was no access for electricity and water. As for the water, we had to buy water regularly from villagers because there was no access to water in our camp. In general, we got water twice in a week. As for the electricity, we had to go to Port - Bergé for transferring data or to recharge the batteries for our electronic equipment. Our third study site BF was also not accessible by a paved road. I had to organize a boat transport across the river Betsiboka for the research team as well as oxcarts to transport us and the luggage to the study site. Here, again, I had to negotiate the stay with the Chef of Village. Due to insecurity, the Chef of the village suggested not to camp in the forest, so we have established the camp near the village. There was also no electricity and we had to organize water from a water well. Only after return to Mahajanga, we were able to transfer data, recharge, batteries etc. Thus, for the two latter study sites, it was necessary to be in villages with electricity to recharge batteries or transfer data to the computer.

Regarding conservation, this project is very important because the people who live near the non-protected forest consider the forest as a resource. Consequently, they destroy and exploit the forest as they need it for example by bushfires for charcoal production (case of Bombetoka forest) and are doing illegally logging (case of Marosely). Before starting, I decided therefore to discuss with the Chef of Village about the purpose of our research, we also mentioned that we will help villagers to conserve the forest and the animals which live there. Villagers are directly motivated because it is not easy to manage the forest when they don't have anyone to help them. We are making a focus group just to explain to the villagers that we are there to help them and we would like to work with them for conserving the nature. For the case of Ankarafantsika National Park, we are working together with the local guides and the Park administration and we encouraged them to protect the forest and the animal and after our research we have written a report to the Responsible of the Park to inform them about the remarks and the results that we got there. But for the case of Marosely forest and Bombetoka forest, we have to explain to the villagers the aim of our study and the way that we bring research for their advantages. Firstly, we mentioned that we will work with them to avoid logging forest or eating animals (Bird, Lemurs etc....), Secondly, we explain about the advantages saving of the nature and the adverse effect when they destroy the forest ( climatic change, lack of rain etc.). They are very interested and they are persuaded that it is very necessary to protect the forest. Finally, we also discussed with them about the punishment for those who practice the logging in this area so after that the Chef of the village had created a commission to manage the conservation of the forest of this area (Marosely forest). During our field study, we have got contact to the villagers by employing them as field assistants, local guides, porters, cookers etc. It is important to mention that during our field study (especially in Marosely), we encountered several people who destroyed trees in the main forest and we noted signs of wood extraction in the forest. People who logged trees destroyed thereby also "sleeping sites" and habitats of mouse lemurs and the other endemic animals of this forest. For this case, we contacted directly the Chef of the village and after that the Commission decides the punishment for these people. During our stay in Marosely, we could notice that the human pressure decreased and the villagers seemed to support the protection of nature. For sustainable protection, it would be best to have researchers continuously monitoring the area.

# **II - MATERIALS AND METHODS**

## II – 1- Study sites:

In 2015, three different field sites were visited to perform my study:

# Ankarafantsika National Park (ANP, April to June 2015):

The ANP with the station Forestier of Ampijoroa (16° 19'S, 46° 48'E) is located 115 km southeast of Mahajanga, including in the rural commune of Marosakoa, district of Marovoay. This site is characterized by dry deciduous forest. The climate is highly seasonal and characterized by a cool dry season from May to October and a very hot and humid rainy season from November to April with heavy rains in January and February. *M murinus* and *M ravelobensis* are known to lives sympatrically in this region.

#### Marosely forest, MF (July to August):

The Marosely forest (15° 49'S, 47° 47'E) area is included in the Massif of Bongolava, situated in Commune of Tsarahasina, district of Port-Berge. The forest of Marosely is characterized by a dry deciduous forest and the climate is seasonal as in ANP.Two species of mouse lemurs: *Microcebus bongolavensis* and *Microcebus murinus* are described from this region.

## Bombetoka forest, BF (September to October):

The Bombetoka forest area(15° 52′S, 46° 48′E) is located 22 km on the south of the District of Mahajanga, including the rural commune of Katsepy, Fokontany of Sankoany and district of Mitsinjo. The climate is seasonal and climate and forest corresponds to the two other sites. Two species of mouse lemurs were described from this forest, *M. myoxinus* and *M. murinus*.

#### II – 2- Trapping of mouse lemurs to get dyads for cage experiments

a) Trapping, morphometry and selection of dyad partners for social encounter experiments At each study site, we captured Microcebus with 90-91 Sherman live animal traps baited with bananas according to established methods (Zimmermann et al. 1998, Olivieri et al. 2007). All traps were set in the late afternoon about 1-2 m above ground at each intersection of an existing rectangular trail system in Ampijoroa and along transects in Marosely and Bombetoka. The traps were checked the next morning (6:00-7:00 a.m.). Captured animals were sexed and measured morphologically according to established protocols (Zimmermann et al. 1998, Olivieri et al. 2007). Small tissues samples from the ear, hair samples, and ectoparasite and intestinal parasite samples were taken of all trapped animals for further genetic and parasitological studies. Tissue samples were stored in a specific solution called "buffer". This buffer is reported to stabilize DNA and inhibit DNAse activity (Hafen et al., 1998). After 6 days of observation per dyad all animals were released at the exact sites of capture. Dyad partners for social encounter experiments were selected based on comparable body mass and of being trapped as far as possible away from each other in order to avoid forming dyads of familiar animals and we also marked one animal in each dyad with a fur cut on the tail to better distinguish them. Animals not needed for social encounter experiments were released at their respective trap site in the early evening. We trapped animals as long as needed to form 12 pairs/study.



Photo: M. bongolavensis during morphometric measurements



Photo: Measurement of the mouse lemur caught by the research team 2015

#### II – 3- Social encounter experiments

We used social encounter experiments of male-male and male-female dyads of mouse lemurs to induce vocalizations. The experimental dyad partners were housed in one cage of about 1 m<sup>3</sup>. Each cage was equipped with wooden bars (2 boards in front are smaller than the 2 board behind) and two shelters/sleeping sites (we closed one of the sleeping boxes that means only one is available for two animals). The cages were placed at the forest floor close to the research camp but >1km away from the capture sites in Ankarafantsika and in Marosely, however, in Bombetoka the cage was placed in the village and therefore far away from the forest. Each animal was fed with banana and we provided water ad lib by a bottle fixed at one side of the cage. Animals had also access to arthropod prey that naturally entered their cages during the night. Each experimentation series for a dyad was done for a six day observation; the two observers divided the task for collecting the data, in which one observer using a Dictaphone recorded behaviours and behavioural interactions by direct observations. Besides, a second observer took notes on paper every 15 seconds. Both observers sat motionless in a 2-4 m distance near the cages and started the protocols as soon as the animals woke up at around 6:30 p.m. Both observers used a headlamp and Maglite torch to obtain better visibility. From day 3 onwards, social encounter experiments took place for 6 days in and the observation started at about 6 p.m. until about 9 p.m. a row for every dyad. The animals were released at their individual capture point after the end of the last observation.

We had recorded the following behaviours:

- 1. Use of shelter (duration of staying in shelter),
- 2. Feeding behaviour was characterized by two parameters: duration of feeding bouts and duration of staying at the feeding bowl. 3. All occurrences of affiliative behaviours: non-agonistic body contact, allogrooming.
- 3. All occurrences of agonistic behaviours: fighting, chasing, attacking and displacing, displacement and avoidance.
- 4. All occurrences of olfactory marking behaviour: urine washing, anogenital marking and substrate rubbing.
- 5. All vocalization

We recorded the location of the animals within the cage with the following precision: compartment in the cage, on the top was the roof, the floor, the shelter, the cage equipped 4 board two above and two below, each board was divided into two zones, left and right.

# II- 4- Recording of the vocalizations

To record vocalizations special equipment to record the vocal activity was needed. Ultrasonic microphones (SMX-II weather-proof microphones, Concord, Massachusetts, USA) were connected to a Song Meter (Wildlife acoustic, Model SM2+, Concord, Massachusetts, USA, frequency range: up to 192 kHz) which then saved the information on 4 SD flash memory cards (Samsung 32 GB SD-Card). The equipment was located on top of the cage and the microphones on each site of the cage (Fig. below). The vocalizations were recorded continuously between 18:00-00:00 (GMT+2). The Song Meter recorded calls from inside the cage and also from mouse lemurs outside the cage.



Photo: Set-up for social encounter experiments and recording of vocalizations

#### **III – PRELIMINARY RESULTS**

#### A-Trapping success and morphometric measurements of focal animals.

## A.1 Ampijoroa, ANP, IRS I

Our first study site of the season was Ampijoroa in the IRS I. The capture success there was very low at the beginning and increased after mid-May (Table I). Unexpectedly, it still rained occasionally in April and even May, and it can be assumed that mouse lemur food was therefore still abundant in the forest, and the traps were not as attractive as in other years during the same time (Zimmermann, Radespiel, pers.com.). We therefore decided to also install some traps around the camp site to increase the animal numbers for *M. ravelobensis* (Table II). In total, we trapped 41 individual mouse lemurs in JBB, 35 of which being *Microcebus ravelobensis* and six of which being *Microcebus murinus*. In the surroundings of the camp, we trapped a total of 5 *M. ravelobensis* and 7*M. murinus*.

Date	Piste	Number of traps sets	Number of animal caught	Remarks
24.04.2015	JBB	90	4	NC
25.04.2015	JBB	90	2	NC
26.04.2015	JBB	90	0	
27.04.2015	JBB	90	2	NC
28.04.2015	JBB	90	1	NC
30.04.2015	JBB	90	0	
0305.2015	JBB	90	0	
04.05.2015	JBB	90	1	NC
06.05.2015	JBB	90	0	
08.05.2015	JBB	90	0	
10.05.2015	JBB	90	0	
16.05.2015	JBB	90	1	NC
27.05.2015	JBB	90	11	8 NC, 3 RC
28.05.2015	JBB	90	13	9 NC, 4 RC
01.06.2015	JBB	90	19	1 NC, 18 RC
06.06.2015	JBB	90	22	2 NC, 18 RC
08.06.2015	JBB	90	23	5 NC, 4RC
12.06.2015	JBB	90	5	1 NC, 4 RC

**<u>Table I</u>**: Trapping in IRS I (Ampijoroa)

NC= New captures (animal is not marked from previous trapping), RC=Recapture;

		.1	<i>с</i> .
Table IIa:	Trapping in	the Ampij	oroa forest

Species	Males	Females	3 Total		
M. murinus	3	3	6		
M. ravelobensis	15	26	41		

Table IIb: Extra trapping near the camp in Ampijoroa

Species	Males	Females	Total
M. murinus	6	1	7

M. ravelobensis	1	3	4

#### Morphometric measurements

In the following, I present the data from the morphometric measurements of the mouse lemurs I used for the social encounter experiments (Table III- V-VII).

#### A.1 Ampijoroa, IRS I

We had 12 dyads, each individual was weighed, and morphometric data were taken, the table below shows only the measurements of the 12 dyads that we observed.

	I abit III.	wiorpi	ionicu	i y OI 12	- uyau	5 (111111)	000000	1400100		DD, III	.01.				
Р	Ι	EL	EW	HD	HW	TL	TC	HFL	BT	LLL	W	Tol	SL	Inter	intra
1	Alain	25,6	12,7	37	23,7	156	18	19,8	75	40,8	50	9,5	4,9	4,5	19,2
1	Annie	15,5	10	35	22,2	147	22	22,2	70	39,5	50	8,8	5,4	4,4	20
2	Ralph	15	11,6	32,1	21	147	17	20,3	63	32,7	48	7,1	8	4,2	20,1
2	Jim	20,7	12,2	37,2	20	154	18	25,5	70	39,2	65	9	6	4	20,5
3	Bob	16,9	11,4	34,8	21,9	150	17	20,5	70	40,1	60	8,3	7,2	5,5	20,4
3	Carter	23,2	15,6	36,1	20,3	147	20	22,4	65	40,6	50	9,3	7,8	7,2	21,6
4	Besady	16,3	12,5	34,1	20	116	22	20	66	38,1	50	7,3	6,5	4,8	12,4
4	Rasoa	17	14	34	18	145	18	21	68	38	48	8	8	9	19
5	Petit	18,8	13,7	34,6	20,9	137	18	20,9	69	33,2	53	7,7	7,4	6	19,8
5	Jane	21	13,7	36,1	20	150	19	24,2	70	39,3	50	7,5	7,6	6,7	19,5
6	Precious	18	12	35,5	24,4	135	22	22,2	78	38,5	65	9,1	6,5	5	20
6	Mike	21	14	38	20	150	19	23	75	42	64	8,8	8	7,2	21,7
7	Roddy	17,3	12	35,4	22,6	145	16	23,7	75	40,5	64	9	6,1	6	19,3
7	Seyah	20,6	13,7	36,6	19,3	154	22	22,7	74	39,5	70	7,6	8	6,4	21
8	John	19	14,7	35	20	160	21	24	75	42	68	9	8	7,6	19
8	Cathy	18	14,2	35,6	18	164	21	23	74	40	63	8,8	7,2	7	20
9	Rayan	16,3	12,5	35,6	20,1	155	18	19	76	39,4	53	9,7	7	6,7	18,9
9	James	18,7	10,2	33,4	20	133	13	23,7	58	35,2	48	10	6,3	4,6	19
10	Kiss	16,7	9,3	36,2	19,5	152	18	19,5	78	39,3	55	9,9	7,4	5	17,9
10	Matteo	19,9	11,4	34,2	21,8	141	19	21,1	72	38,7	50	9,1	5,9	4,8	19,3
11	Bill	19,4	11,6	35,4	21,3	145	18	21,7	76	41,7	53	9,9	5,3	5,8	18,6
11	Coddy	15,4	10,6	34,3	21,3	148	19	21,9	73	37,7	55	9	5,7	5,8	20,6
12	Mario	19,9	10,5	36,6	21,9	141	18	21	82	39,2	70	8,6	4,1	5,1	21,2
12	Cneo	20,8	15,6	36	21,2	152	17	22,6	86	42,2	70	9,5	5,6	6,6	20,8

Table III: Morphometry of 12 dyads (Microcebus ravelobensis) JBB, IRS I.

Abbreviations: P= pair number, I= identification, EL= ear length (mm), EW= ear width (mm), HD=head length (mm), HW= head width (mm), TL= tail length (mm), TC= tail circumference (mm)e, HFL= hind foot length (mm), BT= body tail (mm)l, LLL= lower leg length (mm), W= weight (g), ToL= toe length (mm), SL= snouth, length (mm), Inter= between the outer edges of the eyes (mm), Intra= between the inner corners of the eyes (mm)

#### A.2 Marosely, MF, IRS II

We trapped mouse lemurs between the 09<sup>th</sup> July – 19<sup>th</sup> August 2015, during the observation the weather was always windy. In total, we captured 40 animals in Marosely across all six trails, 35 of which were *Microcebus bongolavensis* and five were *Microcebus murinus* (Table IV).

			Number of			
Date	Piste	Number of trap sets	anımal caught	M.bongolavensis	M.murinus	Remarks
09.07.2015	1, 2, 3	90	8	4F, 3M	1M	NC
13.07.2015	1, 2	80	7	3F, 1M	1F, 2M	NC
15.07.2015	2, 3	80	3		1F, 1M	All RC
17.07.2015	2, 4	50	2	1M	1	NC
18.07.2015	2	30	2	1F, 1M		NC
20.07.2015	2,3	40	13	5F, 3M	2F, 3M	6 NC, 7 RC
24.07.2015	4	60	2	1F, 1M		NC
25.07.2015	3	60	7	3M, 2F	1M, 1F	1NC, 6RC
29.07.2015	1,2,3	90	17	11F, 6M		5NC,12 RC
31.07.2015	1,3	60	8	3F, 2M	1F, 2M	3 NC, 7 RC
01.08.2015	3	42	10	7F,3M		3NC, 7RC
03.08.2015	3	40	3	3F		1NC, 2RC
05.08.2015	2, 4	62	0			
07.08.2015	5,6	90	2	2F		NC
08.08.2015	5	90	1	1M		NC
11.08.2015	1,2	45	4			All RC
12.08.2015	3	50	5			All RC
14.08.2015	4	90	1			RC
15.08.2015	5, 6	80	1			RC
17.08.2015	5,6	80	1			RC

## Table IV: Trapping in IRSII (Marosely)

NC= New (animals marked), RC=Recapture; F=female; M=male

In IRS II (Marosely) we had only 11 dyads. Every day, we did capture but we always got female but not male.

*Microcebus murinus* found in the area relatively high therefore Microcebus *bongolavensis* located in the low altitude, but there was also some places we could find them again in the same altitude when we did the captured.

Table V: Morphometry of 11 dyads (Microcebus bongolavensis) in Marosely, IRS II.

		-						<u> </u>							
Р	Ι	EL	EW	HD	HW	TL	TC	HFL	BT	LLL	W	Tol	SL	Inter	intra
1	M 03-15	22.4	14.2	34	22	161	19	24.5	70	41.8	71g	10	6.3	6.3	21.3
1	F 01-15	21.3	12.2	36.5	21	170	20	21.6	80	39.8	70g	10	6.8	5	17.6
2	M 07-15	22	13.9	35	17.3	165	18	23.2	70	45.3	58g	9	7.5	5.3	19
2	F 02-15	19.5	12.7	34.3	22.4	164	19	25.2	70	42	58g	10.7	6.5	7.6	18.3
3	M 08-15	18.3	12.2	35	19.5	155	18	23.5	70	39	48g	10.5	6.5	6.8	18.8
3	F 13-15	21	14.5	36.8	21	155	20	21.4	71	39	57g	9	6.3	5	18
4	M 15-15	19	14.7	33.7	20	152	18	22.5	70	38.3	43g	10	6	5	16
4	F 11-15	18.5	12.5	35.3	17.5	156	19	23.7	73	39	52g	10	6.3	4.3	16.3
5	M 16-15	18.5	14.4	37.5	20	160	20	25.1	70	42	62g	8.6	6.5	6	20
5	F 21-15	23	11.5	34.8	20	160	19	21.8	74	40	58g	10	7	5.7	19.5
6	M 17-15	19.5	12.3	35.5	20	150	18	22.5	72	39.3	48g	8.1	6.6	7.8	19.14

6	F 20-15	24.3	13.7	34	19.6	140	19	20	70	37.5	50g	10	6.4	6.3	18
7	M 32-15	21	13.5	36	18.3	152	18	23.8	65	40	38g	8.3	7.3	6.6	19.8
7	M 27-15	22	14.8	37.5	19.8	145	18	23.3	65	39.5	48g	8	7	7.5	20.3
8	M 22-15	21.5	12.3	36.5	22.3	170	19	24.2	75	40	46g	10	6	5.3	19.6
8	M 26-15	21	11.5	34	20	155	18	21.2	67	42.6	50g	10.8	6	5	13.3
9	M 36-15	20.3	15.4	35.3	19.2	130	18	23	62	38.3	44g	8.5	8	7.8	18.3
9	M 30-15	22.3	15	37	21.8	153	22	25.1	72	43.8	61g	8.8	7.3	7	20.8
10	M 19-15	21.5	11.7	36.5	18.3	170	18	24	73	43.8	60g	10	7	6.3	20.4
10	M 34-15	21.5	14	36	19.8	152	18	24	68	41	64g	8.8	6.3	6.8	20
11	M 37-15	20.8	15.8	35.5	22.8	151	19	24	70	40.8	70g	8	6.5	7	21.8
11	M 40-15	22	14.5	37.5	19	154	17	21	68	41.8	53g	8.8	7.8	7	20

P= different pair, I= identification, EL= ear length(mm), EW= ear width(mm), HD=head length(mm), HW= head width(mm), TL= tail length(mm), TC= tail circumference (mm), HFL= hind foot length(mm),, BT= body tai(mm)l, LLL= lower leg length(mm), W= weight(g), ToL= toe length(mm), SL= snouth , length(mm), Inter= between the outer edges other eyes(mm), , Intra= between the inner corners of the eyes(mm) length

Note: In Marosely we also encountered several people who destroyed trees in the main forest and signs of wood extraction in the forest. Marosely was the hardest part compared to the other two sites Ampijoroa and Bombetoka because our camp had to be installed at a remote site far from villages and water sources.

During the work we observed many people who logged trees and thereby also "sleeping sites" and habitats of mouse lemurs and the other endemic animals of this National Park.

#### A.3 Bombetoka, BF, IRS 0

In Bombetoka, we captured a total of 51 animals, 33 of which being *Microcebus bongolavensis* and 18 of which being *Microcebus murinus* 

		Ň Ň	Number of			
		Number of	animal			
Date	Piste	trap sets	caught	M.bongolavensis	M.murinus	Remarks
08.09.2015	1	89	4	4F		NC
09.09.2015	1	89	4	1F, 3M		NC
13.09.2015	1	88	1	1M		NC
17.09.2015	1	91	2	1F,1M		NC
18.09.2015	1	89	1	1F		NC
19.09.2015	1	89	3	1F, 2M		1 NC, 2 RC
20.09.2015	1	86	4	1F, 2M	1M	NC
21.09.2015	1	86	6	2F, 2M	1F, 1M	NC
26.09.2015	1	89	18	4F, 3M	5F, 6M	14 N, 4 R
01.10.2015	1,4	89	16	6M, 4F	1F, 5M,	7 N, 13 R
06.10.2015	1,2	75	12	5F, 1M	4M, 2F	1N, 11 R
07.10.2015	1, 2, 3	88	6	1F, 2M	2F,1F	5N, 1R

Table VI: Trapping in IRS 0 (Bombetoka):

NC=new, RC=Recapture

Р	Ι	EL	EW	HD	HW	TL	TC	HFL	BT	LLL	W	Tol	SL	Inter	intra
1	M 06-15	17.5	9	31	19.5	138	16	19.5	73	40	45g	95	5.5	5.75	15.25
1	F 02-15	16	11.25	33	19	139	18	21	64	37.7	44g	10	7	5.5	19.75
2	M 05-15	22.25	12	35	20	138	17	19	66	39.25	41g	9	5	5	18.75
2	F 03-15	19.6	13.2	35.65	17.5	148	17	19.3	68	37.5	40g	9	6	5.4	19.15
3	M 08-15	18	11.25	32.5	18.75	126	16	20.5	78	38.25	37g	9	6.5	5	16.75
3	F 07-15	20	10.25	34	18.75	130	16	20	62	36.25	38g	9.25	6	5.75	17.5
4	M 09-15	19.25	10	34.5	19.5	134	17	21	75	41.75	46g	8	6	5	18.75
4	F 04-15	20.15	12	33.75	19	127	18	20.3	72	38	47g	9	6	5	18
5	M 10-15	18.3	12	34.5	19.8	137	19	21.5	75	38.8	57g	8	7	6	20.8
5	F 01-15	19.15	12.25	35.15	20.57	136	17	18.3	66	37	50g	10.5	6	5.25	19.5
6	M 15-15	17.5	12	33.3	18	142	19	22.8	64	36.8	40g	7.8	8	6.8	19
6	F 13-15	19	12.3	34.5	17.5	150	19	23.3	69	36.5	40g	7.5	7.5	6.5	19.1
7	M 24-15	23.5	12.3	34.3	20.8	135	17	21	65	39	50g	8	6	4	18.3
7	M 19-15	20	13	35.3	18.5	157	20	22.8	70	39.5	50g	7.8	7	6	19
8	M 25-15	17	12.3	31	21.5	115	16	24.5	60	35.6	37g	9	6	6	17.2
8	M 14-15	17.8	12.8	32	18.3	132	17	22	53	37	39g	7.3	7	6.3	19
9	M 39-15	22.3	10	34	20.8	158	18	20.3	63	40.8	53g	9	5	5	18.5
9	M 43-15	22.8	10	35	19	138	18	20	67	39	55g	9	6	5	18.3
10	M 19-15	20	13	35.3	18.5	157	20	22.8	70	39.5	50g	7.8	7	6	19
10	M 34-15	22.3	15.5	34.5	20.3	131	20	22.8	74	39.5	62g	8.5	6.5	6.3	20
11	M 43-15	22.8	10	35	19	138	18	20	67	39	55g	9	6	5	18.3
11	M 42-15	19	10	32.3	17.15	134	16	18.3	65	37	37g	9	6	5	17
12	M 46-15	0.5	9.3	32.8	19	134	17	18	64	39.5	47g	8	5.3	5	19.3
12	M 48-15	19	9	32.3	20	130	18	19.8	72	38.5	46g	9	5.5	6.5	18.8

**Table VII:** Morphometry of 12 dyads (*Microcebus myoxinus*) in Bombetoka, IRS 0

P= different pair, I= identification, EL= ear length(mm), EW= ear width(mm), HD=head length(mm), HW= head width(mm), TL= tail length(mm), TC= tail circumference(mm)e, HFL= hind foot length(mm),, BT= body tai(mm)l, LLL= lower leg length(mm), W= weight(g), , ToL= toe length(mm), SL= snouth , length(mm), Inter= between the outer edges other eyes(mm), , Intra= between the inner corners of the eyes(mm) length.

#### **B.** Social encounter experiments

#### **B.1** Ampijoroa IRS I:

At the first study site (Ampijoroa, IRS I) we had 12 dyads, among which six male – male - and six male – female dyads (Table VII).We observed each dyad for six days during three hours per night, resulting in a total of 24 observation hours per dyad. We succeeded to transfer all protocols from this site into excel sheets.

Table VIII: Mouse lemurs used for social interaction experiments from Ampijoroa, IRS I

SPECIES	SITE	#PAIR	ТҮРЕ	ID1	ID2	START DATE	END DATE
M.ravelobensis	Ampijoroa	1	mf	Alain	Annie	28.04.2015	05.05.2015
M.ravelobensis	Ampijoroa	2	mm	Jim	Ralph	04.05.2015	09.05.2015

M.ravelobensis	Ampijoroa	3	mm	Bob	Carter	06.05.2015	11.05.2015
M.ravelobensis	Ampijoroa	4	mf	Besady	Rasoa	10.05.2015	16.05.2015
M.ravelobensis	Ampijoroa	5	mf	Petty	Jen	12.05.2015	18.05.2015
M.ravelobensis	Ampijoroa	6	mf	Mike	Precious	16.05.2015	21.05.2015
M.ravelobensis	Ampijoroa	7	mf	Roddy	Ceyah	28.05.2015	02.06.2015
M.ravelobensis	Ampijoroa	8	mf	John	Cathy	28.05.2015	02.06.2015
M.ravelobensis	Ampijoroa	9	mm	Jems	Rayan	03.06.2015	08.062015
M.ravelobensis	Ampijoroa	10	mm	Kiss	Matteo	03.06.2015	08.06.2015
M.ravelobensis	Ampijoroa	11	mm	Bill	Coddy	09.06.2015	14.06.2015
M.ravelobensis	Ampijoroa	12	mm	Mario	Cneo	09.06.2015	14.06.2015

m= male, f= female

#### **B.2 Marosely IRS II:**

We collected data from 11 dyads, five male – male - and six male-female dyads for this study ( we have not succeeded to capture a male for the 12 dyads despite of continuous trapping), .We observed each dyad for six nights and 3 hours per night, resulting in 24 observation hours per dyad. We are about to finish to transfer all the protocols from IRS II into excel sheets.

								START	
SPECIES	PISTE	TYPE	PAIR	ID1	Piste	ID2	Piste	DATE	END DATE
M. bongolavensis	Marosely	mf	1	Sely	Ι	Iony	Ι	09.07.2015	14.07.2015
M. bongolavensis	Marosely	mf	2	Nil	Ι	Sofia	III	09.07.2015	14.07.2015
M. bongolavensis	Marosely	mf	3	Ben	II	Yry	II	15.07.2015	20.07.2015
M. bongolavensis	Marosely	mf	4	Rivo	II	Sahara	II	15.07.2015	20.07.2015
M. bongolavensis	Marosely	mf	5	Blaide	II	Criss	III	21.07.2015	26.07.2015
M. bongolavensis	Marosely	mf	6	William	II	belle	Ι	21.07.2015	26.07.2015
M. bongolavensis	Marosely	mm	7	Max	III	Rio	III	29.07.2015	02.08.2015
M. bongolavensis	Marosely	mm	8	Everest	III	Utah	III	29.07.2015	02.08.2015
M. bongolavensis	Marosely	mm	9	Brandy	III	Bien	II	03.08.2015	08.08.2015
M. bongolavensis	Marosely	mm	10	Kero	II	Rainy	III	03.08.2015	07.08.2015
M. bongolavensis	Marosely	mm	11	Surprise	IV	Esperant	III	08.08.2015	12.08.2015

Table IX: Mouse lemurs used for social interaction experiments observed in IRS II

m= male; f= female

#### B.3 Bombetoka:

The third study site was Bombetoka. In Bombetoka we installed 4 trails: Trail 1(mankadala), Trail 2 (lost), Trail 3 (entrance), and Trail 4 (Edge). Here we started trapping on the 8<sup>th</sup> September and started the observations on 09<sup>th</sup> September 2015.

The Bombetoka area was unexpectedly rather unsafe. The area where we trapped did not belong to an Association interested to protect the environment, consequently, people can take what they like from the forest. For security reasons, we had to install our camp in the village. People in this area ate *Microcebus*, unfortunately for that reason, we lost one pair (pair VII) on the 4<sup>th</sup> days of the observations, since it was stolen out of the cage

								START	END
SPECIES	SITE	TYPE	PAIR	NAME	TRAIL	NAME	TRAIL	DATE	DATE
M.myoxinus	Bombetoka	mf	1	Mac	Ι	Dahlia	Ι	09.09.2015	14.09.2015
M.myoxinus	Bombetoka	mf	2	Jacky	Ι	Erica	III	09.09.2015	14.09.2015
M.myoxinus	Bombetoka	mf	3	Mabibo	II	Yry	II	15.09.2015	20.09.2015
M.myoxinus	Bombetoka	mf	4	Rosario	II	Alice	II	15.09.2015	20.09.2015
M.myoxinus	Bombetoka	mf	5	Kango	II	Patricia	III	21.09.2015	26.09.2015
M.myoxinus	Bombetoka	mf	6	Yeoan	II	Jessy	Ι	21.09.2015	26.09.2015
M.myoxinus	Bombetoka	mm	7	Billy	III	Kaira	III	27.09.2015	29.09.2015
M.myoxinus	Bombetoka	mm	8	Tal	III	Dal	III	27.09.2015	02.10.2015
M.myoxinus	Bombetoka	mm	9	Roddy	III	Tatoo	II	01.10.2015	06.10.2015
M.myoxinus	Bombetoka	mm	10	Tonny	II	Romeo	III	03.10.2015	08.10.2015
M.myoxinus	Bombetoka	mm	11	Jah	IV	Poera	III	07.10.2015	12.10.2015
M.myoxinus	Bombetoka	mm	12	Paul	IV	Curtis	III	09.10.2015	14.10.2015

**Table X**: Mouse lemurs used for social interaction experiments observed in IRS 0 m= male; f= female

## <u>C – Preliminary results from audio recording of mouse lemurs at the different sites</u>

I got 97 Gigabyte of audio information which I have to analyse after my next field stay. I have not had time to analyse audio recording so far, because I also got a DAAD-grant to cover my personal salary for this study and thus was in Germany to attend a German language course, just after the field study. Based on our focal animal studies in the social encounter experiments, we heard tsak calls, combinations of tsak and grunt calls and whistle calls According to first sonograms made by the Institute of Zoology (see Figure at the end), and it may be that there are species-specific differences in the tsak calls. However, this has to be verified by quantitative bioacoustic analysis.

In conclusion, the applied methods to get sound recordings at the different study sites was successful and can be continued at the three study sites in 2016. Concerning the analysis of the vocalization, all the calls that we recorded will be cut by a specific software named "Audacity "and analyzed acoustically by "Batsound Pro 3.31" and other sound analysis software.

#### Field study 2016:

After my language course in Deutschland, I will return to Madagascar for the second part of my field study from May 2016 until November 2016.

Time schedule:

May 2016	Back to Madagascar
May to November 2016	Anjajavy forest/Lokobe forest/Sambirano
December 2016	Back to Germany for data analysis

#### Acknowledgements

We thank the Commission Tripartite of the Malagasy government and the Department des Eaux et Forêts, Ankarafantsika National Park (Madagascar), for their permission to work in Ampijoroa, Marosely and Bombetoka and for their logistical support.

I would like to thank the Rufford Small Grant (RSG) for funding this project. I would also like to thank my 2015 research team, my PhD colleague Rina Evasoa Mamy as well as the Master students Laura Mahatoly and Angelo Stephan Andriamendrikaja from the University of Mahajanga and all the local guides for the data collection during my field study.

I would like to thank the German Academic Research Council (DAAD) for supporting my study by a PhD fellowship and the Institute of Zoology at Stiftung Tierärztliche Hochschule Hannover (Prof. Elke Zimmermann), Germany, and the Faculté des Sciences, Université de Mahajanga et Antananarivo (Prof. Blanchard Randrianambinina, Prof Solofonirina Rasoloharijaona, Dr Romule Rakotondravony), Madagascar, for providing supervision and the scientific equipment for trapping, cages and audio recording of mouse lemurs.

#### **References**

-Hafen T, Neveu H, Rumpler Y, Wilden I, Zimmermann E (1998) acoustically dimorphic advertisement calls separate morphologically and genetically homogenous populations of the grey mouse lemur (*Microcebus murinus*). *Folia Primatologica* 

-Mittermeier., Louis. E.E., Richardson ,M. Schwitzer, C. Langrand, O., Rylands, A.B. Hawkins, F.,Rajaobelina, S.,Ratsimbazafy, J. Rasoloarison, R. Ross, C.,P.M.,Mackinnon, J.(2010). Lemurs of Madagascar. Conservation International Tropical Field Guide Series

-Olivieri G. , Zimmermann E., Randrianambinina B. Rasoloharijaona S., Rakotondravony D. Guschanski K, Radespiel U., (2007) The ever-increasing diversity in mouse lemurs: Three new species in north and northwestern Madagascar. Molecular Phylogenetics and Evolution 43 (2007) 309–327Olivieri GL, Sousa V.

-Rasoloarison RM, Goodman SM, Ganzhorn JU (2000) Taxonomic revision of mouse lemurs (*Microcebus*) in the western portions of Madagascar. *International Journal of Primatology*, 21, 963-1019

-Sommer, S., Toto Volahy, A., Seal, US. (2002) A population and habitat viability assessment for the highly endangered giant jumping rat (*Hypogeomys antimena*), the largest extant endemic rodent of Madagascar. *Animal Conservation* 5, 263-273.

-Wilkins R Matthew, Nathalie Seddon, and Rebecca J. Safran (2013) Evolutionary divergence in acoustic signals: causes and consequences. Review Ce Press.

-Zimmermann E, Cepok S, Rakotoarison N, Zietemann V, Radespiel U (1998) Sympatric mouse lemurs in north-west Madagascar: a new rufous mouse lemur species (*Microcebus ravelobensis*). *Folia Primatologica*, 69, 106-114.



Figure: Oscillograms (above) and sonograms (below) of agonistic calls of the three species of mouse lemurs studied in 2015