

Final Report

Rufford Small Grant for Nature Conservation

Project: Population and reproductive biology of *Microcebus* and *Cheirogaleus* in the eastern rain forests of Madagascar.

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Project duration: field work in Madagascar started on October 1st, 2006 and ended on January 25th, 2007. Data analysis was conducted at the University of Massachusetts until May 20th, 2007.

This project represented the first survey on nocturnal lemurs at Tsinjoarivo and a continuation of a long term study of cheirogaleids at Ranomafana National Park. Although both sites are located on the eastern rain forests of Madagascar, environmental conditions as well as the degree of habitat degradation and exposure to ecotourism differ between and within locations. For example, parts that I sampled of the continuous forest of Tsinjoarivo (Vatateza) are located at a higher altitude than the fragmented areas (e.g. Andasivodihazo); during my sampling period, differences between temperature maxima and minima were usually higher in the continuous than fragmented forests, and there were also differences in habitat structure. Forest edge habitat increases significantly under fragmentation. In comparison to Tsinjoarivo, Ranomafana National Park, in particular the Talatakely Trail System where my study was conducted is located at a lower elevation and it is exposed to high levels of ecotourism (Tsinjoarivo is not). Because of these differences among sites, these sites provide an ideal comparative framework for studies of variation in lemur population structure and reproductive biology.

It is well known that reproductive biology in dwarf and mouse lemurs is mainly determined by environmental cues, such as photoperiod. However, variation in the timing of reproductive events (e.g. estrus) has been documented within and between cheirogaleid species, especially in *Microcebus* spp. Although the factors underlying variation in reproductive parameters are not well understood, their ecological significance should not be underestimated, as they may have a significant effect on population dynamics and the probabilities of long term survival of cheirogaleid populations. For example, the number of estrous cycles within a single season as well as the timing of reproductive events (e.g. births) may impact individual reproductive success under highly unpredictable environmental conditions. The latter are known to exist in Madagascan forests, which can vary in the dates and temporal range of the rainy season even from year to year.

This project provided direct reproductive observations of pregnant and possibly lactating female mouse lemurs as well as some pregnant and recently estrous female dwarf lemurs

at Tsinjoarivo during the month of November, which represents the end of the dry season. We also compiled more detailed reproductive observations of female brown mouse lemurs at Ranomafana. Most displayed vaginal openings in October and the beginning of November, and gave birth during the last two weeks in December. Trapping was continued until the end of January, extending the sampling season for *Microcebus* at Ranomafana over those conducted in 2004 and 2005.

An indirect, but no less important aspect of this study was to determine the species status of cheirogaleids at Tsinjoarivo. Despite the relatively short duration of our expedition (17 days) to this site, we succeeded in trapping, for the first time, a number of mouse and dwarf lemurs both in the continuous and fragmented forests (see Results below). Although tissue samples were taken for genetic analysis, these tissues have yet to be studied. However, statistical comparisons of the morphometrics show interesting differences among populations both within and between sites.

The species of cheirogaleids that had been previously identified at Ranomafana are *Microcebus rufus* and *Cheirogaleus major*. However, as was stated in my grant proposal, the cheirogaleids at Tsinjoarivo were previously poorly known, and I expressed the suspicion that the species of *Cheirogaleus* present there might differ from that at Ranomafana. This was based on the observation of a single aberrant individual rescued by Dr. Mitchell Irwin and inspected by the PI, Dr. Laurie Godfrey. In fact, with the trapping of many more individuals, my suspicion has been corroborated. Furthermore, I have determined that there are two species of dwarf lemur at Tsinjoarivo, the second more like that at Ranomafana (if not con-specific). Direct observations as well as dental and morphometric analysis reveal two different “morphs” of dwarf lemurs both of which differ from *Cheirogaleus major* at Ranomafana. The species in the fragmentary forest is much smaller in body size than the other (including individuals in the continuous forest which are in turn smaller than those at Ranomafana). Whether this morphological variation is due to geographical variation (e.g. smaller individuals at a higher elevation in Tsinjoarivo) or it is a result of specific differences between sites, needs to be determined, but we have promising evidence of at least one cheirogaleid species at Andasivodihazo (the fragment surveyed at Tsinjoarivo) that differs from *C. major*. Also interesting is that the fragment- and continuous forest morphs appear to differ in habitat preference at Tsinjoarivo.

Dr. Mitchell Irwin, Dr. Laurie Godfrey, Malagasy student Mamihambola Rakotondratsima and I are currently working on a research paper to present these preliminary findings.

Finally, an important component of this project was, and continues to be, to train Malagasy guides and students. Mamihambola Rakotondratsima is currently working on his DEA at the University of Antananarivo, and he will continue to work on the *Cheirogaleus* project at Tsinjoarivo and Ranomafana National Park. He has been trained in animal handling, radio tracking and nocturnal surveys.

Results: Ranomafana

Microcebus

Population Data

A total of 58 individual mouse lemurs were captured during the study period (from October 2006 to January 2007). As shown in Table 1, a subsample of 29 individuals was captured for the first time and marked with Avid microchips.

Capture percentages were high in October and November, but trapping success dropped to ~10% during the months of December and January at Ranomafana (Table 2). The number of individuals (especially males) that entered traps decreased substantially in December and January (see Tables 3 and 4).

Table 1: Mouse lemur population trapped in Talatakely during study period.

Oct-Jan.	Recaptured	newly tagged	Total	Ratio
Females	17	11	28	1.55
Males	12	18	30	0.67
Total	29	29	58	

Table 2: Sampling schedule and percentage of mouse lemur captures

Month	Trapping nights	N of traps	N of captures	Percentage captures	N of Males	N of Females	Percentage Males	Percentage Females
October	27	742	310	41.78	215	95	69.35	30.65
November	11	242	84	34.71	43	41	51.19	48.81
December	17	584	61	10.45	25	36	40.98	50.02
January	14	478	54	11.30	13	41	24.07	75.93

Tables 3 and 4: Presence/absence of individual females, left, and males, right during trapping period by month.

3. Females	Oct.	Nov.	Dec.	Jan.	4. Males	Oct.	Nov.	Dec.	Jan.
Agatha			X		Andreas	X	X		
Amanda	X	X		X	Andy	X	X	X	X
Andrea		X	X	X	Anthony	X	X		
Anja	X				Carlos	X			
Carla	X				Christopher	X	X		
Ingrid	X	X	X	X	Erik	X	X	X	X
Jaqueline	X	X	X	X	Fernando	X			
Jenna	X				Francisco	X			
Jessica		X			Harley	X		X	
Kathy	X				Ismael	X			
Kerstin	X	X		X	Jeff	X			
Laurie	X				Kerry	X	X		
Lulu	X	X			Lance	X	X		
Mandy		X			Lanto	X	X		
Marcela		X	X	X	Loco	X	X		
Martha	X	X			Mamy	X	X	X	
Medusa	X	X		X	Mark	X	X	X	
Michelle		X	X	X	Mickey	X	X		
Miriam	X	X	X	X	Napoleon	X			
Misa	X	X			Octavio	X			
Patricia	X	X			Olivier			X	
Petra	X				Pascal	X	X	X	
Preciosa	X	X			Paul	X			
Queenie	X	X		X	Pierre	X			
Sarah	X	X	X	X	Ralala	X	X	X	X
Sherry	X				Tsima	X			
Stacey	X	X			Victor	X			
Victoria	X	X	X	X	William	X		X	
total	22	20	9	12	Zaka	X			
					Ziggy	X	X	X	X
					total	29	15	10	4

Reproductive Data

One hypothesis of interest to me is that females in close proximity will tend to cycle together. There was some overlap in the timing of vaginal opening among females (Table 5); however estrus (as opposed to proestrus and metestrus) is restricted to one night per cycle (within a ~7-day period) for each female. Of all females who experienced vaginal openings on the same night (n=7), only two pairs (females Victoria and Ingrid on October 22nd, and Medusa and Michelle, on November 7th) could have been in estrus the same night in the same trapping area (Figure 1). On the basis of trapping data alone, inferences should not be drawn regarding the degree of social communication and exchange of cues during estrus. Nevertheless, it is clear that the proximity hypothesis gains no support from my data. In most cases, females that could have been in estrus during the same night were trapped in different areas of the trail system.

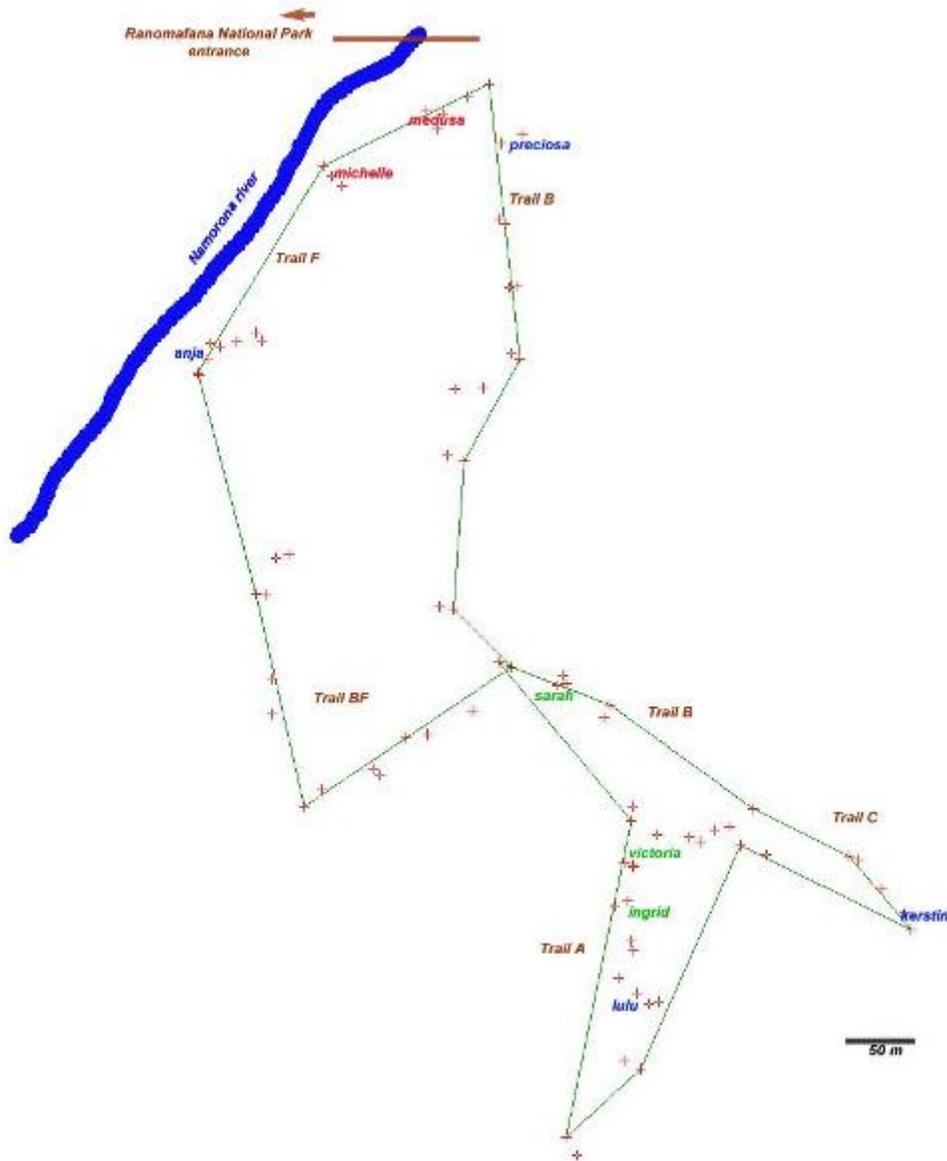


Figure 1 (above) Trapping area (red symbols show individual trap locations based on GPS points) in the Talatakely trail system. A subset of individuals that were captured during the reproductive season of 2006 is shown near their trapping locations. Individual females with potentially synchronous estrous cycles are labeled in identical colors. Polygons enclose the closest and farthest areas from the Park Entrance.

Table 5: Distribution of vaginal swellings and openings in female mouse lemurs during the study period.

females	early oct	late oct	early nov	late nov	early dec	late dec
vag opening	1	11	3			
vag swelling		3	1			1

Another hypothesis to be tested was that there are annual fluctuations in the timing of estrus. When reproductive data collected in 2006 were compared to those collected in the previous year, there was no consistent pattern of change for females observed in both years in the timing of estrus (Table 6). Also tested was the notion that body mass acts as a trigger for estrus, i.e., that heavier females enter estrus earlier than lighter ones, and that such changes explain annual differences in the timing of estrus. However, my data demonstrated no such correlation: females who showed later vaginal openings in 2006 were not necessarily lighter or heavier than during the prior reproductive season in 2005.

Table 6 Comparison of female reproductive schedules in two consecutive years.

Female	Capt. date	W (g)	Obs.	Cap. date	W (g)	Obs.	2006	Days
Medusa	11/13/2005	59	vag. ope.	11/7/2006	56.5	vag. ope.	earlier	-6
Queenie	11/10/2005	52	vag. swe.	10/30/2006	54.5	vag. ope.	earlier	~ -15
Kathy	10/5/2005	43	vag. swe.	10/23/2006	39.5	vag. ope.	later	~ +7
Ingrid	10/15/2005	42	vag. ope.	10/22/2006	44.5	vag. plug	later	+7
Carla	10/14/2005	41	vag. ope.	10/19/2006	41	vag. ope.	later	+5
Kerstin	10/12/2005	51.5	vag. ope.	10/27/2006	45	vag. ope.	later	+15
Jaqueline	10/14/2005	45	vag. ope.	10/7/2006	41.5	vag. ope.	earlier	-7
Stacey	10/25/2005	42	vag. ope.	10/16/2006	45.5	vag. swe.	similar?	
Victoria	10/28/2005	39.5	vag. ope.	10/21/2006	45.5	vag. ope.	earlier	-3
Anja	10/25/2005	53	vag. ope.	10/27/2006	54	vag. ope. (closing)	similar?	

Morphometric Data

To address the question of species identification, the pattern of morphometric variation was examined. There were striking differences in size and proportions among individuals that were unrelated to sex or age. No genetic work has been done to verify or refute any sort of possible genetic separation. However, male and female mouse lemurs could be easily identified as belonging to one of two groups (here called “big” and “small” morphs) on the basis of these differences. A separation could be identified without assigning individuals to groups, on the basis of a principal components analysis. Figure 2 shows the distribution of these individuals in a multivariate space generated via a principal components analysis of these measurements. Also indicated on this plot are the dates of vaginal swellings or openings of the “big” and “small” females. It is noteworthy that, with one exception (Petra), “big” females showed vaginal opening **later** than “small” females. There are two “big” females and five “small” females that show the same pattern, but are not included on the graph due to insufficient morphometric data. These differences suggest that there may be two species of mouse lemur at Ranomafana, a conclusion that had been suspected on the basis of earlier capture work.

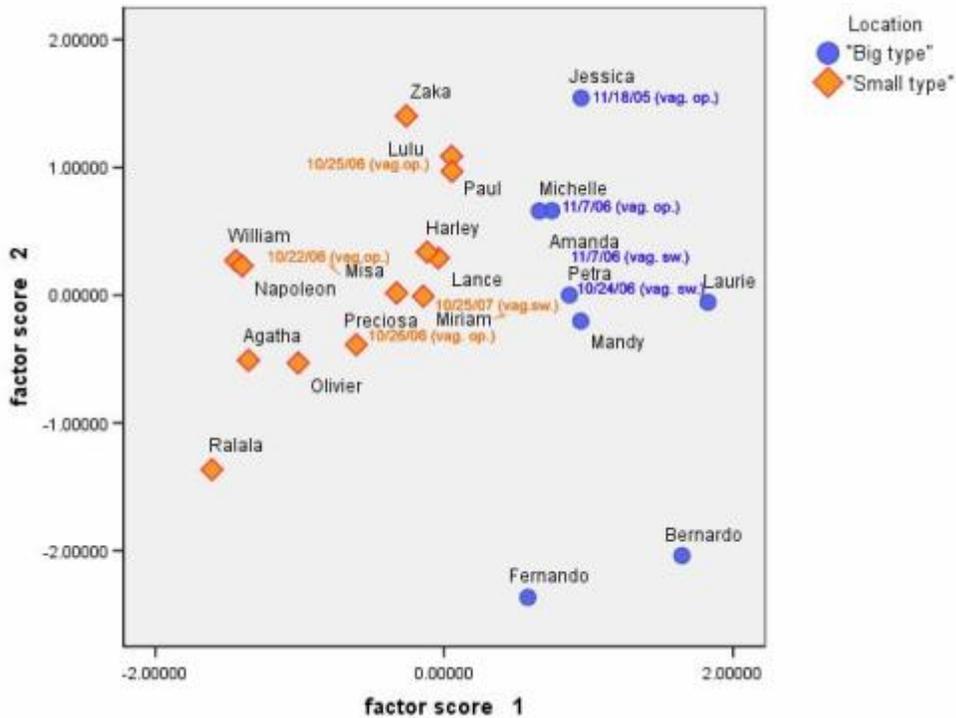


Figure 2: Plot of the first two factors in a Principal Component Analysis, showing “big” and “small” mouse lemurs scoring more positively or negatively respectively on the first axis based on morphometric measurements; reproductive observations were mapped onto the plot. The first component explained 50% of variance and the second one 20%.

Table 7: Structure matrix of the multivariate analysis; individuals with high positive scores on the first axis have longer tails, legs, skulls but relatively small inter-pupil distances.

Component Matrix ^a

	Component	
	1	2
tail (mm)	.828	.040
tail-crown (mm)	.656	-.535
leg (mm)	.855	.102
big toe (mm)	.787	-.318
skull length (mm)	.766	.297
snout length (mm)	.642	.418
interpup.dist. (mm)	.210	.880
skull width (mm)	.727	-.274

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Cheirogaleus

A total of 6 *Cheirogaleus* were also trapped in 2006 in Talatakely. Four of these individuals (3 females and 1 male) were recaptured from 2005. The reproductive status of female dwarf lemurs at capture is shown in Table 8. Individuals captured during the first two weeks in October showed no signs of vaginal opening. Determination of pregnancy

is problematic during the first weeks after conception because nipple swelling will be slight; however a probably-lactating female was captured in mid-December.

Table 8: Reproductive observations in female dwarf lemurs

Female	Cap. date	W (g)	Vag. status	reproductive observations
Gisele	10/11/2006	311.5	closed vagina	no apparent swelling, at least 4 visible very small nipples; pregnant?
Barbara	10/11/2006	341.5	closed vagina	no apparent swelling, 6 small nipples; pregnant?
Laurie	10/12/2006	436.5	closed vagina	6 developed nipples, pregnant?
Patricia	10/14/2006	345	closed vagina	6 visible nipples, pregnant?
Alice	12/18/2006	403	closed vagina	probably lactating?

Results: Tsinjoarivo

During our survey (6 days in Vatateza --the continuous forest-- and 5 days in Andasivodihazo --one of the fragments) we captured a total of 15 mouse lemurs and 10 dwarf lemurs. Capture success was unexpectedly high; given the fact that animals were not habituated to traps (Sherman or Tomahawk) and that there was a high frequency of rodents that were captured, which prevented cheirogaleids from entering the traps (Table 9). Higher trapping success in the fragment may be due to higher densities of cheirogaleids in smaller forested areas as well as their possible affinity to forest edges; trapping locations were in close proximity to the periphery of the fragment, whereas a relatively higher interior area was surveyed in the continuous forest. An affinity of dwarf lemurs to forest edge is counterintuitive, because dwarf lemurs are said to be edge intolerant due to their high degree of frugivory. The possibility that the species in the fragment prefers edge habitat and has shifted its diet accordingly is intriguing, and can only be addressed via focal individual sampling.

Table 9: Schedule of activities at Tsinjoarivo and summary of captures.

Location	Date	Night walk	Trap. night	N of S ¹	N of T ²	N of M ³	N of C ⁴	N of rats	Notes
Vatateza	11/19	1	0	0	0	0	0	0	
Vatateza	11/20		1	20	6	0	0	2	
Vatateza	11/21	2	2	40	10	1	0	6	
Vatateza	11/22	3	3	38	10	0	0	10	
Vatateza	11/23		4	33	10	2	2		

Vatateza	11/24	4	5	39	10	5	1	14	2 recap. (M)
Andasivodihazo	11/27		1	38	10	2	2	0	
Andasivodihazo	11/28	1	2	32	8	1	0	0	
Andasivodihazo	11/29	2	3	36	10	2	3	6	1 recap. (C)
Andasivodihazo	11/30		4	34	8	4	3	16	
Andasivodihazo	12/1	3							

¹number of open Sherman traps; ²number of open Tomahawk traps; ³number of captured mouse lemurs; ⁴number of captured dwarf lemurs

Some reproductive observations were recorded for female mouse lemurs (Table 10) and female dwarf lemurs (Table 11) during the very brief trapping period in both the continuous and fragmented forests of Tsinjoarivo.

Table 10: Female mouse lemur weights and reproductive observations at Tsinjoarivo

Location	Female	Capt. date	W (g)	Vag. Obs.	Reproductive Observation
Vatateza	Fanja	11/21/2006	31.5	closed vagina	remains of blood in vagina, at least 4 visible nipples (perinatal death of offspring or abortion?)
Vatateza	Fanja	11/25/2006	32.5	swollen vagina	Whitish
Vatateza	Chantale	11/23/2006	37	closed vagina	remains of dry blood in vagina; 4 visible nipples but the lower two are more developed, possibly lactating?
Vatateza	Chantale	11/25/2006	34.5	closed vagina	vagina is slightly open but covered with dry blood
Andasivodihazo	Stacy	11/28/2006	60	closed vagina	pregnant, 2 lower nipples are more developed
Andasivodihazo	Jessica	11/30/2006	40.5	closed vagina	vagina was open recently, 4 well developed nipples (lactating?)
Andasivodihazo	Karen	12/1/2006	42.5	closed vagina	six small visible nipples, possibly pregnant?
Andasivodihazo	Lynnette	12/1/2006	44	closed vagina	at least 4 very developed nipples (lactating?)

Table 11: Female dwarf lemur weights and reproductive observations at Tsinjoarivo

Location	Female	Capt. date	W (g)	Vaginal status	Reprod. obs.
Vatateza	Soa	11/23/2006	321.5	closed vagina	4 developed nipples, probably <i>not</i> pregnant
Vatateza	Laurie	11/23/2006	396.5	closed vagina	6 visible nipples, possibly pregnant
Vatateza	Raozy	11/25/2006	361	closed vagina	6 visible nipples, possibly pregnant
Andasivodihazo	Marcia	11/28/2006	226	almost closed vagina	probably recently in estrus
Andasivodihazo	Elisa	11/28/2006	215.5	almost closed vagina	probably recently in estrus
Andasivodihazo	Misa	11/30/2006	305.5	closed vagina	4 visible nipples, possibly pregnant
Andasivodihazo	Marina	11/30/2006	399.5	closed vagina	6 visible nipples,

					pregnant
Andasivodihazo	Justine	1/12/2006	258	closed vagina	4 very small nipples, probably <i>not</i> pregnant
Andasivodihazo	Meline	1/12/2006	377	closed vagina	6 developed nipples, possibly pregnant

The reproductive observations of females “Elisa” and “Marcia” were quite interesting. These females had probably been in estrus soon before they were trapped at the end of November, which would appear to be late. A different female, “Marina”, trapped at about the same time was obviously pregnant (Table 11). The question remains as to whether or not the two “late estrus” females were able to conceive during that reproductive season. It has been reported for *C. medius* in the wild that the first successful pregnancies occur in females 3 years of age or older.

Results: Comparison between Ranomafana and Tsinjoarivo

The species status of cheirogaleids at Tsinjoarivo has not yet been verified. Tissue samples were taken from both mouse and dwarf lemurs and genetic analyses are pending. *A priori*, based on site location and published distribution of cheirogaleid species (although cheirogaleid taxonomy is under revision), we might expect to find at Tsinjoarivo the same species present at Ranomafana: *Microcebus rufus* and *Cheirogaleus major*. Multivariate analysis (Principal Component Analysis -PCA) based on morphometrics, show a great overlap between Tsinjoarivo mouse lemurs and the “small” individuals from Ranomafana (Figures 4 and Table 12). Interestingly enough, dwarf lemurs show a different pattern: individuals from the 3 locations (Tsinjoarivo-Vatateza; Tsinjoarivo-Andasivodihazo; Ranomafana-Talatakely) are spread from the negative to the positive end with no overlap on the first component of the PCA (Figures 5 and Table 13).

Mouse lemur body masses were compared among locations (Table 14). Advanced pregnant females (i.e. those females that could be determined pregnant by palpation and direct observations) were removed from the analysis to avoid overestimation of body weights. For the purpose of this comparison, I included only body weights from individuals captured in November and the beginning of December, to minimize seasonal fluctuation in body weight. Mouse lemurs at Tsinjoarivo fall within the weight range of “small” mouse lemurs at Ranomafana.

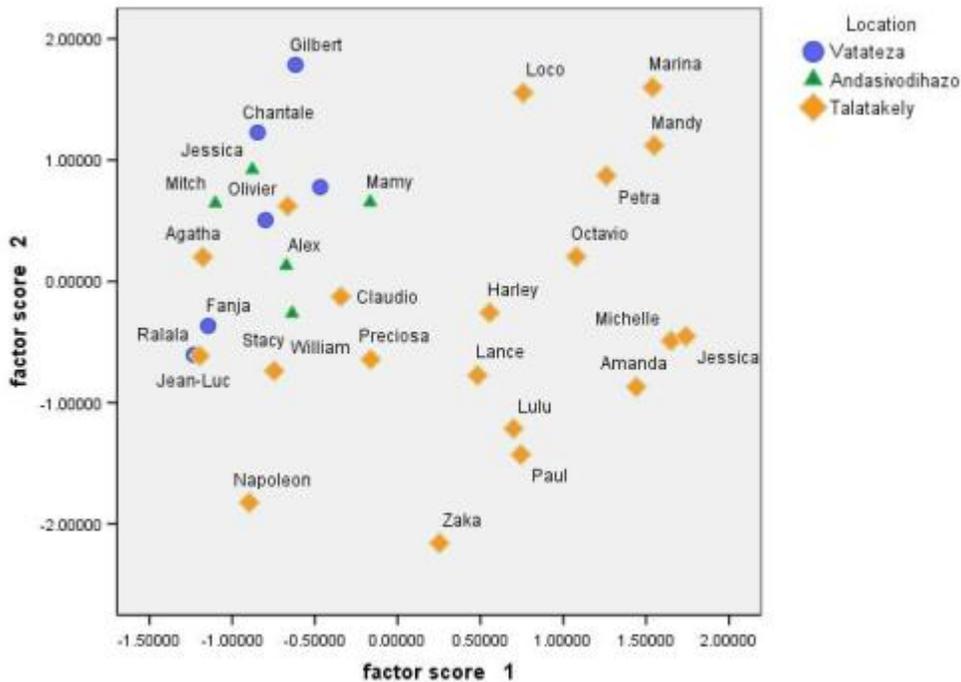


Figure 4: Plot of the first two components of a PCA (the first axis explains 50% of variance, second axis explains 13%) based on mouse lemur morphometrics. All Tsinjoarivo individuals and some “small” Ranomafana mouse lemurs score negatively on the first axis; “big” Ranomafana mouse lemurs tend to score on the positive end of the axis.

Table 12: Structure matrix of the PCA: mouse lemurs scoring on the positive end of the first component have bigger tails, longer skulls and snouts but relatively short bodies and smaller inter-pupil distances.

Component Matrix ^a

	Component	
	1	2
tail-crown (mm)	.460	.537
tail (mm)	.862	.025
leg (mm)	.661	.444
big toe (mm)	.721	.434
arm (mm)	.654	-.295
skull length (mm)	.811	-.296
skull width (mm)	.707	-.111
snout length (mm)	.803	-.040
interpup.dist. (mm)	.603	-.549

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

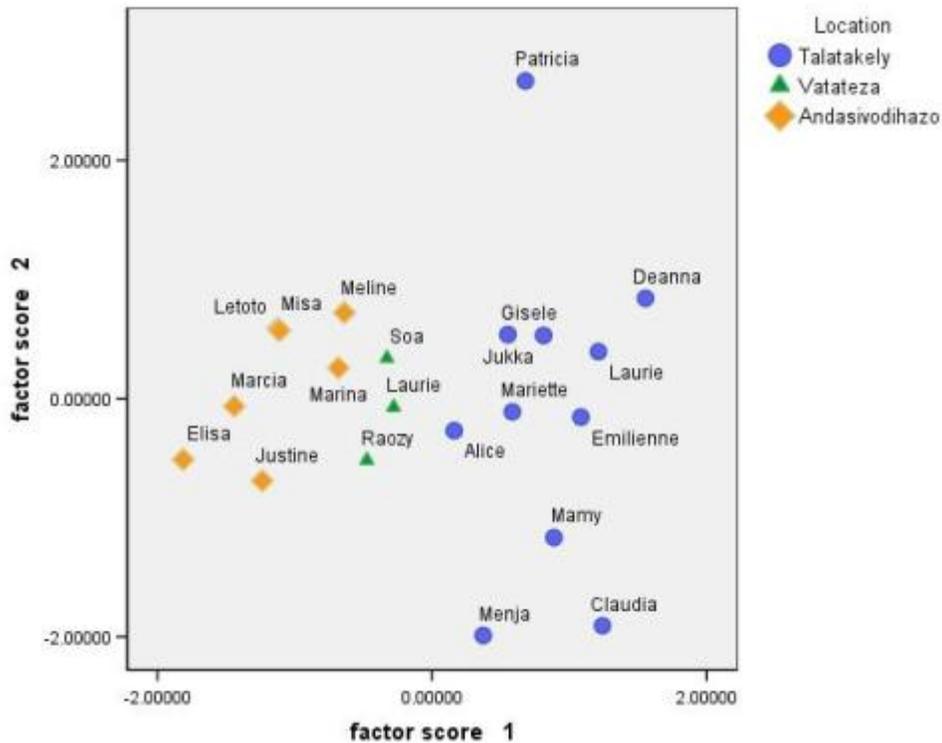


Figure 5: Plot of the first two components of a PCA (the first axis explains 60% of variance, second axis explains 13%) based on dwarf lemur morphometrics.

Table 13: Structure matrix of the PCA: dwarf lemurs scoring highly positive on the first axis have longer tails, longer and wider skulls but shorter ears and relatively smaller inter-pupil distances.

Component Matrix ^a

	Component	
	1	2
tail-crown (cm)	.802	.248
tail (cm)	.952	-.055
leg (cm)	.897	.048
knee to toe (cm)	.620	.237
hindfoot (mm)	.812	-.239
skull length (mm)	.906	-.124
skull width (mm)	.905	-.200
interpup.dist. (mm)	.237	.721
ear length (mm)	-.424	.686
ear width (mm)	.828	.327

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Table 14: Mouse lemur body weights among different locations

Tsinjoarivo	Vatateza	weight (g)	
	N	mean	range
Females	2	34.25	31.5-37
Males	3	42.38	37-44.5
sex combined	5	39.67	31.5-44.5
Tsinjoarivo	Andasivodihazo	weight (g)	
	n	mean	range
Females	3	42.33	40.5-44
Males	5	45.7	40-51
sex combined	8	44.44	40-51
Ranomafana	Talatakely "small"	weight (g)	
	n	mean	range
Females	14	43.25	33.5-51.5
Males	14	42.21	33.5-52.5
sex combined	28	42.73	33.5-52.5
Ranomafana	Talatakely "big"	weight (g)	
	n	mean	range
Females	6	51.25	45-57.5
Males	3	60.5	56-65.5
sex combined	9	54.33	45-65.5

Summary and conclusions

Morphological comparisons between mouse lemurs at Tsinjoarivo and Ranomafana support the claim that the same species may be present at both locations (Figure 6). However, genetic analysis will be necessary to confirm this hypothesis, as a recent discovery of a new species of mouse lemur, *Microcebus lehilahytsara*, differs little, if at all, in morphology from its relative *Microcebus rufus*, which also inhabits the eastern rain forests. More interesting is the apparent intra-population variation within Ranomafana National Park where two sympatric morphs are distinguishable based on their morphometrics. Genetic analysis should help us to determine the significance of these differences in terms of genetic separation. In addition to genetic analysis, however, a comprehensive study integrating multiple areas, e.g. genetics, morphology and reproductive biology, is necessary to better understand the ecological significance of biological variation. The implications of the research thus far is that (1) there are probably two species of dwarf lemurs at Tsinjoarivo and two morphs (perhaps species) of mouse lemurs at Ranomafana; and (2) an ecological separation between the species may exist at Tsinjoarivo, whereas ecological separation is much less clear at Ranomafana, and is indicated only in that the two morphs seem to time their reproductive seasons differently.

Comparing mouse lemurs at Tsinjoarivo and Ranomafana, we can state that the reproductive season in mouse lemurs appears to start earlier in the forests of Tsinjoarivo compared to Ranomafana National Park. There is indirect evidence of some lactating females at Tsinjoarivo at the very end of November, more than a week earlier than the earliest reported lactating female at Ranomafana. Further studies are needed to verify these preliminary findings. It is interesting in this regard that the mouse lemur at

Tsinjoarivo is most similar to the small morph at Ranomafana, and that it is the small morph at Ranomafana that begins its reproductive season earlier than the large morph.

Comparing the dwarf lemurs at Tsinjoarivo and Ranomafana, we can state that multivariate statistics based on morphometric and dental (not shown) measurements clearly distinguish three dwarf lemur groups, each corresponding to a different study site (Tsinjoarivo-continuous, Tsinjoarivo-fragment, Ranomafana). Dwarf lemurs from the fragment, Andasivodihazo and from Ranomafana National Park display the strongest differences. *Cheirogaleus* from the fragment are overall smaller, but they are also different in coat coloration, eye ring thickness, and nose coloration from those in the interior of the continuous forest and at Ranomafana (Figure 7). Genetic analysis must be conducted to confirm or refute our inference that two species are represented here.



Figure 6 (above): mouse lemurs at Tsinjoarivo (left and center from Andasivodihazo; right from Vatateza) and Ranomafana National Park (three individuals that belong to the “small” morph).



Figure 7 (below): three different dwarf lemurs from Tsinjoarivo and Ranomafana National Park. Differences in fur coloration, eyes ring thickness, and nose shape are most visible between Andasivodihazo and Talatakely individuals.

