

RESEARCH ARTICLE

Exceptional Diversity of Mouse Lemurs (*Microcebus* spp.) in the Makira Region With the Description of One New Species

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Although the number of described lemur species has increased considerably over the last 20 years, detailed biogeographic data are still lacking from many geographic regions, in particular in the eastern part of Madagascar. This study investigated mouse lemur species diversity in a previously unstudied Inter-River-System in the eastern Makira region. Three sites were visited and 26 individuals were sampled and characterized with 13 external morphometric measurements. Standard phylogenetic analyses were performed on the basis of sequences of three mitochondrial loci by including representatives of all other published mouse lemur species for comparison. The analyses revealed the presence of three mouse lemur species in one study site, two of which were previously undescribed. The two new species are genetically distinct and belong to the larger-bodied mouse lemur species on the island, whereas the third species, *Microcebus mittermeieri*, belongs to the smaller-bodied mouse lemur species. The study fully describes one of the new species. This study and other lemur inventories suggest that the Makira region is particularly rich in lemur species and the lack of any protected zone in this area should now attract the urgent attention of conservation stakeholders. *Am. J. Primatol.* 70:1033–1046, 2008. © 2008 Wiley-Liss, Inc.

Key words: species richness; Madagascar; *Microcebus macarthurii*; phylogenetic analyses; mtDNA

INTRODUCTION

Madagascar is well known for its exceptional biodiversity and an astonishingly rich endemic fauna in relation to its limited surface area [e.g. Glaw & Vences, 2007; Goodman & Benstead, 2003; Mittermeier et al., 2006]. Intensified research activities on the island during the last 20 years have led to the description of a considerable number of previously unknown species in many taxonomic groups [for amphibians and reptiles see Glaw & Vences, 2007; for primates see Mittermeier et al., 2006; Tattersall, 2007].

For example, the number of described extant lemur species rose from 32 in the year 1994 [Mittermeier et al., 1994] to more than 80 species in the year 2007 [Louis et al., 2006a,b; Olivieri et al., 2007; Tattersall, 2007]. This trend is particularly prominent in two lemur genera, the mouse lemurs (*Microcebus* spp., Cheirogaleidae), which increased from 3 to 15 described species [Andriantompohavana et al., 2006; Kappeler et al., 2005; Louis et al., 2006b; Olivieri et al., 2007; Yoder et al., 2000], and the sportive lemurs (*Lepilemur* spp., Lepilemuridae),

which increased from 7 to 24 described species [Andriaholinirina et al., 2006; Craul et al., 2007; Louis et al., 2006a; Rabarivola et al., 2006].

Although research activities have been greatly intensified and large parts of the island have been systematically inventoried over the last ten years, there are still numerous areas with insufficient taxonomic information. Therefore, it is not yet possible to determine precise species ranges and to understand the biogeographic processes that shaped

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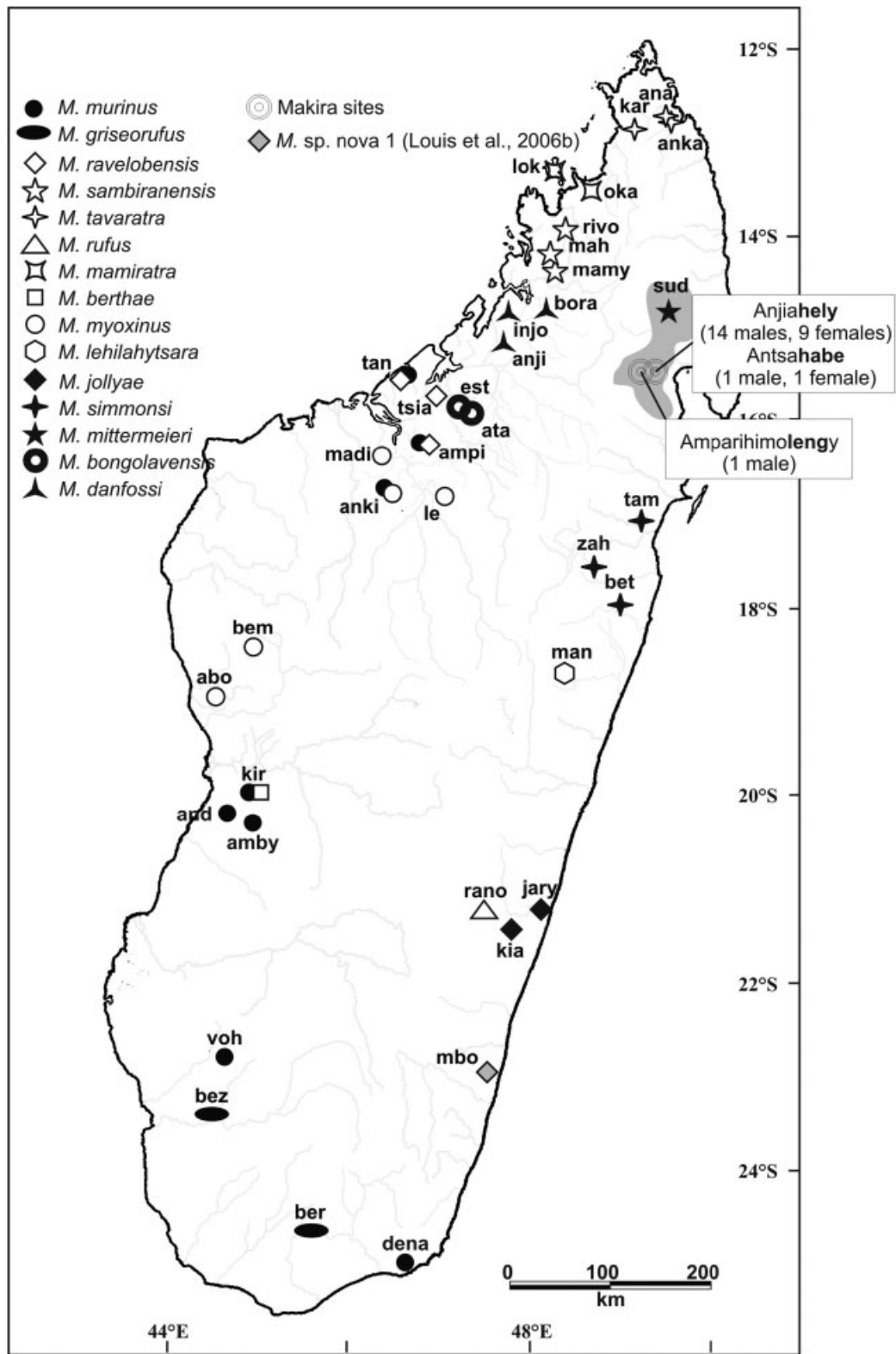


Fig. 1. Map of Madagascar with sites (for abbreviations see Table I) and species included in the phylogenetic analyses. New samples from this study are explained in extra boxes. Gray shaded area indicates the Makira region.

the species diversity on the island. This study aims to fill a biogeographic gap that still exists for *Microcebus* spp. in northeastern Madagascar, in the so-called Makira region (Fig. 1, gray area).

To date, it is known that one or two species of mouse lemurs can occur within a given region [reviewed in Radespiel, 2006]. It has been proposed that large rivers (defined as >50 m wide at 20 km inland) and the mountainous central highlands acted as topographic barriers that induced speciation events and channeled colonization and recolonization events preceding, during and maybe even after Pleistocene climatic and vegetation changes [Craul et al., 2007; Martin, 1995; Olivieri et al., 2007; Wilmé et al., 2006]. Mouse lemurs are small (30–65 g), nocturnal solitary foragers that can be found in all forest habitats of Madagascar [overview in Radespiel, 2006]. However, data on eastern mouse lemur species lag behind the wealth of distribution data that are now available for the western and northwestern species [Andriantompohavana et al., 2006; Kappeler et al., 2005; Louis et al., 2006b; Olivieri et al., 2007; Rasoloarison et al., 2000], despite the presence of large rivers and there being even more prominent mountains in this region than in the western domain.

The aim of this study was therefore to explore the mouse lemur species diversity in a so far understudied remote area of Madagascar, the Makira region (Fig. 1). The Makira region includes the largest still connected block of dense montane evergreen rain forest of 376,156 ha in size [Wildlife Conservation Society, 2004]. The northern part of the Makira region includes the Anjanaharibe-South Special Reserve (sud ★ in Fig. 1) where *Microcebus mittermeieri* occurs [Louis et al., 2006b]. Several large rivers flowing from the eastern mountains into the sea divide the landscape south of this reserve into different Inter-River-Systems (IRSs) [Craul et al., 2008] and could potentially act as species barriers. According to the data available until now, the neighboring species of *M. mittermeieri* to the south is *M. simmonsi*, whose northernmost locality (Tampolo) lies more than 250 km south of the Anjanaharibe-South Special Reserve. More than seven large rivers can be identified between these two sites. The first large river south of the Anjanaharibe-South Special Reserve is the Antainambalana River. During this study, three different sites south of this river were visited to determine the mouse lemur diversity in this first adjacent IRS.

METHODS

Study Sites, Sampling and Morphometric Analyses

Samples were collected from 26 individuals that were captured during two field trips (8 November–13 December 2006 and 27 August–12 September 2007)

in the dense montane evergreen rain forests of the Makira region, about 30–60 km west of Maroantsetra around the villages Anjahely ($n = 23$, S15°24'45", E49°29'37.5", 350–400 m a.s.l.), Antsahabe ($n = 2$, S15°21'33.9", E49°24'23.6", 850–1,200 m a.s.l.) and Amparihimolengy ($n = 1$, S15°24', E49°08', 800–1,200 m a.s.l.) (Fig. 1). Capture sites were situated mostly in the *savoka*, the transitional secondary vegetation after the forest has been cut down for the cultivation of rice.

Animals were trapped either with Sherman Life traps or captured directly by hand during their nightly activity or from their sleeping nests during daytime. Captured animals were anesthetized with an i.m. injection of 0.01 ml of a 5% ketamine solution. Thirteen standard morphometric measurements (*body mass, ear length, ear width, head length, head width, snout length, interorbital distance, intraorbital distance, lower leg length, hind foot length, third toe length, tail length, body length*) were taken from all captured individuals following the techniques of Hafen et al. [1998] and Zimmermann et al. [1998]. Morphometric measures of the sampled animals were compared qualitatively with those found in the literature. Extended quantitative comparisons could not be performed for three reasons: First, it is known from previous work that different investigators may differ slightly in their data collection protocols or handling routines. As a consequence, differences between studies can be partly based on interobserver differences [Olivieri, Radespiel, Randrianambinina, Rasoloharijaona, unpublished results]. Second, published data sets are usually limited to means ± standard deviations and statistical comparisons based on these values are limited. Third, measurements were not available for all variables and all species. Preliminary statistical comparisons were made only with the Mann–Whitney-*U*-test within the data set from this study using the software STATISTICA 6.0 (StatSoft, Tulsa, OK). A Bonferroni correction was performed afterwards.

Small ear biopsies (~2mm²) were taken for subsequent DNA analyses and stored in Queen's lysis buffer [Seutin et al., 1991] until extraction. All animals were released at their individual capture sites at dusk within 24 hr of their capture. All field handling and sampling procedures adhered to the legal requirements of Madagascar and were approved by the Ministry of Water and Forests.

Laboratory Procedures and Phylogenetic Analyses

DNA was extracted from the tissue samples using standard phenol/chloroform extraction techniques [Maniatis et al., 1982]. Three different loci of mitochondrial DNA (partial d-loop region, *cyt b* and COII) were amplified using the primers given in Olivieri et al. [2007]. Polymerase chain reaction

(PCR) conditions and cycling conditions followed Guschanski et al. [2007] for the d-loop/COII and Olivieri et al. [2007] for *cyt b*. PCR products were checked for successful amplification on a 1.5% agarose gel containing 1.3×10^{-4} mg/ml ethidium bromide. PCR products were cleaned using the standard protocol of the Invisorb[®] Spin PCRapid kit (Invitek, Berlin, Germany). Cleaned PCR products were sent to Macrogen Ltd. (www.dna.macrogen.com) for sequencing on an ABI 3730XL automatic DNA sequencer (Applied Biosystems, Foster City, CA).

In order to exclude the potential problem of receiving and analyzing wrong sequence data from nuclear copies of the genes [numts; Thalmann et al., 2004], we performed long-range PCRs on all samples included in later phylogenetic analyses according to the laboratory routines described in Guschanski et al. [2007]. The first PCR amplified a partition of mtDNA over 16,200 bp in length and was followed by a second PCR with the specific primers after purification and dilution. The PCR products were subsequently sequenced and their sequences compared with those obtained with the standard protocol.

Individual sequences were analyzed, edited and aligned using Sequencher[™] 4.0.5 (Gene Codes, Ann Arbor, MI). Preliminary analyses indicated the presence of three genetic groups of sequences in the sample ($n_{\text{group1}} = 22$, $n_{\text{group2}} = 3$, $n_{\text{group3}} = 1$, results not shown). Six haplotypes of group 1, the two haplotypes of group 2 and the haplotype of group 3 were used for phylogenetic reconstructions. In addition, 2–4 sequences per gene per described mouse lemur species were retrieved from GenBank whenever possible for the 15 described mouse lemur species, as well as outgroup sequences from *Mirza zaza* and *Propithecus verreauxi* (Table I).

The final alignment of all sequences was performed for each locus separately with the program Clustal X [Thompson et al., 1997] and checked by eye. Part of the d-loop was too variable to align across taxa. We therefore chose to cut out this region according to the protocol of Olivieri et al. [2007]. The final alignments are available upon request. All three single genes were then combined into concatenated sequences. PAUP 4.0b10 was used for three phylogenetic methods: maximum parsimony (MP), neighbor joining (NJ) and maximum likelihood (ML). Gaps were considered as missing data in ML and NJ, but were treated as fifth character in MP analysis. For MP analysis, we performed a heuristic search with 1,000 random stepwise additions of taxa, tree bisection and reconnection (TBR) and branch swapping. Phylogenetically informative characters were treated as unordered and equally weighted. For ML and NJ, an appropriate nucleotide substitution model was selected using the hierarchical likelihood ratio test (hLRT) as implemented in Modeltest 3.5.mac [Posada

& Crandall, 1998]. In ML, we performed a heuristic search with 30 random stepwise additions of taxa, TBR and branch swapping. Statistical support of the clades was assessed using nonparametric analyses including 100 replicates for ML and 1,000 replicates for each MP and NJ.

The final concatenated sequence of (COII+*cyt b*+d-loop) was 1,298 bp in length. Seven hundred and thirty-one characters were constant, 143 variable characters were parsimony-uninformative and 424 were parsimony-informative. The best-fit model selected by hLRT in Modeltest 3.5.mac was the TrN+I+G model (Base = (0.3477 0.2535 0.1062 0.2926), Nst = 6, Rmat = (1.0000 11.6466 1.0000 1.0000 16.0286), $\alpha = 0.5417$, Pinvar = 0.4528). Published *cyt b* sequences are not yet available for *M. mittermeieri* and *M. jollyae* (Table I). We therefore performed all analyses with a two gene alignment (COII+d-loop) in parallel to determine the phylogenetic position of *M. mittermeieri* and *M. jollyae* in relation to the unknown samples from this study. The concatenated sequence (COII+d-loop) was 991 bp in length. Five hundred and thirty-one characters were constant, 124 variable characters were parsimony-uninformative and 336 were parsimony-informative. The best-fit model selected by hLRT in Modeltest 3.5.mac was the TrN+I+G model (Base = (0.3594 0.2451 0.1008 0.2947), Nst = 6, Rmat = (1.0000 10.8184 1.0000 1.0000 14.5558), $\alpha = 0.4065$, Pinvar = 0.3906).

Finally, relative pairwise distances (% bp changes) were calculated with PAUP 4.0b10 [Swofford, 1998] for the two gene alignment and the three gene alignment, respectively. To determine fixed molecular differences among terminal clades, diagnostic sites for each terminal clade (= species) were identified in all loci on the basis of the three gene alignment using the program MEGA 3.1 [Kumar et al., 2004].

RESULTS

Phylogenetic Analyses

The sequences of the long-range PCR products were identical to those of the standard sequencing procedure in all cases. We therefore assume that all sequences used for phylogenetic analysis represent authentic mtDNA.

All phylogenetic trees with the two gene alignment revealed 15 well-supported terminal clades that coincided with species designations of the previously described mouse lemur species (Fig. 2, ML phylogram shown, MP and NJ trees not shown). Intraspecific sequence divergence varied between 0 and 4.71% (data not shown, largest value: *M. murinus*). Interspecific sequence divergence was always larger than 4.85% (*M. lehilahytsara*–*M. rufus*, Table II).

The samples from the Makira region (this study) did not all cluster together (Fig. 2). Six specimens fell into the terminal clade of *M. mittermeieri* but the

TABLE I. List of Samples Used in the Analysis

Site	Abb.	ST	Species	Individual	COII	Cyt b	d-Loop
Anjiahely	hely	T		001y06hely	EU810353 ^a	EU810362 ^a	EU810371 ^a
		T		002y06hely	EU810349 ^a	EU810358 ^a	EU810367 ^a
		T		003y06hely	EU810354 ^a	EU810363 ^a	EU810372 ^a
		T		005y06hely	EU810347 ^a	EU810356 ^a	EU810365 ^a
		T		006y06hely	EU810346 ^a	EU810355 ^a	EU810364 ^a
		T		001y07hely	EU810352 ^a	EU810361 ^a	EU810370 ^a
Antsahabe	habe	T		007y06habe	EU810351 ^a	EU810360 ^a	EU810369 ^a
		T		008y06habe	EU810350 ^a	EU810359 ^a	EU810368 ^a
Amparihimolengy	leng	T		001y06leng	EU810348 ^a	EU810357 ^a	EU810366 ^a
Ankirihitra	anki	GB	<i>M. murinus</i>	001y03anki	EF065266	EF065183	EF065236
Madirovalo	madi	GB	<i>M. myoxinus</i>	009y03madi	EF065252	EF065182	EF065221
Ampijoroa	ampi	GB	<i>M. ravelobensis</i>	021y00ampi	EF065261	EF065187	EF065229
Tananvaovao	tan	GB	<i>M. ravelobensis</i>	117y03tan	EF065269	EF065196	EF065223
			<i>M. murinus</i>	118y03tan	EF065248	EF065191	EF065224
Tsiaramaso	tsia	GB	<i>M. ravelobensis</i>	370y03tsia	EF065259	EF065198	EF065373
Le Croisement	le	GB	<i>M. myoxinus</i>	001y03le	EF065247	EF065188	EF065213
Mahajamba Est	est	GB	<i>M. bongolavensis</i>	148y03est	EF065254	EF065185	EF065306
Maroakata	ata	GB	<i>M. bongolavensis</i>	345y03ata	EF065263	EF065190	EF065285
			<i>M. murinus</i>	305y03ata	EF065262	EF065186	EF065230
Anjiamangirana	anji	GB	<i>M. danfossi</i>	092y04anji	EF065267	EF065192	EF065235
Bora	bora	GB	<i>M. danfossi</i>	001y02bora	EF065246	EF065203	EF065283
Mahatsinjo	injo	GB	<i>M. danfossi</i>	086y04injo	EF065268	EF065204	EF065234
Ambongomamy	mamy	GB	<i>M. sambiranensis</i>	007y05mamy	EF065264	EF065209	EF065238
Mahilaka	mah	GB	<i>M. sambiranensis</i>	010y02mah	EF065271	EF065180	EF065222
Lokobe	lok	GB	<i>M. mampiratra/lokobensis</i>	003y02lok	EF065245	EF065201	EF065214
Manehoka	oka	GB	<i>M. mampiratra/lokobensis</i>	003y02oka	EF065270	EF065211	EF065215
				013y02oka	EF065250	EF065207	EF065226
Analabe	ana	GB	<i>M. tavaratra</i>	003y03ana	EF065242	EF065206	EF065217
Ankarana	kar	GB	<i>M. tavaratra</i>	007y03kar	EF065239	EF065210	EF065220
Ankavana	anka	GB	<i>M. tavaratra</i>	004y03anka	EF065241	EF065199	EF065218
Mantadia	man	GB	<i>M. lehilahytsara</i>	017y00man	EF065255	EF065200	EF065227
				018y00man	EF065243	EF065181	EF065228
Kirindy	kir	GB	<i>M. murinus</i>	Jorg 33	AF285526	AF285562	AF285485
Berenty	ber	GB	<i>M. griseorufus</i>	YLE 362	AY167064	AY167076	AY167088
Beza Mahafaly	bez	GB	<i>M. griseorufus</i>	RMR 76	AF321180	AF285567	AF285490
Manongarivo	rivo	GB	<i>M. sambiranensis</i>	RMR 37	AF285520	AF285556	AF285479
Ranomafana	rano	GB	<i>M. rufus</i>	YLE 138	AF285508	AF285544	AF285467
				F25	AF285515	AF285551	AF285474
Tampolo	tam	GB	<i>M. simmonsii</i>	YLE 190	AF285516	AF285552	AF285475
Kirindy	kir	GB	<i>M. berthae</i>	SMG 8747	AF285517	AF285553	AF285476
				Jorg 46	AF285507	AF285543	AF285466
Aboalimena	abo	GB	<i>M. myoxinus</i>	Jorg 62	AF285506	AF285542	AF285465
				RMR 82	AF285502	AF285538	AF285461
Bemaraha	bem	GB	<i>M. myoxinus</i>	YLE62	AF285500	AF285536	AF285459
Kianjavato	kia	GB	<i>M. jollyae</i>	KIAN67	AY569183		AY159711
Mananjary	jary	GB	<i>M. jollyae</i>	MANJ11	AY569184		AY159713
Anjanaharibe-Sud	sud	GB	<i>M. mittermeieri</i>	JAR18	AY569182		AY159709
				JAR1	AY569180		AY159705
				JAR12	AY515541		AY159706
				M98	AY515556		AY159712
Manombo	mbo	GB	<i>M. sp. Nova 1</i>	M98	AY515556		AY159712
-	-	GB	<i>Mirza zaza</i>	001y04Mirza	EF122246	EF122248	EF122250
-	-	GB	<i>Propithecus verreauxi</i>	YLE66	AF285492	AF285528	AF285451

Abb.: abbreviation for site; ST: sample type (T: tissue, GB: GenBank).
^aNew sequences from this study.

other three were very distinct from all other previously described mouse lemur taxa. Two of them formed one clade (*M. sp. nova 2*, Fig. 2), which was well supported by the bootstrap analysis, whereas the third specimen (003y06hely) was a genetically

distinct sister taxon (*M. sp. nova 3*) to the two specimens of *M. sp. nova 2*. The specimens from *M. sp. nova 2* differed from *M. mittermeieri* in 9.84–9.96% and from *M. sp. nova 3* in 6.73–6.83% of all base pairs. The specimen from *M. sp. nova 3*

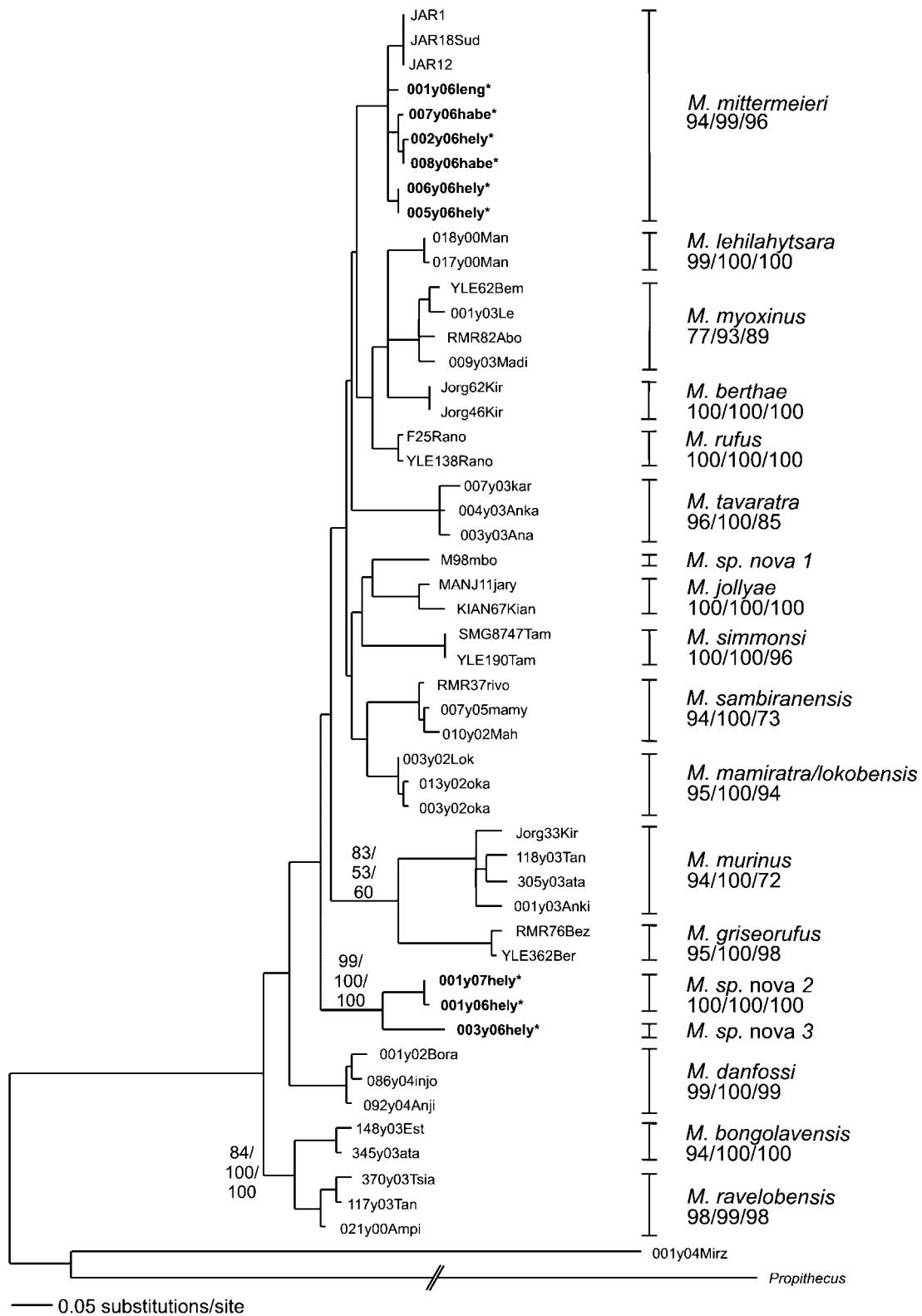


Fig. 2. Maximum likelihood phylogram based on the two gene alignment. New samples from this study are marked with a star. Bootstrap support for the terminal clades (species) is provided below the species name for all three employed methods (order: maximum likelihood/maximum parsimony/neighbor joining).

TABLE II. Minimum (Min) and Maximum (Max) Pairwise Genetic Distances (%) for the Two Gene Alignment (Above Diagonal) and the Three Gene Alignment (Diagonal and Below Diagonal) Among the Sampled Individuals and All Available *Microcebus* Species and the Outgroups

	<i>M. tavaratra</i>	<i>M. lehilahytsara</i>	<i>M. rufus</i>	<i>M. myoxinus</i>	<i>M. berthae</i>	<i>M. sambiranensis</i>	<i>M. simmonsii</i>	<i>M. mamiratra</i>	<i>M. danfossi</i>	<i>M. bongolavensis</i>	<i>M. ravelobensis</i>
<i>M. tavaratra</i>	Min 1.41	7.94	7.33	7.53	8.66	8.06	9.66	7.87	9.51	10.22	9.80
	Max 2.19	8.25	8.05	8.69	9.29	9.41	10.29	8.40	10.22	10.64	10.64
<i>M. lehilahytsara</i>	Min 7.44	0.24	4.55	5.77	5.88	7.04	8.07	5.96	9.09	10.65	9.81
	Max 8.15	0.24	5.26	6.51	5.99	7.45	8.28	6.17	9.29	11.17	10.74
<i>M. rufus</i>	Min 7.05	4.70	0.63	5.47	5.27	7.56	7.75	5.58	9.01	10.01	10.12
	Max 7.92	4.94	0.63	6.20	5.58	7.97	7.86	6.28	9.82	10.53	10.95
<i>M. myoxinus</i>	Min 7.44	5.33	4.94	1.75	5.36	7.86	7.87	5.84	9.52	11.27	10.53
	Max 8.48	5.88	5.64	2.93	6.09	8.60	8.70	7.14	10.54	11.88	11.86
<i>M. berthae</i>	Min 8.06	5.48	4.66	4.93	0.00	8.58	8.49	7.11	10.01	10.64	10.43
	Max 8.85	5.57	5.18	5.49	0.00	9.00	8.60	7.55	10.44	10.96	10.63
<i>M. sambiranensis</i>	Min 8.32	7.07	7.86	8.01	8.79	0.71	7.86	5.53	8.80	10.36	9.32
	Max 9.34	7.38	8.07	8.57	8.95	1.41	8.18	5.85	9.85	11.29	10.04
<i>M. simmonsii</i>	Min 9.20	7.93	7.69	7.77	8.16	7.92	0.08	7.66	9.17	10.22	10.32
	Max 9.76	8.09	7.84	8.40	8.24	8.09	0.08	8.08	9.80	10.44	10.96
<i>M. mamiratra</i>	Min 7.29	5.85	5.94	5.93	6.97	5.94	7.78	0.32	8.22	9.92	9.16
	Max 7.77	6.17	6.18	6.66	7.13	6.17	7.86	0.80	9.17	10.14	10.02
<i>M. danfossi</i>	Min 9.34	8.79	8.87	8.95	9.56	8.64	8.75	7.88	1.49	8.57	8.06
	Max 10.03	8.94	9.65	9.96	9.96	9.59	9.30	8.83	1.96	8.99	8.56
<i>M. bongolavensis</i>	Min 9.96	9.81	9.64	10.44	10.20	10.14	10.19	10.04	8.08	1.56	5.46
	Max 10.59	10.28	10.19	11.06	10.52	10.84	10.43	10.20	8.39	1.56	6.28
<i>M. ravelobensis</i>	Min 9.56	9.10	9.64	9.88	9.96	9.35	10.18	9.15	7.68	4.38	1.72
	Max 10.43	9.88	10.43	10.87	10.12	9.90	10.66	9.95	8.15	5.00	2.19
<i>M. murinus</i>	Min 10.65	10.11	11.04	10.64	10.97	10.03	10.73	9.62	10.81	11.75	11.28
	Max 11.74	10.89	11.66	11.71	11.20	10.75	11.67	10.65	11.52	12.85	11.97
<i>M. griseorufus</i>	Min 11.37	10.92	11.43	11.22	11.60	11.40	11.96	10.51	10.45	11.86	11.13
	Max 12.16	11.15	11.84	12.38	12.16	12.05	12.17	11.25	11.14	12.63	11.52
<i>M. mittermeieri</i>	Min n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Max n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
<i>M. jollyae</i>	Min n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Max n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
001y06leng	Min 8.16	6.04	6.04	6.90	7.37	7.70	7.84	6.57	8.73	10.44	10.28
	Max 8.24	6.11	6.28	7.06	7.38	7.77	7.92	6.89	9.19	11.60	10.44
007y06habe	Min 7.53	5.64	5.49	6.82	7.22	7.70	7.84	6.66	8.97	10.21	10.20
	Max 7.84	5.72	5.88	6.98	7.22	7.77	7.92	6.82	9.27	10.21	10.20
002y06hely	Min 7.53	5.80	5.33	6.66	6.90	7.46	7.52	6.66	8.89	9.97	9.73
	Max 7.84	5.88	5.57	6.82	6.91	7.70	7.60	6.82	9.35	10.27	9.89
008y06habe	Min 7.53	5.88	5.57	6.58	6.82	7.54	7.44	6.58	8.65	10.05	9.97
	Max 7.84	5.96	5.81	6.74	6.83	7.62	7.52	6.74	9.12	10.21	10.13
006y06hely	Min 8.00	5.57	5.88	6.59	7.14	7.85	6.82	6.65	8.73	10.29	9.89
	Max 8.31	5.64	5.96	6.82	7.14	7.93	8.00	6.82	9.04	10.44	10.36
005y06hely	Min 8.00	5.57	5.88	6.59	7.14	6.82	6.82	6.65	8.73	10.29	9.89
	Max 8.31	5.64	5.96	6.82	7.14	7.85	8.00	6.82	9.04	10.44	10.36
001y07hely	Min 9.58	8.71	8.79	9.27	9.26	9.57	9.57	9.31	9.12	10.28	9.33
	Max 10.28	8.71	9.02	9.65	9.27	10.30	10.51	9.55	9.36	10.28	10.28
001y06hely	Min 9.82	8.63	8.79	9.35	9.34	9.81	9.81	9.39	9.04	10.44	9.57
	Max 10.52	8.63	9.03	9.89	9.35	10.31	10.51	9.48	9.28	10.60	10.51
003y06hely	Min 9.73	8.94	9.10	9.66	9.73	9.05	9.05	8.93	9.75	10.22	9.66
	Max 10.05	8.95	9.42	10.17	9.74	9.57	11.00	9.17	10.05	10.46	10.21
<i>Mirza zaza</i>	Min 18.05	17.21	17.99	17.22	17.99	17.09	18.19	17.08	17.90	18.38	17.82
	Max 18.45	17.36	18.07	17.44	18.01	17.57	18.28	17.40	18.61	18.53	18.05
<i>Propithecus v.</i>	Min 23.52	24.09	24.03	24.17	24.39	23.23	23.35	23.53	24.85	24.37	24.38
	Max 24.48	24.25	24.27	24.65	24.40	23.54	23.43	23.77	25.06	24.74	24.76

Table II. Continued

	<i>M. murinus</i>	<i>M. griseorufus</i>	<i>M. mittermeieri</i>	<i>M. jollyae</i>	001	007	002	008	006	005	001	001	003	Mirza zaza	<i>Propithecus v.</i>
<i>M. tavaratra</i>	Min 10.00	10.94	8.17	9.30	8.47	7.74	7.85	7.75	8.37	8.37	9.52	9.93	10.14	18.62	24.38
	Max 11.14	11.58	8.49	9.35	8.47	8.06	8.16	8.06	8.68	8.68	10.55	10.87	10.45	19.24	25.74
<i>M. lehilahysara</i>	Min 10.11	10.46	5.59	7.28	5.68	5.26	5.58	5.58	5.16	5.16	8.99	8.89	9.51	17.91	25.65
	Max 10.74	10.77	5.79	7.96	5.78	5.37	5.68	5.68	5.26	5.26	8.99	8.89	9.51	18.12	25.87
<i>M. rufus</i>	Min 10.31	10.51	5.48	7.36	5.68	5.06	4.96	5.17	5.58	5.58	8.68	8.69	9.30	18.64	25.15
	Max 11.02	10.95	5.80	8.08	5.89	5.48	5.17	5.58	5.58	5.58	8.89	8.99	9.61	18.86	25.57
<i>M. myoxinus</i>	Min 9.90	10.23	6.53	7.75	6.82	6.22	6.71	6.50	6.50	6.50	9.72	9.84	10.24	17.72	25.55
	Max 11.21	11.76	7.04	8.89	7.02	7.23	7.12	6.92	7.02	7.02	10.23	10.55	10.93	18.12	26.20
<i>M. berthae</i>	Min 10.53	10.63	6.52	9.10	7.33	7.23	7.12	6.92	7.13	7.13	9.41	9.52	10.03	18.85	25.95
	Max 10.83	11.37	6.63	9.32	7.34	7.24	6.93	6.72	7.13	7.13	9.42	9.53	10.04	18.87	25.97
<i>M. sambiranensis</i>	Min 9.61	11.19	7.05	7.46	6.93	6.83	6.62	6.83	7.04	7.04	10.05	10.07	9.34	17.14	24.31
	Max 10.44	11.84	7.57	7.99	7.14	7.25	7.04	7.04	7.45	7.45	10.05	10.28	9.55	17.87	24.62
<i>M. simmonsii</i>	Min 9.99	11.70	7.56	7.25	7.43	7.54	7.01	7.01	7.75	7.75	10.54	10.56	11.31	18.61	24.72
	Max 11.46	11.83	7.77	8.46	7.54	7.65	7.12	7.12	7.86	7.86	10.54	10.56	11.42	18.72	24.83
<i>M. mamiratra</i>	Min 9.25	10.43	6.61	6.33	6.27	6.49	6.38	6.38	6.49	6.49	9.49	9.60	8.98	17.32	24.96
	Max 10.52	10.99	6.72	7.08	6.70	6.71	6.60	6.60	6.71	6.71	9.81	9.71	9.31	17.65	25.28
<i>M. danfossi</i>	Min 10.34	10.46	9.24	7.87	8.81	9.02	8.82	8.61	8.92	8.92	9.53	9.43	10.58	18.82	26.55
	Max 11.67	11.26	9.86	10.09	9.53	9.53	9.53	9.53	9.43	9.43	9.94	9.84	10.98	19.67	26.93
<i>M. bongolavensis</i>	Min 12.07	11.80	10.05	9.53	10.76	10.55	10.45	10.45	10.66	10.66	11.69	11.90	11.81	19.24	26.51
	Max 13.20	12.91	10.67	11.72	11.07	10.66	10.76	10.55	10.97	10.97	11.79	12.21	12.02	19.34	27.23
<i>M. ravelobensis</i>	Min 11.34	11.06	10.56	10.24	10.75	10.24	10.44	10.44	10.34	10.34	10.64	10.96	10.87	18.71	26.03
	Max 12.25	11.77	11.08	11.16	11.17	10.96	10.65	10.86	11.17	11.17	11.47	11.79	11.49	18.72	26.33
<i>M. murinus</i>	Min 2.65	9.26	10.34	10.56	10.22	10.32	9.91	9.70	10.12	10.12	11.79	11.90	12.10	18.49	23.78
	Max 3.98	10.91	10.86	11.72	10.74	11.25	10.83	10.63	10.84	10.84	12.60	13.11	12.92	19.31	25.04
<i>M. griseorufus</i>	Min 8.75	1.33	10.75	11.29	11.05	10.87	10.73	10.73	10.74	10.74	11.89	12.00	11.68	19.57	25.09
	Max 10.09	1.33	10.88	12.57	11.18	10.94	10.87	10.87	11.08	11.08	12.24	12.55	12.12	19.80	25.70
<i>M. mittermeieri</i>	Min n.a.	n.a.	n.a.	6.94	2.27	2.58	2.58	2.69	1.86	1.86	2.58	2.58	18.69	18.69	26.03
	Max n.a.	n.a.	n.a.	7.98	2.79	2.38	2.69	2.69	1.96	1.96	9.95	9.96	10.16	18.79	26.13
<i>M. jollyae</i>	Min n.a.	n.a.	n.a.	n.a.	7.05	7.15	7.15	7.15	7.25	7.25	9.74	10.06	10.16	18.10	25.82
	Max n.a.	n.a.	n.a.	n.a.	7.57	7.78	7.89	7.89	7.78	7.78	10.48	10.48	10.28	18.90	27
001y06leng	Min 10.97	11.21	n.a.	n.a.	n.a.	2.27	2.78	2.58	1.86	1.86	9.62	9.42	9.41	18.35	25.78
	Max 11.36	11.31	n.a.	n.a.	n.a.	n.a.	1.34	1.13	1.65	1.65	10.03	10.04	9.31	18.25	25.36
007y06habe	Min 11.13	11.15	n.a.	n.a.	n.a.	1.10	n.a.	0.21	2.17	2.17	9.41	9.43	9.10	18.04	25.05
	Max 11.83	11.21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
002y06hely	Min 10.89	11.13	n.a.	n.a.	n.a.	n.a.	0.24	1.96	1.96	1.96	9.31	9.32	9.00	17.94	24.94
	Max 11.44	11.23	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
008y06habe	Min 10.65	11.05	n.a.	n.a.	n.a.	0.86	1.88	1.64	1.96	1.96	9.31	9.31	9.11	18.56	25.67
	Max 11.36	11.15	n.a.	n.a.	n.a.	1.41	1.88	1.64	0.00	0.00	9.31	9.11	9.72	18.56	25.67
006y06hely	Min 10.97	10.90	n.a.	n.a.	n.a.	1.41	1.88	1.64	0.00	0.00	9.31	9.11	9.72	18.56	25.67
	Max 11.52	11.15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
005y06hely	Min 10.97	10.90	n.a.	n.a.	n.a.	1.41	1.88	1.64	0.00	0.00	9.31	9.11	9.72	18.56	25.67
	Max 11.52	11.15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
001y07hely	Min 11.85	11.93	n.a.	n.a.	n.a.	10.21	9.66	9.66	9.50	9.50	1.03	1.03	6.73	19.29	26.74
	Max 12.31	12.03	n.a.	n.a.	n.a.	n.a.	9.66	9.66	9.35	9.35	0.78	0.78	6.83	19.71	26.85
001y06hely	Min 11.93	12.01	n.a.	n.a.	n.a.	10.21	9.66	9.66	9.50	9.50	1.03	1.03	6.83	19.71	26.85
	Max 12.70	12.27	n.a.	n.a.	n.a.	n.a.	9.66	9.66	9.35	9.35	0.78	0.78	6.83	19.71	26.85
003y06hely	Min 12.01	11.77	n.a.	n.a.	n.a.	9.19	9.11	8.95	9.50	9.50	6.52	6.60	19.04	20.13	26.64
	Max 12.71	12.10	n.a.	n.a.	n.a.	n.a.	9.11	8.95	9.50	9.50	6.52	6.60	19.04	20.13	26.64
<i>Mirza zaza</i>	Min 17.95	18.54	n.a.	n.a.	n.a.	18.07	17.77	17.77	18.09	18.09	18.25	18.56	19.04	24.18	25.37
	Max 18.66	18.72	n.a.	n.a.	n.a.	n.a.	17.77	17.77	18.09	18.09	18.25	18.56	19.04	24.18	25.37
<i>Propithecus v.</i>	Min 23.31	24.22	n.a.	n.a.	n.a.	24.82	24.51	24.51	24.98	24.98	24.75	24.83	24.83	24.83	25.37
	Max 23.78	24.68	n.a.	n.a.	n.a.	n.a.	24.51	24.51	24.98	24.98	24.75	24.83	24.83	24.83	25.37

differed from *M. mittermeieri* in 9.0–9.72% of all base pairs. These two previously unknown genetic clades were more distinct from any previously described species (>8.68% sequence divergence) than 46 of 105 pairwise species comparisons in the two gene analysis (Table II, upper half matrix). Furthermore, they were more distinct from each other than 17 of 105 pairwise species comparisons. However, the deep nodes and thereby the general topologies of the two gene trees were not very well resolved in any of the phylogenetic tree methods.

The three gene alignment was used to improve the resolution of the tree topologies. All species formed distinct terminal clades in the trees (4.7–12.85% sequence divergence, Table II, lower matrix). All three phylogenetic methods supported the monophyly of *M. murinus* and *M. griseorufus* (bootstrap values: ML: 91, MP: 81, MJ: 86) as well as the monophyly of the three northwestern reddish mouse lemur species *M. danfossi*, *M. bongolavensis* and *M. ravelobensis* (bootstrap values: ML: 66, MP: 71, MJ: 98). Furthermore, all analyses revealed the phylogenetic proximity between the four species *M. lehilahytsara*, *M. rufus*, *M. myoxinus* and *M. berthae*, although the specific branching pattern was identical in only two out of three methods. *M. mittermeieri* was the sister taxon of these four species in all analyses. The remaining branching events and in particular the deeper nodes in the trees were not highly resolved. The deeply rooted separation of the three divergent specimens from the Makira region from all previously described mouse lemur species, however, was again well supported. They formed two terminal branches within one monophyletic clade (all bootstrap values at 100), and were clearly distinct from all other species (8.63–12.71%, Table II). In particular, they were more distinct from *M. mittermeieri* (8.95–10.21%) than 21 of 78 available species pairs (Table II). Furthermore, they were more distinct from each other (6.6–6.52%) than 10 of 78 available species pairs.

The analysis of diagnostic sites of the final concatenated sequences revealed that all species possessed unique molecular traits that varied from 0 to 8 bp in the COII locus, from 0 to 4 bp in the *cyt b* locus and from 0 to 8 bp in the d-loop (for details see supplementary electronic material). Taken together, species had between 2 (*M. lehilahytsara*, *M. myoxinus*, *M. bongolavensis*) and 16 (*M. griseorufus*) unique and therefore diagnostic sites. *M. sp. nova 2* and *M. sp. nova 3* had 9 and 11 diagnostic sites, respectively, which is clearly above the mean (mean = 7.31) of all species.

Morphometric Comparisons

Morphometric measurements were taken from 22 (14 males, 8 females) *M. mittermeieri*, 3 individuals (1 male, 2 females) of *M. sp. nova 2* and 1 individual

male of *M. sp. nova 3* in this study. These measurements were listed in comparison with published data from all other described mouse lemur species (Table III). The *M. mittermeieri* from this study ($n = 22$) and the 3 individuals from *M. sp. nova 2* differed statistically in 7 out of 13 variables. Whereas the variable *ear width* was smaller in *M. sp. nova 2* than in *M. mittermeieri*, the contrary was true for the variables *head length*, *lower leg length*, *hind foot length*, *tail length*, *body length* and *body mass* (for a comparison of the variables *head length*, *tail length* and *body mass* among the eastern species, see Fig. 3). These differences, however, were no longer significant after Bonferroni correction, which was probably owing to the small sample size of *M. sp. nova 2*. The overall qualitative cross-species comparisons revealed that *M. sp. nova 2* belongs to the group of larger-bodied mouse lemur species (>50 g body mass) that also includes *M. simmonsii*, *M. mairatra/lokobensis*, *M. tavaratra*, *M. danfossi*, *M. bongolavensis*, *M. ravelobensis*, *M. jollyae*, *M. murinus* and *M. griseorufus* (Table III, for a comparison of all eastern species, see Fig. 3). It also has a long tail as do most of the larger species excluding *M. jollyae* and *M. murinus*. In contrast, *M. mittermeieri* belongs to the group of smaller-bodied species (<50 g body mass) that includes *M. sambiranensis*, *M. myoxinus*, *M. berthae*, *M. lehilahytsara* and *M. rufus*. Its morphometry seems to be most similar to the latter two (for a comparison of the eastern species, see Fig. 3). *M. sp. nova 3* is represented by one individual only and interspecific comparisons therefore remain preliminary. This particular animal, however, was also large and had a long tail (Table III, Fig. 3). Its body mass was even larger than the average of all published species.

DISCUSSION

The results of this study support the presence of three mouse lemur species in the Makira region, two of which were previously undescribed. The vast majority of captured individuals ($n = 22$) clustered with *M. mittermeieri* from the Anjanaharibe-South Special Reserve and can therefore be assigned to this species. However, two further genetic lineages were identified, consisting of three individuals and one individual, respectively. These lineages were genetically distinct not only from *M. mittermeieri* but also from all other known species. Further, they were also genetically distinct from each other, although they jointly formed a monophyletic clade in all phylogenetic trees. The genetic distance among these two new groups and previously described species was well within the range of other species pairs. Furthermore, they possessed a relatively large number of diagnostic sites each. It should therefore be concluded that these four individuals did not belong to a previously described species but to two new species, one of which will be subsequently

TABLE III. Morphometric Characteristics of the Sampled Individuals and, for Comparison, All Mouse Lemur Species Described in the Literature (Mean ± SD, Alternatively SE in Two Species)

Variables (in mm)	<i>M. mittermeieri</i> from Anjanaharibe-Sud (<i>n</i> = 5) ^a	<i>M. mittermeieri</i> (<i>n</i> = 22), this study	<i>M. sp. nova 2</i> (<i>n</i> = 2-3), this study	<i>M. sp. nova 3</i> (<i>n</i> = 1), this study	<i>M. simmonsii</i> (<i>n</i> = 6) ^c	<i>M. tacaratra</i> (<i>n</i> = 31-33) ^b	<i>M. mamiratra/lokobensis</i> (<i>n</i> = 17) ^b	<i>M. sambiranensis</i> (<i>n</i> = 28) ^b	<i>M. danfossi</i> (<i>n</i> = 72-76) ^b
Ear length	n.a.	18.26 ± 1.39	19.00 ± 0.44	18.00	n.a.	21.58 ± 1.45	19.81 ± 1.07	17.40 ± 2.20	22.56 ± 1.53
Ear width	n.a.	12.09 ± 0.83	10.50 ± 0.52*	12.00	n.a.	13.59 ± 0.90	12.10 ± 0.64	11.38 ± 0.92	13.89 ± 1.04
Head length	33 ± 0.0	34.27 ± 0.82	36.07 ± 0.84*	37.50	36 ± 1	35.05 ± 1.74	35.25 ± 2.04	33.28 ± 1.09	37.27 ± 1.38
Head width	n.a.	19.59 ± 1.38	19.27 ± 0.75	19.80	n.a.	20.72 ± 1.18	20.25 ± 1.01	19.86 ± 1.90	22.09 ± 1.48
Snout length	n.a.	7.80 ± 0.87	8.27 ± 0.15	8.00	n.a.	7.45 ± 0.59	7.55 ± 0.85	7.78 ± 1.54	9.48 ± 1.11
Interorbital distance	n.a.	20.58 ± 1.07	21.57 ± 0.45	25.30	n.a.	20.79 ± 1.29	21.06 ± 1.14	20.17 ± 1.24	21.55 ± 1.20
Intraorbital distance	n.a.	6.82 ± 1.29	6.50 ± 0.36	6.80	n.a.	5.83 ± 0.67	6.20 ± 0.64	6.29 ± 0.85	7.25 ± 0.64
Lower leg length	n.a.	35.36 ± 1.49	38.87 ± 0.76*	40.20	n.a.	38.98 ± 1.60	36.14 ± 1.60	35.13 ± 1.51	41.72 ± 2.48
Hind foot length	n.a.	20.17 ± 1.30	22.13 ± 1.18*	21.60	n.a.	22.45 ± 0.93	21.38 ± 1.06	20.60 ± 0.86	25.45 ± 1.05
Third toe length	n.a.	7.90 ± 0.49	8.37 ± 0.38	8.20	n.a.	9.18 ± 0.77	8.24 ± 0.58	8.42 ± 1.39	9.44 ± 0.96
Tail length	113 ± 2	121.55 ± 8.34	146.50 ± 6.36*	142.00	142 ± 1.0	158.33 ± 6.56	155.29 ± 9.02	138.00 ± 5.71	160.97 ± 8.77
Body length	87 ± 2	71.77 ± 6.13	80.67 ± 5.13*	85.00	92 ± 1.0	79.55 ± 7.01	78.53 ± 6.32	43.20 ± 34.44	81.69 ± 23.17
Body mass (g)	44.1 ± 7.4	45.09 ± 6.23	53.67 ± 4.04*	68.00	64.7 ± 17.5	51.73 ± 8.38	57.76 ± 15.32	40.43 ± 6.98	62.80 ± 12.23
Ear length	<i>M. bongolavensis</i> (<i>n</i> = 37-38) ^b	21.90 ± 1.83	18.82 ± 2.39	17.9 ± 0.14	<i>M. rufus</i> (<i>n</i> = 15) ^a	16.9 ± 0.67	n.a.	<i>M. murinus</i> (<i>n</i> = 89) ^b	<i>M. griseorufus</i> (<i>n</i> = 6) ^c
Ear width	13.04 ± 1.12	13.49 ± 1.35	11.83 ± 0.85	16.9 ± 0.3	n.a.	10.9 ± 0.26	n.a.	21.66 ± 1.99	23.7 ± 0.81
Head length	36.38 ± 1.09	35.76 ± 1.72	35.00 ± 1.35	13.3 ± 0.2	n.a.	33.5 ± 0.4	36 ± 1	14.00 ± 1.33	n.a.
Head width	21.29 ± 1.07	21.14 ± 1.45	20.48 ± 1.28	31.0 ± 0.23	33 ± 1	19.7 ± 0.5	n.a.	34.63 ± 1.59	n.a.
Snout length	9.33 ± 0.98	8.13 ± 1.70	8.00 ± 1.38	17.9 ± 0.14	n.a.	n.a.	n.a.	21.13 ± 1.28	n.a.
Interorbital distance	20.34 ± 1.14	19.95 ± 1.22	20.84 ± 1.12	n.a.	n.a.	n.a.	n.a.	8.12 ± 0.97	n.a.
Intraorbital distance	7.75 ± 0.49	6.64 ± 1.13	6.97 ± 0.91	n.a.	n.a.	n.a.	n.a.	20.58 ± 0.98	n.a.
Lower leg length	41.43 ± 1.72	39.94 ± 2.15	37.27 ± 1.28	n.a.	n.a.	n.a.	n.a.	7.33 ± 0.73	n.a.
Hind foot length	25.45 ± 1.09	23.54 ± 1.23	21.70 ± 1.38	29.6 ± 0.27	n.a.	20.0 ± 0.39	n.a.	38.47 ± 2.37	n.a.
Third toe length	8.88 ± 0.51	8.41 ± 1.14	8.50 ± 0.86	n.a.	n.a.	9.5 ± 0.54	n.a.	22.48 ± 1.14	32.5 ± 0.82
Tail length	157.11 ± 8.54	155.48 ± 7.57	141.50 ± 8.27	133.4 ± 1.27	117 ± 8	117.9 ± 2.63	122 ± 1	8.42 ± 0.68	9.0 ± 0.0
Body length	86.95 ± 7.10	84.02 ± 8.52	78.08 ± 7.04	n.a.	86 ± 3	72.7 ± 1.79	93 ± 3	130.81 ± 6.15	142.8 ± 5.85
Body mass (g)	53.76 ± 8.86	51.82 ± 11.14	45.23 ± 7.02	30.2 ± 0.86	43.7 ± 4.2	46.2 ± 2.19	61.3 ± 4.5	53.16 ± 6.87	62.6 ± 16.36

n.a.: values not available.
^aFrom Louis et al. [2006b].
^bFrom Olivieri et al. [2007].
^cFrom Rasoloarison et al. [2000].
^dFrom Atsals et al. [1996].
^eFrom Zimmermann et al. [1998].
 *Significant difference ($P < 0.05$) to *M. mittermeieri* (this sample).

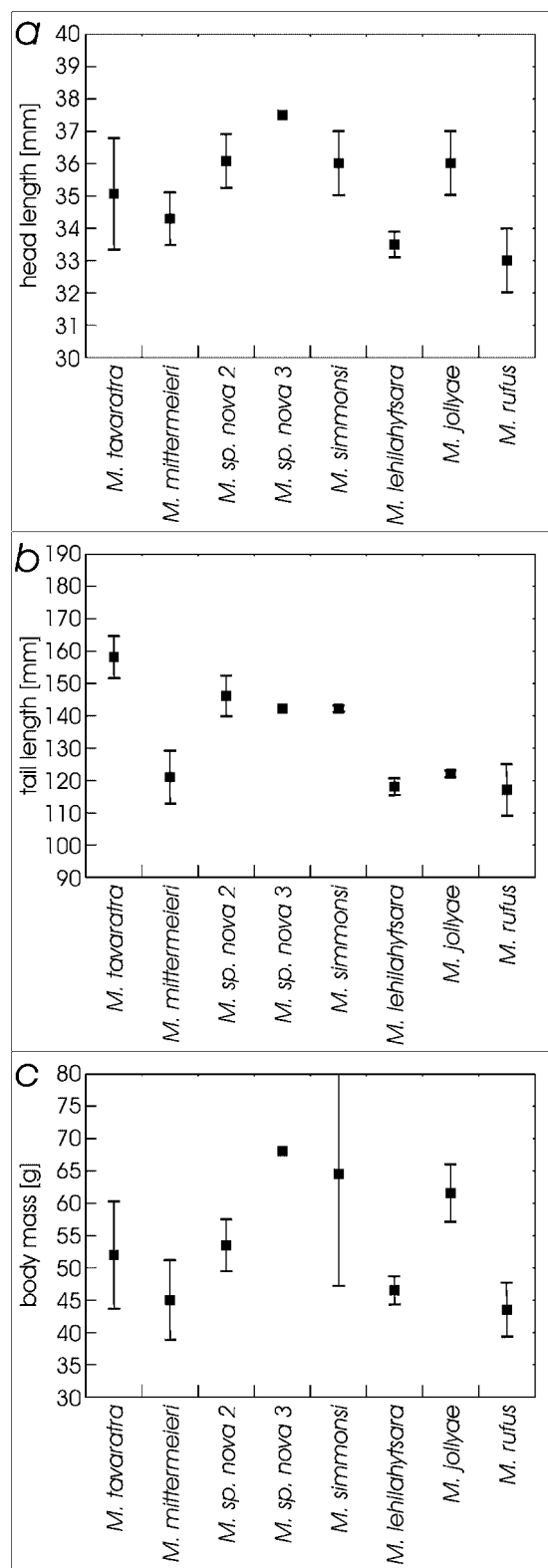


Fig. 3. Comparison of morphometric measurements of all described eastern mouse lemur species in geographic order. Northernmost species: to the left, southernmost species: to the right, (a) head length, (b) tail length and (c) body mass.

described (*M. sp. nova 2* = *M. macarthurii*). The other one is merely proposed (*M. sp. nova 3*) owing to the small sample size ($n = 1$) and lack of photographic material.

Genetic differences were also reflected in morphometric differences, although thorough statistical testing was not possible owing to small sample sizes. The available data, however, indicate that both new species belong to the group of larger-bodied mouse lemur species. Further, they were quite distinct from the smaller-sized and lighter *M. mittermeieri*, their sympatric congener. Interestingly, *M. mittermeieri* is phylogenetically close to four other small-sized mouse lemur species, *M. myoxinus*, *M. berthae*, *M. lehilahytsara* and *M. rufus*. It is therefore likely that small body size is a homologous trait for this closely related group of sister species [Louis et al., 2006b].

It is notable that *M. mittermeieri* and the two new species all occurred within the same IRS, between the Antainambalana River and the Rantabe River further to the south. This is the first report of three mouse lemur species living in the same area of Madagascar. All three species were captured less than 1 km from each other in the vicinity of the village Anjahely, although a maximum of only two species were captured in exactly the same location. These were *M. mittermeieri* together with *M. sp. nova 2* in one location and *M. sp. nova 2* together with *M. sp. nova 3* in another location. A lemur inventory has recently been completed in Anantaka, 5 km away from Anjahely [Rasolofoson et al., 2007a,b]. This inventory has revealed the presence of 14 lemur species in this forest area (including two forms of *Microcebus* sp.). Among these were three diurnal species (*Indri indri*, *Varecia variegata subcincta*, *Haplemur griseus griseus*), one cathemeral species (*Eulemur fulvus albifrons*) and ten nocturnal species (*Avahi laniger*, *Lepilemur seali*, *Cheirogaleus major*, *C. rufus*, *C. sibreei*, two species of *Microcebus*, *Allocebus trichotis*, *Phaner furcifer* and *Daubentonia madagascariensis*). If the forests of Anjahely were similarly rich and even contained three instead of two mouse lemur species, they would have the highest lemur species richness ($n = 15$) of any forest habitat in Madagascar so far reported [Ganzhorn et al., 1999]. Presently, it is unknown which ecological factors may explain this exceptional lemur species richness of the Makira region. Therefore, the Makira region warrants and needs intensified and urgent research and conservation efforts, as anthropogenic pressures in the form of hunting, deforestation, slash-and-burn cultivation and mining activities are prevalent in many areas [Rasolofoson et al., 2007a]. Unfortunately, no effective protection level for this unique region has been established yet.

Finally, we can conclude from this study that the Antainambalana River does not act as a strict species barrier for *Microcebus* sp. Although we cannot be

sure whether the two new species occur north of this river, *M. mittermeieri* is now confirmed to occur south of it. Similar findings were recently described for the genus *Lepilemur* [Craul et al., 2008]. *L. seali* has also been confirmed to occur on both sides of the river. The presence of *L. seali* on both sides was suggested to be the result of a broad altitudinal range of this species, enabling animals to circumnavigate the headwaters of this large river and to maintain gene flow at least until recent times [Craul et al., 2008; see also Goodman & Ganzhorn, 2004]. The same could in principle apply to *Microcebus* sp. More research, however, is needed to establish the altitudinal range of the three mouse lemur species of this region and to understand their ecological plasticity in varying habitats. Moreover, the southern distribution limits and thereby the

biogeography of all three species still need to be established by sampling the IRSs south of the Rantabe River.

SPECIES DESCRIPTION

M. macarthurii, sp. nov. (Fig. 4, left)

Type

001y07hely, adult female captured on 4 September 2007, by D. R., B. R., S. R. and O. R. in Anjiahely (S15°24'22.5", E49°29'54.3"), in a *savoka* at about 380 m a.s.l., close to the village Anjiahely, about 26 km west of Maroantsetra, Province of Antsirana, Madagascar. Tissue and hair samples as well as pictures of the animal are stored at the Institute of Zoology of the University of Veterinary Medicine Hannover in Germany.



Fig. 4. Photo of *Microcebus macarthurii* on the left and *M. mittermeieri* on the right. Ventral fur color differences and size differences are prominent in this picture. For a color representation of this figure, the reader is referred to the web version of this article.

Measurements of type

Morphometric measurements (all lengths measured in mm): *ear length*: 19.5; *ear width*: 11.1; *head length*: 35.1; *head width*: 19.7; *snout length*: 8.3; *interorbital distance*: 21.6; *intraorbital distance*: 6.4; *lower leg length*: 38.7; *hind foot length*: 23.5; *third toe length*: 8.2; *body length*: 85.0; *body mass*: 53 g.

Paratypes

- 001y06hely, adult male captured in Anjiahely on 11 November 2006. Tissue and hair samples as well as morphometric measurements are stored at the Institute of Zoology of the University of Veterinary Medicine Hannover in Germany.
- 004y06hely, adult female captured in Anjiahely on 9 November 2006. Tissue and hair samples as well as morphometric measurements are stored at the Institute of Zoology of the University of Veterinary Medicine Hannover in Germany.

Diagnosis

M. macarthurii can be distinguished from its sympatric congener *M. mittermeieri* by morphometric and genetic differences, although the morphometric differences are still to be tested with a larger sample size. *M. macarthurii* appears to be generally larger than *M. mittermeieri*. It is heavier and has a significantly longer head, lower leg, hind foot, tail and body length than *M. mittermeieri*. It differs genetically from *M. mittermeieri* in 9.35–10.21% of the three mtDNA genes analyzed in this study and from its southern neighbor, *M. simmonsii*, in 9.57–10.51% of the genes. *M. macarthurii* possesses nine unique diagnostic sites over all three loci (4 bp in COII, 2 bp in *cyt b*, 3 bp in d-loop, for details see supplementary electronic material) and is therefore genetically distinct from all other mouse lemur species.

Description

M. macarthurii is a larger-bodied reddish-orange mouse lemur. The fur is dense and short. The head is rufous colored, which turns orange on the cheeks. It is dark brownish around the eyes but has a distinct white stripe between the eyes. The ears are darker rufous. The dorsum is reddish-brown from head to tail and has a broad darker rufous line along the midline. A lighter reddish color extends toward the outer upper legs and arms. The tail is densely furred and reddish-brown in coloration, darker on the dorsal than the ventral side and middle brown toward its tip. The ventrum is yellowish-orange with a creamy-white coloration on the ventral throat and the genital region. Hands and feet are sparsely haired and these hairs are whitish-gray. The skin on the palmar and plantar surfaces of hands and feet is pink to slightly brownish.

Notes

The distribution of *M. macarthurii* is so far limited to the collection site Anjiahely where it has been captured in two different locations about 850 m apart. This locality is not yet part of any protected zone. Considering the presumably small distribution of this species and the present rates of deforestation in Madagascar, it is of utmost importance to create protected areas in the Makira region.

Etymology

M. macarthurii is named after the MacArthur Foundation that generously financed this study and the inventory program in the Makira region. By naming this species after the foundation, we also acknowledge the importance of this foundation for the work of Groupe d'Étude et de Recherche sur les Primates de Madagascar. Its support has helped not only to detect the unexpected species richness present in the Makira region but also to stimulate and support young Malagasy researchers and to strengthen Malagasy biodiversity research in general.

Vernacular name

English name: MacArthur's mouse lemur, French name: microcèbe de MacArthur, German name: MacArthur's Mausmaki.

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