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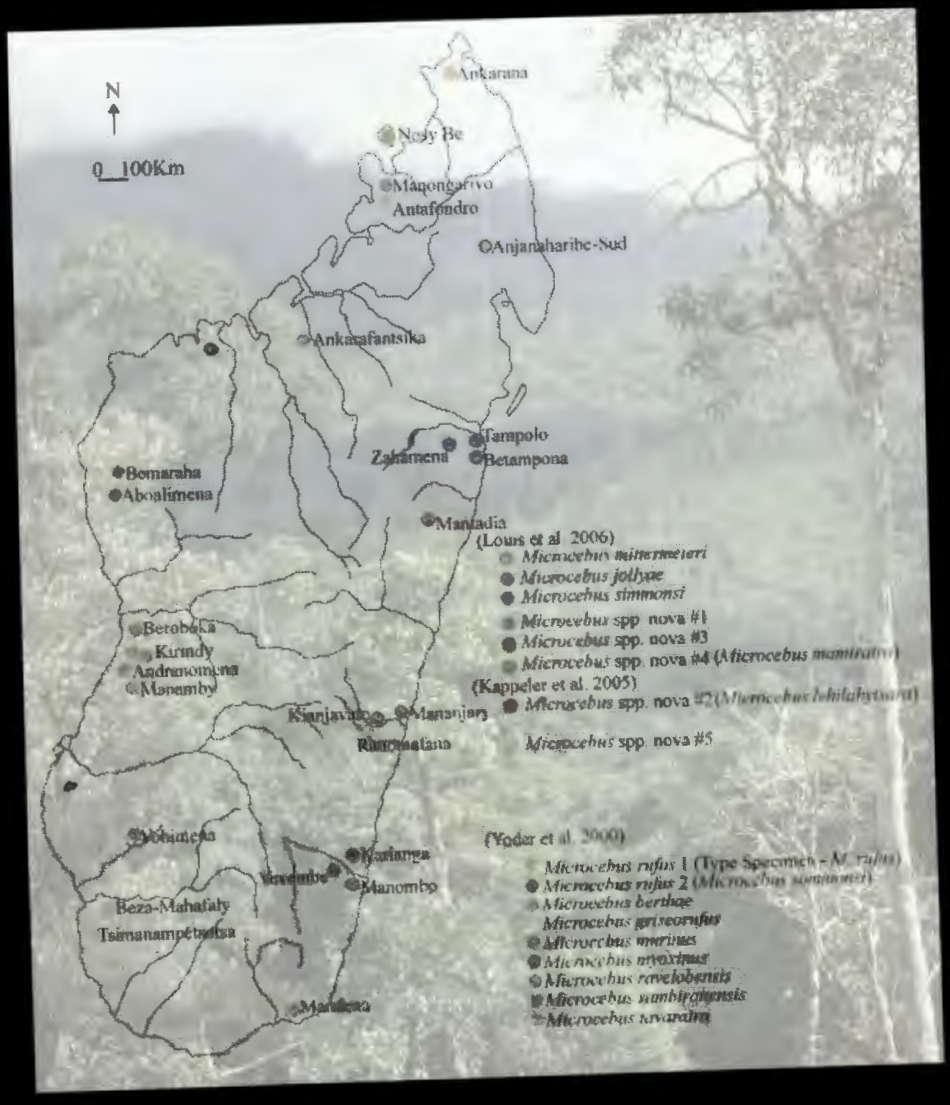

Mouse Lemurs of Northwestern Madagascar with a Description of a New Species at Lokobe Special Reserve

Editorial Comment. Occasional Paper #259 by Rambinintsoa Andriantompohavana and his colleagues reviews the systematics and biodiversity of the mouse lemurs *Microcebus* of northwestern Madagascar. The fauna of Madagascar has always fascinated biologists. Madagascar is one of those places that mammalogists dream of visiting to see aye-ayes, tenrecs, sucker-footed bats, flying foxes, etc. So when I received the program for the Texas Genetics Society meeting in Galveston (April 2006), I was excited to see that Dr. Ed Louis, Jr. was presenting a paper on the lemurs of Madagascar. Dr. Louis's presentation included a DNA sequence data set in which he described substantially more diversity than had been recognized previously using morphology. Robert Bradley and I were finishing a paper on the Genetic Species Concept in mammals, and he and I had spent considerable effort defining what is a "species" using genetic data (Baker and Bradley, 2006, Journal of Mammalogy 87:643-662). The data set that Dr. Louis presented seemed to be a fascinating opportunity to think about how many species of lemurs were currently unrecognized and how those data could be used to transform biodiversity in genetics into a Linnaean format. After his talk, I approached Dr. Louis and asked him what was the status of describing these previously unrecognized species, and I encouraged him to explore the Occasional Papers of The Museum of Texas Tech University as a possible source of publication of description of new taxa. This paper, OP #259, is our first result of that discussion. Efforts by Andriantompohavana et al. and our editorial board have been to define the species from genetic data and morphological observations of live specimens, and to determine a means by which the available data can be archived in museums at a level acceptable to the classical systematic community. From a museum perspective, there needs to be an intensive dialogue defining procedures and standards to be employed to use genetic databases to describe biodiversity within the framework of the International Code of Zoological Nomenclature. To accomplish this goal, it will be necessary to embrace descriptions defined by base pair and amino acid order as diagnoses, tissue samples with or without classical museum specimens as holotypes, e-vouchers, and a rethinking of the role of museums to accommodate complex data sets for which there are limited classical museum specimens. Papers discussing the issues associated with this subject include Jones et al. (Science 308:1161-1164, 20 May 2005), and letters to the editor regarding that paper (Timm et al., Landry, and Polaszek et al., Science 309:2163-2166, 30 September 2005). We interpret the description of the mouse lemur (*Microcebus*) in OP #259 as meeting the requirements of the Code of Zoological Nomenclature because there is a designated holotype. Holotype tissues are catalogued and curated in Madagascar at the Parc Bontanique et Zoologique de Tsimbazaza. Further, there are photographic images of the holotype and paratypes archived in museums (PBZT and TTU) (e-vouchers as defined in Monk and Baker, 2001, Museology 10:1-8).

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Front cover: Current distribution map of the Genus *Microcebus* confirmed from molecular data analysis.

Mouse Lemurs of Northwestern Madagascar with a Description of a New Species at Lokobe Special Reserve

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Abstract

Implementing a phylogenetic analysis of mitochondrial DNA (mtDNA) sequence data (ca. 3000 bp), the molecular variation of northwestern mouse lemurs (*Microcebus* species) was compared, providing support for the formal description of a new species from Nosy Be, Madagascar. Additionally, there is initial support for a proposed new species from Antafondro Classified Forest. Phylogenetic inference and tree topology of the variation observed in mitochondrial DNA sequence data was generated from 42 individuals. This represents the 12 currently recognized species of *Microcebus* and clearly distinguishes the current taxonomy as well as a newly described species and a newly proposed species. The localities of the newly identified species are from within the original distribution of *Microcebus sambiranensis*. A formal description established from molecular genetic and morphologic variation is presented for the newly named species from Nosy Be Island.

Key words: D-loop, Madagascar, *Microcebus*, mouse lemur, prosimian, systematics

Introduction

Due to its unique species biodiversity and to the continued pressure from human encroachment, Madagascar has been placed at the top of conservation priority lists, or hotspots (Myers 2000). Distributed throughout the island, lemurs are particularly susceptible to extinction risks such as stochastic and deterministic factors due to their relatively small fragmented geographic ranges (Jernvall and Wright 1998). The taxonomic revision of species and distributions warrants the need to consistently re-evaluate the conservation protection status of lemurs as new information becomes available (Louis et al. 2006). For instance, recent molecular genetic and morphological studies within the genera *Avahi*, *Cheirogaleus, Lepilemur,* and *Microcebus* have led to a significant increase in the number of recognized species (Andriaholinirina et al. 2006; Groves 2000; Kappeler et al. 2005; Louis et al. 2006; Rasoloarison et al. 2000; Thalmann and Geissmann 2005; Zimmermann et al. 1998). Currently, all lemurs are protected under the Convention of International Trade of Endangered Species (CITES) and are designated by the IUCN/SSC Red List Categories from critically endangered to threatened depending on the species (IUCN/SSC 1999). Thus, it is critical that we accurately define species and subspecies to better evaluate conservation risks and make appropriate recommendations for the management of wild populations, especially when considering the ranges of newly recognized species of lemurs in light of previously recognized taxa.

Until recently, the northwestern mouse lemurs have been represented by the Northern Mouse Lemur, *Microcebus tavaratra*, found at Ankarana National Park, and the Sambirano Mouse Lemur, *Microcebus sambiranensis*, found at Manongarivo Special Reserve (Rasoloarison et al. 2000). Based on phylogenetic inference of mitochondrial DNA sequence data, Louis et al. (2006) presented evidence for three new *Microcebus* species for eastern Madagascar, along with proposing a new *Microcebus* species (*Microcebus* species nova #4) for Nosy Be Island and Lokobe Special Reserve. In this paper, the authors present a comparative phylo-

genetic analysis of the northwestern mouse lemurs and molecular support for the proposed *Microcebus* species #4 (Louis et al. 2006), and accordingly, a formal description for this new species from Nosy Be Island.

The endangered status of lemurs, all of which have been designated CITES appendix I, along with the digital and molecular technological capabilities of the twenty-first century, has been shown to justify the designation of new primate species with the use of only morphologic data, detailed photographs, and tissue samples (Thalmann and Geissmann 2005). Although adopting this methodology does not preclude the utility of a whole voucher, whole vouchers from the wild can later supplement the process as they become available opportunistically (e.g., raptor nests or remains of Fossa [*Cryptoprocta ferox*] predation). Therefore, the individual designated as the holotype for this newly described *Microcebus* species is a captive "live voucher" curated at Parc Botanique et Zoologique de Tsimbazaza. Holotype tissues are catalogued and archived as PBZT130 at the Museum of Parc Botanique et Zoologique de Tsimbazaza. After this specimen dies, it will be prepared as a voucher specimen and will be available upon formal request through this institute's museum. Total genomic DNA for three paratype specimens are currently curated at The Museum of Texas Tech University (TK125580/TTU-M104431; TK125581/TTU-M104432; TK125582/TTU-M104433). Additionally, an electronic database that includes all *Microcebus* field data and photographs, including data for the holotype specimen, is curated at The Museum of Texas Tech University under TTU-M104430. This database is stored in the Type Specimen Collection in multiple media formats. This collection of field data and photographs, as well as additional tables and figures, also are available online at the website of Omaha's Henry Doorly Zoo. See Appendix I for a directory of appropriate website addresses.

METHODS

Sampling.—All lemurs in this molecular study were free-ranging, wild-caught, adults (Table 1; Figure 1; Louis et al. 2006). The mouse lemurs were live-trapped or hand-caught and subsequently immobilized using 2.0-3.0 mg of Telazol (Fort Dodge Laboratories, Inc.; Ft. Dodge, Iowa), and two 2.0 mm biopsies were collected and stored in room temperature tissue preservative (Longmire et al. 1992). The lemurs designated as outgroups were immobilized with a CO_2 projection rifle or blowgun with 10mg/kg of Telazol (Fort Dodge Laboratories Inc.; Ft. Dodge, Iowa; Table 1), and four 2.0 mm biopsies were collected and stored in room temperature tissue preservative (Longmire et al. 1992). The location of each immobilized lemur was recorded using a global positioning system (Table 1; Appendix Ia). All measurements were taken on sedated animals as follows: weight (weight measured to within ±0.1 g), ear length (total length from tip of the ear to the base ±0.1 mm), ear width (total width across widest portion of the ear pinna ±0.1 mm), muzzle length (total length from the tip of the nose [soft tissue of the nose is not included] to the medial corner of the eye ±0.1 mm), head crown (total length from tip of the nose [soft tissue of the nose not included] to the occipital crown ±0.1 cm), crown body length (total length of body from the occipital crown of the head to the base of tail ±0.1 cm), and tail length (total length from base of tail to the end of the last caudal vertebra ±0.1 cm). For presentation purposes, the authors present the weight, head crown, body length, and tail length in this publication following the guidelines of Smith and Jungers (1997; Table 2).

Data Collection.—Genomic DNA was extracted from a 2.0 mm ear punch using a phenol-chloroform extraction (Sambrook et al. 1989). From these samples, the following regions of the mitochondrial DNA (mtDNA) were amplified: D-loop or control region (D-loop; Baker et al. 1993; Louis et al. 2006; Wyner et al. 1999); a fragment of the cytochrome oxidase subunit III gene (COIII); NADH-dehydrogenase subunits 3, 4L, and 4 (ND3, ND4L, and ND4); and the $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$ genes (PAST; Louis et al. 2006; Pastorini et al. 2000). Using 50 ng of genomic DNA, the D-loop (487-522 bp) and the PAST fragments (2,366-2,367 bp) were amplified by the polymerase chain reaction (PCR) using the following conditions: 94°C for 30s, a primer-specific annealing temperature for 1 min, 72°C

*Table 1. Samples (*n = *42) from free–ranging lemurs used in the present genetic study and taxonomic revision of the Genus* Microcebus *from northern Madagascar. [a]Mitochondrial DNA sequence data for each sample are available from GenBank under the listed accession numbers and Appendix Ia. Global Position System designates the locality or site where the animal was immobilized. [b]PBZT are the designated vouchers for the new* Microcebus *species and are maintained as live vouchers at Parc Botanique et Zoologique de Tsimbazaza (Louis et al. 2006). [c]TK numbers are representative samples curated at The Museum of Texas Tech University.*

Identification Number	TK number[c]	Species Designation	Location	Global Position System (GPS)	Microchip Number	D Loop[a]	PAST[a]
CAR5.1	TK125567	*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'55.4" - E049°08'02.4"	46164F2673	DQ534951	DQ534982
CAR5.2	TK125568	*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'40.7" - E049°07'58.3"	467D594E7B	DQ534952	DQ534983
CAR5.3		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'54.3" - E049°08'00.9"	467826276E	DQ534953	DQ534984
CAR5.4		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'51.6" - E049°07'44.4"	46754E4C56	DQ534954	DQ534985
CAR5.5		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°58'01.5" - E049°08'05.9"	4639174C17	DQ534955	DQ534986
CAR5.6		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'33.3" - E049°07'52.0"	46266E4F3A	DQ534956	DQ534987
CAR5.7		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°58'04.5" - E049°08'18.2"	467606575C	DQ534957	DQ534988
CAR5.8		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'40.0" - E049°07'57.2"	4660012C7B	DQ534958	DQ534989
CAR5.9		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'58.7"- E049°08'17.1"	4633664C64	DQ534959	DQ534990
CAR5.10		*Microcebus tavaratra*	Ankarana (Mahamasina)	S12°57'59.0" - E049°08'17.1"	462F453B79	DQ534960	DQ534991
LOKO4.26	TK125580	*Microcebus mamiratra*	Lokobe	S13°24'25.8" - E048°18'10.4"	4427241664	DQ535012	DQ535017
LOKO4.37	TK125581	*Microcebus mamiratra*	Lokobe	S13°24'16.9" - E048°18'11.2"	None	DQ535013	DQ535018
LOKO4.38	TK125582	*Microcebus mamiratra*	Lokobe (Marodoka)	S13°24'16.9" - E048°18'11.2"	None	DQ535014	DQ535019
PBZT130[b]		*Microcebus mamiratra*	Lokobe (Ambatozavavy)	S13°29'48.1" - E048°14'07.1"	471B665671	DQ535015	DQ535020
ANT5.1		*Microcebus species nova#5*	Antafondro	S14°02'56.4" - E048°13'17.7"	465F50502A	DQ535016	DQ535021
ANK7		*Microcebus ravelobensis*	Ankarafantsika	S16°21'72.4" - E046°45'99.7"	None	AY159695	AY582545
GAR6		*Microcebus sambiranensis*	Manongarivo	S14°01'25.3" - E048°16'20.4"	None	AY159704	AY582636
MOR62		*Microcebus murinus*	Beroboka	S19°58'59.9" - E044°39'99.2"	None	AY159714	AY582654
PET41		*Microcebus griseorufus*	Tsimanampetsotsa	S24°05'16.8" - E043°45'14.9"	None	AY159719	AY582659
PBZT115[b]		*Microcebus mittermeieri*	Anjanaharibe-Sud	S14°47'77.2" - E049°28'41.1"	442A03452E	AY254491	AY582642
PBZT117[b]		*Microcebus simmonsi*	Betampona	S17°55'87.1" - E049°12'20.0"	4428175773	AY254490	AY582671
TAD23		*Microcebus lehilahytsara*	Mantadia	S18°48'49.0" - E048°25'47.8"	None	AY159726	AY582662
PBZT114[b]		*Microcebus jollyae*	Kianjavato	S21°22'70.0" - E047°52'10.0"	4422523846	AY254492	AY582646
PBZT116[b]		*Microcebus rufus*	Ranomafana	S21°15'75.2" - E047°25'34.9"	442A24324F	AY255104	AY582653
ANAL5		*Lepilemur ankaranensis*	Analamera	S12°48'58.2" - E049°31'98.9"	None	AY769363	AY582564

Table 1 (cont.)

Identification Number	Bar Code[e]	Species Designation	Location	Global Position System (GPS)	Microchip Number	D Loop[f]	PAST[f]
RANO45		*Eulemur fulvus rufus*	Ranomafana	S21°14'90.0" - E047°25'26.6"	None	AY585738	AY582561
FAN21		*Varecia v. variegata*	Fandriana	S20°23'40.5" - E047°38'06.6"	None	AY584494	AY582555
RANO332		*Propithecus edwardsi*	Ranomafana	S21°14'90.0" - E047°25'26.6"	None	AY585739	AY582556
MOR68		*Propithecus verreauxi*	Beroboka	S19°58'59.9" - E044°39'99.2"	None	AF354712	AY582557
RANO261		*Avahi laniger*	Ranomafana	S21°14'90.0" - E047°25'26.6"	None	AY584496	AY582559
RANO67		*Avahi laniger*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY584495	AY582558
ANK33		*Avahi occidentalis*	Ankarafantsika	S16°21'72.4" – E046°45'99.7"	None	AY584497	AY582560
GAR9		*Hapalemur g. occidentalis*	Manongarivo	S14°01'17.1" – E048°16'30.0"	433B7F0445	AY584492	AY582553
ANAL2.23		*Hapalemur g. occidentalis*	Analamera	S21°48'07.0" – E049°22'18.2"	433C40687F	AY584493	AY582554
RANO61		*Hapalemur g. griseus*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY584490	AY582551
RANO62		*Hapalemur g. griseus*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY584491	AY582552
RANO351		*Hapalemur aureus*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY584489	AY582549
RANO352		*Hapalemur aureus*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY254048	AY582550
RANO338		*Hapalemur simus*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY254049	AY582547
KIAN124		*Hapalemur simus*	Kianjavato	S21°22'68.6" – E047°52'11.4"	None	AY584488	AY582548
GAR8		*Cheirogaleus medius*	Manongarivo	S14°01'25.3" – E048°16'20.4"	4333500470	AY584498	AY582562
RANO229		*Cheirogaleus major*	Ranomafana	S21°14'90.0" – E047°25'26.6"	None	AY254050	AY582563

for 5 min for 35 cycles. Since the molecular data from the previous studies reflect specific biases to regions of the mitochondrial DNA by each researcher, data sets based on independent vouchers or sample sets are difficult to correlate without generating the sequence data sets for these regions (Pastorini et al. 2003; Wyner et al. 1999; Yoder et al. 2000). Since all possible species have not been collected, much less the entire range of all *Microcebus* species, accessioned sequences were used to correlate and augment the datasets to expand the current taxonomic knowledge of the genus *Microcebus* (Louis et al. 2006; Pastorini et al. 2000, 2001, 2002, 2003; Yoder et al. 2000). To exclude the potential amplification of nuclear insertions, the PCR products were subsequently generated with a quick, efficient species-independent technique that is derived from the degenerate oligonucleotide-primed PCR method (DOP-PCR; Telenius et al. 1992). Adapting the long products from low quantity DOP-PCR methodology (LL-DOP-PCR), the sequence data generated from overlapping segments was verified for the D-loop and PAST PCR fragments. PCR products were confirmed visually on a 1.2% agarose gel and purified using QIAquick PCR purification kit (Qiagen; Valencia, California). Using the BigDye terminator cycle sequencing ready reaction kit by Applied Biosystems, the sequence was generated with a 7% polyacrylamide gel by an ABI 377 automated sequencer (Applied Biosystems, Inc; Foster City, California). Two internal sequencing primers for the PAST sequence data, MicHDZSec1F (5'-TCTGCTCGTCTACCMTTCTCC-3') and MicHDZSec1R (5'-ATGGAGAAKGGTAGACGAGC-3'), were designed and utilized to confirm and generate the consensus sequence. Additional sequencing and amplification primers were used to generate the sequence data (Louis et al. 2006). The sequence fragments were aligned to generate a consensus sequence using Sequencher (Gene Corp; Ann Arbor, Michigan), and the consensus sequences were aligned using ClustalX (Thompson et al. 1997). The consensus sequences were submitted to GenBank and Accession Numbers are listed in Table 1. The taxa used as outgroups for each of the sequence data sets are listed in Table 1.

Phylogenetic Analysis.—To examine the genetic diversity of the mouse lemurs of northern Madagascar, maximum-parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) analyses were implemented for the D-loop, PAST, and combined (D-loop

Figure 1. Range map of northern *Microcebus* species.

and PAST) sequence data with PAUP software (Swofford 2001). The trees described in this paper are all consensus trees except for the bootstrap analysis (all trees were presented as phylograms for presentation purposes only). Bootstrap analyses were accomplished with 1000, 3000, and 4000 pseudoreplicates with the D-loop, PAST, and combined sequence files, respectively, with 10 random addition heuristic searches per pseudoreplicate option selected. Only nodes with greater than 50% support were reported. The D-loop NJ tree was generated using the Tamura-Nei model (Tamura and Nei 1993; Saitou and Nei 1987). The stepwise addition option was selected for MP and ML analyses, and corrections for nucleotide sequence data suggested by Kimura (1980) were used with the NJ analyses. Gaps were considered as a fifth character in MP analyses, whereas gaps were treated as missing data in the NJ analyses. The ML trees were estimated via the heuristic search. For the substitution model, the transition/transversion ratios were estimated in MacClade (Maddison and Maddison 1992), and a discrete approximation to gamma distribution was estimated for among site rate variation. The default settings were maintained for all other settings, thus yielding the equivalent of the HKY model (Hasegawa et al. 1985). In addition to character-based phylogenetic analysis of DNA sequences, PAUP software (Swofford 2001) was also used to calculate uncorrected pairwise distances ('p') and Kimura distance measures for the D-loop and PAST fragments.

As described in Davis and Nixon (1992), Louis et al. (2006), Mayor et al. (2004), and Wyner et al. (1999), we utilized MacClade 3.01 (Maddison and Maddison 1992) and MEGA version 2.0 (Kumar et al. 1993) in a diagnostic search to designate evolutionarily sig-

*Table 2. Morphological data taken from immobilized animals. *Head and body length measurement taken from Rasoloarison et al. (2000). **The morphological data taken from Louis et al. (2006).*

Species Name	Common Name	N	Weight (g)	Head Crown (cm)	Body Length (cm)	Tail Length (cm)
*Microcebus berthae**	Berthe's Mouse Lemur	3	30.6 ± 0.57	N/A	9.2 ± 2.65	N/A
*Microcebus sambiranensis**	Sambirano Mouse Lemur	6	44.1 ± 5.91	N/A	11.7 ± 4.14	N/A
Microcebus sambiranensis	Sambirano Mouse Lemur	1	48.0 ± 0.0	2.6 ± 0.0	8.3 ± 0.0	14.0 ± 0.0
*Microcebus mittermeieri***	Mittermeier's Mouse Lemur	5	44.1 ± 7.4	3.3 ± 0.0	8.7 ± 0.2	11.3 ± 0.2
*Microcebus myoxinus**	Pygmy Mouse Lemur	15	49.0 ± 6.32	N/A	12.4 ± 4.76	N/A
Microcebus murinus	Grey Mouse Lemur	10	65.5 ± 4.2	3.4 ± 0.2	9.3 ± 0.7	13.0 ± 1.0
Microcebus ravelobensis	Golden-Brown Mouse Lemur	10	65.9 ± 12.5	3.7 ± 0.1	9.6 ± 0.7	14.5 ± 0.3
*Microcebus simmonsi***	Simmons's Mouse Lemur	6	64.8 ± 17.5	3.6 ± 0.1	9.2 ± 1.0	14.2 ± 1.0
*Microcebus jollyae***	Jolly's Mouse Lemur	3	61.3 ± 4.5	3.6 ± 0.1	9.3 ± 0.3	12.2 ± 0.1
*Microcebus griseorufus**	Reddish Grey Mouse Lemur	6	62.6 ± 5.91	N/A	12.3 ± 6.4	N/A
Microcebus griseorufus	Reddish Grey Mouse Lemur	3	43.7 ± 2.5	3.3 ± 0.1	8.7 ± 0.3	13.9 ± 1.3
Microcebus rufus	Rufous Mouse Lemur	5	43.7 ± 4.2	3.3 ± 0.1	8.6 ± 0.3	11.7 ± 0.8
*Microcebus tavaratra**	Northern Mouse Lemur	6	61.1 ± N/A	N/A	12.6 ± N/A	15.5 ± N/A
Microcebus tavaratra	Northern Mouse Lemur	10	52.3 ± 7.2	3.4 ± 0.3	9.0 ± 0.8	14.6 ± 1.0
Microcebus mamiratra	Claire's Mouse Lemur	4	60.8 ± 8.3	3.4 ± 0.1	9.4 ± 0.5	15.8 ± 1.1
Microcebus lehilahytsara	Goodman's Mouse Lemur	5	39.6 ± 3.3	3.2 ± 0.1	8.3 ± 0.6	10.7 ± 0.7

nificant units (ESU) for the *Microcebus* species using a population aggregate analysis (PAA) of the D-loop (487-522 bp) and the PAST (2,366-2,367 bp) sequence data. With the sequential addition of each individual without an *a priori* species designation, a PAA distinguishes attributes or apomorphic characters according to the smallest definable unit (Davis and Nixon 1992; Louis et al. 2006; Mayor et al. 2004; Ravaoarimanana et al. 2004).

RESULTS

Mitochondrial DNA sequence data were completed for two fragments, D-loop and PAST (approximately 3000 bp), for 42 individuals, representing all 12 recognized species of mouse lemurs from a total of 15 sites (Figure 1; Table 1). All new mtDNA sequences generated for this study were deposited in GenBank and can be acquired through the accession number (Table 1; Appendix Ia). The sequence alignments for the data sets are available from the first author upon request. Based on the phylogenetic inferences of the NJ, MP, and ML analyses of three sequence alignments (D-loop, PAST, and combined), the 12 recognized *Microcebus* species are differentiated (Figures 2-7). In order to verify that our samples are indicative of the recognized species in Kappeler et al. (2005), Louis et al. (2006), Rasoloarison et al. (2000), Thalmann and Geissmann (2005), and Zimmermann et al. (1998), we used GenBank to BLAST D-loop sequence generated for our data set. This comparison confirmed that the samples described in this study are representative of those species (GenBank Accession AF285457-AF285458, AF285463, AF285477-AF285479, and AF285490 are included in the D-loop analyses [Yoder et al. 2000] and AF224636 is included in the PAST analyses [Pastorini et al. 2001]). Additionally, two distinct subpopulations were identified from regions formerly based on the proposed distribution of the recognized *Microcebus* species, *M. sambiranensis* (Figures 2-7). The results from the population aggregate analysis of the D-loop and PAST sequence data are presented in Appendices II and III, respectively. Multiple diagnostic characters differentiate each *Microcebus* species, along with the newly described species at Nosy Be (Tables 3 and 4). In addition, these multiple diagnostic characters define another population at Antafondro. Until additional samples are available to reliably confirm the current information, the species status of the Antafondro mouse

(text continued on page 14)

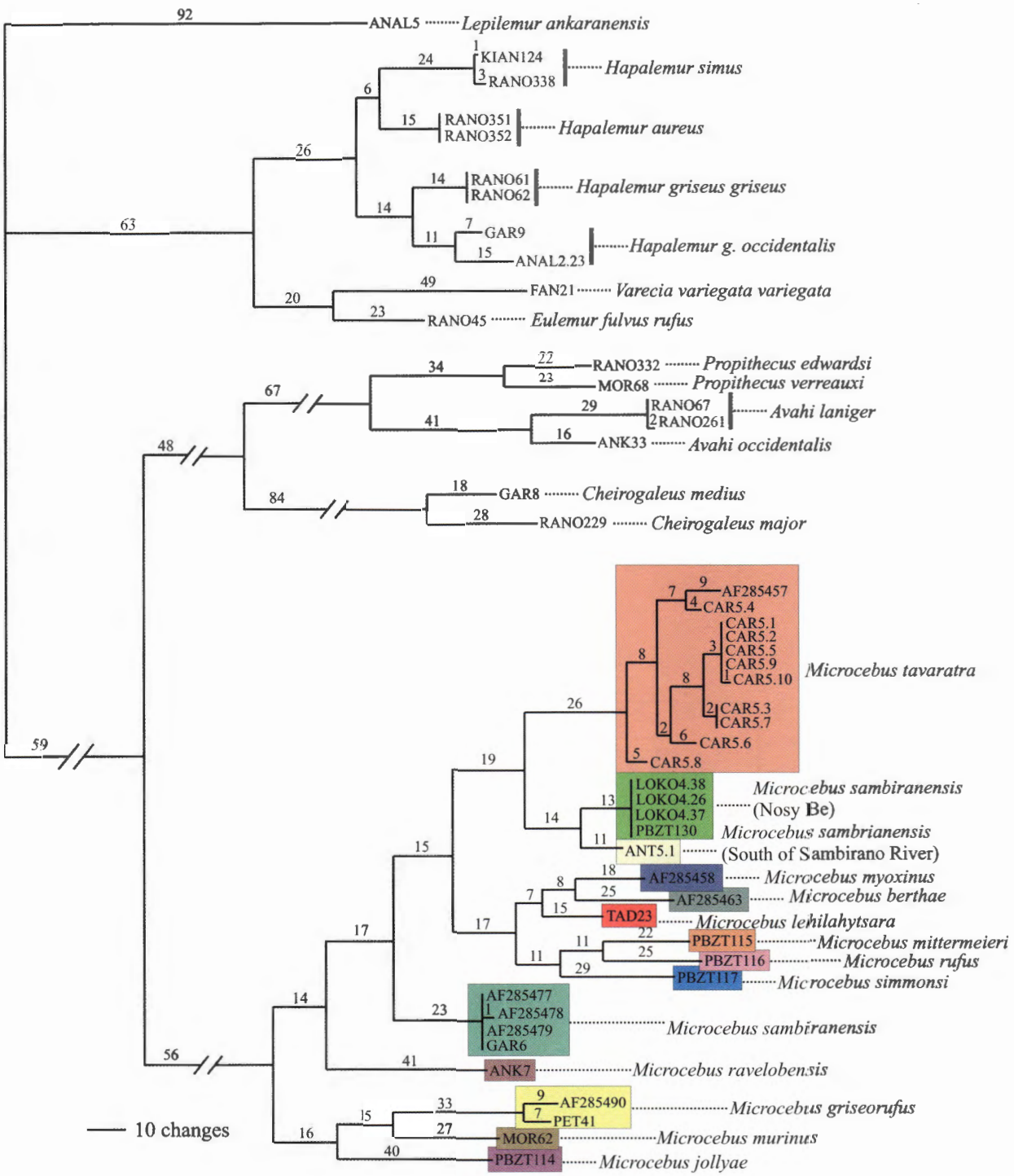

Figure 2. Phylogeny derived from D-loop fragment sequence data from 48 *Microcebus* individuals (one of four most parsimonious trees). Values above branches indicate number of changes between nodes. Length = 1,476; CI = 0.1729; RI = 0.7630; RC = 0.3608; HI = 0.5271.

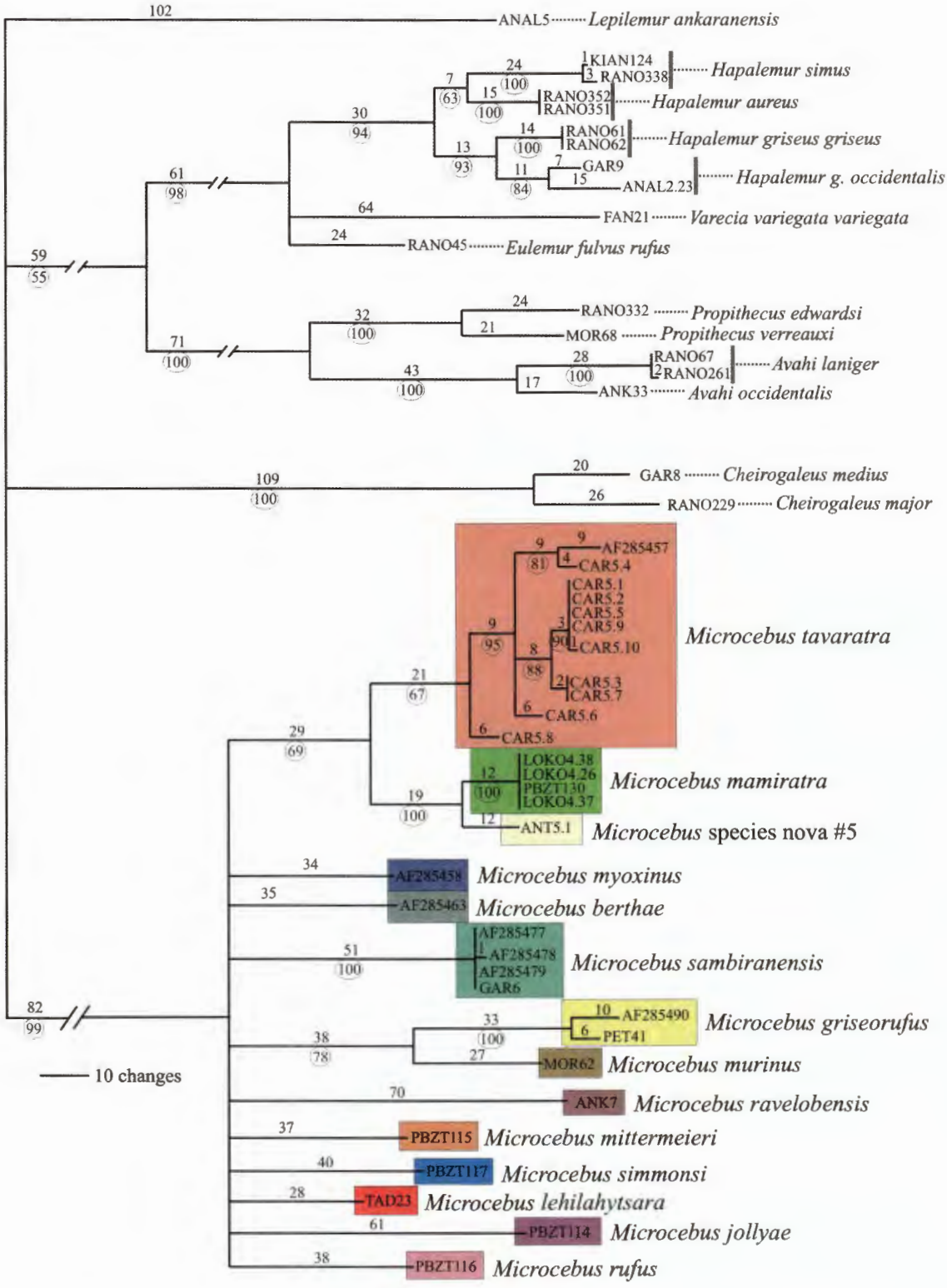

Figure 3. Neighbor-joining phylogram derived from the D-loop DNA sequence data from the 48 *Microcebus* individuals. Species designated according to the distribution in the current literature; therefore, LOKO and ANT individuals were initially designated *M. sambiranensis* notwithstanding Louis et al. (2006) proposing Nosy Be mouse lemurs as a new species (Mittermeier et al. 2006). Values above branches indicate number of changes between nodes. Values below branches within circles indicate support of bootstrap pseudoreplicates.

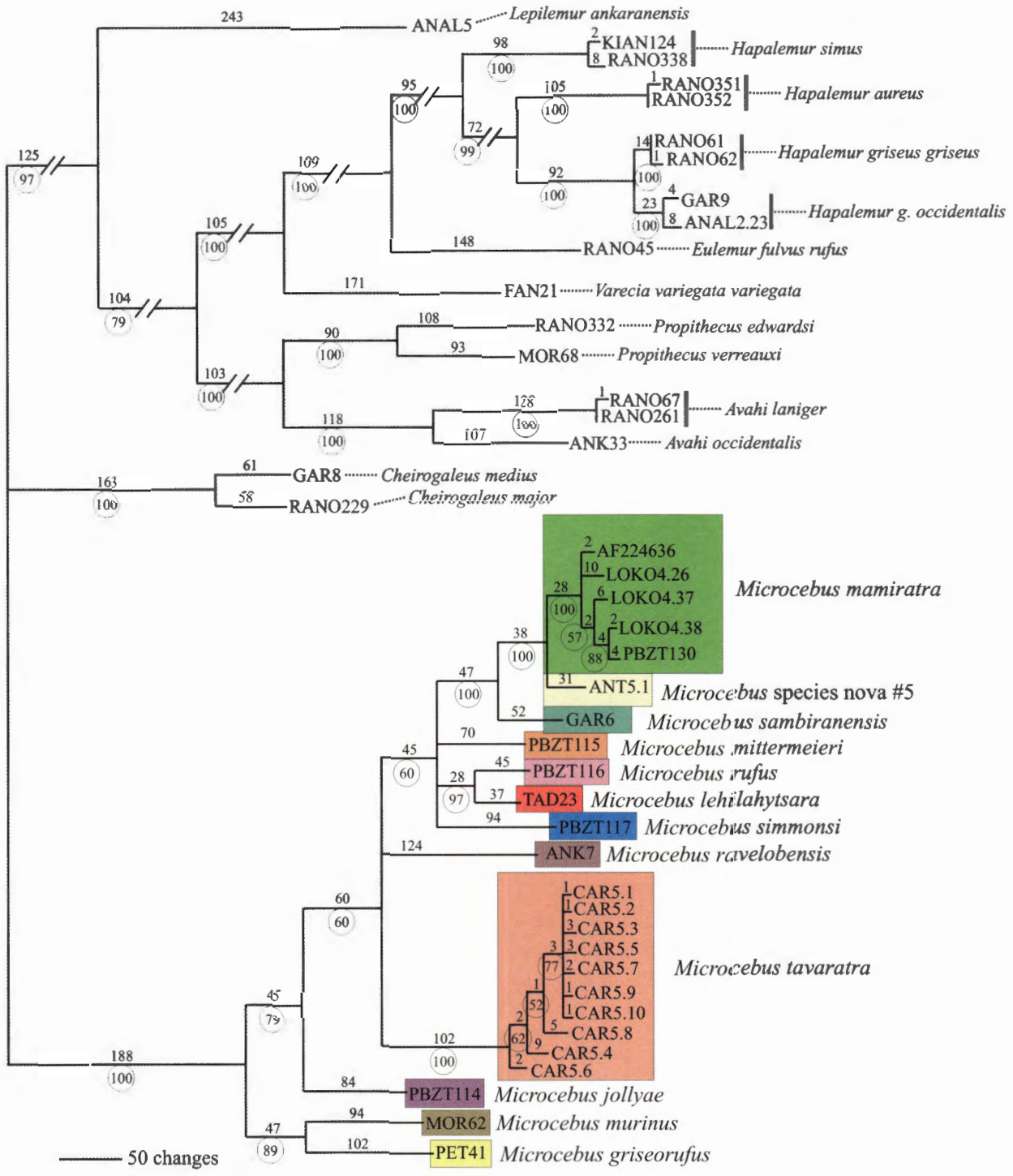

Figure 4. Neighbor-joining phylogram derived from the PAST DNA sequence data from 43 *Microcebus* individuals. Species designated according to the distribution in the current literature (Louis et al. 2006; Mittermeier et al 2006). Values above branches indicate number of changes between nodes. Values below branches indicate support of bootstrap pseudoreplicates. Length = 3,492; CI = 0.4323; RI = 0.7717; RC = 0.3336; HI = 0.5677.

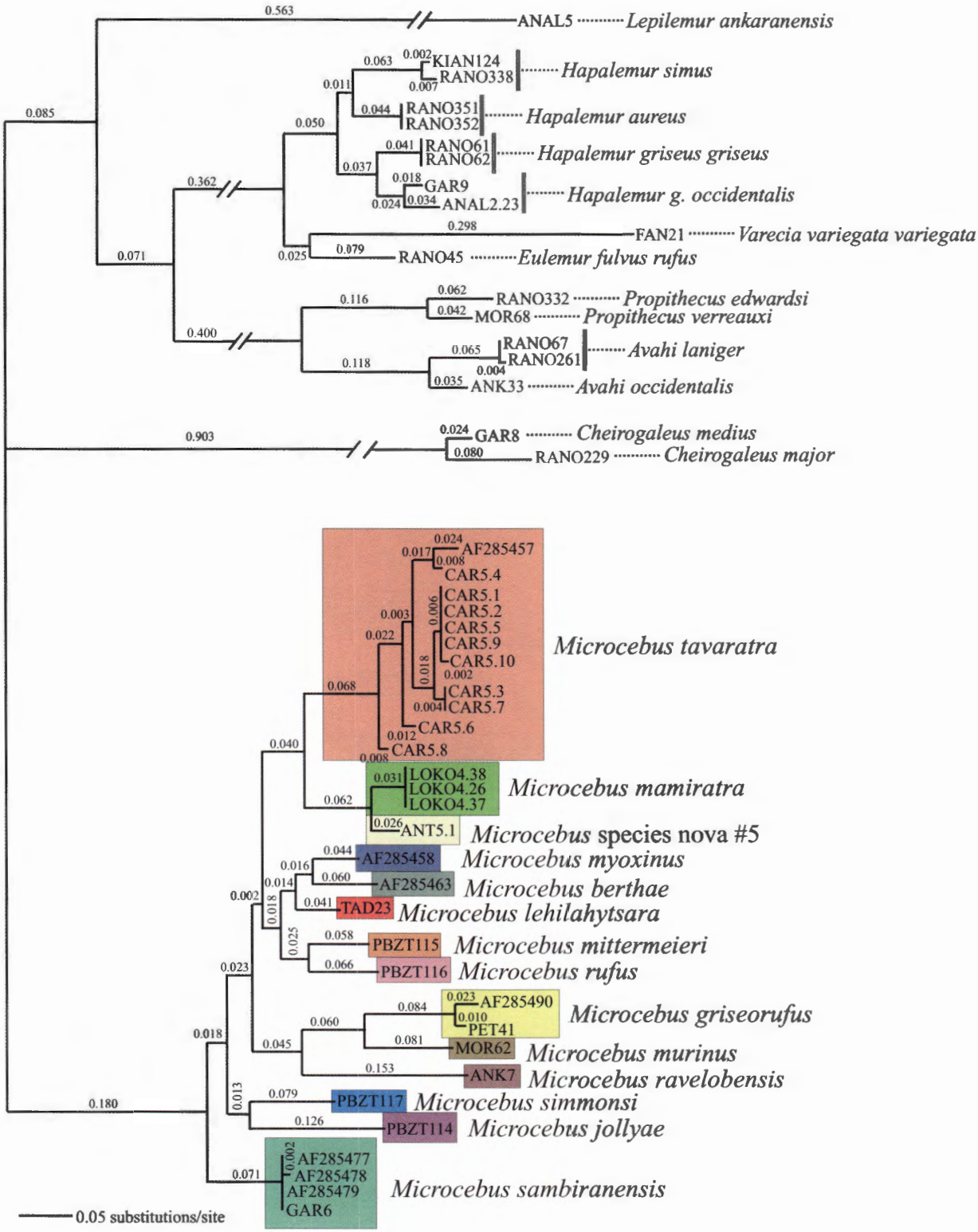

Figure 5. Maximum-likelihood phylogram derived from the PAST fragment sequence data from 48 *Microcebus* individuals. The phylogram presented with support of 1000 bootstrap pseudoreplicates (values specified on the branches).

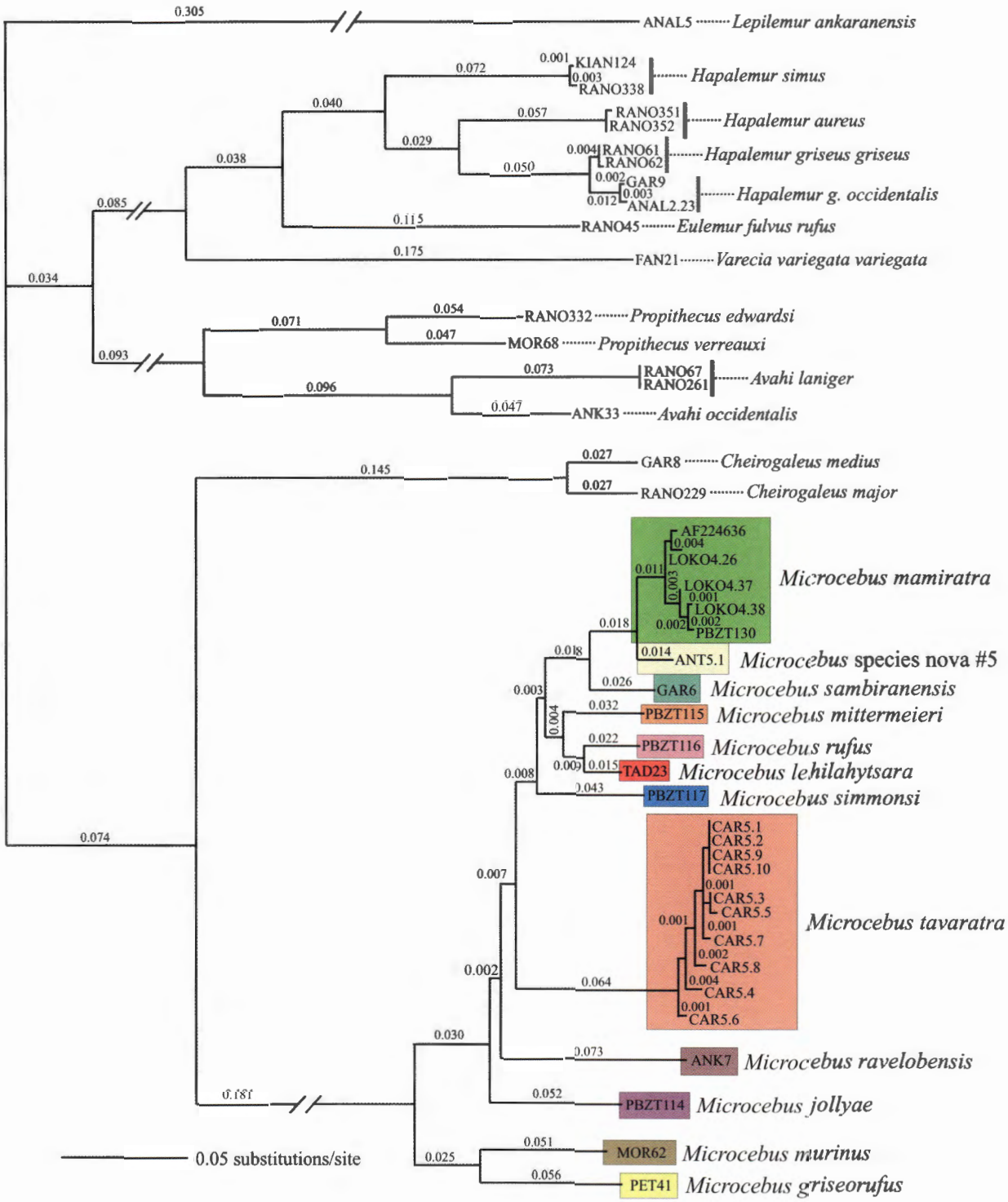

Figure 6. Maximum-likelihood phylogram derived from the PAST fragment sequence data from 43 *Microcebus* individuals. The phylogram presented with support of 3000 bootstrap pseudoreplicates (values specified on the branches).

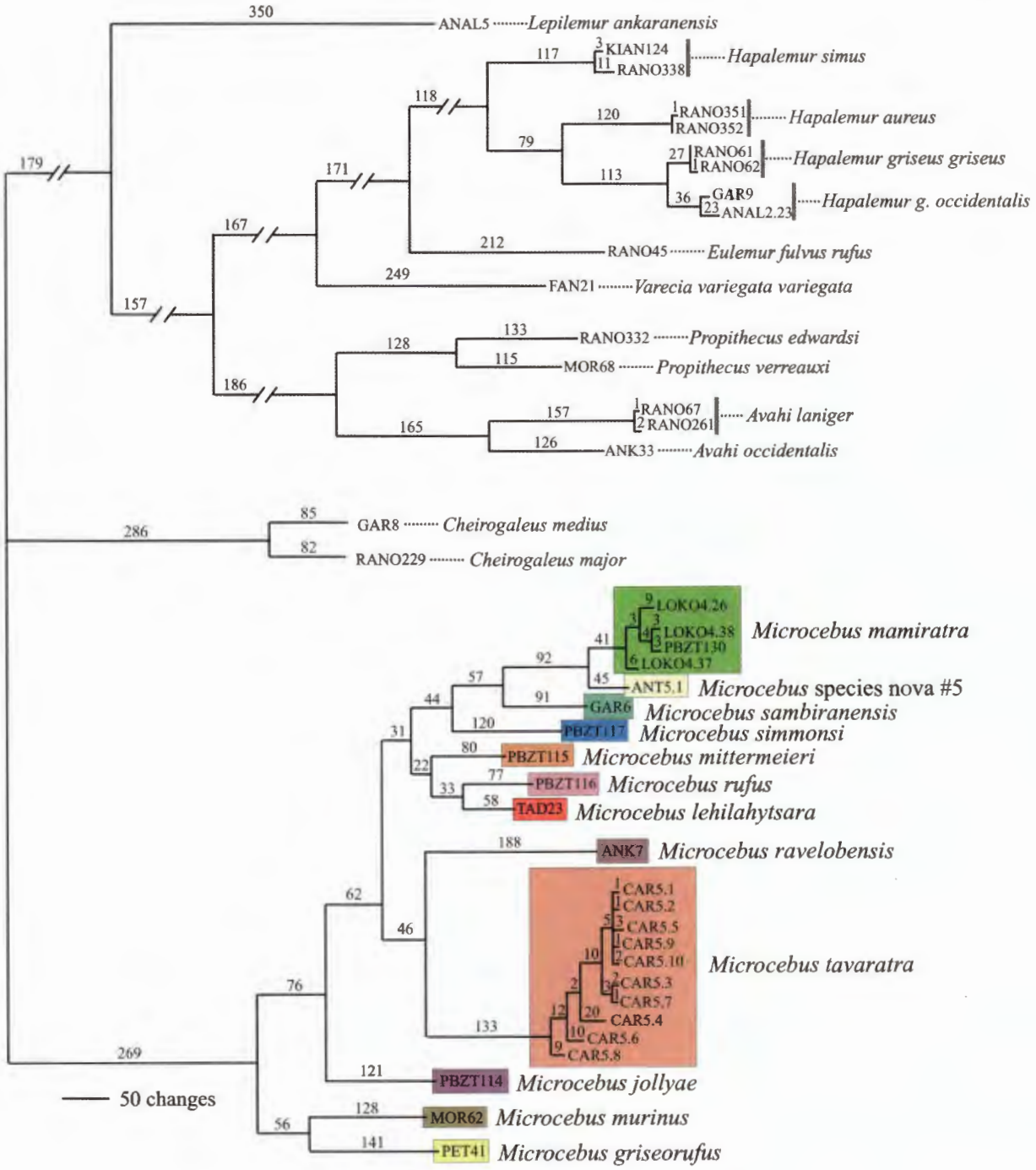

Figure 7. Phylogeny derived from combined D-loop and PAST fragment sequence data from 24 *Microcebus* individuals (one of 4 most parsimonious trees). Values above branches indicate number of changes between nodes. Length = 5,732; CI = 0.4552; RI = 0.7731; RC = 0.3519; HI = 0.5448.

Table 3. Summary of Population Aggregate Analysis (PAA) D-loop diagnostic sites for Microcebus *species. *Designates no diagnostic sites within this gene.*

Species	Fragment Size (bp)	PAA base pair location
Microcebus griseorufus	526	42, 149, 159, 192, 195, 243, 292, 323, 324, 335, 337, 411, 436, 490, 500, 515
Microcebus murinues	531	150, 159, 164, 165, 195, 244, 246, 250, 307, 315, 427, 500, 511, 514, 515
Microcebus ravelobensis	520	26, 76, 147, 169, 244, 246, 247, 248, 254, 258, 261, 285, 299, 300, 301, 336, 444, 397, 399, 409, 444, 454, 474, 476, 477, 480, 482, 485, 501, 511, 512, 513, 514
Microcebus sambiranensis	513-514	164, 262, 281, 286, 332, 432
Microcebus simmonsi	489	132, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 253, 263, 297, 333, 336, 337, 392, 437, 480
Microcebus jollyae	518	190, 194, 195, 279, 323, 335, 414, 415, 473, 488, 489, 497, 500, 506, 520
Microcebus rufus	522	256, 306, 328, 354
Microcebus lehilahytsara	522	*
Microcebus berthae	521	39, 73, 124, 159, 370, 513
Microcebus myoxinus	520	222, 286, 510
Microcebus mittermeieri	518	125, 238, 307, 328, 347, 511, 520
Microcebus species nova #5	490	221, 326, 370, 490
Microcebus mamiratra	487	250, 326, 370, 476, 479
Microcebus tavaratra	515	214, 279, 281, 365, 481, 482, 493

Table 4. Summary of Population Aggregate Analysis (PAA) Pastorini fragment diagnostic sites for Microcebus *species.*

Species	Fragment Size (bp)	PAA base pair location
Microcebus griseorufus	2366	115, 160, 290, 547, 577, 605, 618, 644, 647, 673, 772, 785, 828, 845, 874, 979, 994, 1006, 1040, 1055, 1069, 1075, 1090, 1168, 1319, 1358, 1366, 1486, 1546, 1552, 1583, 1585, 1597, 1601, 1619, 1711, 1738, 1810, 1828, 2026, 2087, 2173, 2234
Microcebus murinus	2366	46, 202, 403, 502, 507, 508, 547, 551, 602, 626, 653, 659, 744, 746, 750, 772, 791, 871, 944, 994, 1018, 1030, 1076, 1099, 1142, 1144, 1207, 1222, 1282, 1317, 1359, 1510, 1696, 1838, 1992, 2005, 2008, 2047, 2295, 2323
Microcebus ravelobensis	2366	26, 29, 133, 143, 187, 211, 226, 304, 394, 487, 526, 539, 560, 563, 599, 633, 716, 727, 780, 797, 917, 919, 931, 991, 1097, 1122, 1171, 1187, 1204, 1243, 1259, 1261, 1273, 1312, 1322, 1348, 1402, 1957, 2032, 2035, 2038, 2041, 2087, 2164, 2176, 2239, 2260
Microcebus sambiranensis	2366	6, 223, 232, 366, 562, 656, 659, 683, 764, 2212, 2308
Microcebus simmonsi	2367	172, 208, 449, 561, 578, 614, 657, 869, 873, 932, 1034, 1147, 1237, 1273, 1368, 1501, 1640, 1819, 1825, 1921, 2095, 2150, 2230
Microcebus jollyae	2367	47, 82, 84, 87, 121, 126, 139, 170, 187, 238, 337, 436, 474, 476, 495, 527, 567, 570, 740, 838, 844, 892, 924, 1000, 1108, 1222, 1246, 1301, 1343, 1717, 1906, 1957, 1990, 2071, 2122, 2242, 2309
Microcebus rufus	2366	103, 208, 283, 309, 450, 843, 873, 972, 1008, 1198, 1231, 1342, 1420, 1618, 1669, 2112
Microcebus lehilahytsara	2366	14, 450, 638, 773, 972, 1357, 1563, 1771
Microcebus mittermeieri	2366	274, 376, 457, 641, 656, 705, 855, 859, 907, 1054, 1093, 1115, 1177, 1316, 1504, 1804, 1906, 1954, 1983, 1984, 2002, 2069, 2230
Microcebus species nova #5	2366	285, 380, 815, 865, 992, 1024, 1093, 1249, 1292, 1633, 1672, 1786, 2295
Microcebus mamiratra	2366-2367	142, 672, 743, 1075, 1150, 1219, 2126
Microcebus tavaratra	2366	111, 134, 142, 202, 238, 406, 430, 549, 650, 835, 1063, 1267, 1289, 1291, 1292, 1304, 1350, 1355, 1356, 1367, 1400, 1552, 1567, 1591, 1594, 1597, 1615, 1645, 1651, 1660, 1765, 1849 ,1855,1867, 1894, 2068, 2155, 2274

lemur will be designated as a proposed new species, *Microcebus* species nova #5. A review of the morphometric data for the 12 recognized species of mouse lemurs is presented in Table 2 (see Appendix Ia for detailed morphological measurements of the individual animals). The complete uncorrected 'p' distance and the Kimura two-parameter distance measures are presented in Appendix Ib. There is high bootstrap support for the MP and NJ analyses with respect to the topology of the genera and species (Figures 3 and 4).

DISCUSSION

The persistent and rapid loss of habitat and the resulting fragmentation of panmictic populations have compelled wildlife and conservation agencies to take protective action according to existing guidelines and information with the ultimate goal of prioritizing species and/or sites. The explosive rate of deforestation in Madagascar, however, has eliminated many of the available options and has left many species susceptible to stochastic and deterministic factors. The burden to conserve Madagascar's biodiversity remains with a need to identify rapidly and reliably all species and distributions of the seemingly infinite Malagasy taxa. Once identified, wildlife and conservation agencies can then overlay the distributions of all endemic taxa, creating a viable template in which to organize and manage the existing biodiversity. With this in mind, the authors present another revision of the Genus *Microcebus*, introducing a new species from Nosy Be and Lokobe Special Reserve.

The phylogenetic inference of the two mtDNA regions, D-loop and PAST fragments, distinguished the 12 recognized species of mouse lemurs. However, the topologies of the two mtDNA data sets were incongruent, reflecting the inability to include all known species in both files (Figures 2, 5-7). By sequentially including a unique *Microcebus* species that was not represented in each previous data set, Louis et al. (2006) demonstrated the plasticity of the tree topology for *Microcebus*. The plasticity and lack of bootstrap support for the sister taxa is symptomatic of variable data sets used in the analyses due to inclusion of different species within each dataset, despite the use of multiple genes (Figures 3-4, 7; Louis et al. 2006). More importantly, tree topology will continue to change until a complete sample set that is representative of all *Microcebus* populations is obtained.

In this paper, the current *Microcebus* taxonomy for northwestern Madagascar was examined according to the Phylogenetic Species Concept (PSC) sensu (Wheeler and Platnick 2000; Louis et al. 2006; Mayor et al. 2004). The diagnostic characters or attributes define evolutionarily significant units (ESUs). Several authors suggest that ESUs are equivalent to species as determined through the Phylogenetic Species Concept (Amato et al. 1998; Barrowclough and Flesness 1996; Cracraft 1983). The constant addition of samples to the PAA data set will continue to test the distinction of these characters. The identification of a new species in the following description establishes the essential need for extensive as well as detailed sample collections across Madagascar to determine geographic ranges for all of the mouse lemurs.

Microcebus mamiratra, New Species

Type Series.—PBZT130; adult female (pregnant at the time of capture); live voucher and tissues are curated at Parc Botanique et Zoologique de Tsimbazaza. Additional tissue samples are stored at Omaha's Henry Doorly Zoo. A microchip pit tag was placed subcutaneously between scapulas and recorded as 471B665671. PBZT130 was collected by Richard Randriamampionona, Richard Rakotonomenjanahary, Gilbert Rakotoarisoa, Jean Amié Andriamihaja, John R. Zaonarivelo, and Edward Louis on 10 November 2005. Measurements (in centimeters and grams) recorded in field catalog on 8 February 2006: weight: 50.0 g; head crown: 3.2 cm; body length: 9.5 cm; tail length: 14.3 cm; muzzle length: 11.0 mm; ear length: 16.6 mm; and ear width: 9.6 mm. Whole total genomic DNA (50 ng/µl) for LOKO4.26 (TK125580), adult female; LOKO4.37 (TK125581), adult female; and LOKO4.38 (TK125582), adult female; are stored and curated at Museum of Texas Tech University. LOKO4.26, LOKO4.37, and LOKO4.38 were collected by Richard Randriamampionona, Jean C. Randriamanana, Gerard Nalanirina, Rambinintsoa Andriantompohavana, and John Zaonarivelo on 8 July 2004, 9 July 2003, and 9

July 2004, respectively. Individual measurements, e-voucher photos, and collection data are given in Appendix Ia and are archived in the Type Specimen Collection of The Museum of Texas Tech University (TTU-M104430).

Type Locality.—MADAGASCAR: Province de Antsiranana, Nosy Be, Lokobe Special Reserve (approximately 13°24'16.9"S, 048°18'11.2"E at 13m.).

Description.—*Microcebus mamiratra* is a medium-sized mouse lemur (60.8 g). The pelage is a light reddish-brown dorsally, becoming a brighter reddish-brown on the dorsum or cap of the head. A whitish midline stripe is found on the anterior portion of the muzzle, becoming wider and more diffuse between the eyes. The tail is uniformly light reddish-brown as the rest of the body. The venter is white to cream color. There is occasionally a light grayish-brown diffuse midline dorsal stripe present in some individuals (Figure 8).

Diagnosis.—In the D-loop and PAST sequence fragments, *M. mamiratra* differs from its closest relatives by both genetic and geographic distance, *M. tavaratra*, *M. sambiranensis*, *M. ravelobensis*, and *M.* species nova #5, by 10%±1.4% (64 informative sites), 13.4%±1.8% (58 informative sites), 18.1%±2.1% (75 informative sites) in D-loop; 8.8 %±0.5% (211 informative sites), 5.1%±0.5% (122 informative sites), 10.2%±0.8% and 222 informative sites), and 2.7%±0.3% (65 informative sites) in PAST.

Figure 8. *Microcebus mamiratra*, Claire's Mouse Lemur, at Lokobe Special Reserve.

Distribution.—*Microcebus mamiratra* is currently known from Nosy Be Island.

Comparisons and Remarks.—In Louis et al. (2006), the authors proposed that the mouse lemurs from Nosy Be should be considered a separate species (*Microcebus* species nova #4), based on the PAST sequence fragment from one individual from Pastorini et al. (2001) that was included in the analyses (no measurements or description were available). Of the recognized mouse lemurs that are in the adjacent regions of "mainland" Madagascar, *Microcebus mamiratra* (60.8 g) is larger in size than *M. sambiranensis* (48.0 g), and *M. tavaratra* (52.3 g), but smaller than *M. ravelobensis* (65.9 g).

Etymology.—The name *mamiratra* is derived from the Malagasy language and means "clear and bright" and is proposed for Claire Hubbard and the Theodore F. and Claire M. Hubbard Family Foundation. The Hubbard Foundation has provided generous support over the past five years, providing the Malagasy graduate students the opportunity to conduct conservation genetics projects in the field and in the laboratory.

Vernacular Names.—Claire's Mouse Lemur.

The new species described in this paper, *M. mamiratra*, follows its initial proposal in Louis et al. (2006). With the description of the new species at Lokobe and the proposed new species at Antafondro, the former distribution of *Microcebus sambiranensis* is apportioned and is currently restricted to the Manongarivo Special Reserve, north of the Sambirano River. Additional samples, representing the entire Sambirano region, will be needed to demonstrate the validity of *Microcebus* species nova #5, and to support the observed trend of rivers acting as barriers to species across Madagascar.

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APPENDIX I

The following Appendices to this publication are available online at the indicated website addresses:

a. *Microcebus* Field Data (Individual data file for each *Microcebus*, including morphometrics, photos, sequence accessions, global position system, microchip data, gender, and location):

http://www.omahazoo.com/ccr/genetics/papers/microcebus.pdf

b. Pairwise Data (Complete pairwise matrices):

http://www.omahazoo.com/ccr/genetics/papers/pairwisetable1.pdf

c. D-loop Maximum Parsimony Tree:

http://www.omahazoo.com/ccr/genetics/papers/nosybetree1.pdf

d. PAST Maximum Parsimony Tree:

http://www.omahazoo.com/ccr/genetics/papers/nosybetree2.pdf

Appendix II

Diagnostic nucleotide sites from the D-loop Pairwise Aggregate Analysis (PAA) of Microcebus *Nosy Be population.*

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                 1111111111111122222222222222222222222222222222223333333333333333333333444444444444444444444445555555555
                 2347722344556669999011111111112234444455555666788899900001222233333445679990111233345777778888889990001111112
                 6923645279094590245901234567891283467803468123915627901675346823567474502799145726744346790125890370160123450

PET41            GCATA-CAGTA-GG-ACCGGTACGATCAATTACGA-AGTATTTTCTTAGG-GGTTTTTTTCAGTGAATCTAATG-TG---GAAGCAGCAAGGCTTGGTGTATTATATGT
AF285490         .T..G-....G-..-..........C.........-.............-.AC...........G........-..---............................
MOR62            ..G.G-...GTGAA-.T.C...........C..TCT..A.........T.T.....GAG-T...TGT......A-.A--T.T......GG......T.AC...C..AT.
ANK7             A.G.--..TG.T..G.T.T......C.......ATGGT..A.-A....-.A.TAG...G-T...TCTC.....TGAA---.T.TT.CGCGA.-A..T.AAC..-----.
GAR6             ..G.G-A..GGTT.-.TTT.............-.-.........T..-.CT.-.....G-T.--TTT.......-.A---AT.......G..G-.AT..A.-.....--
AF285477         ..G.G-A..GGTT.-.TTT.............-.-.........T..-.CT.-.....G-T.--TTT.......-.A---AT.......G..G-.AT..A.-.....--
AF285478         ..G.G-A..GGTT.-.TTT.............-.-.........T..-.CT.-.....G-T.--TTT.......-.A---AT.......G..G-.AT..A.-.....--
AF285479         ..G.G-A..GGTT.-.TTT.............-.-.........T..-.CT.-.....G-T.--TTT.......-.A---AT....-..G..G-.AT..A.-.....--
PBZT117          ..G.G-AG.G.T..-.T-------------...-.-...T.....C.G.TTT-.....A-T..GT--.....AA-.A---.TG.....G.TAG-.AT.AA.-.....-.
PBZT114          ..G.G--..GGT..-GTGA............C.-G-...GC.....A.ATT.-..G..--T...CTT......A-.ACT-.T...-..GG.AG-ATT.T-.C.....-A
PBZT116          ..G.G--..G.T..-.TTT.......T......TG-.....C......T-T.-..C..G-TT..TGT..C....-.A---.T......GG..G-.AT.AA.......-.
TAD23            ..G.G-...G.T..-.TTT.......T.......-.-...GC........TT.-.....G-T...TGT......A-.A---.T......GG.AG-.AT..A.....G.-.
AF285463         .-GC.A...G.C..-.TTT.......T...C..-G-...........GATT.-.....G-T...T.T....G.A-.A---.T.A....GG..G-.AT.AA.....C.-.
AF285458         ..G.G-...G.T..-.TTT.......T....T.-.-....C......G.AT.-.....G-T.A.T.......-.A---.T......GG..G-..T.AA..C....-.
PBZT115          ..G.G-T..G.T..-.TTT.......T.....T-G-............TT.-...C.G-TG..TTT.T....A-.A---.T.A....G..AG-.AT..A...G...-C
ANT5.1           ..G.G--..GGT..-----.......T...A..T--........-...GT-AA......G-G...TTT....C.A-.A---.T......GG..G-.AC..A.G.....-.
PBZT130          ..G.G--..GGT..-----.............T---.G.C..-...GT-AA-.....G-A..-TTT....T.A-.A---.T.....TGT..G-.AT..A.G...G.-.
LOKO4.26         ..G.G--..GGT..-----.............T---.G.C..-...GT-AA-.....G-A..-TTT....T.A-.A---.T.....TGT..G-.AT..A.G...G.-.
LOKO4.37         ..G.G--..GGT..-----.............T---.G.C..-...GT-AA-.....G-A..-TTT....T.A-.A---.T.....TGT..G-.AT..A.G...G.-.
LOKO4.38         ..G.G--..GGT..-----.............T---.G.C..-...GT-AA-.....G-A..-TTT....T.A-.A---.T.....TGT..G-.AT..A.G...G.-.
CAR5.1           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CTA-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.2           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CTA-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.3           ..G.G-...G.T..-.TTT.....G......C.A--.......-..CT.-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.4           ..G.G-...G.T..-.TTT.....G.T....C.A--.......-..CT.-AA-..G..G-..A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
AF285457         ..G.G-...G.T..-.TTT.....G.T....C.-.-.......G..CT..T.-..G..G-..A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....--
CAR5.5           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CTA-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.6           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CT.-AA-..G..G-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.7           ..G.G-...G.T..-.TTT.....G......C.A--.......-..CT.-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.8           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CT.-AA-..G..G-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.9           ..G.G-...G.T..-.TTT.....G.T....C.A--.........CTA-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
CAR5.10          ..G.G-...G.T..-.TTT.....G.T....C.A--.........CTA-AA-..G..A-T.A.TTT...T..A-.A---.T......GG.TA-.ATA.A.-.....-.
```

APPENDIX III

Diagnostic nucleotide sites from the PAST Pairwise Aggregate Analysis (PAA) of Microcebus *Nosy Be population.*

```
                  1111111111111122222222222233333334444444444455555555555555555566666666666666666667
                122448880112233344677800012233788900367890033455778900022344566666777900112334445555577 80
                646967247315163492300272581362843504976604360690746752786797910123705892548638147036792335

PET41           GCAAATAACTCGTTTAACCTTAATCCTTCATAATTACCCCCGTCAA-AA-AAGAATCCTCTATGCCCCAAAAGACCTAACCATCCAACAT
MOR62           ....T......A.......C...C..........C.......A...-..-...GTC...T.G......C..GA.TT...TT.C..T.T..
ANK7            ..CC.......A..C...AC..G...C.T.....CG.....A....-..-.G....TACA..C..A..C.G.A.T.C..TT......T..
GAR6            A..........A.......C.......C.G....C...TT...T..-..-.......A.A....T...C...A.T....TT..T.GCTG.
PBZT117         ...........A.......C.G...T........C...........C..-.......A.A...A....TG..ACT....TT...T..T..
PBZT114         .....CGGT..ACC..G..CC.C.......A...C..T.....T.G-..AG.A....G.A......ATC...A.T....TT......T..
PBZT116         ....G....C.A......TC.....A......C.C.G.........-C.-.......A.A........T...A.T....TT......T..
TAD23           .T..G......A......TC.............C...........-T.-.......A.A........C...A.T..C.TT......T..
PBZT115         ...........A......TC...........G..C....A......-.G-.......A.A........C...A.T...GTT..A...T.C
ANT5.1          ...........A.......C.............CC.....T.....-..-.......A.A........C...A.T....TT.....CT..
PBZT130         ...........A.....A.C.............C....T......-..-.......A.A........C...A.T....TT.....TT..
LOKO4.26        ...........A.....A.C.............C....T......-..-.......A.A........C...A.T....TT.....TT..
LOKO4.37        ...........A.....A.C.............C...........-..-.......A.A........C...A.T....TT.....TT..
LOKO4.38        ...........A.....A.C.............C...........-..-.......A.A........C...A.T....TT.....TT..
AF224636        ...........A.....A.C.............C...........-..-.......A.A........C...A.T....TT.....TT..
CAR5.1          ..........AA...G.T.C....T.....C...C........AG.-..-.........AC.......C...A.T....TTG.....T..
CAR5.2          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.3          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.4          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.5          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.6          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.7          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.8          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.9          ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
CAR5.10         ..........AA...G.T.C....T.....C...C........AG.-..-.......A.AC.......C...A.T....TTG.....T..
```

APPENDIX III (CONT.)

Continuation of diagnostic nucleotide sites from the PAST Pairwise Aggregate Analysis (PAA) of Microcebus *Nosy Be population.*

```
                                                         11111111111111111111111111111111111111111111111111111111
               77777777777777888888888888888999999999999000000000000000000011111111111122222222222222222333333
               12444456778899123344455667779011233477999000123345566779999901244456778900123344456678899001111
               62034604230517585834559591342779412429124069840404539560367985224708177847921736991732912142679

PET41          CACTTACACCCTTTCCATTACTACATCAAACTCATATTACTACAAACCGATTGCTGACTAATTAACCCTTTCTTATAACACCATCCTATCAGAAA
MOR62          T...CGT.T..CC..T....T....C.C.......G.C..C.T.G.T.A.C.ATCA...G...TG..T.....C.A.........A.......TT
ANK7           AT.C....A.TC.C.T.C..T......C..AC.T...CG.A.T.....A.C.AT.A..C...C....TC.C.C.....T..TG.T......T..T
GAR6           T..C...GA..C...T.C..T......C.....C...C..A.T.....A.C.AT.A...........T.......................A..T
PBZT117        T..C....A..C...T....T...G.AC.....CC..C..A.T....TA.C.AT.A.........ATT.........T......A......A..T
PBZT114        T.TC....A..C...T.A.GT......CG...TC...C..AGT.....A.C.AT.A....G.....TT.......C...G.........T.A..T
PBZT116        T..C....A..C...T.CG.T.....TC..T..C..AC..A.TT....A.C.AT.A...........T...T....G..............A..T
TAD23          T..C....AT.C...T.C..T......C..T..C..CC..A.T.....A.C.AT.A...........T.......................A..T
PBZT115        T..C....A..C...T.C..TCG....C.G...C...C..A.T.....AGC.AT.AG....C.....T.C.....................AG.T
ANT5.1         T..C....A..C..TT....T..T...C.....C...C.TA.T..G..A.C.AT.A.T.........T............T.......C..A..T
PBZT130        T..A....A..C...T....T......C.....C...C..A.T.....A.C.AA.A..........AT.......................A..T
LOKO4.26       T..A....A..C...T....T......C.....C...C..A.T.....A.C.AA.A..........AT.......................A..T
LOKO4.37       T..A....A..C...T....T......C.....C...C..A.T.....A.C.AA.A..........AT.......................A..T
LOKO4.38       T..A....A..C...T....T......C.....C...C..A.T.....A.C.AA.A..........AT.......................A..T
AF224636       T..A....A..C...T....T......C.....C...C..A.T.....A.C.AA.A..........AT.......................A..T
CAR5.1         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.2         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.3         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.4         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.5         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.6         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.7         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.8         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.9         ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
CAR5.10        ...C....A..C...TT...T......C.....C...C..A.T.....A.CCAT.A...........T......G........C..CGA.GA..T
```

APPENDIX III (CONT.)

Continuation of diagnostic nucleotide sites from the PAST Pairwise Aggregate Analysis (PAA) of Microcebus *Nosy Be population.*

```
            1111111111111111111111111111111111111111111111111111111111111111111111112222222222222222222222222222222222222
            3333333333333444455555555555566666666666677777788888888899999999000000000000000011111111222222223333
            2444555555666002800145668899901113445667911367801122345690255889900023334466788891225567713334679002
            2238056789678020614062373514715893051092617851640958795746147340225862581789167952260543620492045893

PET41       CAGAATGAACCATTATCCTCTTAATCTTAACAGATAAACTATTCACATTTCTAATAATTATTTTTCTTCTCTACTAACATCAAACCCCACTACAAATAAG
MOR62       ........TTT.....T..TCC..GA..TT..A.......GACT....C..CG...........C.CCT....T...T.........T...G....A..A
ANK7        A..G....T.T...G.T...CC..GG..TTT.A........ACT....CC.C......C.C.......TGTCG....T.C.......TTG..GT.G.....
GAR6        ........T.T.....T...CC..GA..TC..A........ACT.G..CC.C......C.........T.........T.........T.A.G.....G..
PBZT117     ........T.T.C...TT..CC..GG..TTT.A.C......ACT....CATC......A.........T........T..T...T.AT..CG........
PBZT114     ..C.....T.T.....T...CC..GA..TC..A........AAT....CC.C.....CC.A..C....T.......GT....G....T...G.G....T.
PBZT116     .G......T.T....CT...CC..GA..TT.GA.....T..A.T....CC.C......C.........TC.......T...G.....T...G........
TAD23       .......GT.T.....T...CCG.GA..TT..A........ACT.T..CC.C......C.........TC.......T.........T...G........
PBZT115     ........T.T.....T.C.CC..GA..TT..A........ACT...CCC.C.....ACG.CC..T..T......T.TG........T..AG........
ANT5.1      ........T.T.....T...CC..GA..TT..AG.....C.ACT.AG.CC.C.G....C.........T........T.........T...G....C...
PBZT130     ........T.T.....T...CC..GA..TTT.A........ACT.A..CC.C.G....C.........T........T.....T...T...G........
LOKO4.26    ........T.T.....T...CC..GA..TTT.A........ACT.A..CC.C.G....C.........T........T.....T...T...G........
LOKO4.37    ........T.T.....T...CC..GA..TTT.A........ACT.A..CC.C.G....C.........T........T.....T...T...G........
LOKO4.38    ........T.T.....T...CC..GA..TTT.A........ACT.A..CC.C.G....C.........T........T.....T...T...G........
AF224636    ........T.T.....T...CC..GA..TTT.A........ACT.A..CC.C.G....C.........T........T.....T...T...G........
CAR5.1       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.2       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.3       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.4       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.5       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.6       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.7       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.8       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.9       ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
CAR5.10      ...TCT.T.TC.C..T...CA.TGACCCTA.A..GGG...A.TTG..C..C.TCGG.C.........T.....C..T.......TAT...G...G....
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