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Revision of the Mouse Lemurs, *Microcebus* (Primates, Lemuriformes), of Northern and Northwestern Madagascar with Descriptions of Two New Species at Montagne d'Ambre National Park and Antafondro Classified Forest

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Abstract: Molecular genetic sequence variation of northern and northwestern mouse lemurs (*Microcebus*) was examined during a phylogenetic analysis of mitochondrial DNA (mtDNA) sequence data (*c*. 3,000 bp) for the entire genus. Phylogenetic inference of the mitochondrial DNA sequence data was generated from 132 individuals, representing 15 species of mouse lemurs. The database distinguished the 15 described *Microcebus* species and also provided diagnostic evidence for two further species. A comparison of the data for two mouse lemur species described from Nosy Be confirmed the existence of just one for this island population. The localities of the newly identified species are within the distributions previously recognized for *Microcebus sambiranensis* and *Microcebus tavaratra*. Formal descriptions, drawn from molecular genetic data, are presented for the two newly named species: one from Antafondro Classified Forest and the other from Montagne d'Ambre National Park. We revise the Inter-River-System hypothesis concerning the biogeographic patterns of the distributions of the northern and northwestern mouse lemurs according to our findings concerning the two species described here.
Key words: *Microcebus*, mouse lemur, systematics, Madagascar, prosimian, biogeography

Introduction

Due to its unique species biodiversity and to the continued pressure from human encroachment, Madagascar is among the highest conservation priorities worldwide (Myers *et al.* 2000). With 40% of the forest cover lost between the 1950s and 2000, rapid and comprehensive surveys of the remaining forest are essential (Harper *et al*. 2007). Dufils (2003) estimated that 90% of Madagascar's biodiversity is found exclusively in forest or woodland tracts, making these research efforts more urgent still. Recent molecular genetic and morphological studies of lemurs, particularly the mouse lemurs (*Microcebus*) and sportive lemurs (*Lepilemur*), have led to a great increase in the number of recognized species (Andriantompohavana *et al*. 2006; Craul *et al*. 2007; Kappeler *et al*. 2005; Louis *et al*. 2006a, 2006b; Olivieri *et al*. 2007; Radespiel *et al.* 2008). Even with these taxonomic revisions and the consequent realignments of the distributions of the species, regular re-evaluations are needed to monitor the conservation status of each taxon (Louis Jr. *et al*. 2006b).

All lemurs are currently protected under the Convention on International Trade in Endangered Species (CITES). Forty-one lemurs (43% of the 96 species and subspecies listed) were categorized on the *2008 IUCN Red List of Threatened Species* as threatened (IUCN 2008). The status of a further 43 lemurs (45%) were, however, too poorly known to be assessed and were classified as Data Deficient. Distributed throughout the island, lemurs are particularly susceptible to extinction from stochastic and deterministic factors due to their relatively small and fragmented geographic ranges (Jernvall and

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Wright 1998). Mouse lemurs are adaptable, being found in secondary or otherwise degraded forest tracts, even along roads. They live in small social units, being solitary or forming small family groups (Guschanski *et al*. 2007), and are limited in their capacity to disperse because they are nocturnal and small (30–80 g) and have small home ranges of 0.3–1.5 ha (Schwab 2000; Weidt *et al*. 2004; Louis Jr. *et al*. 2006a).

Until recently, the northern and northwestern mouse lemurs were 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 (mtDNA) sequence data, Andriantompohavana *et al*. (2006) presented evidence for a new species of mouse lemur they named *M. mamiratra* in northwestern Madagascar at Nosy Be Island, and also indicated the probability of another, which they referred to as *Microcebus* sp. *nova* #5, at Antafondro Classified Forest. Olivieri *et al*. (2007) presented a biogeographic model for the northern mouse lemurs, and described three new species, including one, *M. lokobensis*, from Lokobe Special Reserve on Nosy Be Island and Manehoka on the mainland of Madagascar.

Three biogeographic models have been proposed for the distribution patterns of mouse lemurs, based on different relative contributions of factors that include large rivers (>50 m wide at 20 km inland), retreat dispersion watersheds, and topographical barriers such as mountains (Martin 1995; Wilmé *et al*. 2006; Craul *et al*. 2007; Olivieri *et al*. 2007). Olivieri *et al*. (2007) and Craul *et al*. (2008) presented biogeographic models which defined "centers of endemism" based on the isolation effects of paired rivers, or Inter-River-Systems (IRS; Fig. 1). During the course of a number of biogeographic reviews of northern and northwestern Madagascar, the number of Inter-River-Systems has increased from four (Martin 1995), to five (Wilmé *et al*. 2006) to nine (Craul *et al*. 2008).

In this paper, we present a comparative phylogenetic analysis of the northern and northwestern mouse lemurs. With comprehensive sampling in this region (novel samples and sites, along with accessioned published sequences), we re-evaluate the biogeographic partitions, define the relationship between *Microcebus mamiratra* and *Microcebus lokobensis* described independently from the island of Nosy Be, and provide descriptions of two mouse lemurs that we consider to be distinct species; one from Antafondro Classified Forest and the other from Montagne d'Ambre National Park.

Methods

Sample collection

All lemurs in this molecular study were free-ranging, wild-caught, adults (Fig. 1; Table 1; Appendix I(a)). All mouse lemurs were hand-caught and subsequently immobilized using 1.0–3.0 mg of Telazol® (Fort Dodge). Two 2.0-mm biopsies and 0.01–0.05 cc of whole blood 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; Appendix I(a)), and four 2.0-mm biopsies and 1.0 ml/kg of blood were collected and stored in room temperature tissue preservative (Longmire *et al*. 1992). Genomic DNA was extracted from a 2.0-mm ear punch using a phenol-chloroform extraction (Sambrook *et al*. 1989). All measurements were taken on sedated animals as described in Andriantompohavana *et al*. (2006). We measured the weight (±0.1 g), head crown (total length from the tip of the nose [soft tissue of the nose is not included] to the occipital crown ±0.1 cm), body length (total length of body from the occipital condyle to the base of the tail ±0.1 cm), tail length (total length from the base of the tail to the end of the last caudal vertebra ±0.1 cm), ear length (total length from the tip of the ear to the base ±0.1 mm), ear width (total width across the widest portion of the pinna ±0.1 mm), and 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). For presentation purposes we provide the weight, head crown, body length, and tail length following the guidelines of Smith and Jungers (1997). (See Table 1. Appendices I(a–b).)

Data generation

To compare our data with previously published molecular studies, we analyzed the following regions of the mitochondrial DNA (mtDNA): D-loop or control region (D-loop; Baker *et al*. 1993; Wyner *et al*. 1999); and a fragment of the cytochrome oxidase subunit III gene (COIII); NADH-dehydrogenase subunits 3, 4L, and 4 (ND3, ND4L, and ND4); as well as the $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$ genes (PAST; Pastorini *et al*. 2000; Louis Jr. *et al*. 2006a). Using 50 ng of genomic DNA, the D-loop (487-531 base pairs (bp)) and the PAST fragments (2367 bp) were amplified by the polymerase chain reaction (PCR) using the following conditions: 94°C for 30 s, a primer-specific annealing temperature for 1 min, and 72°C for 5 min for 35 cycles. Since all potential sites or populations of mouse lemurs have not been collected, accessioned sequences were used to compare and augment the datasets to evaluate the current taxonomic knowledge of the genus *Microcebus* (Andriantompohavana *et al*. 2006; Yoder *et al*. 2000; Louis Jr. *et al*. 2006a; Olivieri *et al*. 2007; see Table 1; Appendix III(a)). The species described by Radespiel *et al*. (2008) were not included in these analyses since sequence fragments could not be compared at this time. To evaluate the two described species of Nosy Be, *Microcebus mamiratra* and *M. lokobensis*, representative sequences for the D-loop were added to the data file (Appendix III(a)).

PCR products were confirmed visually on a 1.2% agarose gel, and purified using QIAquick PCR purification kit (Qiagen, Valencia, CA). Using the BigDye terminator cycle sequencing ready reaction kit by Applied Biosystems, the sequence was generated with a 7% polyacrylamide gel by an ABI 3100 automated sequencer (Applied Biosystems, Inc; Foster City,

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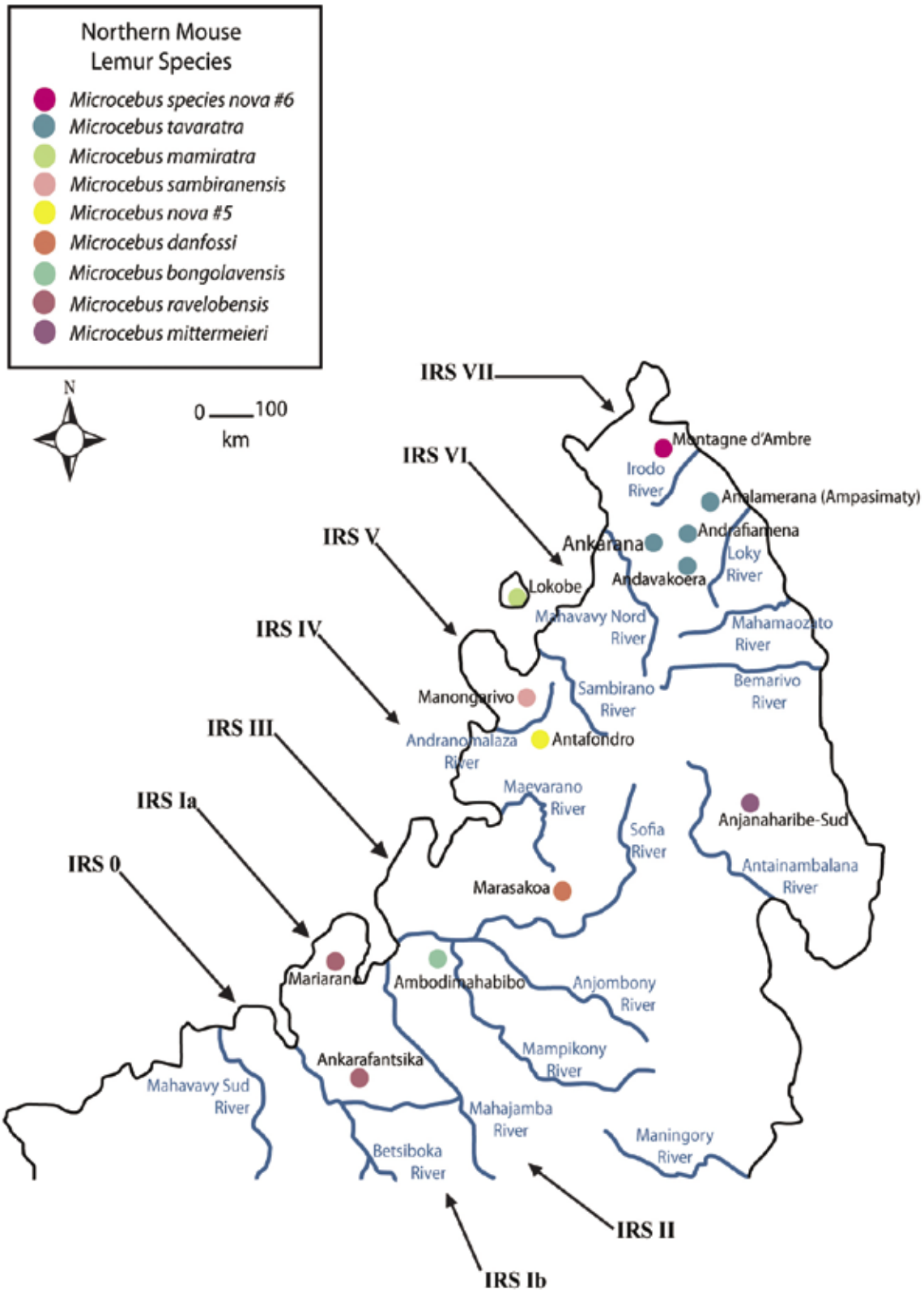


Figure 1. Distribution map of the mouse lemur (genus *Microcebus*) samples of northern and northwestern Madagascar. Each sample site is color-coded to a specific *Microcebus* species. The Inter-River-System (IRS) data is based on Olivieri *et al*. (2007).

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CA). The sequence fragments were aligned to generate a consensus sequence using Sequencher (Gene Corp; Ann Arbor, MI), 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 (see Appendix I(a)). The sequence alignments for the data sets are available from the first author upon request.

Phylogenetic analysis

To examine the genetic diversity of the mouse lemurs of the northern region of Madagascar, maximum-parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) analyses were implemented for the D-loop and PAST, and combined (D-loop//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 D-loop/PAST combined sequence files, respectively, with 10 random addition heuristic searches per replicate 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). The stepwise addition option was selected for MP 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 best-fit model selected by the hierarchical likelihood ratio test (hLRT) in ModelTest3.5 (Posada and Crandall 1998). The best-fit model selected by the hLRT criteria was the TrN+I+G model [(0.2750 0.0996 0.2552), Nst=6, Rmat=(1.0000 13.5199 1.0000 1.0000 8.4486), Gamma=1.0731, Pinvar=0.4333]. 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 D-loop and PAST fragments.

Bayesian inference analyses were conducted using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The model of evolution was selected by using MrModeltest 2.2, a modified version of Modeltest 3.6

Table 1. Samples (27 total) from free-ranging mouse lemurs (*Microcebus*) used in this study. MtDNA sequence data for each mouse lemur sample are available from GenBank under the listed accession numbers. The TK number is the catalogue of the paratype DNA sample stored at the Museum of Texas Tech University, Lubbock, Texas. Global Positioning System (GPS) shows the site where the animal was immobilized. The samples not listed in this manuscript are available in Louis *et al*. (2006a) and Andriantompohavana *et al*. (2006).

Accession number	TK Number	Species designation	Location	Global Positioning System (GPS)	D-loop fragment	PAST fragment
FIA5.30		*Microcebus tavaratra*	Andrafiamena (Anjakely)	S12°54'52.0" – E049°18'49.6"	DQ534961	DQ534992
MATY5.22		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°45'56.0" – E049°29'00.5"	DQ534962	DQ534993
MATY5.23		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°45'56.0" – E049°29'00.5"	DQ534963	DQ534994
MATY5.24		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°45'56.0"– E049°29'00.5"	DQ534964	DQ534995
MATY5.25		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°45'09.5" – E049°29'01.4"	DQ534965	EF175219
MATY5.35		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°45'47.3"– E049°29'06.9"	DQ534966	DQ534996
MATY5.38		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°46'10.1" – E049°29'00.5"	DQ534967	DQ534997
MATY5.39		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°46'11.7" – E049°29'03.0"	DQ534968	DQ534998
MATY5.41		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°46'11.7" – E049°29'00.9"	DQ534969	DQ534999
MATY5.43		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°46'18.0" – E049°29'00.9"	DQ534970	DQ535000
MATY5.44		*Microcebus tavaratra*	Analamera (Ampasimaty)	S12°46'18.0" – E049°29'00.9"	DQ534971	DQ535001
KOER6.5		*Microcebus tavaratra*	Andavakoera	S13°07'16.8" – E049°13'42.3"	EF175269	EF175220
AMB5.24		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°31'28.1" – E049°10'22.8"	DQ534972	DQ535002
AMB5.25		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°31'34.1" – E049°10'30.0"	DQ534973	DQ535003
AMB5.26		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°31'05.8" – E049°10'33.0"	DQ534974	DQ535004
AMB5.33		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°30'44.7" – E049°11'23.3"	DQ534975	DQ535005
AMB5.38		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°28'43.7" – E049°12'58.2"	DQ534976	DQ535006
AMB5.39	TK145310	*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°28'43.7" – E049°12'58.2"	DQ534977	DQ535007
AMB5.40	TK145311	*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°30'28.2" – E049°11'38.1"	DQ534978	DQ535008
AMB5.41	TK145312	*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°28'38.2" – E049°13'20.8"	DQ534980	DQ535009
AMB5.42		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°28'40.1" – E049°13'04.1"	DQ534981	DQ535010
AMB5.43		*Microcebus* sp. *nova* #6	Montagne d'Ambre	S12°30'44.6" – E049°11'21.5"	DQ534979	DQ535011
TAFO6.1	TK145314	*Microcebus* sp. *nova* #5	Antafondro (Maromiandra)	S14°02'44.5" – E048°13'23.4"	EF175273	EF175224
TAFO6.2	TK145315	*Microcebus* sp. *nova* #5	Antafondro (Maromiandra)	S14°02'35.7" – E048°13'21.7"	EF175274	EF175225
TAFO6.5		*Microcebus* sp. *nova* #5	Antafondro (Maromiandra)	S14°02'44.5" – E048°13"23.4"	EF175275	EF175226
TAFO6.6		*Microcebus* sp. *nova* #5	Antafondro (Maromiandra)	S14°02'48.8" – E048°13'10.3"	EF175276	EF175227
TAFO6.7		*Microcebus* sp. *nova* #5	Antafondro (Maromiandra)	S14°02'48.7" – E048°13'09.7"	EF175277	EF175228

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(Nylander 2004; Posada and Crandall 1998). A Markov Chain Monte Carlo (MCMC) run with four simultaneous chains and 1,000,000 generations was performed. Every hundredth generation, the tree with the best likelihood score was saved, resulting in 4000 trees. The 4000 trees were condensed in a majority rule consensus tree using PAUP Version 4.0b10 software (Swofford 2001). Branch supports were assigned as posterior probabilities on the consensus tree. The pattern of sequence evolution was estimated by conducting a minimum spanning network generated with the program NETWORK Version 4.11 (Bandelt *et al*. 1999; Forster *et al*. 2001; Gonzales *et al*. 1998) and Arlequin, Version 2.0 (Schneider *et al*. 2000).

As described in Andriantompohavana *et al*. (2006), Davis and Nixon (1992), Wyner *et al*. (1999), Mayor *et al*. (2004), and Louis Jr. *et al*. (2006a, 2006b), we used MacClade 3.01 (Maddison and Maddison 1992) and MEGA version 2.0 (Kumar *et al*. 1993) in a diagnostic search to designate Evolutionary Significant Units (ESU) for the *Microcebus* species using a Population Aggregate Analysis (PAA) of the D-loop (487-531 bp) and PAST (2367 bp) sequence data. In this paper, the current *Microcebus* taxonomy for northern and northwestern Madagascar was examined according to the Phylogenetic Species Concept (PSC) *sensu* (Wheeler and Platnick *et al*. 2000; Louis Jr. *et al*. 2006; Mayor *et al*. 2004). 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 (Andriantompohavana *et al*. 2006; Davis and Nixon 1992; Mayor *et al*. 2004; Louis Jr. *et al*. 2006a, 2006b; Ravaoarimanana *et al*. 2004).

Results

Mitochondrial DNA sequence data were completed for two fragments, D-loop and PAST (approximately 3,000 bp), for 121 individuals, representing all 15 recognized species of mouse lemurs from a total of 32 sites (Figs. 1–4, Appendices II(a–e)). Based on the phylogenetic inferences of the NJ, MP, and ML analyses of three sequence alignments (D-loop, PAST, and combined), the 15 *Microcebus* species were represented in 15 well-supported terminal clades (Figs. 2–4; the newly described species by Radespiel *et al*. (2008) were not included in these analyses since sequence fragments could not be correlated). All three phylogenetic methods corroborate the monophyly of *M. griseorufus* and *M. murinus* and the monophyly of *M. bongolavensis*, *M. danfossi*, and *M. ravelobensis* as presented in Radespiel *et al.* (2008). Additionally, the sister relationship between *M. myoxinus*, *M. berthae*, *M. lehilahytsara*, and *M. rufus* exists with all three methods for the D-loop sequence fragment, but cannot be confirmed for the PAST or D-loop/PAST concatenated due to the unavailability of samples sets for *M. bongolavensis* and *M. danfossi*. The mouse lemur samples from the island of Nosy Be, comprising *Microcebus* sp. *nova* #4 from Louis Jr. *et al*. (2006a), *M. mamiratra* from Andriantompohavana *et al*. (2006), and *M. lokobensis* from the IRS VI in Olivieri *et al*. (2007; Lokobe Special Reserve on Nosy Be and Manehoka from mainland Madagascar) were found to form a single terminal clade (Figs. 1 and 2). The minimum spanning network for the *Microcebus* D-loop haplotypes reveal a similar evolutionary pattern as the three phylogenetic methods (Fig. 5). Interestingly, *Microcebus jollyae*, an east coast reddish morph, is aligned intermediately between the *M. griseorufus* and *M. murinus* group, west coast gray forms, and the *M. mittermeieri* and *M. simmonsi*, east central coast reddish morphs. The samples from Nosy Be, representing the two described species, *M. mamiratra* and *M. lokobensis*, along with the samples from Manehoka (mainland Madagascar), clustered together as one well-supported terminal clade. Furthermore, all three phylogenetic methods support two distinct subpopulations, *Microcebus* sp. *nova* #5 (Antafondro) and *Microcebus* sp. *nova* #6 (Montagne d'Ambre; Figs. 1–4; Appendices II(a–e)).

A review of the morphometric data for 13 described species of mouse lemurs are presented in Table 2 (detailed morphological measurements of the novel individual mouse lemurs are available in Appendix I(b)). No extensive quantitative analyses were conducted on the morphometric data. Inherent inconsistencies found or produced within morphologic data sets prevent a statistically reliable conclusion. Numerous factors such as small sample sets, independent data sets, multiple data collectors, the variance between live, sedated individuals versus processed museum vouchers, along with seasonal and age differences of individual mouse lemurs, currently restrict any comprehensive analysis of the genus *Microcebus*. With that said, this morphometric information is provided as supplemental data, only complementing the partitioning of unique biodiversity (Table 2).

The results from the population aggregate analysis of the D-loop and PAST sequence data are presented in Tables 3 and 4, respectively (Appendices III(b–e)). Multiple diagnostic characters distinguish each established *Microcebus* species, along with *Microcebus* sp. *nova* #5 and *Microcebus* sp. *nova* #6 at Antafondro and Montagne d'Ambre, respectively (Tables 3 and 4; Appendices III(b–c)). *Microcebus* sp; *nova* #5 had seven diagnostic sites, whereas *Microcebus* sp. *nova* #6 had nine. The complete uncorrected 'p' distance and the Kimura two-parameter distance measures are presented in Tables 5a and 5b. The absolute pairwise distances generated between undefined terminal clades and described mouse lemur species corresponds to the observed interspecific values found between described species (Andriantompohavana *et al*. 2006; Louis Jr. *et al.* 2006a; Olivieri *et al*. 2007). Although the absolute pairwise distance between *M. mamiratra* and *Microcebus* sp. *nova* #5 is the smallest percentage between the terminal clades, the geographic distance between sampling sites is also reduced (Appendix II(h)). Values ranged mostly from 10% to 15% with the lowest percentage found between *Microcebus* sp. *nova* #5 and *M. mamiratra* (4.9% and 2.5%, D-loop and PAST, respectively) and the highest percentage was found between *M. ravelobensis* and *M. jollyae* (24.3% and 10.7%, D-loop and PAST, respectively).

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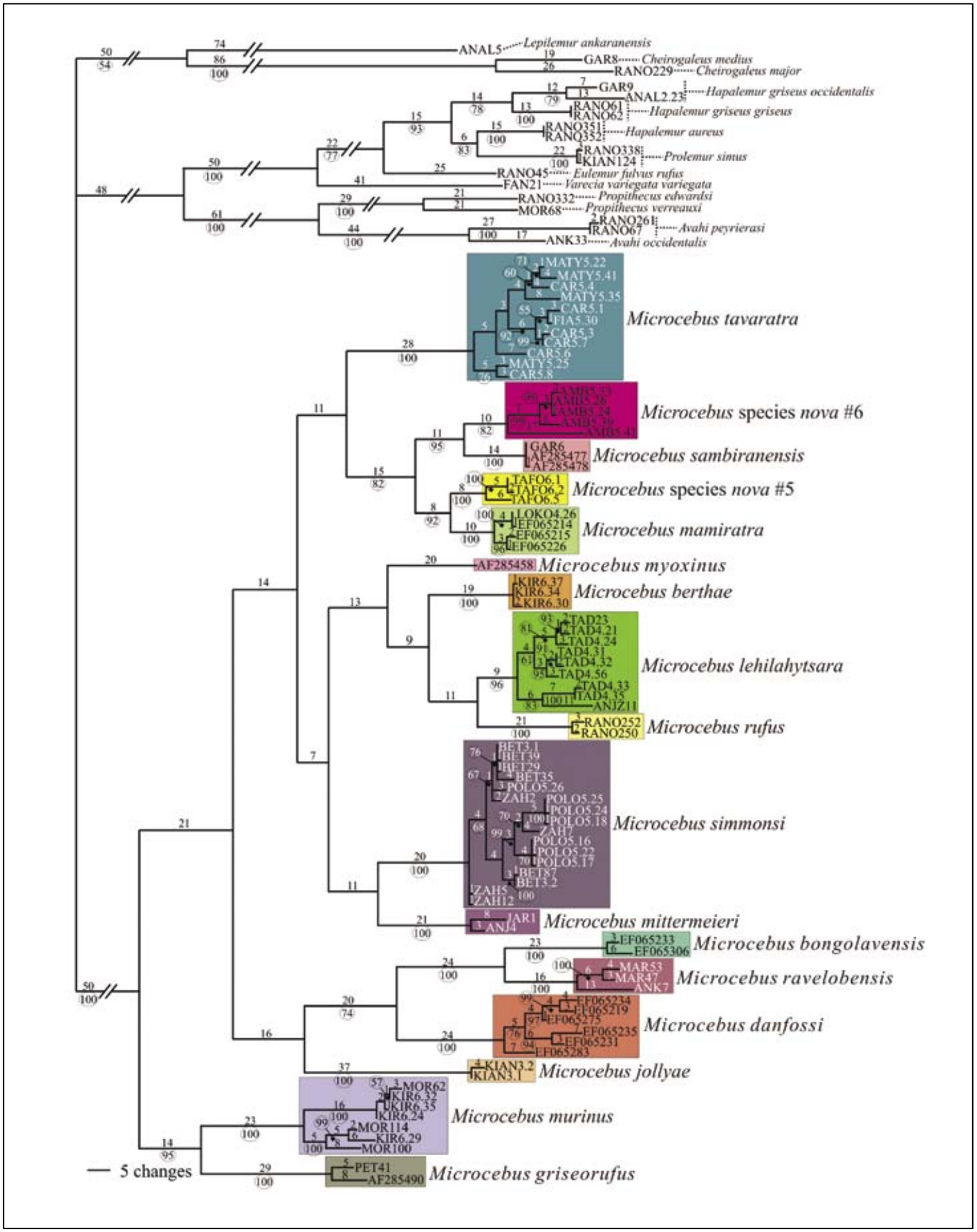


Figure 2. Neighbor-joining phylogram derived from the D-loop DNA sequence data from 82 *Microcebus* individuals with 18 out-group taxa. Species designated according to the distribution in the current literature (Andriantompohavana *et al*. 2006; Louis Jr. *et al*. 2006a; Mittermeier *et al*. 2006; Olivieri *et al*. 2007). Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates.

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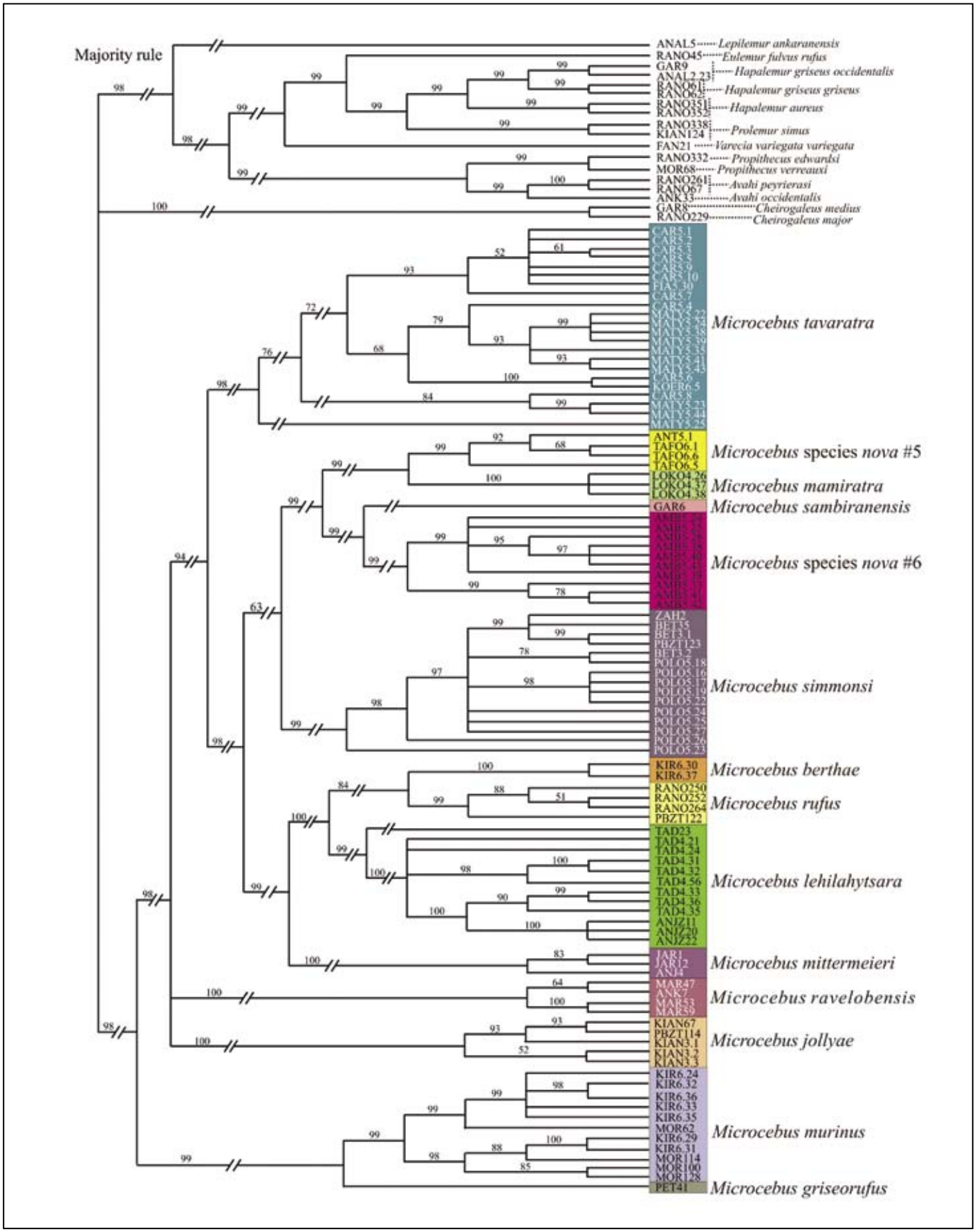


Figure 3. Fifty percent majority-rule consensus phylogenetic tree from the Bayesian analysis derived from the PAST sequence data from 97 haplotypes from 121 *Microcebus* individuals with 18 out-group taxa reconstructed using the computer program package MrBayes. Branches without posterior probability values are supported by less than 50% of the sampled trees.

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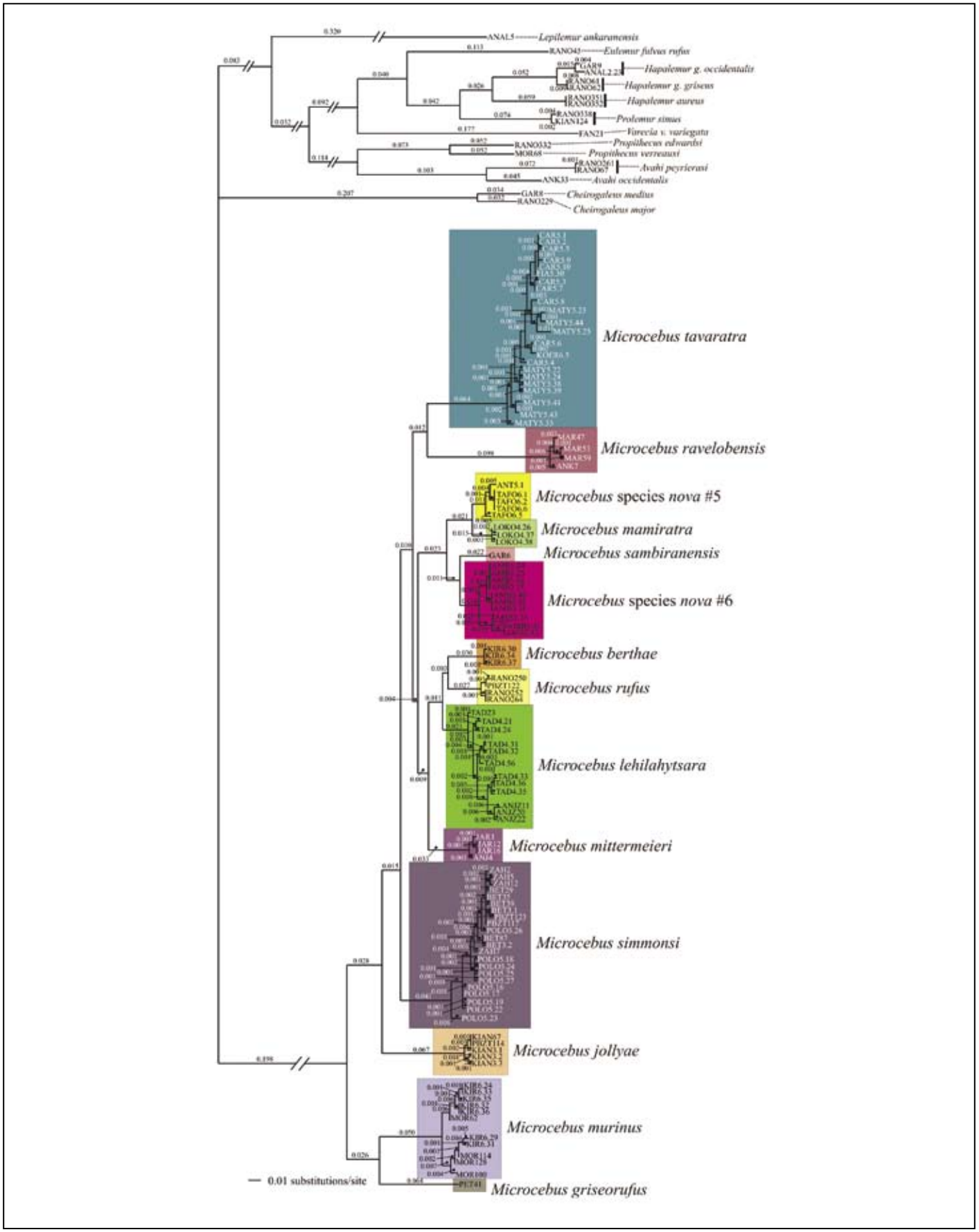


Figure 4. Maximum-likelihood phylogram derived from concatenated D-loop and PAST sequence data from 107 *Microcebus* haplotypes with 18 out-group taxa. The phylogram is presented with branch lengths proportional to the number of changes (values specified on the branches). We obtained the maximum likelihood phylogram (-ln likelihood = 4921.54) from the D-loop and PAST concatenated alignment (K = 7) and γ shape parameter of 1.07.

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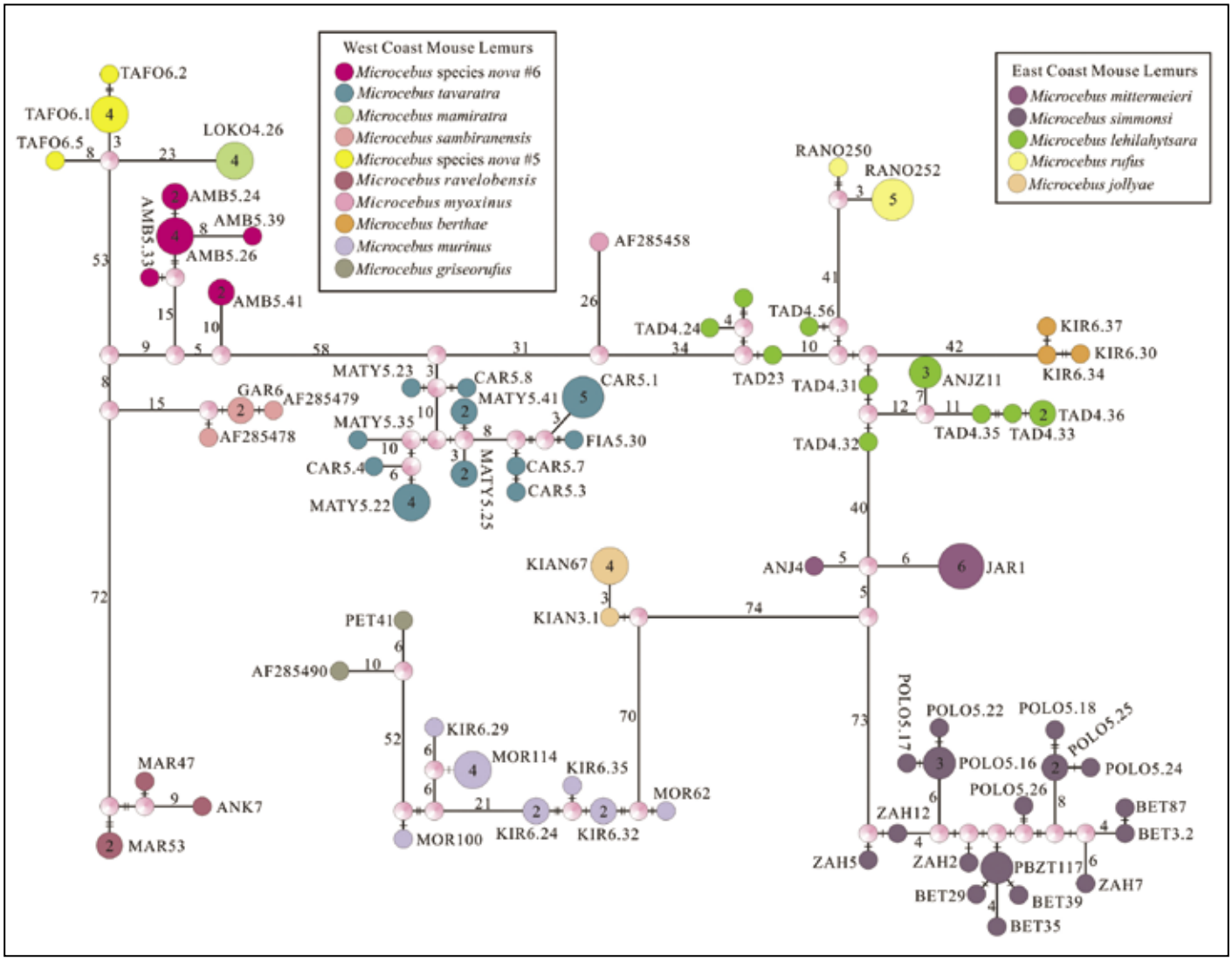


Figure 5. Minimum spanning network of *Microcebus* D-loop haplotypes calculated using Arlequin 2.0 and Network 4.11. Identification numbers denote unique haplotypes. The minimum number of mutational steps separating matriarchal lines is indicated above the branches. Nucleotide substitutions are indicated by dashes. The number of nucleotide differences (more than two) in their connecting lines of the network is indicated by the number at each connecting link. Missing intermediates are indicated by conical pink circles. The size of circles approximates the number of individuals with matching haplotypes corresponding to information in Appendix III(d) (circles without any number represent one individual).

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Table 2. Morphometric data collected from sedated *Microcebus* individuals. (Individual morphological data available online; see Appendix I). Morphological data taken from immobilized animals.

Species	Common name	N	Weight (gm)	Head crown (cm)	Body length (cm)	Tail length (cm)
*Microcebus berthae**	Berthe's mouse lemur	3	30.6±0.6	N/A	9.2±0.3	N/A
Microcebus berthae	Berthe's mouse lemur	3	21.1±1.3	2.8±0.0	6.2±0.3	11.6±0.3
*Microcebus sambiranensis**	Sambirano mouse lemur	6	44.1±5.9	N/A	11.7±0.4	N/A
Microcebus sambiranensis	Sambirano mouse lemur	1	48.0	2.6	8.3	14.0
Microcebus mamiratra	Claire's or Nosy Be 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
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±6.3	N/A	12.4±0.5	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' 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±0.6	N/A
Microcebus griseorufus	Reddish grey mouse lemur	3	43.7±3.1	3.3±0.1	8.7±0.4	13.9±1.6
Microcebus rufus	Brown or rufous mouse lemur	15	43.7±4.2	3.3±0.1	8.6±0.3	11.7±0.8
*Microcebus tavaratra**	Northern rufous mouse lemur	6	61.1±N/A	N/A	12.6±N/A	15.5±N/A
*Microcebus tavaratra**	Northern rufous mouse lemur	20	52.3±7.2	3.4±0.3	9.0±0.8	14.6±1.0
Microcebus sp. *nova* #5	-	10	41.0±14.0	3.1±0.4	7.4±1.8	13.2±2.2
Microcebus sp. *nova* #6	-	6	49.7±18.0	3.1±0.2	8.2±1.1	12.1±1.5

*Head and body length measurements are taken from Rasoloarison *et al*. (2000). Head crown is the total length from tip of the nose (soft tissue of the nose is not included) to the occipital crown (±0.1 cm); body length is from the occipital condyle to the base of the tail (±0.1 cm), and the tail length is from the base of the tail to the last caudal vertebra (±0.1 cm). All values (±) calculated as standard deviation.
**The data include mouse lemurs that are considered juveniles.

Table 3. Summary of Population Aggregate Analysis (PAA) D-Loop diagnostic sites for the genus *Microcebus*. Refer to Appendix III(b).

Species	Fragment size (bp)	PAA base pair location
M. tavaratra	515	367, 513, 514, 515, 517
M. ravelobensis	520	26, 146, 160, 161, 162, 166, 170, 171, 172, 173, 257, 261, 265, 266, 267, 268, 271, 272, 273, 274, 278, 279, 290, 294, 303, 306, 307, 311, 399, 401, 411, 446, 456, 476, 480, 481, 483, 484, 488, 490, 491, 493, 500, 501, 502, 509
M. sp. *nova* #5	490	490
M. sambiranensis	513-514	246, 281, 434, 523
M. sp. *nova* #6	515	476
M. mamiratra	487	199, 478, 481
M. berthae	521	73, 158, 506, 516
M. murinus	527-531	150, 158, 163, 164, 244, 245, 429, 497, 503
M. rufus	522	123, 244, 308, 356, 494
M. simmonsi	489	188, 189, 190, 191, 192, 198, 199, 200, 201, 202, 203, 204, 253, 337, 439, 480, 482
M. mittermeieri	518	124, 238, 349, 503, 522
M. jollyae	518	166, 190, 194, 195, 299, 327, 331, 418, 419, 475, 486, 487, 505, 508, 522
M. lehilahytsara	522	*
M. griseorufus	526	42, 149, 158, 192, 195, 220, 244, 325, 339, 438, 506, 517
M. myoxinus	520	122, 222, 289

*No character or attribute is available for this fragment.

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Table 4. Summary of Population Aggregate Analysis (PAA) Pastorini fragment diagnostic sites for the genus *Microcebus*. Refer to Appendix III(c).

Species	Fragment size (bp)	PAA base pair location
M. tavaratra	2366	111, 134, 238, 834, 1062, 1218, 1266, 1290, 1291, 1303, 1349,1354, 1355, 1366, 1399, 1551, 1566, 1590, 1593, 1596, 1614, 1644, 1650, 1659, 1764, 1848, 1854, 1866, 1893, 2067, 2154, 2273
M. ravelobensis	2366	133, 143, 187, 211, 226, 313, 317, 335, 365, 376, 379, 525, 538, 559, 562, 598, 632, 715, 721, 779, 916, 918, 930, 990, 1121, 1170, 1186, 1258, 1260, 1321, 1434, 1956, 2031, 2034, 2037, 2040, 2088, 2175, 2238, 2259
M. sp. *nova* #5	2366	380, 814, 864, 1291, 1632, 1785
M. sambiranensis	2366	561, 658, 682, 763, 2307
M. sp. *nova* #6	2366	310, 503, 1479, 1491, 1898, 1992, 2001, 2243
M. mamiratra	2367	340, 671, 742, 1074, 2125, 2292
M. berthae	2366	907, 921, 1317, 1435, 1488, 1521, 1705, 1998, 2097, 2235
M. murinus	2366	46, 202, 304, 502, 506, 507, 546, 601, 652, 742, 743, 745, 749, 771, 790, 870, 943, 993, 1017, 1029, 1075, 1098, 1141, 1206, 1221, 1316, 1358, 1434, 1509, 1836, 1981, 1991, 2004, 2046, 2097, 2295, 2322
M. rufus	2366	103, 283, 376, 450, 872, 971, 1008, 1197, 1230, 1341, 1419, 1617, 1668, 2111
M. simmonsi	2367	172, 403, 449, 577, 613, 656, 868, 1639, 1818, 1824, 1920, 2229
M. mittermeieri	2366	274, 704, 1092, 1114, 1176, 1315, 1503, 1803, 1905, 1953, 1982, 1983, 2086, 2229
M. jollyae	2367	47, 82, 84, 121, 139, 187, 377, 436, 476, 495, 526, 566, 569, 739, 891, 923, 999, 1107, 1221, 1245, 1300, 1342, 1716, 1905, 1965, 1989, 2070, 2121, 2241, 2308
M. lehilahytsara	2366	14, 337, 1356, 1562
M. griseorufus	2366	115, 290, 366, 546, 574, 592, 604, 617, 643, 646, 672, 742, 771, 784, 827, 844, 873, 993, 1005, 1039, 1054, 1068, 1074, 1089, 1318, 1357, 1365, 1431, 1485, 1536, 1540, 1545, 1551, 1582, 1584, 1596, 1600, 1618, 1710, 1737, 1749, 1809, 1827, 1933, 2025, 2085, 2233, 2249

Table 5a. Genetic distance matrix for D-loop sequence data for the genus *Microcebus*. 1. *M. tavaratra*; 2. *M. ravelobensis*; 3. *M.* sp. *nova* #5; 4. *M. sambiranensis*; 5. *M.* sp. *nova* #6; 6. *M. mamiratra*; 7. *M. berthae*; 8. *M. murinus*; 9. *M. rufus*; 10. *M. simmonsi*; 11. *M. mittermeieri*; 12. *M. jollyae*; 13. *M. lehilahytsara*; 14. *M. griseorufus*; and 15. *M. myoxinus*. Genetic distance based on absolute differences is displayed above the diagonal, and genetic distance as a percentage is displayed below the diagonal.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		111	73	81	91	72	80	114	80	98	84	101	85	94	69
2	19.1±2.2		95	93	109	88	107	128	105	107	104	116	122	105	102
3	10.2±1.5	20.3±2.4		42	57	23	52	89	55	80	58	75	73	75	61
4	12.5±1.7	20.2±2.4	8.8±1.4		53	46	69	98	66	80	68	88	83	84	68
5	11.0±1.5	20.0±2.5	7.9±1.3	6.4±1.2		60	86	115	82	92	80	94	93	98	82
6	10.4±1.4	19.8±2.4	3.7±1.0	9.6±1.5	8.4±1.4		57	90	56	78	62	72	74	73	53
7	11.3±1.7	20.6±2.4	10.1±1.6	13.1±1.6	13.2±1.7	10.6±1.6		97	52	84	58	91	66	79	48
8	17.0±2.0	22.9±2.6	15.4±1.9	17.0±1.9	17.8±2.1	15.0±1.8	15.2±1.8		103	94	106	93	119	74	110
9	11.0±1.6	19.8±2.6	11.2±1.8	13.1±2.0	13.0±2.0	10.5±1.7	10.2±1.9	15.6±2.0		79	51	78	63	82	45
10	15.0±2.0	21.1±2.4	14.8±2.3	14.8±2.1	14.1±2.1	14.1±2.2	15.2±2.0	15.2±1.8	13.8±2.0		77	79	92	76	94
11	12.5±1.7	20.7±2.7	11.8±1.7	13.2±1.9	13.5±1.9	12.4±1.8	10.6±1.6	18.0±2.1	8.5±1.5	14.6±2.1		80	65	81	60
12	16.2±1.7	24.3±2.7	15.9±2.0	16.7±2.1	15.1±1.8	14.9±2.0	16.7±2.1	14.6±2.0	13.7±1.8	14.4±1.9	13.9±1.6		95	72	76
13	10.0±1.4	20.9±2.3	10.4±1.5	12.5±1.8	13.1±1.8	10.4±1.6	8.5±1.4	16.6±2.0	8.2±1.4	13.6±1.8	9.4±1.4	14.1±1.7		101	66
14	15.4±1.9	21.9±2.6	16.1±2.0	17.2±2.2	16.2±2.1	16.2±2.0	14.7±2.0	10.5±1.4	15.8±2.2	15.7±2.0	16.4±2.2	14.1±2.0	16.2±2.1		91
15	8.7±1.5	19.7±2.3	11.5±1.8	12.9±1.8	12.9±1.8	10.1±1.6	7.4±1.3	16.6±2.0	8.3±1.6	14.7±1.9	11.8±2.0	13.9±1.9	8.0±1.3	15.4±1.9	

Table 5b. Genetic distance matrix for PAST fragment sequence data for the genus *Microcebus*. 1. *M. tavaratra*; 2. *M. ravelobensis*; 3. *M.* sp. *nova* #5; 4. *M. sambiranensis*; 5. *M.* sp. *nova* #6; 6. *M. mamiratra*; 7. *M. berthae*; 8. *M. murinus*; 9. *M. rufus*; 10. *M. simmonsi*; 11. *M. mittermeieri*; 12. *M. jollyae*; 13. *M. lehilahytsara*; and 14. *M. griseorufus*. Genetic distance based on absolute differences is displayed above the diagonal, and genetic distance as a percentage is displayed below the diagonal.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		243	227	178	233	220	200	324	200	220	194	228	227	245
2	10.6±0.7		242	216	240	229	209	304	222	235	201	234	246	276
3	9.7±0.7	11.1±0.8		104	134	54	163	259	162	181	164	217	171	197
4	9.7±0.7	10.5±0.8	5.1±0.4		50	114	148	215	153	121	142	187	119	247
5	9.2±0.7	10.2±0.7	5.1±0.5	3.5±0.4		130	170	281	170	182	157	220	183	236
6	9.6±0.6	10.5±0.8	2.5±0.3	5.2±0.5	5.0±0.4		154	262	154	174	151	207	171	255
7	8.6±0.6	9.5±0.7	7.4±0.6	6.7±0.6	6.8±0.6	7.1±0.6		271	94	171	115	187	113	258
8	14.0±0.8	13.3±0.8	11.1±0.7	11.0±0.7	11.4±0.8	11.4±0.8	12.0±0.8		277	277	270	272	277	181
9	8.5±0.6	10.1±0.7	7.3±0.6	7.0±0.6	6.7±0.6	7.0±0.6	4.2±0.4	12.1±0.8		168	124	191	114	251
10	9.1±0.7	10.2±0.7	7.9±0.6	7.2±0.6	6.9±0.5	7.6±0.6	7.3±0.6	11.7±0.8	7.1±0.6		159	202	177	225
11	8.3±0.6	9.1±0.6	7.4±0.6	6.5±0.6	6.2±0.6	6.9±0.6	5.2±0.5	11.9±0.8	5.5±0.5	6.8±0.6		189	137	252
12	10.0±0.7	10.7±0.6	10.0±0.7	8.7±0.6	9.2±0.6	9.6±0.7	8.6±0.6	11.9±0.8	8.7±0.6	8.8±0.6	8.6±0.7		205	255
13	9.0±0.6	10.5±0.7	7.1±0.6	6.9±0.6	6.7±0.6	7.1±0.6	4.3±0.4	11.5±0.7	4.3±0.4	6.6±0.5	5.4±0.4	8.7±0.6		228
14	13.4±0.8	13.6±0.9	12.4±0.9	11.5±0.8	12.0±0.8	12.1±0.8	12.2±0.9	9.2±0.7	11.8±0.9	12.1±0.8	12.0±0.9	12.1±0.8	12.2±0.8	

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Discussion

The persistent and rapid loss of habitat and the resulting fragmentation of panmictic populations have compelled wildlife and conservation agencies to define management decisions according to existing guidelines and data with the ultimate goal of prioritizing species and/or sites (Wilmé *et al*. 2006; Kremen *et al*. 2008). Many studies have shown that molecular genetics technology offers a reliable and rapid method of identifying unique and cryptic biodiversity (Louis Jr. *et al*. 2006a; Olivieri *et al*. 2007; Radespiel *et al*. 2008). With this in mind, we present another revision of the genus *Microcebus*, concentrating on the biogeographic distribution of the mouse lemurs in northern and northwestern Madagascar. Through the analyses of accessioned and novel sample sets, we found that each described mouse lemur clusters in distinct and well-supported terminal clades.

Since Radespiel *et al.* (2008) demonstrated the same result with an alternative data set, a singular terminal clade for both described mouse lemur species from the island of Nosy Be, we have established that *Microcebus mamiratra* has precedence over *M. lokobensis* Andriantompohavana *et al*. 2006, which should consequently be regarded as a junior synonym. Furthermore, the distribution of *M. mamiratra* not only extends throughout the island of Nosy Be, but also exists on mainland Madagascar, occupying IRS VI (Olivieri *et al*. 2007; see Fig. 1).

In addition to the well-supported terminal clades of the 15 acknowledged mouse lemur species, the data revealed a distinct clade for the mouse lemur initially proposed in Andriantompohavana *et al*. (2006) at Antafondro Classified Forest, and also showed a remarkable cryptic diversity from Montagne d'Ambre National Park (Figs. 2–4). Three main criteria provide support for the definition of the two new species indicated, as follows: molecular genetic parameters, geographic and topographic barriers, and relative partitions between species.

By providing the initial criterion for the justification of species-level status for the two undefined mouse lemur taxa, molecular genetic data and inference offers the first line of argument. According to the Phylogenetic Species Concept (PSC) *sensu* Wheeler and Platnick (2000; Groves 2001; Louis Jr. *et al*. 2006a), diagnostic characters or attributes define Evolutionary Significant Units (ESUs). Several authors suggest that ESUs are equivalent to species and reflect species barriers (Cracraft 1983). Given this criterion, the two undefined species had multiple molecular diagnostic sites (Tables 3 and 4). The constant addition of samples to the PAA data set will continue to test the distinction and diagnostic ability of these characters; and, therefore, the ongoing status of each species.

The second line of argument is as follows. The two undefined mouse lemur taxa, *Microcebus* sp. *nova* #5 and *Microcebus* sp. *nova* #6, have distributions defined by geographic and topographic barriers. Following the initial proposal in Andriantompohavana *et al*. (2006), *Microcebus* sp. *nova* #5 is bounded by the Andranomalaza River to the northwest, the Sambirano River to the northeast, and the Maevarano River to the south (Fig. 1). Although the Andranomalaza River does not meet the large river criterion (>50 m wide, 20 km inland), geographic barriers in combination with the small size of mouse lemurs and limited dispersal ability essentially could drive allopatric speciation (Wilmé *et al*. 2006). With the distribution of *M. mamiratra* extended to mainland Madagascar (directly east of the island of Nosy Be in IRS VI), the topographic presence of Tsaratanana, one of the three mountains in Madagascar with an altitude above 2,000 m, could create a significant geographic barrier to *Microcebus* sp. *nova* #5 just north of the Sambirano River. *Microcebus* sp. *nova* #6 is found in the montane rainforest of Montagne d'Ambre National Park, north of the Irodo River. Ankarana National Park and Analamerana Special Reserve establish the southern boundary to this undefined mouse lemur's range. As a limestone plateau and tsingy formation intermixed with dry deciduous forest, Ankarana and Analamerana could be acting as a significant barrier to dispersal (Fig. 1). Additionally, the Bobakindro River courses along the northern margin of Analamerana Special Reserve. Again, the Irodo and Bobakindro Rivers do not meet the criterion of a major river barrier, however the topographic features and habitat differences offer strong support for the uniqueness of this undefined species.

Third, each undefined mouse lemur is found paired geographically (smallest geographic distance) with a defined species that is also segregated by an Inter-River-System but is not its genetically most proximal sister taxon (Fig. 1). All three phylogenetic analyses, along with the spanning network, demonstrated the phylogenetic proximity between *Microcebus* sp. *nova* #5 and *M. mamiratra*, on the one hand, and *Microcebus* sp. *nova* #6 and *M. sambiranensis*, on the other. With *Microcebus* sp. *nova* #5 at Antafondro, the distribution of *M. sambiranensis* would be limited to the Manongarivo Special Reserve, north of the Andranomalaza River and south of the Sambirano River, placing its range in between the distribution of *M. mamiratra* and the undefined species. Similarly, the distribution of *M. tavaratra* in Ankarana, Andrafiamena, Analamerana, and Andavakoera and *M. mamiratra* in Manehoka provide a significant species barrier between *M. sambiranensis* and its genetically closest sister taxa *Microcebus* sp. *nova* #6.

Species Descriptions

Microcebus margotmarshae new species

Formerly *Microcebus* sp. *nova* #5; initially proposed in Andriantompohavana *et al*. (2006). See Fig. 6, Appendix II(f).

Holotype. TAFO6.1; adult female captured in Antafondro Classified Forest on 21 May 2006. Material: Total genomic DNA (50 ng/µl) for TAFO6.1 (Bar Code 145314), adult female. Total genomic DNA materials are stored and curated at the Museum of Texas Tech University, Lubbock,

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Texas, USA. Two 2.0-mm biopsies from ear pinna tissue are stored at Henry Doorly Zoo, Omaha, Nebraska, USA. A microchip pit tag was placed subcutaneously between scapulas and recorded as 4722607B5D. TAFO6.1 was collected by Francois Randrianasolo, Richard Rakotonomenjanahary, Jean Amié Andriamihaja, and Rambinintsoa Andriantompohavana on 21 May 2006.

Paratypes. TAFO6.2 (Bar Code 145315), adult female and ANT5.1 (Bar Code 145313), adult male; captured in Antafondro Classified Forest. Total genomic DNA (50 ng/µl) TAFO6.2 (Bar Code 145315), adult female; and ANT5.1 (Bar Code 145313), adult male; are stored and curated at the Museum of Texas Tech University, Lubbock, Texas, USA. Two 2.0-mm biopsies from ear pinna tissues are stored at Henry Doorly Zoo, Omaha, Nebraska, USA. Individual measurements, e-voucher photos, and collection data are given in Appendix I(b) and are available at the Museum of Texas Tech University, Lubbock, Texas, USA. Francois Randrianasolo, Richard Rakotonomenjanahary, Jean Amié Andriamihaja, and Rambinintsoa Andriantompohavana collected TAFO6.2 and ANK5.1 on 21 May 2006 and 4 October 2005, respectively.

Type Locality. Madagascar: Province de Antsiranana, Antafondro Classified Forest Special Reserve (approximately 14°02'44.5"S, 48°13'23.4"E, 134 m above sea level).

Measurements of holotype. Recorded in the field catalog on 21 May 2006: weight: 49.0 g; head crown: 3.2 cm; body length: 8.4 cm; tail length: 14.3 cm; muzzle length: 9.5 mm; ear length: 15.4 mm; and ear width: 8.7 mm.

Description. *Microcebus margotmarshae* is a small mouse lemur (41.0 g). The dorsal and tail pelage is predominantly reddish-orange with gray undertones, (Fig. 6; Appendix II(g)). The ventral fur is white to cream. The head is largely bright reddish-orange. The ears are small. The muzzle and the area surrounding the eyes are light brown, and there is a small, bright white spot on the nose ridge between the eyes.

Diagnosis. In the D-loop and PAST sequence fragments, *M. margotmarshae* differs from its closest relatives, *M. tavaratra*, *M. sambiranensis*, *M. mamiratra* and *M. arnholdi*, by both genetic and geographic distance by 12.3% ±1.6% (73 informative sites), 9.5%±1.4% (42 informative sites), 4.9%±1.0% (23 informative sites) and 9.5%±1.3% (57 informative sites); 9.7%±0.7% (227 informative sites), 5.1%±0.5% (132 informative sites), 2.5%±0.3% (54 informative sites) and 5.1%±0.5% (134 informative sites), respectively. Even though *M. margotmarshae* is a rufous-type mouse lemur as *M. mamiratra* (genetically the closest related), *M. margotmarshae* (41.0 gm) is significantly smaller than *M. mamiratra* (60.8 gm).

Distribution. *Microcebus margotmarshae* is known from the Antafondro Classified Forest, south of the Andranomalaza River and north of the Maevarano River, Madagascar.

Comparisons and remarks. Andriantompohavana *et al*. (2006) proposed that the mouse lemurs from Antafondro Classified Forest should be considered a separate species (*Microcebus* sp. *nova* #5), based on the PAST sequence fragment from one individual that was included in the analyses (Table 3 and 4; Appendix III(b–c)). Of the recognized mouse lemurs that are in the adjacent regions of Madagascar, *Microcebus margotmarshae* (41.0 gm) is approximately the same size as *M. sambiranensis* (44.0 gm), but smaller than *M. mamiratra* (60.8 gm), *M. tavaratra* (52.3 gm), and *M. ravelobensis* (65.9 gm). Additional samples from the entire region south of the Andranomalaza River and north of the Maevarano River are needed to define the distribution of *M. margotmarshae*. Olivieri *et al*. (2007) presented the course of the Maevarano River in an east to west direction, when, in fact, this river travels in more of a northwest to southeast direction, increasing as such the size of IRS V (Fig. 1). Samples should be collected from mouse lemurs from Tsaratanana Special Reserve. It is possible that mouse lemurs can be found at high altitudes there.

Etymology. *Microcebus margotmarshae* is named in honor of the late Margot Marsh, who during her lifetime contributed very generously to primate conservation initiatives in many different countries, including the publication of the first edition of the field guide *Lemurs of Madagascar* in 1994 (Mittermeier *et al*. 1994). The Margot Marsh Biodiversity Foundation was created after her death in 1995, thus continuing support for efforts that help safeguard the future of threatened primates.

Vernacular names. Margot Marsh's mouse lemur or Antafondro mouse lemur.

Microcebus arnholdi new species

Formerly *Microcebus* sp. *nova* #6 (Fig. 7, Appendix II(g)).

Holotype. AMB5.39; adult female; collected on 27 November 2005, captured at Montagne d'Ambre National Park. Material: Total genomic DNA (50 ng/µl) for AMB5.39 (Bar Code 145310), adult female stored and curated at the Museum of Texas Tech University, Lubbock, Texas, USA. Two 2.0-mm biopsies from ear pinna, and 0.07 cc of whole blood tissues stored at Henry Doorly Zoo, Omaha, Nebraska, USA. A microchip pit tag was placed subcutaneously between the scapulas and recorded as 4657027B18. AMB5.39 was collected by Richard Randriamampionona, Richard Rakotonomenjanahary, Jean Amié Andriamihaja, Fidelis Razafimananjato Tsirivaliniaina, John R. Zaonarivelo, and Edward Louis Jr. on 27 November 2005.

Paratypes. AMB5.40 (Bar Code 145311), adult female; and AMB5.43 (Bar Code 145312), adult female; captured at Montagne d'Ambre National Park. Material: Total genomic DNA (50 ng/µl) for each are stored and curated at the Museum of Texas Tech University, Lubbock, Texas, USA. Two 2.0-mm biopsies from ear pinna, and 0.07 cc of whole blood tissues stored at Henry Doorly Zoo, Omaha, Nebraska, USA. Individual measurements, e-voucher photos, and collection data are given in Appendix I(b) and are available at the Museum of Texas Tech University, Lubbock, Texas, USA. Richard Randriamampionona, Richard Rakotonomenjanahary, Jean Amié

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Figure 6. *Microcebus margotmarshae*, Margot Marsh's or Antafondro mouse lemur, at Antafondro Classified Forest (Maromiandra). Photo by Rambinintsoa Andriantompohavana.

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Figure 7. *Microcebus arnholdi*, Arnhold's or Montagne d'Ambre mouse lemur, at Montagne d'Ambre National Park and Classified Forest. Photo by Edward E. Louis Jr.

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Andriamihaja, Fidelis Razafimananjato Tsirivaliniaina, John R. Zaonarivelo, and Edward Louis Jr. collected AMB5.40 and AMB5.43 on 28 November 2005.

Type Locality. Madagascar: Province de Antsiranana, Montagne d'Ambre National Park and Montagne d'Ambre Special Reserve (approximately 12°31'28.1"S; 049°10'22.8"E, 990 m above sea level).

Measurements of holotype. AMB5.39; adult female. Recorded in the field catalog on 21 November 2005. Weight 71.0 grams; head crown 3.3 cm; body length 8.1 cm; tail length 12.9 cm; muzzle length 9.4 mm; ear length 17.8 mm; and ear width 10.1 mm.

Description. *Microcebus arnholdi* is a medium-sized mouse lemur (49.7 gm). The overall dorsal pelage is a mixture of dark brown, red and gray (Fig. 7; Appendix II(g)). There is a dark brown midline dorsal stripe that runs down to the base of the tail. The tail is dark brown near the tip. The ventral fur is white to cream, with gray undertones. The head is predominately red, with dark brown on the muzzle and surrounding the eyes and with a white nose ridge that stops at the distal end of the muzzle. The ear length of *M. arnholdi* (17.5±0.4 mm) is smaller than *M. tavaratra* (21.7±0.7 mm).

Diagnosis. In the D-loop and PAST sequence fragments, *M. arnholdi* differs from its closest relatives, *M. tavaratra*, *M. sambiranensis*, *M. mamiratra* and *M. margotmarshae*, in both genetic and geographic distance, by 12.6%±1.5% (91 informative sites), 6.9%±1.1% (53 informative sites), 9.6%±1.3% (60 informative sites) and 9.5%±1.3% (57 informative sites); 9.2%±0.7% (233 informative sites), 3.5%±0.4% (113 informative sites), 5.0%±0.5% (151 informative sites) and 5.1%±0.5% (134 informative sites, respectively). Of the recognized mouse lemurs that are in the adjacent regions of Madagascar, *Microcebus arnholdi* (49.7 gm) is smaller than *M. tavaratra* (52.3 gm), and

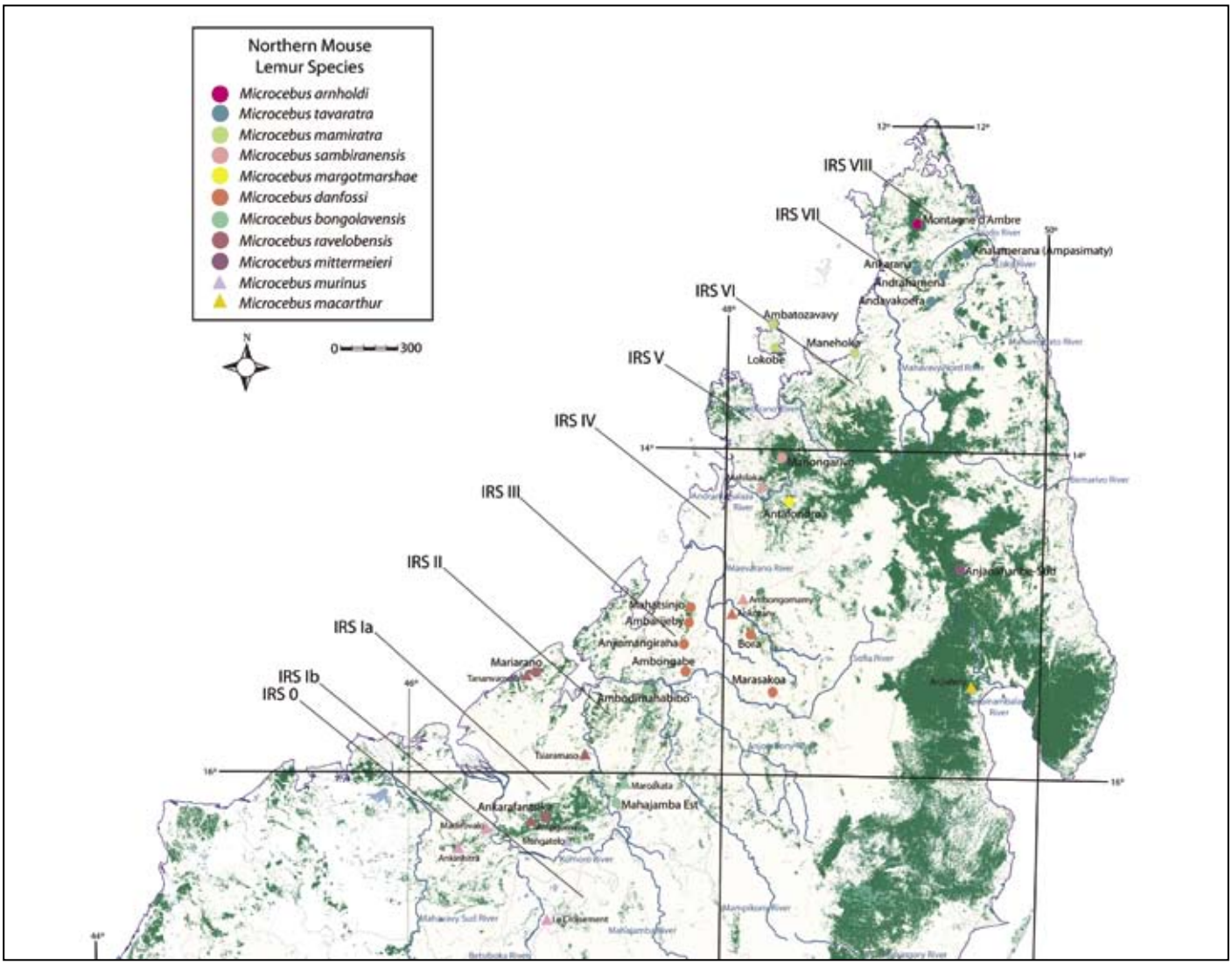


Figure 8. Distribution of the mouse lemurs (genus *Microcebus*) of northern and northwestern Madagascar. Color-coded circles represent the samples (sites) that were included in the analyses (these samples include accessioned GenBank sequences and the colors are species specific (see Appendices II(h) and III(a)). The map was modified from an image provided by Conservation International, Arlington, VA (Harper *et al*. 2007), and incorporates the Landsat Enhanced Thematic Mapper Plus (ETM+) data from 1999-2001, predominantly from 2000. Colored triangles represent accessioned samples not used in this study, but the color of the triangle is representative of a specific *Microcebus* species.

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Microcebus mamiratra (60.8 gm). Even though *M. arnholdi* is a rufous type mouse lemur as *M. mamiratra*, the pelage of *M. arnholdi* is more grayish brown.

Distribution. *Microcebus arnholdi* is known from the Montagne d'Ambre National Park and Special Reserve, northwest of the Irodo River, Madagascar.

Comparisons and remarks. *Microcebus arnholdi* can be found in montane rainforest, whereas *M. tavaratra* occupies the dry deciduous forest in the Ankarana and Analamerana IRS VII; Fig. 8). As shown in Figure 8, *M. arnholdi* is a new species in a new Inter-River-System (IRS) VIII; the tenth IRS in northern and northwest Madagascar. Figure 8 also illustrates the need for comprehensive sampling in this intensely researched region of Madagascar, a detailed distribution map of the species sampled that correlates to the existing forest tracts, and accurate mapping of the course of all river systems. The distributions of other genera in the region should be overlaid to provide us with a better understanding the biogeography of lemurs in general. Lastly, molecular genetic data should be generated for the all lemur holotypes, and included in the phylogenetic inferences and diagnostic evaluations of lemur taxonomy. Of the recognized mouse lemurs that are in the adjacent regions of Madagascar, *Microcebus arnholdi* (49.7 gm) is slightly larger than *M. sambiranensis* (48.0 gm), but smaller than *M. tavaratra* (52.3 gm), *Microcebus mamiratra* (60.8 gm), and *M. ravelobensis* (65.9 gm).

Etymology. The name *arnholdi* honors Henry Arnhold of New York, who has supported conservation efforts throughout the developing world, with a particular focus on linking the well-being of the people with the protection of their environment. Conservation International's Healthy Communities Initiative and Conservation Stewards' Program has come into existence because of Mr. Arnhold's commitment to linking the well-being of people with the protection of critically important biodiversity hotspots. Madagascar has been among the places that have benefited substantially from the support that Henry Arnhold has provided. By naming this species after him, we recognize his great commitment and express the appreciation of the conservation community for all that he has done to further the cause for biodiversity conservation in Madagascar and around the world.

Vernacular names. Arnhold's mouse lemur or Montagne d'Ambre mouse lemur.

Note

As discussed in Andriantompohavana *et al*. (2006, 2007), Louis Jr *et al*. (2006a, 2006b), and Thalmann and Geissmann (2005), the use of whole vouchers as the designated holotype for a new species is not a prerequisite for describing an undefined species. Opportunistic collection, however, can later supplement morphological, and/or molecular data in combination with curated blood and/or tissue samples. Total genomic DNA for the holotypes and paratypes of the newly described *Microcebus margotmarshae* and *Microcebus arnholdi*, along with e-vouchers and field data, are currently curated at the Museum of Texas Tech University, Lubbock, Texas, USA, under the following catalogue numbers: TK145310; TK145311; TK145312; and TK145313; TK145314; TK145315, respectively; Appendix I(a).

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Appendix I

http://10.10.10.3/ccr/genetics/lemur/index.asp?page=ccr/genetics/lemur/appendixInorthernmouselemur.htm

Appendix I(a). *Microcebus* table of individual samples and corresponding information for each sample (bar code number, site, original species designation, current species designation, GenBank accession numbers of sequence data).

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Appendix I(b). Field notes for *Microcebus margotmarshae* (formerly *Microcebus* sp. *nova* #5) and *Microcebus arnholdi* (formerly *Microcebus* sp. *nova* #6)

Appendix II

http://10.10.10.3/ccr/genetics/lemur/index.asp?page=ccr/genetics/lemur/appendixIInorthernmouselemurMS.htm

Appendix II(a). Maximum parsimony phylogram derived from the D-loop sequence data from 82 *Microcebus* individuals with 18 out-group taxa (one of 3886 most parsimonious trees). Values above branches indicate number of changes between nodes. Values within the circles along the branches indicate support of bootstrap pseudoreplicates. Length=2052; CI = 0.4235; RI = 0.8460; RC = 0.3583; HI = 0.5765.

Appendix II(b). Part A. Neighbor-joining phylogram derived the D-loop DNA sequence data from the 121 *Microcebus* individuals with18 out-group taxa. Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates. Solid black circle indicates the branch that connects in-group taxa to the out-group taxa (displayed on next page (Part B)).

Appendix II(b). Part B. Neighbor-joining phylogram derived the D-loop DNA sequence data from the 121 *Microcebus* individuals with18 out-group taxa. Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates. Solid black circle indicates the branch that connects to the in-group taxa (displayed on previous page (Part A)).

Appendix II(c). Maximum parsimony phylogram derived from the D-loop sequence data from 77 *Microcebus* haplotypes with 18 out-group taxa (one of 364 most parsimonious trees). Values above branches indicate number of changes between nodes. Values within the circles along the branches indicate support of bootstrap pseudoreplicates. Length=2138; CI = 0.4574; RI = 0.8578; RC = 0.3924; HI = 0.5426.

Appendix II(d). Fifty percent majority-rule consensus phylogenetic tree from the Bayesian analysis derived from the D-loop sequence data from 77 *Microcebus* individuals with 18 out-group taxa reconstructed using the program MrBayes. Branches without posterior probability values (PP) are supported by less than 50% of the sampled trees.

Appendix II(e). Maximum parsimony phylogram derived from the D-loop and PAST sequence data from 89 *Microcebus* individuals with 18 out-group taxa (one of 4112 most parsimonious trees). Values above branches indicate number of changes between nodes. Values within the circles along the branches indicate support of bootstrap pseudoreplicates. Length=6539; CI = 0.4271; RI = 0.8755; RC = 0.3739; HI = 0.5729.

Appendix II(f). *Microcebus margotmarshae*, Margot Marsh's or Antafondro mouse lemur at Antafondro Classified Forest (Maromiandra). Photo by Rambinintsoa Andriantompohavana.

Appendix II(g). *Microcebus arnholdi*, Arnhold's or Montagne d'Ambre mouse lemur at Montagne d'Ambre National Park and Classified Forest. Photo by Edward E. Louis Jr.

Appendix II(h). Distribution map of the mouse lemurs of Madagascar. Designated sites and species are based on molecular genetic data. The species legend corresponds to the color coded to the sites.

Appendix III

http://10.10.10.3/ccr/genetics/lemur/index.asp?page=ccr/genetics/lemur/appendixIIInorthernmouselemurMS.htm

Appendix III(a). Summary of the acronyms and GenBank accessioned sequences used in this study.

Appendix III(b). Table 1A. Diagnostic nucleotide sites from the D-loop Pairwise Aggregate Analysis (PAA) of genus *Microcebus*.

Appendix III(c). Table 1B. Diagnostic nucleotide sites from the PAST Pairwise Aggregate Analysis (PAA) of genus *Microcebus*.

Appendix III(d). Haplotypes for *Microcebus* D-loop Sequences

Appendix III(e). Haplotypes for *Microcebus* PAST Sequences.

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