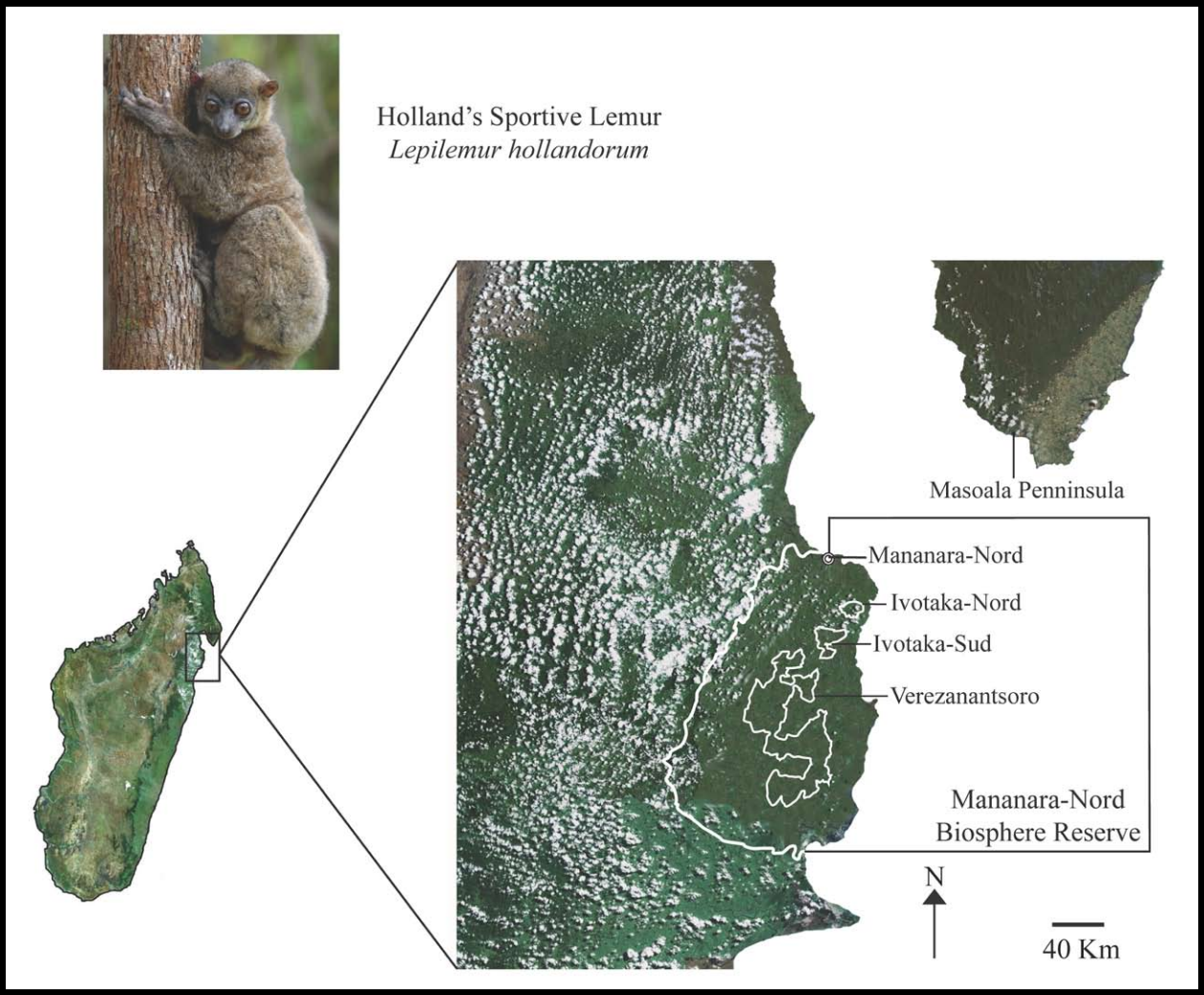
TEXAS TECH UNIVERSITY
Natural Science Research Laboratory

Occasional Papers

Museum of Texas Tech University
Number 286 8 April 2009



Sportive Lemur Diversity at Mananara-Nord Biosphere Reserve, Madagascar

Front cover: Description of a new sportive lemur, Holland's or Mananara-Nord sportive lemur, from Mananara-Nord Biosphere Reserve, Madagascar. Created by Lisa Kimmel.

Sportive Lemur Diversity at Mananara-Nord Biosphere Reserve, Madagascar

Boromé Ramaromilanto, Runhua Lei, Shannon E. Engberg, Steig E. Johnson, Brandon D. Sitzmann, and Edward E. Louis, Jr.

Abstract

Molecular genetic sequence variation among the sportive lemurs (genus *Lepilemur*) of Madagascar was investigated utilizing mitochondrial DNA (mtDNA) sequence data (*ca*. 3,000 base pairs). We infer *Lepilemur* phylogenetic relationships based on the topology of mtDNA trees generated with 225 individuals from 24 currently recognized species of the genus from 43 field sites, along with a previously undefined taxon from Mananara-Nord Biosphere Reserve. We formally describe this new species based on genetic analyses and biogeographic information (including the recent Inter-River-Systems model), supplemented with morphological data.

Key words: biogeography, *Lepilemur*, Madagascar, Mananara-Nord Biosphere Reserve, phylogenetics, sportive lemur

Introduction

Madagascar's remarkable species diversity and high levels of endemism are persistently under threat from anthropogenic pressures (Mittermeier et al. 2006; Harper et al. 2007). Consequently, the island has been ranked among the world's most important biodiversity hotspots, underscoring the need for coordinated conservation efforts (Myers 2000; Groombridge and Jenkins 2002). While most of the Malagasy fauna is susceptible to extinction risk, lemurs are especially vulnerable due to their relatively small and often fragmented geographic ranges (Jernvall and Wright 1998). As a result, lemurs are protected under the Convention on International Trade in Endangered Species (CITES) and many species are red-listed as Critically Endangered, Endangered or Vulnerable by the IUCN SSC (IUCN 2008). Many species are still data deficient (IUCN 2008), and their number is likely to increase due to the recent rapid expansion in the number of recognized species (e.g., Andriaholinirina et al. 2006; Louis et al. 2006b; Rabarivola et al. 2006; Craul et al. 2007, 2008; Lei et al. 2008). In addition, the description of new species often reduces the geographic range of traditionally recognized taxa (Louis et al. 2006b). Thus, frequent re-evaluation of the conservation status of all lemur species is necessary, using newly available information on taxonomy, biogeography, ecology, ethology, and current threats.

Among the most widely distributed lemur groups, the sportive lemurs (genus *Lepilemur*) are medium-sized nocturnal lemurs that were originally thought to consist of only two species, *L. mustelinus* from the eastern rainforests and *L. ruficaudatus* from the western and southern dry forests of the island (Schwarz 1931; Hill 1953). Recent investigations using molecular genetic, cytogenetic, and morphological data have greatly expanded the diversity of this genus (Louis et al. 2006b; Rabarivola et al. 2006; Craul et al. 2007, 2008; Lei et al. 2008). These developments in *Lepilemur* taxonomy are a salient example of the need for verifying unique diversity in order to assess the conservation status of a group. Currently, there is one vulnerable, one endangered, and one critically endangered *Lepilemur* species, while 21 of the listed species (most of them recently described) remain data deficient (IUCN 2008).

Northeastern Madagascar is one region where sportive lemur taxonomy is currently in revision. *L. mustelinus* was formerly the only recognized taxon between the Bemarivo and Mangoro Rivers (Petter et

al. 1977; Tattersall 1982). More recently, two previously undefined species have been described from this region. Louis et al. (2006b) described *L. seali* from Anjanaharibe-Sud Special Reserve, while Lei et al. (2008) introduced *L. scottorum* from the Masoala Peninsula (Fig. 1). Furthermore, Craul et al. (2008) extended the distribution of *L. seali* south of the Antainambalana River. Louis et al. (2006b) assigned the sportive lemur from Mananara-Nord Biosphere Reserve (MNBR) to *L. seali* although the available molecular data suggested that this population would eventually be described, pending further field studies. With only a single representative from MNBR available, Lei et al. (2008) established it as an undefined species, *Lepilemur* species *nova* #2. By incorporating sequence data from Louis et al. (2006b), Craul et al. (2008) subsequently reconfirmed the uniqueness of the population at MNBR.

Three biogeographic models have been proposed based on different relative contributions of factors including large rivers (>50 m wide at 20 km inland), retreat dispersion watersheds, and topographical barriers, i.e. mountains (Martin 1995; Wilmé et al. 2006; Craul et al. 2007; Olivieri et al. 2007). In Olivieri et al. (2007) and Craul et al. (2008) the authors presented biogeographic models in which "centers of endemism" were defined based on the isolation effects of paired rivers, or Inter-River-System (IRS; Fig. 1). During the course of several biogeographic revisions of northern and northwestern Madagascar, the number of IRS has increased from four (Martin 1995), to five (Wilmé et al. 2006), and to nine (Craul et al. 2008). In Craul et al. (2008), an initial IRS model was presented to delineate the ranges of the sportive lemurs of northeastern Madagascar. We aim to add to the baseline understanding of the diversity in the greater Antongil Bay region, which includes Anjanaharibe-Sud Special Reserve, Masoala National Park, Makira Forest, Mananara-Nord Biosphere Reserve, and adjacent habitats. By revising the known biodiversity of sportive lemurs for this region, we present an amended IRS model to one of the largest remaining tracts of intact forest in Madagascar (Fig. 1).

Historically, the Biological Species Concept (BSC), emphasizing reproductive isolation, has been the most common approach to define species (Mayr 1942). However, when a putative species is geographically isolated from closely related species, this concept is difficult to implement. The Phylogenetic Species Concept (PSC) employs a cladistic perspective, incorporating evolutionary patterns of ancestry and descent as it defines species operationally as the smallest diagnosable, distinct cluster of individuals (Cracraft 1983; Wheeler and Platnick 2000; Groves 2001a, b). It is also useful in defining conservation units (Vogler and DeSalle 1994). In this paper, we present a taxonomic analysis of the genus *Lepilemur* using the PSC, including a formal description of a new species from MNBR.

As previously discussed in Andriantompohavana et al. (2006), Louis et al. (2006a, b) and Thalmann and Geissmann (2005), the utilization of whole vouchers as the designated holotype for a new species is not a prerequisite for species descriptions; opportunistic collections can later supplement morphological, vocalization, and/or molecular data in combination with curated blood and/or tissue samples (Jones et al. 2005). The sportive lemurs are a prime candidate for this methodology because the highly folivorous dietary requirements of this group currently preclude any attempts to curate "live vouchers" (Thalmann and Geissmann 2005). Total genomic DNA for the three paratype specimens is currently curated at the Museum of Texas Tech University (TK125726; TK125727; TK125728). Additionally, an electronic database that contains all *Lepilemur* field data and photographs, including data for the paratype specimens, is curated at the Museum of Texas Tech University. The 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, is also available online at the website of Omaha's Henry Doorly Zoo. See Appendices I-III for a directory of appropriate website addresses.

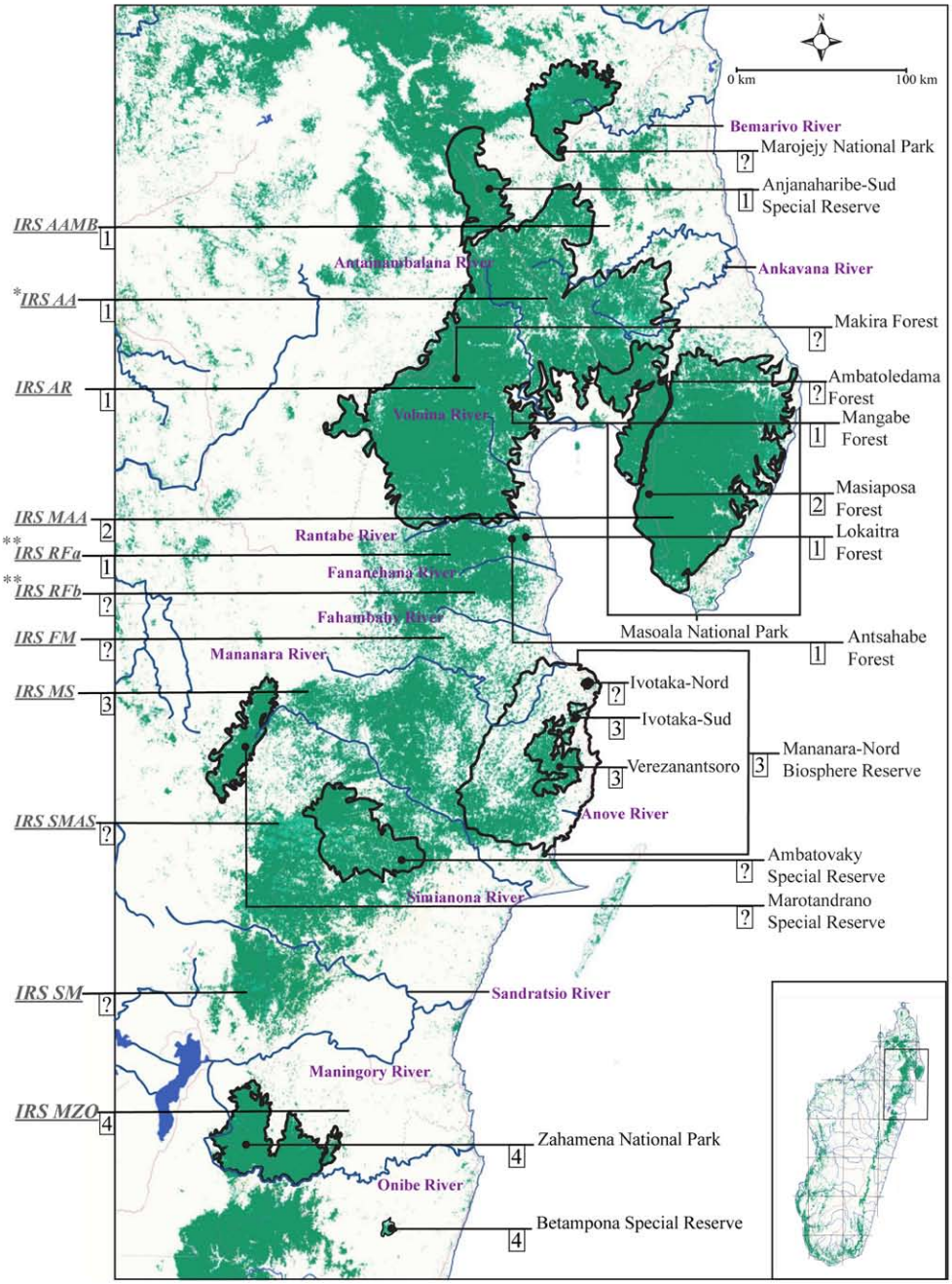


Figure 1. Map of northeastern Madagascar. This regional map features the potential boundaries and forest tracts for the sportive lemurs (genus *Lepilemur*) in northeastern Madagascar. Samples collected in the Ivontaka-Sud and Verezanantsoro parcels of Mananara-Nord Biosphere Reserve, Madagascar, were evaluated, along with sequence data from Louis et al. (2006b) and Lei et al. (2008). The Inter-River-System (IRS) model was adapted from Craul et al. (2008). IRS A and IRS RF from Craul et al. (2008) were revised to IRS AA and IRS RFa and RFb, respectively. IRS SMAS, IRS SM, and IRS MZO were inserted to define the potential boundaries for *L.* species *nova* #2 (Lei et al. 2008) and *L. mustelinus*. IRS AAMB was inserted to define the potential northern boundary for *L. seali*. IRS MAA was inserted to define the potential boundaries for *L. scottorum*. The boxed numbers found below the horizontal lines indicate what sportive lemur is designated for that site or IRS as follows: [1] specifies *L. seali*; [2] specifies *L. scottorum*; [3] specifies *L.* sp. *nova* #2; [4] specifies *L. mustelinus*; and [?] specifies undefined species or data unavailable.

METHODS

Sampling.—All lemurs investigated in this study were wild-caught, free-ranging individuals immobilized with a CO_2 projection rifle or blowgun with 10 mg/kg of Telazol (Fort Dodge Animal Health; Overland Park, Kansas; Fig. 1; Table 1). Four 2.0 mm biopsies and 1.0 cc per kilogram of whole blood were collected from each sedated animal and immediately stored in room temperature storage buffer (Longmire et al. 1992). A HomeAgain microchip (Schering-Plough Veterinary Corp.; Kenilworth, New Jersey) was placed subcutaneously between the scapulae of each lemur (Appendix I(a)). This procedure was used to field-catalog each animal with a unique recognition code in order to re-identify all captured individuals during any future immobilizations. In addition, morphometric measurements were taken. For presentation purposes, we present the weight, head crown, body length, and tail length in this publication following the guidelines of Smith and Jungers (1997; Appendices I(a-b)). Field data, including all measurements and e-voucher photographs, are available in Appendix I(b), Louis et al. (2006b), and Lei et al. (2008).

Data Collection.—We recorded the location of all immobilized lemurs using a global positioning system (GPS; Appendix I(a-b)). Genomic DNA was extracted from samples using a phenol-chloroform/isoamyl extraction (Sambrook et al. 1989). We recorded the location of all immobilized lemurs using a global positioning system (GPS; Appendix I(a-b)). From these samples, the following regions of mtDNA were amplified: the displacement loop or control region (D-loop; Baker et al. 1993; 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), as well as the $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$ genes (subsequently referred to as the PAST fragment; Louis et al. 2006b). The accessioned sequences of *L. seali* and *L. mittermeieri* of Craul et al. (2008) and Rabarivola et al. (2006), respectively, were not compatible with this data set so were not utilized in this study. Although available as accession sequences in GenBank, we would have had to truncate and eliminate two-thirds of our generated sequence data to include their *L. seali* and *L. mittermeieri* accession fragments in these analyses. Using 50 nanograms of genomic DNA, the D-loop (555 bp) and the PAST (2,378 bp) fragments were amplified using the following conditions: 94°C for 30s, 47°C for 45s, and 72°C for 45s for 34 cycles. Since potential nuclear insertions or mitochondrial pseudogenes within the nuclear genome can be amplified inadvertently, we minimized the likelihood by amplifying both mitochondrial DNA regions as intersecting or overlapping segments and confirming these segments with the degenerate oligonucleotide-primed PCR methodology (Telenius et al. 1992; Zhang and Hewitt 1996; Louis et al. 2006b). The samples were electrophoresed on a 1.2% agarose gel to verify the PCR product and purified with Exonuclease I and Shrimp Alkaline Phosphatase (EXOSAP; Silva et al. 2001).

The purified products were cycle-sequenced using a BigDye terminator sequencing kit (Applied Biosystems; Foster City, California). The sequences were analyzed by capillary electrophoresis with an Applied Biosystems Prism 3130 genetic analyzer. The PCR and sequencing primer suite from Louis et al. (2006b) and Lei et al. (2008) were used to generate the D-loop and PAST fragment sequences. The sequence fragments were aligned to generate a consensus sequence using Sequencher 4.8 (Gene Codes Corporation; Ann Arbor, Michigan), and the consensus sequences were aligned using ClustalX 1.83 (Thompson et al. 1997). All aligned sequences are available from the first author upon request. All sequences have been deposited in GenBank, and the sequence data and information are available from the referenced accession numbers (Table 1; Appendix I(a); Louis et al. 2006b; Lei et al. 2008).

Phylogenetic Analysis.—Maximum likelihood (ML) analyses for the D-loop and PAST fragment sequence data were performed under the GTRCAT algorithm implemented in the parallel Message Passing Interface (MPI) version of RAXML-VI-HPC (Stamatakis 2006; software available at http://icwww.epfl.ch/~stamatak). The best-scoring ML-trees were searched and saved in PAUP* 4.0b10 (Swofford 2001). Bayesian inference analyses of the D-loop and PAST fragment sequence data were conducted using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). A Markov Chain Monte Carlo (MCMC) run with four simultaneous chains and 1,000,000 generations was performed.

Table 1. Samples (225 Lepilemur *and 29 outgroups total) used in the present genetic study and taxonomic revision of the genus* Lepilemur. *TK Number*[α] *is referenced voucher curated at the Museum of Texas Tech University. Mitochondrial DNA sequence data for PAST (Pastorini Fragment), and D-loop (D-loop or control region) for each sample are available from GenBank under the listed accession numbers*[ϕ].

Catalog Number	TK Number[α]	Site	Original Species Designation	Current Species Designation	D loop[ϕ]	PAST[ϕ]
NARA4.20	TK125726	Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	DQ529552	DQ529694
NARA8.5	TK125727	Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	EU779982	EU779966
NARA8.7	TK125728	Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	EU779983	EU779967
NARA8.8		Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	EU779984	EU779968
NARA8.11		Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	EU779985	EU779969
NARA8.12		Mananara-Nord (Ivontaka-Sud)	*Lepilemur mustelinus*	*Lepilemur hollandorum*	EU779986	EU779970
BIBO7.1		Ambodimahabibo	*Lepilemur edwardsi*	*Lepilemur otto*	EU779987	EU779971
BIBO7.3		Ambodimahabibo	*Lepilemur edwardsi*	*Lepilemur otto*	EU779988	EU779972
BIBO7.6		Ambodimahabibo	*Lepilemur edwardsi*	*Lepilemur otto*	EU779989	EU779973
BIBO7.7		Ambodimahabibo	*Lepilemur edwardsi*	*Lepilemur otto*	EU779990	EU779974
TVY7.23		Ambatovy	*Allocebus trichotis*	*Allocebus trichotis*	EU779975	EU779959
ZOMB10		Zombitse	*Phaner furcifer pallescens*	*Phaner pallescens*	EU779976	EU779960
ANT5.3		Antafondro (Ambohimiandra)	*Mirza coquereli*	*Mirza zaza*	EU779977	EU779961
ZOMB54		Zombitse	*Mirza coquereli*	*Mirza coquereli*	EU779978	EU779962
MER1		Analamerana	*Eulemur coronatus*	*Eulemur coronatus*	EU779979	EU779963
HIH6		Anjiamangirana	*Daubentonia madagascariensis*	*Daubentonia madagascariensis*	EU779980	EU779964
REN31		Berenty	*Lemur catta*	*Lemur catta*	EU779981	EU779965

The model of evolution was selected for the ML inferences by using Mrmodeltest 2.2, a modified version of Modeltest 3.6 (Posada and Crandall 1998; Nylander 2004). It was performed with HKY+I+G Model for D-loop and GTR+I+G Model for PAST, for 1,000,000 generations. Every hundredth generation, the tree with the best likelihood score was saved, resulting in 4,000 trees. These were condensed in a majority rule consensus tree using PAUP* 4.0b10 (Swofford 2001), and clade posterior probabilities (PP) were computed. The pattern of sequence evolution was estimated by conducting a minimum spanning network generated with the program NETWORK version 4.500 (Bandelt et al. 1999) and Arlequin version 2.0 (Schneider et al. 2000). MEGA 3.1 (Kumar et al. 2004) was used to calculate uncorrected pairwise distances ('p') and Kimura distance measures (Kimura 1980) for D-loop and PAST fragments.

We utilized MacClade 3.01 (Maddison and Maddison 1992) and MEGA version 3.1 (Kumar et al. 2004) in a diagnostic search to designate evolutionary significant units (ESU) using population aggregate analysis (PAA) of the D-loop (550 bp) and PAST (2,378 bp) sequence data for genus *Lepilemur* (Davis and Nixon 1992; Louis et al. 2006a, b; Lei et al. 2008). 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; Vogler and DeSalle 1994; Groves 2001a, b; Louis et al. 2006b; Lei et al. 2008).

RESULTS

Mitochondrial DNA sequence data were completed for two fragments, D-loop and PAST (*ca*. 3,000 bp), for 225 individuals representing 24 recognized species of sportive lemurs collected from 43 field sites (Table 1; Louis et al. 2006b; Lei et al. 2008). Due to different mtDNA fragments utilized in Rabarivola et al. (2006), congruent sequence data were not available and thus, *L. mittermeieri* was not included in this study. Based on the phylogenetic inferences of the ML and Bayesian analyses of D-loop and PAST sequence alignments, 24 *Lepilemur* species were represented in 24 distinct and well-supported terminal clades. These terminal clades could be partitioned into four geographic regions (Figs 2-3; Appendices II(a-h)). In general, Section A consists of sportive lemurs from northern and northwestern Madagascar as follows: *L. ankaranensis*, *L. milanoii*, *L. septentrionalis*, *L. tymerlachsoni*, *L. dorsalis*, *L. sahamalazensis*, and *L. ahmansonorum*. Section B is associated with northwestern Madagascar: *L. otto, L. edwardsi,* and *L. grewcockorum*. Section C corresponds to southern and west central Madagascar as follows: *L. hubbardorum*, *L. ruficaudatus*, *L. aeeclis*, *L. randrianasoli*, *L. leucopus*, and *L. petteri*. With the exception of *L. microdon*, Section D incorporates the sportive lemurs of eastern Madagascar as follows: *L. mustelinus*, *L. jamesorum*, *L. betsileo*, *L. fleuretae*, *L. wrightae*, *L. seali*, *L. scottorum*, and *L.* species *nova* #2. Furthermore, all phylogenetic methods support the uniqueness of the subpopulation, *Lepilemur* species *nova* #2 from MNBR (Figs. 2-3).

All methods revealed the same phylogenetic proximity between regions and among sportive lemur species, resulting in distinguishable eastern and western clades with the exception of *L. microdon* (Figs. 2-3; Appendices II(a-h)). The phylogenetic association of *L. microdon* to Sections B or C varied according to which mtDNA sequence fragment was analyzed. Based on either phylogenetic method, *L. microdon* clusters with the northwestern sportive lemurs (Section B) for the D-loop sequence fragment. However, for the PAST sequence fragment, this east coast sportive lemur's association shifts to the species located in west central and southern Madagascar (Section C; Figs. 2-3; Appendices (a-h)).

The complete uncorrected 'p' distance and the Kimura two-parameter distance measures for the genus *Lepilemur* are presented for D-loop and PAST fragments in Appendices III(c-d). Values ranged from 7.0% to 10.9% and from to 4.2% to 11.0% for D-loop and PAST, respectively, for the three sportive lemur species geographically closest to *L.* species *nova* #2. The minimum spanning network diagrammatically presents the relative evolutionary associations among 25 sportive lemur species (Fig. 4).

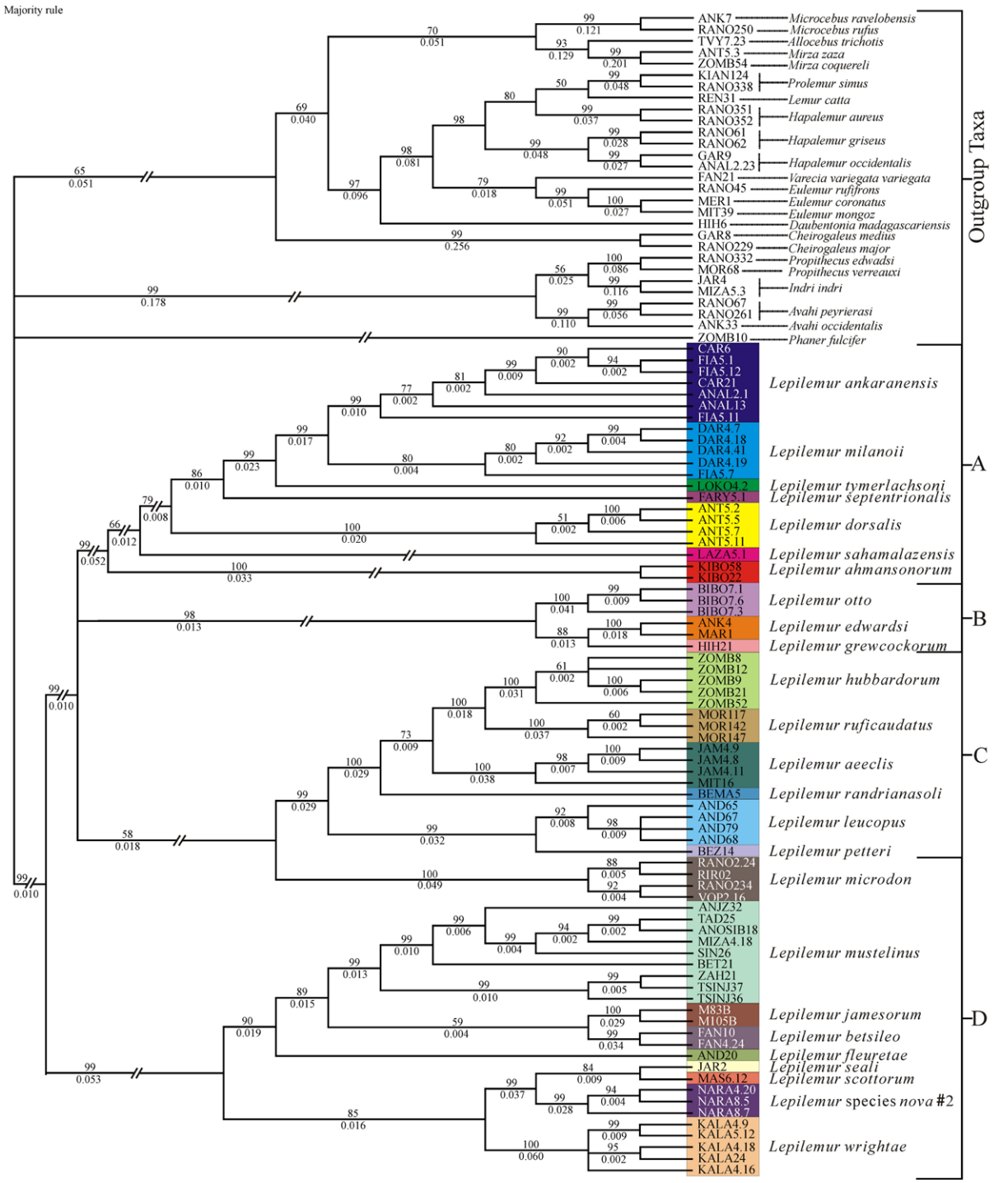


Figure 2. Phylogenetic relationships between *Lepilemur* species inferred from the maximum likelihood and Bayesian approaches for the D-loop sequence data from 73 haplotypes from the 225 *Lepilemur* individuals with 29 outgroup taxa. Numbers above the branches represent posterior probability support. Numbers below the branches represent ML values. We obtained the maximum likelihood tree (-Ln likelihood=12,404.88) from the D-loop alignment using a transition/transversion ratio of 2 (k=3.99). Section A consists of sportive lemurs from northern and northwestern Madagascar. Section B consists of sportive lemurs from northwestern Madagascar. Section C consists of sportive lemurs from west central and southern Madagascar. Section D consists of sportive lemurs except for *L. microdon* (associated with Section C) from eastern Madagascar. Outgroup taxonomy based on Mittermeier et al. (2008).

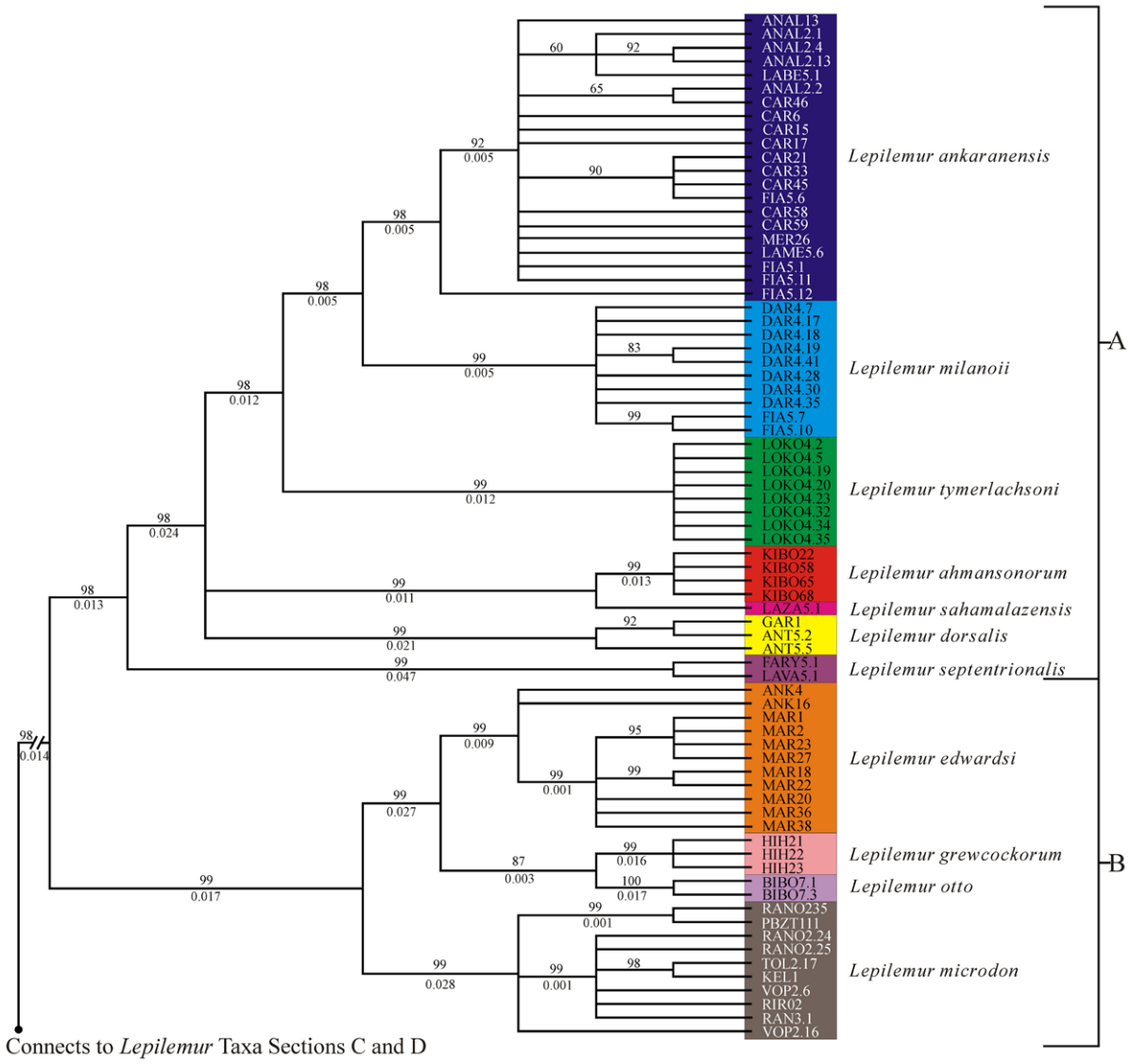


Figure 3a. Phylogenetic relationships between *Lepilemur* species inferred from the maximum likelihood and Bayesian approaches of PAST fragment sequence data from 161 haplotypes from the 225 *Lepilemur* individuals with 29 outgroup taxa. Numbers above the branches represent posterior probability support. Numbers below the branches represent ML values. We obtained the maximum likelihood tree (-Ln likelihood=38,278.20) from the PAST alignment using a transition/transversion ratio of 2 (k=4.23). Section A consists of sportive lemurs from northern and northwestern Madagascar. Section B consists of sportive lemurs from northwestern Madagascar.

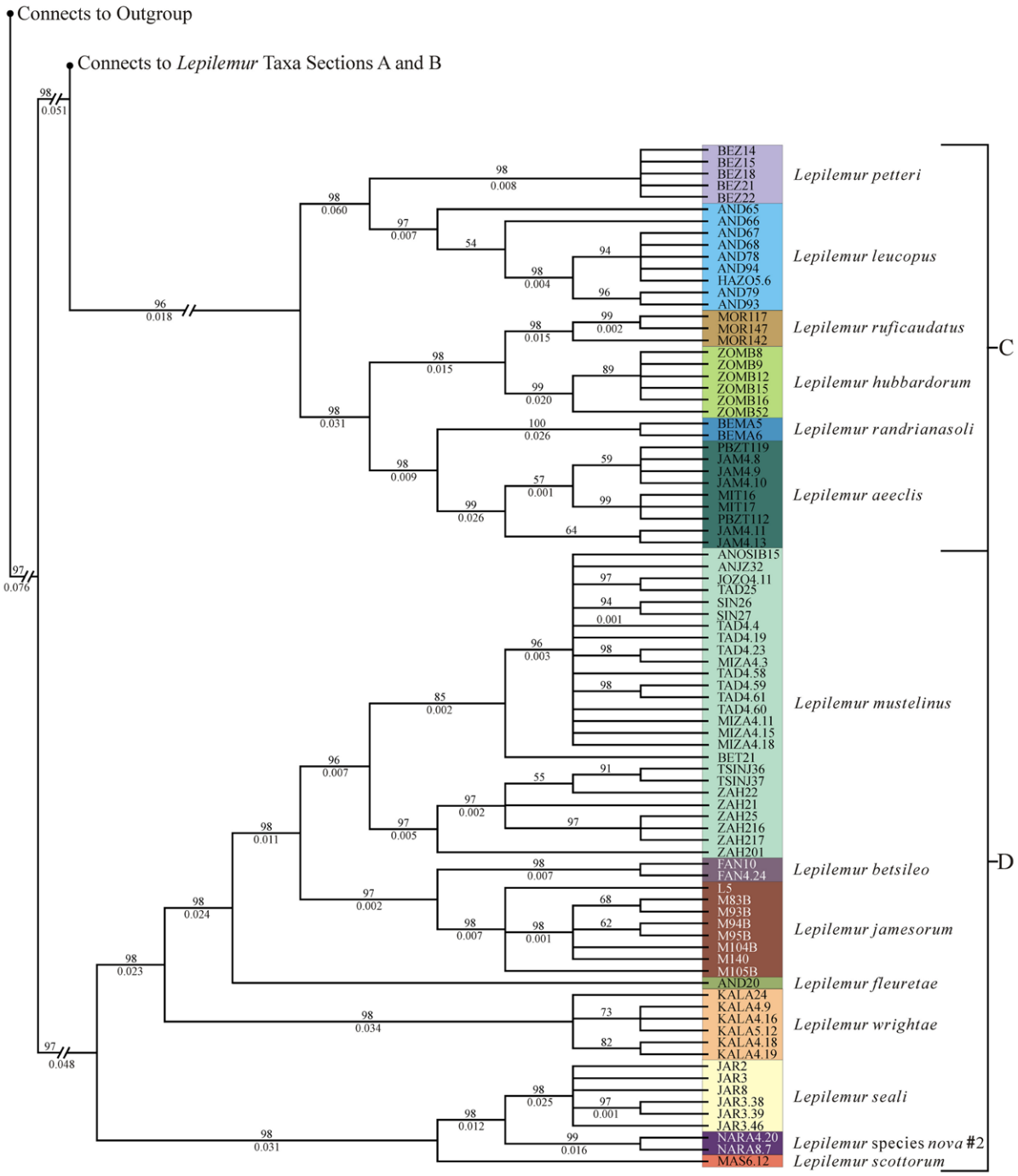


Figure 3b. Phylogenetic relationships between *Lepilemur* species inferred from the maximum likelihood and Bayesian approaches of PAST fragment sequence data from 161 haplotypes from the 225 *Lepilemur* individuals with 29 outgroup taxa. Numbers above the branches represent posterior probability support. Numbers below the branches represent ML values. Section C consists of sportive lemurs from west central and southern Madagascar. Section D consists of sportive lemurs except for *L. microdon* (associated with Section B) from eastern Madagascar.

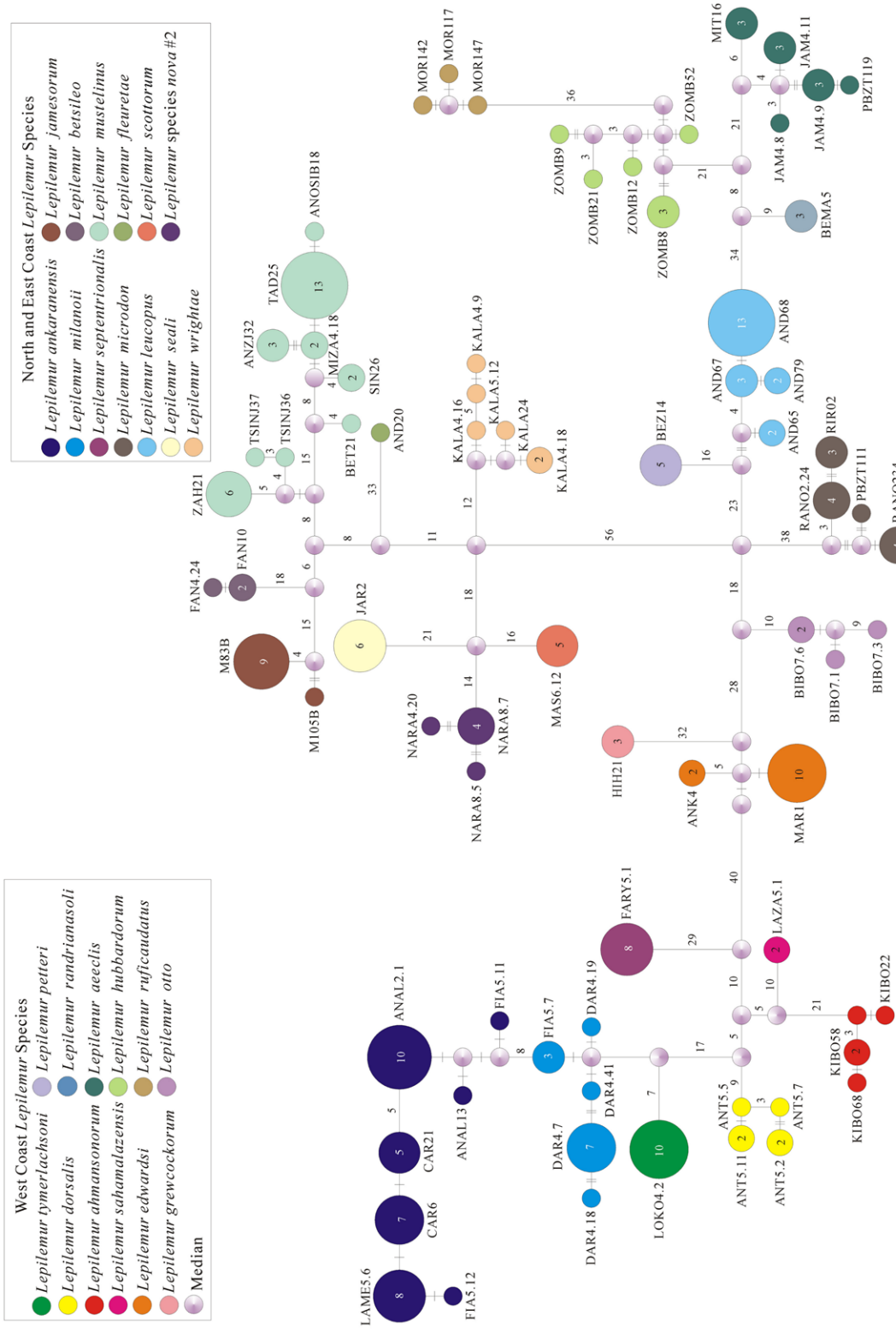


Figure 4. Minimum spanning network of *Lepilemur* haplotypes calculated using Arlequin Version 2 and Network Version 4.11. Identification numbers denote haplotypes corresponding to Table 1 (Appendix III(e)). The minimum number of mutational steps separating matriarchal lines is indicated. Nucleotide substitutions are indicated by dashes. The number of nucleotide differences in their connecting lines is indicated when there are more than two. Missing intermediates are indicated by gray circles. The size of the circles is representative of the number of individuals with matching haplotypes (circles without any number represent one individual).

DISCUSSION

Anthropogenic pressure has resulted in the fragmentation of panmictic populations, which has compelled wildlife and conservation agencies to make management priorities according to the current understanding of taxonomy, historical biogeography and present distribution of these now isolated populations (Wilmé et al. 2006; Kremen et al. 2008). Many studies have shown that molecular genetics offers a reliable and rapid method of identifying unique and/or cryptic biodiversity (e.g., Louis et al. 2006b; Craul et al. 2007, 2008). Through the analyses of accessioned and novel sample sets, we found that each described sportive lemur clusters in distinct and well-supported terminal clades, along with the undefined individuals from MNBR. With this aim, we present here a revision of the genus *Lepilemur*, concentrating on the distribution of the sportive lemur species in northeastern Madagascar.

Ganzhorn et al. (2006) described the importance of rivers as corridors referring to cytogenetic reconstructions of the genus *Lepilemur*, specifically the sister relationship of a western sportive lemur, *L. edwardsi* (Ankarafantsika National Park) and an eastern sportive lemur, *L. microdon* (Ranomafana National Park; Ishak et al. 1992). The current phylogenetic reconstructions from sequence-based data of the genus *Lepilemur* have substantiated this finding (Louis et al. 2006b; Lei et al. 2008). However, incongruence between the D-loop and PAST inferences in regard to the phylogenetic relationships of *L. microdon* to the northwestern sportive lemurs (Section B) and the west central and southern sportive lemurs (Section C) presents an alternative system of river corridor dispersal (Figs. 2-3). This finding illustrates the importance of developing and assessing novel nuclear DNA based sequence fragments to examine further the significance or function of river corridors in speciation.

The population aggregate analysis (PAA) results from the D-loop and PAST sequence fragments are presented in Tables 2A and 2B, respectively (Appendix III(a-b)). Multiple diagnostic characters distinguish each described sportive lemur, along with the undefined sportive lemur from Mananara-Nord Biosphere Reserve. *Lepilemur* species *nova* #2 had 12 diagnostic sites for D-loop and PAST fragments combined. According to the Phylogenetic Species Concept (*sensu* Wheeler and Platnick 2000), diagnostic characters or attributes define Evolutionary Significant Units (ESUs). Several authors suggest that ESUs are equivalent to species and reflect species barriers (Cracraft 1983; Groves 2001a). *Lepilemur* species *nova* #2 had multiple molecular diagnostic sites (Tables 2A and 2B; Appendices III(a-b)) and, given this criterion, represents a distinct ESU. The continuous addition of sequence from novel samples and sites to the PAA data set will dynamically test the distinction and diagnostic ability of these characters, and, therefore, the ongoing status of this and related species.

A summary of the morphometric data for the three sportive lemur species of northeastern Madagascar, along with the proposed species, *Lepilemur* species *nova* #2, are presented in Tables 3A and 3B (detailed morphological measurements of the novel sportive lemur are available in Appendix I(b)). No extensive quantitative analyses were conducted on the morphometric data at this point (Table 3). Therefore, this morphometric information is provided as supplemental data, complementing the molecular data used to partition unique biodiversity. Nevertheless, it can be seen that the new species most resembles *L. mustelinus* in size, but has a distinctly longer tail and shorter pollex and hallux. Meanwhile, we describe the new species as follows:

***Lepilemur hollandorum*, New Species**

Lepilemur species *nova* #2 of Lei et al. (2008)

Type Specimen.—NARA4.20 (TK125726; TTU-M 109031), adult female; collected on 8 August 2004, captured at Mananara-Nord Biosphere Reserve. Material: Total genomic DNA (50ng/µl) for NARA4.20 (TK 125726), adult female, stored and curated at the Museum of Texas Tech University, Lubbock, Texas, USA. Two 2.0 mm biopsies from ear pinna, and 0.007 cc of whole blood tissues stored at Henry Doorly Zoo, Omaha, Nebraska, USA. A microchip pit tag was placed subcutaneously between the scapulae and recorded as 4556420944. NARA4.20 was collected by Edward E. Louis, Jr., Richard Randriamampionona,

Table 2A. Summary of Population Aggregate Analysis (PAA) D-loop diagnostic sites for Lepilemur. *Refer to Appendix III(a). The locality of* Lepilemur *species* nova *#2 is Mananara-Nord Biosphere Reserve. *No character or attribute is available for this fragment.*

Species	Fragment Size (bp)	PAA base pair location
Lepilemur ankaranensis	540	*
Lepilemur milanoii	540	130
Lepilemur tymerlachsoni	538	*
Lepilemur septentrionalis	536	33, 43, 111, 113, 249
Lepilemur dorsalis	540	536, 537
Lepilemur sahamalazensis	542	*
Lepilemur petteri	534	*
Lepilemur leucopus	535	19
Lepilemur ruficaudatus	535	103, 126, 310
Lepilemur hubbardorum	535	242, 253, 270, 302
Lepilemur randrianasoli	538	33, 272
Lepilemur edwardsi	545-546	127
Lepilemur grewcockorum	544	139, 195, 357
Lepilemur ahmansonorum	542	*
Lepilemur aeeclis	537-538	21
Lepilemur mustelinus	552-553	*
Lepilemur jamesorum	552	132
Lepilemur betsileo	553	272, 273, 286
Lepilemur fleuretae	550	10, 24, 37, 200, 287, 288, 314, 317, 330
Lepilemur microdon	530	25, 34, 107, 110, 120, 121, 123, 124, 125, 127, 137, 139, 396
Lepilemur wrightae	551	55, 58, 275, 301, 476, 493
Lepilemur seali	550	54, 159, 221
Lepilemur species *nova* #2	550	87, 195, 231, 327, 475
Lepilemur scottorum	551	24, 30, 140, 187, 266
Lepilemur otto	545-547	160, 162

Table 2B. Summary of Population Aggregate Analysis (PAA) PAST fragment diagnostic sites for Lepilemur. *Refer to Appendix III(b). The locality of* Lepilemur *species* nova *#2 is Mananara-Nord Biosphere Reserve.*

Species	Fragment Size (bp)	PAA base pair location
Lepilemur ankaranensis	2359-2360	364, 858, 1315, 1804
Lepilemur milanoii	2359	342, 769, 1896
Lepilemur tymerlachsoni	2359	152, 1309, 1378, 1861, 1898, 1995
Lepilemur septentrionalis	2360	44, 113, 211, 214, 274, 353, 354, 533, 551, 555, 576, 674, 734, 1103, 1174, 1231, 1347, 1399, 1448, 1492, 1550, 1582, 1603, 1630, 1777, 2144, 2146, 2363
Lepilemur dorsalis	2361	579, 717, 746, 1525, 1780, 2163, 2168, 2177, 2236
Lepilemur sahamalazensis	2360	204, 539, 737, 749, 770, 803, 1358
Lepilemur petteri	2360	337, 578, 779, 957, 1615
Lepilemur leucopus	2360-2361	220, 719, 836, 1960
Lepilemur ruficaudatus	2360	94, 127, 235, 365, 776, 1074, 1370, 1783, 1835, 1867, 1921, 2068
Lepilemur hubbardorum	2361	350, 543, 566, 629, 681, 1012, 1015, 1240, 1396, 1559, 1906, 1907, 2111
Lepilemur randrianasoli	2360	191, 699, 849, 923, 982, 1018, 1035, 1053, 1219, 1432, 1444, 1753, 1981, 1988, 2250, 2267
Lepilemur edwardsi	2360	1018, 1474, 1979
Lepilemur grewcockorum	2360	406, 888, 896, 988, 1114, 1226, 1354, 1537
Lepilemur ahmansonorum	2360	46, 304, 350, 1096, 1097, 1818, 2141, 2170
Lepilemur aeeclis	2360	535, 548, 563, 581, 975, 1357, 1368, 1423, 1442, 1990, 2089, 2107
Lepilemur mustelinus	2358-2359	85
Lepilemur jamesorum	2359	2144
Lepilemur betsileo	2359	8, 1057
Lepilemur fleuretae	2359	29, 103, 269, 358, 533, 534, 546, 553, 664, 1124, 1574, 2013, 2023
Lepilemur microdon	2360	146, 510, 581, 596, 826, 829, 1171, 1954, 1991, 2077, 2164
Lepilemur wrightae	2359	55, 133, 663, 691, 871, 907, 942, 1105, 1117, 1120, 1837, 1856, 1936, 2041, 2096, 2181, 2185, 2331
Lepilemur seali	2360	124, 147, 290, 626, 665, 692, 722, 1302, 1313, 1371, 1679, 1875, 1879, 1969
Lepilemur species *nova* #2	2360	86, 567, 1157, 1337, 1483, 1606, 2165
Lepilemur scottorum	2360	72, 256, 1033, 1112, 1167, 1237, 1336, 1538, 1902
Lepilemur otto	2360	115, 196, 328, 379, 702, 988, 1004, 1955

Table 3A. Morphometric data collected from the four geographically closest species of Lepilemur *from northeast Madagascar. (Individual morphological data available at Appendix I(b).) *Only adult individuals were included (based on body weight and canine length).*

Species Name	Common Name	*N	Weight (kg)	Head Crown (cm)	Body Length (cm)	Tail Length (cm)
Lepilemur mustelinus	Weasel sportive lemur	27	0.99±0.21	7.9±1.4	25.8±4.0	25.2±2.1
Lepilemur seali	Seal's sportive lemur	5	0.95±0.09	7.5±0.9	27.5±1.4	26.0±1.4
Lepilemur scottorum	Scott's sportive lemur	5	0.88±0.14	6.7±0.6	26.6±0.9	27.8±2.0
Lepilemur species *nova* #2		5	0.99±0.02	7.5±0.5	24.0±1.7	28.1±1.3

Table 3B. Morphometric data collected from the four geographically closest species of Lepilemur *from northeast Madagascar. (Individual morphological data available online; see Appendix I(b).) *Only adult individuals were included (based on body weight and canine length).*

	Forelimb					Hindlimb				
Species Name	Pollex Length (cm)	LD Length (cm)	Hand Length (cm)	Ulna/ Radius Length (cm)	Humerus Length (cm)	Hallux Length (cm)	LD Length (cm)	Foot Length (cm)	Tibia Length (cm)	Femur Length (cm)
Lepilemur mustelinus	2.6±0.3	3.6±1.4	6.2±0.4	8.0±0.7	6.4±0.7	4.8±0.7	2.9±0.4	9.1±0.4	9.7±0.9	10.9±0.9
Lepilemur seali	2.2±0.3	3.2±0.4	6.2±0.2	8.4±0.3	6.2±0.3	3.9±1.2	3.2±0.6	9.2±0.3	10.4±0.8	10.6±0.8
Lepilemur scottorum	2.1±0.3	2.9±0.2	5.9±0.3	8.4±0.4	6.3±0.3	3.5±0.2	2.8±0.3	8.3±1.3	9.3±2.0	8.7±0.5
Lepilemur species *nova* #2	1.8±0.5	3.1±0.2	6.3±0.2	7.9±0.7	5.9±0.3	3.2±0.7	2.9±0.6	9.4±0.4	9.7±0.8	10.8±0.5

Richard Rakotonomenjanahary, Jean C. Randriamanana, Justin Andrianasolo, Jean Claude Rakotoniaina, Jean Freddy Ranaivoarisoa, Boromé Ramaromilanto, Fidelis Razafimanjato, and Jean Aimé Andriamihaja on 8 August 2004.

Type Series.—Whole blood for NARA4.20 (TK125726; TTU-M 109031), adult female; NARA8.5 (TK125727; TTU-M 109032), adult male; and NARA8.7 (TK125728; TTU-M 109033), adult female; are stored and curated at the Museum of Texas Tech University. Individual measurements, e-voucher photos, and collection data are given in Appendix I(b). NARA4.20, NARA8.5, and NARA8.7 were collected by Edward E. Louis, Jr., Brandon D. Sitzmann, Richard Randriamampionona, Richard Rakotonomenjanahary, Jean C. Randriamanana, Justin Andrianasolo, Jean Claude Rakotoniaina, Jean Freddy Ranaivoarisoa, Boromé Ramaromilanto, Fidelis Razafimanjato, and Jean Aimé Andriamihaja on 8 August 2004, 21 February 2008, and 21 February 2008, respectively (Appendix I(b)).

Type Locality.—Mananara-Nord Biosphere Reserve, Toamasina Province, Madagascar (approximately S16˚18'22.6", E049˚47'03.9").

Description.—*Lepilemur hollandorum* is a large-sized sportive lemur (0.99 kg; Table 3). The pelage on the head, along the shoulders down to the mid back is mottled reddish-gray, where the coat then transitions to a lighter grayish-brown down to the pygal region of the tail (Fig. 5; Appendix I(b)). The head has a faint dark brown to black middle dorsal stripe or inverted Y-shaped pattern. The dorsal mid-line or stripe progresses from the head to the lower half of the back. Ears protrude out and are fleshy. The ventral coat is primarily light gray, with darker undertones. The venter and face are generally gray while the neck area close to the ears and chin are lighter brown to blonde. The hands and feet are grayish-brown, and the tail is dark brown to black towards the distal end.

Diagnosis.—In the D-loop and PAST sequence fragments, *Lepilemur hollandorum* differs from its closest relatives by genetic and geographic distances, *L. seali*, *L. scottorum* and *L. mustelinus*, as follows: 7.5%±1.2% (43 informative sites), 7.0%±1.2% (39 informative sites), and 10.9%±1.4% (68 informative sites), respectively; and 4.2%±0.4% (98 informative sites), 4.2%±0.4% (95 informative sites), and 11.0%±0.8% (253 informative sites), respectively. *Lepilemur hollandorum* has 12 diagnostic attributes (five attributes for D-loop and seven attributes for PAST fragment).

Distribution.—Currently, *L. hollandorum* is known only from Mananara-Nord Biosphere Reserve, in the Ivontaka-Sud and Verezanantsoro (Ambinanibeorana) parcels in IRS MS (Fig. 1). The Ivontaka-Sud and Verezanantsoro (Ambinanibeorana) parcels are lowland rainforest fragments. The northern boundary of *L. hollandorum* has not been defined but is expected to be either the Fahambahy or Mananara River (Craul et al. 2008). Furthermore, the lack of comprehensive samples between Zahamena National Park and Mananara-Nord Biosphere Reserve prevents definitive resolution of the southern boundary. Therefore, additional survey work is required to establish the sportive lemur biodiversity of IRS SMAS (Marotandrano and Ambatovaky Special Reserves), IRS SM, and IRS FM (Figs. 1 and 6). Additionally, IRS AAMB, IRS A, IRS AR, and IRS RFa are proposed as the distribution of *L. seali*, but additional survey work should be initiated to define the northern boundary of this sportive lemur (Figs. 1 and 6). Furthermore, the Ankavanana River is proposed as the northern boundary of *L. scottorum* (IRS MAA; Figs. 1 and 6).

Comparisons and Remarks.—*Lepilemur hollandorum* (0.99 kg) is larger in weight than *L. seali* (0.95 kg) and *L. scottorum* (0.88 kg), but it is similar in weight to *L. mustelinus* (0.99 kg; Tables 3A and 3B). Despite the geographic proximity of *L. hollandorum* to *L. mustelinus*, the genetic proximity of *L. seali*, *L. hollandorum*, *L. scottorum*, and *L. wrightae* versus that of *L. mustelinus* to the southeastern sportive lemurs provides further support for species status of *L. hollandorum* (Figs. 2-3; Appendices II(a-i)).

Etymology.—The name *hollandorum* is proposed in honor of Dick and Mary Holland for their philanthropic support for art, science, education, and research, including providing opportunities for young Malagasy scientists.

Vernacular Names.—Holland's or Mananara-Nord sportive lemur.

Figure 5. *Lepilemur hollandorum*, Holland's or Mananara-Nord sportive lemur. Photo by Edward E. Louis, Jr.

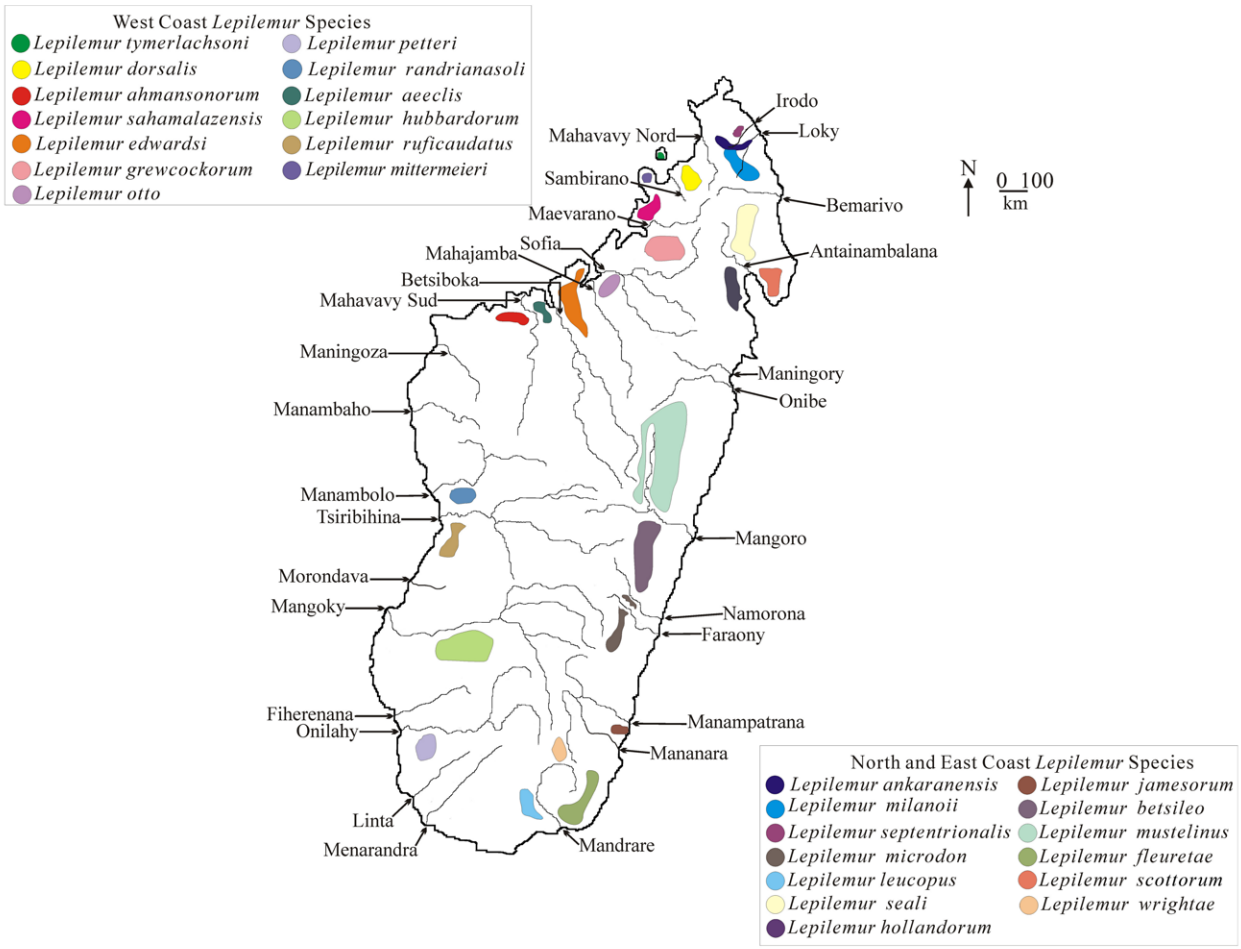


Figure 6. Revised distribution map of the sportive lemurs (genus *Lepilemur*) of Madagascar.

Despite the well-supported divergence of northeastern sportive lemur species based on genetic characters, firm evidence for geographic isolation of these taxa is presently lacking. *Lepilemur hollandorum* may be delimited by rivers acting as barriers to dispersal although the specific rivers have not been defined (Craul et al. 2008). We propose that the Fahambahy or Mananara Rivers bound the northern distribution of the MNBR sportive lemur (Fig. 1). *Lepilemur seali* is located north of this region (IRS RFa), at least as far south as the Fananehana River (Craul et al. 2008; Fig. 1). Furthermore, we propose the Simianona, Sandratsio, or Maningory River as the southern boundary. *L. mustelinus* is found to the south of these river systems (Fig. 1; Mittermeier et al. 2006; Louis et al. 2006b). Field surveys between each of these rivers will be needed to refine the distribution and Inter-River-System model of the sportive lemurs of northeastern Madagascar (Fig. 1).

ACKNOWLEDGMENTS

We want to acknowledge the Association Nationale pour la Gestion des Aires Protégées (ANGAP), the Ministère de l'Environnement des Eaux et Forêts of Madagascar, U. S. Fish & Wildlife Service (USFWS), and Parc Botanique et Zoologique de Tsimbazaza (PBZT). This project would not have been possible without the support of the staff, guides, and drivers of Henry Doorly Zoo's Madagascar Biodiversity and Biogeography Project (MBP-HDZ). We acknowledge the generosity of Bill and Berniece Grewcock, the Ahmanson Foundation, the Theodore F. and Claire M. Hubbard Family Foundation, and the James Family for their long-term support and commitment. We also acknowledge Omaha's Henry Doorly Zoo Center for Conservation and Research (CCR) Genetics Department staff, especially graphics specialist Lisa Kimmel for creating the web page and documents. We would also like to acknowledge John Callahan, Michael Bosetti, Nikola Miljkovic, Peter Kiewit Institute, Holland Computing Center, University of Nebraska Foundation, and the University of Nebraska at Omaha for their enthusiastic support for seeing this project through to completion. Finally, we appreciate the reviewers' constructive criticism, comments, and suggestions.

LITERATURE CITED

Andriaholinirina, N., J-L. Fausser, C. Roos, C. Rabarivola, I. Ravarimanana, D. Zinner, U. Thalmann, J. U. Ganzhorn, B. Meier, R. Hilgartner, L. Walter, A. Zaramody, C. Langer, T. Hahn, E. Zimmermann, U. Radespiel, M. Craul, J. Tomiuk, I. Tattersall, and Y. Rumpler. 2006. Molecular phylogeny and taxonomic revision of the sportive lemurs (*Lepilemur*, Primates). BioMed Central Evolutionary Biology 6:1-13.

Andriantompohavana, R., J. R. Zaonarivelo, S. E. Engberg, R. Randriamampionona, S. M. McGuire, G. D. Shore, R. Rakotonomenjanahary, R. A. Brenneman, E. E. Louis, Jr. 2006. The mouse lemurs of northwestern Madagascar with a description of a new species at Lokobe Special Reserve. Occasional Papers, Museum of Texas Tech University 259:1-23.

Baker, C. S., A. Perry, J. L. Bannister, M. T. Weinrich, R. B. Abernethy, J. Calambokidis, R. H. Lien, J. U. Lambersen, O. Ramirez, P. Vasquez, J. Clapham, A. Alling, S. J. O'Brien, and S. R. Palumbi. 1993. Abundant mitochondrial DNA variation and worldwide population structure in humpback whales. Proceedings of the National Academy of Sciences 90:8239-8243.

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

Cracraft, J. 1983. Species concepts and speciation analysis. Pp. 159-187 in Current ornithology, Vol. 1 (R. F. Johnston, ed.). Plenum Press, New York, New York.

Craul, M., E. Zimmermann, S. Rasoloharijaona, B. Randrianambinina, and U. Radespiel. 2007. Unexpected species diversity of Malagasy primates (*Lepilemur* spp.) in the same biogeographical zone: a morphological and molecular approach with the description of two new species. BioMed Central Ltd. Evolutionary Biology 83:1-15.

Craul, M., U. Radespiel, D. W. Rasolofoson, G. Rakotondratsimba, O. Rakotonirainy, S. Rasoloharijaona, B. Randrianambinina, J. Ratsimbazafy, F. Ratelolahy, T. Randrianamboavaonjy, and L. Rakotozafy. 2008. Large rivers do not always act as species barriers for *Lepilemur* sp. Primates 49:211-218.

Davis, J. I., and K. C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41:421-435.

Ganzhorn, J. U., S. M. Goodman, S. Nash, and U. Thalmann. 2006. Lemur biogeography. Pp. 229-254 in Primate biogeography, progress and prospects (S. M. Lehman and J. G. Fleagle, eds.). Springer Press+Business Media, LLC, New York, New York.

Groombridge, B., and M. Jenkins. 2002. World atlas of biodiversity: earth's living resources in the 21st century. University of California Press, Berkeley.

Groves, C. P. 2001a. Primate taxonomy. Pp. 26-53; 86-92. Smithsonian Institution, Washington, D.C.

Groves, C. P. 2001b. Why taxonomic stability is a bad idea, or why are there so few species of primates (or are there?). Evolutionary Anthropology 10:192-198.

Harper, G. J., M. K. Steininger, C. J. Tucker, D. Juhn, and F. Hawkins. 2007. Fifty years of deforestation and forest fragmentation in Madagascar. Environmental Conservation 34:1-9.

Hill, W. C. O. 1953. Primates: comparative anatomy and taxonomy. VIII. Strepsirhini. Edinburgh University Press, Edinburgh.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

Ishak, B., S. Warter, B. Butrillaux, and Y. Rumpler. 1992. Chromosomal rearrangements and speciation of Sportive Lemurs (*Lepilemur* species). Folia Primatologica 58:121-130.

IUCN. 2008. IUCN Red List of threatened species: www.iucnredlist.org. Downloaded on 09 October 2008.

Jernvall, J., and P. C. Wright. 1998. Diversity components of impending primate extinctions. Proceedings of the National Academy Sciences 95:11279-11283.

Jones, T., C. L. Ehardt, T. M. Butynski, T. R. B. Davenport, N. E. Mpunga, S. J. Machaga, and D. W. De Luca. 2005. The highland mangabey *Lophocebus kipunji*: A new species of African monkey. Science 308:1161-1164.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

Kremen, C., A. Cameron, A. Moilanen, S. Phillips, C. D. Thomas, H. Beentje, J. Dransfeld, B. L. Fisher, F. Glaw, T. Good, G. Harper, R. J. Hijmans, D. C. Lees, E. Louis, Jr., R. A. Nussbaum, A. Razafimpahanana, C. Raxworthy, G. Schatz, M. Vences, D. R. Vietes, P. C. Wright, and M. L. Zjhra. 2008. Aligning conservation priorities across taxa in Madagascar, a biodiversity hotspot, with high-resolution planning tools. Science 321:340-342.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment Briefings. Bioinformatics 5:150-163.

Lei, R., S. E. Engberg, R. Andriantompohavana, S. M. McGuire, R. A. Mittermeier, J. R. Zaonarivelo, R. A. Brenneman, and E. E. Louis, Jr. 2008. Nocturnal lemur diversity at Masoala National Park. Special Publications, Museum of Texas Tech University 53:1-41.

Longmire, J. L., G. F. Gee, C. L. Hardekoff, and G. A. Mark. 1992. Establishing paternity in whooping cranes (*Grus americana*) by DNA analysis. The Auk 109:522-529.

Louis, E. E., Jr., M. S. Coles, R. Andriantompohavana, J. A. Sommer, S. E. Engberg, J. R. Zaonarivelo, M. I. Mayor, and R. A. Brenneman. 2006a. Revision of the mouse lemurs (Primates, *Microcebus*) of eastern Madagascar. International Journal of Primatology 27:347-389.

Louis, E. E., Jr., S. E. Engberg, R. Lei, H. Geng, J. A. Sommer, R. Randriamampionona, J. C. Randriamanana, J. R. Zaonarivelo, R. Andriantompohavana, G. Randriamanana, Prosper, B. Ramaromilanto, G. Rakotoarisoa, A. Rooney, and R. A. Brenneman. 2006b. Molecular and morphological analyses of the sportive lemurs (Family Megaladapidae: Genus *Lepilemur*) reveals 11 previously unrecognized species. Special Publications, Museum of Texas Tech University 49:1-47.

Maddison, W. P., and D. R. Maddison. 1992. MacClade: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts.

Martin, R. D. 1995. Prosimians: from obscurity to extinction? Pp. 535-563 in Creatures of the dark: the nocturnal prosimians (L. Alterman, G. A. Doyle, and M. K. Izard, eds.). Plenum Press, New York.

Mayr, E. 1942. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York, New York.

Mittermeier, R. A., W. R. Konstant, F. Hawkins, E. E. Louis, O. Langrand, J. Ratsimbazafy, R. Rasoloarison, J. U. Ganzhorn, S. Rajaobelina, I. Tattersall, and D. M. Meyers. 2006. Mouse Lemurs. Pp. 183-208 in Lemurs of Madagascar, 2nd Edition. Conservation International Tropical Field Guide Series, Washington, D.C.

Mittermeier, R. A., J. U. Ganzhorn, W. R. Konstant, K. Glander, I. Tattersall, C. P. Groves, A. B. Rylands, A. Hapke, J. Ratsimbazafy, M. I. Mayor, E. E. Louis, Jr., Y. Rumpler, C. Schwitzer, and R. M. Rasoloarison. 2008. Lemur diversity in Madagascar. International Journal of Primatology 29:1607-1656.

Myers, N. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853-858.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Olivieri, G., E. Zimmermann, B. Randrianambinina, S. Rasoloharijaona, D. Rakotondravony, K. Guschanski, and U. Radespiel. 2007. The ever-increasing

diversity in mouse lemurs: three new species in north and northwestern Madagascar. Molecular Phylogenetics and Evolution 43: 309-327.

Petter J.-J., R. Albignac, and Y. Rumpler. 1977. Faune de Madagascar 44: mammifères lémuriens (Primates Prosimiens). ORSTROM/CNRS, Paris.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818.

Rabarivola, C., A. Zaramody, J.-L. Fausser, N. Andriaholinirina, C. Roos, D. Zinner, H. Marcel, and Y. Rumpler. 2006. Cytogenetic and molecular characteristics of a new species of sportive lemur from northern Madagascar. Lemur News 11:45-49.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Sambrook, J., E. F. Fritch, and T. Maniatus. 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Press, New York, New York.

Schneider, S., D. Roessli, and L. Excofier. 2000. Arlequin: a software for population genetics data analysis. Version 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Schwarz, E. 1931. A revision of the genera and species of Madagascar Lemuridae. Proceeding of the Zoological Society of London 1931:399-426.

Silva, W. A., Jr., M. C. R. Costa, V. Valente, J. de Freitas Sousa, M. L. Pacó-Larson, E. M. Espreafico, S. S. Camargo, E. Monteiro, A. de Jesus, M. A. Holanda, M. A. Zago, A. J. G. Simpson, and E. D. Neto. 2001. PCR template preparation for capillary DNA sequencing. BioTechniques 30:537-542.

Smith, R. J., and W. L. Jungers. 1997. Body mass in comparative primatology. Journal of Human Evolution 32:523-559.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-2690.

Swofford, D. L. 2001. PAUP*. Phylogenetic Analyzing Using Parsimony (*and Other Methods). Version 4.0b5. Sinauer Associates, Sunderland, Massachusetts.

Telenius, H., N. P. Carter, C. E. Bebb, M. Nordenskjöb, A. J. Ponder, and A. Tunnacliffe. 1992. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718-725.

Tattersall, I. 1982. The Primates of Madagascar. Columbia University Press, New York.

Thalmann, U., and T. Geissmann. 2005. New species of woolly lemur *Avahi* (Primates: Lemuriformes) in Bemaraha (central western Madagascar). American Journal of Primatology 67:371-376.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX-Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876-4882.

Vogler, A. P., and R. DeSalle. 1994. Diagnosing units of conservation management. Conservation Biology 8:354-363.

Wheeler, Q. D., and N. I. Platnick. 2000. The Phylogenetic Species Concept (*sensu* Wheeler and Platnick). Pp. 55-69 in Species concepts and phylogenetic theory: a debate (Q. D. Wheeler and R. Meier, eds.). Columbia University Press, New York, New York.

Wilmé, L., S. M. Goodman, and J. U. Ganzhorn. 2006. Biogeographic evolution of Madagascar's microendemic biota. Science 312:1063-1065.

Wyner, Y. M., G. Amato, and R. DeSalle. 1999. Captive breeding, reintroduction, and the conservation genetics of black and white ruffed lemurs, *Varecia variegata variegata*. Molecular Ecology 8:S107-S115.

Zhang, D.-X., and G. M. Hewitt. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology and Evolution 11:247-251.

Addresses of authors:

BOROMÉ RAMAROMILANTO

Parc Botanique et Zoologique de Tsimbazaza
BP 4096
Antananarivo 101, Madagascar
ramaro_boro@yahoo.fr

RUNHUA LEI

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
leir@omahazoo.com

SHANNON E. ENGBERG

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
genetics@omahazoo.com

STEIG E. JOHNSON

Department of Anthropology
University of Calgary
2500 University Drive N.W.
Calgary, AB, T2N 1N4, Canada
steig.johnson@ucalgary.ca

BRANDON D. SITZMANN

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
brandons@omahazoo.com

EDWARD E. LOUIS, JR.

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
edlo@omahazoo.com

The following Appendices are available online at the indicated website addresses as portable document format (pdf) documents.

APPENDIX I

(a). Appendix I(a). Table of all *Lepilemur* individual samples and corresponding information including TK number, site, original species designation, current species designation, GenBank accession number of sequence data.

(b). Appendix I(b). Individual field data information for each novel *Lepilemur*, including morphometrics, photos, global position system, microchip data, gender, and location.

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

APPENDIX II

(a). Appendix II(a). *Lepilemur* D-loop fragment haplotypes Bayesian analysis cladogram.

(b). Appendix II(b). *Lepilemur* PAST fragment haplotypes Bayesian analysis cladogram.

(c). Appendix II(c). *Lepilemur* D-loop fragment haplotypes maximum likelihood phylogram.

(d). Appendix II(d). *Lepilemur* PAST fragment haplotypes maximum likelihood phylogram.

(e). Appendix II(e). *Lepilemur* D-loop fragment total maximum likelihood phylogram.

(f). Appendix II(f). *Lepilemur* D-loop fragment total Bayesian analysis cladogram.

(g). Appendix II(g). *Lepilemur* PAST fragment total maximum likelihood phylogram.

(h). Appendix II(h). *Lepilemur* PAST fragment total Bayesian analysis cladogram.

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

APPENDIX III

(a). Table 1A. Diagnostic nucleotide sites from the D-loop fragment Pairwise Aggregate Analysis (PAA) of Lepilemur.

(b). Table 1B. Diagnostic nucleotide sites from the PAST fragment Pairwise Aggregate Analysis (PAA) of Lepilemur.

(c). Table 4A. Genetic distance matrix for D-Loop fragment sequence data for *Lepilemur* species.

(d). Table 4B. Genetic distance matrix for PAST fragment sequence data for *Lepilemur* species.

(e). Haplotype Table 1 (Summary of designated haplotypes for the genus *Lepilemur* from all localities for D-loop and PAST fragments).

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

PUBLICATIONS OF THE MUSEUM OF TEXAS TECH UNIVERSITY

Institutional subscriptions are available through the Museum of Texas Tech University, attn. NSRL Publications Secretary, Box 43191, Lubbock, TX 79409-3191. Individuals may also purchase separate numbers of the Occasional Papers directly from the Museum of Texas Tech University.

Layout and Design: Lisa Bradley
Cover Design: Lisa Kimmel

This book was set in Times New Roman and printed on acid-free paper that meets the guidelines for permanence and durability of the Committee on Production Guidelines for Book Longevity of the Council on Library Resources.

Printed: 8 April 2009

Library of Congress Cataloging-in-Publication Data
Occasional Papers, Museum of Texas Tech University, Number 286
Series Editor: Robert J. Baker

SPORTIVE LEMUR DIVERSITY AT MANANARA-NORD BIOSPHERE RESERVE, MADAGASCAR

Boromé Ramaromilanto, Runhua Lei, Shannon E. Engberg, Steig E. Johnson, Brandon D. Sitzmann, and Edward E. Louis, Jr.

ISSN 0149-175X

Museum of Texas Tech University
Lubbock, TX 79409-3191 USA
(806)742-2442

The Museum of Texas Tech University has a catalog of Occasional Papers which may be viewed online at www.nsrl.ttu.edu. To do so, you must have Adobe Acrobat installed on your computer. If you have difficulty downloading Occasional Papers, please contact the Webmaster. If there is continued difficulty, contact the Webmaster and a single hard copy can be provided to you via mail at no charge.