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Large rivers do not always act as species barriers for *Lepilemur* sp.

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Abstract Sportive lemurs constitute a highly diverse endemic lemur family (24 species) for which many biogeographic boundaries are not yet clarified. Based on recent phylogeographic models, this study aims to determine the importance of two large rivers (the Antainambalana and Rantanabe) in northeastern Madagascar as species barriers for *Lepilemur seali*. The Antainambalana River was previously assumed to act as the southern border of its distribution. A total of 1,038 bp of the mtDNA of four individuals stemming from two adjacent inter-river systems south of the Antainambalana River was sequenced and compared to sequences of 22 described *Lepilemur* species. The phylogenetic reconstruction did not find support for either of the two rivers as species barrier for *Lepilemur*, as all captured individuals clustered closely with and therefore belonged to *L. seali*. However, a previously published sequence of an individual from a site south of our study sites belongs to a separate species. The southern boundary of *L. seali* must therefore be one of two large rivers further south of our study sites. The results suggest that *L. seali* may possess a relatively large altitudinal range that enabled this species to migrate around the headwaters of the Antainambalana and Rantanabe Rivers. Previous phylogeographic models need to be refined in order to incorporate these findings, and more species-specific altitudinal range data are urgently needed in order to fully understand the biogeographic patterns of lemurs on Madagascar.

Keywords Biogeography · Lemurs · Sportive lemurs · Speciation

Introduction

Lemurs show a remarkable species diversity, both numerically and in terms of adaptations (Mittermeier et al. 2006; Tattersall 2007). During the last decade, the number of described lemur species has increased enormously (e.g., Louis et al. 2006; Craul et al. 2007; Olivieri et al. 2007). In relation to the small surface area of Madagascar, species diversity within this primate radiation is quite high. Three major models have been proposed to explain the diversity of Malagasy mammals (Martin 1972, 1995; Wilmé et al. 2006; Olivieri et al. 2007; Craul et al. 2007). All three emphasize the general importance of rivers as biogeographic barriers that enabled founder populations to differentiate and speciate in allopatry. However, incongruence exists among the models concerning the number of effectively isolating rivers and thereby the number of "centers of endemism" on the island. For example, the number of effectively isolated geographic zones in western to northern Madagascar increased from four (Martin 1995)

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and five (Wilmé et al. 2006) to as many as nine (Andriaholinirina et al. 2006; Louis et al. 2006; Craul et al. 2007; Olivieri et al. 2007) as a consequence of fine-scale geographic sampling of different lemur taxa within the last few years.

Sportive lemurs all belong to an endemic monogenic primate family, the Lepilemuridae, which probably split off from the monophyletic phylogeny of Malagasy lemurs between 30 and 45 million years ago (Yoder and Yang 2004). They are middle-sized (0.6–1.2 kg, Louis et al. 2006) and nocturnal and inhabit a large variety of different forest habitats on the island (Mittermeier et al. 2006). Due to cryptic coloration and inconspicuous morphology, the number of extant species was long underestimated and rose only recently from six described species (Petter et al. 1977; Tattersall 1982) to 25 described species (Andriaholinirina et al. 2006; Louis et al. 2006; Rabarivola et al. 2006; Craul et al. 2007; Lei et al. 2008). Since sampling intensities varied greatly among different geographic regions, further efforts are needed to fill in geographic gaps and to test the significance of large rivers as biogeographic barriers under different climatic and topographic conditions. Large rivers have previously been operatively defined as being wider than 50 m at a distance of 20 km inland (Olivieri et al. 2007; Craul et al. 2007).

Lepilemur seali (Seal's Sportive Lemur) is one of the larger sportive lemur species with a uniform light chocolate-brown to reddish-brown coloration. The ventral side is of lighter brown–gray, and the pelage is extremely long and thick throughout the body (Louis et al. 2006). The Seal's sportive lemur is currently known from the Anjanaharibe-South Special Reserve, and the large Antainambalana River was proposed as the southern limit of its distribution range (Louis et al. 2006) (Fig. 1, inter-river system A, short: IRS A). The closest available sampling location to the south is Mananara-North, which is about 170 km south of the Anjanaharibe-South Special Reserve (Fig. 1, IRS M). Louis et al. (2006) still assigned the sportive lemurs from Mananara-North to *L. seali*, although molecular data already suggested that this population should rather be addressed as a separate species. Following the above given definition of a large river, the Anjanaharibe-South Special Reserve and Mananara-North are separated by altogether four large rivers, the Antainambalana, Rantabe, Fahambahy, and Mananara (Fig. 1), but their importance as biogeographic barriers has not yet been evaluated.

The aim of this study is to investigate the biogeographic pattern of *Lepilemur* sp. in this region and to test the significance of two of the four large rivers as biogeographic barriers (Antainambalana, Rantabe) for *L. seali*. Three localities were visited in two adjacent inter-river systems (IRS AR and IRS RF), and genetic samples were taken and analyzed with standard phylogenetic methods in comparison to previously published sportive lemur species.

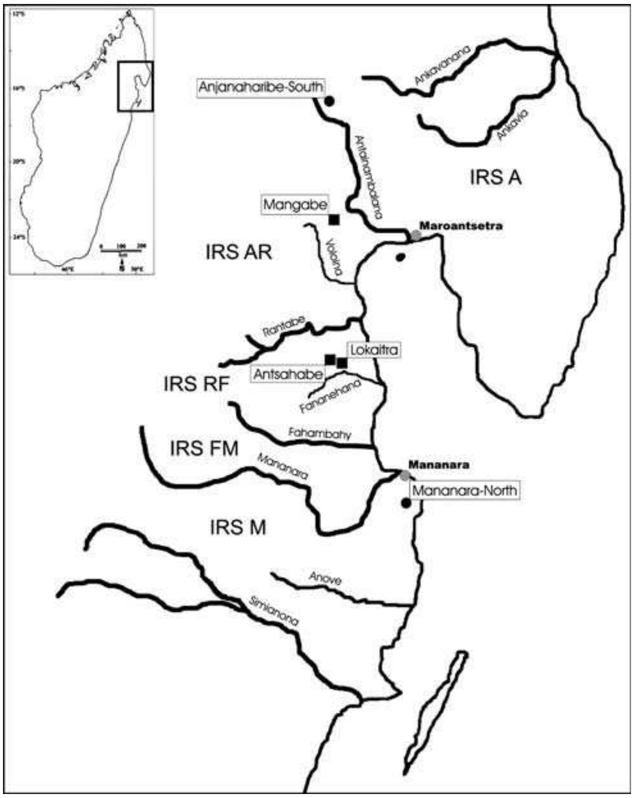

Fig. 1 Map of sampling sites (*black squares* origin of *Lepilemur* individuals from this study; *black circles* type locality of *L. seali* (Anjanaharibe-South) and of individual *L. seali* 5 (Mananara-North) (from Louis et al. 2006)

Methods

In November and December 2006, *Lepilemur* sp. were captured at three different localities from two adjacent IRSs in northeastern Madagascar southeast of the large river Antainambalana and north of the large river Mananara (IRS AR, IRS RF, Fig. 1). All visited sites contained dense montane evergreen rain forest at varying altitudes [Mangabe (S15°18′ E49°30′): 500–950 m, Antsahabe (S15°48′ E49°30′): 850–1,200 m, Lokaitra (S15°49′ E49°30′): 400–1,100 m]. A small biopsy from one or both pinnae was taken as tissue sample from each individual. Tissue samples were stored in Queen's lysis buffer (Seutin et al. 1991) for later DNA extraction and genetic analyses.

DNA from the tissue of four individuals was isolated with a standard proteinase K digestion followed by a Phenol/Chloroform protocol (Sambrook et al. 1989) and stored at −20°C (Table 1, bottom). We sequenced the mitochondrial genes *D-loop* and parts of the PAST-fragment (Louis et al. 2006) containing the complete genes of

Table 1 Origin and GenBank accession numbers of the samples used for this study

Species	Origin	Original sample code	Citation	D-loop	ND3/ND4L
L. aeeclis 1	Anjahamena	PBZT119	Louis et al. (2006)	DQ529528	DQ529670
L. aeeclis 2	Anjahamena	JAM4.8	Louis et al. (2006)	DQ529529	DQ529671
L. aeeclis 4	Anjahamena	MIT16	Louis et al. (2006)	AY769403	AY582604
L. aeeclis 3	Anjahamena	PBZT112	Louis et al. (2006)	AY769405	AY582606
L. ahmansoni 1	Tsiombikibo	KIBO58	Louis et al. (2006)	DQ529461	DQ529603
L. ahmansoni 2	Tsiombikibo	KIBO68	Louis et al. (2006)	DQ529463	DQ529605
L. ahmansoni 3	Tsiombikibo	KIBO22	Louis et al. (2006)	DQ529460	DQ529602
L. ankaranensis 1	Analamera	LABE5.17	Louis et al. (2006)	DQ529601	DQ529743
L. ankaranensis 2	Ankarana	CAR59	Louis et al. (2006)	AY769381	AY582582
L. ankaranensis 3	Andrafiamena	FIA5.1	Louis et al. (2006)	DQ529572	DQ529714
L. ankaranensis 4	Andrafiamena	FIA5.5	Louis et al. (2006)	DQ529573	DQ529715
L. betsileo 1	Fandriana	FAN4.24	Louis et al. (2006)	DQ529550	DQ529692
L. dorsalis 1	Lokobe	Lok1	Craul et al. (2007)	EF686740	EF686654
L. dorsalis 3	Manehoka	Oka2	Craul et al. (2007)	EF686742	EF686656
L. dorsalis 4	Manehoka	Oka3	Craul et al. (2007)	EF686743	EF686657
L. dorsalis5	Manehoka	Oka1	Craul et al. (2007)	EF686744	EF686658
L. edwardsi 1	Ankarafantsika	ANK16	Louis et al. (2006)	AY769385	AY582586
L. edwardsi 2	Ankarafantsika	ANK4	Louis et al. (2006)	AY769384	AY582585
L. edwardsi 3	Ankarafantsika	MAR1	Louis et al. (2006)	DQ529464	DQ529606
L. grewcockorum 1	Anjiamangirana	HIH23	Louis et al. (2006)	DQ529476	DQ529618
L. grewcockorum 2	Anjiamangirana	HIH22	Louis et al. (2006)	DQ529475	DQ529617
L. grewcockorum 3	Anjiamangirana	HIH21	Louis et al. (2006)	DQ529474	DQ529616
L. hubbardi 1	Zombitse	ZOMB52	Louis et al. (2006)	DQ529527	DQ529669
L. hubbardi 2	Zombitse	ZOMB15	Louis et al. (2006)	DQ529524	DQ529666
L. hubbardi 3	Zombitse	ZOMB21	Louis et al. (2006)	DQ529526	DQ529668
L. hubbardi 4	Zombitse	ZOMB12	Louis et al. (2006)	DQ529523	DQ529665
L. hubbardi 5	Zombitse	ZOMB16	Louis et al. (2006)	DQ529525	DQ529667
L. jamesi 1	Manombo	L5	Louis et al. (2006)	AY769420	AY582620
L. leucopus 1	Andohahela	HAZO5.23	Louis et al. (2006)	DQ529568	DQ529710
L. leucopus 2	Andohahela	AND66	Louis et al. (2006)	DQ529488	DQ529630
L. leucopus 3	Andohahela	AND65	Louis et al. (2006)	DQ529487	DQ529629
L. microdon 1	Ranomafana	RAN3.1	Louis et al. (2006)	DQ529558	DQ529700
L. microdon 2	Ranomafana	TOL2.17	Louis et al. (2006)	AY769398	AY582599
L. microdon 3	Ranomafana	KEL1	Louis et al. (2006)	DQ529540	DQ529682
L. milanoii 1	Daraina	DAR4.17	Louis et al. (2006)	DQ529478	DQ529620
L. milanoii 2	Daraina	DAR4.18	Louis et al. (2006)	DQ529479	DQ529621
L. milanoii 3	Daraina	DAR4.23	Louis et al. (2006)	DQ529481	DQ529623
L. milanoii 4	Daraina	DAR4.19	Louis et al. (2006)	DQ529480	DQ529622
L. mustelinus 1	Maromizaha	MIZA11	Louis et al. (2006)	DQ529554	DQ529696
L. mustelinus 2	Maromizaha	MIZA3	Louis et al. (2006)	DQ529553	DQ529695
L. mustelinus 3	Zahamena	ZAH216	Louis et al. (2006)	AY769430	AY582630
L. otto 1	Ambodimahabibo	Bibo1	Craul et al. (2007)	EF686762	EF686676
L. petteri 1	Beza-Mahafaly	BEZ15	Louis et al. (2006)	AY769387	AY582588
L. petteri 2	Beza-Mahafaly	BEZ18	Louis et al. (2006)	AY769388	AY582589
L. randrianasoli 1	Tsingy de Bemaraha	BEMA6	Louis et al. (2006)	DQ529519	DQ529661
L. randrianasoli 2	Tsingy de Bemaraha	BEMA7	Louis et al. (2006)	DQ529520	DQ529662
L. ruficaudatus 1	Beroboka	MOR142	Louis et al. (2006)	AY769401	AY582602
L. ruficaudatus 2	Beroboka	MOR117	Louis et al. (2006)	AY769400	AY582601

Table 1 continued

Species	Origin	Original sample code	Citation	D-loop	ND3/ND4L
L. ruficaudatus 3	Beroboka	MOR147	Louis et al. (2006)	AY769402	AY582603
L. ruficaudatus 4	Kirindy	Kir1	Craul et al. (2007)	EF686766	EF686680
L. ruficaudatus 5	Kirinidy	Kir2	Craul et al. (2007)	EF686767	EF686681
L. sahamalazensis 1	Sahamalaza	Sah2	Craul et al. (2007)	EF686751	EF686665
L. seali 1	Anjanaharibe-South	JAR3.46	Louis et al. (2006)	DQ529502	DQ529644
L. seali 2	Anjanaharibe-South	JAR8	Louis et al. (2006)	AY769394	AY582595
L. seali 3	Anjanaharibe-South	JAR3.38	Louis et al. (2006)	DQ529500	DQ529642
L. seali 4	Anjanaharibe-South	JAR3	Louis et al. (2006)	AY769393	AY582594
L. seali 5	Mananara-North	NARA4.20	Louis et al. (2006)	DQ529552	DQ529694
L. septentrionalis 1	Sahafary	LAVA5.4	Louis et al. (2006)	DQ529589	DQ529731
L. tymerlachsoni 1	Lokobe	LOKO4.32	Louis et al. (2006)	DQ529513	DQ529655
L. wrighti 1	Kalambatritra	KALA5.12	Louis et al. (2006)	DQ529569	DQ529711
L. wrighti 2	Kalambatritra	KALA4.9	Louis et al. (2006)	DQ529504	DQ529646
L. wrighti 3	Kalambatritra	KALA4.16	Louis et al. (2006)	DQ529505	DQ529647
L. wrighti 4	Kalambatritra	KALA24	Louis et al. (2006)	DQ529499	DQ529641
L. wrighti 5	Kalambatritra	KALA4.18	Louis et al. (2006)	DQ529506	DQ529648
L. sp. (*L. seali*)	Antsahabe	01-06Ant	This study	EU810338	EU810342
L. sp. (*L. seali*)	Mangabe	03-06Man	This study	EU810340	EU810344
L. sp. (*L. seali*)	Mangabe	05-06Man	This study	EU810341	EU810345
L. sp. (*L. seali*)	Lokaitra	08-06Lok	This study	EU810339	EU810343

$tRNA^{Gly}$ and $tRNA^{Arg}$, as well as the NADH-dehydrogenase subunits 3 (ND3, complete) and 4L (ND4L, partial), because reference sequences from 22 recognized species were available from GenBank for these genes. We used the same laboratory methods as described by Craul et al. (2007). The purified PCR products were sent to South Korea and sequenced on an ABI 3730XL automatic DNA sequencer by Macrogen (http://www.macrogen.com/english/index.html). The respective sequences were deposited in GenBank (Table 1).

For a comprehensive phylogenetic analysis of the sequence data, we compared our data set of four unknown specimen with five published *L. seali* sequences from the Louis et al. (2006) study and with 59 different reference sequences of 21 other sportive lemur species (Table 1). *Avahi laniger* and *A. occidentalis* were selected as outgroups for phylogenetic tree reconstructions. Sequences were aligned using the program CLUSTAL_X (Thompson et al. 1997) and checked by eye. Tree reconstructions were performed with the maximum-parsimony (MP), neighbor-joining (NJ), and maximum-likelihood (ML) algorithms as implemented in PAUP4.0b10 (Swofford 1999). Throughout the analyses, all characters were treated as unordered and equally weighted. Gaps were considered as missing data in NJ and ML, but were treated as fifth character in MP analysis. The NJ and ML trees were constructed using the best-fit model selected by the hierarchical likelihood ratio test (hLRT) in Modeltest3.5.mac (Posada and Crandall 1998). Relative support of internal nodes was provided by bootstrap analyses with 1,000 replications for MP and NJ and 100 replications for ML. In order to reconstruct the phylogenetic relationships within the genus *Lepilemur*, we combined the two loci to one concatenated sequence, 1,038 bp in length. Four hundred and eighty-nine characters were constant, 221 variable characters were parsimony-uninformative, and 328 were parsimony-informative. The best-fit model selected by hLRT in Modeltest 3.5.mac was the TrN+I+G model [base = (0.3213 0.2779 0.1183), Nst = 6 Rmat = (1.0000 17.5780 1.0000 1.0000 12.7019), Alpha = 0.6013, Pinvar = 0.3220]. Finally, absolute pairwise distances were calculated using PAUP4.0b10 for the total sample of 68 sequences in order to estimate and differentiate intraspecific variability from interspecific differentiation.

Results

Figure 2 shows the maximum likelihood tree based on the concatenated sequences of the two loci. All reference sequences of each species clustered together in well-supported terminal clades and different species were separated from each other in all but one case. This case concerns the reference sequences from *L. dorsalis* and *L. tymerlachsoni* that clustered together in all three phylogenetic methods. However, this is not due to an inefficient resolution of the

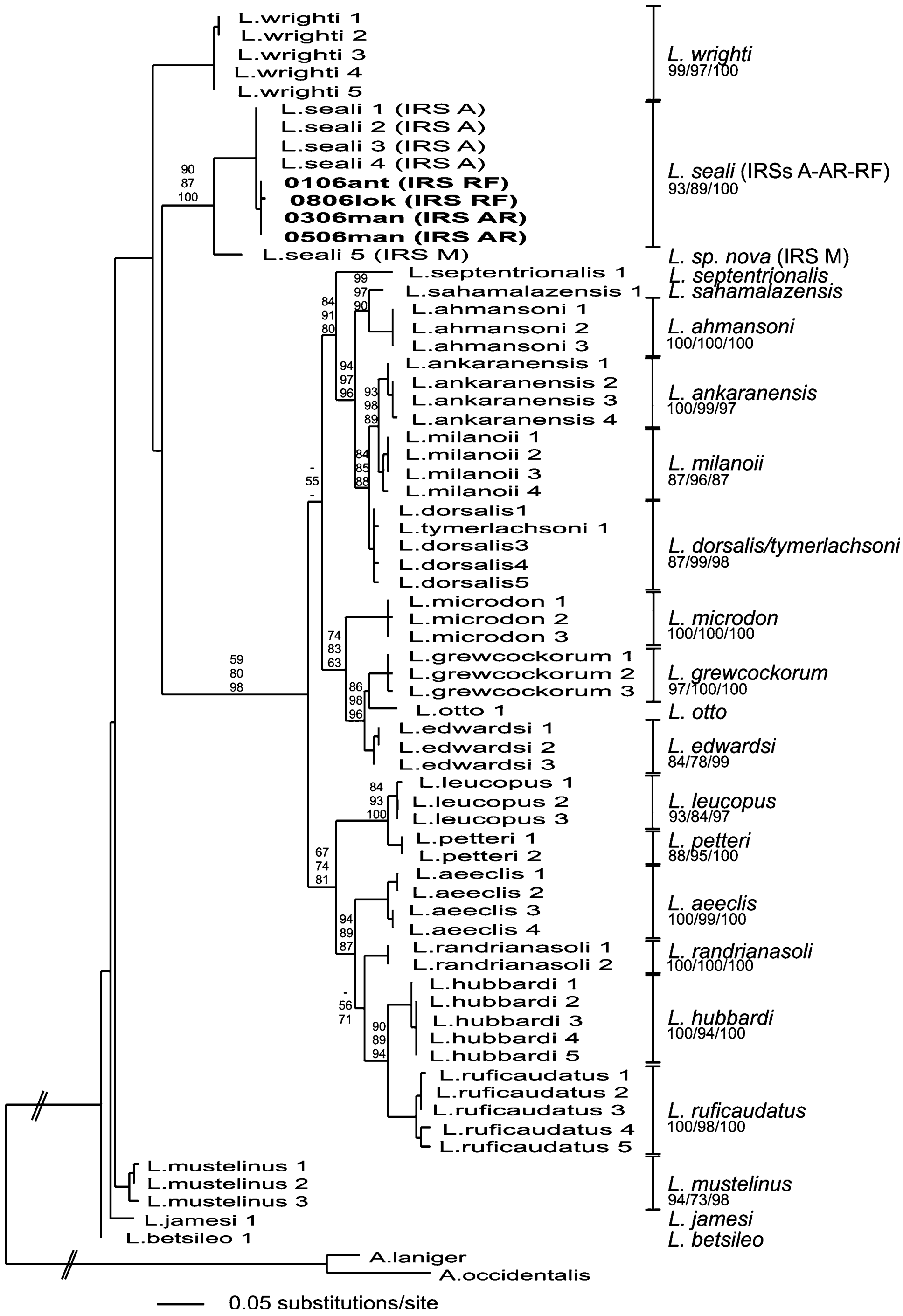

Fig. 2 Maximum likelihood tree based on the concatenated sequences of the two loci. The branch lengths indicate the number of substitutions; the numbers above the branches indicate bootstrap values for internal branches (*top* ML, *middle* NJ, *lower* MP). Bootstrap values for terminal clades are provided on the right below species names (ML, NJ, MP). In *bold* unknown samples from this study. *IRS* inter-river system, for details of the geographic setting see Fig. 1

tree, but to the fact that *L. tymerlachsoni*, living on the island of Nosy Bé and newly described by Louis et al. (2006), probably represents a synonym for *L. dorsalis* that has been found both on the island Nosy Bé as well as in the opposing inter-river system on the mainland of Madagascar (Craul et al. 2007).

The four individuals from this study, 01-06Ant, 08-06Lok (both IRS RF), 03-06Man, and 05-06Man (both IRS AR), clustered closely together with four reference sequences of *L. seali* (*L. seali* 1–4) from the IRS A. The remaining *L. seali* 5 sequence from Mananara-North (IRS M) diverged strongly from the rest of the *L. seali* clade, being more different from *L. seali* than several other closely related species among themselves.

The absolute pairwise distances between 01-06Ant, 03-06Man, 05-06Man, 08-06Lok and the individuals from Anjanaharibe-South ranged from 0.30 to 0.49% (Table 2). These differences resembled the typical intraspecific

Table 2 Minimum (min) and maximum (max) absolute pairwise distances (%) among the sampled individuals and all available *Lepilemur* species (–: no value as sample size = 1)

		L. wrighti	*L. seali* (Anjanaharibe-Sud)	*L.* sp. (*L. seali*, this study)	NARA4.20 (Mananara-Nord)	*L. mustelinus*	*L. betsileo*	*L. jamesi*	*L. septentrionalis*	*L. sahamalazensis*	*L. ahmansoni*	*L. ankaranensis*	*L. milanoii*	*L. dorsalis*	*L. microdon*	*L. grewcockorum*	*L. edwardsi*	*L. otto*	*L. leucopus*	*L. petteri*	*L. aeeclis*	*L. randrianasoli*	*L. hubbardi*	*L. ruficaudatus*
L. wrighti	min	0,10																						
	max	0,79																						
L. seali (Anjanaharibe-Sud)	min	8,85	0,00																					
	max	9,05	0,00																					
L. sp. (*L. seali*, this study)	min	8,95	0,30	0,00																				
	max	9,34	0,49	0,59																				
NARA4.20 (Mananara-Nord)	min	9,14	5,70	5,99	-																			
	max	9,44	5,70	5,99	-																			
L. mustelinus	min	7,76	8,95	8,95	8,75	0,10																		
	max	8,35	9,15	9,44	9,54	1,77																		
L. betsileo	min	7,66	9,24	9,34	8,75	2,65	-																	
	max	8,06	9,34	9,64	8,85	3,05	-																	
L. jamesi	min	7,47	9,54	9,64	8,85	3,44	2,55	-																
	max	7,76	9,54	9,83	8,85	3,54	2,65	-																
L. septentrionalis	min	13,38	14,67	14,77	14,87	13,59	13,39	12,80	-															
	max	13,78	14,67	14,87	14,87	14,18	13,49	12,80	-															
L. sahamalazensis	min	12,79	14,37	14,47	13,69	12,21	11,32	11,61	7,28	-														
	max	12,99	14,37	14,67	13,69	12,69	11,42	11,61	7,28	-														
L. ahmansoni	min	12,39	14,07	14,17	14,17	12,59	11,70	11,70	7,57	2,95	0,10													
	max	13,08	14,26	14,56	14,27	12,98	11,90	11,80	7,77	3,05	0,39													
L. ankaranensis	min	13,18	14,07	14,17	14,07	12,79	12,19	12,39	7,08	4,42	4,52	0,10												
	max	13,57	14,36	14,66	14,27	13,86	12,49	12,59	7,28	5,01	5,01	0,69												
L. milanoii	min	12,98	13,67	13,77	13,87	12,39	11,70	12,19	6,59	4,33	4,42	1,18	0,10											
	max	13,37	13,87	14,17	13,97	13,08	11,90	12,29	6,69	4,42	4,81	1,77	0,49											
L. tymerlachsoni/dorsalis	min	12,99	14,47	14,57	13,88	12,99	12,30	12,60	6,59	3,34	3,93	2,16	2,26	0,10										
	max	13,67	14,87	15,16	14,08	13,77	12,70	12,89	6,89	3,64	4,52	3,05	2,65	0,49										
L. microdon	min	13,16	12,87	12,97	13,76	12,28	11,88	12,28	8,36	7,96	8,44	7,95	7,76	8,45	0,10									
	max	13,65	12,97	13,27	13,96	13,16	12,28	12,67	8,46	8,06	8,74	8,74	8,05	8,84	0,69									
L. grewcockorum	min	12,57	13,17	13,27	13,76	12,78	12,18	12,28	9,14	7,96	8,25	8,74	8,84	8,26	5,99	0,10								
	max	13,16	13,37	13,66	13,96	13,16	12,47	12,47	9,34	8,16	8,54	9,33	9,13	8,85	6,28	0,29								
L. edwardsi	min	12,47	12,88	12,97	12,88	11,79	11,20	11,20	8,26	6,49	6,58	7,27	7,36	7,08	5,89	2,85	0,49							
	max	13,06	13,17	13,46	13,17	12,67	11,59	11,49	8,65	6,88	7,17	7,86	7,76	7,77	6,38	3,34	0,59							
L. otto	min	13,16	13,76	13,86	14,15	13,56	12,86	12,77	9,63	8,26	8,15	9,23	9,33	8,75	7,26	3,93	3,24	-						
	max	13,45	13,76	14,05	14,15	13,95	12,96	12,77	9,63	8,26	8,25	9,33	9,43	9,24	7,36	4,12	3,44	-						
L. leucopus	min	14,28	15,95	16,05	15,85	13,60	13,89	13,49	9,84	10,14	10,13	9,34	9,15	9,15	9,93	10,33	9,15	10,62	0,10					
	max	15,07	16,24	16,54	16,04	14,28	14,08	13,89	10,44	10,63	10,92	10,23	9,64	10,04	10,23	10,82	9,74	10,72	0,79					
L. petteri	min	13,78	15,06	15,16	15,26	13,40	13,50	13,49	10,24	10,04	11,02	10,03	9,74	9,55	10,03	10,52	9,54	10,62	1,77	0,30				
	max	14,38	15,36	15,65	15,55	14,28	13,89	13,79	10,53	10,33	11,41	10,52	10,13	10,14	10,62	11,01	10,23	10,72	2,56	0,30				
L. aeeclis	min	12,70	15,05	15,05	15,05	13,29	13,10	13,49	9,55	9,44	9,83	8,55	8,46	8,86	9,63	8,35	8,06	9,73	8,46	8,46	0,10			
	max	13,39	15,45	15,74	15,45	14,07	13,49	13,88	9,84	9,94	10,42	9,83	9,34	9,45	10,52	9,14	9,14	10,22	9,15	9,15	1,38			
L. randrianasoli	min	13,77	15,56	15,65	15,56	13,29	13,48	13,28	8,86	10,13	9,74	9,34	9,24	8,85	9,83	9,44	8,95	10,42	7,97	8,56	4,92	0,10		
	max	14,17	15,65	15,95	15,66	13,77	13,68	13,38	8,96	10,23	9,93	9,64	9,64	9,24	10,03	9,74	9,44	10,52	8,17	8,95	5,22	0,10		
L. hubbardi	min	14,07	15,16	15,26	15,56	14,07	13,97	13,88	9,35	11,01	10,62	10,13	9,64	9,74	9,93	9,63	9,24	10,42	8,86	9,45	6,60	5,61	0,10	
	max	14,66	15,46	15,76	15,86	14,46	14,27	14,07	9,55	11,31	11,01	10,43	10,23	10,32	10,32	10,03	9,73	10,72	9,64	9,94	7,18	5,90	0,39	
L. ruficaudatus	min	13,18	14,77	14,67	14,58	13,29	13,58	13,38	10,43	10,62	10,72	10,82	10,42	10,32	9,73	10,03	9,54	10,52	9,84	9,64	6,69	6,20	5,11	0,10
	max	14,27	15,26	15,56	15,26	13,87	14,17	13,58	10,82	10,91	11,31	11,41	11,11	10,91	10,32	10,72	10,23	10,82	10,63	10,63	7,48	6,79	5,80	2,56

variability that could be observed within this genus (Table 2, on diagonal). The absolute pairwise distance between the samples from this study and the individual from Mananara-North (*L. seali* 5) equaled 5.99% (Table 2). Finally, the *L. seali* 5 sequence was more different from the *L. seali* sequences (5.70%) than 24 of the analyzed species pairs in Table 2.

Discussion

In all phylogenetic analyses, the sampled individuals clustered closely together with *L. seali* from the Anjanaharibe-South Special Reserve. The absolute pairwise distances between *L. seali* and the specimen from this study are within the range of intraspecific variation. This finding strongly suggests that the sampled individuals belonged to the species *L. seali*. In contrast, the individual from Mananara-North did not cluster closely with these sequences. The absolute pairwise distances between all *L. seali* sequences and the individual from Mananara-North were in the range of those observed among *Lepilemur* species and in other lemur genera, such as *Mirza* (Kappeler et al. 2005), *Microcebus* (Kappeler et al. 2005; Louis et al. 2006; Olivieri et al. 2007), *Hapalemur* (Fausser et al. 2002; Pastorini et al. 2002), or *Propithecus* (Mayor et al. 2004).

This suggests that the southern limit of *L. seali* is not the Antainambalana River, as was proposed by Louis et al. (2006). The next large southern river, the Rantabe River, could also not be confirmed as a species barrier for *Lepilemur* sp., as *L. seali* could be found south of it in the sites Antsahabe and Lokaitra (IRS AR). The results indicate that either the Fahambahy or the Mananara River should act as a biogeographic barrier for *L. seali*, since the sportive lemur from Mananara-North already belonged to a different taxon. Study sites between those two rivers will be needed to clarify their respective role as isolating barriers.

The findings of this study differ not only from the study of Louis et al. (2006), but also from the predictions made by Wilmé et al. (2006). Wilmé et al. (2006) suggested the Antainambalana watershed as a zone of retreat and dispersion during times of paleoclimatic periods of fluctuating aridification. However, this scenario should subsequently have led to separate centers of endemism on either side of the river. At least for the genus *Lepilemur*, this model could not be confirmed by this study. The lack of isolating effects of the Antainambalana River is surprising, as this river is still about 30 m wide even 100 km inland (http://earth.google.com) and has been shown to separate even larger congeneric taxa, such as *Varecia v. variegata* from *V. v. rubra* (Goodman and Ganzhorn 2004).

Altitudinal stratification has been suggested as another important factor limiting the migratory potential of lemur species. Goodman and Ganzhorn (2004) suggested that the potential for river crossings depends on the elevational range of a given species in comparison to the altitude of the headwaters of the river under study. The headwaters of the Antainambalana River do not exceed 1,500 m (source at 1,450 m, Goodman and Ganzhorn 2004). The elevational range of *L. seali* is not yet known, but its congeners *L. mustelinus* and *L. microdon* have been observed at altitudes higher than 1,600 m (Goodman and Rasolonandrasana 2001; Goodman and Ganzhorn 2004). If *L. seali* would possess the same altitudinal tolerance, they could have migrated around the headwaters of the Antainambalana River. Further studies are needed to establish the elevational range of *L. seali* in order to be able to fully understand the biogeographic pattern of lemurs in this region.

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