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RESEARCH ARTICLE

Phylogeny and Phylogeography of Squirrel Monkeys (Genus *Saimiri*) Based on Cytochrome *b* Genetic Analysis

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Squirrel monkeys (genus *Saimiri*) are distributed over a wide area encompassing the Amazon Basin: French Guiana, Suriname, and Guyana, together with Western Panama and Western Costa Rica. The genus *Saimiri* includes a complex of species and subspecies displaying considerable morphological variation. Taxonomic and systematic studies have identified, in this genus, one to seven species comprising up to 16 subspecies. The phylogenetic relationships between these taxa are poorly understood. Molecular markers have yielded a consistent framework for the systematics of Central and South American *Saimiri*, identifying four distinct clades: *S. oerstedii*, *S. sciureus*, *S. boliviensis*, and *S. ustus*. Here, we reconsider the phylogenetic and biogeographic history of *Saimiri* on the basis of mitochondrial (mtDNA) sequence data, focusing mostly on individuals originating from the Amazon Basin. We studied 32 monkeys with well-defined geographic origins and inferred the phylogenetic relationships between them on the basis of full-length cytochrome *b* gene nucleotide sequences. The high level of gene diversity observed (0.966) is consistent with the high level of behavioral and morphological variation observed across the geographic range of the genus: 20 mtDNA haplotypes were identified with a maximum divergence of 4.81% between *S. b. boliviensis* and *S. ustus*. In addition to confirming the existence of the four clades previously identified on the basis of molecular characters, we suggest several new lineages, including *S. s. macrodon*, *S. s. albigena*, *S. s. cassiquiarensis*, and *S. s. collinsi*. We also propose new patterns of dispersion and diversification for the genus *Saimiri*, and discuss the contribution of certain rivers and forest refuges to its structuring. Am. J. Primatol. 72:242–253, 2010.

Key words: cytochrome *b*; phylogeny; phylogeography; *Saimiri*

INTRODUCTION

After the colonization of South America by an ancestral African monkey stock at the end of the Eocene, platyrrhine radiation occurred early in the Miocene [Poux et al., 2006; Schrago, 2007]. Nevertheless, the biogeography of many South American primates remains a matter of debate [Cortes-Ortiz et al., 2003; Ruiz-Garcia et al., 2006, 2007], and the taxonomy and systematics of many genera, including *Saimiri*, remain incomplete [Cropp, 1998; Cropp et al., 1999; Medeiros et al., 1997].

The genus *Saimiri* has a wide geographical distribution, extending from latitudes 10°N to 17°S and encompassing the Amazon Basin: Brazil, Colombia, Ecuador, Peru, Bolivia, Venezuela, Guyana, Suriname, and French Guiana, together with the Pacific part of Costa Rica and Panama in Central America (Fig. 1). Paleontological data have yielded various estimates of divergence times for the genus *Saimiri*, ranging from 12 to 20 million years ago (MYA). A divergence time estimate of 16.9 MYA between the *Cebus* and *Saimiri* genera has been proposed on the basis of the maximum likelihood procedure, whereas a Bayesian approach gave a

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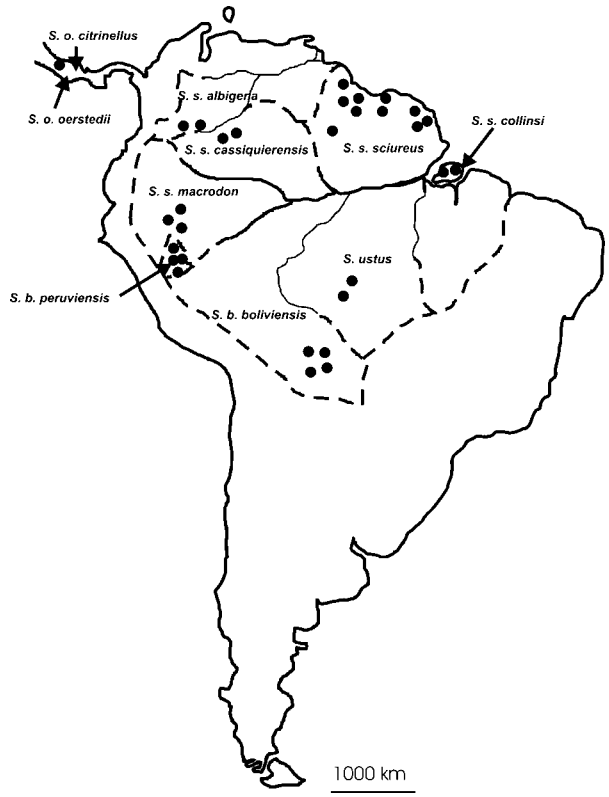

Fig. 1. Geographical distribution of the species and subspecies of *Saimiri* based on Hershkovitz's classification [1984]. Dots indicate localities of origin of the squirrel monkeys samples.

TABLE I. Main Classifications of Squirrel Monkeys, Based on Morphological Criteria

Hershkovitz [1984]	Thorington [1985]	Costello [1993]
Roman type		
S. boliviensis	*S. sciureus*	*S. sciureus* (all South American taxa)
S. b. boliviensis	*S. s. boliviensis*	
S. b. peruviensis	*S. s. sciureus*	
	S. s. cassiquiarensis	
Gothic type	*S. s. oerstedii*	
S. sciureus		
S. s. sciureus		
S. s. macrodon		
S. s. albigena		
S. s. cassiquiarensis		
S. oerstedii		*S. oerstedii* (Central American forms)
S. o. oerstedii		
S. o. citrinellus		
S. ustus	*S. madeirae* (= *S. ustus*)	
*S. vanzolinii**	*S. vanzolinii**	

*after Ayres, 1985.

divergence time estimate between these genera of 19.5 MYA (SD: 1.5 MYA) [Opazo et al., 2006].

The taxonomy and phylogeny of the current squirrel monkey population also remain unclear. Indeed, squirrel monkeys display significant phenotypic variability, with 1–7 species and up to 16 subspecies recognized on the basis of morphological criteria [Costello et al., 1993; Hershkovitz, 1984; Thorington, 1985] (Table I). Molecular characters for classification have also recently been investigated [Boinski & Cropp, 1999; Cropp & Boinski, 2000]. An analysis of nuclear and mitochondrial genes identified four major clades supporting Hershkovitz's classification: (i) *S. oerstedii* with two subspecies, *S. o. oerstedii* and *S. o. citrinellus*; (ii) *S. boliviensis* with two subspecies, *S. b. boliviensis* and *S. b. peruviensis*; (iii) *S. ustus*; and (iv) *S. sciureus*. However, the relationships between the Amazonian subspecies of *S. sciureus* remain unresolved. For instance, there is currently no molecular evidence to support the taxa *macrodon* reported in Ecuador, Peru, and Colombia, *albigena* in Colombia, *cassiquiarensis* in Colombia, Venezuela, and Brazil, and *collinsi* at the mouth of the Amazon. We carried out a molecular study on squirrel monkeys from the Amazon region to decipher the evolutionary relationships between the various species belonging to the genus *Saimiri*. We used the sequence of the mitochondrial cytochrome *b* gene as a marker for this study of the phylogeography, biosystematics, and genetic structure of the genus. Cytochrome *b* has often been used to investigate evolutionary significant units and resolve taxonomic conflicts in various mammalian groups [Avise, 2000; Wan et al., 2004]. This marker has been used, for example, to infer phylogeographic patterns in numerous small neotropical mammals [Mustrangi & Patton, 1997; Patton & Da Silva, 1998]. This marker has also been used to investigate the biogeographic history of *Alouatta* species [Bonvicino et al., 2001; Cortes-Ortiz et al., 2003; Nascimento et al., 2005].

Our primary objective in this study was to investigate the phylogeography of the genus *Saimiri*, and to identify patterns of dispersal and diversification. Indeed, the current organization, diversification of clades, and evolution of population sizes in Amazonia have been influenced by different historical and ecological factors during the Quaternary period. These events include tectonic uplift or subsidence of certain areas, development and presence of river barriers, and climatic fluctuations leading to changes in the vegetation cover. A variety of nonexclusive hypotheses has been proposed to explain patterns of speciation in the Quaternary period [Rull, 2006]: (i) The Paleogeography hypothesis is related to tectonic movements and/or sea-level fluctuations; (ii) The River hypothesis and its variants concern the effect of Amazonian rivers as barriers to dispersal; (iii) The Refuge hypothesis

argues that a combination of alternating climatic changes, caused by Milankovitch cycles and tectonic uplift of the Andes and other higher elevation terrain, allowed for the formation of humid forest refugia interspersed by areas of dry forest and open grasslands [Haffer, 1997]; (iv) The Disturbance–Vicariance hypothesis proposes that alternating cold–warm cycles during the Quaternary caused the current tropical Amazon forest to shrink, leaving dry habitat on the periphery thought to be areas of high disturbance and faunal turnover, leading to high levels of speciation [Colinvaux et al., 2000]; and (v) The Gradient hypothesis imagines that parapatric speciation has occurred across steep environmental gradients without separation of the representative populations. The signatures of these different factors have already been identified in several taxa, including trees [e.g. Aide & Rivera, 1998; Dick & Heuertz, 2008], fishes [Hubert et al., 2007; Renno et al., 2006], reptiles [Pearse et al., 2006], birds [Hayes & Sewlal, 2004], and mammals [Cortes-Ortiz et al., 2003; Patton & Da Silva, 1998; Ruiz-Garcia et al., 2007]. They would also be expected to have an impact on *Saimiri* phylogenetic history.

Our secondary objective was to use molecular data to test the validity of the clades proposed by Hershkovitz [1984], which remain the most commonly supported classification of modern squirrel monkeys [Da Silva et al., 1987; VandeBerg et al., 1987]. This classification proposes recognizing two morphological types based on the shape of the black-pigmented arch of the eyebrows: the Roman type with one species corresponding to *S. boliviensis* with two subspecies (*S. b. boliviensis* and *S. b. peruviensis*) and the Gothic type including three species corresponding to *S. sciureus* with four subspecies (*S. s. sciureus, S. s. macrodon, S. s. albigena, and S. s. cassiquiarensis*), *S. oerstedii* with two subspecies (*S. o. oerstedii* and *S. o. citrinellus*), and *S. ustus*.

METHODS

Mitochondrial cytochrome *b* gene sequences from 18 squirrel monkeys belonging to nine different *Saimiri* taxa (including species and subspecies) were obtained from liver or muscle tissue, hair or ear samples. Fourteen other squirrel monkey cytochrome *b* sequences already deposited in public databases [Lavergne et al., 2003] were included. Samples were collected from captive animals of known origin kept legally in recognized zoos, or directly from animals killed in the field by indigenous hunters for their own purposes, with the full consent of the hunters. Sampling from live animals was carried out in accordance with French animal care regulations and laws, and also adhered to the American Society of Primatology Principles for the Ethical Treatment of Non-Human Primates. The full data set for analysis comprised 32 *Saimiri* sequences and six sequences from other species used as outgroups (Table II). The number of samples representing each putative species of squirrel monkey was (i) *S. boliviensis* (including *S. b. boliviensis* and *S. b. peruviensis*) ($n = 9$), (ii) *S. sciureus* (including *S. s. sciureus*, *S. s. collinsi, S. s. cassiquiarensis, S. s. albigena, S. s. macrodon*) ($n = 19$), (iii) *S. ustus* ($n = 3$), and (iv) *S. o. oerstedii* ($n = 1$). Samples were assigned to taxa on the basis of their provenance, according to the distributions identified by Hershkovitz [1984] (Fig. 1). Tissues were preserved in 95% ethanol and genomic DNA was extracted by the classical phenol-chloroform method [Sambrook et al., 1989]. Two overlapping fragments of the cytochrome *b* gene, each about 800 bp, were amplified by polymerase chain reaction (PCR), as previously described [Montgelard et al., 1997]. The full-length cytochrome *b* sequence was 1,140 bp long. PCR products were directly sequenced on both strands, using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science, Ohio). Primers used for amplification and sequencing are given in the Appendix. All new sequences were deposited in the GenBank database (accession numbers EU232694 to EU232713).

All new sequences were aligned with other previously published squirrel monkey sequences, using the ED editor option in MUST [Philippe, 1993] (Table II), and alignments were checked manually. Cytochrome *b* sequences were translated into amino acid sequences, and both nucleotide and amino acid sequences were checked for irregularities potentially indicative of nuclear homologs [Arctander, 1995]. Samples of *Aotus nancymaae*, *A. seniculus*, *C. albifrons*, and *Callithrix jacchus* were included and *A. seniculus* was used as an outgroup. DNASP version 4.10.4 software [Rozas et al., 2003] was used to assess genetic diversity by calculating the number of mitochondrial haplotypes, the gene haplotype diversity H_E [Nei, 1987], polymorphic sites, and nucleotide diversity p_i (i.e. the mean number of nucleotide differences between pairs of sequences). The phylogenetic relationships between samples were evaluated using distance, parsimony, and maximum likelihood procedures. The MODELTEST 3.7 program [Posada & Crandall, 1998] was used to determine the optimal model of nucleotide evolution for the data set used for distance and maximum likelihood analyses. The HKY model [Hasegawa et al., 1985], with a gamma (G) distribution and a proportion (I) of invariant sites was identified as the best nucleotide substitution model. The estimated nucleotide frequencies were: A = 0.3186, C = 0.3211, G = 0.1051, T = 0.2552. The $-\ln L$ value was 4744.62 and the estimated cytochrome *b* transition/transversion ratio (ti/tv) was 7.21. One thousand iterations were performed for distance and 100 replicates were used for maximum likelihood analysis. For

TABLE II. Animals Analyzed for the Cytochrome *b* Marker, Including (i) Their Taxonomic Identification Based on Hershkovitz Classification (1984); (ii) Individual Identification Number; (iii) Trapping Locations (for Wild Animals); (iv) Accession Number of New and Previously Published Sequences; and (v) Identification of Cytochrome *b* Haplotype

Species/subspecies	Identification number	Origin (geographic, accession number, or collection sites)	Haplotype Cyt *b*
S. b. boliviensis	*S. boliviensis 53582*	Bolivia, Santa Cruz (U53582)	H1
	S. boliviensis B16	Bolivia, Santa Cruz (AJ315387)	H1
	S. boliviensis 1179	Unknown (AJ315385)	H1
	S. boliviensis 38273	Unknown (SSU38273)	H2
	S. boliviensis B14	Bolivia, Santa Cruz (AJ315388)	H3
S. b. peruviensis	*S. b. peruviensis 5323*	Loreto region. Peruvian Amazon. Pacaya-Samiria National Park (EU232696)	H4
	S. b. peruviensis SS47	Loreto region. Peruvian Amazon. Pacaya-Samiria National Park (EU232711)	H4
	S. b. peruviensis 1604	Loreto region. Peruvian Amazon. Ucayali River (EU232698)	H4
	S. b. peruviensis 5325	Loreto region. Peruvian Amazon. Ucayali River (EU232697)	H5
S. s. sciureus	*S. s. sciureus T1862*	French Guiana (AJ315393)	H6
	S. s. sciureus T1901	French Guiana (AJ315394)	H6
	S. s. sciureus 1781	Guyana (AJ315398)	H7
	S. s. sciureus 1479	Guyana Shield (AJ489754)	H7
	S. s. sciureus 1543	Guyana Shield (AJ489755)	H7
	S. s. sciureus 1212	Guyana (AJ315396)	H8
	S. s. sciureus 22	Guyana (AJ315392)	H8
	S. s. sciureus 14	Guyana (AJ 315391)	H8
	S. s. sciureus SSB4	French Guiana (EU232704)	H9
	S. s. sciureus 1	Guyana (AJ315390)	H10
S. s. collinsi	*S. s. collinsi ATAID*	Centre of Primatology Belém, Marajo, Brazil (EU232710)	H11
	S. s. collinsi ATAEQ	Centre of Primatology Belém, Marajo, Brazil (EU232705)	H11
S. ustus	*S. ustus BBAAH*	Fiocruz breeding colony, Brazil (EU232706)	H12
	S. ustus E19	Fiocruz breeding colony, Brazil (EU232707)	H12
	S. ustus 180	Fiocruz breeding colony, Brazil (EU232708)	H12
S. o. oerstedii	*S. o. oerstedii SMO1*	Unknown (EU232702)	H13
S. s. cassiquiarensis	*S. s. cassiquiarensis 7*	Colombian Eastern Llanos frontier with Venezuela, Vichada Department (EU232695)	H14
	S. s. cassiquiarensis 26	Colombian Eastern Llanos frontier with Venezuela, Vichada Department (EU232694)	H15
S. s. albigena	*S. s. albigena 5327*	Transition between the Eastern Andean foothills and Eastern Llanos in Colombia, Meta Department (EU232700)	H16
	S. s. albigena 5326	Transition between the Eastern Andean foothills and Eastern Llanos in Colombia, Meta Department (EU232701)	H17
S. s. macrodon	*S. s. macrodon SS-15*	Loreto region. Peruvian Amazon. Napo River (EU232703)	H18
	S. s. macrodon 5324	Loreto region. Peruvian Amazon. Nanay River (EU232699)	H19
	S. s. macrodon SB1707	Loreto region. Peruvian Amazon. Canal del Puhinauva. Ucayali River (EU232709)	H20
C. albifrons	*C. albifrons*	(AJ309866)	
Callithrix jacchus	*C. jacchus*	(AY434079)	
Alouatta seniculus	*A. seniculus T1485*	French Guiana (AJ489759)	
	A. seniculus T1992	French Guiana (EU232713)	
Aotus nancymaae	*A. nancymaae M35*	Colombia, Leticia (AJ489745)	
	A. nancymaae M26	Colombia, Leticia (AJ489746)	

parsimony analysis, trees were generated using the heuristic search option in PAUP*4.0b8 [Swofford, 2002] with tree bisection, reconnection branch swapping, and the addition of 100 random taxa. The robustness of the resulting topology was assessed by nonparametric bootstrapping [Felsenstein, 1985]. One thousand iterations were performed for the parsimony analyses.

A Bayesian approach was carried out with MrBAYES 3.1.0 [Ronquist & Huelsenbeck, 2003], to infer phylogenetic relationships. Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations with four simultaneous chains, using a sample frequency of 100 and a burn-in of 2,500 runs. The MrMODELTEST 2.2 [Nylander, 2004] program was used to determine the optimal model of nucleotide evolution and the HKY model, with its gamma (G) distribution and proportion (I) of invariant sites was identified and used for the Bayesian approach. Inferences were validated by checking that the standard deviation of splits frequencies was less than 0.01 (value of 0.002).

We calculated the divergence time for the different clades of *Saimiri*, by including an additional four sequences from Old World primates in the analyses: *Macaca mulatta*, *Gorilla gorilla*, *Pan troglodytes*, and *Homo sapiens*. Bayesian analysis in BEAST version 1.4.7 [Drummond & Rambaut, 2007] was used to estimate rates, using three calibration points in this larger dataset: Platyrrhines–Catarrhines, 34–40 MYA [Poux et al., 2006]; *Homo–Pan*, 6.5–10 MYA [Benton & Donoghue, 2007]; *Saimiri–Cebus*, 12–20 MYA. We considered the upper constraint (most recent) to be represented by the stem lineage *Neosaimiri*, which was discovered by Stirton in Colombia in 1951 and dates to 12–15 MYA [Fleage & Brown, 1983; Rosenberger, 1979; Stirton, 1951]. The lower constraint was considered to be represented by the crown lineage of *Saimiri*, *Dolichocebus gaimansis* [Szalay & Delson, 1979], which dates to around 20 MYA [Kay et al., 2008]. We then used BEAST to calculate divergence times between the different clades of *Saimiri* recognized in various phylogenetic analyses. Bayesian factors (BF) were used to select a molecular clock model. The harmonic mean of the model likelihood, f (X|Mi), corresponding to the stationary phase, was compared between molecular clock strategies (constant clock, uncorrelated lognormal clock, uncorrelated exponential clock), using BF for the 2LnB10 equation in TRACER version 1.4 [Newton & Raftery, 1994; Rambaut & Drummond, 2007]. A BF > 10 was considered to provide strong evidence of a relaxed molecular clock, with the rate for each branch drawn from an exponential distribution. Rates were estimated under an HKY model of nucleotide substitution, with a gamma-distributed rate variation between sites and six rate categories selected with MrMODELTEST 2.2. We assumed a constant population size of over 20,000,000 generations, with the first 2,000,000 generations discarded as the burn-in and parameter values sampled every 500 generations, and lognormal calibration dates. The effective sample size for parameter estimates and the convergence of the chains were obtained and checked with TRACER version 1.4 [Rambaut & Drummond, 2007].

RESULTS

Molecular Diversity and Mitochondrial DNA Haplotype Distribution and Variation

We sequenced a total of 1,140 bp of the cytochrome *b* gene from 18 *Saimiri* individuals and from one other neotropical primate species (*A. seniculus*). Analyses were carried out with 32 *Saimiri* (18 new sequences and 14 previously published sequences) and six other New World primate species sequences (Table II). The cytochrome *b* sequence began with an ATG start codon in all individuals analyzed, except for *A. seniculus,* for which the start codon has not been determined (the obtained sequence begins at position 12). All sequences ended with a TAA codon, except for that from *C. jacchus*, which terminated with a TAG. Each sequence thus encoded a 380-amino acid peptide. *Saimiri* sequences presented 138 variable sites and 108 informative sites (S), with 35 transitions and only two transversions. Most of the transitions concerned the third position in the codon (25 of the 35); only six concerned the first position and four the second position. The two transversions concerned the third position. Cytochrome *b* sequence comparisons revealed the presence of 20 different haplotypes among the 32 *Saimiri* studied (Table II). Gene diversity (H_E) was 0.966, and nucleotide diversity (p_i) was 0.0325. The 20 haplotypes formed nine main groups (Table II). Five haplotypes were recorded in the *S. s. sciureus* group from Guianas. One to three haplotypes were recorded for the other species and subspecies (Table II). Within each cluster of haplotypes, mean genetic distances (based on the Kimura 2-parameter algorithm) were low, ranging from 0 for *S. s. ustus* and *S. s. collinsi* to 1.97 (SD = 0.32) for *S. s. macrodon* (Table III). Within the genus, among the nine clusters, the mean divergence ranged from 0.53 (SD = 0.20) between *S. s. cassiquiarensis* and *S. s. albigena* to 4.81 (SD = 0.68) between *S. b. boliviensis* and *S. ustus* (Table III).

Phylogenetic Analyses

The phylogenetic relationships between the 20 *Saimiri* haplotypes and outgroups are presented in Figure 2. Four different methods (distance, maximum likelihood, parsimony, Bayesian approach), based on different assumptions, were used for phylogenetic reconstructions. All produced similar trees, in terms of both branching and the robustness

TABLE III. Mean Pairwise Divergence and Standard Errors, Based on the Kimura 2-Parameter Algorithm [Kimura, 1980], between the Different Species and Subspecies of Squirrel Monkeys (32 *Saimiri* Individuals from Nine Different Clades)

	% sequence divergence between groups									
	S. b. boliviensis	*S. b. peruviensis*	*S. s. sciureus*	*S. o. oerstedii*	*S. ustus*	*S. s. collinsi*	*S. s. cassiquiarensis*	*S. s. albigena*	*S. s. macrodon*	% within-group sequence divergence
S. b. boliviensis										0.07 ± 0.05
S. b. peruviensis	0.90 ± 0.28									0.04 ± 0.04
S. s. sciureus	4.76 ± 0.65	4.57 ± 0.61								0.36 ± 0.11
S. o. oerstedii	4.42 ± 0.61	4.36 ± 0.59	3.79 ± 0.54							nc
S. ustus	4.81 ± 0.68	4.56 ± 0.65	4.52 ± 0.62	4.20 ± 0.59						0
S. s. collinsi	3.76 ± 0.59	3.56 ± 0.55	3.98 ± 0.63	3.72 ± 0.58	2.42 ± 0.46					0
S. s. cassiquiarensis	3.71 ± 0.56	3.65 ± 0.57	3.59 ± 0.54	3.77 ± 0.55	3.30 ± 0.50	2.47 ± 0.44				0.09 ± 0.08
S. s. albigena	4.00 ± 0.60	3.94 ± 0.61	3.97 ± 0.56	4.15 ± 0.57	3.21 ± 0.49	2.38 ± 0.43	0.53 ± 0.20			0.18 ± 0.13
S. s. macrodon	4.24 ± 0.58	4.18 ± 0.57	4.62 ± 0.60	4.36 ± 0.56	3.11 ± 0.45	2.64 ± 0.41	2.72 ± 0.41	2.63 ± 0.40		1.97 ± 0.32
C. albifrons	22.81 ± 1.64	22.67 ± 1.63	24.54 ± 1.73	23.77 ± 1.69	23.11 ± 1.70	23.49 ± 1.71	22.88 ± 1.71	22.78 ± 1.72	23.02 ± 1.65	

*nc: not calculated.

Fig. 2. Phylogram (rooted on *Alouatta*) of the 32 cytochrome *b* sequences of *Saimiri* spp. representing the 20 different haplotypes obtained in maximum likelihood analysis, using the HKY model with a gamma distribution and proportion I of invariable sites. This tree has an ln of −4744.62. Robustness values were obtained by bootstrapping (1000 replicates for distance and MP, 100 replicates for ML). Bootstrap values are indicated above (Dist/MP) and below (ML) branches, together with the posterior probabilities obtained with the Bayesian approach. Individual identification numbers are given in Table I.

of the nodes. The genus *Saimiri* is monophyletic and diverges from other neotropical primates with a bootstrap value of 100% and a posterior probability value of 1. Within the genus, four main clades can be recognized: (i) *S. s. sciureus* and *S. o. oerstedii*, (ii) *S. s. albigena*, *S. s. cassiquiarensis*, and *S. s. macrodon*, (iii) *S. s. collinsi* and *S. ustus*, and (iv) *S. b. boliviensis* and *S. b. peruviensis*. These four groups are supported with posterior probability values of 0.92, 0.61, 0.73, and 0.98, respectively, in the Bayesian approach, but the relationships between them remain poorly resolved. Nevertheless, as shown in Figure 2, the phylogenetic relationships between different species and subspecies can be linked to their geographical distribution. Indeed, animals of the Bolivian clade (*S. b. boliviensis*) are related to animals originating from Peru, clustering with these animals in the tree (Fig. 2). Furthermore, animals from Eastern Colombia, represented by *S. s. albigena* and *S. s. cassiquiarensis*, formed two clusters related to distribution areas: the Andean foothills vs. the transition area between the Eastern Llanos and the northern part of the Colombian Amazon.

Bayesian analysis was used to estimate the times at which the major clades diverged (Fig. 3). Three ancestral groups were identified: (i) *S. boliviensis*, (ii) *S. sciureus sciureus*/*S. oerstedii*, and (iii) other *S. sciureus* subspecies and *S. ustus*. The divergence between third group (composed of the other *S. sciureus* species and *S. ustus*) and the other *Saimiri* was found to be the most ancient (2.6 MYA, 95% highest posterior density (HPD): 1.3–4.3 MYA). Within the second lineage, *S. sciureus* from the Guianas diverged from *S. oerstedii* 2.3 MYA (95% HPD: 1–4.1 MYA). The

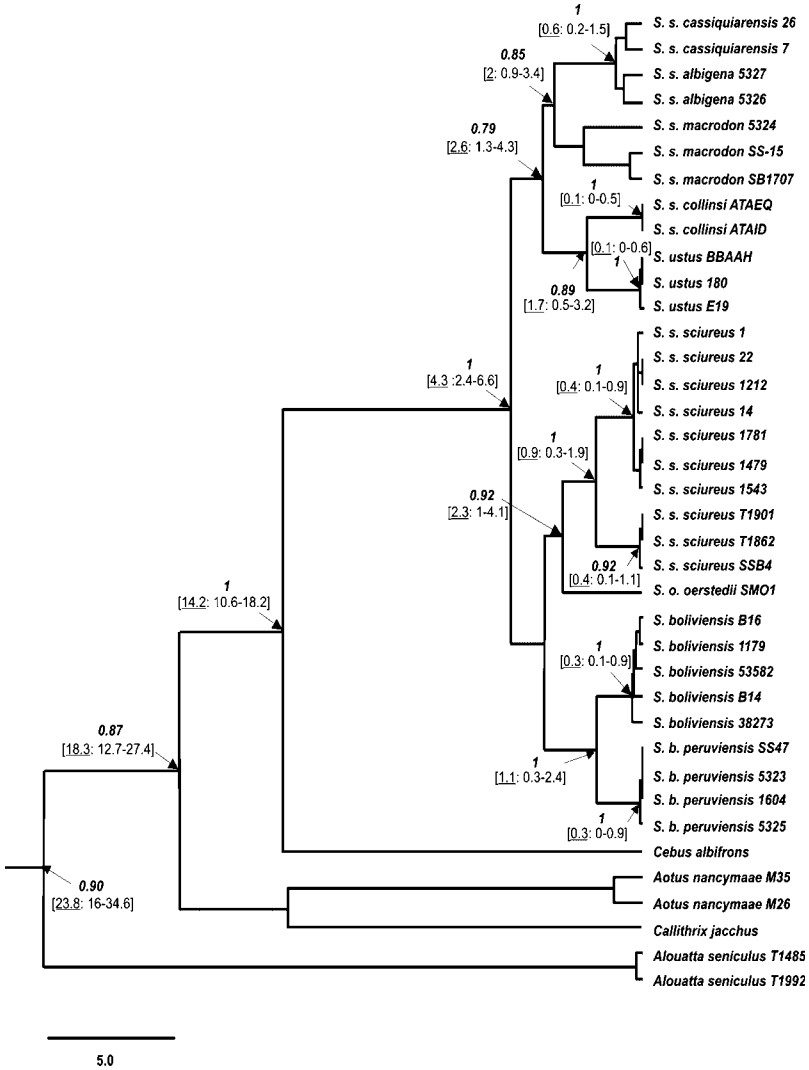

Fig. 3. Consensus tree of 32 cytochrome *b* sequences of *Saimiri* spp. in the relaxed molecular clock analysis with an HKY model of nucleotide substitution with a gamma-distributed rate variation between sites and six-rate categories. Each internal node is labeled with the posterior probability (in bold typeface and italics) for monophyly of the corresponding clade. Values in brackets correspond to the median (underlined) and 95% highest posterior density intervals for each divergence time, expressed in millions of years.

first group (composed of the two subspecies of *S. boliviensis*) diverged from the others about 1.1 MYA (95% HPD: 0.3–2.4 MYA). Intraspecific divergences in *S. boliviensis* and *S. sciureus* occurred 0.3–0.9 MYA.

DISCUSSION

Our phylogenetic investigation of the genus *Saimiri* was based on 32 complete cytochrome *b* sequences from animals sampled throughout most of the Amazonian region. Despite a rather limited sample size and the use of only one mitochondrial marker, our study addresses several persistent taxonomic questions and contributions to our understanding of the phylogeography and diversification of squirrel monkeys.

Taxonomic Considerations

This work provides support for several widely accepted clades potentially related to some of the species and subspecies described by Hershkovitz [1984] and for some of them by Cropp and Boinski [2000]: *S. b. boliviensis*, *S. b. peruviensis*, *S. oerstedii*, *S. s. sciureus*, *S. s. collinsi*, *S. s. macrodon*, *S. s. albigena, S. s. cassiquiarensis*, and *S. ustus*.

Saimiri boliviensis

Our molecular data support the existence of a monophyletic *S. boliviensis* clade, consistent with classifications based on behavioral and biochemical patterns [Boinski & Cropp, 1999; Vandeberg et al., 1990], susceptibility to malaria [Whiteley et al., 1987], acoustic structure [Boinski & Newman, 1988], reproductive physiology [Coe et al., 1985; Walker et al., 1981], and genetic distances for several protein-encoding loci [Da Silva et al., 1993]. Cropp and Boinski [2000] timed the divergence between *S. boliviensis* and the other *Saimiri* taxa to 4.05–6.42 MYA on the basis of mitochondrial D Loop (mtDNA) data, and to 1.14–1.81 MYA on the basis of data for nuclear DNA. They placed the divergence between *S. b. boliviensis and S. b. peruviensis* at 1.2–2 MYA to 0.5–0.7 MYA, on the basis of mitochondrial and nuclear DNA data, respectively. Our data, suggesting a divergence 1.1 MYA (95% HPD: 0.3–2.4 MYA) between *S. b. boliviensis and S. b. peruviensis* are consistent with these findings. However, we were unable to time with confidence the divergence between *S. boliviensis* and the other *Saimiri* taxa (*P* posterior value: 0.48).

The Saimiri sciureus Subspecies Complex

Animals classified as *S. sciureus* (Table II) did not form a monophyletic clade (Fig. 2). With the exception of the association of *S. s. albigena* and *S. s. cassiquiarensis*, the phylogenetic associations between *S. sciureus* subspecies were not well supported. *S. s. collinsi* clustered with *S. ustus* and the *S. sciureus* from Central and Western Amazon and the Eastern Llanos, but not with *S. s. sciureus*. Thus, if we assume that *S. ustus*, *S. oerstedii*, and *S. boliviensis* are different species [Hershkovitz, 1984] then *S. s. sciureus* would represent a single species, whereas *S. ustus*, *S. s. collinsi*, *S. s. macrodon*, *S. s. albigena*, and *S. s. cassiquiarensis* could instead be assigned to a single species (*S. ustus*) with five subspecies: *S. u. ustus*, *S. u. macrodon*, *S. u. albigena*, *S. u. cassiquiarensis*, *and S. u. collinsi*. More gene sequences will be necessary for firm conclusions to be drawn concerning the complex of *S. sciureus* subspecies.

Saimiri oerstedii

Together with information relating to private protein alleles and differences in teeth between *S. oerstedii* and other *Saimiri* taxa [Boinski & Cropp, 1999; Costello et al., 1993; Cropp & Boinski, 2000], the genetic divergence of *S. oerstedii* supports the acceptance of full species status for this clade.

Hypotheses Concerning the Origin and Routes of Dispersion of *Saimiri*

Western Amazonia has already been identified as a possible key area in the diversification of other primates, such as spider monkeys [Collins & Dubach, 2000; Ruiz-Garcia et al., 2006] and howler monkeys [Cortes-Ortiz et al., 2003]. On the basis of phylogenetic relationships and divergence times inferred in this study, a Western Amazonian origin of the genus *Saimiri* can be suggested. An ancestral form related to *S. boliviensis* or *S. s. macrodon* may have been the original point of diversification from which the other current Western Amazonian form, *S. s. macrodon* or *S. boliviensis* (Peru, Ecuador, and Colombia), emerged. *S. s. albigena* and *S. s. cassiquiarensis* would then have emerged on the northern bank of the Amazon, followed by *S. ustus,* which would have evolved from *S. s. macrodon* on the southern bank. *S. s. collinsi* subsequently emerged from *S. ustus,* whereas *S. s. sciureus* probably originated from the ancestral *S. s. albigena* and *S. s. cassiquiarensis* complex (Fig. 4). This hypothesis is supported by the presence in *S. b. boliviensis* and *S. s. macrodon* of the same six pairs of acrocentric chromosomes and higher levels of genetic diversity for these two taxa compared to the other clades

The Central American species, *S. oerstedii*, may have originated from the ancestral *S. s. sciureus* clade (Median height 2.3 MYA) since the completion of the Panama land bridge [2.8–3.1 MYA; Coates & Obando, 1996]. Such migration toward Central America from Guianas and north-eastern Brazil along the Atlantic–Caribbean coast, and possibly via some of the Caribbean islands, has been suggested for neotropical primates, such as howler monkeys [Cortes-Ortiz et al., 2003] and *Ateles hybridus* spider monkeys along the Atlantic–Caribbean coast in Colombia and Venezuela,

Fig. 4. Proposed routes for the dispersion and diversification of squirrel monkeys in the Amazon region. Grey dots indicate the original point of diversification from which the other current forms emerged, and arrows underline routes of dispersal. See text for more explanations.

which are thought to have originated from the migration of *A. paniscus* of the Guianas [Medeiros et al., 1997].

Evolutionary and Ecological Causes of *Saimiri* Diversification

Current *Saimiri* taxa diverged more recently than other neotropical primates. In *Alouatta* and *Ateles*, intense differentiation occurred in the upper Miocene period (5.5–10 MYA), probably through Andean vicariance [Cortes-Ortiz et al., 2003; Ruiz-Garcia et al., 2006], coinciding roughly with the formation of the modern Amazon River [Lundberg et al., 1998]. The divergence between *S. ustus* on the southern side of the Amazon River and *Saimiri* taxa on the northern side occurred much more recently, about 1.7 MYA. The Amazon clearly played a role in this process, given the western Amazonian origin of the genus. Dispersion routes and the diversification associated with them may have occurred in parallel on both sides of the Amazon, accentuating the differences between *S. ustus* and *S. s. sciureus*, on the southern and northern banks of the lower part of the river, respectively. More locally, other large rivers and fast running blackwater rivers may also have played a role in the dispersion of subspecies. Like *A. paniscus* and *A. macconnelli* [Cortes-Ortiz et al., 2003; Ruiz-Garcia et al., 2006], *S. s. sciureus* has been restricted to the north-eastern Amazon area by blackwater rivers, and the Jurua River may have separated *S. b. boliviensis* and *S. s. macrodon*. Similarly, the Rio Xingu may have separated *S. ustus* and *S. s. collinsi*, and the Colombian Apaporis River may have separated *S. s. macrodon* and *S. s. cassiquiarensis*. Nevertheless, squirrel monkeys are found throughout the Amazon Basin, implicating that river barriers have not been powerful obstacles for their dispersion [Ferrari, 2004]. Furthermore, squirrel monkeys would also likely be insensitive to the potential effect of river barriers owing to their preferred distribution along watercourses [Thorington, 1985]. Indeed, *Saimiri* phylogeography may also have been shaped by other elements, including ecological gradients [Endler, 1982], flood-plain dynamics [Salo et al., 1986], and ecological heterogeneities [Tuomisto et al., 1995], and intergeneric competition could have reinforced distributions initially shaped by physical barriers [Ferrari, 2004]. The distribution and abundance of fruits greatly vary between the Central American forest where *S. oerstedii* occurs, the Guyana shield forest (*S. s. sciureus*), and the Peruvian rainforests (*S. boliviensis*), and determine the social structure of these *Saimiri* species throughout sexual selection that models the social behavior of males and females [Boinski & Cropp, 1999]. Thus, the current structure of *Saimiri* populations is thought to have resulted from concurrent geographic and ecologic constraints.

Most estimates of divergence times between *Saimiri* taxa lie between 1.1 and 2.3 MYA, so the splits between the main *Saimiri* species (*S. boliviensis* vs. all the other *Saimiri*; *S. oerstedii* vs. *S. sciureus*; *S. ustus* vs. *S. sciureus*) occurred during the Pliocene. Other splits within the *S. boliviensis* and *S. sciureus* complex occurred more recently, during the Quaternary period. Milankovitch's cycles, with cold and dry phases, generated refuges in the Amazon [Haffer, 1997]. These Pleistocene forest refuges may have increased the genetic distances between populations after their dispersion in the Amazon, and may be responsible for the emergence of the current *Saimiri* taxa within *S. boliviensis* (within the Eastern Peruvian refuges) and within the *S. ustus–macrodon–albigena–cassiquiarensis* complex (within the Madeira–Tapajos, Napo, and Imeri refuges, respectively).

The diversification of squirrel monkeys thus contrasts with the diversification of larger monkeys in the Amazon region. Squirrel monkeys are generalists [Janson & Boinski, 1992], and this characteristic may have strongly influenced their population history because it is probably linked to their ability

to disperse. Unlike for more specialized species, such as spider monkeys and howler monkeys, considerable variation in forest cover density during the Quaternary period in Amazonia [Colinvaux et al., 2000; Cowling et al., 2001] may have had a more limited effect on the diversification of squirrel monkey populations, with associated delayed evidences of speciation and rather low levels of genetic divergence within and among clades when compared to *Alouatta* species [Bonvicino et al., 2001; Nascimento et al., 2005]. With the notable exceptions of large primates [Cortes-Ortiz et al., 2003; Cropp et al., 1999; Ruiz-Garcia et al., 2006] and small mammals [Patton et al., 2000], the phylogeography of many neotropical mammals remains poorly investigated. Nevertheless, the crab-eating fox *Cerdocyon thous* [Tchaika et al., 2007] and the fruit bat *Carollia perspicillata* [Ditchfield, 2000], both of which are generalist mammals, have dispersion patterns and divergence times similar to those of *Saimiri*. This suggests that bioecology plays a more important role than taxonomic status in the phylogeographic patterns observed for genera and species.

To conclude, this study helps us understand the life history and the current diversity of the genus *Saimiri*. Nevertheless, a more complete evaluation of the phylogenetic history of squirrel monkeys would benefit from the inclusion of additional sequences and of additional and more variable markers, such as nuclear microsatellite sequences and/or the mtDNA. This next step will be necessary to more complete relationships among *S. sciureus* subspecies.

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REFERENCES

Aide TM, Rivera E. 1998. Geographic patterns of genetic diversity in *Poulsenia armata* (Moraceae): implications for the theory of Pleistocene refugia and the importance of riparian Forest. J Biogeogr 25:695–705.

Arctander P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. Proc Biol Sci 262:13–19.

Avise JC. 2000. Phylogeography—the history and formation of species. London, England: Harvard University Press.

Benton MJ, Donoghue PCJ. 2007. Paleontological evidence to date the tree of life. Mol Biol Evol 24:26–53.

Boinski S, Cropp SJ. 1999. Disparate data sets resolve squirrel monkey (*Saimiri*) taxonomy: implications for behavioral ecology and biomedical usage. Int J Primatol 20:237–256.

Boinski S, Newman JD. 1988. Preliminary observations on squirrel monkey (*Saimiri oerstedii*) vocalizations in Costa Rica. Am J Primatol 14:329–343.

Bonvicino CR, Lemos B, Seuanez HN. 2001. Molecular phylogenetics of howler monkeys (*Alouatta*, Platyrrhini). A comparison with karyotypic data. Chromosoma 110: 241–246.

Coates AG, Obando JA. 1996. The geologic evolution of the Central American isthmus. In: Jackson JBC, Budd AF, Coates AG, editors. Evolution and environment in tropical America. Chicago: The University of Chicago Press. p 21–56.

Coe CL, Smith ER, Levine S. 1985. The endocrine system of the squirrel monkey. In: Rosenblum LA, Coe CL, editors. Handbook of squirrel monkey research. New York: Plenum Press. p 191–218.

Colinvaux PA, De Oliveira PE, Bush MB. 2000. Amazonian and Neotropical plant communities on glacial time scales, the failure of the aridity and refuge hypothesis. Quaternary Sci Rev 19:141–169.

Collins AC, Dubach JM. 2000. Biogeographic and ecological forces responsible for speciation in *Ateles*. Int J Primatol 21:421–444.

Cortes-Ortiz L, Bermingham E, Rico C, Rodriguez-Luna E, Sampaio I, Ruiz-Garcia M. 2003. Molecular systematics and biogeography of the neotropical monkey genus, *Alouatta*. Mol Phylogenet Evol 26:64–81.

Costello RK, Dickinson C, Rosenberger AL, Boinski S, Szalay FS. 1993. A multidisciplinary approach to squirrel monkey (genus *Saimiri*) species taxonomy. In: Kimbel W, Martin L, editors. Species, species concepts, and primate evolution. New York: Plenum Press. p 177–237.

Cowling SA, Maslin MA, Sykes MT. 2001. Paleovegetation simulations of Lowland Amazonia and implications for Neotropical allopatry and speciation. Quaternary Res 55: 140–149.

Cropp S. 1998. Evolutionary diversification among tamarins (genus *Saguinus*). Dissertation Abstract Int B 58:6433.

Cropp S, Boinski S. 2000. The Central American squirrel monkey (*Saimiri oerstedii*): introduced hybrid or endemic species? Mol Phylogenet Evol 16:350–365.

Cropp SJ, Larson A, Cheverud JM. 1999. Historical biogeography of tamarins, genus *Saguinus*: the molecular phylogenetic evidence. Am J Phys Anthropol 108:65–89.

Da Silva BTF, Sampaio MIC, Schneider MPC, Schneider H, Villavicencio H, Montoya H, Encarnación F, Salzano FM. 1987. Preliminary analysis of genetic distance between squirrel monkeys. Int J Primatol 8:528.

Da Silva BTF, Sampaio MIC, Schneider H, Schneider MPC, Montoya E, Encarnacion F, Callegari-Jacques SM, Salzano FM. 1993. Protein electrophoretic variability in *Saimiri* and the question of its species status. Am J Primatol 29:183–193.

Dick CW, Heuertz M. 2008. The complex biogeographic history of a widespread tropical tree species. Evolution 62: 2760–2774.

Ditchfield AD. 2000. The comparative phylogeography of neotropical mammals: patterns of intraspecific mitochondrial

DNA variation among bats contrasted to nonvolant small mammals. Mol Ecol 9:1307–1318.
Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214.
Endler JA. 1982. Pleistocene forest refuges: fact or fancy? In: Prance GT, editor. Biological diversification in the tropics. New York: Columbia University Press. p 641–657.
Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.
Ferrari SF. 2004. Biogeography of Amazonian Primates. In: Mendes SL, Chiarello AG, editors. A Primatologia no Brasil no 8, IPEMA/SBPr, Vitoria. p 101–122.
Fleage JC, Brown TM. 1983. New primate fossils from late Oligocene (Colhuehuapian) localities of Chubut Province, Argentina. Folia Primatol 41:240–266.
Haffer J. 1997. Alternative models of vertebrate speciation in Amazonia: an overview. Biodiv Cons 6:451–476.
Hasegawa M, Kishino H, Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174.
Hayes FE, Sewlal JAN. 2004. The Amazon river as a dispersal barrier to passerine birds: Effects of river width, habitat and taxonomy. J Biogeogr 31:1809–1818.
Hershkovitz P. 1984. Taxonomy of Squirrel Monkey genus *Saimiri* (Cebidae, Platyrrhini): a preliminary report with description of hitherto unnamed form. Am J Primatol 7:155–210.
Hubert N, Duponchelle F, Nunez J, Garcia Davila C, Paugy D, Renno JF. 2007. Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the neotropical ichthyofauna. Mol Ecol 16:2115–2136.
Janson CH, Boinski S. 1992. Morphological and behavioral adaptations for foraging in generalist primates: the case of the cebines. Am J Phys Anthropol 88:483–498.
Kay RF, Fleagle JG, Mitchell TRT, Colbert M, Bown T, Powers DW. 2008. The anatomy of *Dolichocebus gaimanensis*, a stem platyrrhines monkey from Argentina. J Human Evol 54:323–382.
Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120.
Lavergne A, Catzeflis F, Lacôte S, Barnaud A, Bordier M, Mercereau–Puijalon O, Contamin H. 2003. Genetic analysis of the *Saimiri* breeding colony of the Pasteur Institute (French Guiana): development of a molecular typing method using a combination of nuclear and mitochondrial DNA markers. J Med Primatol 32:330–340.
Lundberg JG, Marshall LG, Guerrero J, Horton B, Malabarba MCSL, Wesselingh F. 1998. The stage for neotropical fish diversification: a history of tropical South American rivers. In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS, editors. Phylogeny and classification of neotropical fishes. Porto Alegre: Edipucrs. p 13–48.
Medeiros MA, Barros RMS, Pieczarka JC, Nagamachi CY, Ponsa M, Garcia M, Garcia F, Egozcue J. 1997. Radiation and speciation of spider monkeys, genus *Ateles*, from the cytogenetic viewpoint. Am J Primatol 42:167–178.
Montgelard C, Catzeflis FM, Douzery E. 1997. Phylogenetic relationships of artiodactyls and cetaceans as deduced from the comparison of cytochrome *b* and 12S rRNA mitochondrial sequences. Mol Biol Evol 14:550–559.
Mustrangi MA, Patton JL. 1997. Phylogeography and systematics of the slender mouse opossum marmosops (Marsupialia, Didelphidae). Berkeley, CA: University of California Publications. 130p.
Nascimento FF, Bonvicino CR, da Silva FC, Schneider MP, Seuanez HN. 2005. Cytochrome *b* polymorphisms and population structure of two species of *Alouatta* (Primates). Cytogenet Genome Res 108:106–111.
Nei M. 1987. Molecular evolutionary genetics. New York: Colombia University Press.
Newton MA, Raftery AE. 1994. Approximate Bayesian inference with the weighted likelihood bootstrap (with discussion). J Royal Stat Soc Ser B 56:3–48.
Nylander JAA. 2004. MrModeltest v2 program distributed by the author. Evolutionary Biology Centre, Uppsala University.
Opazo JC, Wildman DE, Prychitko T, Johnson RM, Goodman M. 2006. Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates). Mol Phylogenet Evol 40:274–280.
Patton JL, Da Silva MNF. 1998. Rivers, refuges and ridges: the geography of speciation of Amazonian mammals. In: Howard DJ, Berlocher SH, editors. Endless forms: species and speciation. Oxford: Oxford University Press. p 202–213.
Patton JL, da Silva MNF, Malcolm JR. 2000. Mammals of the Rio Jurua and the evolutionary and ecological diversification of Amazonia. Bull Amer Mus Nat Hist 244: 1–306.
Pearse DE, Arndt AD, Valenzuela N, Miller BA, Cantarelli V, Sites Jr JW. 2006. Estimating population structure under nonequilibrium conditions in a conservation context: continent-wide population genetics of the giant Amazon river turtle, *Podocnemis expansa* (Chelonia; Podocnemidae). Mol Ecol 15:985–1006.
Philippe H. 1993. MUST, a computer package for management utilitarians for sequences and trees. Nucleic Acids Res 21:5264–5272.
Posada D, Crandall A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
Poux C, Chevret P, Huchon D, de Jong WW, Douzery EJ. 2006. Arrival and diversification of caviomorph rodents and platyrrhine primates in South America. Syst Biol 55: 228–244.
Rambaut A, Drummond AJ. 2007. Tracer v1.4, available from http://beast.bio.ed.ac.uk/Tracer.
Renno JF, Hubert N, Torrico JP, Duponchelle F, Nunez Rodriguez J, Garcia Davila C, Willis SC, Desmarais E. 2006. Phylogeography of *Cichla* (Cichlidae) in the upper Madera basin (Bolivian Amazon). Mol Phylogenet Evol 41:503–510.
Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.
Rosenberger AL. 1979. Cranial anatomy and implications of *Dolichocebus*, a late Oligocene ceboid primate. Nature 279:416–418.
Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.
Ruiz-Garcia M, Parra A, Romero-Aleán N, Escobar-Armel P, Shostell JM. 2006. Genetic characterization and phylogenetic relationships between the *Ateles* species (Atelidae, primates) by means of DNA microsatellite markers and craniometric data. Primate Report 73:3–47.
Ruiz-Garcia M, Escobar-Armel P, Alvarez D, Mudry M, Ascunce M, Gutierrez-Espeleta G. 2007. Genetic variability in four *Alouatta* species measured by means of nine DNA microsatellite markers: genetic structure and recent bottlenecks. Folia Primatol 78:73–87.
Rull V. 2006. Quaternary speciation in the neotropics. Mol Ecol 15:4257–4259.
Salo J, Kalliola R, Hakkinen I, Marinen Y, Niemela P, Puhakka M, Coley PD. 1986. River dynamics and the diversity of Amazon lowland forest. Nature 322:254–258.
Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. 2nd edition. New York: Cold Spring Harbor Laboratory Press.
Schrago CG. 2007. On the time scale of New World primate diversification. Am J Phys Anthropol 132:344–354.

Stirton RA. 1951. Ceboid monkeys from the Miocene of Columbia. Bull Univ California Publ Geol Sci 28: 315–356.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods) Version 4. Sunderland, Massachusetts: Sinauer.

Szalay FS, Delson VE. 1979. Evolutionary history of the primates. New York: Academic Press.

Tchaika L, Eizirk E, de Oliveira TG, Candido JF, Freitas TRO. 2007. Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). Mol Ecol 16:819–838.

Thorington RW. 1985. The taxonomy and distribution of squirrel monkeys (*Saimiri*). In: Rosenblum RA, Coe CL, editors. The handbook of squirrel monkey research. New York: Plenum Press. p 1–33.

Tuomisto H, Ruokolainen K, Kalliola R, Linna A, Danjoy W, Rodriguez Z. 1995. Dissecting Amazonian biodiversity. Science 269:63–69.

Vandeberg JL, Cheng ML, Moore CM, Abee CR. 1987. Genetics of the squirrel monkeys (genus *Saimiri*): implications for taxonomy and research. Int J Primatol 8:423.

VandeBerg JL, Moore CM, Williams-Blangero S, Min-Lee C, Abee CR. 1990. Genetic relationships among three squirrel monkey types: implications for taxonomy, biomedical research, and captive breeding. Am J Primatol 22:101–111.

Walker LC, Kaack B, Brizzee KR, Walker ML. 1981. Prenatal ionizing irradation and early postnatal growth of Colombian and Bolivian squirrel monkeys (*Saimiri sciureus*). Am J Primatol 1:379–387.

Wan QH, Wu H, Fujihara T, Fang SG. 2004. Which genetic marker for which conservation genetics issue? Electrophoresis 25:2165–2176.

Whiteley HE, Everitt JI, Kakoma I, James MA, Ristic J. 1987. Pathologic changes associated with fatal *Plasmodium falciparum* infection in the Bolivian squirrel monkey (*Saimiri sciureus boliviensis*). Am J Trop Med Hyg 37:1–8.