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Phylogeny of the titi monkeys of the *Callicebus moloch* group (Pitheciidae, Primates)

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Running Title: Phylogeny of the *C. moloch* group

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ABSTRACT

Callicebus is a Neotropical primate genus of the family Pitheciidae, which currently comprises 34 recognized species. Based on their morphological traits and geographic distribution, these species are currently assigned to five groups: the *C. moloch*, *C. cupreus, C. donacophilus, C. torquatus*, and *C. personatus* groups, although in the past, alternative arrangements have been proposed based on the analysis of morphological data. The principal disagreements among these arrangements are related to the composition of the *C. moloch* group. In the present study, we tested the different taxonomic proposals for the *C. moloch* group, based on the molecular analysis of nuclear markers (*Alu* insertions and flanking regions) and three mitochondrial genes (16S, COI and Cyt *b*), with a total of approximately 7 kb of DNA sequence data. Phylogenetic reconstructions based on maximum likelihood and Bayesian inference methods indicated that the species of the current *C. cupreus* group should be reintegrated into the *C. moloch* group. In addition, our results corroborated previous studies suggesting that the species of the current *C. personatus* group form a distinct species group. We also observed a relatively subtle level of divergence between *C. dubius* and *C. caligatus.* While the known diversity of *Callicebus* is considerable, these findings indicate that the relationships among groups and species may still not be completely understood, highlighting the need for further research into the biological, geographic and genetic variability of these primates, which will be fundamental to the effective conservation of the genus.

Key words: *Callicebus moloch* group, species group, taxonomy, new species.

INTRODUCTION

Callicebus Thomas, 1903 is one of the four Neotropical primate genera of the family Pitheciidae [Schneider and Sampaio 2015]. In the first taxonomic review of this genus, Elliot [1913] recognized 22 monotypic species. Almost a half century later, Hill [1960] published a comprehensive review of the social structure, reproduction, behavior, parasitology, geographic distribution and systematics of the subfamilies Callicebinae, Aotinae, Pitheciinae and Cebinae. In that work, his arrangement of the genus *Callicebus* included only six species, but 34 subspecies. Hershkovitz [1963] identified only two species, from the Amazon (*Callicebus moloch*) and Orinoco (*C. torquatus*) basins, but later revised this number to 13 [Hershkovitz 1988; 1990]. These species were allocated to four species groups, based on cranial and post-cranial morphology and pelage coloration: (i) the *Callicebus modestus* group, with one species, which Hershkovitz [1988] identified as an "isolated relict species"; (ii) the *C. donacophilus* group, with three species; (iii) the *C. moloch* group with eight species, and (iv) the *C. torquatus* group, with a single species. Subsequently, Kobayashi [1995] using meristic cranial characters, pelage, geographical distribution and karyotypes, suggested five species groups: (i) *C. donacophilus*; (ii) *C. moloch*; (iii) *C. cupreus*; (iv) *C. personatus* and (v) *C. torquatus* (Table 1). In that study, the *C. moloch* group was divided into three species (*C. moloch*, *C. cupreus* and *C. personatus*), while *C. modestus* was incorporated into the *C. donacophilus* group.

van Roosmalen et al. [2002] followed the proposal of Kobayashi [1995], but raised all subspecies to the species level, based on the phylogenetic species concept. Since then, nine new putative species have been discovered and incorporated into

these groups based on morphological, ecological and biogeographical criteria [Dalponte et al., 2014; Silva Júnior et al., 2013; Vermeer and Tello-Alvarado, 2015].

The genus *Callicebus* is widely distributed in tropical South America. Three of the species groups (*C. torquatus, C. cupreus* and C. *moloch* groups) are found in the Amazon and Orinoco basins [Kobayashi, 1995], the *C. donacophilus* group is found primarily in the dry Chaco region, while the *C. personatus* group is centered on the Brazilian Atlantic Forest biome, Cerrado and Caatinga (Fig. 1).

The composition of the *C. moloch* group has changed a number of times, from a maximum of 14 taxa (species and subspecies) in Hershkovitz [1988, 1990] to six in the most recent proposal [van Roosmalen et al., 2002]. The purpose of the present study is to clarify the taxonomic arrangement of the *Callicebus moloch* group based on molecular data obtained from both nuclear and mitochondrial regions.

METHODS

Samples and molecular markers

A total of 64 samples were obtained from blood or muscle tissue preserved in absolute ethanol. These samples were obtained from the following Brazilian institutions: the Goeldi Museum (MPEG), National Institute of Amazonian Research (INPA), Federal University of Pará (UFPA), Federal University of Rondônia (UNIR), Federal University of Amazonas (UFAM), Rio de Janeiro Primate Center (CPRJ-INEA), and the National Primate Center (CENP) in Ananindeua, Pará. This research adhered to the legal requirements of Brazil legislation as well as to "Principles for the Ethical Treatment of Non Human Primates" of the American Society of Primatologists (ASP).

For this study, putative species identifications were based on morphological and pelage coloration (*sensu* van Roosmalen et al. [2002]). We included specimens of species assigned to the *C. moloch* group by van Roosmalen et al's [2002] classification, as well as those falling into van Roosmalen et al's [2002] *C. cupreus,* and *C personatus* groups because some of those species were considered part of the *moloch* group by others authors (e.g., Groves [2001], Hershkovitz [1988, 1990]). We also included samples of individuals from newly described species *C. vieirai* and *C. miltoni* [Gualda-Barros et al., 2012; Dalponte et al., 2014]. The sample codes, sources and localities are shown in Table 2, and the localities are plotted in Fig. 1. Samples of the other pitheciid genera (*Pithecia*, *Chiropotes* and *Cacajao*) were used as the outgroup for the phylogenetic analyses. Our phylogenetic inferences were based on ten nuclear and three mitochondrial markers (Table S1). The three mtDNA genes were rRNA16S (543 bps), cytochrome oxidase subunit I – COI (605 bps) and cytochome *b* - CYT *b* (1074 bps). The nuclear regions correspond to sites including mobile *Alu* elements, other repetitive sequences, and their flanking regions.

Extraction, amplification and sequencing of DNA

Total DNA was obtained with Promega's Wizard Genomic kit, according to the manufacturer's protocol. The mitochondrial and nuclear regions were amplified by polymerase chain reaction (PCR). For the PCRs, a final volume of 15 µl was used, containing about 30 ng of genomic DNA, 2.4 µl of dNTPs (1.25mM), 1.5 µl of 10X Buffer (200 mM Tris-HCl, 500 mM KCl), 1 µl of $MgCl_2$ (25 mM), 1 µl of each primer (0.2 µM), and 1 U of Taq DNA polymerase. The amplification protocol was initiated with four minutes of denaturation at 95°C, followed by 35 cycles of three stages: (i) denaturation at 95°C for 30 s, (ii) annealing at a specific temperature (see

Table S1), and (iii) extension at 72°C for 30 seconds. After completion of the 35 cycles, there was a final extension stage at 72°C for seven minutes. The PCR products were then purified using polyethylene glycol and ethanol [Paithankar and Prasad, 1991]. The sequencing reactions were run using the BigDye Terminator Sequencing kit v. 3.1 (Life Technologies) and the reaction products were separated and visualized using an ABI 3500xl automatic sequencer (Life Technologies).

Sequence alignment, identification of Alus and phylogenetic analyses

The DNA sequences were aligned initially using ClustalW [Thompson et al., 1994] and then corrected manually using the BioEdit v. 7.2.5 software [Hall, 1999]. Saturation was assessed using DAMBE version 5.3.109 [Xia, 2013]. We used the software PartitionFinder [Lanfear et al., 2012] to test different partition schemes and select the most appropriate evolutionary model. We were particular concerned with evaluating whether evolutionary rates differed among the three types of markers (nuclear Alu elements, regions flanking Alu sites, and mitochondrial genes) (see Table S2). For PartitionFinder analyses, we set the search method to "greedy", allowed unlinked branch lengths, and evaluated results based on Bayesian information criterion (BIC). Our analysis suggested that the best scheme for our data set was to separate it into two partitions (nuclear and mitochondrial regions). The regions containing interspaced repeats (SINEs and LINEs) were identified using the software RepeatMasker (http://www.repeatmasker.org).

Phylogenetic reconstruction were made using both the maximum likelihood (ML) method, run in RaxML v.8 [Stamatakis, 2014] with 1000 bootstrap replicates and Bayesian inference (BI) as implemented in MrBayes v. 3.2.1 [Ronquist and Huelsenbeck, 2003]. In MrBayes, the analysis of substitution model parameters was

unlinked across partitions. Two independent runs were initiated simultaneously with four independent Markov-Chain Monte Carlo (MCMC) chains (1 cold and 3 heated). The MCMC algorithm was based on 500,000 cycles (generations), sampled every 5000 cycles, with 25% of the samples being discarded as burn-in. Convergence was assessed by comparing the two runs. The MCMC output was visualized and diagnosed in Tracer v. 1.6 [Rambaut et al., 2014]. The run was considered satisfactory when, for all traces, the Effective Sample Size (ESS) values were over 200. For interspecific comparisons, matrices of genetic distances based on the K2P model [Kimura, 1980] were generated for each marker in the MEGA v. 6.0 software [Tamura et al., 2013]. Given the large number of specimens analyzed, genetic distances were also estimated using only two specimens of each species for visualization purposes.

We also perform a Bayesian multispecies coalescent analysis in *BEAST [Heled, Drummond, 2010] with two runs of 300 million generations each. The nucleotide substitution model chosen for the concatenated nuclear regions, and the mitochondrial genes CytB, COI and 16S were respectively: GTR+Gamma; GTR+Gamma; HKY+Gamma; GTR+Gamma. For the clock model, both strict and correlated relaxed clock were tested. For species tree and population Size model, Yule and Piecewise linear and constant root were the priors used, respectively. For model parameters and statistics, the default priors were used.

The logs of these two runs were visualized in Tracer to check if the ESS values were above 200. When considered adequate, the logs were combined in LogCombiner v. 1.8.3 and after a 20% burn-in the trees were summarized in the TreeAnnotator v. 1.8.3. All trees (ML, BI, and species tree) were visualized and edited in FigTree v. 1.4.2 [Rambaut, 2012].

RESULTS

Data and missing data

A total of 747 sequences were generated, which correspond to 88.4% of the number of possible sequences (see S3 for details). The total sequence of 7121 bps included 4899 bps of nuclear markers and 2222 bps of the mitochondrial markers (Table S2). Gaps in the data arose due to the lack of biological material in some samples or the failure of the PCR amplification.

Saturation, phylogenetic analysis, species tree and genetic divergences

No saturation was detected in any of the markers (data not shown). The Maximum Likelihood and Bayesian approaches generated well supported topologies for the majority of the nodes (Fig. 2). A clear and significant division was found between the species of the *C. personatus* group (Atlantic Forest) and the remaining (Amazonian) species analyzed in this study. In the Amazonian group, *C. hoffmannsi* appears to have diverged first, followed by a trichotomy of groups – (i) *C. cupreus, C. brunneus, C. caligatus* and *C. dubius*, (ii) *C. cinerascens* and *C. miltoni*, and (iii) *C. moloch, C. vieirai* and *C. bernhardi*.

The species tree inferred using *BEAST had the same topology as that reconstructed under maximum likelihood using RAxML and Bayesian inference as implemented in MrBayes, regardless of whether a constant or relaxed molecular clock was applied. All currently recognized species were assigned to well-supported clades, with the exception of *C. moloch*, which consistently appeared paraphyletic, with individuals collected near to Alta Floresta, left bank of the Tapajós river identified as *C. moloch*2 forming a distinct clade, sister to other *C. moloch*1 individuals and *C. vieirai* (Fig. 3).

Pairwise genetic distances (K2P) were estimated between clades in the whole dataset, as well as between species in a reduced dataset. Genetic divergence between *C. bernhardi* and *C. cinerascens* varied from 4.7% to 4.9% (Table S4), which is consistent with the genetic distances between the *C. moloch* and *C. cupreus* groups recognized by Kobayashi [1995]. Based on the topology obtained in the present study, five clades were identified: (**M**) *C. moloch, C. vieirai* and *C. bernhardi*; (**Ci**) *C. cinerascens* and *C. miltoni*; (**Cu**) *C. cupreus, C. brunneus, C. caligatus* and *C. dubius*; (**H**) *C. hoffmannsi*, and (**P**) the species of the *C. personatus* group.

We estimated genetic distances within and between these five clades for both mitochondrial sequences only (COI, 16S and CYT b) and for concatenated mitochondrial and nuclear sequences. Intra-clades distances were lowest for clade **H** and highest for clade **P**. Inter-clade distances were, overall, much higher between clade **P** and the remaining clades, while clades **M**, **Cu**, **Ci** and **H** all had similar genetic distances from one another (Table 3).

DISCUSSION

As mentioned previously, the configuration of *Callicebus* species groups has been the subject of much discussion, although there are two basic proposals for the *C. moloch* group. One is that of Kobayashi [1995], which includes *C. moloch*, *C. cinerascens*, *C. brunneus*, *C. hoffmannsi*, and *C. baptista*, and is similar to the proposal of van Roosmalen et al. [2002]. The second proposal is that of Groves [2001], which is in fact similar to that of Hershkovitz [1990]. Groves [2001] added *C. cupreus* (and its subspecies) and *C. personatus* to the *C. moloch* group, in addition to the species suggested by Kobayashi [1995] and Kobayashi and Langguth [1999].

The results of the present study nevertheless indicate emphatically that the *C. personatus* clade from the Brazilian Atlantic Forest is a group quite distinct from the Amazonian forms. This is supported by the greater genetic distances between the *C. personatus* and the Amazonian clades of 6.6–7.2% for the nuclear sequences and more than 13% for the mitochondrial ones (CytB = 13.0% and COI = 13.7%). A similar conclusion was reached by Perelman et al. [2011] who also observed that the Atlantic species are very distantly related to the Amazonian ones, estimating a separation time of approximately 10 Ma. This result contrasts with Hershkovitz's [1990] and Groves's [2001] hypotheses that placed the titi monkeys of the Atlantic Forest inside the *C. moloch* group.

In the Amazonian group, the results of the present study identified a monophyletic clade including *C. cupreus, C. brunneus, C. caligatus* and *C. dubius*, which was supported strongly by bootstrap and Bayesian credibility values, with *C. moloch* in a sister clade together with *C. cinerascens, C. miltoni, C. bernhardi,* and *C. vieirai.* This is incompatible with the proposal of Kobayashi [1995] and Kobayashi and Langguth [1999], which is also followed by van Roosmalen et al. [2002], which

placed *C. brunneus* more closely related *C. moloch* and *C. cinerascens* than with *C. cupreus.*

The result of the present study indicate that the groups proposed by Kobayashi [1995], Kobayashi & Languth [1999] and van Roosmalen et al. [2002] are not monophyletic, and are incompatible with the genetic similarity between species of the *C. cupreus* and *C. moloch* groups. Until further confirmatory research, then, we would recommend adopting an arrangement similar to that proposed by Groves [2001], in which the *C. moloch* group would include the following species (species in brackets were not analyzed in the present study): *C. moloch*, *C. hoffmannsi*, *C. cinerascens*, *C. brunneus*, [*C. baptista*], *C. bernhardi*, *C. vieirai*, *C. miltoni, C. cupreus, C. caligatus, C. dubius,* [*C. discolor*], [*C. ornatus*], [*C. stephennashi*], [*C. aurepalatti*], [*C. caquetensis*], [*C. toppini*] and [*C. urubambensis*].

Kobayashi (1995) pointed out that the morphological differences between the species of the *C. moloch* and *C. cupreus* groups are extremely subtle, although their parapatric geographic distribution, divided by the Madeira River, was considered to be decisive to consider them as distinct taxonomic groups. The Madeira is a major geographic barrier for a number of taxa, and separates two Amazonian centers of endemism – the Inambari and Rondônia centers [Da Silva et al., 2005]. Even so, a number of other primate taxa (*Saguinus weddelli*, *Saimiri ustus, Lagothrix cana* and *Ateles chamek*) are found on both banks of the upper Madeira, suggesting the occurrence of gene flow (active or passive) between the margins of this river.

In addition, the topology obtained in the present study indicate that the specimens collected near to Alta Floresta, left bank of the Tapajós river identified as *C. moloch*2, they are a distinct taxon of others *C. moloch* here studied (*C. moloch*1) and also of *C. vieirai*. This suggests that the specimens of *C. moloch*2 may represent an undescribed

species of the *C. moloch* group; even though this area is within the known geographic distribution *C. moloch* or that the differences between both *C. moloch* groups (1 and 2) and *C. vieirai* represents the extremes of a gradient of variation within *C. moloch*, that due to the scattered nature of the sampling in this study is impossible to evaluate.

The results of the present study also indicate that *C. hoffmannsi* is one of the most basal within the *C. moloch* group, rather than *C. dubius*, as suggested by Hershkovitz [1988]. As no samples of *C. baptista* were available for analysis, it was not possible to evaluate its relationship with *C. hoffmannsi*, which is generally considered to be its sister species. With regard to the two most recently-described species, *C. miltoni* and *C. vieirai*, the results provided some important insights. While it is morphologically similar to *C. bernhardi* in its pelage, for example, *C. miltoni* is closely related, in genetic terms, to *C. cinerascens*. By contrast, a close genetic relationship was found between *C. vieirai* and *C. moloch*, which was expected, given the occurrence of *C. vieirai* between the Iriri and Xingu rivers, an area surrounded by the geographical distribution of *C. moloch.* One other interesting finding was the close relationship between *C. dubius* and *C. caligatus*, which was in fact the smallest genetic divergence found between any two species. This supports the position of Groves [2001], who concluded that *C. dubius* is a geographical variant of *C. caligatus*, rather than a valid species.

We hope that these new insights into the considerable diversity of the titi monkeys will contribute to the definition of the taxonomic arrangement of the genus. Further research into their diversity, biogeography, and genetic variability of these primates will be fundamental to a more complete understanding of their phylogeny, and the effective conservation of the genus.

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Table 1. Taxonomic arrangements proposed recently for the genus *Callicebus*.

Hershkovitz (1988, 1990)	Kobayashi (1995), Kobayashi & Langguth (1999)	Groves (2001)	Van Roosmalen, van Roosmalen, Mittermeier (2002)
C. modestus	***C. donacophilus***	***C. modestus***	***C. donacophilus***
	C. d. pallescens		*C. d. pallescens*
C. donacophilus	*C. modestus*	***C. donacophilus***	*C. modestus*
C. d. pallescens	*C. olallae*	*C. d. pallescens*	*C. olallae*
C. oenanthe		*C. d. oenanthe*	
C. olallae	***C. cupreus***	*C. olallae*	***C. cupreus***
	C. c. discolor		*C. caligatus*
C. moloch	*C. c. ornatus*	***C. moloch***	*C. discolor*
C. cinerascens		*C. cinerascens*	*C. ornatus*
C. cupreus cupreus	***C. moloch***	*C. cupreus cupreus*	*C. dubius*
C. c. discolor	*C. cinerascens*	*C. c. discolor*	*C. stephennashi*
C. c. ornatus	*C. brunneus*	*C. c. ornatus*	
C. caligatus	*C. hoffmannsi hoffmannsi*	*C. brunneus*	***C. moloch***
C. brunneus	*C. h. baptista*	*C. hoffmannsi*	*C. cinerascens*
C. hoffmannsi hoffmannsi		*C. baptista*	*C. brunneus*
C. h. baptista	***C. personatus***	*C. personatus personatus*	*C. hoffmannsi*
C. dubius	*C. melanochir*	*C. p. melanochir*	*C. baptista*
C. personatus personatus	*C. nigrifrons*	*C. p. nigrifrons*	*C. bernhardi*
C. p. melanochir	*C. barbarabrownae*	*C. p. barbarabrownae*	*C. miltoni**
C. p. nigrifrons	*C. coimbrai*	*C. coimbrai*	*C. vieirai**
C. p. barbarabrownae			
	C. torquatus	***C. torquatus***	***C. personatus***
C. torquatus	*C. t. lugens*	*C. t. lugens*	*C. melanochir*

C. t. lugens	*C. t. lucifer*	*C. t. lucifer*	*C. nigrifrons*
C. t. lucifer	*C. t. purinus*	*C. t. purinus*	*C. barbarabrownae*
C. t. purinus	*C. t. regulus*	*C. t. regulus*	*C. coimbrai*
C. t. regulus	*C. t. medemi*	*C. medemi*	
C. t. medemi			***C. torquatus***
			C. lugens
			C. lucifer
			C. purinus
			C. regulus
			C. medemi

* Species described after van Roosmalen, van Roosmalen, Mittermeier [2002] were placed into the *C. moloch* group

Table 2. Details of the *Callicebus* specimens analyzed in the present study, including their origin and collecting locality.

	Species	Code	Origin	Coordinates		Locality
				Latitude	Longitude	
01	*C. bernhardi*	FR26	INPA	05°76'S	60°26'W	Left bank of the Aripuanã River, Amazonas, Brazil
02	*C. bernhardi*	CCM173	INPA	08°60'S	62°41'W	Mariepauá River, tributary of the Madeira River, Amazonas, Brazil
03	*C. bernhardi*	UFRO354	UNIR	12°06'S	60°67'W	UHE Rondon II, Pimenta Bueno, Rondônia, Brazil
04	*C. bernhardi*	42960	MPEG	12°17'S	63°19'W	São Francisco do Guaporé Biological Reserve, Rondônia, Brazil
05	*C. bernhardi*	42961	MPEG	12°17'S	63°19'W	São Francisco do Guaporé Biological Reserve, Rondônia, Brazil
06	*C. bernhardi*	42964	MPEG	12°17'S	63°19'W	São Francisco do Guaporé Biological Reserve, Rondônia, Brazil
07	*C. moloch*	RVR22	INPA	09°53'S	56°01'W	Novo Horizonte community, Alta Floresta, Mato Grosso, Brazil
08	*C. moloch*	RVR68	INPA	09°53'S	56°01'W	Novo Horizonte, community, Alta Floresta, Mato Grosso, Brazil
09	*C. moloch*	RVR73	INPA	09°53'S	56°01'W	Novo Horizonte, community, Alta Floresta, Mato Grosso, Brazil
10	*C. moloch*	1103	UFPA	04°16'S	49°48'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
11	*C. moloch*	1229	UFPA	04°26'S	49°35'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
12	*C. moloch*	299	UFPA	04°29'S	49°39'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
13	*C. moloch*	309	UFPA	04°19'S	49°48'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
14	*C. moloch*	590	UFPA	04°20'S	49°37'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
15	*C. moloch*	1516	UFPA	04°15'S	49°34'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
16	*C. moloch*	1690	UFPA	04°16'S	49°50'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
17	*C. moloch*	308	UFPA	04°22'S	49°52'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
18	*C. moloch*	857	UFPA	04°25'S	49°30'W	UHE Tucuruí, left bank of the Tocantins River, Pará, Brazil
19	*C. moloch*	MCB63	UFPA	02°45'S	51°53'W	Senador José Porfírio, right bank of the Xingu River, Pará, Brazil
20	*C. moloch*	MCB64	UFPA	02°45'S	51°53'W	Senador José Porfírio, right bank of the Xingu River, Pará, Brazil
21	*C. moloch*	MCB79	UFPA	02°50'S	51°50'W	Senador José Porfírio, right bank of the Xingu River, Pará, Brazil

22	*C. moloch*	CTGAM420	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
23	*C. moloch*	CTGAM421	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
24	*C. moloch*	CTGAM433	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
25	*C. moloch*	SANTAR	UFPA	02°30'S	54°40'W	Santarém, Igarapé Mararu, right bank of the Tapajós River, Pará, Brazil
26	*C. vieirai*	2465	CNRJ	09°50'S	53°28'W	Right bank of the Iriri River, Mato Grosso, Brazil
27	*C. vieirai*	2694	CNRJ	09°50'S	53°28'W	Right bank of the Iriri River, Mato Grosso, Brazil
28	*C. cinerascens*	FR123	INPA	NI	NI	NI
29	*C. cinerascens*	FR31	INPA	06°41'S	59°56'W	Novo Aripuanã, right bank of the Aripuanã River, Amazonas, Brazil
30	*C. cinerascens*	FR50	INPA	NI	NI	NI
31	*C. cinerascens*	UFRO195	UNIR	12°06'S	60°64'W	UHE Rondon II, Pimenta Bueno, Rondônia, Brazil
32	*C. miltoni*	42991	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
33	*C. miltoni*	42992	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
34	*C. miltoni*	42993	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
35	*C. brunneus*	2220	UFPA	08°47'S	63°15'W	UHE Samuel, right bank of the Jamari River, Rondônia, Brazil
36	*C. brunneus*	2394	UFPA	08°43'S	63°28'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
37	*C. brunneus*	2397	UFPA	08°41'S	63°32'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
38	*C. brunneus*	2422	UFPA	08°43'S	63°31'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
39	*C. brunneus*	4346	UFPA	08°49'S	63°32'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
40	*C. brunneus*	4505	UFPA	08°47'S	63°14'W	UHE Samuel, right bank of the Jamari River, Rondônia, Brazil
41	*C. brunneus*	UFRO541	UNIR	08°47'S	63°54'W	Porto Velho, right bank of the Madeira River, Rondônia, Brazil
42	*C. brunneus*	UFRO327	UNIR	08°46'S	62°45'W	Manoa Farm, Cujubim, Rondônia, Brazil
43	*C. caligatus*	CTGAM181	UFAM	05°37'S	63°10'W	Tapauá, Igarapé do Jacinto, right bank of the Purus River, Amazonas, Brazil
44	*C. caligatus*	CTGAM182	UFAM	05°37'S	63°10'W	Tapauá, Igarapé do Jacinto, right bank of the Purus River, Amazonas, Brazil
45	*C. caligatus*	MVR58	INPA	NI	NI	NI

46	*C. dubius*	UFRO403	UNIR	08°43'S	63°55'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
47	*C. dubius*	UFRO427	UNIR	08°43'S	63°55'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
48	*C. dubius*	UFRO544	UNIR	08°42'S	63°56'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
49	*C. dubius*	FR75	INPA	06°46'S	64°22'W	Canutama, left bank of the Mucuim River, Amazonas, Brazil
50	*C. cupreus*	4982	UFPA	NI	NI	NI
51	*C. cupreus*	4986	UFPA	NI	NI	NI
52	*C. cupreus*	AAM15	INPA	03°50'S	64°00'W	RESEX Catuá-Ipixuna Coari, Ipixuna Lake, Amazonas, Brazil
53	*C. cupreus*	CTGAM210	UFAM	05°22'S	63°15'W	Rebio Abufari, Tapauá, left bank of the Purus River, Amazonas, Brazil
54	*C. cupreus*	JLP15920	INPA	05°18'S	69°23'W	RESEX Alto Jurua, left bank of the Juruá River, Amazonas, Brazil
55	*C. hoffmannsi*	02CNP	CENP	NI	NI	NI
56	*C. hoffmannsi*	CTGAM248	UFAM	03°20'S	55°24'W	Cametá community, left bank of the Tapajós River, Pará, Brazil
57	*C. hoffmannsi*	CTGAM290	UFAM	03°20'S	55°24'W	Cametá community, left bank of the Tapajós River, Pará, Brazil
58	*C. hoffmannsi*	JTI	UFPA	03°04'S	55°15'W	Pau da Letra community, left bank of the Tapajós River, Pará, Brazil
59	*C. melanochir*	2329	CNRJ	NI	NI	Eunápolis, Bahia, Brazil
60	*C. personatus*	2466	CNRJ	NI	NI	Aracruz, Espirito Santo, Brazil
61	*C. nigrifrons*	04	PUC	NI	NI	Minas Gerais, Brazil
62	*Chiropotes albinasus*	CTGAM5663	UFPA	NI	NI	NI
63	*Cacajao calvus*	CTGAM5666	UFPA	NI	NI	NI
64	*Pithecia pithecia*	Pit22	UFPA	NI	NI	NI

UNIR = Federal University of Rondônia; MPEG = Museu Paraense Emílio Goeldi; UFPA = Federal University of Pará; UFAM = Federal University of Amazonas; CPRJ = Rio de Janeiro Primate Center; INPA = National Institute for Amazonian Research; CENP = National Primate Center, Ananindeua–Pará, NI= no information, UHE = Hydroelectric Plant.

Table 3. K2P distances (%) between the five major clades generated from the three mitochondrial genes and the ten concatenated nuclear regions.

	M				**Cu**				**Ci**				**H**				**P**			
	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.
M	**2.3**	**0.7**	**2.8**	**1.0**																
Cu	4.8	1.5	4.9	1.9	**2.3**	**1.1**	**3.2**	**1.1**												
Ci	3.7	1.2	4.1	1.8	4.5	1.3	5.0	2.0	**0.7**	**0.5**	**1.3**	**0.5**								
H	5.5	2.2	4.9	2.3	5.3	2.3	5.3	2.2	5.5	2.4	5.5	2.6	**0.5**	**0.2**	**0.2**	**0.2**				
P	13.2	7.8	13.7	6.8	13.3	7.8	13.1	6.6	13.0	7.8	13.7	7.2	13.4	7.0	13.1	7.0	**6.4**	**3.8**	**7.4**	**3.8**

M= *C. moloch* clade; **Cu**= *C. cupreus* clade; **Ci**= *C. cinerascens* clade; **H**= *C. hoffmmansi* clade and **P**= *C. personatus* clade; Nuc.=Nuclear.

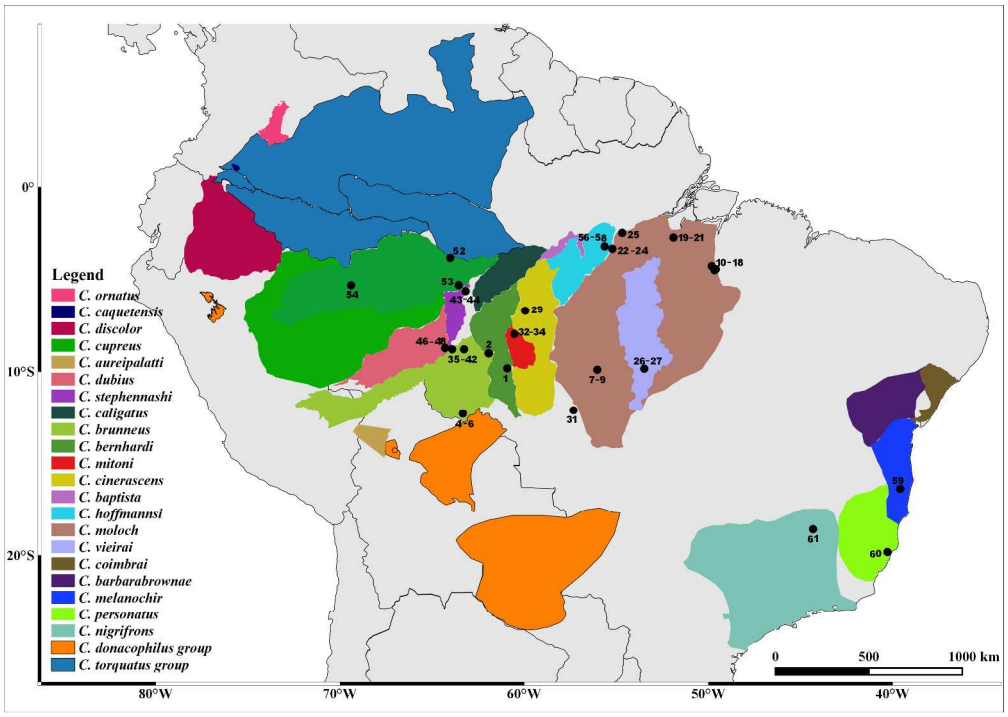

Figure 1. Geographic distribution of the genus *Callicebus* and map of South America showing the sites from which the specimens were obtained for analysis in the present study. The different colors represent each *Callicebus* species, and the site numbers correspond to those in Table 2. The hatched areas represent the ranges of four of the species groups (*C. torquatus*, *C. cupreus*, *C. donacophilus*, and *C. personatus*), while the species of the *C. moloch* group are represented by colored polygons.
296x209mm (300 x 300 DPI)

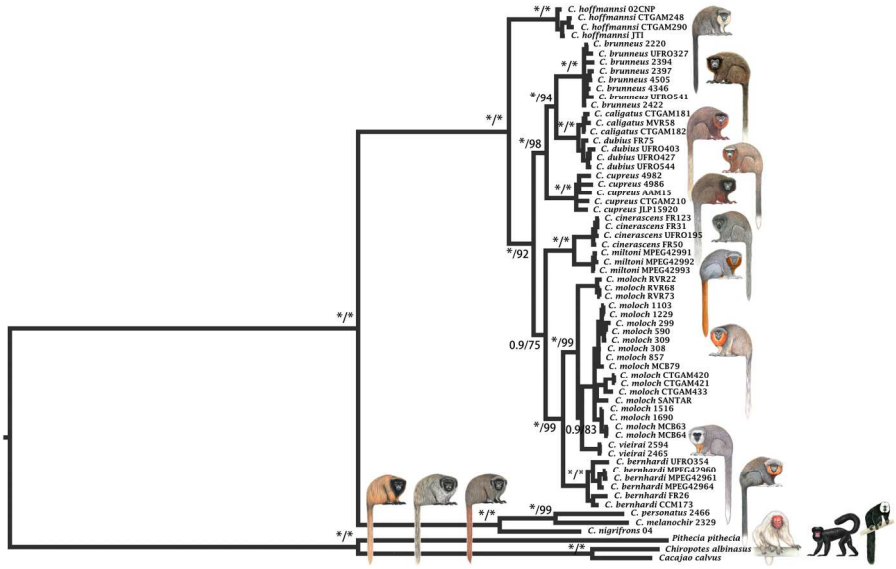

Figure 2. Phylogenetic tree based on a combined ~7 Kb sequence of nuclear and mitochondrial regions. Bootstrap support/posterior probability for the Maximum Likelihood and Bayesian inference analyses are shown at each node. Asterisks represent maximum support of values. Source *Callicebus* drawings Stephen Nash.
677x381mm (72 x 72 DPI)

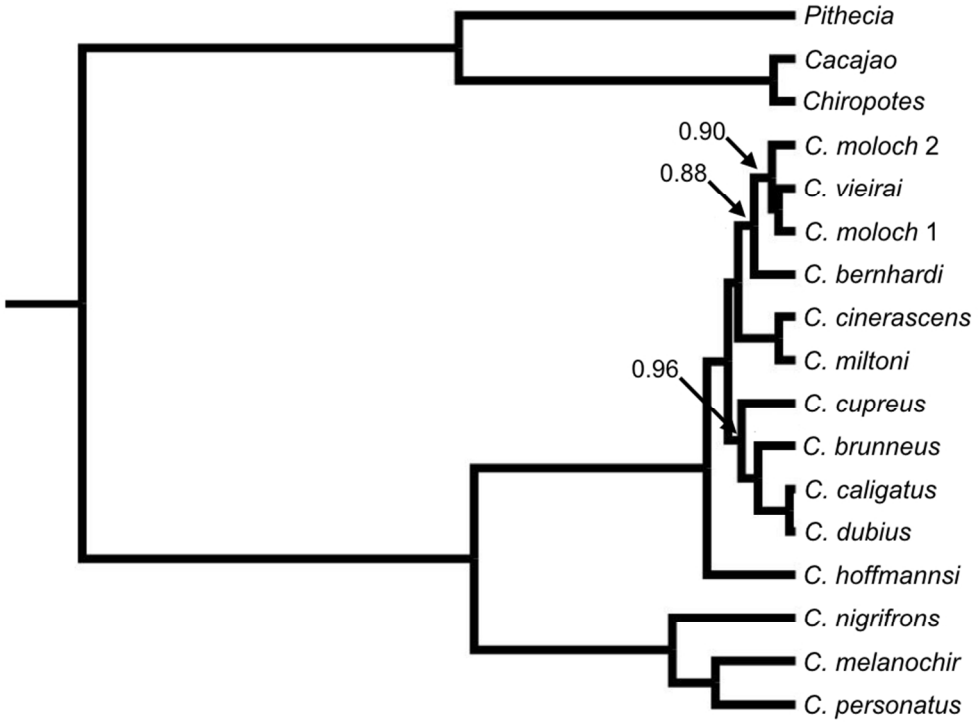

Figure 3. Phylogenetic tree obtained in BEAST v. 1.8.1. (Drummond et al., 2012). Only nodes with posterior probabilities below 1 are shown (see arrows).
361x270mm (72 x 72 DPI)