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

Carneiro, J, Sliva Junior, J, Sampaio, I, Pissinatti, A, Hrbek, T, Messias, M, Rohe, F,  
Farias, I, Boubli, JP and Schneider, H

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

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Manuscripts

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3 1 **Phylogeny of the titi monkeys of the *Callicebus moloch* group (Pitheciidae,**  
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5 2 **Primates)**  
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25 **ABSTRACT**

26 *Callicebus* is a Neotropical primate genus of the family Pitheciidae, which  
27 currently comprises 34 recognized species. Based on their morphological traits  
28 and geographic distribution, these species are currently assigned to five  
29 groups: the *C. moloch*, *C. cupreus*, *C. donacophilus*, *C. torquatus*, and *C.*  
30 *personatus* groups, although in the past, alternative arrangements have been  
31 proposed based on the analysis of morphological data. The principal  
32 disagreements among these arrangements are related to the composition of the  
33 *C. moloch* group. In the present study, we tested the different taxonomic  
34 proposals for the *C. moloch* group, based on the molecular analysis of nuclear  
35 markers (*Alu* insertions and flanking regions) and three mitochondrial genes  
36 (16S, COI and *Cyt b*), with a total of approximately 7 kb of DNA sequence  
37 data. Phylogenetic reconstructions based on maximum likelihood and  
38 Bayesian inference methods indicated that the species of the current *C.*  
39 *cupreus* group should be reintegrated into the *C. moloch* group. In addition,  
40 our results corroborated previous studies suggesting that the species of the  
41 current *C. personatus* group form a distinct species group. We also observed a  
42 relatively subtle level of divergence between *C. dubius* and *C. caligatus*.  
43 While the known diversity of *Callicebus* is considerable, these findings  
44 indicate that the relationships among groups and species may still not be  
45 completely understood, highlighting the need for further research into the  
46 biological, geographic and genetic variability of these primates, which will be  
47 fundamental to the effective conservation of the genus.

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

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5 51 **INTRODUCTION**

6  
7 52 *Callicebus* Thomas, 1903 is one of the four Neotropical primate genera of the  
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10 53 family Pitheciidae [Schneider and Sampaio 2015]. In the first taxonomic review of  
11  
12 54 this genus, Elliot [1913] recognized 22 monotypic species. Almost a half century  
13  
14 55 later, Hill [1960] published a comprehensive review of the social structure,  
15  
16 56 reproduction, behavior, parasitology, geographic distribution and systematics of the  
17  
18 57 subfamilies Callicebinae, Aotinae, Pitheciinae and Cebinae. In that work, his  
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20 58 arrangement of the genus *Callicebus* included only six species, but 34 subspecies.  
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22  
23 59 Hershkovitz [1963] identified only two species, from the Amazon (*Callicebus*  
24  
25 60 *moloch*) and Orinoco (*C. torquatus*) basins, but later revised this number to 13  
26  
27 61 [Hershkovitz 1988; 1990]. These species were allocated to four species groups, based  
28  
29 62 on cranial and post-cranial morphology and pelage coloration: (i) the *Callicebus*  
30  
31 63 *modestus* group, with one species, which Hershkovitz [1988] identified as an “isolated  
32  
33 64 relict species”; (ii) the *C. donacophilus* group, with three species; (iii) the *C. moloch*  
34  
35 65 group with eight species, and (iv) the *C. torquatus* group, with a single species.  
36  
37 66 Subsequently, Kobayashi [1995] using meristic cranial characters, pelage,  
38  
39 67 geographical distribution and karyotypes, suggested five species groups: (i) *C.*  
40  
41 68 *donacophilus*; (ii) *C. moloch*; (iii) *C. cupreus*; (iv) *C. personatus* and (v) *C. torquatus*  
42  
43 69 (Table 1). In that study, the *C. moloch* group was divided into three species (*C.*  
44  
45 70 *moloch*, *C. cupreus* and *C. personatus*), while *C. modestus* was incorporated into the  
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47 71 *C. donacophilus* group.

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52 72 van Roosmalen et al. [2002] followed the proposal of Kobayashi [1995], but  
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54 73 raised all subspecies to the species level, based on the phylogenetic species concept.  
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56 74 Since then, nine new putative species have been discovered and incorporated into  
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3 75 these groups based on morphological, ecological and biogeographical criteria  
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5 76 [Dalponte et al., 2014; Silva Júnior et al., 2013; Vermeer and Tello-Alvarado, 2015].  
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8 The genus *Callicebus* is widely distributed in tropical South America. Three of  
9  
10 78 the species groups (*C. torquatus*, *C. cupreus* and *C. moloch* groups) are found in the  
11  
12 79 Amazon and Orinoco basins [Kobayashi, 1995], the *C. donacophilus* group is found  
13  
14 80 primarily in the dry Chaco region, while the *C. personatus* group is centered on the  
15  
16 81 Brazilian Atlantic Forest biome, Cerrado and Caatinga (Fig. 1).  
17

18 The composition of the *C. moloch* group has changed a number of times, from a  
19  
20 82 maximum of 14 taxa (species and subspecies) in Hershkovitz [1988, 1990] to six in  
21  
22 83 the most recent proposal [van Roosmalen et al., 2002]. The purpose of the present  
23  
24 84 study is to clarify the taxonomic arrangement of the *Callicebus moloch* group based  
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26 85 on molecular data obtained from both nuclear and mitochondrial regions.  
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## 31 88 **METHODS**

### 32 89 **Samples and molecular markers**

33  
34 90 A total of 64 samples were obtained from blood or muscle tissue preserved in absolute  
35  
36 91 ethanol. These samples were obtained from the following Brazilian institutions: the  
37  
38 92 Goeldi Museum (MPEG), National Institute of Amazonian Research (INPA), Federal  
39  
40 93 University of Pará (UFPA), Federal University of Rondônia (UNIR), Federal  
41  
42 94 University of Amazonas (UFAM), Rio de Janeiro Primate Center (CPRJ-INEA), and  
43  
44 95 the National Primate Center (CENP) in Ananindeua, Pará. This research adhered to  
45  
46 96 the legal requirements of Brazil legislation as well as to “Principles for the Ethical  
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48 97 Treatment of Non Human Primates” of the American Society of Primatologists  
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50 98 (ASP).  
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3 99 For this study, putative species identifications were based on morphological and  
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5 100 pelage coloration (*sensu* van Roosmalen et al. [2002]). We included specimens of  
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7 101 species assigned to the *C. moloch* group by van Roosmalen et al's [2002]  
8  
9 102 classification, as well as those falling into van Roosmalen et al's [2002] *C. cupreus*,  
10  
11 103 and *C personatus* groups because some of those species were considered part of the  
12  
13 104 *moloch* group by others authors (e.g., Groves [2001], Hershkovitz [1988, 1990]). We  
14  
15 105 also included samples of individuals from newly described species *C. vieirai* and *C.*  
16  
17 106 *miltoni* [Gualda-Barros et al., 2012; Dalponte et al., 2014]. The sample codes, sources  
18  
19 107 and localities are shown in Table 2, and the localities are plotted in Fig. 1. Samples of  
20  
21 108 the other pitheciid genera (*Pithecia*, *Chiropotes* and *Cacajao*) were used as the  
22  
23 109 outgroup for the phylogenetic analyses. Our phylogenetic inferences were based on  
24  
25 110 ten nuclear and three mitochondrial markers (Table S1). The three mtDNA genes  
26  
27 111 were rRNA16S (543 bps), cytochrome oxidase subunit I – COI (605 bps) and  
28  
29 112 cytochrome *b* - CYT *b* (1074 bps). The nuclear regions correspond to sites including  
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31 113 mobile *Alu* elements, other repetitive sequences, and their flanking regions.  
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#### 115 **Extraction, amplification and sequencing of DNA**

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41 116 Total DNA was obtained with Promega's Wizard Genomic kit, according to the  
42  
43 117 manufacturer's protocol. The mitochondrial and nuclear regions were amplified by  
44  
45 118 polymerase chain reaction (PCR). For the PCRs, a final volume of 15  $\mu$ l was used,  
46  
47 119 containing about 30 ng of genomic DNA, 2.4  $\mu$ l of dNTPs (1.25mM), 1.5  $\mu$ l of 10X  
48  
49 120 Buffer (200 mM Tris-HCl, 500 mM KCl), 1  $\mu$ l of MgCl<sub>2</sub> (25 mM), 1  $\mu$ l of each  
50  
51 121 primer (0.2  $\mu$ M), and 1 U of Taq DNA polymerase. The amplification protocol was  
52  
53 122 initiated with four minutes of denaturation at 95°C, followed by 35 cycles of three  
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55 123 stages: (i) denaturation at 95°C for 30 s, (ii) annealing at a specific temperature (see  
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3 124 Table S1), and (iii) extension at 72°C for 30 seconds. After completion of the 35  
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5 125 cycles, there was a final extension stage at 72°C for seven minutes. The PCR products  
6  
7 126 were then purified using polyethylene glycol and ethanol [Paithankar and Prasad,  
8  
9 127 1991]. The sequencing reactions were run using the BigDye Terminator Sequencing  
10  
11 128 kit v. 3.1 (Life Technologies) and the reaction products were separated and visualized  
12  
13  
14 129 using an ABI 3500xl automatic sequencer (Life Technologies).  
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### 19 131 **Sequence alignment, identification of Alus and phylogenetic analyses**

20  
21 132 The DNA sequences were aligned initially using ClustalW [Thompson et al.,  
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23 133 1994] and then corrected manually using the BioEdit v. 7.2.5 software [Hall, 1999].  
24  
25 134 Saturation was assessed using DAMBE version 5.3.109 [Xia, 2013]. We used the  
26  
27 135 software PartitionFinder [Lanfear et al., 2012] to test different partition schemes and  
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29 136 select the most appropriate evolutionary model. We were particular concerned with  
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31 137 evaluating whether evolutionary rates differed among the three types of markers  
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33 138 (nuclear Alu elements, regions flanking Alu sites, and mitochondrial genes) (see  
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35 139 Table S2). For PartitionFinder analyses, we set the search method to “greedy”,  
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37 140 allowed unlinked branch lengths, and evaluated results based on Bayesian information  
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39 141 criterion (BIC). Our analysis suggested that the best scheme for our data set was to  
40  
41 142 separate it into two partitions (nuclear and mitochondrial regions). The regions  
42  
43 143 containing interspaced repeats (SINEs and LINEs) were identified using the software  
44  
45 144 RepeatMasker (<http://www.repeatmasker.org>).  
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49 145 Phylogenetic reconstruction were made using both the maximum likelihood (ML)  
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51 146 method, run in RaxML v.8 [Stamatakis, 2014] with 1000 bootstrap replicates and  
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53 147 Bayesian inference (BI) as implemented in MrBayes v. 3.2.1 [Ronquist and  
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55 148 Huelsenbeck, 2003]. In MrBayes, the analysis of substitution model parameters was  
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3 149 unlinked across partitions. Two independent runs were initiated simultaneously with  
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5 150 four independent Markov-Chain Monte Carlo (MCMC) chains (1 cold and 3 heated).  
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7 151 The MCMC algorithm was based on 500,000 cycles (generations), sampled every  
8  
9 152 5000 cycles, with 25% of the samples being discarded as burn-in. Convergence was  
10  
11 153 assessed by comparing the two runs. The MCMC output was visualized and  
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13 154 diagnosed in Tracer v. 1.6 [Rambaut et al., 2014]. The run was considered  
14  
15 155 satisfactory when, for all traces, the Effective Sample Size (ESS) values were over  
16  
17 156 200. For interspecific comparisons, matrices of genetic distances based on the K2P  
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19 157 model [Kimura, 1980] were generated for each marker in the MEGA v. 6.0 software  
20  
21 158 [Tamura et al., 2013]. Given the large number of specimens analyzed, genetic  
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23 159 distances were also estimated using only two specimens of each species for  
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25 160 visualization purposes.

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29 161 We also perform a Bayesian multispecies coalescent analysis in \*BEAST [Heled,  
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31 162 Drummond, 2010] with two runs of 300 million generations each. The nucleotide  
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33 163 substitution model chosen for the concatenated nuclear regions, and the mitochondrial  
34  
35 164 genes CytB, COI and 16S were respectively: GTR+Gamma; GTR+Gamma;  
36  
37 165 HKY+Gamma; GTR+Gamma. For the clock model, both strict and correlated relaxed  
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39 166 clock were tested. For species tree and population Size model, Yule and Piecewise  
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41 167 linear and constant root were the priors used, respectively. For model parameters and  
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43 168 statistics, the default priors were used.

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47 169 The logs of these two runs were visualized in Tracer to check if the ESS values  
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49 170 were above 200. When considered adequate, the logs were combined in LogCombiner  
50  
51 171 v. 1.8.3 and after a 20% burn-in the trees were summarized in the TreeAnnotator v.  
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53 172 1.8.3. All trees (ML, BI, and species tree) were visualized and edited in FigTree v.  
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55 173 1.4.2 [Rambaut, 2012].

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3 175 **RESULTS**

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5 176 **Data and missing data**

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7 177 A total of 747 sequences were generated, which correspond to 88.4% of the  
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9 178 number of possible sequences (see S3 for details). The total sequence of 7121 bps  
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11 179 included 4899 bps of nuclear markers and 2222 bps of the mitochondrial markers  
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13 180 (Table S2). Gaps in the data arose due to the lack of biological material in some  
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15 181 samples or the failure of the PCR amplification.  
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21 183 **Saturation, phylogenetic analysis, species tree and genetic divergences**

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23 184 No saturation was detected in any of the markers (data not shown). The  
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25 185 Maximum Likelihood and Bayesian approaches generated well supported topologies  
26  
27 186 for the majority of the nodes (Fig. 2). A clear and significant division was found  
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29 187 between the species of the *C. personatus* group (Atlantic Forest) and the remaining  
30  
31 188 (Amazonian) species analyzed in this study. In the Amazonian group, *C. hoffmannsi*  
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33 189 appears to have diverged first, followed by a trichotomy of groups – (i) *C. cupreus*, *C.*  
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35 190 *brunneus*, *C. caligatus* and *C. dubius*, (ii) *C. cinerascens* and *C. miltoni*, and (iii) *C.*  
36  
37 191 *moloch*, *C. vieirai* and *C. bernhardi*.

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40 192 The species tree inferred using \*BEAST had the same topology as that  
41  
42 193 reconstructed under maximum likelihood using RAxML and Bayesian inference as  
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44 194 implemented in MrBayes, regardless of whether a constant or relaxed molecular clock  
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46 195 was applied. All currently recognized species were assigned to well-supported clades,  
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48 196 with the exception of *C. moloch*, which consistently appeared paraphyletic, with  
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50 197 individuals collected near to Alta Floresta, left bank of the Tapajós river identified as  
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52 198 *C. moloch2* forming a distinct clade, sister to other *C. moloch1* individuals and *C.*  
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54 199 *vieirai* (Fig. 3).  
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3 200 Pairwise genetic distances (K2P) were estimated between clades in the whole  
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5 201 dataset, as well as between species in a reduced dataset. Genetic divergence between  
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7 202 *C. bernhardi* and *C. cinerascens* varied from 4.7% to 4.9% (Table S4), which is  
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9  
10 203 consistent with the genetic distances between the *C. moloch* and *C. cupreus* groups  
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12 204 recognized by Kobayashi [1995]. Based on the topology obtained in the present study,  
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14 205 five clades were identified: (**M**) *C. moloch*, *C. vieirai* and *C. bernhardi*; (**Ci**) *C.*  
15  
16 206 *cinerascens* and *C. miltoni*; (**Cu**) *C. cupreus*, *C. brunneus*, *C. caligatus* and *C. dubius*;  
17  
18 207 (**H**) *C. hoffmannsi*, and (**P**) the species of the *C. personatus* group.  
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20  
21 208 We estimated genetic distances within and between these five clades for both  
22  
23 209 mitochondrial sequences only (COI, 16S and CYT b) and for concatenated  
24  
25 210 mitochondrial and nuclear sequences. Intra-clades distances were lowest for clade **H**  
26  
27 211 and highest for clade **P**. Inter-clade distances were, overall, much higher between  
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29 212 clade **P** and the remaining clades, while clades **M**, **Cu**, **Ci** and **H** all had similar  
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31 213 genetic distances from one another (Table 3).  
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215 **DISCUSSION**

216 As mentioned previously, the configuration of *Callicebus* species groups has  
217 been the subject of much discussion, although there are two basic proposals for the *C.*  
218 *moloch* group. One is that of Kobayashi [1995], which includes *C. moloch*, *C.*  
219 *cinerascens*, *C. brunneus*, *C. hoffmannsi*, and *C. baptista*, and is similar to the  
220 proposal of van Roosmalen et al. [2002]. The second proposal is that of Groves  
221 [2001], which is in fact similar to that of Hershkovitz [1990]. Groves [2001] added *C.*  
222 *cupreus* (and its subspecies) and *C. personatus* to the *C. moloch* group, in addition to  
223 the species suggested by Kobayashi [1995] and Kobayashi and Langguth [1999].

224 The results of the present study nevertheless indicate emphatically that the *C.*  
225 *personatus* clade from the Brazilian Atlantic Forest is a group quite distinct from the  
226 Amazonian forms. This is supported by the greater genetic distances between the *C.*  
227 *personatus* and the Amazonian clades of 6.6–7.2% for the nuclear sequences and  
228 more than 13% for the mitochondrial ones (CytB = 13.0% and COI = 13.7%). A  
229 similar conclusion was reached by Perelman et al. [2011] who also observed that the  
230 Atlantic species are very distantly related to the Amazonian ones, estimating a  
231 separation time of approximately 10 Ma. This result contrasts with Hershkovitz's  
232 [1990] and Groves's [2001] hypotheses that placed the titi monkeys of the Atlantic  
233 Forest inside the *C. moloch* group.

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

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3 240 placed *C. brunneus* more closely related *C. moloch* and *C. cinerascens* than with *C.*  
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5 241 *cupreus*.

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7 242 The result of the present study indicate that the groups proposed by Kobayashi  
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9 243 [1995], Kobayashi & Languth [1999] and van Roosmalen et al. [2002] are not  
10  
11 244 monophyletic, and are incompatible with the genetic similarity between species of the  
12  
13 245 *C. cupreus* and *C. moloch* groups. Until further confirmatory research, then, we would  
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15 246 recommend adopting an arrangement similar to that proposed by Groves [2001], in  
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17 247 which the *C. moloch* group would include the following species (species in brackets  
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19 248 were not analyzed in the present study): *C. moloch*, *C. hoffmannsi*, *C. cinerascens*, *C.*  
20  
21 249 *brunneus*, [*C. baptista*], *C. bernhardi*, *C. vieirai*, *C. miltoni*, *C. cupreus*, *C. caligatus*,  
22  
23 250 *C. dubius*, [*C. discolor*], [*C. ornatus*], [*C. stephennashi*], [*C. aurepalatti*], [*C.*  
24  
25 251 *caquetensis*], [*C. toppini*] and [*C. urubambensis*].

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28  
29 252 Kobayashi (1995) pointed out that the morphological differences between the  
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31 253 species of the *C. moloch* and *C. cupreus* groups are extremely subtle, although their  
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33 254 parapatric geographic distribution, divided by the Madeira River, was considered to  
34  
35 255 be decisive to consider them as distinct taxonomic groups. The Madeira is a major  
36  
37 256 geographic barrier for a number of taxa, and separates two Amazonian centers of  
38  
39 257 endemism – the Inambari and Rondônia centers [Da Silva et al., 2005]. Even so, a  
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41 258 number of other primate taxa (*Saguinus weddelli*, *Saimiri ustus*, *Lagothrix cana* and  
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43 259 *Ateles chamek*) are found on both banks of the upper Madeira, suggesting the  
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45 260 occurrence of gene flow (active or passive) between the margins of this river.

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49 261 In addition, the topology obtained in the present study indicate that the specimens  
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51 262 collected near to Alta Floresta, left bank of the Tapajós river identified as *C. moloch*<sub>2</sub>,  
52  
53 263 they are a distinct taxon of others *C. moloch* here studied (*C. moloch*<sub>1</sub>) and also of *C.*  
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55 264 *vieirai*. This suggests that the specimens of *C. moloch*<sub>2</sub> may represent an undescribed

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3 265 species of the *C. moloch* group; even though this area is within the known geographic  
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5 266 distribution *C. moloch* or that the differences between both *C. moloch* groups (1 and  
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7 267 2) and *C. vieirai* represents the extremes of a gradient of variation within *C. moloch*,  
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10 268 that due to the scattered nature of the sampling in this study is impossible to evaluate.

11 269 The results of the present study also indicate that *C. hoffmannsi* is one of the most  
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13 270 basal within the *C. moloch* group, rather than *C. dubius*, as suggested by Hershkovitz  
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15 271 [1988]. As no samples of *C. baptista* were available for analysis, it was not possible to  
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17 272 evaluate its relationship with *C. hoffmannsi*, which is generally considered to be its  
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19 273 sister species. With regard to the two most recently-described species, *C. miltoni* and  
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21 274 *C. vieirai*, the results provided some important insights. While it is morphologically  
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23 275 similar to *C. bernhardi* in its pelage, for example, *C. miltoni* is closely related, in  
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25 276 genetic terms, to *C. cinerascens*. By contrast, a close genetic relationship was found  
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27 277 between *C. vieirai* and *C. moloch*, which was expected, given the occurrence of *C.*  
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29 278 *vieirai* between the Iriri and Xingu rivers, an area surrounded by the geographical  
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31 279 distribution of *C. moloch*. One other interesting finding was the close relationship  
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33 280 between *C. dubius* and *C. caligatus*, which was in fact the smallest genetic divergence  
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35 281 found between any two species. This supports the position of Groves [2001], who  
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37 282 concluded that *C. dubius* is a geographical variant of *C. caligatus*, rather than a valid  
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39 283 species.  
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45 284 We hope that these new insights into the considerable diversity of the titi  
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47 285 monkeys will contribute to the definition of the taxonomic arrangement of the genus.  
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49 286 Further research into their diversity, biogeography, and genetic variability of these  
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51 287 primates will be fundamental to a more complete understanding of their phylogeny,  
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53 288 and the effective conservation of the genus.  
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301

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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)
<b><u>C. modestus</u></b>	<b><u>C. donacophilus</u></b>	<b><u>C. modestus</u></b>	<b><u>C. donacophilus</u></b>
	<i>C. d. pallescens</i>		<i>C. d. pallescens</i>
<b><u>C. donacophilus</u></b>	<i>C. modestus</i>	<b><u>C. donacophilus</u></b>	<i>C. modestus</i>
<i>C. d. pallescens</i>	<i>C. olallae</i>	<i>C. d. pallescens</i>	<i>C. olallae</i>
<i>C. oenanthe</i>		<i>C. d. oenanthe</i>	
<i>C. olallae</i>	<b><u>C. cupreus</u></b>	<i>C. olallae</i>	<b><u>C. cupreus</u></b>
	<i>C. c. discolor</i>		<i>C. caligatus</i>
	<i>C. c. ornatus</i>		<i>C. discolor</i>
<b><u>C. moloch</u></b>		<b><u>C. moloch</u></b>	<i>C. ornatus</i>
<i>C. cinerascens</i>	<b><u>C. moloch</u></b>	<i>C. cinerascens</i>	<i>C. dubius</i>
<i>C. cupreus cupreus</i>	<i>C. cinerascens</i>	<i>C. cupreus cupreus</i>	<i>C. stephennashi</i>
<i>C. c. discolor</i>	<i>C. brunneus</i>	<i>C. c. discolor</i>	
<i>C. c. ornatus</i>	<i>C. hoffmannsi hoffmannsi</i>	<i>C. c. ornatus</i>	<b><u>C. moloch</u></b>
<i>C. caligatus</i>	<i>C. h. baptista</i>	<i>C. brunneus</i>	<i>C. cinerascens</i>
<i>C. brunneus</i>		<i>C. hoffmannsi</i>	<i>C. brunneus</i>
<i>C. hoffmannsi hoffmannsi</i>	<b><u>C. personatus</u></b>	<i>C. baptista</i>	<i>C. hoffmannsi</i>
<i>C. h. baptista</i>	<i>C. melanochir</i>	<i>C. personatus personatus</i>	<i>C. baptista</i>
<i>C. dubius</i>	<i>C. nigrifrons</i>	<i>C. p. melanochir</i>	<i>C. bernhardi</i>
<i>C. personatus personatus</i>	<i>C. barbarabrownae</i>	<i>C. p. nigrifrons</i>	<i>C. miltoni*</i>
<i>C. p. melanochir</i>	<i>C. coimbrai</i>	<i>C. p. barbarabrownae</i>	<i>C. vieirai*</i>
<i>C. p. nigrifrons</i>		<i>C. coimbrai</i>	
<i>C. p. barbarabrownae</i>			
	<b><u>C. torquatus</u></b>	<b><u>C. torquatus</u></b>	<b><u>C. personatus</u></b>
<b><u>C. torquatus</u></b>	<i>C. t. lugens</i>	<i>C. t. lugens</i>	<i>C. melanochir</i>

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*C. t. lugens*  
*C. t. lucifer*  
*C. t. purinus*  
*C. t. regulus*  
*C. t. medemi*

*C. t. lucifer*  
*C. t. purinus*  
*C. t. regulus*  
*C. t. medemi*

*C. t. lucifer*  
*C. t. purinus*  
*C. t. regulus*  
*C. medemi*

*C. nigrifrons*  
*C. barbarabrownae*  
*C. coimbrai*

**C. torquatus**  
*C. lugens*  
*C. lucifer*  
*C. purinus*  
*C. regulus*  
*C. medemi*

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

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

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6	22	<i>C. moloch</i>	CTGAM420	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
7	23	<i>C. moloch</i>	CTGAM421	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
8	24	<i>C. moloch</i>	CTGAM433	UFAM	03.21'S	55°12'W	Belterra, right bank of the Tocantins River, Pará, Brazil
9	25	<i>C. moloch</i>	SANTAR	UFPA	02°30'S	54°40'W	Santarém, Igarapé Mararu, right bank of the Tapajós River, Pará, Brazil
10	26	<i>C. vieirai</i>	2465	CNRJ	09°50'S	53°28'W	Right bank of the Iriri River, Mato Grosso, Brazil
11	27	<i>C. vieirai</i>	2694	CNRJ	09°50'S	53°28'W	Right bank of the Iriri River, Mato Grosso, Brazil
12	28	<i>C. cinerascens</i>	FR123	INPA	NI	NI	NI
13	29	<i>C. cinerascens</i>	FR31	INPA	06°41'S	59°56'W	Novo Aripuanã, right bank of the Aripuanã River, Amazonas, Brazil
14	30	<i>C. cinerascens</i>	FR50	INPA	NI	NI	NI
15	31	<i>C. cinerascens</i>	UFRO195	UNIR	12°06'S	60°64'W	UHE Rondon II, Pimenta Bueno, Rondônia, Brazil
16	32	<i>C. miltoni</i>	42991	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
17	33	<i>C. miltoni</i>	42992	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
18	34	<i>C. miltoni</i>	42993	MPEG	07°44'S	60°31'W	Novo Aripuanã, left bank of the Aripuanã River, Amazonas, Brazil
19	35	<i>C. brunneus</i>	2220	UFPA	08°47'S	63°15'W	UHE Samuel, right bank of the Jamari River, Rondônia, Brazil
20	36	<i>C. brunneus</i>	2394	UFPA	08°43'S	63°28'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
21	37	<i>C. brunneus</i>	2397	UFPA	08°41'S	63°32'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
22	38	<i>C. brunneus</i>	2422	UFPA	08°43'S	63°31'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
23	39	<i>C. brunneus</i>	4346	UFPA	08°49'S	63°32'W	UHE Samuel, left bank of the Jamari River, Rondônia, Brazil
24	40	<i>C. brunneus</i>	4505	UFPA	08°47'S	63°14'W	UHE Samuel, right bank of the Jamari River, Rondônia, Brazil
25	41	<i>C. brunneus</i>	UFRO541	UNIR	08°47'S	63°54'W	Porto Velho, right bank of the Madeira River, Rondônia, Brazil
26	42	<i>C. brunneus</i>	UFRO327	UNIR	08°46'S	62°45'W	Manoa Farm, Cujubim, Rondônia, Brazil
27	43	<i>C. caligatus</i>	CTGAM181	UFAM	05°37'S	63°10'W	Tapauá, Igarapé do Jacinto, right bank of the Purus River, Amazonas, Brazil
28	44	<i>C. caligatus</i>	CTGAM182	UFAM	05°37'S	63°10'W	Tapauá, Igarapé do Jacinto, right bank of the Purus River, Amazonas, Brazil
29	45	<i>C. caligatus</i>	MVR58	INPA	NI	NI	NI
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46	<i>C. dubius</i>	UFRO403	UNIR	08°43'S	63°55'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
47	<i>C. dubius</i>	UFRO427	UNIR	08°43'S	63°55'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
48	<i>C. dubius</i>	UFRO544	UNIR	08°42'S	63°56'W	Porto Velho, left bank of the Madeira River, Rondônia, Brazil
49	<i>C. dubius</i>	FR75	INPA	06°46'S	64°22'W	Canutama, left bank of the Mucuim River, Amazonas, Brazil
50	<i>C. cupreus</i>	4982	UFPA	NI	NI	NI
51	<i>C. cupreus</i>	4986	UFPA	NI	NI	NI
52	<i>C. cupreus</i>	AAM15	INPA	03°50'S	64°00'W	RESEX Catuá-Ipixuna Coari, Ipixuna Lake, Amazonas, Brazil
53	<i>C. cupreus</i>	CTGAM210	UFAM	05°22'S	63°15'W	Rebio Abufari, Tapauá, left bank of the Purus River, Amazonas, Brazil
54	<i>C. cupreus</i>	JLP15920	INPA	05°18'S	69°23'W	RESEX Alto Jurua, left bank of the Juruá River, Amazonas, Brazil
55	<i>C. hoffmannsi</i>	02CNP	CENP	NI	NI	NI
56	<i>C. hoffmannsi</i>	CTGAM248	UFAM	03°20'S	55°24'W	Cametá community, left bank of the Tapajós River, Pará, Brazil
57	<i>C. hoffmannsi</i>	CTGAM290	UFAM	03°20'S	55°24'W	Cametá community, left bank of the Tapajós River, Pará, Brazil
58	<i>C. hoffmannsi</i>	JTI	UFPA	03°04'S	55°15'W	Pau da Letra community, left bank of the Tapajós River, Pará, Brazil
59	<i>C. melanochir</i>	2329	CNRJ	NI	NI	Eunápolis, Bahia, Brazil
60	<i>C. personatus</i>	2466	CNRJ	NI	NI	Aracruz, Espírito Santo, Brazil
61	<i>C. nigrifrons</i>	04	PUC	NI	NI	Minas Gerais, Brazil
62	<i>Chiropotes albinasus</i>	CTGAM5663	UFPA	NI	NI	NI
63	<i>Cacajao calvus</i>	CTGAM5666	UFPA	NI	NI	NI
64	<i>Pithecia pithecia</i>	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.



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

	<b>M</b>				<b>Cu</b>				<b>Ci</b>				<b>H</b>				<b>P</b>			
	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.	CytB	16S	COI	Nuc.
<b>M</b>	<b>2.3</b>	<b>0.7</b>	<b>2.8</b>	<b>1.0</b>																
<b>Cu</b>	4.8	1.5	4.9	1.9	<b>2.3</b>	<b>1.1</b>	<b>3.2</b>	<b>1.1</b>												
<b>Ci</b>	3.7	1.2	4.1	1.8	4.5	1.3	5.0	2.0	<b>0.7</b>	<b>0.5</b>	<b>1.3</b>	<b>0.5</b>								
<b>H</b>	5.5	2.2	4.9	2.3	5.3	2.3	5.3	2.2	5.5	2.4	5.5	2.6	<b>0.5</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>				
<b>P</b>	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	<b>6.4</b>	<b>3.8</b>	<b>7.4</b>	<b>3.8</b>

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

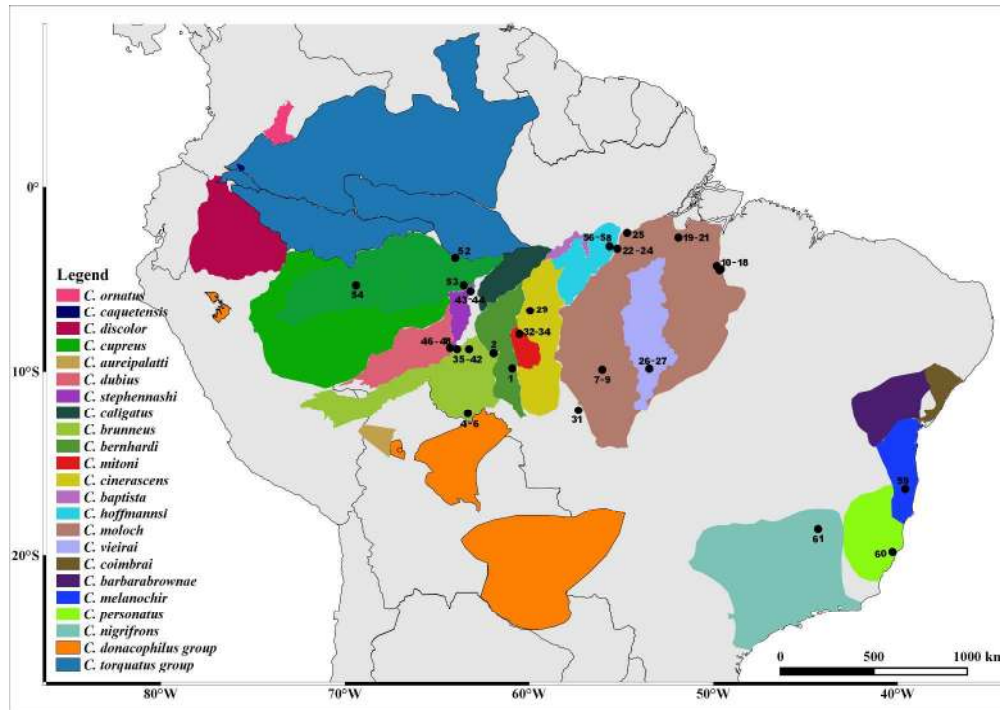


Figure 1. Geographic distribution of the genus *Callicebus* and map of South America showing the sites from which the specimens were obtained for analysis in the present study. The different colors represent each *Callicebus* species, and the site numbers correspond to those in Table 2. The hatched areas 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)



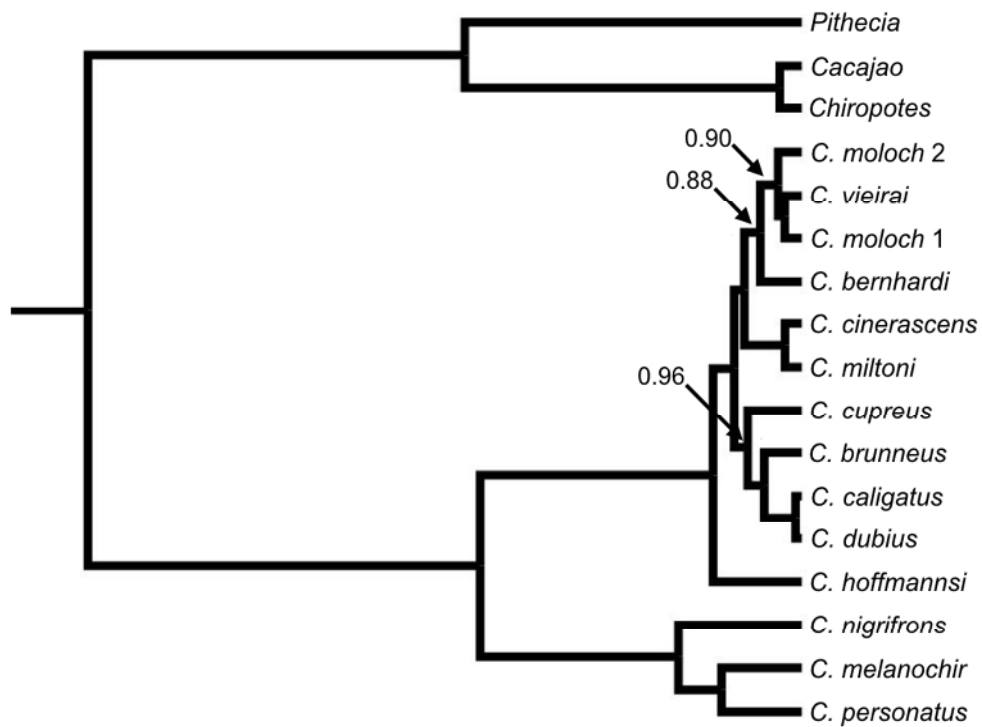


Figure 3. Phylogenetic tree obtained in BEAST v. 1.8.1. (Drummond et al., 2012). Only nodes with posterior probabilities below 1 are shown (see arrows).

361x270mm (72 x 72 DPI)