Understanding the Relationship Between Alouatta ululata and Alouatta belzebul (Primates: Atelidae) Based on Cytogenetics and Molecular Phylogeny

Article *in* Oecologia Australis · October 2015

DOI: 10.4257/oeco.2015.1901.11

CITATION
1

READS
299

6 authors, including:

Maria Carolina Viana
University of Campinas
13 PUBLICATIONS **29** CITATIONS
SEE PROFILE

Cibele Rodrigues Bonvicino
Brazilian National Cancer Institute
372 PUBLICATIONS **2,941** CITATIONS
SEE PROFILE

Leandro Jerusalinsky
Instituto Chico Mendes de Conservação da Biodiversidade
90 PUBLICATIONS **245** CITATIONS
SEE PROFILE

Alfredo Langguth
Universidade Federal da Paraíba
45 PUBLICATIONS **895** CITATIONS
SEE PROFILE

Oecologia Australis
19(1): 173-182, 2015
10.4257/oeco.2015.1901.11

UNDERSTANDING THE RELATIONSHIP BETWEEN *ALOUATTA ULULATA* AND *ALOUATTA BELZEBUL* (PRIMATES: ATELIDAE) BASED ON CYTOGENETICS AND MOLECULAR PHYLOGENY

*Maria C. Viana[1], Cibele R. Bonvicino[1,2], Juliana G. Ferreira[3], Leandro Jerusalinsky[4], Alfredo Langguth[5], Héctor Seuánez[1]**

1 Instituto Nacional de Câncer, Coordenação de Pesquisa, Divisão de Genética, Rua André Cavalcante, 37, 4° andar, 20231-050, Rio de Janeiro, RJ, Brazil
2 FIOCRUZ, Instituto Oswaldo Cruz, Pavilhão Lauro Travassos, Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Av. Brasil, 4365, Manguinhos, 21045-900, Rio de Janeiro, RJ, Brazil.
3 Coordenação Regional 11, Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Lagoa Santa, Minas Gerais, Brazil.
4 Centro Nacional de Pesquisa e Conservação de Primatas Brasileiros (CPB), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), João Pessoa, Paraíba, Brazil.
5 Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia, Campus Universitário 58059-970, João Pessoa, PB, Brazil.
E-mails: mcarolviana@gmail.com, cibelerb@inca.gov.br, leandro.jerusalinsky@icmbio.gov.br, juliana.ferreira@icmbio.gov.br, boninomvd@hotmail.com, hseuanez@inca.gov.br

ABSTRACT

The genus *Alouatta* Lacépède 1799 comprises a group of neotropical primates distributed from southern Mexico to northern Argentina. Ten species of *Alouatta* occur in Brazil, including *Alouatta belzebul* (Linnaeus, 1766) and *A. ululata* Elliot, 1912; this latter being considered a full species, subspecies or a junior synonymous of *A. belzebul*. In order to clarify the relationship of *A. ululata* with *A. belzebul* and infer their relationships with other *Alouatta* species, karyotype and mitochondrial DNA data were analyzed. Phylogenetic analyses were carried out with a 801 bp fragment of cytochrome *b* DNA of one *A. ululata* sample and 33 sequences of *A. belzebul, A. caraya, A. fusca, A. nigerrima, A. seniculus,* and *A. macconnelli* available in GenBank, with *Brachyteles arachnoides* as outgroup. The G-band karyotype of a male *A. ululata* showed a diploid number of 49, similar to the one reported for *A. belzebul,* with the same pattern of autosome heteromorphism, apparently resulting from a Y-autosome translocation. Maximum-likelihood and Bayesian analyses and median joining network did not show an internal structure among *A. belzebul* haplotypes and placed *A. ululata* haplotype within *A. belzebul* clade. Karyotypic and molecular analyses herein carried out did not allow the separation of *A. ululata* from *A. belzebul*. However, additional analysis with larger sample sizes may provide relevant information for this question.

Keywords: *Alouatta;* karyotype; *MT-CYB;* phylogeny.

INTRODUCTION

The genus *Alouatta* Lacépède 1799 comprises a group of large neotropical primates with a wide geographic distribution ranging from southern Mexico to northern Argentina. A region of eastern Amazonia and northeastern Brazil, south of the Amazonas river and above 10º latitude, is inhabited by several *Alouatta* forms. A study of geographic variation of coat color

(Bonvicino *et al*. 1989) recognized the single species *A. belzebul* (Linnaeus, 1766) with four subspecies *A. b. belzebul*, *A. b. nigerrima* Lönnberg, 1941, *A. b. discolor* (Spix, 1823) and *A. b. ululata* Elliot, 1912. *A. nigerrima* was raised to species level (Bonvicino *et al*. 2001) while the other forms have been maintained as subspecies. Groves (2001, 2005) synonymized *A. b. discolor* and *A. b. ululata* under *A. belzebul*. Gregorin (2006), in a study of Brazilian species of this genus, raised *A. b. discolor* and *A. b. ululata* to the species level.

The taxonomy of *Alouatta* is mainly based on pelage color (Bonvicino *et al*. 1989, Gregorin 2006). Previous studies classified the pelage of *Alouatta belzebul* in different patterns (Bonvicino *et al*. 1989:141), based on the extent of rufous or yellowish areas over a black background in their dorsal coloration. Based on patterns of pelage color, on absence of records (hiatus of distribution) or on geographic isolation by semi-arid environment, different areas of occurrence were identified, with specimens from areas 1 to 3 identified as *A. b. belzebul* and those of area 4 identified as *A. b. ululata* (Bonvicino *et al.* 1989, Figure 1). Howlers in areas 1 to 3 inhabit humid tropical forest contrary to howlers of area 4, comprising more mesic regions with higher trees surrounded by semiarid vegetation (Bonvicino *et al*. 1989). Area 1 is the northeastern portion of the Atlantic Forest from Paraíba to Alagoas states, with all specimens with the same pelage pattern; area 2 is the region of east Pará and Maranhão states, with specimens with two pelage pattern; area 3 is the region of the middle/lower Rio Tocantins basin, with highly variable specimens, with five pelage patterns; and area 4 is the region on the northern part of Maranhão, Piauí and Ceará states, from the Rio Mearim in Maranhão westward to Serra de lbiapaba in Ceará, with specimens with three pelage patterns (Bonvicino *et al*. 1989). Sexual dimorphism is characteristic of *A. b. ululata;* males are black, with rufous areas on limbs, tail and back, while females are yellowish-brown, olive brown or gray.

Some taxonomic consideration was also carried out based on karyotypic data. The genus *Alouatta* shows a wide variability in chromosome number and in their sex chromosome systems (Torres and Ramírez 2003). The karyotypes of *A. belzebul* 2n = 50, XX in females and 2n = 49 in males, carrying a Y-autosome translocation, was reported by Armada *et al*. (1987) and Lima and Seuánez (1989). The karyotype of *A. b. ululata,* however, is still unknown.

Discrepant classifications based on morphological data pointed to the need of revising the taxonomic status of *Alouatta belzebul* and *A. b. ululata*. This is particularly important since both taxa are listed as threatened of extinction in the national (MMA, 2014) and international (IUCN, 2008) red lists, and therefore specific conservation strategies, including management measures, should consider the taxonomic status and relatedness of these populations. With this purpose we report in this study the karyotype of *A. b. ululata* and a molecular phylogeny to understand the relationship between *A. b. ululata* and *A. belzebul*.

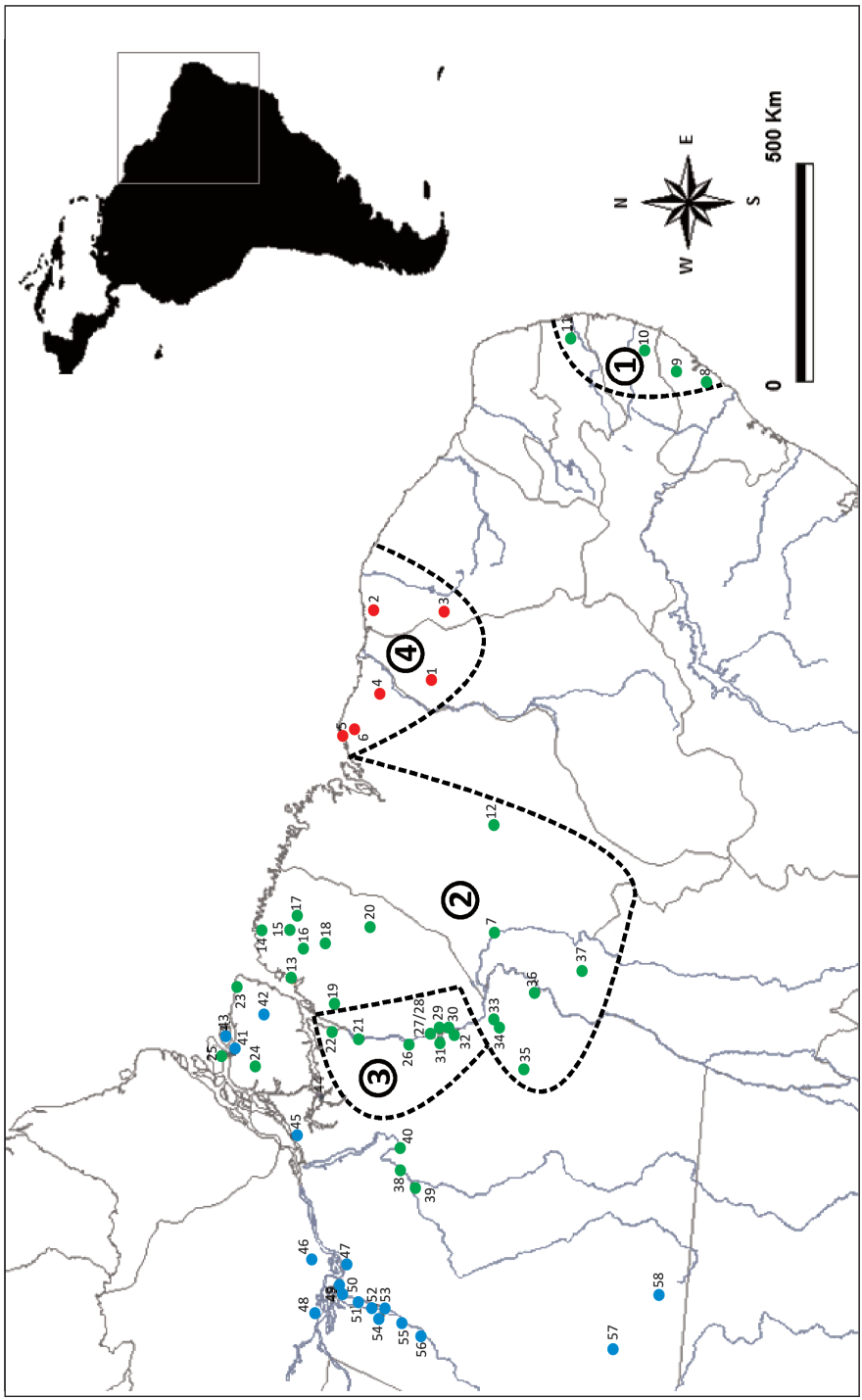

Figure 1. Areas of occurrence *of Alouatta belzebul belzebul* (1 to 3) and *Alouatta belzebul ululata* (4) defined by Bonvicino *et al.* (1989). Dots refer to localities investigated by these authors.

MATERIAL AND METHODS

Samples and karyotypic analysis

One Alouatta belzebul ululata (CPB71) was captured in Campo Maior, Piauí state, Brazil. Species identifitication were carried out by L. Jerusalinsky and G. Ferreira based on pelage coloration, and the specimen was housed in the CETAS, IBAMA/PB, with supervision of Centro Nacional de Pesquisa e Conservação de Primatas Brasileiros (CPB), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). This specimen showed the tradicional colour of *A. b. ululata*, with an overall black coloration with rufous hairs on hands, feet and anterior part of dorsum.

Chromosome preparations were obtained from peripheral blood cultures with 80% RPMI 1640, 20% fetal calf serum, ethidium bromide (5 mg/ml) and colchicine (10^{-6}) for 72 hours at 37°C, following by hypotonic shock with KCl (0.075M) for 30 minutes, pre-fixation and fixation with Carnoy solution (3 methyl alcohol:1 acetic acid). Conventional staining was carried out with 4% Giemsa in 0,1M phosphate buffer. G-banding was carried out by trypsin digestion for 30 seconds followed by Giemsa staining. Chromosomes were paired according to morphology, size and banding patterns and estimates of fundamental number were restricted to autosome pairs. Karyotypic comparisons were carried out with *A. belzebul*.

DNA isolation and molecular analysis

Peripheral blood of *A. b. ulutata* specimen CPB71 was collected and DNA was isolated with the phenol-chloroform protocol (Sambrook *et al.* 1989). *Cytochrome b* gene (*MT-CYB*; ca. 801 bp) was PCR amplified with primers L14724 (Irwin *et al.* 1991) and MVZ16 (Silva and Patton 1993), with a pre-denaturation step at 94ºC for 2 min; 35 cycles of denaturation at 94ºC for 30 sec, annealing at 50ºC for 30 sec, extension at 72ºC for 60 sec, and final extension of 72ºC for 2 min. The mitochondrial cytochrome *b* gene was referred as *MT-CYB* following HGNC rules (Eyre *et al*. 2006, HGNC 2009).

Amplicons were purified with GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Brazil). Sequencing reactions were performed with L14724, cit-alo (Bonvicino *et al*. 2001), AloAotR (Nascimento *et al*. 2007), AloAotF (Nascimento *et al*. 2005). Amplicons were labeled with XL and BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and loaded to an ABI Prism™ 3130 platform.

Sequences were edited and assembled with ChromasPro (version 1.7.5; available at www.technelysium.com.au/chromas.html). Phylogenetic analyses included 26 sequences of *A. belzebul* and from seven other Alouatines deposited in GenBank (Table 1). *Brachyteles arachnoides* (GenBnk JX262672) was used as outgroup.

Genetic distance estimates were carried out with complete deletion using Kimura's two parameters, with MEGA (version 5; Tamura *et al.* 2011). The HKY+G was selected as the DNA substitution model with MODELGENERATOR (version 0.85; Keane *et al.* 2006) using the

Table 1. List of *Cytochrome b* sequences used in phylogenetic analyses, with haplotypes number (H), GenBank accession number (GB nº) and collection locality. Brazilian states (BR) are Amazonas (AM), Pará (PA), Piauí (PI), Paraíba (PB), Goiás (GO), Mato Grosso (MT), and Santa Catarina (SC).

H	Taxa	GB nº	Collection site
1	*Alouatta b. ululata*	CPB71	BR: PI, Campo Maior
2	*Alouatta belzebul*	AF289511	BR: PA, Tucuruí
3	*Alouatta belzebul*	DQ387025, AY374344, AY374347, AY374350, AY374353, AY374354	BR: PA, Tucuruí
4	*Alouatta belzebul*	AY374349	BR: PA, Tucuruí
5	*Alouatta belzebul*	DQ387044	BR: PB, unknown locality
5	*Alouatta belzebul*	DQ398008, AF289515, DQ398009	BR: PB, Sapé
6	*Alouatta belzebul*	DQ387042, AY374351	BR: PA, Tucuruí
7	*Alouatta belzebul*	AY374343, T4-6a1998	BR: PA, Tucuruí
8	*Alouatta belzebul*	AY374352	BR: PA, Tucuruí
9	*Alouatta belzebul*	AF289512	BR: PA, Tucuruí
10	*Alouatta belzebul*	AF289513	BR: PA, Tucuruí
11	*Alouatta belzebul*	AY374348	BR: PA, Tucuruí
12	*Alouatta belzebul*	AY374346	BR: PA, Tucuruí
13	*Alouatta belzebul*	AY374341, AY374355	BR: PA, Tucuruí
14	*Alouatta belzebul*	AY374345	BR: PA, Tucuruí
15	*Alouatta belzebul*	DQ387028	BR: PA, Tucuruí
16	*Alouatta belzebul*	AF289514	BR: PA, Tucuruí
	Alouatta caraya	DQ350637	BR: GO, Padre Bernardo
	Alouatta caraya	AY374357	BR: MT, Chapada dos Guimarães
	Alouatta fusca	DQ679784	BR: SC, Lages
	Alouatta macconnelli	AJ489759	French Guiana
	Alouatta macconnelli	CRB2010	BR: AM, Barcelos
	Alouatta nigerrima	AF289985	BR: unknown locality
	Alouatta seniculus	AF289983	BR: AM, Barcelos

Bayesian information criterion (BIC) for phylogenetic reconstructions.

Phylogenetic reconstructions based on maximum likelihood (ML) were carried out with PHYML (version 3.0; Guindon *et al*. 2010). Branch support was calculated with the approximate likelihood ratio test (aLRT) with SH-like interpretation, a procedure that is conservative and accurate as bootstrapping but less computationally intensive (Anisimova and Gascuel 2006, Guindon *et al*. 2010). Bayesian analysis (BA) was carried out with MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). DNAsp 5 was used for haplotype estimates and nucleotide diversity (Librado and Rozas 2009).

NETWORK (version 4.6.1.1; available at http://www.fluxus-engineering.com) was used for reconstructing a median-joining (MJ) network (Bandelt *et al*. 1999) to evaluate sub-population structure and geographic distribution patterns. MJ was calculated using variable sites only.

RESULTS

Karyotype

Karyotypic analysis of a single male *Alouatta b. ululata* (CPB71) showed 2n = 49, the chromosome complement comprised 11 metacentric or submetacentric pairs and 13 acrocentric pairs (Figure 2). G- banding allowed the identification of all chromosomes pairs (Figure 2).

Molecular phylogeny

Alouatta belzebul ululata together with 26 *A. belzebul* sequences retrieved from GenBank accounted for 16 haplotypes, 11 of which being unique and five were shared by different individuals (Table 1). Haplotype H3 from Pará (Table 1) was the most frequent one. The ML topology was similar to the one obtained by Bayesian analysis (BA; Figure 3), except in relation to the position of *A. fusca*. ML placed *A. fusca* as the most basal offshoot, without support, while BA placed *A. caraya* as the most basal offshoot. ML and BA were coincident in grouping (*A. seniculus* (*A. nigerrima, A. macconnelli*)), with low support and *A. belzebul* and *A. b. ululata*, with high support. The *A. b. ululata* haplotype grouped within the clade of *A. belzebul* (Figure 3).

MJ analyses (Figure 4) showed a similar result to ML and BA analysis in relation to *A. b. ululata* and *A. belzebul*, placing *A. b. ululata* haplotype directly connected with haplotypes from Tocantins (H2).

DISCUSSION

Karyotype

The *Alouatta belzebul ululata* karyotype was similar to the one described for *A. belzebul* by Armada *et al*. (1987) in morphology and the G-band patterns (Figure 2). The Y chromosome of *Alouatta b. ululata*, as in *Alouatta belzebul*, was apparently translocated to one member of a heteromorphic autosome chromosome pair (Nº 17). Conversely, in females, this translocation was not present (Lima and Seuánez 1989). Based on chromosome similarity, *A. belzebul* and *A. b. ululata* are likely to belong to the same species.

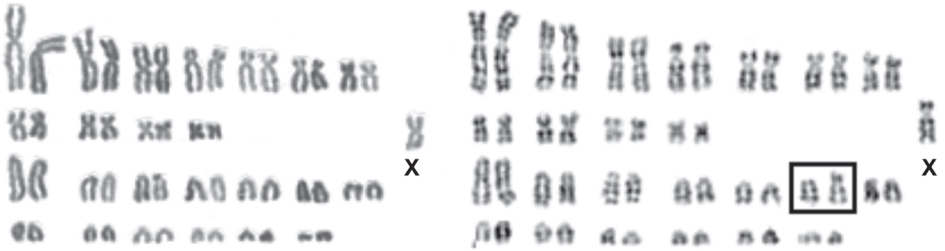

Figure 2. Karyotype of a male specimen (CPB 71) of *Alouatta belzebul ululata*. Conventional staining (left) showed 2n = 49 and absence of a free Y chromosome. G-band karyotype (right) showing a heteromorphic pair (boxed). X = X chromosome. For comparisons with *A. b. belzebul* see Armada *et al*. (1987).

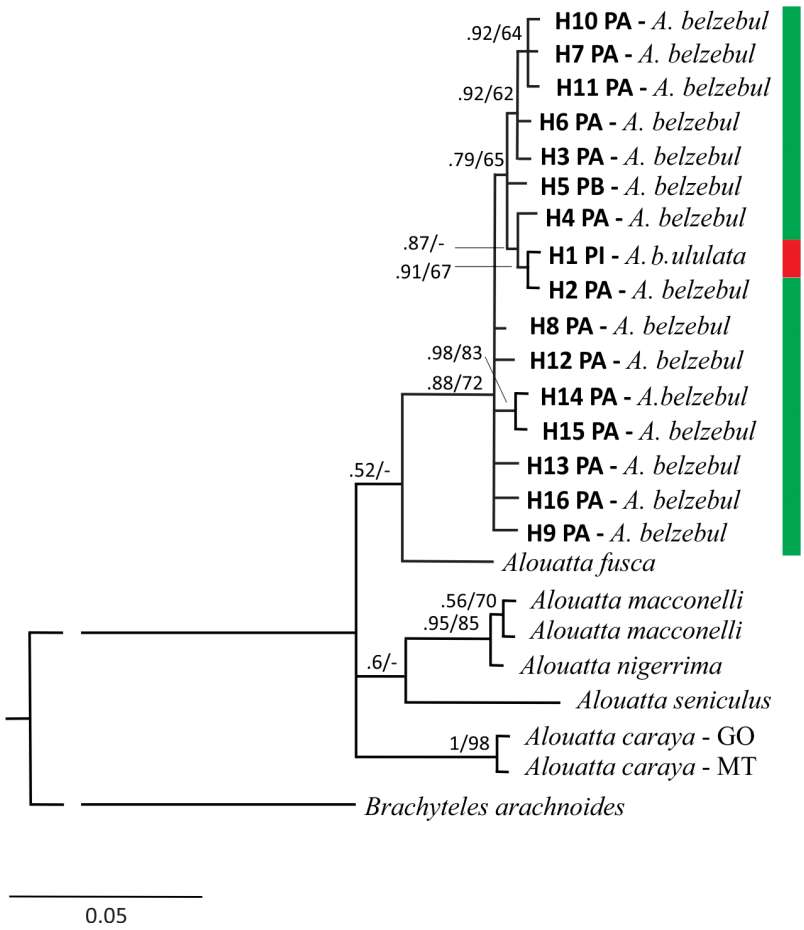

Figure 3. Phylogeny of *Alouatta* based on *Cytochrome b* (Bayesian analysis). Numbers at nodes represent posterior probability values (left) and aLRT estimates (right).

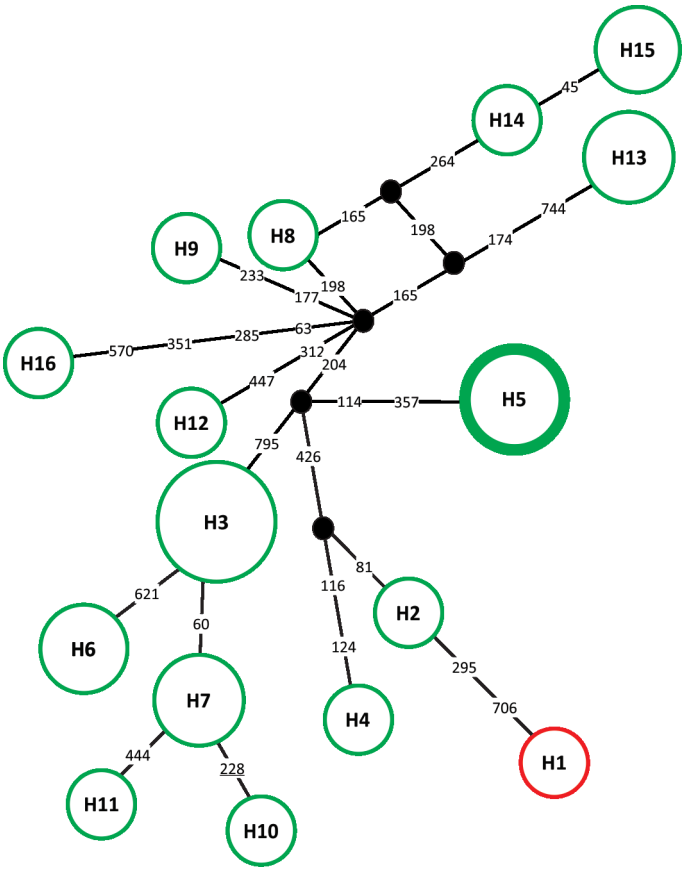

Figure 4. Median-joining network of *Alouatta* from Pará, Paraíba (green circles, areas 1 and 3 of Fig. 1), and Piauí (red circle, area 4 of Fig.1) states. Numbers inside circles indicates haplotypes listed in Table 1. Circle size denote number of individuals sharing a given haplotype. Numbers in connecting branches denote nucleotide substitutions (transition = not underlined; transversions = underlined).

Phylogenetic relationships

Molecular analysis showed the close relationship between *A. seniculus*, *A. macconnelli* and *A. nigerrima*, corroborating previous reports based on *Cytochrome b* gene (Bonvicino *et al.* 2001), and mitochondrial and nuclear genes (Cortés-Ortiz *et al.* 2003). The close affinity between *A. fusca* and *A. belzebul,* herein recovered by BA analysis, was also shown in previous reports based on *g1-globin* pseudogene (Meireles *et al.* 1999), *Cytochrome b* gene (Bonvicino *et al.* 2001) and several mitochondrial and nuclear genes (Cortés-Ortiz *et al.* 2003). This close relationship, however, was not evident by multicolor, cross-species chromosome painting (de Oliveira *et al.* 2002).

The *A. belzebul* clade showed a poor resolution with several polytomies. This was probably due to the predominant composition of *A. belzebul* from Pará state (area 2 and 3), contrary to *A. b. ululata* (area 4, H1), and one specimen from the Atlantic Forest (area 1, H5). Specimens from area 3 from the Rio Tocantins basin are extremely polymorphic (in pelage coloration and MT-CYB) and likely to be from both banks or from river islands (Bonvicino *et al*. 1989). MJ analyses corroborated ML analysis showing lack of structure between *Alouatta belzebul* and *Alouatta b. ululata* haplotypes and indicated that, in this case, *Cytochrome b* analysis did not provide evidence to draw taxonomic conclusions. This analysis was carried out with a single molecular marker, incomplete lineage sorting may result in a different *MT-CYB* tree in relation to species tree.

Morphology

Color pattern of rufous mid dorsum (pattern D4 of Bonvicino *et al.* 1989) is common to areas 2 in Pará and 4 in Ceará state. Two other patterns also with rufous mid dorsum (D3 and D1) are common to areas 4 in Maranhão and 3 in Pará (Tocantins), while three other patterns of black dorsum (C1, C3 and C4) are unique to area 4 in Ceará and Maranhão states (*A. b. ululata*). Since black body may be considered a plesiomorphic character (shared by all areas), we may hypothesize that gene flow took place from west to east supporting the hypothesis of conspecificity. The role of sexual dimorphism found in some specimens from area 4 as a reproductive barrier should be investigated.

Phylogeographic analyses will require a larger number of specimens to show relationships between populations. Special attention should be paid to the basin of the middle/lower course of Rio Tocantins to explain its large howler diversity and to determine whether the remaining populations dispersed from this region.

ACKNOWLEDGEMENTS

Work supported by CNPq grant 307669/2013-0 and FAPERJ grant e26/102.956/2011 to CRB. The authors are grateful to Flavia Casado for karyotyping, Plautino Laroque (CPB/ICMBio) for helping in field work, and Marcelo Weksler for several comments that increased the quality of this manuscript.

REFERENCES

Anisimova, M., and O. Gascuel. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Systematics Biology 55:539-552.

Armada, J. L. A., C. M. L. Barros, M. M. C. Lima, J. Augusto, P. C. Muniz, and H. N. Seuánez. 1987. Chromosome Studies in *Alouatta belzebul*. American Journal of Primatology 13:283-296.

Bonvicino, C. R., A. Langguth, and R. A. Mittermeier. 1989. A study of pelage color and geography distribution in *Alouatta belzebul* (Primates, Cebidae). Revista Nordestina de Biologia 6(2):139-148.

Bonvicino, C. R., B. Lemos, and H. N. Seuánez. 2001. Molecular phylogenetics of howler monkeys (*Alouatta*, Platyrrhini): A comparison with karyotypic data. Chromosoma 110:241-246.

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

Cortés-Ortiz L., E. Bermingham, C. Rico, E. Rodríguez-Luna, I. Sampaio, and M. Ruiz-

Garcıa. 2003. Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Molecular Phylogenetics and Evolution 26:64-81.

Eyre, T. A., F. Ducluzeau, T. P. Sneddon, S. Povey, E. A. Bruford, and M.J. Lush. 2006. The HUGO Gene Nomenclature Database, 2006 updates. Nucleic Acids Research 34:319-321.

Gregorin, R. 2006. Taxonomia e variação geográfica das espécies do gênero *Alouatta* Lacépède (Primates, Atelidae) no Brasil. Revista Brasileira de Zoologia 23(1):64-144.

Groves, C. 2001. Primate taxonomy. Washington DC: Smithsonian Institution Press. 350 p.

Groves, C. P. 2005. Family Atelidae. Pages 148-151 in D.E. Wilson and D.M. Reeder (Eds). Mammal Species of the World. A taxonomic and geographic reference. Baltimore, The Johns Hopkins University Press, 3rd ed.

Guindon S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307-321.

HGNC. 2009. HGNC Database, HUGO Gene Nomenclature Committee (HGNC). EMBL Outstation - Hinxton, European Bioinformatics Institute, Welcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK.

Irwin, D. M., T.D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome b gene of mammals. Journal of Molecular Evolution 32:128-144.

Keane, T. M., C.J. Creevey, M. M. Pentony, T. J. Naughton, and J. O. McLnerney. 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evolutionary Biology 6:29.

Librado, P., and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Lima, M. M. C., and H. N. Seuánez. 1989. Cytogenetic characterization of *Alouatta belzebul* with atypical pelage coloration. Folia Primatologica 52:97-101.

Meireles, C. M., J. Czelusniak, S. F. Ferrari, M. P. C. Schneider, and M. Goodman. 1999. Phylogenetic relationships among brazilian howler monkeys, genus *Alouatta* (Platyrrhini, Atelidae), based on g1-globin pseudogene sequences. Genetics and Molecular Biology 22(3):337-344.

Nascimento, F. F. do, C. R. Bonvicino, F. C. D. da Silva, M. P. C. Schneider, and H. N. Seuánez. 2005. Cytochrome b polymorphisms and population structure of two species of *Alouatta* (Primates: Alouattinae). Cytogenetics and Genome Research (Guest Editor: S Müller, Munich) 108:106-111.

Nascimento, F. F. do, C. R. Bonvicino, and H. N. Seuánez. 2007. Population studies of *Alouatta caraya* (Alouattinae, Primates): inferences on geographic distribution and ecology. American Journal of Primatology 69:1093-1104.

de Oliveira, E. H. C., M. Neusser, W. B. Figueiredo, C. Nagamachi, J. C. Pieczarka, I. J. Sbalqueiro, J. Wienberg, and S. Müller. 2002. The phylogeny of howler monkeys (*Alouatta*, Platyrrhini): Reconstruction by multicolor cross-species chromosome painting. Chromosome Research 10:669-683.

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

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Silva, M. N. F., and J. L. Patton. 1993. Amazonian phylogeography: mtDNA sequences variation in arboreal echimyid rodents (Caviomorpha). Molecular Phylogenetics and Evolution 2:243-255.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA 5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731-2739.

Torres, O. M., and C. Ramírez. 2003 Estudio Citogenético de *Alouatta palliata* (Cebidae). Caldasia 25(1):193-198.

Submetido em 30/06/2014
Aceito em 27/07/2015