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Cytogenetic Characterization of Brown Howler Monkeys, *Alouatta guariba clamitans* (Atelidae, Platyrrhini): Meiotic Confirmation of an $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y_1Y_2$ Sex Chromosome System

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Key Words

Alouatta guariba clamitans · Meiosis · Multiple sex chromosome systems · Sex chromosome pentavalent

Abstract

For brown howler monkeys (*Alouatta guariba clamitans*), diploid chromosome numbers varying from 2n = 45 to 2n = 52, with XX/XY, $X_1X_1X_2X_2/X_1X_2Y$, and $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y_1Y_2$ sex chromosome systems have been described by mitotic studies but still await confirmation by meiotic analyses. We analyzed 3 male individuals sampled in the wild (in the municipality of Santa Maria, RS, Brazil) as well as 1 male and 1 female individual in captivity at the São Braz breeding center. Peripheral blood samples and testicular biopsies were taken. We found different diploid numbers for both sexes in somatic cells, $2n = 45,X_1X_2X_3Y_1Y_2$ in males and $2n = 46,X_1X_1X_2X_2X_3X_3$ in females, with 4 metacentric (9–12), 7 submetacentric (1–6, 8), and 9 acrocentric autosomal chromosome pairs (13–20, 22). X_1 and X_2 were submetacentric chromosomes, while X_3, Y_1, and Y_2 were acrocentric ones. Spermatocyte microspreads were examined for synaptonemal complexes. Pachytene spermatocyte analysis was done to verify the chromosome number and morphologies observed in mitotic karyotypes. Immunodetection was performed using anti-SMC3 and anti-CREST antibodies. The presence of a sex chromosome pentavalent $X_1X_2X_3Y_1Y_2$ in the males was confirmed by C-banding in metaphase I and by immunodetection in prophase I by the clear identification of 5 centromeres. The G-banded karyotype corresponded to that previously described for *A. g. clamitans* in the south of Brazil (Curitiba, Parana State, and Blumenau, Santa Catarina State) and for the Misiones Province, Argentina.

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Alouatta guariba clamitans (formerly *A. fusca clamitans*) is one of the 2 subspecies currently recognized for brown howler monkeys [Groves, 2001; Rylands and Mittermeier, 2009; Cortés-Ortiz et al., 2015]. Its geographic distribution ranges from southeast and south Brazil from the State of Espírito Santo through the State of Rio Grande do Sul and the Province of Misiones in Argentina [Groves, 2001; Cortés-Ortiz et al., 2015]. This subspecies of brown howler monkey shows sexual dichromatism: the pelage coloration of adult males ranges from dark rufous to yellowish rufous dorsally, with darker arms, legs, and tail, whereas adult females are fully dark brown or reddish brown [Gregorin, 2006]. Adult male and female fur color patterns show a latitudinal gradient. Adult males from southern populations have lighter pelage coloration than the ones from northern populations, whereas adult females show an opposite trend in which darker individuals occur at higher latitudes. The inverse trends result in a more striking dichromatism in populations living near the southern border of the specific range.

Along the geographic distribution of this subspecies, different diploid numbers were reported. They varied from 2n = 45 for males and 2n = 46 for females in the south, to 2n = 49 and 2n = 52 for males and 2n = 50 for females in the northern populations [Koiffmann and Saldanha, 1974; de Oliveira et al., 1995, 1998, 2002; Gifalli-Iughetti, 2008; Steinberg, 2011; Cardoso Coimbra, 2015], showing a high degree of intraspecific chromosomal variability. Regarding the sex chromosomes, the presence of XX/XY, $X_1X_1X_2X_2/X_1X_2Y$, and $X_1X_1X_2X_2X_3X_3/_1X_2X_3Y_1Y_2$ systems was described by mitotic studies, but awaits confirmation by meiotic analyses [de Oliveira et al., 1995, 1998, 2000, 2002; Gifalli-Iughetti, 2008; Steinberg, 2011; Cardoso Coimbra, 2015]. In the São Paulo State, Brazil, several karyomorphs with various numbers of biarmed chromosomes were found, differing from one another by pericentric inversions (resulting in different biarmed/acrocentric ratios between the karyomorphs). A similar scenario was observed in rodents, where the presence of metacentrics in a heterozygous state was associated with abnormalities of chromosome pairing which influences the progression of meiosis in mice [Garagna et al., 2014]. Depending on the degree and type of heterozygosity, these detrimental effects (together with errors of segregation) may lead to subfertility or sterility, potentially contributing to the increasing reproductive isolation of chromosomal races and, in time, to speciation. Therefore, the karyological variability observed in *A. g. clamitans* has led some authors to suggest a gradient of chromosomal evolution, with a reduction of chromosome number in a north to south direction. They indicate that these might be populations in different stages of speciation and probably reproductively isolated due to meiotic disturbance [de Oliveira et al., 2000; Gifalli-Iughetti, 2008; Cardoso Coimbra, 2015]. These data are supported by molecular genetic studies that show that there are 2 distinct haplogroups corresponding to the northern and southern populations, with both haplogroups occurring in sympatry within the São Paulo State in Brazil [Harris et al., 2005; de Mello Martins et al., 2010; Bonvicino et al., 2015].

All the previous data highlight the importance of genetic studies in the genus, including both cytogenetic and molecular genetic analyses, as well as the need of more sampling localities, in order to contribute to a more complete characterization of the wide diversity within the species. For this reason, in the present contribution, we provisionally retain the conservative subspecies classification, *A. g. clamitans* [Cortés-Ortiz et al., 2015], until a new taxonomic revision has been done.

We performed chromosome analyses in 4 adult males, both in somatic and germ cells, and in an adult female, somatic cells only, from the Santa Maria region, RS, Brazil to provide a first description of specimens from this region of *the A. g. clamitans* geographic distribution range.

Materials and Methods

Samples

Three males were captured in the wild in the Santa Maria Municipality, Rio Grande do Sul, Brazil (Fig. 1), and 1 male and 1 female were sampled while in captivity at the São Braz breeding center (Santa Maria, RS, Brazil). The animals were photographed to record their pelage coloration, weighed, and their morphometric measurements (body length, thoracic and head circumferences, and tail length) were taken. Peripheral blood samples were obtained with disposable heparinized syringes (Sodic Heparine, Phada Pharma, Buenos Aires, Argentina) for karyotyping, and a testicular biopsy was performed under anesthesia using a combination of ketamine S(+) (9 mg/kg) and midazolam (0.9 mg/kg) with a Telinject (remote injection) system [Chagas et al., 2010]. After the individuals had recovered from the anesthesia, they were released at the same location where they had been captured.

Mitotic Studies

Lymphocyte cultures from peripheral blood samples were performed following standard techniques [Steinberg et al., 2014a]. At least 50 metaphases per animal were analyzed at 1000× to determine the diploid number [Mudry, 1983]. Metaphase spreads were treated by G-Wright banding [Steinberg et al., 2014a]. At least 10 G-banded metaphases were photographed per individual. Photomicrographs were taken using a Leica DMLB microscope equipped with a Leica DFC 340 FX camera. Images were processed, and

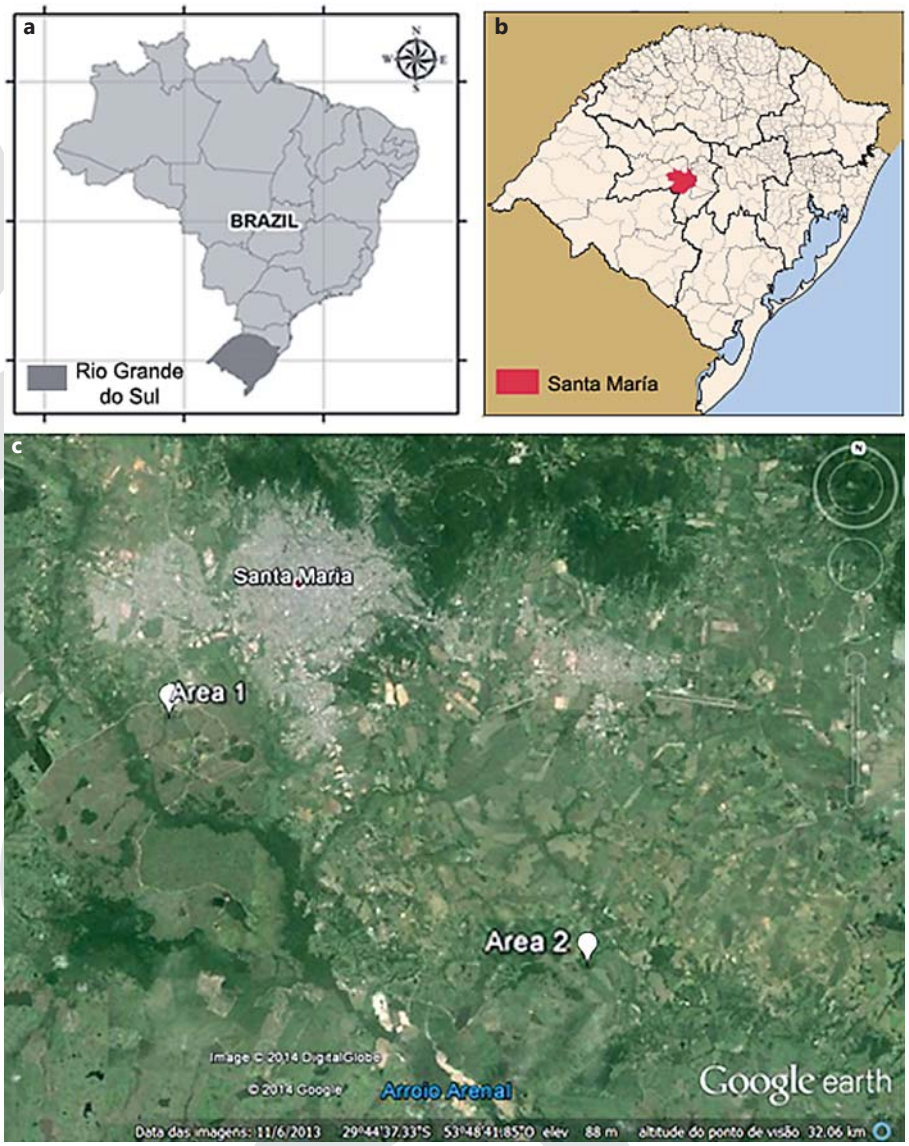

Fig. 1. Sampling sites of the *Alouatta guariba clamitans* specimens. **a** Map of Brazil indicating the Rio Grande do Sul State. **b** Map of Rio Grande do Sul State indicating the position of the Santa Maria Municipality. **c** Areas where samples were collected in the wild.

karyograms were arranged employing the Adobe Photoshop CS5® program using a Wacom Bamboo pen tablet CTL460L.

Meiotic Studies

The testicular tissue obtained from the biopsies was divided into 2 smaller pieces (3 mm^3 each) for 2 different approaches: the spermatocyte microspread technique for synaptonemal complexes (SC) [Garcia-Cruz et al., 2009] and the air-drying technique for the analysis of the pentavalent in metaphase I [Evans et al., 1964] (modified for primates [Steinberg et al., 2014a]). At least 30 diakineses/metaphases I per individual were analyzed. The C-banding technique was applied on air-dried spreads to identify the centromeres in the multivalent and to confirm its structure [Steinberg et al., 2014b]. Immunodetection was performed in spermatocyte microspreads using the primary antibodies rabbit anti-SMC3 (1:50; Merck Millipore, MA, USA) and human CREST serum (1:200; Laboratorios IFI Buenos Aires, Argentina) for the detection of the cohesin axes (SC) and the kinetochores, respectively. The incubation was done overnight at 37°C in a humid chamber. After washing, the following secondary antibodies were applied for 2 h at room temperature: rhodamine-labeled goat anti-rabbit (1:50) and FITC-labeled anti-human gamma globulin IgG (1:50). Slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (0.2 µg/mL, to aid in the identification of the spermatocytes at low magnification) and mounted in glycerol with 1,4-diazobicyclo-(2,2,2)-octane (DABCO) antifade. All the spermatocyte microspreads were examined using a LEICA DM microscope (Leica Microsystems, Wetzlar, Germany) and photographed with a Leica DFC 300 FX digital camera (Cambridge, UK). The separate images were superimposed using Adobe Photoshop CS (Adobe Systems Inc., San Jose, CA, USA).

Fig. 2. Pelage coloration pattern of the *Alouatta guariba clamitans* from the sampling sites in Rio Grande do Sul, Brazil. **a** The females have a red to light brown uniform pelage coloration. **b** The males have an orange to light red uniform pelage coloration.

Table 1. Morphometric measurements of the 4 male and the female *Alouatta guariba clamitans* analyzed

Specimen, sex	Sampling area (Fig. 1)	Body length, cm	Thoracic circumference, cm	Head circumference, cm	Tail length, cm	Weight, kg
1, M	1	41	30	23	54	3.45
2, M	2	52	38	27	57	5.88
3, M	2	60	42	29.5	60	8.38
4, M	São Braz	60	40	30	60	6.20
5, F	São Braz	44	35.5	25	55	3.80

Results

Pelage Coloration Pattern and Morphometric Measurements

The male brown howler monkeys analyzed in this study showed an orange to light red uniform pelage coloration, and the female showed a red to light brown uniform pelage (Fig. 2). The morphometric measurements obtained for all specimens are listed in Table 1.

Mitotic Studies

In 72% of the mitotic metaphases analyzed in the female, 2n = 46,$X_1X_1X_2X_2X_3X_3$ was observed. In the males, 2n = 45,$X_1X_2X_3Y_1Y_2$ was detected in 74 ± 7.28% of the analyzed mitotic metaphases. The fundamental number was 70 for the female and 67 for males due to the presence of a multiple sex determination system in males (see below).

The autosomal complement in both sexes was composed of 4 metacentric (9–12), 7 submetacentric (1–6, 8), and 9 acrocentric pairs (13–20, 22). The G-banding pattern (Fig. 3) allowed us to identify a multiple sex chromosome system ($X_1X_2X_3Y_1Y_2$) in males, originated by Y chromosome translocations. X_1 corresponds to the ancestral X chromosome. Chromosomes X_2 and X_3 in the males correspond to one of the elements of pair 7 and pair 21 in the female, respectively. Y_1 is formed by a portion of the ancestral Y and an autosomal region, and Y_2 corresponds to the other chromosome involved in the translocations (see Discussion).

Meiotic Studies

Analysis of spermatocytes in metaphase I was used to corroborate the presence of the multiple sex chromosome system detected in the mitotic analyses and to confirm the number and morphology of the chromosomal elements. In the air-dried preparations, the 20 autosomal bivalents and the sex chromosome pentavalent observed in at least 10 metaphases I per individual corresponded to 2n = 45,$X_1X_2X_3Y_1Y_2$. When the C-banding technique was applied in metaphase I, the location of the C-positive heterochromatic regions evidenced the presence of 5 centro-

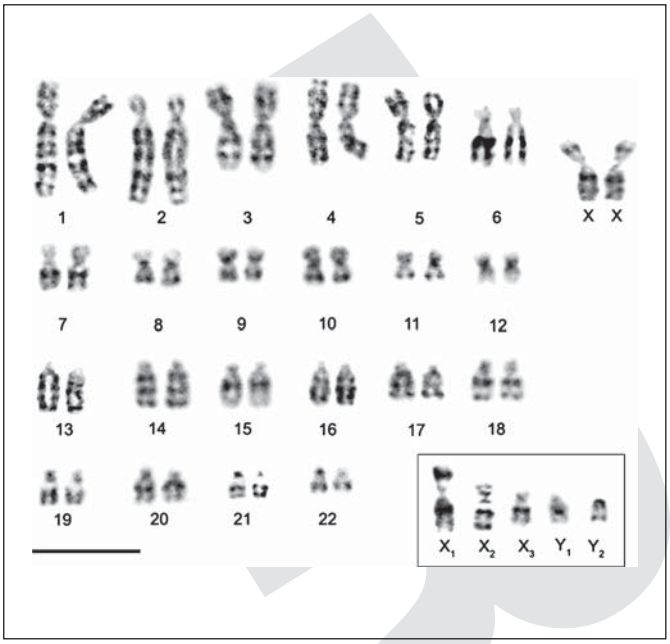

Fig. 3. G-banded karyotype of the female *Alouatta guariba clamitans*. Scale bar, 10 μm. **Inset** Sex chromosomes of a male *A. g. clamitans*.

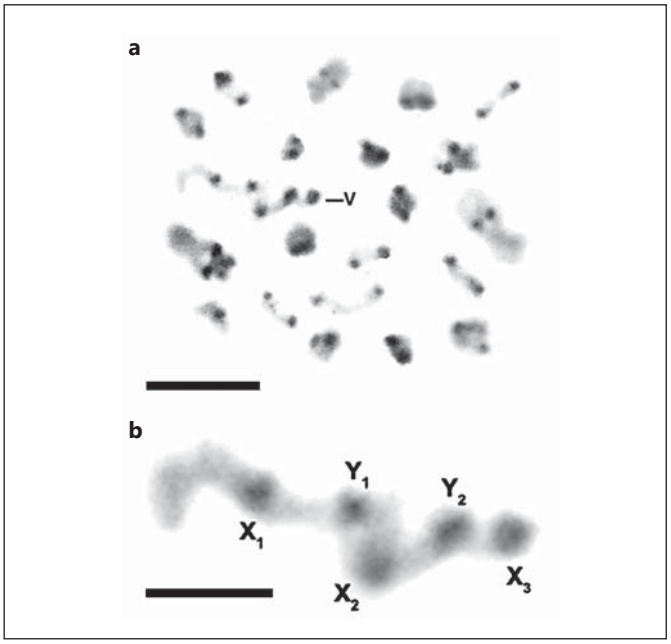

Fig. 4. a C-banded spermatocyte in metaphase I. Meiotic analysis confirmed the presence of a sex chromosome pentavalent ($X_1X_2X_3Y_1Y_2$) in the males, indicated by a line (V). Scale bar, 10 μm. **b** Enlarged pentavalent, with chromosomes indicated next to every centromere. Scale bar, 5 μm.

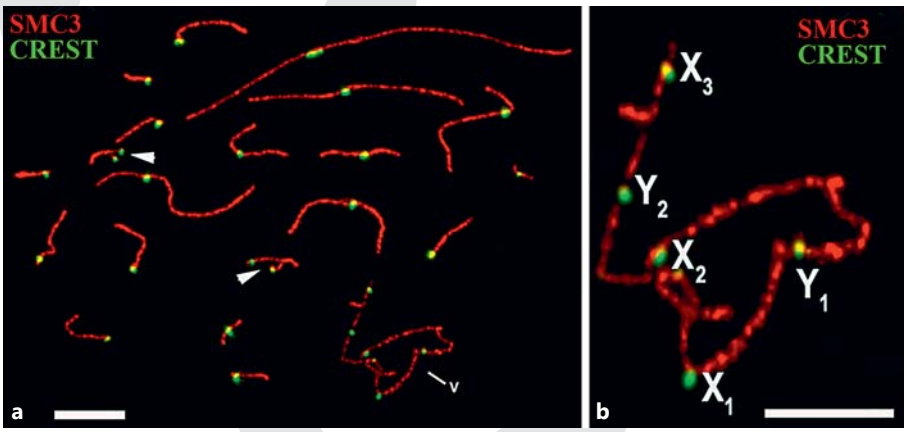

Fig. 5. Immunodetection in a late zygotene spermatocyte. Red, cohesin axes (SMC3). Green, centromeres (CREST). V, pentavalent. Scale bars, 10 μm. **a** The sex chromosome pentavalent is fully synapsed although some of the autosomal bivalents are still completing their synapsis (indicated by arrow heads). **b** Enlarged sex chromosome pentavalent, with chromosomes indicated next to every centromere.

meres, thus confirming the proposed structure of a sex chromosome pentavalent (Fig. 4). We also performed immunodetection in prophase I to study the multivalent behavior during this stage, and also observed the clear presence of 5 centromeres in the multivalent (Fig. 5). Our observations show that the sex chromosome pentavalent in *A. g. clamitans* completes synapsis before the autosomal bivalents do (Fig. 5) and folds on itself during the progress of the pachytene stage (Fig. 6).

Discussion

Sex Chromosome Systems in Alouatta

In primates, the most widespread sex chromosome system is XX/XY [Solari, 1993], observed in approximately 98% of the described species [Mittermeier et al., 2013; Steinberg and Mudry, 2016]. In neotropical primates, infraorder Platyrrhini, multiple sex chromosome systems originated by Y-autosome translocations so far were ob-

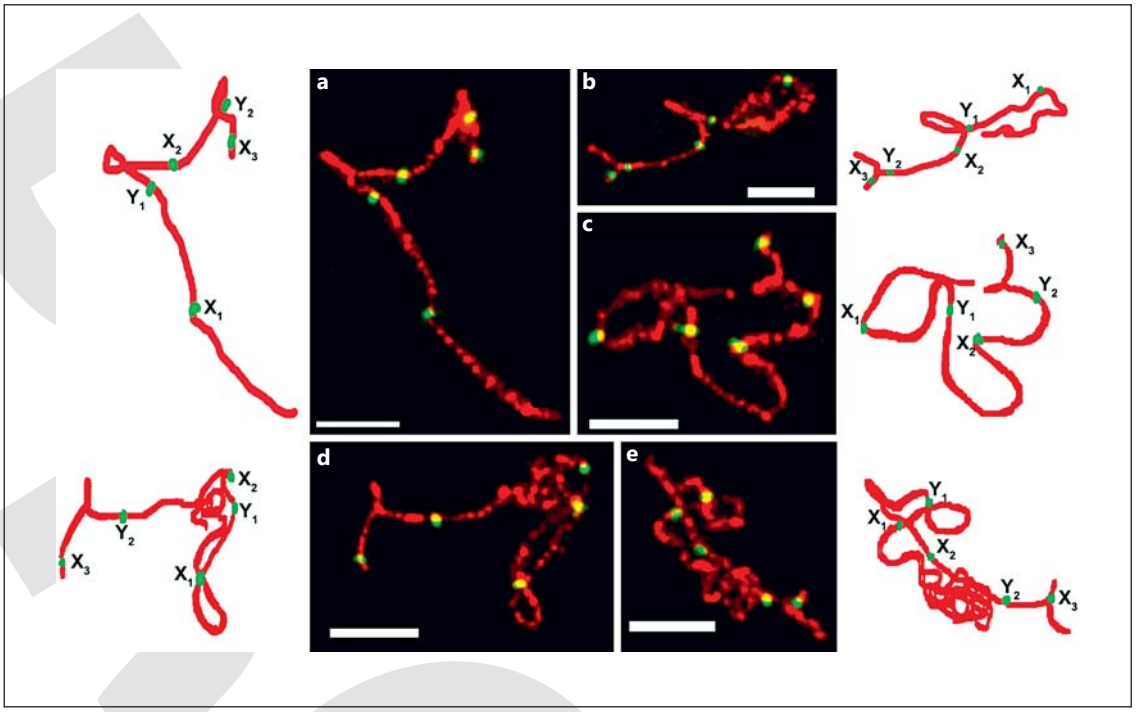

Fig. 6. Immunodetection of the sex chromosome pentavalent in prophase I. Red, cohesin axes (SMC3). Green, centromeres (CREST). Scale bars, 5 μm. Next to each figure, the corresponding schematic representation is shown. **a–c** Early pachytene. **d**, **e** Late pachytene.

served in 8 species, corresponding to different genera: *Alouatta*, *Aotus*, *Callimico*, and *Cacajao* [Steinberg and Mudry, 2016]. Among howler monkeys, several multiple sex chromosome systems were confirmed by meiotic analyses in males: sex chromosome trivalents X_1X_2Y in *A. belzebul* [Armada et al., 1987] and *A. palliata* [Solari and Rahn, 2005] and quadrivalents $X_1X_2Y_1Y_2$ in *A. seniculus stramineus* [Lima and Seuánez, 1991], *A. caraya* [Rahn et al., 1996; Mudry et al., 1998, 2001], and *A. pigra* [Steinberg et al., 2008]. These sex chromosome systems have different chromosomal origins in the Mesoamerican and the South American howler species, having formed independently from different autosomes in each group [Steinberg et al., 2014b]. The chromosome involved in the Y-autosome translocation in the South American species (*A. caraya*, *A. macconnelli*, *A. sara*, and *A. seniculus arctoidea*) is always the same one, is homeologous to regions of human chromosomes 3 and 15 [Consigliere et al., 1996, 1998; Mudry et al., 2001; de Oliveira et al., 2002], and corresponds to chromosome 7 in *A. g. clamitans* females. On the other hand, the chromosome involved in the Y-autosome translocation in the Mesoamerican species (*A. pigra* and *A. palliata*) shares homeology with human chromosome 7 [Steinberg et al., 2014b] and corresponds to chromosome 6 in *A. g. clamitans* females.

In the present contribution, we have for the first time confirmed the presence of a sex chromosome pentavalent in males of *A. g. clamitans* by meiotic analysis. The sex pentavalent in *A. g. clamitans* is fully synapsed even before the autosomal bivalents finish to do so. The X chromosome folds on itself during the progress of the pachytene stage, in accordance with the observations in the quadrivalent of *A. caraya* [Mudry et al., 2001; Garcia-Cruz et al., 2011].

This pentavalent sex chromosome system could have been formed from a quadrivalent ($X_1X_2Y_1Y_2$) system. To form the pentavalent sex chromosome system from this quadrivalent (Fig. 7a), simultaneous breaks in Y_2q_{int} and p_{prox} of an autosome (A, that corresponds to chromosome 21 in females of *A. g. clamitans* and shares homeology with human chromosome 1), followed by the translocation of most of the A chromosome to Y_2q, gave rise to the new Y_2 chromosome. The rest of the autosome (Ap) is lost, and the Y_2 fragment is either lost or could have remained as a microchromosome in some howler species that show this characteristic (e.g., *A. seniculus*

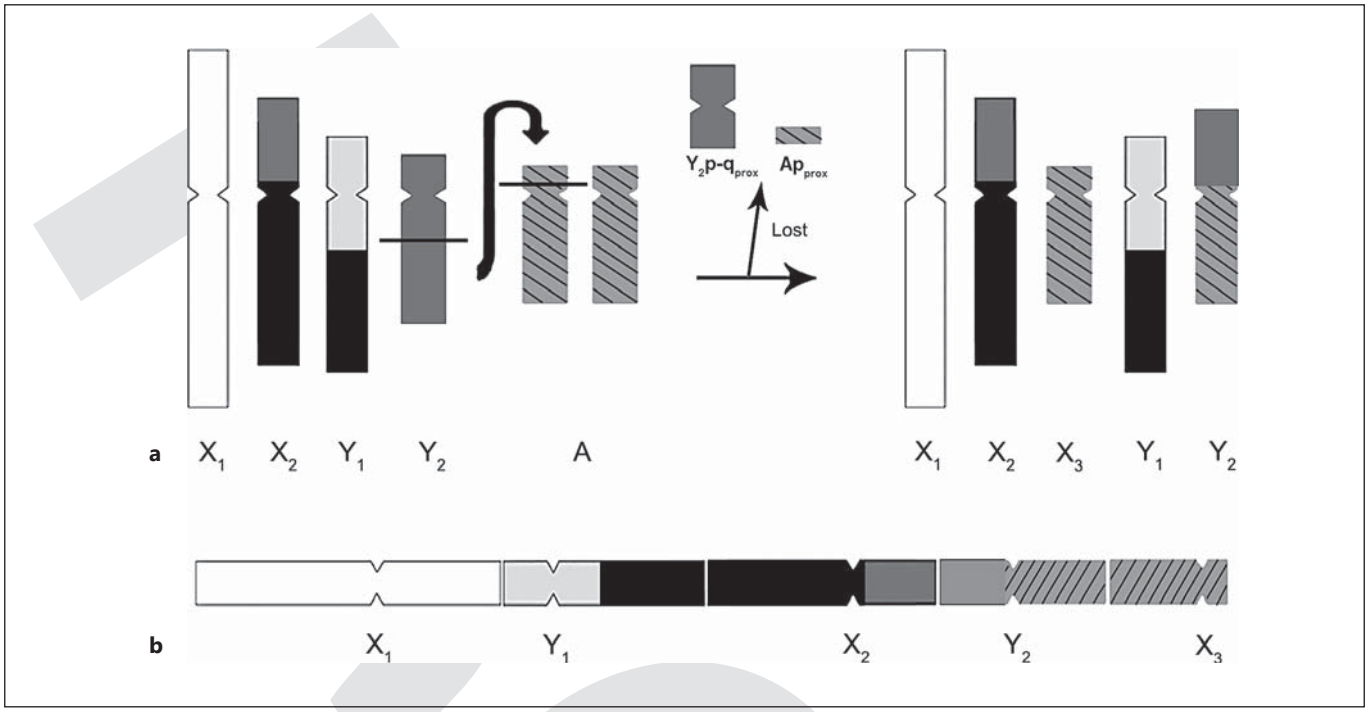

Fig. 7. a Possible origin of the $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y_1Y_2$ sex chromosome system in the genus *Alouatta*. The ancestral X chromosome is shown in white, the ancestral Y chromosome in light gray, the portions with homeology to human chromosome 3 in black, the portions with homeology to human chromosome 15 in dark gray, and the portions with homeology with human chromosome 1 in dark gray with stripes. Simultaneous breaks occur in Y_2q_{int} and p_{prox} of an autosome (A). This autosome corresponds to chromosome 21 in females of *A. g. clamitans*. These breaks are followed by the translocation of most of the A chromosome to Y_2q, giving rise to the new Y_2 chromosome. The rest of the autosome (Ap_{prox}) is lost and the Y_2 fragment either is lost or could remain as a microchromosome in some howler species. The chromosome homologous to the autosome in question (A) becomes X_3. **b** Structure of the pentavalent during prophase and metaphase I.

[Yunis et al., 1976; Lima and Seuánez, 1991; Torres and Leibovici, 2001], *A. sara* [Minezawa et al., 1985], and *A. macconnelli* [Lima et al., 1990]). The chromosome homologous to the autosome A not involved in the translocation became X_3. Considering our results, the order of the configuration observed during prophase and metaphase I (schematized in Fig. 7b), the sex chromosomes' G-banding pattern (Fig. 3), as well as the homeologies with human chromosomes previously described by other authors [de Oliveira et al., 2002; Cardoso Coimbra, 2015], this seems to be the most feasible hypothesis.

Taxonomic Implications

A compilation of all the cytogenetic characterizations performed to this date for *A. g. clamitans* is shown in Table 2. There are 2 distinct karyomorphs in the north and in the south of this subspecies' geographic distribution. Our G-banded karyotype corresponded to the ones described for the south of Brazil (Curitiba, Parana State [de Oliveira et al., 2002] and Blumenau, Santa Catarina State [Gifalli-Iughetti, 2008; Cardoso Coimbra, 2015]), and the Misiones Province, Argentina [Steinberg, 2011]. Both pelage coloration patterns as well as morphometrical measurements were in agreement with the ones previously reported for *A. g. clamitans* from the south portion of its geographic distribution [Rowe, 1996; Groves, 2001; Gregorin, 2006].

These karyomorphs have $2n = 45,X_1X_2X_3Y_1Y_2$ in males and $2n = 46,X_1X_1X_2X_2X_3X_3$ in females, with an autosomal complement of 22 biarmed chromosomes and 18 acrocentric chromosomes. The other karyomorph described for the southern portion of the geographic distribution area also possessed 2n = 45 in males and 2n = 46 in females, but had 22 biarmed chromosomes and 20 acrocentric ones, with a trivalent sex determination system $X_1X_1X_2X_2/X_1X_2Y$ [de Oliveira et al., 1995]. It is difficult to characterize a multiple sex chromosome system by mitotic analysis alone. Analysis in somatic cells does not

Table 2. Mitotic analyses performed up to now in *Alouatta guariba clamitans*. The data are organized according to the geographical origin (from north to south) of the specimens studied in these contributions

Geographical provenance	2n	Autosomes, *n*		Sex chromosome system	Reference
		biarmed	acrocentric		
Espirito Santo, Brazil	♂52	22	28	XY	de Oliveira et al. [1995]
Rio de Janeiro, Brazil	♂49	14	32	X_1X_2Y	de Oliveira et al. [1995, 1998]
Rio de Janeiro, Brazil	♂49	14	32	X_1X_2Y	Gifalli-Iughetti [2008]
Floresta da Cantareira, São Paulo State, Brazil	♀/♂50 ♂49	20 18	28 28	XX/XY X_1X_2Y	Koiffmann and Saldanha, [1974]
Several localities within the São Paulo State, Brazil (Parelheiros, Serra da Cantareira, Horto Forestal, Grajaú, Mairiporã, Pq. Est. Fontes do Ipiranga, Tremembé, Bragança Paulista, Jabaquara, Itapecerica da Serra Embu-Guaçu)	♀50 ♂49	16 18 14 15[b] 17[b] 19	28 26 30 29[b] 27[b] 25	$X_1X_1X_2X_2X_3X_3$ $X_1X_2X_3Y_1Y_2$	Gifalli-Iughetti [2008]; Cardoso Coimbra [2015]
Iguape, São Paulo State, Brazil	♀48[a]	19	23	$X_1X_1X_2X_2X_3X_3$	Cardoso Coimbra [2015]
Curitiba, Paraná State, Brazil	♀46 ♂45	22	18	$X_1X_1X_2X_2X_3X_3$ $X_1X_2X_3Y_1Y_2$	de Oliveira et al. [2002]
Paraná and Santa Catarina, Brazil	♀46 ♂45	22	20	$X_1X_1X_2X_2$ X_1X_2Y	de Oliveira et al. [1995]
Pomerode, Santa Catarina State, Brazil	♀46	21[b]	19[b]	$X_1X_1X_2X_2X_3X_3$	Cardoso Coimbra [2015]
Blumenau, Santa Catarina State, Brazil	♀46	22	18	$X_1X_1X_2X_2X_3X_3$	Gifalli-Iughetti [2008]
Santa María, Rio Grande do Sul State, Brazil	♀46 ♂45	22	18	$X_1X_1X_2X_2X_3X_3$ $X_1X_2X_3Y_1Y_2$	this study
Province of Misiones, Argentina	♂45	22	18	$X_1X_2X_3Y_1Y_2$	Steinberg [2011]

[a] Putative hybrid between 2 different karyomorphs (2n = 45/46 and 2n = 49/50).
[b] These specimens are structural heterozygotes for pericentric inversions.

have enough resolution power, since even with a good G-banding pattern a multiple sex chromosome system could be confused with an XY sex system [Mudry et al., 2001]. Only by meiotic studies, the sex chromosome system can be confirmed unequivocally. Therefore, it is possible that, if meiotic analyses in these $X_1X_1X_2X_2/X_1X_2Y$ specimens were performed, we would find a pentavalent sex chromosome system and an autosomal complement of 22 biarmed and 18 acrocentric chromosomes.

In the north of the *A. g. clamitans* geographic distribution range, fewer cytogenetic characterizations were performed, and only males were studied [de Oliveira et al., 1995, 1998; Gifalli-Iughetti, 2008]. Karyomorphs with 2n = 49,X_1X_2Y were described in several specimens with 14 biarmed and 32 acrocentric chromosomes in Rio de Janeiro (Brazil) [de Oliveira et al., 1995, 1998; Gifalli-Iughetti, 2008]. Additionally, there is a description of a male with 2n = 52,XY, with 22 biarmed and 28 acrocentric chromosomes from the Espirito Santo State [de Oliveira et al., 1995].

In the middle of the previously described northern and southern regions, São Paulo State males have 2n = 49,$X_1X_2X_3Y_1Y_2$ and females have 2n = 50,$X_1X_1X_2X_2X_3X_3$, but there are descriptions of karyomorphs that differ in the biarmed/acrocentric ratio, with many individuals being structural heterozygotes for pericentric inversions [Gifalli-Iughetti, 2008; Cardoso Coimbra, 2015]. These individuals came from 11 different populations (Table 2) in close geographic proximity, separated by an average distance of only 54.7 km (SD = 45.0 km; median = 39.0 km, Google Earth®). Within this region, in the Floresta da Cantareira, an early study in the subspecies described males and females with 2n = 50 and an XX/XY sex chromosome system, along with a male with 2n = 49 and a multiple sex chromosome system X_1X_2Y [Koiffmann and Saldanha, 1974]. The specimens in this region seem to

have high chromosomal variability. As stated in the introduction, molecular studies found that in this Brazilian state the haplotypes of the southern and northern population coexist [Harris et al., 2005; de Mello Martins et al., 2010; Bonvicino et al., 2015], but no specimens with the 2n = 45/46 karyomorph have been described in the São Paulo State so far.

A hybrid between the 2 karyomorphs, 2n = 45/46 and 2n = 49/50, which differ by 2 robertsonian translocations, would require the formation of 2 trivalents, which could have seriously impaired its meiosis. A karyological hybrid was indeed described [Cardoso Coimbra, 2015] in a female specimen captured in Iguape, São Paulo State, Brazil. This specimen had 2n = 48,$X_1X_1X_2X_2X_3X_3$, with a karyotype of 19 biarmed and 23 acrocentric chromosomes.

The karyotypical differences observed between the northern and southern populations of *A. g. clamitans* are identical to those found between the 2 subspecies, *A. g. clamitans* and *A. g. guariba*. Gregorin [2006] suggested that *A. g. clamitans* and *A. g. guariba* should be elevated to full species, based on pelage coloration patterns, since *A. g. clamitans* shows sexual dichromatism while *A. g. guariba* does not. The only karyological description of *A. g. guariba* [de Oliveira et al., 2002] showed 2n = 49/50, $X_1X_2X_3Y_1Y_2/X_1X_1X_2X_2X_3X_3$, with 18 biarmed and 22 acrocentric chromosomes. However, more genetic studies on a larger number of specimens along its geographic distribution are needed for a more thorough taxonomic revision.

Adding to all the previously described complexity, putative hybrids between *A. g. clamitans* and *A. caraya* were reported in the wild, both in the south of Brazil as well as in the Province of Misiones, Argentina [Aguiar et al., 2007; Agostini et al., 2008; Fortes and Bicca-Marques, 2008; Dias et al., 2015]. These specimens were regarded as possible hybrids because of their mixed pattern of pelage coloration, but to this date no genetic analyses were performed to corroborate their hybrid status. Therefore, a more detailed genetic characterization of *A. guariba* is fundamental for the conservation and management of the species.

All the previous data emphasize the need of a systematic revision of the species, linking geographic distribution, pelage coloration pattern, and genetic data in a "Total Evidence" framework [Kluge, 1989] to clarify the complex taxonomy of *A. guariba*. In this context, this report takes a higher relevance because it provides the first meiotic description in *A. g. clamitans* and the first confirmation of a pentavalent sex chromosome system in the Order Primates.

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Statement of Ethics

All research reported in this manuscript followed the guide for care and use of experimental animals as promulgated by the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates (October 2, 2001; https://www.asp.org/society/resolutions/EthicalTreatmentOfNonHumanPrimates.cfm). The authors followed the "Code of best practices for field primatology" as promulgated by the American Society of Primatology (ASP).

Disclosure Statement

The authors declare no conflicts of interest.

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