*Chromosome Research* **10**: 669–683, 2002.
© 2002 *Kluwer Academic Publishers. Printed in the Netherlands*


# The phylogeny of howler monkeys (*Alouatta*, Platyrrhini): Reconstruction by multicolor cross-species chromosome painting

Edivaldo H. C. de Oliveira[1,2,3], Michaela Neusser[1], Wilsea B. Figueiredo[4], Cleusa Nagamachi[2], Julio Cesar Pieczarka[2], Ives J. Sbalqueiro[3], Johannes Wienberg[1,5] & Stefan Müller[1]*
[1]*Department Biologie II, Humangenetik, Ludwig-Maximilians-Universität, Richard-Wagner-Strasse 10, D-80333 Munich, Germany; Tel: 49 (0)89 21806728; Fax: 49 (0)89 21806719; E-mail: S.Mueller@lrz.uni-muenchen.de;* [2]*Laboratório de Citogenética, Depto de Genética, Universidade Federal do Pará, Belém, PA, Brazil;* [3]*Laboratório de Citogenética Animal, Depto de Genética, Universidade Federal do Paraná, Curitiba, PR, Brazil;* [4]*Universidade Federal do Pará, Campus de Santarém, Santarém, PA, Brazil;* [5]*Institute of Human Genetics, TU Munich and GSF Forschungszentrum für Umwelt und Gesundheit, Germany*
**Correspondence*

Received 14 August 2002. Received in revised form and accepted for publication by Pat Heslop-Harrison 27 September 2002

*Key words: Alouatta*, chromosomal phylogeny, comparative molecular cytogenetics, fluorescence *in-situ* hybridization, Primates

## Abstract

We performed multidirectional chromosome painting in a comparative cytogenetic study of the three howler monkey species *Alouatta fusca*, *A. caraya* and *A. seniculus macconnelli* (Atelinae, Platyrrhini) in order to reconstruct phylogenetic relationships within this genus. Comparative genome maps between these species were established by multicolor fluorescence *in-situ* hybridization (FISH) employing human, *Saguinus oedipus* and *Lagothrix lagothricha* chromosome-specific probes. The three species included in this study and previously analyzed howler monkey species were subjected to a phylogenetic analysis on the basis of a data matrix comprised of 98 discrete molecular cytogenetic characters. The results revealed that howler monkeys represent the genus with the most extensive karyotype diversity within Platyrrhini so far analyzed with high levels of intraspecific chromosomal variability. Two different multiple sex chromosome systems were identified. The phylogenetic analysis indicated that *Alouatta* is a monophyletic clade which can be derived from a proposed ancestral Atelinae karyotype of $2n = 62$ chromosomes by a chromosome fusion, a fission, a Y-autosomal translocation and a pericentric inversion. Following these suggestions, the genus *Alouatta* can be divided into two distinct species groups: the first includes *A. caraya* and *A. belzebul*, the second *A. s. macconnelli*, *A. sara*, *A. s. arctoidea* and *A. fusca*.

## Introduction

A number of conflicting hypotheses about evolutionary relationships among neotropical primates (Platyrrhini) have been recently produced, based upon morphological, chromosomal and molecular data (for reviews see Hugot 1998, Schneider 2001). Nevertheless, authors unanimously agree on the monophyly of Atelinae, comprising the large and prehensile-tailed genera *Alouatta*, *Ateles*, *Lagothrix*

and *Brachyteles*. Within Atelinae, howler monkeys (genus *Alouatta*) are placed as the most basal genus by several authors (Rosenberger 1981, Ford 1986, Kay 1990, Schneider *et al.* 1993, Harada *et al.* 1995, Schneider *et al.* 1996, Meireles *et al.* 1999a, Schneider *et al.* 2001).

Members of the genus *Alouatta* show a geographic distribution between southern Mexico and northern Argentina. Until today, the taxonomy and classification of *Alouatta* as well as phylogenetic relationships within this genus remain controversial. Hershkovitz (1949) divided the five species that were accepted at that time into three groups, based on morphological studies of the hyoid bone: (1) *Alouatta seniculus* group (*A. seniculus*, *A. belzebul* and *A. fusca*); (2) *A. palliata* group (*A. palliata*); and (3) *A. caraya* group (*A. caraya*). In further detailed morphological and biogeographical analysis, two further species – *A. pigra* and *A. coibensis* – were recognized and included in the *A. palliata* group (Mittermeier *et al.* 1981, Gregorin 1996). Both authors placed *A. palliata* as the most basal genus, followed by *A. caraya* and the clade formed by *A. fusca* and the Amazonian species, *A. seniculus* and *A. belzebul* (Figure 1a). More recently, comparative sequence analyses produced a different phylogeny. Analyses of seven Brazilian *Alouatta* species based on cytochrome *b* DNA sequence data grouped *A. belzebul* and *A. fusca* in one clade while another well-supported clade contained *A. seniculus* as the most basal offshoot, followed by *A. nigerrima* as a sister lineage of *A. macconnelli*/*A. stramineus* (Bonvicino *et al.* 2001). The same result was obtained by comparative analysis of the $\gamma^1$-globin pseudogene sequence: *A. caraya* was allocated to a basal position, followed by *A. seniculus* as the sister-group of the most derived clade *A. belzebul*/*A. fusca* (Meireles *et al.* 1999b, Figure 1b).

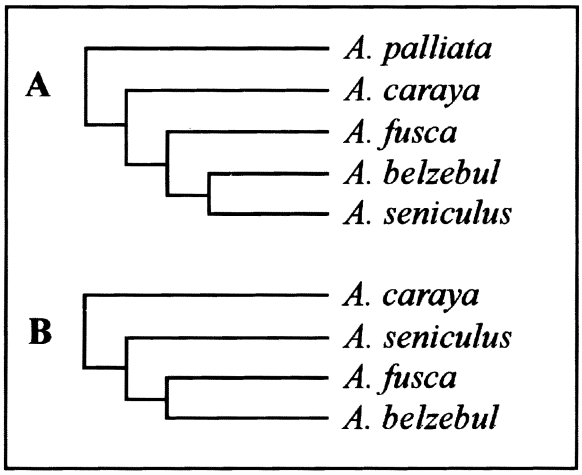



*Figure 1.* Two alternative phylogenetic arrangements of *Alouatta* species, based on: (**A**) morphological and biogeographic studies (Mittermeier *et al.* 1981, Gregorin 1996) and (**B**) DNA data sets (Meireles *et al.* 1999b, Bonvicino *et al.* 2001).

*Alouatta* gained special interest because of the high chromosomal variability and complex sex chromosome systems found within this genus (Ma *et al.* 1975, Armada *et al.* 1987, Lima *et al.* 1990, Lima & Seuánez 1991, Mudry *et al.* 1994, Stanyon *et al.* 1995, de Oliveira 1996, Mudry *et al.* 1998, de Oliveira *et al.* 1998, 2000). In recent years, comparative genome maps between human and several Platyrrhini species have been established by cross-species chromosome painting (Sherlock *et al.* 1996, Richard *et al.* 1996, Consigliére *et al.* 1996, Morescalchi *et al.* 1997, Consigliére *et al.* 1998, Garcia *et al.* 2000, Stanyon *et al.* 2000, 2001, Müller *et al.* 2001, Neusser *et al.* 2001). Among the species analyzed were three *Alouatta* taxa (*A. sara*, *A. seniculus arctoidea* and *A. belzebul*; Consigliére *et al.* 1996, 1998). Comparative molecular cytogenetic analysis confirmed drastic karyotypic differences between *Alouatta* species. In *A. sara* and *A. seniculus arctoidea*, which were traditionally classified as *A. seniculus* subspecies, a conservative estimate of karyotypic differences included two Robertsonian translocations, five tandem translocations and five intra-chromosomal rearrangements (Consigliére *et al.* 1996).

Here we report on multidirectional chromosome painting (Müller *et al.* 1999) in three further *Alouatta* species. In a multicolor FISH approach, we employed human, *Saguinus oedipus* and *Lagothrix lagothricha* chromosome painting probes to establish comparative genome maps. We compared this set of chromosomal homology data with previous publications on other Platyrrhini species based on chromosome painting. A binary character matrix was constructed and used for computer-assisted maximum parsimony analysis. This approach allowed us to propose the hypothetical ancestral Atelinae and *Alouatta* karyotypes. It provided further insights into the

succession of chromosomal rearrangements that occurred during the evolution in this genus, including the analysis of the Y-autosome translocations that gave origin to the multiple sex chromosome systems. Finally, it allowed us to draw new conclusions about phylogenetic relationships among *Alouatta* species.

## Materials and methods

### *Cell samples*

Chromosome preparations for *in-situ* hybridization experiments were obtained from whole-blood cultures of one male and one female *A. caraya* from Paraná (Brazil) and Corrientes (Argentina), respectively, two male *A. fusca clamitans*, kept at Passeio Público de Curitiba (Paraná, Brasil), and one male and one female *A. fusca fusca*, kept at Fundação Zoo-Botânica de Belo Horizonte (Minas Gerais, Brazil). Preparations from one male and one female *A. seniculus macconnelli* captured on the Eastern bank of Jari River and from one male captured on the north bank of the Uatumã River (Pará, Brazil) were kindly provided by Dr. Margarida Lima (Universidade Federal do Pará). One male *Cebus apella* kept at the Jardim Zoológico de Curitiba (Paraná, Brazil) was used as phylogenetic outgroup.

### *Composition and labeling of multicolor chromosome painting probe sets*

Human and New World monkey chromosome-specific painting probes were the same as described before (Stanyon *et al.* 2001, Müller *et al.* 2001). Human, *S. oedipus* and *L. lagothricha* multicolor probe sets H1-H4, S1-S4 and L1 were labeled in Boolean combinations (Neusser *et al.* 2001). Probe labeling was performed by DOP-PCR (Telenius *et al.* 1992) in the presence of Biotin-dUTP, Digoxigenin-dUTP (Roche) and TAMRA-dUTP (Applied Biosystems/PE).

### *G-banding,* in-situ *hybridization and probe detection*

G-banding with Wright stain prior to hybridization experiments was performed as described in Garcia *et al.* (1999). Slides were incubated in $2 \times$ SSC at 60°C for 90–150 s and in Wright stain diluted 1 : 3 in Sorensen buffer (pH 6.8). After microscopic inspection and registration of the metaphase coordinates, slides were incubated in $4 \times$ SSC/0.2% Tween for 60 min, 70% ethanol for 15 min and methanol/acetic acid (3/1; v/v) for 15 min, followed by serial ethanol dehydration (70%, 90%, and 100%). *In-situ* hybridization and probe detection were carried out exactly as described by Neusser *et al.* (2001). Biotinylated DNA probes were detected by Avidin-Cy5 (Vector Laboratories); Digoxigenin-labeled probes were visualized by sheep-anti-Dig-FITC antibodies (Roche). Chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindol, Sigma).

### *Microscopy and image analysis*

Metaphase images were captured with a cooled CCD camera (Photometrics C250/A equipped with a KAF1400 chip, Kodak) coupled to a Zeiss Axiophot microscope. Cell repositioning was performed by a programmable motorized stage (Ludl Electronics). Microscope and camera control, digital image acquisition, subsequent merging of metaphase images and false color assignment was performed by SmartCapture VP software (Digital Scientific).

### *Phylogenetic analysis*

Table 1 summarizes the *Alouatta* species included in this phylogenetic analysis. A data matrix was established (Tables 2 and 3), based on the presence or absence of discrete chromosomal homology characters in the species included in our study, in those *Alouatta* species previously analyzed by Consigliére *et al.* (1996, 1998) and in the phylogenetic outgroups, *L. lagothricha* (Stanyon *et al.* 2001) and *Cebus apella* (Garcia *et al.* 2000). These characters were exclusively comprised of conserved syntenic segments detectable by chromosome painting and associations thereof (Table 2). The character nomenclature for human homologous segments detected by human and *S. oedipus* paint probes followed Neusser *et al.* (2001) and was further refined in this study by the incorporation of *L. lagothricha* probes (see below). These data were

*Table 1.* Taxa of the genus *Alouatta* included in this phylogenetic study.

| Species | 2n | Chromosome nomenclature | Cross-species FISH |
|---|---|---|---|
| *A. belzebul* | 49–50 | Armada *et al.* 1987 | Consigliére *et al.* 1998 |
| *A. seniculus arctoidea* | 40 | Consigliére *et al.* 1996 | Consigliére *et al.* 1996 |
| *A. seniculus sara** | 48 + 0–3 B chr. | Consigliére *et al.* 1996 | Consigliére *et al.* 1996 |
| *A. caraya* | 52 | Mudry *et al.* 1998 | This study |
| *A. seniculus macconnelli* | 46 + 0–3 B chr. | Lima *et al.* 1990 | This study |
| *A. fusca fusca* | 45, 46, 49, 50, 52 | de Oliveira *et al.* 1998 | This study |
| *A. fusca clamitans* | 45, 46, 49, 50, 52 | de Oliveira *et al.* 1998 | This study |

**A. seniculus sara* was recently elevated to species status, *A. sara*

subjected to maximum parsimony analysis (PAUP 4.0 software; Phylogenetic Analysis Using Parsimony; Swofford 1998), using the exhaustive search option. All characters had the same weight, based on the premise that chromosome rearrangements occur by equal chance. The relative stability of nodes was assessed by bootstrap estimates based on 200 iterations. Each bootstrap replicate involved a heuristic parsimony search with 10 random taxon additions and tree-bisection-reconnection (TBR) branch swapping.

## Results

In the present experiments, all human and *S. oedipus* chromosome-specific paints were hybridized to metaphase preparations of *A. caraya*, *A. seniculus macconnelli*, *A. fusca fusca* and *A. fusca clamitans* in sets of 6 or 7 combinatorially labeled probes. Additionally, a 7-color probe set was hybridized, composed of *L. lagothricha* painting probes derived from chromosomes differing from *S. oedipus* homologues by fissions and a translocation (Stanyon *et al.* 2001). The latter probe set provided further subchromosomal definition of human chromosome 1p, 4/15 and 5 homologous segments in howler monkeys. Respective human homologous chromosome segments detected by these probes were defined as $1a_1$ (HSA 1p21-pter) $1a_2$ (HSA 1p12-21), 4a (HSA 4q31.3-qter), 4b (HSA 4q23-q31.2)/$15a_2$ (HSA 15q13-q21.2), 4c (HSA 4pter-q22), 5a (HSA 5q31.3-qter) and 5b (HSA 5pter-q31.2) (Stanyon *et al.* 2001) according to the nomenclature used by Neusser *et al.* (2001). All probes delivered reproducible results except for Y-chromosome probes. A Y chromosome was only detected in *C. apella* by the *S. oedipus* Y-specific probe. Centromeres as well as some other heterochromatic chromosome regions were not hybridized. Identification and verification of segments involved in chromosomal rearrangements among all species included in this study were feasible since reciprocal painting data between human, *L. lagothricha* and *S. oedipus* were previously published (Stanyon *et al.* 2001, Müller *et al.* 2001). Figure 2 illustrates representative FISH experiments with human, *S. oedipus* and *L. lagothricha* multicolor probe sets. Figures 3 and 4 summarize the assignment of human and *S. oedipus* chromosome-specific probes to G-banded karyotypes of the *Alouatta* species investigated. The findings in *C. apella* (data not shown) agreed with previous reports (Garcia *et al.* 2000) and were further confirmed by *S. oedipus* probes.

### *Alouatta caraya*

A male and a female individual was analyzed, with $2N = 52, X_1X_2/Y_1Y_2$ chromosomes in the male and $2N = 52, X_1X_1/X_2X_2$ in the female. Human autosome probes detected 36 homologous segments per haploid set. Six chromosomes were entirely conserved between human and *A. caraya* (HSA 6, 11, 12, 13, 17, 19 and X). *S. oedipus* probes revealed that the genomes of *A. caraya* and *S. oedipus* shared 14 conserved chromosomes (SOE 3–5, 8, 12–15, 17, 18, 20–22 and X). *L. lagothricha* probes allowed the identification of segments homologous to human chromosome 1p, 4 and 5 (see above). Hence, $1a_1$ and 4a were conserved between *A. caraya* and *L. lagothricha* whereas all other homologues were rearranged. $X_2$, $Y_1$ and $Y_2$ chromosomes were defined by chromosome painting as

*Table 2.* Electronic supplementary material: Summary of characters (homologous chromosomes and syntenic association of homologous chromosome segments) that were used to assemble the binary data matrix.

| | | | | | |
|---|---|---|---|---|---|
| 1. | $1a_1/1a_2$ | 34. | $14/15a_1$ | 67. | $1a_2/8a$ |
| 2. | $(5a/7a)_1$ | 35. | $(16a/10a)_2/(16a/10a)_1$ | 68. | $7b/14/15a_1$ |
| 3. | 5b/5a | 36. | 15b/Y | 69. | $(16a/10a)_2$ |
| 4. | 2b/16b | 37. | 2b | 70. | $11/(16a/10a)_2$ |
| 5. | 4a/4b | 38. | 16b | 71. | $(16a/10a)_1$ |
| 6. | 4b/4c | 39. | 3c/15b | 72. | $(16a/10a)_1/4c$ |
| 7. | $(16a/10a)_3$ | 40. | $(11/5b)_1$ | 73. | $10b/(16a/10a)_1/4c$ |
| 8. | 6 | 41. | 5b/7a | 74. | $10b/(16a/10a)_1$ |
| 9. | 8a/18a | 42. | 12/9 | 75. | 19/13 |
| 10. | $15a_1/15a_2$ | 43. | $1a_1/2b$ | 76. | 19/22 |
| 11. | 15b | 44. | 16b/4c | 77. | $22/1a_1$ |
| 12. | 7b | 45. | 22/14 | 78. | $19/22/1a_1$ |
| 13. | 8b | 46. | 2a/20 | 79. | 2a/4b |
| 14. | 12 | 47. | 2a/4b | 80. | $6/1a_2$ |
| 15. | 11 | 48. | 8b/2a | 81. | Y/15b/3c |
| 16. | 13 | 49. | 7a/8b | 82. | $1a_2/5b/7a/5a/7a$ |
| 17. | 9 | 50. | 17/2b | 83. | 9/22 |
| 18. | 3b | 51. | 17/2b/12 | 84. | 17/11 |
| 19. | 3c | 52. | 2b/12 | 85. | 3b/8a |
| 20. | 1b | 53. | 1b/11 | 86. | $15a_1/1b$ |
| 21. | 1c | 54. | $1b/(11/5b)_2$ | 87. | $16b/15a_1/1b$ |
| 22. | 3a/21 | 55. | $(11/5b)_2$ | 88. | $4c/1a_2$ |
| 23. | 10b | 56. | 18/14 | 89. | 14 |
| 24. | 22 | 57. | $3b/15a_1$ | 90. | 2b/4c |
| 25. | X | 58. | $3b/15a_1/16b$ | 91. | $15a_2/7a/5a/7a$ |
| 26. | Y | 59. | $15a_1/16b$ | 92. | $6/15a_1$ |
| 27. | 5b | 60. | 17/10b | 93. | $14/1a_2$ |
| 28. | $(5a/7a)_2$ | 61. | 17/10b/19 | 94. | $6/15a_1/14/1a_2$ |
| 29. | $1a_1$ | 62. | 10b/19 | 95. | 17/8a/18 |
| 30. | $1a_2$ | 63. | 22/20 | 96. | $22/(16a/10a)_1$ |
| 31. | 4a | 64. | 22/20/1c | 97. | 2a/11 |
| 32. | $4b/15a_2$ | 65. | 20/1c | 98. | $(16a/10a)_2/1c/20$ |
| 33. | 4c | 66. | $(11/5b)_3$ | | |

The nomenclature of chromosome segments is following Neusser *et al.* (2001).

translocations of chromosome segments homologous to HSA 3 and HSA 15. Human Y homologous material was not identified but is most probably located on the short arm of *A. caraya* chromosome $Y_2$ which was not hybridized by any probe. The G-banded karyotype of *A. caraya* together with the summary of the chromosome painting results is shown in Figure 3A.

*A. seniculus macconnelli*

Two different karyotypes (Jari and Uatumã karyotypes; see Material and methods) with the same diploid number were found for the *A. seniculus macconnelli* samples. Human probes produced a total of 43 signals in each sample. Human chromosome 12, 17 and X paints hybridized entire chromosomes in both samples, providing evidence that these syntenic groups are conserved. A total of 38 signals were found when applying the *S. oedipus* probes to *A. s. macconnelli* metaphases. *S. oedipus* chromosomes 2–4, 12, 17, 20 and X homologs were conserved in both species. *L. lagothricha* probes showed that in *A. s. macconnelli* human 4a and 5b homologs are conserved as individual chromosomes. Complex hybridization patterns were observed with human chromosome 3 and 15 probes (*S. oedipus* chromosome 9 and 19 probes, respectively), indicating the same Y-autosomal translocation as the origin of the $X_1X_2/Y_1Y_2$

*Table 3.* Electronic supplementary material: Binary character matrix used in the parsimony analysis, comprised of 98 characters (0 = absent, 1 = present, nd = not defined, since a female individual was analyzed).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. apella* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *L. lagothricha* | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. belzebul* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. caraya* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. s.arctoidea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. sara* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. s. macconnelli* U | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. s. macconnelli* J | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. fusca fusca* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. fusca clamitans* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. apella* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| *L. lagothricha* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| *A. belzebul* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | nd | 1 | 1 | 0 | 1 |
| *A. caraya* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| *A. s.arctoidea* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| *A. sara* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| *A. s. macconnelli* U | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| *A. s. macconnelli* J | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| *A. fusca fusca* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| *A. fusca clamitans* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |

| | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. apella* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *L. lagothricha* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. belzebul* | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. caraya* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s.arctoidea* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. sara* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| *A. s. macconnelli* U | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s. macconnelli* J | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fusca fusca* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fusca clamitans* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. apella* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *L. lagothricha* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. belzebul* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. caraya* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s.arctoidea* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *A. sara* | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *A. s. macconnelli* U | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. s. macconnelli* J | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *A. fusca fusca* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fusca clamitans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Table 3.* (Continued).

| | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. apella* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *L. lagothricha* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. belzebul* | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. caraya* | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s.arctoidea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. sara* | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s. macconnelli* U | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. s. macconnelli* J | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *A. fusca fusca* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| *A. fusca clamitans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |

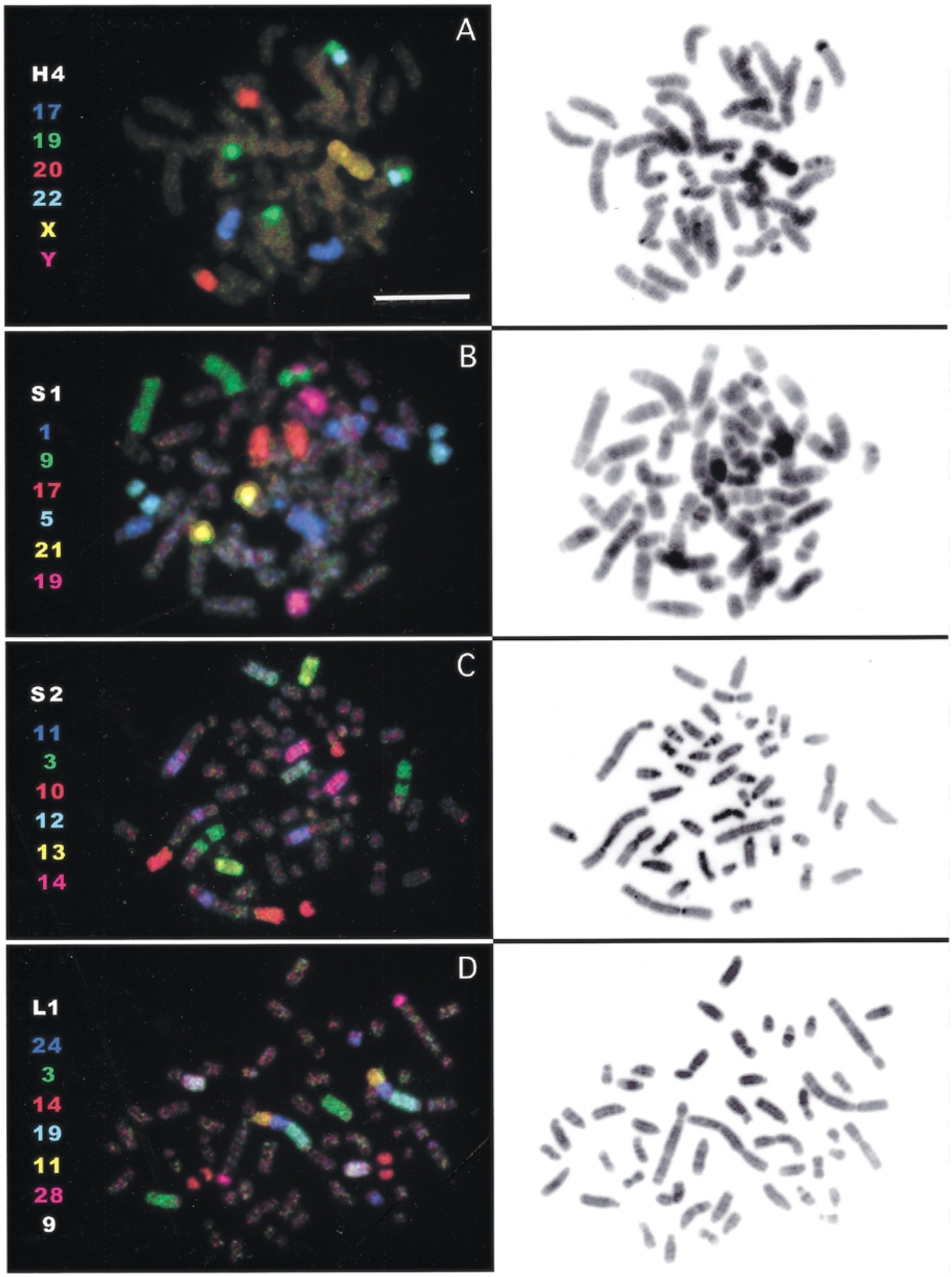
chromosome system as found in *A. caraya*. The different Jari and Uatumã karyotypes are the consequence of two independent Robertsonian translocations or a centric fission–fusion event. The Uatumã river karyotype showed chromosome forms 2p/2q and 16, the Jari karyotype 2q and 2p/16. As described by Lima & Seuánez (1991), both individuals showed zero to three B-chromosomes per cell which were not hybridized by any human or *S. oedipus* probe. The assignment of human and *S. oedipus* homologous chromosome regions is illustrated in Figure 3B.

### *Alouatta fusca*

Different diploid chromosome numbers were found in the *A. fusca* subspecies analyzed. Individuals of *A. fusca clamitans* (AFC) showed a diploid number of 2N = 45 in the two males as described by de Oliveira *et al.* (2000) for specimens from Southern Brazil. In *A. fusca fusca* (AFF), a male with 2N = 49 and a female with 2N = 50 were analyzed. They had similar karyotypes compared to specimens obtained from Southeastern Brazil (Rio de Janeiro state, within the area of distribution of *A. f. clamitans*; de Oliveira *et al.* 1998). Although the two subspecies differ in chromosome numbers the total number of hybridization signals was the same. Their karyotypes differed by Robertsonian rearrangements without changing the number of conserved segments. The chromosomal homology maps of the two *A. fusca* subspecies are illustrated in Figure 4A and B. Forty homologous segments were found by hybridization with human chromosome-specific probes compared with 38 for *S. oedipus* probes. The hybridization with human probes revealed six fully conserved autosomal syntenies (comprised of chromosomes homologous to HSA 9, 11, 12, 13, 19 and 20) in *A. f. fusca*, compared with only three (HSA 13, 19 and 20) in *A. f. clamitans*. The remaining three segments are involved in two Robertsonian type rearrangements resulting in association of HSA 9/12 and HSA 2/11 homologous chromosomal material (Figure 4B). Accordingly, *S. oedipus* probes detected nine entirely conserved autosomal syntenies in *A. f. fusca* (comprised of chromosomes homologous to SOE 4, 5, 12–14, 17, 18, 21 and 22) and seven in *A. f. clamitans* (SOE 4, 5, 13, 17, 18, 21, and 22). *L. lagothricha* probes revealed that homologs to human 4a, $1a_1$, 1b and 1c are conserved on individual chromosomes between the two *A. fusca* subspecies. The short arms of AFC 6, 9, 11, $X_3$ and AFF 7 were not hybridized by any probe.

The translocation of segments homologous to HSA 3 and 15 (SOE 9 and 19) involved in the complex sex chromosome system were similar to that found in *A. caraya*. However, no signal from the human 3c (SOE 19) homolog was observed on

*Figure 2.* Representative multicolor FISH experiments with human, *S. oedipus* and *L. lagothricha* painting probe sets to *Alouatta* metaphases (left image). Beside each metaphase the respective probe composition and false color assignment is given. The right image illustrates the chromosomal counterstain (inverted DAPI). (**A**) Human probe set H4 in *A. seniculus macconnelli*; (**B**) *S. oedipus* probe set S1 in *A. caraya*; (**C**) *S. oedipus* probe set S2 in *A. fusca*, 2n = 49; and (**D**) the *L. lagothricha* probe set L1 in *A. fusca*, 2n = 49. Scale bar = 10 μm.


A
H4
17
19
20
22
X
Y
B
S1
1
9
17
5
21
19
C
S2
11
3
10
12
13
14
D
L1
24
3
14
19
11
28
9

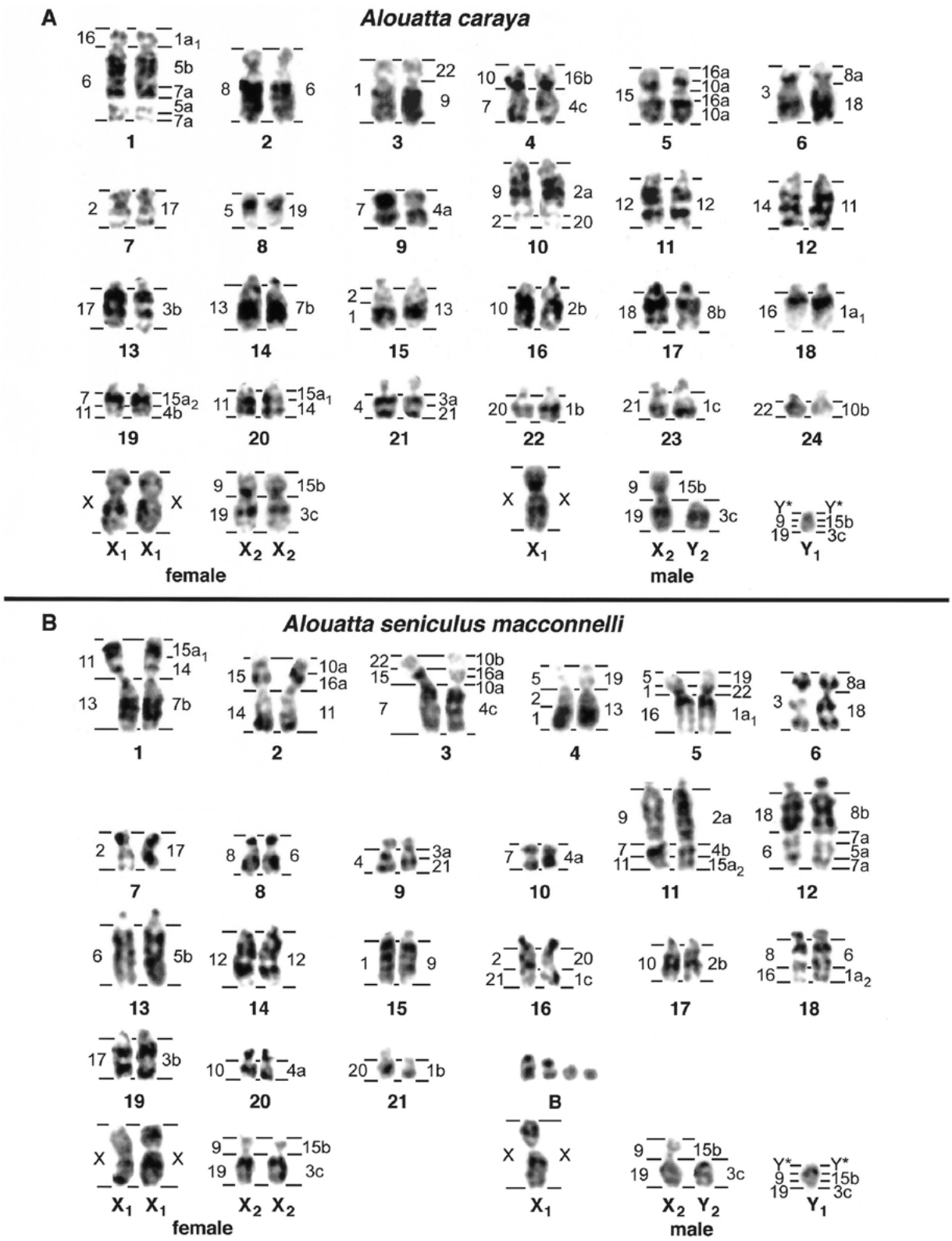



*Figure 3.* G-banded karyotype of (**A**) *Alouatta caraya*; (**B**) *Alouatta seniculus macconnelli*, together with the assignment of human and *S. oedipus* chromosome-specific painting probes. Chromosome numbering (below each chromosome) follows citations given in Table 1. Numbers beside chromosomes indicate human (right) and *S. oedipus* (left) homologous chromosome regions, horizontal bars the borders of homologous regions, respectively. Chromosomal regions outside these borders were not painted by any probe and represent terminal heterochromatin.

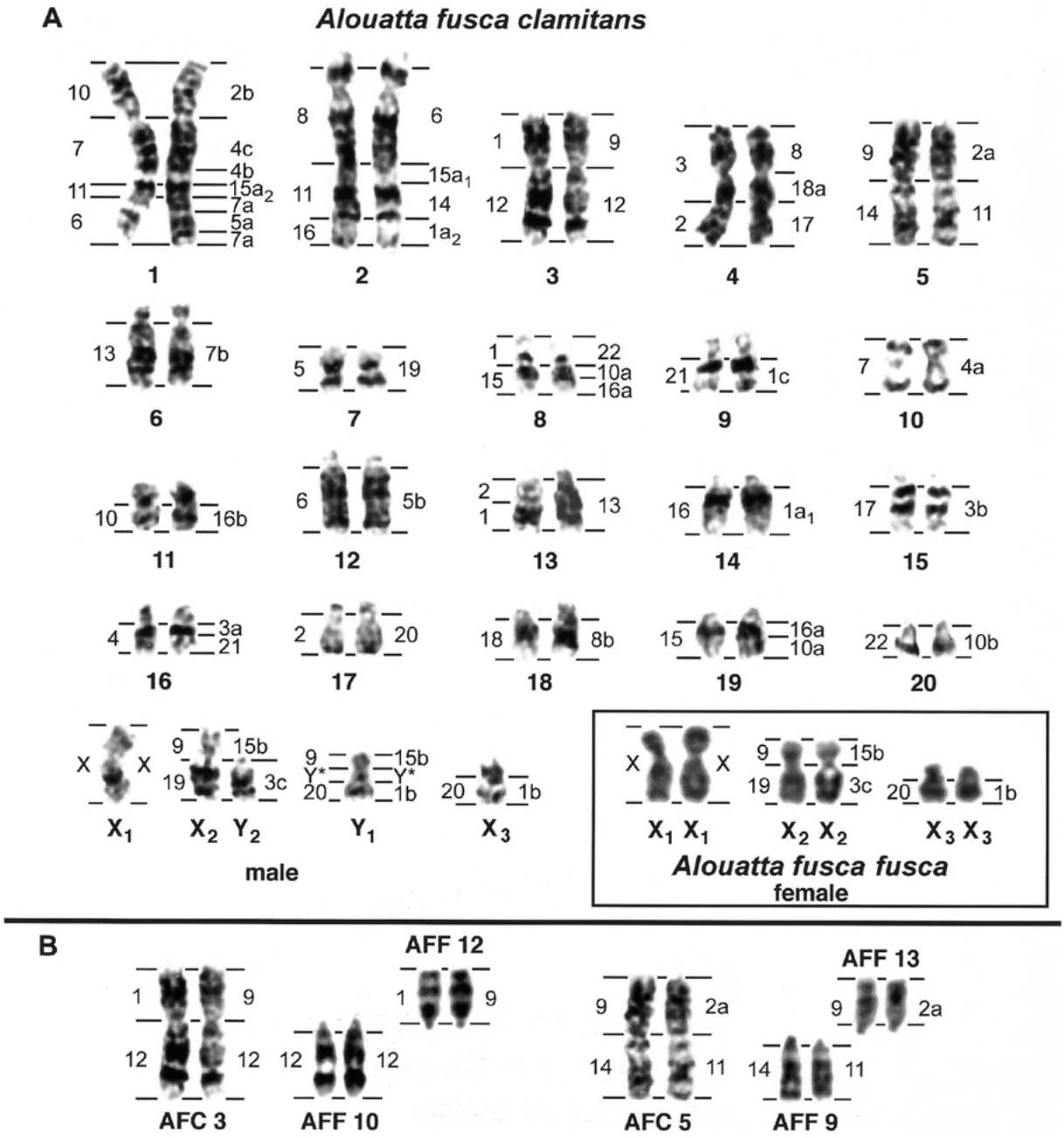



*Figure 4.* (**A**) G-banded karyotype of *Alouatta fusca clamitans* together with the assignment of human (right) and *S. oedipus* (left) homologous chromosome regions (inset: sex chromosomes observed in the female *A. fusca fusca*). (**B**) Summary of those chromosomes that differ between *A. f. clamitans* (AFC, 2n = 45) and *A. f. fusca* (AFF, 2n = 49) by Robertsonian type rearrangements.

$Y_1$ and one more segment homologous to HSA 1/SOE 20 was involved. Thereby the sex chromosome system was further modified to $X_1X_2X_3Y_1Y_2$ in the male (Figure 4A).

*Phylogenetic analysis*

Homologous chromosomes and chromosome segments that were identified by multidirectional

chromosome painting were translated into numerical characters according to Table 2. The resulting binary data matrix with all species included in this study is given in Table 3. Maximum parsimony analysis resulted in only one most parsimonious tree (80 steps long, consistency index = 0.92; retention index = 0.89). High bootstrap values and three synapomorphies support the monophyly of genus *Alouatta*, that is subsequently split in two main lineages. The first lineage is represented by *A. caraya* and *A. belzebul* which share three synapomorphies. The second lineage is supported by two different synapomorphies. This clade is arranged as follows: *A. fusca* {*A. seniculus macconnelli* (*A. sara* and *A. seniculus arctoidea*)}. *A. seniculus macconnelli*, *A. sara* and *A. seniculus arctoidea* share three synapomorphies. Finally, *A. sara* and *A. seniculus arctoidea* exclusively share six derived chromosome forms. This maximum parsimony tree is illustrated in Figure 5.

## Discussion

This comparative study allowed the reconstruction of chromosomal phylogenies between six howler monkey species, three of them yet uncharacterized by molecular cytogenetics. For the first time, a consistent phylogenetic analysis with binary characters extracted from cross-species chromosome painting experiments was performed. The number of informative characters obtained (98 characters) was comparable to that used in previous morphological studies and was sufficiently high to produce a single most parsimonious tree.

### *Chromosomal rearrangements as cladistic markers for phylogenetic linkage*

Evolutionary chromosome rearrangements can be considered 'rare genomic changes' (for review see Rokas & Holland 2000), with very low levels of convergence. However, homoplasies by reversal to ancestral chromosome forms, especially centric fissions, may be found in moderate numbers. In contrast, convergent rearrangements resulting in identical derived traits are extremely rare. In our study, six out of 62 evolutionary chromosomal rearrangements by which extant howler monkeys differ from the inferred Platyrrhini ancestor appeared to be reversals: the putative ancestral howler monkey lost the ancestral Platyrrhini chromosome form 2b/16b, resulting in 2b and 16b. The derived centric fission resulting in chromosome forms $(16a/10a)_1$ and $(16a/10a)_2$ that are shared by *A. seniculus macconnelli*, *A. sara* and *A. fusca* is reversed to 16a/10a/16a/10a in *A. s. arctoidea*. Further, the ancestral Platyrrhini chromosome 8a/18 was fissioned in *A. s. arctoidea* and the common primate association 14/15 was not found in *A. sara* and *A. s. arctoidea* (Consigliére *et al.* 1996). Finally, the fission of 3c/15b in *A. belzebul* (Consigliére *et al.* 1998) and the fusion of 4b/4c in *A. fusca* represent reversals of ancestral *Alouatta* or Atelinae traits, respectively. More relevant than secondary losses of derived traits are convergent gains of derived traits in genus *Alouatta*. There is only one out of 62 rearrangments (the association 9/12 in *A. fusca clamitans* and *A. belzebul*) which can be assumed to be convergent. Another apparently convergent rearrangement (association 5/11), that was observed in *A. belzebul*, *A. sara* and *A. s. arctoidea*, is, however, most likely the result of two evolutionary different rearrangements: a reciprocal translocation shared by *A. sara* and *A. s. arctoidea* and a centric fusion in *A. belzebul*.

### *Reconstruction of chromosomal phylogenies in the genus* Alouatta

When comparing chromosomes observed in *Alouatta* to the inferred ancestral Platyrrhini karyotype (Neusser *et al.* 2001), 8 out of 28 ancestral chromosomes are conserved in *A. seniculus macconnelli*, 11 in *A. fusca clamitans*, 14 in *A. fusca fusca* and 15 in *A. caraya*. In addition, a high level of species-specific (autapomorphic) rearrangements were observed, reflecting the extensive karyological variation within this genus. The number varies between two in *A. caraya* and 10 in *A. s. macconnelli* (Figure 5).

Howler monkeys, however, also share several derived chromosome forms with other Atelinae. Two separate fragments homologous to human chromosome 1a, 5 and 15a and three for chromosome 4 (resulting in chromosomes $14/15a_1$, 5a/7a, 5b, 4a, $4b/15a_2$, 4c, $1a_1$ and $1a_2$, Figure 5) are also present in *Ateles geoffroy* (Morescalchi *et al.* 1997) and *L. lagothricha* (Stanyon *et al.* 2001). Hybridization of the respective *L. lagothricha*

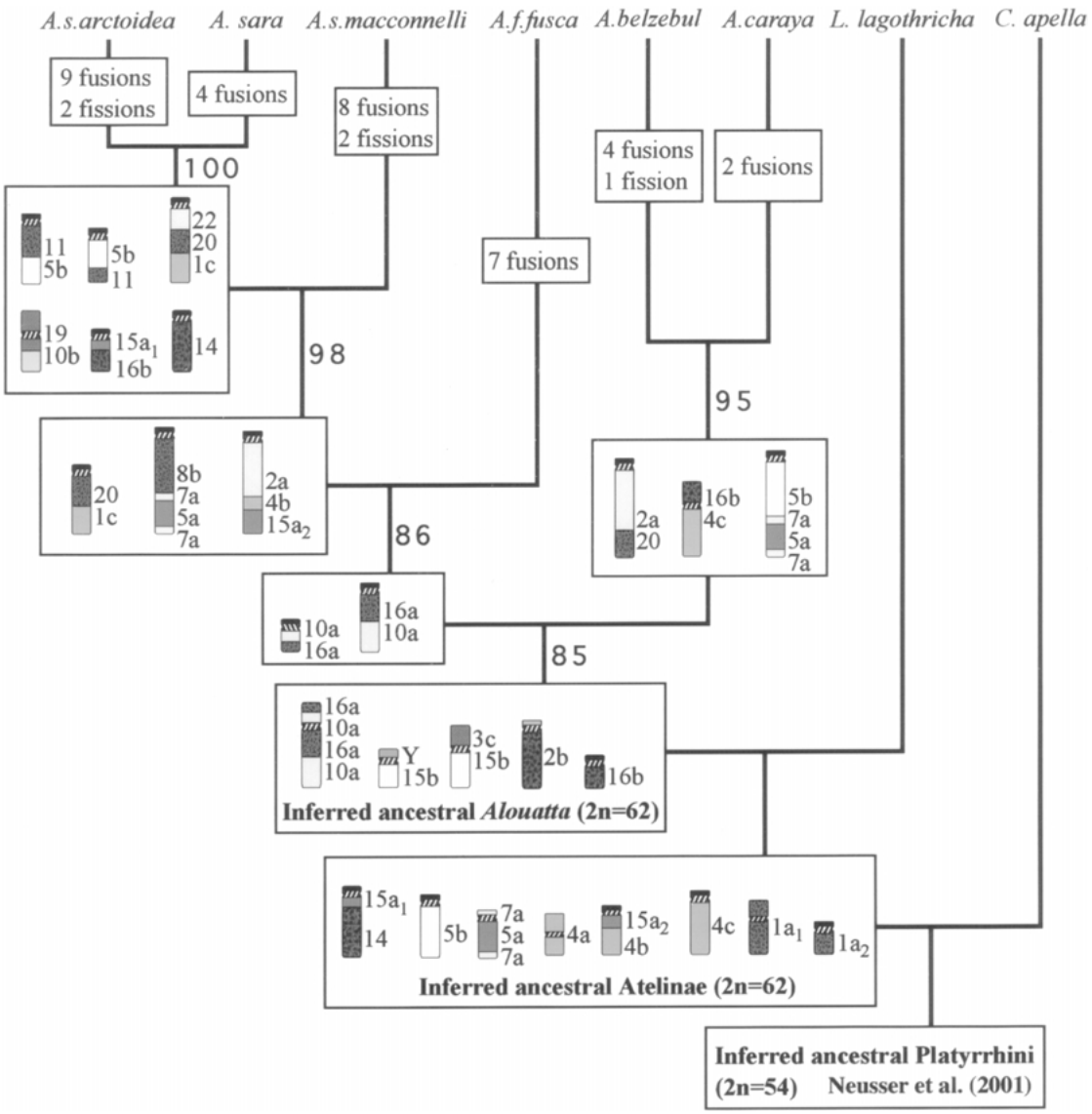



*Figure 5.* The reconstruction of chromosomal changes in genus *Alouatta* lead to one most parsimonious phylogenetic tree. Bold numbers indicate bootstrap values. The chromosome nomenclature followed Neusser *et al.* (2001) and refers to homology with human chromosomes.

probes to *Alouatta* metaphases confirmed that these chromosomes are truly homologous. They were derived by fissions and one translocation and most likely represent the ancestral Atelinae condition with 2N = 62 chromosomes, which may have been conserved in *L. lagothricha*. This observation gives support for the monophyly of Atelinae.

Associations 3c/15b and Y/15b were found in all *Alouatta* species so far analyzed, except for *A. belzebul* (Consigliére *et al*. 1998). Furthermore, inversion of the 10a/16a homolog and the absence of a 2b/16b association are probably shared by all howler monkeys and are unique among Atelinae. The resulting chromosomes 3c/15b, Y/15b, 2b, 16b and 16a/10a/16a/10a are most probably ancestral *Alouatta* chromosomes. In agreement with proposals made by Consigliére *et al*. (1998), the inferred ancestral *Alouatta* karyotype would

have had 2N = 62 chromosomes. Following the nomenclature proposed by Neusser *et al.* (2001), the ancestral *Alouatta* karyotype would be constituted of homologs to human autosomes $1a_1$, $1a_2$, 1b, 1c, 2a, 2b, 3a/21, 3b, 4a, $4b/15a_2$, 4c, 5a/7a, 5b, 6, 7b, 8a/18, 8b, 9, 16a/10a/16a/10a, 10b, 11, 12, 13, $14/15a_1$, 16b, 17, 19, 20, 22 and a multiple sex chromosome system involving $X_1$ (X), $X_2$ (3c/15b), $Y_1$ (Y/15b), $Y_2$ (3c) (see below).

The associations of 4c/16b, 2a/20 and 5b/7a/5a/7a constitute synapomorphies for *A. caraya* and *A. belzebul*. The second lineage within genus *Alouatta* is characterized by a derived fission of the 16a/10a/16a/10a chromosome. The resulting chromosome forms phylogenetically link the *A. seniculus* group with *A. fusca*. In a similar way, derived associations 8b/7a/5a/7a, $2a/4b/15a_2$ and 20/1c are exclusively shared by the *Alouatta seniculus* subspecies analyzed. *A. sara* shares 6 additional synapomorphies with *A. s. arctoidea* that were not observed in *A. s. macconnelli*. These observations place *A. s. macconnelli* most basal within the *A. seniculus* group (Figure 5).

When comparing the most parsimonious chromosomal phylogeny presented in our study (Figure 5) with phylogenies based on recent molecular, mophological and biogeographical studies (Gregorin 1996, Meireles *et al.* 1999b, Bonvicino *et al.* 2001; Figure 1), the most basal position of *A. caraya* and the grouping of *A. fusca* with the Amazonian species, *A. belzebul* and *A. seniculus*, is in consensus. In contrast to our grouping {(*A. seniculus* and *A. fusca*) *A. belzebul*} (Figure 5), Meireles *et al.* (1999b) and Bonvicino *et al.* (2001) classified *A. fusca* with *A. belzebul*, having *A. seniculus* as sister clade. Gregorin (1996), on the other hand, placed *A. belzebul* and *A. seniculus* in one clade with *A. fusca* as sister-group (Figure 1). Since comparative studies of morphology, biogeography, DNA sequence and cytogenetics still provide different phylogenies, further detailed work will be necessary to reach a final conclusion.

### *Multiple sex chromosome systems in* Alouatta *species*

Multiple sex chromosome systems due to Y-autosome translocations have been observed in all the species of *Alouatta* by classical banding techniques: *A. palliata* (Ma *et al.* 1975), *A. belzebul* (Armada *et al.* 1987, Lima & Seuánez 1989) and *A. fusca clamitans* (Koiffmann 1977, de Oliveira *et al.* 1998). Although an XX/XY was reported for *A. caraya* (Mudry *et al.* 1994, Bonvicino *et al.* 2001), synaptonemal complex and banding pattern analysis indicated that the rearrangements in *A. caraya* resulted in a $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ sex system (Rahn *et al.* 1994, Mudry *et al.* 1998). Similar cases were described in *A. seniculus macconnelli*, *A. sara* and *A. seniculus arctoidea* (Yunis *et al.* 1976, Minezawa *et al.* 1985, Lima *et al.* 1990, Stanyon *et al.* 1995). Consigliére *et al.* (1996), using human chromosome-specific probes in *A. s. arctoidea* and *A. sara*, showed that the $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ sex system is conserved. In both species, the autosome involved in this rearrangement corresponds to the association 3c/15b, as found in our study for *A. caraya* and *A. s. macconnelli*. We demonstrated that the sex chromosome systems found in *A. s. macconnelli* and *A. caraya* are identical, whereas in *A. fusca* one further rearrangement involving the human chromosome 1b homologous segment was observed. Thus, the ancestral sex chromosome system for all *Alouatta* species should consist of $X_1X_2/Y_1Y_2$ chromosomes, where $X_1$ would correspond to the homolog to the human X, $X_2$ to 3c/15b, $Y_1$ to Y/15b, and $Y_2$ to 3c. A comprehensive reconstruction of the sequence of Y-autosomal rearrangements in howler monkeys, however, will require further detailed studies with region-specific probes.

### *Taxonomic implications*

The taxonomy of howler monkeys is one of the most controversial within the New World primates, with the number of species ranging from five to ten (Gregorin 1996). On the basis of our findings we hope to elucidate some open questions regarding the taxonomy of *Alouatta* species. Based upon the following considerations, we propose a revision of the present species/subspecies status. Our analysis of *A. s. macconnelli* individuals from the Uatumã and Jari river and previous G-banding data (Lima & Seuánez 1991) revealed distinct karyotypes due to a centric fission–fusion event. It is unclear whether one chromosomal difference already has the potential to act as a profound post-mating isolating mechanism. However, our results on the

*A. fusca* subspecies, *A. f. fusca* and *A. f. clamitans*, showed that they differ by two Robertsonian translocations. A hybrid between the respective cytotypes, 2n = 49/50 and 2n = 45/46, would require the formation of two trivalents, which could have serious effects during meiosis. These findings suggest that the *A. fusca* subspecies investigated may consist of at least two valid species instead of a single one. Our data revealed that *A. s. macconnelli* and *A. s. arctoidea* differ by multiple translocations which may further contribute to reproductive isolation. Moreover, a closer phylogenetic relationship between *A. seniculus arctoidea* and *A. sara* in comparison to *A. seniculus macconnelli* is indicated. Since '*A. seniculus sara*' was recently elevated to species status, our chromosome data indicate that the taxonomic status of *A. s. macconnelli* and *A. s. arctoidea* should be reconsidered as well.

## Acknowledgements

The authors would like to thank Dan Davison and Terrence Demos for improvements in the English of the manuscript; John Bates for comments and discussions and Jorge Rissino (UFPA) for technical support. We are indebted to Margarida Lima for providing chromosome preparations of *A. seniculus macconnelli*, and to Dr. Carlyle Mendes and Dr. Valéria Pereira (Fundação Zoobotânica de Belo Horizonte), Zoológico Municipal de Curitiba (Curitiba-PR-Brazil), and Centro Argentino de Primates (Corrientes, Argentina), for providing blood samples of *A. caraya* and *A. fusca*. The work was supported by Deutsche Forschungsgemeinschaft, DAAD, CAPES, UFPA and UFPR.

## References

Armada JLA, Barroso CML, Lima MMC, Muniz JAP, Seuánez HN (1987) Chromosome studies in *Alouatta belzebul*. *Am J Primatol* **13**: 283–296.

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

Consigliére S, Stanyon R, Koehler U, Agoramoorthy G, Wienberg J (1996) Chromosome painting defines genomic rearrangements between red howler monkey subspecies. *Chromosome Res* **4**: 264–270.

Consigliére S, Stanyon R, Koehler U, Arnold N, Wienberg J (1998) *In-situ* hybridisation (FISH) maps chromsomal homologies between *Alouatta belzebul* (Platyrrhini, Cebidae) and other primates and reveals extensive interchromosomal rearrangements between howler monkey genomes. *Am J Primatol* **46**: 119–133.

Ford SM (1986) Systematics of New World monkeys: In: Swindeler DR, Erwin J, eds. *Comparative Primate Biology*, Volume I: *Systematics, Evolution and Anatomy*. New York: Alan R. Liss, pp 73–135.

Garcia F, Nogués C, Garcia M, Egozcue J, Ponsà M (1999) Characterization of constitutive heterochromatin in *Cebus apella* (Cebidae, Primates) and *Pan troglodytes* (Hominidae, Primates): Comparison to human chromosomes. *Am J Primatol*. **49**(3): 205–221.

Garcia F, Nogués C, Garcia M *et al.* (2000) Chromosomal homologies between human and *Cebus apella* (Primates) revealed by ZOO-FISH. *Mamm Genome* **11**: 399–401.

Gregorin R (1996) *Variação Geográfica e Taxonomia das Espécies Brasileiras do gênero Alouatta Lacépède, 1799 (Primates, Atelidae)*. Masters Dissertation, Universidade de São Paulo, SP, Brazil.

Harada ML, Schneider H, Schneider MPC, Sampaio IM, Czelusniak J, Goodman M (1995) DNA evidence on the phylogenetic systematics of New World monkeys: Support for the sister-grouping of *Cebus* and *Saimiri* from two unlinked nuclear genes. *Mol Phyl Evol* **4**: 331–349.

Hershkovitz P (1949) Mammals of the Northern Colombia. Preliminary report n. 4: Monkeys (primates with descriptions of four new forms. *Folia Primatol* **4**: 381–395.

Hugot JP (1998) Phylogeny of Neotropical monkeys: the interplay of morphological, molecular and parasitological data. *Mol Phylogenet Evol* **9**: 408–413.

Kay RF (1990) The phyletic relationships of extant and fossil Pithecinae (Platyrrhini). J Hum Evol **19**: 175–208.

Koiffmann CP (1977) *Variabilidade cromossômica na família Cebidae (Platyrrhini, Primates)*. Doctoral thesis, Instituto de Biologia, USP, São Paulo.

Lima MMC, Seuánez HN (1989) Cytogenetic characterization of *Alouatta belzebul* with atypical pelage coloration. *Folia Primatol* **52**: 97–101.

Lima MMC, Seuánez HN (1991) Chromosome studies in the red howler monkey, *Alouatta seniculus stramineus* (Platyrrhini, Primates): description of an $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex chromosome system and karyological comparisons with other subspecies. *Cytogenet Cell Genet* **57**: 151–156.

Lima MM, Sampaio MIC, Schneider MPC, Scheffrahn W, Schneider H, Salzano FM (1990) Chromosome and protein variation in red howler monkeys. *Rev Brasil Genet* **13** (04): 789–802.

Ma NSF, Jones TC, Thorington RW, Miller A, Morgan L (1975) Y-autosome translocation in the howler monkeys, *Alouatta palliata*. *J Med Primatol* **4**: 299–307.

Meireles CM, Czelusniak J, Schneider MPC *et al.* (1999a) Molecular phylogeny of Ateline New World monkeys (Platyrrhini, Atelinae) based on $\gamma$-globin pseudogene sequences: evidence that *Brachyteles* is the sister group of *Lagothrix*. *Mol Phyl Evol* **12**:10–30.

Meireles CM, Czelusniak J, Ferrari SF, Schneider MPC, Goodman M (1999b) Phylogenetic relationships among

Brazilian howler monkeys, genus *Alouatta* (Platyrrhini, Atelidae), based on $\gamma^1$-globin pseudogene sequences. *Genet Mol Biol* **22**: 337–344.

Minezawa M, Harada M, Jordan OC, Borda CJV (1985) Cytogenetics of Bolivian endemic red howler monkeys (*Alouatta seniculus sara*): Accessory chromosome and Y-autosome translocation related numerical variations. *Kyoto Univ Overseas Res Rep New World Monkeys* **5**: 7–16.

Mittermeier RA, Coimbra-Filho AF (1981) Systematics: Species and subspecies. In: Coimbra-Filho AF, Mittermeier RA, eds. *Ecology and Behaviour of Neotropical Primates*. Rio de Janeiro, Academia Brasileira de Ciencias.

Morescalchi MA, Schempp W, Wienberg J, Stanyon R (1997) Chromosome painting in the New World monkey, *Ateles geoffroyi*, the black-handed spider monkey. *Chromosome Res* **5**: 527–536.

Mudry M, Ponsa M, Borell A, Egozcue J, Garcia M (1994) Prometaphase chromosomes of the howler monkey (*Alouatta caraya*): G, C, NOR and restriction enzyme (Res) banding. *Am J Primatol* **33**: 121–134.

Mudry MD, Rahn M, Gorostiaga M, Hick A, Merani MS, Solari AJ (1998) Revised karyotype of *Alouatta caraya* (Primates, Platyrrhini) and *A. seniculus macconnelli* on synatonemal complex and banding analysis. *Hereditas* **128** (1): 9–16.

Müller S, Stanyon R, O'Brien PC, Ferguson-Smith MA, Plesker R, Wienberg J (1999) Defining the ancestral karyotype for all primates by multidirectional chromosome painting between tree shrews, lemurs and humans. *Chromosoma* **108**: 393–400.

Müller S, Neusser M, Stanyon R, Wienberg J (2001) Molecular cytogenetic characterization of EBV producing cell line B95-8 (*S. oedipus*, Platyrrhini) by flow cytometry and multicolour cross species chromosome painting. *Chromosome Res* **9**: 689–693.

Neusser M, Wienberg J, Stanyon, R, Müller S (2001) Molecular cytotaxonomy of Platyrrhini – comparative analysis of five species by multicolour reciprocal chromosome painting gives evidence for a classification of *Callimico goeldii* with Callitrichidae. *Cytogenet Cell Genet* **94**: 206–215.

de Oliveira EHC (1996) *Estudos citogenéticos e evolutivos nas espécies brasileiras e argentinas do gênero* Alouatta *(Atelidae, Platyrrhini)*. Masters Thesis. Universidade Federal do Paraná, Curitiba, PR, Brasil.

de Oliveira EHC, Sbalqueiro IJ, Lima MMC, Pissinati A (1998) The karyotype of *Alouatta fusca* from Rio de Janeiro, Brazil: Evidence of a Y-autosome translocation. *Genet Mol Biol* **21** (3): 361–364.

de Oliveira, EHC, Suemitsu E, da Silva AF, Sbalqueiro IJ (2000) Geographical variation of chromosomal number in *Alouatta fusca clamitans* (Primates, Atelidae). *Caryologia* **53**: 163–168.

Rahn MI, Mudry M, Merani MS, Solari AJ (1996) Meiotic behavior of the $X_1X_2Y_1Y_2$ quadrivalent of the primate *Alouatta caraya*.*Chromosome Res* **4**: 350–356.

Richard F, Lombard M, Dutrillaux B (1996) ZOO-FISH suggests a complete homology between human and Capuchin monkey (Platyrrhini) euchromatin. *Genomics* **36**: 417–423.

Rokas A, Holland WH (2000) Rare genomic changes as a tool for phylogenetics. *Trends Ecol Evol* **15**: 454–459.

Rosenberger AL (1981) Systematics: the higher taxa. In: Coimbra-Filho AF, Mittermeier R, eds. *Ecology and Behavior of Neotropical Primates*, Vol. I. Rio de Janeiro: Acad Brasileira de Ciencias, pp 9–27.

Schneider H, Schneider MPC, Sampaio MIC, Harada ML, Stanhope M, Goodman M (1993) Molecular phylogeny of the New World monkeys (Platyrrhini, Primates). *Mol Phylogenet Evol* **2**: 225–242.

Schneider H, Sampaio I, Harada ML *et al.* (1996) Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two unlinked nuclear genes: IRBP intron 1 and ε-globin sequences. *Am J Phys Anthropol* **100**: 153–179.

Schneider H, Canavez FC, Sampaio I, Moreira MA, Tagliaro CH, Seuánez HN (2001) Can molecular data place each neotropical monkey in its own branch? *Chromosoma* **109**: 515–523.

Sherlock JK, Griffin DK, Delhanty JDA, Parrington JM (1996). Homologies between human and Marmosett (*Callitrix jacchus*) chromosomes revealed by comparative chromosome painting. *Genomics* **33**: 214–219.

Stanyon R, Tofanelli S, Morescalchi MA, Agoramoorthy G, Ryder OA, Wienberg J (1995) Cytogenetic analysis shows extensive genomic rearrangements between red howler (*Alouatta seniculus*, Linnaeus) subspecies. *Am J Primatol* **35**: 171–183.

Stanyon R, Consigliére S, Müller S, Morescalchi A, Neusser M, Wienberg J (2000) Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey. *Am J Primatol* **50**: 95–107.

Stanyon R, Consigliére S, Bigoni F, Ferguson-Smith M, O'Brien PC, Wienberg J (2001) Reciprocal chromosome painting between a New World primate, the woolly monkey, and humans. *Chromosome Res* **9**: 97–106.

Swofford D (1998) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Telenius H, Pelmear AHP, Tunnacliffe A *et al.* (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* **4**: 257–263.

Yunis EJ, Torres de Caballero OM, Ramirez C, Ramirez ZE (1976) Chromosomal variation in the primate *Alouatta seniculus seniculus*. *Folia Primatol* **25**: 215–224.