Karyotypic characteristics of hybrid marmosets of the genus Callithrix (Erxeleben, 1777) suggest the participation of three parental species

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RESUMO: (Características cariotípicas de saguis híbridos do gênero Callithrix (Erxeleben, 1777) sugerem participação de três espécies parentais) O gênero Callithrix possui seis espécies distribuídas no bioma Mata Atlântica, desde o nordeste brasileiro até o norte do estado de São Paulo, e regiões do cerrado e caatinga. Híbridos já foram registrados em condições de cativeiro e em zonas de contato entre algumas das espécies do gênero. O objetivo deste trabalho foi analisar citogeneticamente animais provenientes da Ilha D’Água no estado do Rio de Janeiro, considerados híbridos das espécies introduzidas C. jacchus e C. penicillata, usando técnicas de coloração convencional, Ag-NOR e bandeamento C e hibridação com sondas de DNA repetitivo. Os espécimes apresentaram 2n=46, com 5 pares de cromossomos metacêntricos (1, 2, 3, 4 e 5), 9 pares de cromossomos submetacêntricos (6, 7, 8, 9, 10, 11, 12, 13 e 14), um par subtelocêntrico (15) e 7 pares de cromossomos telocêntricos (16, 17, 18, 19, 20, 21 e 22). O sistema cromossômico de determinação sexual é XX/XY, sendo os cromossomos X de morfologia submetacêntrica e o Y de morfologia telocêntrica. O número diploide foi consistente com o padrão descrito para o gênero. A morfologia telocêntrica do cromossomo Y foi semelhante à esperada em C. aurita, a qual era nativa na região. A coloração Ag-NOR marcou o braço curto de dois pares de cromossomos telocêntricos, semelhante ao padrão já descrito para as outras espécies exceto em C. jacchus, que apresenta também

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marcações de NOR no cromossomo Y. O bandeamento C marcou a região centromérica de todos os cromossomos, corroborando a homogeneidade observada em outras espécies do gênero. A marcação dos di e tri-nucleótidos revelaram blocos em apenas alguns cromossomos para as sondas sondas CA\(_{15}\), GAG\(_{10}\) e CGG\(_{10}\), e CAT\(_{10}\) revelou uma distribuição relativamente dispersa em todos os cromossomos. Os caracteres cariotípicos sugerem que os híbridos apresentam mais de duas espécies parentais.

**Palavras-chave:** cromossomos, hibridismo, FISH, primatas, Mata Atlântica.

**ABSTRACT:** The *Callithrix* genus has six species distributed in the Atlantic Forest biome, from northeastern Brazil to the north of São Paulo and parts of the Cerrado and Caatinga. Hybrids have been recorded in captivity and in contact zones between some of the species of the genus. The objective of this study was to cytogenetically analyze animals from Ilha D’Água, in the state of Rio de Janeiro regarded as hybrids of the introduced species *C. jacchus* e *C. penicillata*, using the techniques of conventional staining, Ag-NOR and C-banding and staining with fluorochrome repetitive DNA probes. The specimens had 2n = 46, with 5 pairs of metacentric chromosomes (1, 2, 3, 4, 5), 9 pairs of submetacentric chromosomes (6, 7, 8, 9, 10, 11, 12, 13 and 14), a pair subtelocentric (15) and telocentric chromosome 7 pair (16, 17, 18, 19, 20, 21 and 22). The chromosomal sex determination system is XX / XY; with a submetacentric X chromosome and a telocentric Y chromosome. The diploid number was consistent with the pattern described for the genus. The telocentric Y chromosome morphology is similar to that expected in *C. aurita*, which was the native species of this region. The Ag-NOR color marked the short arm of two pairs of telocentric chromosomes, similar to the pattern already reported for other species except *C. jacchus*, which also features NOR markings on the Y chromosome. The C-banding marked the centromeric region of all chromosomes, confirming the homogeneity observed in other species of the genus. The marking patterns of di- and tri-nucleotides showed only a few blocks chromosomes when CA\(_{15}\), GAG\(_{10}\) and CGG\(_{10}\) probes were used, and CAT\(_{10}\) revealed a relatively sparse distribution in all chromosomes. The karyotypic characters suggest that the hybrids have more than two parental species, and that the native *C. aurita* was involved in the colonization process.

**Keywords:** chromosomes, hybridization, FISH, primates, Atlantic Forest.

**Introduction**

The genus *Callithrix* (Erxeleben, 1777) has six species that occur in eastern Brazil, from the Northeast to the northern state of São Paulo, with the
species *C. penicillata* (Geoffroy Saint-Hilaire, 1812) also occurring in Cerrado (Rylands et al., 2009). The species *C. flaviceps* (Thomas, 1903) and *C. geoffroyi* (Humboldt, 1812) show the contact areas in the state of Espírito Santo and contact zones between *C. flaviceps* and *C. aurita* (Geoffroy Saint-Hilaire 1812) have been found in state of Minas Gerais (Mendes, 1997). Hybridization zones are also known for the species *C. geoffroyi* and *C. penicillata*, probably arising from the destruction of forests, leading to an increase in the distribution area of *C. penicillata* (Passamani et al., 1997).

Hybrids of captive individuals of *C. geoffroyi* and *C. jacchus* (Linnaeus, 1758) were described in 1975, in Rio de Janeiro (Coimbra-Filho & Maia, 1976). The interspecific mating can lead to abnormal conditions such as stillborn pups or the abortion of embryos at various developmental stages (Coimbra-Filho, 1970), but the reproduction of the species of the genus *Callithrix* has successfully generated puppies exhibiting intermediate morphological patterns (Coimbra-Filho & Maia, 1976; Coimbra-Filho, 1993).

The introduction of the species *C. jacchus* and *C. penicillata* in several municipalities in the state of Rio de Janeiro (RJ) has produced hybrid offspring with intermediate morphology between these two species (Ruiz-Miranda et al., 2011). Some of these hybrid populations compete with *Leontopithecus rosalia* populations. The latter species was reintroduced in Atlantic Forest fragments of private farms in São João River, RJ (Ruiz-Miranda et al., 2006; Ruiz-Miranda et al., 2010; Ruiz-Miranda et al., 2011; Kierulff et al., 2012).

Chromosomal changes may represent the beginning of the formation of new taxonomic groups (Eberle, 1975). Species of the genus *Callithrix* are karyotypically homogeneous, with 2n=46 to the species *C. jacchus*, *C. penicillata*, *C. geoffroyi*, *C. aurita*, and *C. kuhli*; the karyotype of *C. flaviceps* has not been described yet (Nagamachi et al., 1997; Nogueira et al., 2011). These species differ in the morphology of the Y chromosome, where *C. kuhli* presents metacentric morphology for the Y chromosome, *C. aurita* has telocentric morphology, *C. penicillata* presents metacentric and submetacentric morphology for the Y chromosome, *C. geoffroyi* has metacentric morphology and *C. jacchus* presents the metacentric morphology, submetacentric and telocentric. *C. jacchus* is only species which shows NOR on the Y chromosome (Nagamachi 1982, Nagamachi e Ferrari, 1986, Ardito et al., 1983, 1987).

Previous cytogenetic studies with individuals, possibly hybrids, developed with captive animals in the state of Rio de Janeiro found the same 2n=46 chromosomes, with the morphology of chromosome Y being similar to the one described for *C. aurita* (Nogueira et al., 2011). The aim of this study was to carry on a karyotypical analysis on individuals considered as putative hybrids by answering the following questions: i - The karyotype of these individuals.
match the chromosome number and karyotypic formula of the putative parental species? ii – Do they have Ag-NOR and C-band patterns that match the expected patterns of the parental species? iii - What is the fluorescence in situ hybridization (FISH) using repetitive DNA probes?

**Material and Methods**

**Study area.** Seven marmosets, four males and three females (C. jacchus and C. penicillata hybrids), were obtained from removal programs authorized under license No. 33965-1, issued by SISBIO-ICMBio-MMA, from Ilhad’Água in Baía de Guanabara - RJ region where these species does not occur naturally. Ilhad’Água belongs to TRANSPETRO and it is located in Baía de Guanabara, the city and state of Rio de Janeiro. The marmosets were considered a problem for the security of workers in this site and their removal was required. An agreement between TRANSPETRO and the Universidade Estadual do Norte Fluminense (UENF) was signed to remove these animals from the locality, being allocated to the Department of Ethology, Breeding and Conservation of Wild Animals (SERCAS) of UENF, for health evaluation and research.

**Chromosomatal analysis.** Colchicine (0.05%) was injected intraperitoneally into each animal for a period of one hour and after this time, they were anesthetized and euthanized. All used protocols and procedures were conducted according to the Brazilian CONCEA legislation (Conselho Nacional de Experimentação Animal). Metaphase material was obtained from bone marrow (Ford & Hamerton, 1956). The chromosomes were stained with Giemsa, images of metaphases were obtained by CellSens Dimensions, measured using Image Pro Plus ® software and classified using arm ratio (RB) in metacentrics (m), submetacentrics (sm), subtelocentrics (st), and telocentrics (t) (Levan et al., 1964). The nucleolar organizer regions (NORs) were identified by impregnation with silver nitrate (Howell & Black, 1980). The patterns of constitutive heterochromatin were obtained using the C-banding technique (Sumner, 1972). The repetitive sequence were identified using CA(15), CAT(10), GAG(10) and CGG(10) probe following Kubat et al. (2008) modified by Cioffi et al (2010). The probes for the microsatellite motifs were synthesized and labeled with the fluorochrome Cy3 on the 5’ end (Sigma, St. Louis, MO, USA). The preparations were counterstained using 4’6-diamidine-2’-phenylindole dihydrochloride (DAPI) and were mounted in Vectashield (Vector, Burlingame, CA, USA). The chromosomes and signals were observed using an Olympus microscope BX53 equipped with a fluorescence lamp and the appropriate filters. Grey-scale
images were captured using a Olympus XM10 cooled digital camera and were processed using Adobe Photoshop CS7 software.

**Results**

The karyotypes of the individuals analyzed presented $2n=46$ chromosomes, with 5 pairs of metacentric chromosomes (1, 2, 3, 4, 5), 9 pairs of submetacentric chromosomes (6, 7, 8, 9, 10, 11, 12, 13 and 14), a pair subtelocentric (15) and 7 telocentric chromosome pairs (16, 17, 18, 19, 20, 21 and 22). The chromosomal sex determination system is XX / XY, with a

![Karyotype Diagram](image)

**Figure 1.** (a) Karyotype of a female individual, in Giemsa staining with $2=46$ and XX sex chromosomes. The inset highlights staining of chromosomes using impregnation of silver nitrate Ag-NOR with four stained chromosomes, (b) karyotype of a male individual with $2n=46$ and XY sex chromosome.
submetacentric X chromosome and a telocentric Y chromosome. (Figure 1a and b). The Ag-NOR stained the short arm of two pairs of telocentric (Figure 1a). The heterochromatin occurred as small C-bands in the centromeric region of all chromosomes (results not shown).

Labeling of the di and tri-nucleotide arrays revealed clusters for probes CA_{15}, GAG_{10} and CGG_{10}. The CA_{15} probe marked 3 pairs of telocentric chromosomes and only one homologue of a pair of submetacentric chromosomes (Figure 2a). The CAT_{10} probe revealed a dispersed distribution on all of the chromosomes.

![Image of fluorescence in situ hybridization (FISH)](image)

**Figure 2.** Fluorescence in situ hybridization (FISH), (a) CA_{15} probe of repetitive DNA marking 3 pairs of telocentric chromosomes. Note the marking in only one homologue of a pair of submetacentric chromosomes, (b) CAT_{10} probe revealed a dispersed distribution on all of the chromosomes, (c) GAG_{10} probe marking one pair of chromosomes telocentrics, (d) CGG_{10} probe marking 3 pairs of telocentric chromosomes. Barra 10µm.
chromosomes (Figure 2b). The GAG\(_{10}\) probe marked one pair of telocentrics (Figure 2c). The CGG\(_{10}\) probe marked 3 pairs of telocentric chromosomes (Figure 2d). However, due to slight differences in these chromosomes, it was not possible to precisely identify them.

**Discussion**

The chromosome number of animals considered as hybrids was \(2n=46\) with 5 pairs of metacentric chromosomes (1, 2, 3, 4, 5), 9 pairs of submetacentric chromosomes (6, 7, 8, 9, 10, 11, 12, 13 and 14), a pair subtelo-centric (15) and 7 telocentric chromosome pairs (16, 17, 18, 19, 20, 21 and 22). The chromosomal sex determination system is XX / XY, with a submetacentric X chromosome and a telocentric Y chromosome. The structure and number of chromosomes is evolutionarily relevant because they limit the group size of linkage affecting the average recombination and rearrangements (Eberle, 1975); they also act as potential indicators of environmental mutagens (Delimitreva et al., 2013). Differences in the number of chromosomes between closely related species are mainly due to Robertsonian translocations, as in *C. emiliae* (Thomas, 1920) \((2n=44)\) and *C. jacchus* \((2n=46)\), where the telocentric pairs number 16 and 22 suffered this translocation, differentiating the karyotype of these two groups of marmosets (Barros et al., 1990).

In this context, it is relevant to indicate that the genus *Callithrix* is a homogeneous clade and the diagnostic character between species karyotypes is the Y chromosome morphology, which occurs in *C. jacchus* in metacentric, submetacentric and telocentric forms (Nagamachi & Ferrari, 1984; Nagamachi et al., 1997) whereas in *C. penicillata* presents in metacentric and submetacentric forms (Nagamachi et al., 1997). In the specimens analyzed, all males showed Y telocentrics, following the morphology found in *C. jacchus* (Nagamachi et al., 1997), which is one of the possible parents described. However, Y telocentric is also found in *C. aurita* (Nagamachi et al., 1992), which is a native species in the studied region (Rylands et al., 2009). Thus, based on Y morphology, we infer that although the coat coloration is intermediate between *C. jacchus* and *C. penicillata*, *C. aurita* might also be one the possible parental species, based on the telocentric morphology of the Y chromosome. Moreover, the Y chromosome lacks nucleolar organizer region (Ag-NOR), a characteristic that would be expected in *C. jacchus* (Nagamachi & Ferrari, 1984). Ag-NORs present in two chromosome pairs was similar to what be expected in *C. geoffroyi* (Nagamachi et al., 1997).

The C-banding pattern restricted to the centromeric regions of
chromosomes, was similar to the general pattern reported for *Callithrix*. This pattern differs from the group the “argentata” marmoset, in the Amazon, which has a large region of heterochromatin. The accumulation of constitutive heterochromatin in marmosets occurred after the separation of the genus *Callithrix* and *Mico*, featuring a recent phenomenon (Canavez et al., 1996). The *C. jacchus* species presents a nearest basal pattern since it has the lowest amount of heterochromatin (Nagamachi et al., 1992). The interspecific homogeneity of distribution of constitutive heterochromatin is also found in other genus of the *Callithrichidae* family and the genus *Leontopithecus*. Thus, the C-banding was uninformative to separate hybrids from parentals.

The FISH technique is potentially informative since it may reveal homologous chromosome regions, characteristics of each species at the DNA level (Sherlock et al., 1996). There are no previous studies of patterns of probes for repetitive DNA in marmosets, and this work is the of its kind for the genus. The chromosomal mapping has shown that DNA repetitive sequences participate in the origin of new sex determination in Orthoptera systems and accumulation of these sequences are related to the increase in genome size (Palacio-Gimenez et al., 2015). These sequences accumulate both A complement of chromosomes as in the supernumerary chromosomes, it is found in both heterochromatin as has euchromatin (Milani e Cabral-de-Melo, 2014). The labeling of only a chromosome in pair can indicate that these chromosomes would come from distinct species and that these had different distribution patterns of CA_{(15)}-specific repetitive DNA probe. In this sense, future studies with species considered as “pure” using specific and other repetitive DNA probes may be a tool to determine hybridization within Callithrichids.

The callitrichids represent a karyotypically conserved group, in which the homologies found in its chromosomes are present in a common ancestor before the divergence between *Cebidae* and *Callithrichidae*. Hybridization with the production of fertile individuals and maintenance of hybrid populations among species of the genus *Callithrix* may be indicative of homogeneity in their karyotypes.

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**Literature Cited**


Callithrix (Callitrichidae: Primates) in the Atlantic forest of the state of Rio de Janeiro, Brazil. Iheringia, 101:156-160.


