Cytogenetic study in natural hybrids of *Callithrix* (Callitrichidae: Primates) in the Atlantic forest of the state of Rio de Janeiro, Brazil.

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ABSTRACT. In the Atlantic forest of Rio de Janeiro, *Callithrix aurita* (É. Geoffroy in Humboldt, 1812) is a native species vulnerable to extinction and *C. jacchus* (Linnaeus, 1758) and *C. penicillata* (É. Geoffroy, 1812) are invasive species. The major threats to the native species are habitat degradation and hybridization, although there are currently no genetic data about natural hybrids available. Previous studies have revealed that species of the *Callithrix* genus are extremely homogeneous in their karyotypes with the exceptions of the morphology and size of the Y chromosome and its nucleolar organizer region (NOR) banding pattern. Three male marmosets captured in the wild in Guapimirim municipality, Rio de Janeiro, Brazil, considered as possible hybrids between *C. aurita* and *C. jacchus* or *C. penicillata* on the basis of pelage pattern, were cytogenetically studied. Metaphase chromosomes were obtained by using short-term lymphocyte cultures and Ag-NOR staining was performed. The hybrids karyotypes were 2n=46, 14 uni- and 30 bi-armed autosomes, a median size submetacentric X and NOR bearing autosomes, being compatible with that observed for the genus. In the three individuals studied, Y chromosomes were similar to those found for *C. aurita*, without NORs. The data obtained suggest the involvement of *C. aurita* in natural hybridization with one of the invasive species. We discuss the possible consequences of this hybridization.

KEYWORDS. Marmosets, Y-chromosome, karyotype, hybridization.

RESUMO. Estudo citogenético em híbridos naturais de *Callithrix* (Callitrichidae: Primatas) na Mata Atlântica do Estado do Rio de Janeiro, Brasil. No estado do Rio de Janeiro, em área de Mata Atlântica, *Callithrix aurita* (É. Geoffroy in Humboldt, 1812) é a espécie nativa considerada vulnerável à extinção e *C. jacchus* (Linnaeus, 1758) e *C. penicillata* (É. Geoffroy, 1812) são espécies invasoras. As principais ameaças à espécie nativa são a degradação do habitat e a hibridação, embora não haja, até o momento, dados genéticos que confirmem a hibridação natural. Estudos anteriores revelaram que as espécies do gênero *Callithrix* são extremamente homogêneas em seus cariótipos, diferindo quanto à morfologia do cromossomo Y e padrão de marcação da região organizadora de nucléolo (NOR). No presente estudo, três saguis machos capturados na natureza no município de Guapimirim, distrito de Teresópolis, RJ, considerados como possíveis híbridos entre *C. aurita* e *C. jacchus* ou *C. penicillata*, com base no padrão de pelagem, foram estudados citogeneticamente. Os cromossomos metafásicos foram obtidos pela técnica de cultura de linfócitos e a coloração Ag-NOR foi realizada. Os cariótipos dos híbridos, 2n=46, continham 14 autossomos com um braço e 30 com dois braços, um cromossomo X de tamanho médio submetacêntrico e marcação NOR nos autossomos, sendo compatíveis com aquele observado para o gênero. Nos três casos, o cromossomo Y foi similar ao observado em *C. aurita*, sem marcação NOR. Os dados obtidos sugerem a participação de *C. aurita* em hibridação natural com as espécies invasoras e suas prováveis consequências são discutidas.

PALAVRAS-CHAVE. Saguis, cromossomo y, cariótipo, híbridicação.

The South American primate genus *Callithrix* Erxleben, 1777 belongs to the family Callithrichidae, which occurs in the Atlantic forest and neighboring *Caatinga* and *Cerrado* biomes of central and northeastern Brazil (Sena *et al.*, 2002). The genus comprises six parapatric taxa that originally occupied areas of ecological transition (Veracini *et al.*, 2002): *Callithix jacchus* (Linnaeus, 1758), *C. penicillata* (É. Geoffroy, 1812), *C. geoffroyi* (Humboldt, 1812), *C. flaviceps* (Thomas, 1903), *C. aurita* (É. Geoffroy in Humboldt, 1812) and *C. kuhli* (Coimbra-Filho, 1985) (Mittermeier *et al.*, 1992; Rylands *et al.*, 1993; Roosmalen *et al.*, 1998).

The common marmoset *C. jacchus* originally inhabited dry thorn scrub (*Caatinga*) and nearby regions of Atlantic forest in northeastern Brazil (Mittermeier *et al.*, 1988; Rylands *et al.*, 1993), south to the state of Bahia. For at least one century it was introduced in other regions of the Atlantic forest, particularly in southeastern Brazil, establishing itself in areas occupied by other *Callithrix* species (Coimbra-Filho, 1990; Brandão & Develley, 1998; Ruiz-Miranda *et al.*, 2000).

Callithrix penicillata occurs in seasonal environments of gallery forests, forest patches and savanna (*Cerrado*) in central Brazil (Hershkovitz, 1977). This species, as in the previous case, is invading and possibly replacing other species in many regions of the east and south, in the states of Minas Gerais, Espírito Santo, São Paulo and Rio de Janeiro (Rylands *et al.*, 1993; Rocha *et al.*, 2004). The other four species occur in the Atlantic forest of eastern and southeastern Brazil: *C. kuhli* and *C. geoffroyi* inhabit lowlands while *C. aurita* and *C. flaviceps* occur in the mountainous rain forests above 400–500m.

Typical of the Atlantic forest of Rio de Janeiro, *C. aurita* also occurs in the east and northeast of São Paulo and in the southern part of Minas Gerais (Coimbra-Filho, 1986; Brandão & Develley, 1998). This species is considered vulnerable, according to the International

Union for Conservation of Nature (IUCN, 2008), and is also listed in the Convention on International Trade in Endangered Species (CITES, 2009) and in the Brazilian Institute for the Environment's list of nationally threatened species (IBAMA, 2003), due to its small population size and ongoing decline. The widespread degradation of the Atlantic forest and the hybridization with invasive species (*C. jacchus* and *C. penicillata*) are the major threats to *C. aurita*, although the extent of the latter remains obscure (Rylands *et al.*, 2009). Coimbra-Filho *et al.* (1993) reported the occurrence of experimental hybridization in captivity among *Callithrix* species which resulted in fertile individuals with several distinct phenotypes. There is also evidence of natural hybridizations at the distributional limits of the *Callithrix* species or due to introduction (in the case of *C. jacchus* and *C. penicillata*) (Rylands *et al.*, 2000). The present study examines three male *Callithrix* captured in the wild in Guapimirim municipality, state of Rio de Janeiro, Brazil, which were considered possible hybrids between *C. aurita* and *C. jacchus* or *C. penicillata* according to indistinct pelage.

Many invasive species take opportunistic advantage of other forms of ecosystem change, such as habitat disturbance, rather than being the direct cause of the decline of native species (Didham *et al*., 2005). For the establishment of conservation management plans to protect the vulnerable species, different causal mechanisms that lead to interspecific breeding must be distinguished. The genetic confirmation of the hybridization is imperative to clarify if there is a directional pattern in the hybridization and also in the survival and reproduction of the hybrids.

From the cytogenetic aspect, the *Callithrix* species represent a homogeneous clade with the exceptions of morphology and size of the Y chromosome, and their Ag-NOR pattern (Nagamachi & Ferrari, 1984; Ardito *et al*., 1987).

Based on chromosomal differences already reported for *Callithrix* species, our objectives were: to analyze the morphology of the Y chromosome of the three possible hybrids, comparing its morphology with that previously observed for *C. aurita*, *C. jacchus* and *C. penicillata*; to identify the paternal species involved in hybridization; and to examine if the Y chromosome of the individuals studied present secondary constriction similar to that reported for *C. jacchus*, which would indicate paternity by a male of this species.

MATERIAL AND METHODS

Three male marmosets considered as possible hybrids between *Callithrix aurita* and *C. jacchus* or *C. penicillata* on the basis of pelage pattern were cytogenetically studied. The individuals were captured in the wild in Guapimirim (22°32'14" S, 42°58'55" W), in the state of Rio de Janeiro at an altitude of 40 m, in the forest near the Rio de Janeiro Primate Centre (CPRJ/INEA), Brazil, where these species are kept in captivity. The capture location is part of the Atlantic forest, a highly threatened ecosystem, classified as a global hotspot of biodiversity due to its exceptional concentration of endemic species and the loss of more than 70% of its primary vegetation (Myers *et al.*, 2000).

Metaphase chromosomes were obtained by using the lymphocyte culture method (Moorhead *et al.*, 1960). Blood (2mL) was collected at CPRJ/INEA by femoral puncture in heparinized syringes. Chromosomes were analyzed by conventional staining with 3% Giemsa in phosphate buffer pH 6.8 and examined at a 100x magnification. Nucleolar organizing region (NOR) characterization was performed following Howell & Black (1980). The karyotypes of the three possible hybrids were compared to those previously described by Nagamachi (1995) and Nagamachi *et al.* (1997) (Fig. 1).

RESULTS

The karyotypes of the three possible hybrid marmosets showed the same pattern observed in the genus *Callithrix*. They presented 2n=46, with 14 uni- and 30 bi-armed autosomal pairs, a median submetacentric X and a diminutive uni-armed Y chromosome, similar to that found in *C. aurita* (Fig. 2).

The Ag-NOR staining marked secondary constrictions located in the short arms of six acrocentric autosomes (Fig. 3). Active NOR was not found on the Y chromosome differing from that observed in *C. jacchus* (Bedard *et al.*, 1978; Nagamachi & Ferrari, 1984; Ardito *et al*., 1987).

DISCUSSION

The five *Callitrix* species, except *C. flaviceps* (not studied), present 2n=46, 14 acrocentric and 30 bi-armed autosomes, a median size submetracentric X chromosome, and differ only in the morphology of the Y chromosome (Fig. 1; Nagamachi *et al.*, 1997), which in the case of *C. jacchus* was variable even between populations. The Y chromosome is subtelocentric in individuals from Bahia and metacentric in those from Pernambuco, probably due to a pericentric inversion (Nagamachi & Ferrari, 1984).

Analysis of the G- and C-banding patterns demonstrated a lack of any chromosomal rearrangements to differentiate their karyotypes (Nagamachi *et al.*, 1997).

Ag-NOR staining marked secondary constrictions of the small arms of the acrocentric chromosomes of all *Callithrix* species studied. *Callithrix jacchus* differs from its congeners by being the only species that shows Ag-NOR staining on the Y chromosome (Bedard *et al.*, 1978; Nagamachi & Ferrari, 1986; Ardito *et al*., 1987).

The karyotype of the three individuals studied

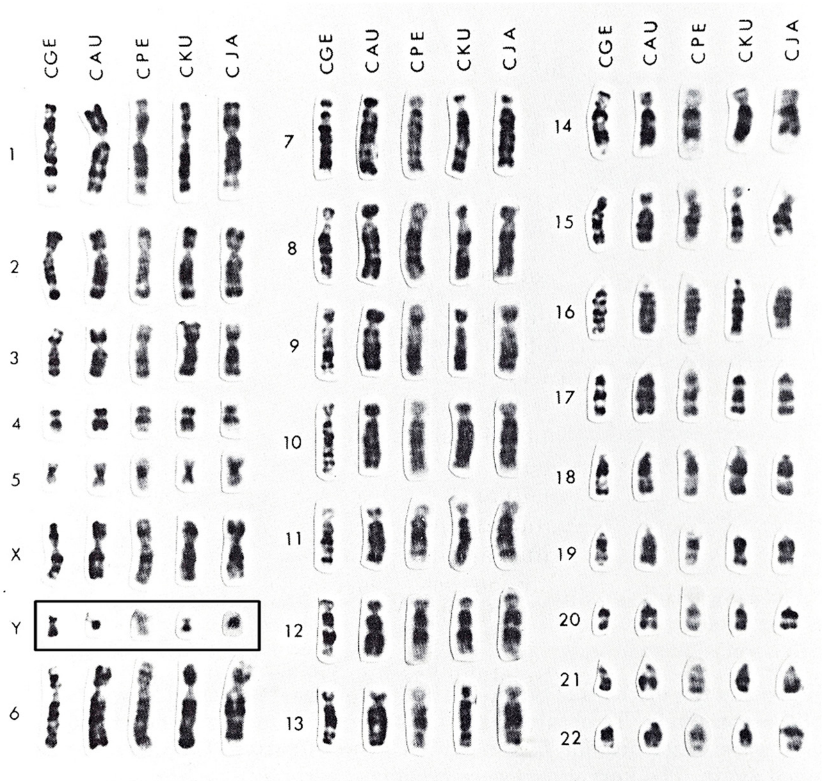

Fig. 1. Comparison of G-banding pattern of the five *Callithrix* species according to NAGAMACHI *et al.* (1997): CGE, *C. geoffroyi*; CAU, *C. aurita*; CPE, *C. penicillata*; CKU, *C. kuhlii*; CJA, *C. jacchus*. The black box emphasizes the morphology of the Y chromosomes of the five species.

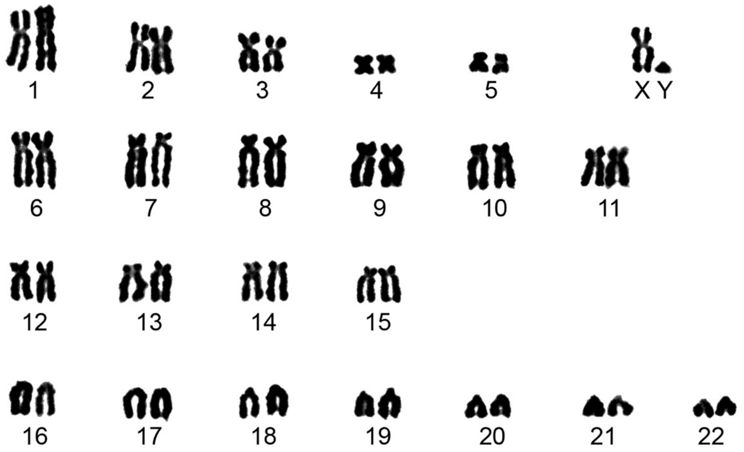

Fig. 2. Karyotypic pattern found in all *Callithrix* hybrids with 2n=46, XY and the diminutive Y chromosome similar to *C. aurita* as described by NAGAMACHI *et al.* (1997).

was compatible with that described for the genus. The Y chromosome similar to that of *C. aurita* and the absence of NOR in this chromosome suggest that the paternal species of the three possible hybrids is *C. aurita*, and that one of the invaders, *C. jacchus* or *C. penicillata*, is the maternal species.

Cytogenetic and molecular analyses conducted so far indicate that the *Callithrix* genus constitute a homogeneous clade with little genetic divergence among the species, which suggests that the speciation in this group is recent (SCHNEIDER, 2000) and that they can still be in a process of speciation (VERACINI *et al.*, 2002).

One piece of evidence of the close phylogenetic relation of the taxa is the fact that all species hybridize at the distributional limits of the various forms (*C. penicillata* x *C. geoffroyi*, *C. penicillata* x *C.kuhlii*, *C. geoffroyi* x *C. flaviceps*, *C. aurita* x *C. flaviceps*) or due to the introduction of one (or both) forms (*C. jacchus* and *C. penicillata*) (COIMBRA-FILHO *et al.*, 1993). In the last case, the occurrence of those species together with

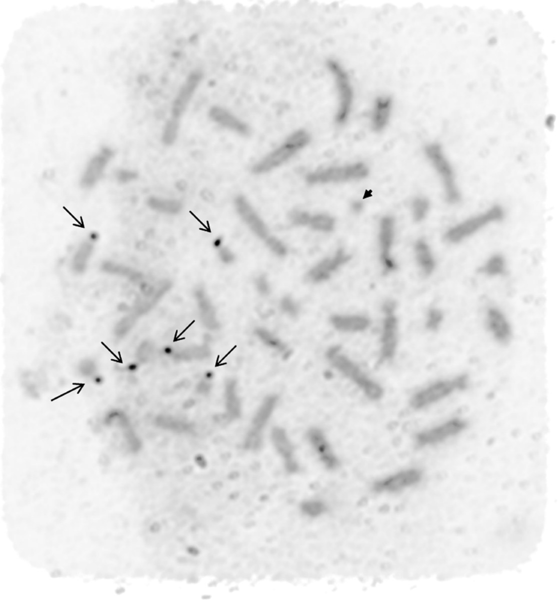

Fig. 3. Ag-NOR stained metaphase of *Callithrix* sp. All the hybrids have similar NOR banding pattern. The arrows indicate six NOR-bands in the short arms of acrocentric chromosomes. The head of the arrow indicates the diminutive Y chromosome without NOR.

C. aurita allied with its population decline can result in interspecific breeding and generation of natural hybrids.

Since the only karyotypic difference among the species is represented by the Y chromosome morphology, it does not interfere with synapse during the meiosis, allowing for the production of normal gametes. The great genetic similarity contributes to the viability and fertility of the hybrid descendents observed in captivity (Coimbra-Filho, 1978); however, its fitness in the wild in terms of survival and reproductive success has yet to be determined.

Although there are few studies on the breeding and social behavior of *C. aurita*, polygyny was observed as a mating system for one group studied in São Paulo, southeastern Brazil (Coutinho & Corrêa, 1995). Due to the population decline, hybridization can be viewed in the case of the threatened species as an alternative strategy to carry on reproduction. Otherwise, by "hybrid vigor", the hybrids can represent an additional competitor to the vulnerable species, possibly superior to the invasive species, *C. jacchus* and *C. penicillata*, which have already established themselves in the new territory.

Biological invasion is now considered to be one of the "big five" environmental issues of public concern (Sala *et al.*, 2000), since a significant emphasis has been placed on invasive species as one of the leading causes of species decline and loss of biodiversity (Wilcove *et al.*, 1998; Vitousek *et al.*, 1996). The data presented here represents the first attempt to confirm the involvement of *C. aurita* in interspecific breeding, although further genetic analyses are necessary to identify the other species involved and the magnitude of the involvement.

When management strategies to control biological invasion are being debated, including invasive species eradication (Zavaleta *et al.*, 2001), genetic analysis can help understand the type and the extension of interactions between native and invasive species to direct conservation action in order to accomplish effective restoration goals.

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