

# Chiroptera Neotropical



Dr. Adriano Lúcio Peracchi working at the American Museum of Natural History, New York, 2010 Photo by Marcelo R. Nogueira.

## CONTRIBUTIONS IN HONOR OF ADRIANO LÚCIO PERACCHI

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## Karyotypic composition of bats from the Brazilian nuclear power plant, state of Rio de Janeiro

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### Abstract

Chromosomes have been a useful tool to address systematical issues of Neotropical bats and can be applied to evaluate certain degrees of radiation exposure, but some basic cytogenetical data is needed in this case. Nothing is known about cytogenetics of bats from the zone of influence of nuclear power plants around the world. In this study we present the karyotypic composition of the first bat sample collected in the surroundings of the only Brazilian nuclear complex, which is located in a very important remnant of Atlantic Forest in southwestern Rio de Janeiro. The following diploid numbers and fundamental numbers of autosomal arms were found: *Artibeus lituratus*; 31, 56, *Artibeus obscurus*; 31, 56, *Carollia perspicillata*; 21, 36, *Platyrrhinus lineatus*; 30, 56, *Sturmira lilium*; 30, 56, *Eptesicus brasiliensis*; 50, 48. Silver stained nucleolar organizer regions (Ag-NORs) are located in short arm telomers of submetacentric or subtelocentric autosomes in all cases, excluding *C. perspicillata*, which showed Ag-NORs in the X chromosome. Constitutive heterochromatin in all species except *E. brasiliensis* is mostly pericentromeric, but telomeric and interstitial bands are also found, as well as completely heterochromatic sex chromosomes. New C-banding patterns were detected for the two species of *Artibeus* improving the known karyotypical variability in these species. This data is a first step to a better understanding about the bat fauna of this very distinctive place.

**Key words:** Ag-NORs, Atlantic Forest, C-bands, Phyllostomidae, Vespertilionidae.

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### Introduction

Despite bats play a key role in Neotropical rainforest mammal communities, supplanting all other mammalian orders in number of species by locality, many questions about its taxonomy, karyology, and phylogeny still need to be answered (Varella-Garcia et al. 1989; Emmons 1997).

Chromosomes have been considered very useful tool in chiropteran systematic because they are not affected by adaptation to different feeding niches as cranial and general gross anatomies (Baker 1970). The systematical usage of karyological data needs, first of all, basic knowledge about chromosomal variability, which means analyzing the karyotypes of the highest number of species from the most diverse collection sites as possible. In Brazil, approximately 30% of species of bats do not have the diploid and fundamental number known and the intraspecific karyological variability of the other 70% is poorly understood (Moratelli and Morielle-Versute 2007). A better knowledge about Brazilian bats' karyotypes can improve our understanding about the systematic of Neotropical bats as whole.

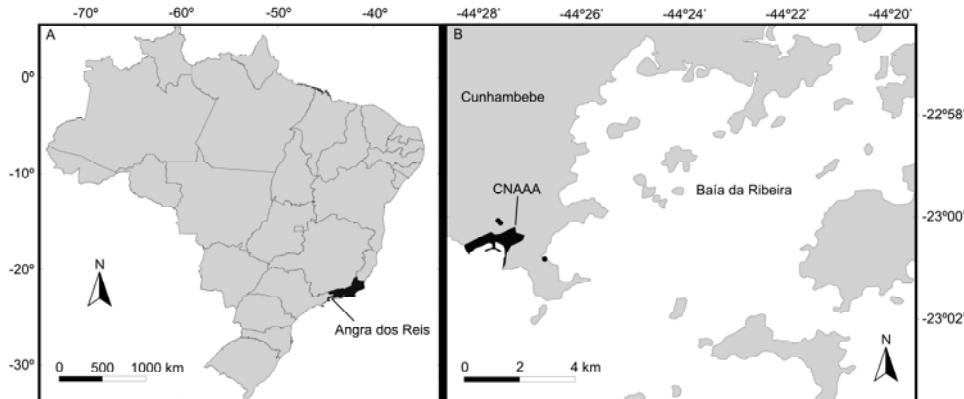
Few studies with small mammals have been conducted at the zone of influence of nuclear

power plants around the world. A remarkable example is the extensive survey made by Robert J. Baker and his team in the surroundings of Chernobyl power plant after the disaster in 1986. They did not find genetic, chromosomal, or morphological abnormalities in animals from this area (Baker et al. 1996, 1997; Rodgers et al. 2001). On the other hand, morphological anomalies were found in small mammals collected close to a power plant in central Italy that suffered several minor accidents (Amarena et al. 1994). It is noteworthy these studies were conducted only after some kind of malfunctioning in power plants and no special attention was previously given to these fauna. Although no chromosomal damage was observed in small mammals inhabiting the surroundings of nucleapower plants after disaster, no study have been developed to monitor small mammal fauna that inhabits these environments. Nevertheless, long term laboratory experiments revealed that chromosomal aberrations can be found on these animals when they are exposed to radiation (Cooper and Hsu 1971; Jacquet et al. 2001).

In the beginning of 1970's the Brazilian government started the licensing process and the search for a suitable place to build the country's first nuclear complex (Rosa et al. 1991). Although

this initiative had raised much criticism from distinct society segments, the government

continued its project, and chose Itaorna Beach in Cunhambebe, Municipality of Angra dos Reis,



**Figure 1** – Map of the study area. A) Location of the Municipality of Angra dos Reis in Rio de Janeiro State (black area), Brazil; B) Bat collection sites (black circles) around CNAAA in the district of Cunhambebe, Angra dos Reis.

State of Rio de Janeiro, to install this complex. Currently, this place harbors the only Brazilian nuclear complex, named Central Nuclear Almirante Álvaro Alberto (CNAAA), which is composed by two operational power plants; a third one must be finished up to 2015 (ELETRONUCLEAR 2009).

Concerning CNAAA, many studies have been conducted to investigate its influence over marine fauna (e.g., Manso and Absalão 1988; Andreato et al. 2002), but nothing similar exists for the small mammals inhabiting the area. More attention should be paid to these organisms because CNAAA is included in one of the largest continuous remnants of Atlantic Forest from Rio de Janeiro, which is in turn included in Serra do Mar Corridor, an extremely biodiversity-rich area in the Atlantic Forest (Aguilar et al. 2005; SOS Mata Atlântica and INPE 2008). Among mammals, bats can be a good option to access environmental conditions around the CNAAA, because they have long life spans and exploit several trophic resources (Fenton et al. 1992).

During 2002, a small mammal survey was performed in the surroundings of CNAAA for the very first time. It was part of a larger preliminary faunal inventory that preceded the environmental impact assessment for the construction of the third power plant of the nuclear complex. Herein, we present the results of cytogenetic analyses of bat specimens collected in that occasion. Our aim is to provide the karyotypic composition of these animals, as a first approach in the study of the small mammals from this peculiar place. The methods applied here were not specifically designed to investigate chromosomal anomalies nor did we expect to find them. However, our data can be very useful in further studies. Additionally, our results improve the understanding of the

karyotypical variability of Brazilian and, in a larger scale, of Neotropical bats.

#### Material and Methods

We analyzed six species of bats, collected in May and September of 2002, in Atlantic Forest around CNAAA (Fig. 1), district of Cunhambebe, municipality of Angra dos Reis, State of Rio de Janeiro, Brazil. This area is classified as coastal tropical rainforest *sensu* Hueck (1972). Voucher specimens, including fluid preserved individuals and skulls, are deposited at Museu Nacional, Rio de Janeiro, Brazil. Scientific names follow Simmons (2005). The Appendix contains museum numbers of all specimens analyzed and the coordinates of collection sites.

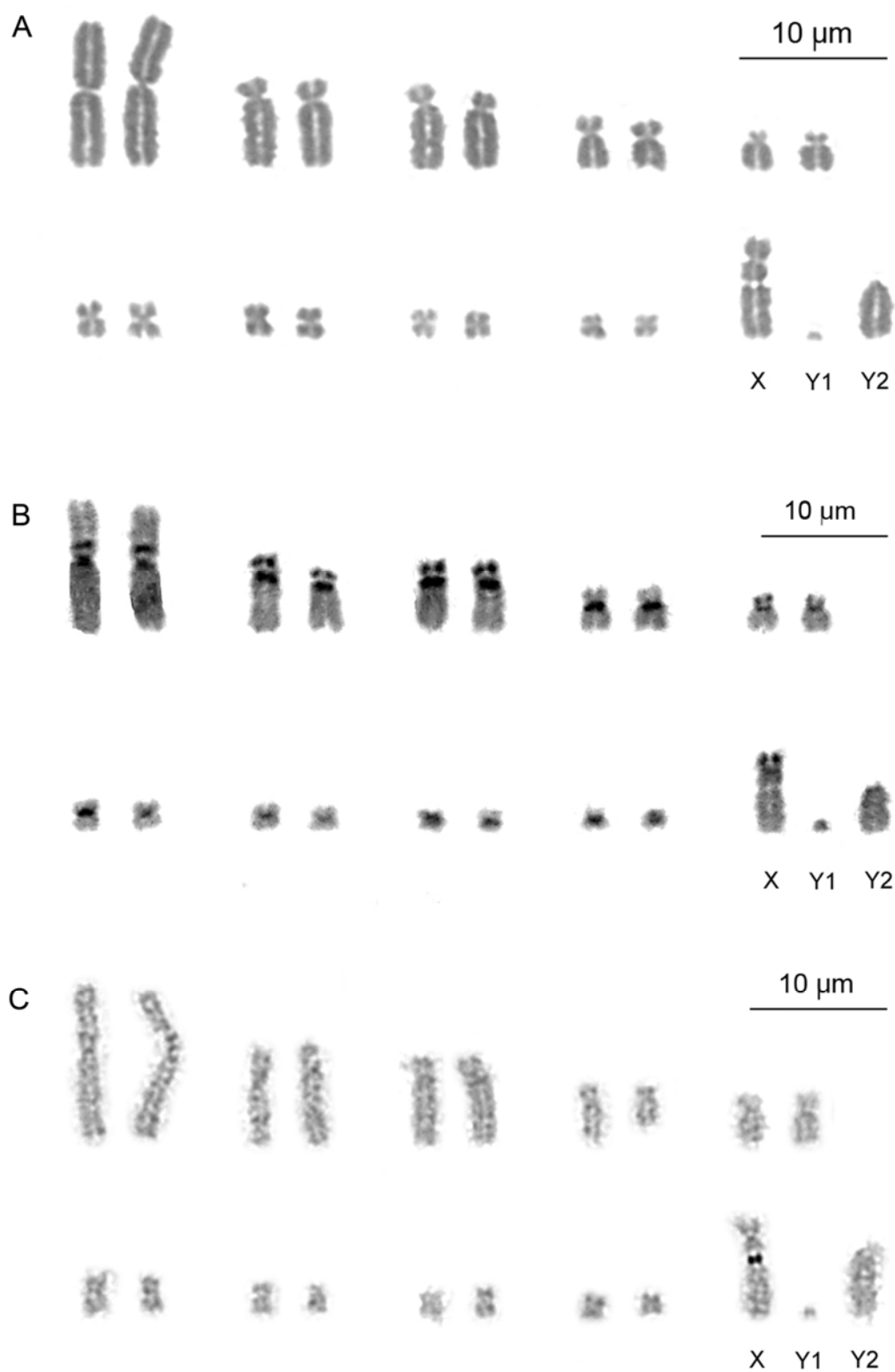
At least one individual of each species was karyotyped. We employed the technique of Ford and Hamerton (1956) for mitotic preparations. C-banding and Ag-NOR sites were detected employing the techniques of Sumner (1972) and Howell and Black (1980), respectively. Photomicrographs were taken with an Optronics digital camera coupled on a Zeiss Axioscope microscope at the Laboratório de Tecnologia e Processamento de Imagens from the Instituto de Biologia, Universidade Federal do Rio de Janeiro, and images were edited with Adobe Photoshop® CS. A minimum of 20 metaphases per individual were analyzed to define diploid and fundamental number of autosomal arms (2n and FN<sub>a</sub>, respectively). Banding patterns were defined based on the analysis of five metaphases per individual at least. The chromosomal nomenclature follows Levan et al. (1964).

#### Results

We sampled 25 specimens representing six species of three families. Five species were

**Table 1** – Karyotypic composition of bat species from the surroundings of CNAAA, Rio de Janeiro. 2n = diploid number; FNa = fundamental number of autosomal arms; X = sex chromosome; Y<sub>1</sub> = sex chromosome; Y<sub>2</sub> = sex chromosome; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; Pc = pericentromeric.

Species	2n	FNa	X	Y <sub>1</sub>	Y <sub>2</sub>	Ag-NORs	C-Bands
<i>C. perspicillata</i>	21	36	ST	A	A	Secondary constriction of X	Pc in all chromosomes but Y <sub>1</sub> ; interstitial in short arms of 1 M autosomal pair; telomeric in short arms of 2 ST autosomal pairs and X
<i>A. lituratus</i>	31	56	SM	A	A	Telomers' short arms of 3 SM autosomal pairs	Pc in all autosomes and X; Y <sub>1</sub> completely heterochromatic; telomeric in short arms of 1 SM autosomal pair
<i>A. obscurus</i>	31	56	SM	A	A	Telomers' short arms of 2 SM autosomal pairs	Pc in all autosomes and X; Y <sub>1</sub> completely heterochromatic; interstitial in long arms of 1 M autosomal pair
<i>P. lineatus</i>	30	56	SM	-	-	Telomers' short arms of 1 ST autosomal pair	Pc in 5 M, 3 SM, and 1 ST autosomal pairs; telomeric in short arms of X
<i>S. lilium</i>	30	56	ST	-	-	Telomers' short arms of 1 ST autosomal pair	Pc, interstitial, and telomeric in autosomes
<i>E. brasiliensis</i>	50	48	SM	A	-	-	-



**Figure 2** – A representative karyotype of a male specimen of *Carollia perspicillata* (MN72057) collected around CNAAA, Rio de Janeiro, Brazil. A) Giemsa staining; B) C-banding; C) Ag-NOR staining.

included in two subfamilies within Phyllostomidae as follow: *Carollia perspicillata* (Linnaeus, 1758) represents the subfamily Carollinae and *Artibeus lituratus* (Olfers, 1818), *Artibeus obscurus* (Schinz, 1821), *Platyrrhinus lineatus* (É. Geoffroy, 1810), and *Sturnira lilium* (É. Geoffroy, 1810) represent the subfamily Stenodermatinae. The other species analyzed, *Eptesicus brasiliensis* (Desmarest, 1819), represents the family Vespertilionidae.

The karyotype description for each species is given below. After, a summary of the results is presented in Table 1.

*Carollia perspicillata*;  $2n = 21$ ,  $FN_a = 36$ . Ten specimens were analyzed, being five females and five males. The autosomal complement consists in five pairs of metacentrics, one large and four small-sized, two pairs of medium-sized submetacentrics, and two pairs of small submetacentrics. The sex chromosome system of males is  $XY_1Y_2$ . The X chromosome is a large submetacentric with secondary constrictions on its long arms and both Y chromosomes are acrocentrics,  $Y_1$  is small and  $Y_2$  is medium-sized (Fig. 2A). Pericentromeric constitutive heterochromatin is found in all chromosomes but  $Y_1$ . Non-centromeric heterochromatin is present as interstitial bands on the short arms of the large metacentric autosomes and as telomeric bands on the two pairs of submetacentric autosomes and on the X chromosome (Fig. 2B). The X chromosome presents Ag-NORs, which are located at its secondary constrictions (Fig. 2C).

*Artibeus lituratus*;  $2n = 31$ ,  $FN_a = 56$ . Six specimens were analyzed, being two females and four males. The autosomal complement consists in a graded series of 10 pairs of metacentrics, ranging in size from large to small, and four pairs of medium-sized submetacentrics. The sex chromosome system of males is  $XY_1Y_2$ . The X chromosome is a medium-sized than  $Y_2$  (Fig. 3A). Pericentromeric constitutive heterochromatin is found in all autosomes and in the X chromosome. The  $Y_1$  chromosome is completely heterochromatic. Non-centromeric constitutive heterochromatin is found in the telomers of one pair of submetacentric autosomes (Fig. 3B). Three pairs of submetacentric autosomes present Ag-NORs on the telomers of their short arms (Fig. 3C).

*Artibeus obscurus* –  $2n = 31$ ,  $FN_a = 56$ . Two male specimens were analyzed. The autosomal complement consists in a series of 10 pairs of metacentrics, ranging in size from large to small, and four pairs of medium-sized submetacentrics. The sex chromosome system is  $XY_1Y_2$ . The X chromosome is a medium-sized submetacentric and both Y chromosomes are small acrocentrics, with  $Y_1$  smaller than  $Y_2$  (Fig 4A).

When observed under Giemsa staining, the karyotype of this species is very similar to that described for *A. lituratus* above (Fig. 3A). However, C-banding and Ag-NOR staining revealed some differences between these karyotypes. In *A. obscurus* non-centromeric constitutive heterochromatin is found only as proximal interstitial bands in the long arms of the largest pair of metacentric autosomes (Fig. 4B), and only two pairs of submetacentric autosomes present Ag-NORs on the telomers of their short arms (Fig. 4C).

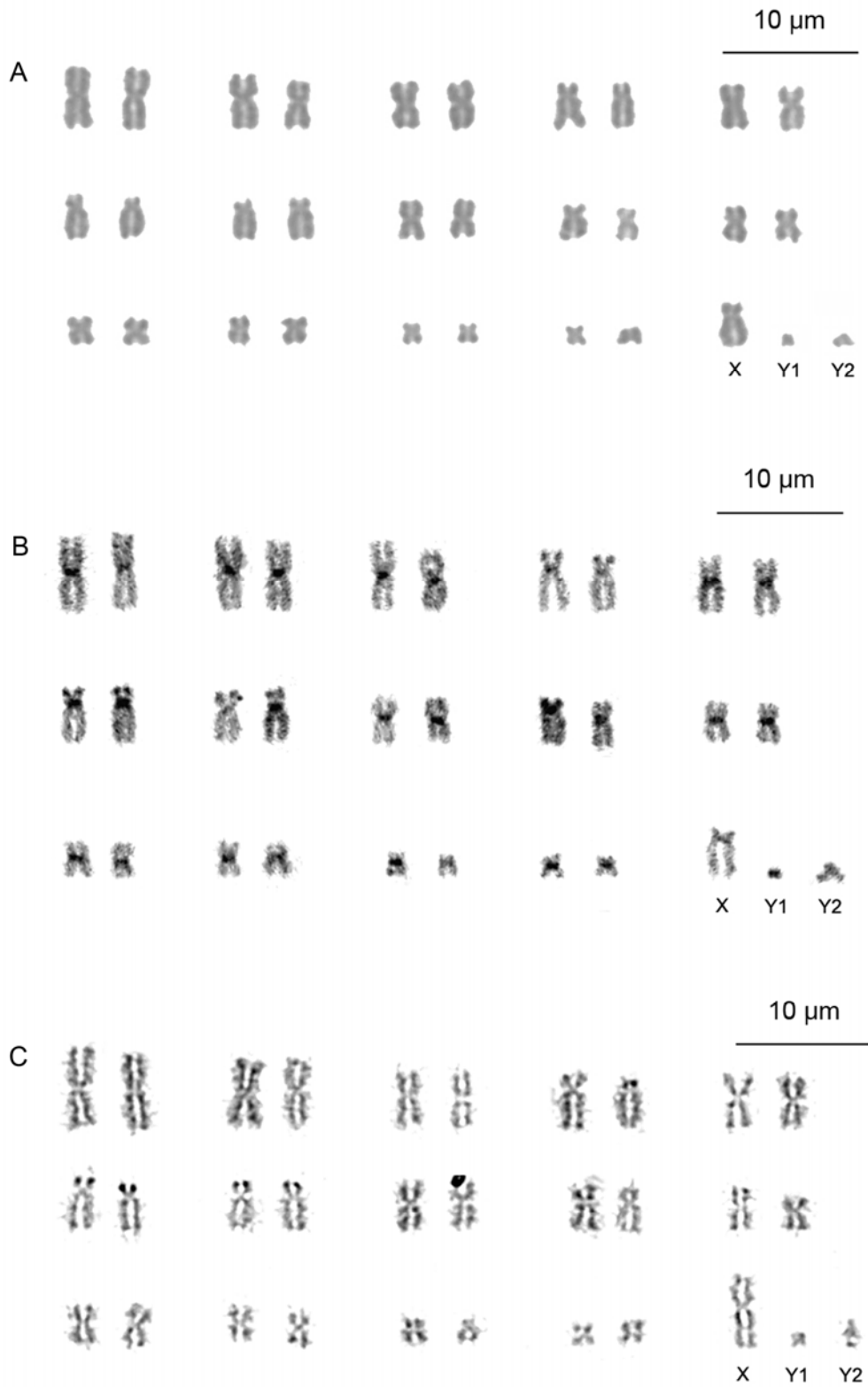
*Platyrrhinus lineatus*;  $2n = 30$ ,  $FN_a = 56$ . Two female specimens were analyzed. The autosomal complement consists in a graded series of 10 pairs of metacentrics, ranging in size from large to small, three pairs of medium-sized submetacentrics, and one pair of medium-sized submetacentrics. The X chromosome is a medium-sized submetacentric (Fig. 5A). Pericentromeric constitutive heterochromatin is found in the centromers of five pairs of metacentric autosomes, ranging from large to medium-size, and in all submetacentric and submetacentric pairs of autosomes. Non-centromeric C-bands are present on the telomers of the short arms of X chromosome (Fig. 5B). The pair of submetacentric autosomes presents Ag-NORs on the telomers of their short arms (Fig. 5C).

*Sturnira lilium*;  $2n = 30$ ,  $FN_a = 56$ . Four specimens were analyzed, being three females and one male. The autosomal complement consists in a graded series of 10 pairs of metacentrics, ranging in size from large to small, three pairs of medium-sized submetacentrics, and one pair of medium-sized submetacentrics. The X chromosome is a medium-sized submetacentric (Fig. 6A). The mitotic preparations of *S. lilium* did not permit an accurate analysis of constitutive heterochromatin. We just could note that there are pericentromeric, telomeric, and interstitial C-bands in autosomes (Fig. 6B). One pair of submetacentric autosomes presents Ag-NORs on the telomers of their short arms (Fig. 6C).

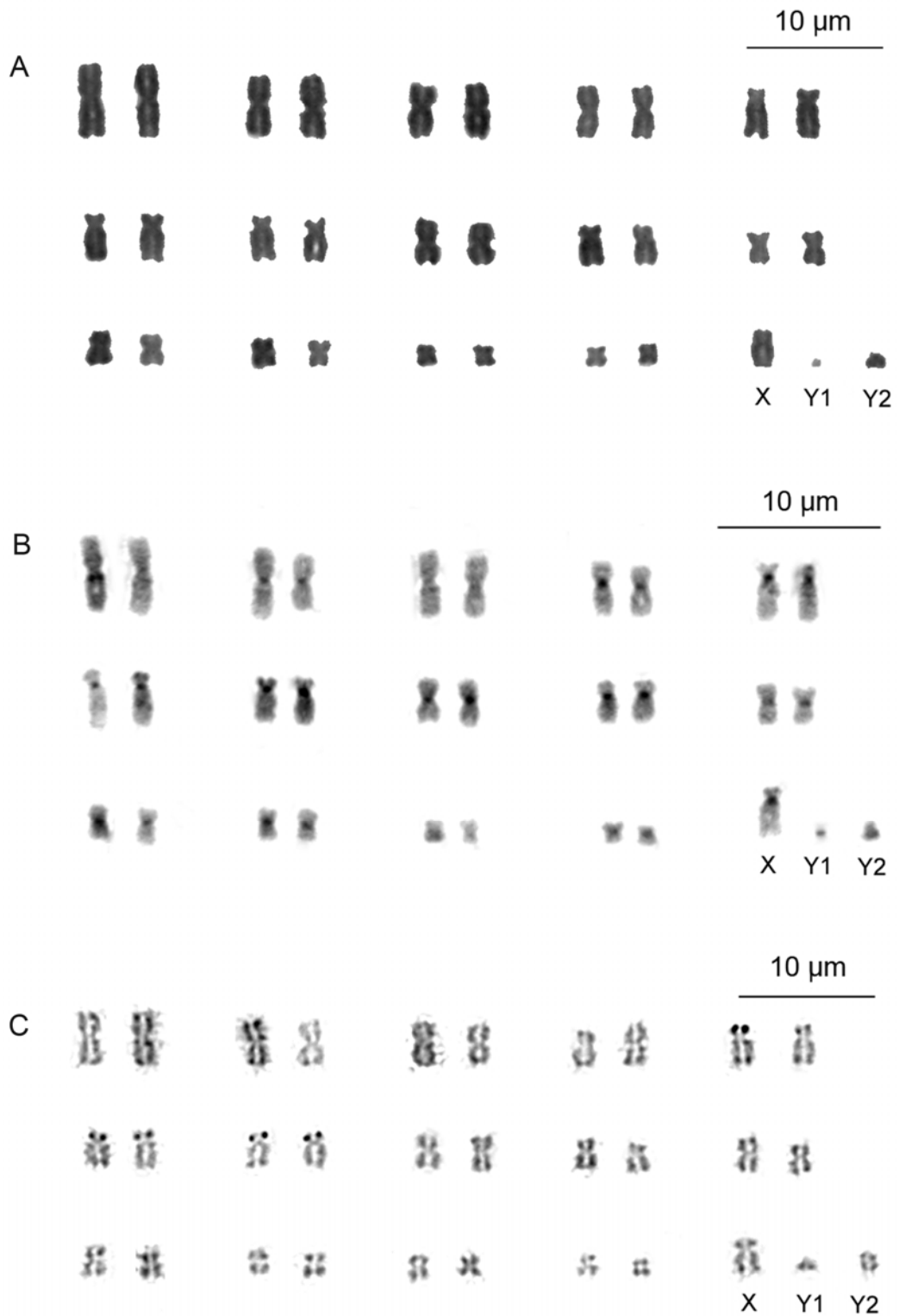
*Eptesicus brasiliensis* –  $2n = 50$ ,  $FN_a = 48$ . One male specimen was analyzed. The autosomal complement consists in a graded series of 24 pairs of acrocentrics, ranging in size from medium to small. The sex chromosome system of male is XY. The X chromosome is a medium-sized submetacentric and the Y is a small acrocentric (Fig. 7). Banding techniques applied to mitotic preparations of *E. brasiliensis* did not permit any analysis.

## Discussion

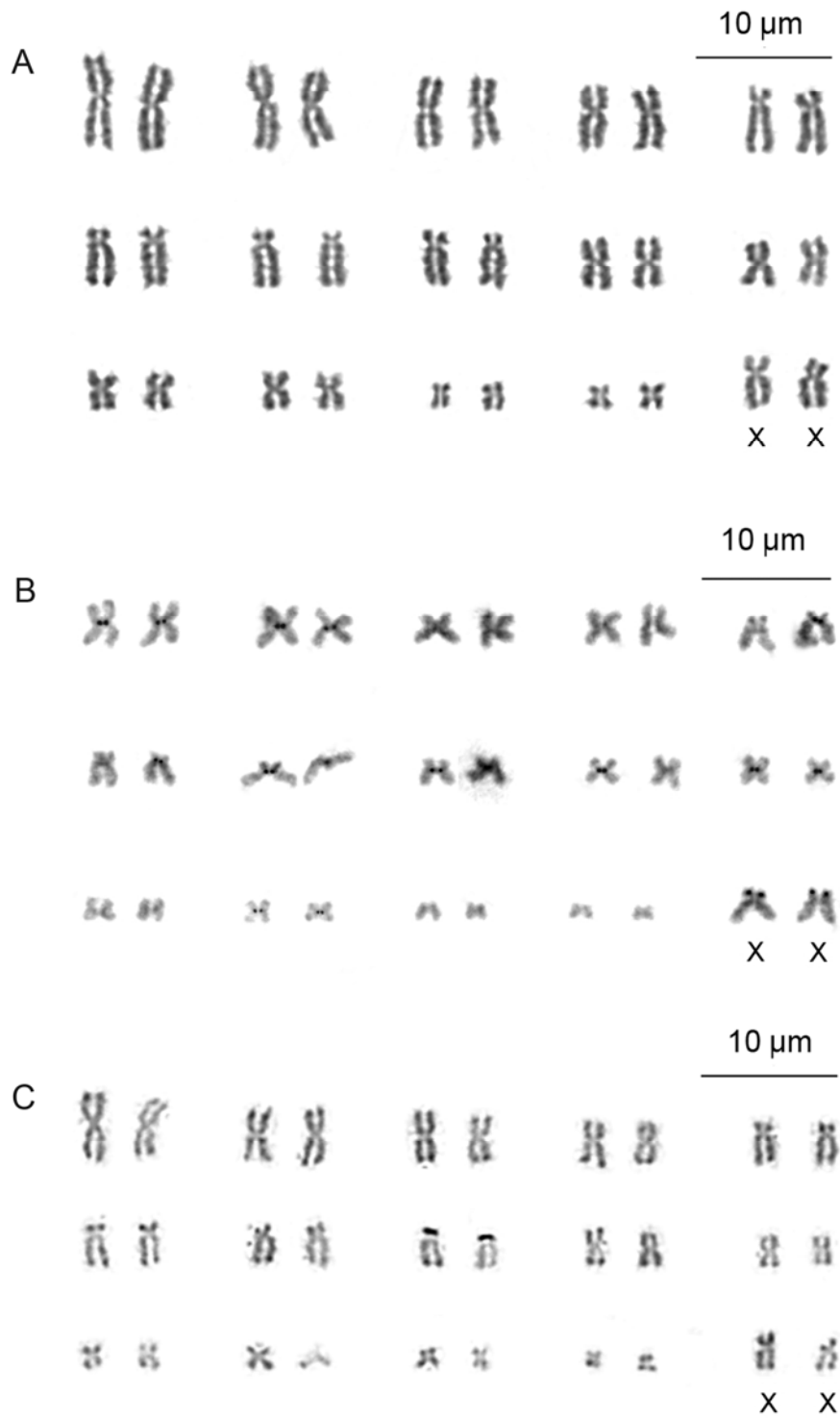
Cytogenetic data can be helpful to understand part of the influence of Central Nuclear Almirante Álvaro Alberto over small mammal local fauna.



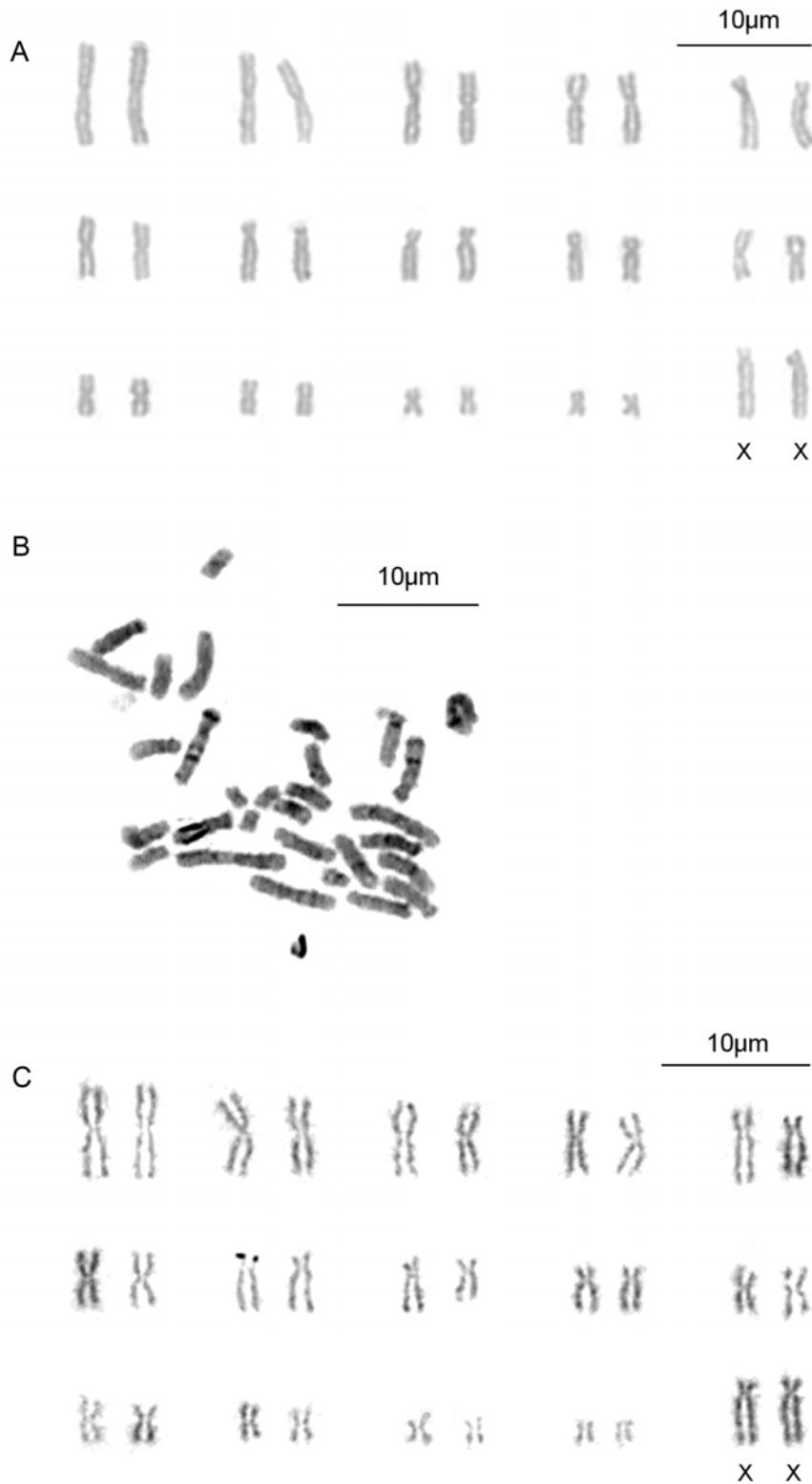
**Figure 3** – A representative karyotype of a male specimen of *Artibeus lituratus* (MN72107) collected around CNAAB, Rio de Janeiro, Brazil. A) Giemsa staining; B) C-banding; C) Ag-NOR staining.



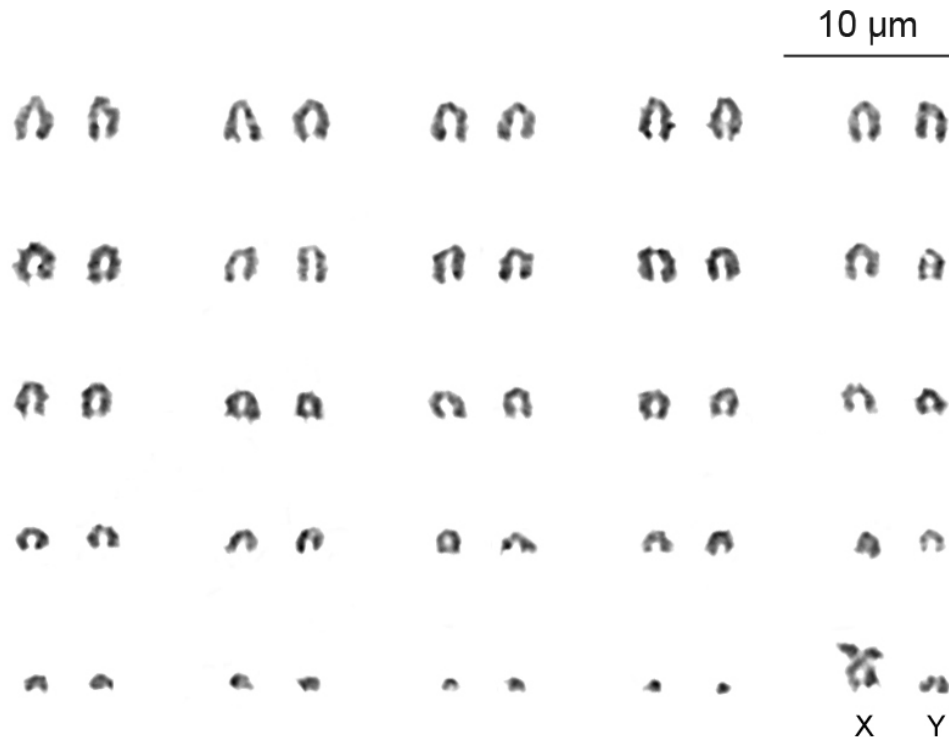
**Figure 4** – A representative karyotype of a male specimen of *Artibeus obscurus* (MN72055) collected around CNAAB, Rio de Janeiro, Brazil. A) Giemsa staining; B) C-banding; C) Ag-NOR staining.



**Figure 5** – A representative karyotype of a female specimen of *Platyrrhinus lineatus* (MN72112) collected around CNAAB, Rio de Janeiro, Brazil. A) Giemsa staining; B) C-banding; C) Ag-NOR staining.



**Figure 6** – A representative karyotype of a female specimen of *Sturnira lilium* (MN72104) collected around CNAAB, Rio de Janeiro, Brazil. A) Giemsa staining; B) C-banding; C) Ag-NOR staining.



**Figure 7** – A representative karyotype of a male specimen of *Eptesicus brasiliensis* (MN72140) collected around CNAAA, Rio de Janeiro, Brazil (Giemsa staining).

While non-volant small mammals present short life spans, with many species living less than one year under field conditions (Bourlière 1975), bats present an extreme longevity, with life spans, on average, at least threetimes as long as those of non-volant small mammals (Neuweiler 2000). Therefore, to investigate the occurrence of unexpected radioactive emissions from nuclear complex, bats could be used to access long-term exposure, a similar situation to that of the nuclear complex staff. The efficiency of cytogenetical markers to detect long-term effects of low dose exposure to radiation was already confirmed in the vespertilionid bat *Rhinolophus capensis* (Meehan et al. 2004).

However, to achieve such goals, firstly is necessary to build up a detailed database of karyological traits of the bat fauna that inhabits the surroundings of the CNAAA, which currently does not exist. In this regard, this study can be considered an initial step to fill out this gap.

Additionally, our results reveal new karyotypical variability in some of the studied species. Under the conventional and Ag-NOR staining no significant differences were found

between our results and those in literature, but under C-banding, the species of *Artibeus* showed differences in distribution patterns of the constitutive heterochromatin (Morielle and Varella-Garcia 1988; Varella-Garcia et al. 1989).

*Artibeus lituratus* and *A. obscurus* from southern, southeastern, and northeastern Brazil present pericentromeric C-bands on all autosomes and on the sex chromosomes X and Y<sub>2</sub>, plus telomeric C-bands on four autosomal pairs (Varella-Garcia et al. 1989; Souza and Araujo 1990; Althoff 1996). In the surroundings of CNAAA, both species do not present pericentromeric C-bands on the Y<sub>2</sub>. Besides, *A. obscurus* does not present telomeric C-bands either and presents interstitial bands on the long arms of the largest autosomal pair, which are reported for the first time in this study. However, these interstitial bands were already known for *A. planirostris* (Souza and Araujo 1990), which is considered the sister group of *A. obscurus* in the recent phylogenies available (Lim et al. 2004; Redondo et al. 2008).

As pointed out by Souza and Araujo (1990) the location of constitutive heterochromatin in

Stenodermatinae bats shows great variability; concerning Brazilian species, intraspecific variation in C-banding pattern was already reported for *Platyrrhinus lineatus* (Varella-Garcia et al. 1989). More cases, like our findings about *A. obscurus*, may appear as we increase our knowledge about bat karyological data.

The lack of data about radionuclide emission in the surroundings of CNAEA in addition to the scarcity of studies about intraspecific variation in constitutive heterochromatin distribution in *A. obscurus*, preclude an association between our findings and the presence of the nuclear power plant. Therefore, further studies about *A. obscurus* cytogenetics are needed within the zone of influence of the CNAEA, as well as through the total range of species distribution.

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#### Appendix

Voucher specimens analyzed in this study are deposited in the Museu Nacional (MN), Rio de Janeiro, Brazil. The cell suspension samples are deposited in the Laboratório de Mastozoologia, Departamento de Zoologia – IB/UF RJ.

*Carollia perspicillata*: MN72056-72058, MN72061-72063, MN72076, MN72078, MN72080, MN72082; *Artibeus lituratus*: MN72060, MN72084, MN72085, MN72105, MN72107, MN72108; *Artibeus obscurus*: MN72055, MN72059; *Platyrrhinus lineatus*: MN72083, MN72112; *Sturnira lilium*: MN72081, MN72104, MN72106, MN72130; *Eptesicus brasiliensis*: MN72140.

Coordinates of bat collection sites showed in Figure 1 are, from right to left, respectively: 23°00'49.1"S, 44°26'43.8"W; 23°00'06.9"S, 44°27'35.2"W; 23°00'04.2"S, 44°27'38.1"W.

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