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Identification of regions of constitutive heterochromatin and sites of ribosomal DNA (rDNA) in *Rhogeessa hussoni* (Genoways & Baker, 1996) (Chiroptera; Mammalia; Vespertilionidae)

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Abstract. There is scarce information about the geographical distribution, biological and cytogenetic data from the *Rhogeessa hussoni*. This study aims to characterize its chromosome composition through chromosome bandings to visualize regions of constitutive heterochromatin (CGB bands) and sites of ribosomal DNA (rDNA) in *R. hussoni*'s karyotype. A female specimen of *R. hussoni* was collected in the “Parque Municipal Mário Viana” Conservation Unit, Nova Xavantina, Mato Grosso, Central-West region of Brazil. The karyotype constitution was $2n=52$ and $NF=54$. The CBG bands evidenced a sex X chromosome nearly completely constituted by heterochromatin. The *Rhogeessa hussoni* has two sites of rDNA located in a single pair (pair 25) of autosomal chromosomes. We carried out the first cytogenetic characterization of *R. hussoni*, supplementing knowledge about regions of heterochromatin and ribosomal DNA in this species, thus contributing to future elucidations about the genetic diversification in the genus *Rogeessa*.

Keywords: bats, cytogenetics, X chromosome, heterochromatin, ribosomal DNA.

INTRODUCTION

The family Vespertilionidae is constituted by the genera *Eptesicus*, *Histiotus*, *Lasiurus*, *Myotis*, and *Rhogeessa*. The genus *Rhogeessa* is composed by eleven species: *R. tímida*, *R. aeneus*, *R. parvula*, *R. mira*, *R. minutilla*, *R. genowaysi*, *R. bickhami*, *R. alleni*, *R. gracilis*, *R. io* and *R. hussoni*. The species occur exclusively in neotropical regions (Laval 1973; Genoways and Baker 1996; Ramiréz et al. 2014), and among them, *R. minutilla*, *R. hussoni*, and *R. io* are restricted to regions of South America (Gardner 2008). The *Rhogeessa hussoni* occurs in Suriname and Brazil and, in the latter, covers the states of

Maranhão, Bahia (Gardner 2008). Recently had its distribution extended to the states of Sergipe (Mikalauskas et al. 2011), Mato Grosso, Minas Gerais, and Pará (Aires et al. 2011).

Despite this broad spatial distribution, accurate taxonomic resolution in the genus *Rhogeessa* is still required (Baird et al. 2009). Identifications based only in external morphological features led for some time to incongruences in distinctions of some species grouped in the complex *Rhogeessa tumida-parvula* (Laval 1973; Gardner 2008; Baird et al. 2009). The use of molecular and cytogenetic markers provided more conclusive information to distinguish some species (Baird et al. 2009), and cytogenetic markers showed singularities in the chromosomal constitution of the following species: *R. genowaysi* (2n=42) (Roots and Baker 1998), *R. parvula* (2n=44) (Roots and Baker 2007), *R. tumida* (2n=34), *R. aenus* (2n=32) (Bickham and Baker 1977), *R. io* (2n=30) (Gardner 2008), and *R. hussoni* (2n=52) (Genoways and Baker 1996; Gardner 2008). An explanation for this diverse karyotype within the genus *Rhogeessa* is the occurrence of chromosomal rearrangements by means of centric fusions and fissions (Baker et al. 1985; Baird et al. 2009).

Despite the efforts to acknowledge the variety of species of the genus *Rhogeessa*, the information about the *R. hussoni* and *R. io* occurring in the Brazilian territory is still scarce (Nogueira et al. 2014), with overlapping areas in the state of Mato Grosso (Gardner 2008; Aires et al. 2011). *Rhogeessa hussoni* and *R. io* are considered cryptic species, because of the difficult taxonomic designation through external morphological data (Gardner 2008; Gurgel-Filho et al. 2015). However, it is possible to distinguish the two species through their basic diploid number, being *R. hussoni* 2n=52 and *R. io* 2n=30 (Bickham and Baker 1977; Genoways and Baker 1996). The existing cytogenetic information about *R. hussoni* come from a specimen in the region of Suriname (Genoways and Baker 1996; Gardner 2008), and the authors presented only the basic karyotype of the species. However, there are no cytogenetic studies with mappings of specific chromosome regions for this species. In this study, we characterized the chromosome composition of a specimen of *R. hussoni* captured in Brazil, by identifying the sites of ribosomal DNA (rDNA) and the regions of constitutive heterochromatin (CBG bands) in this specimen's karyotype.

MATERIAL AND METHODS

The capture of a female specimen of *Rhogeessa hussoni* was made using mist nets (7.0 x 3.0) in the “Parque

Municipal Mário Viana” Conservation Unit, located in the municipality of Nova Xavantina, state of Mato Grosso, Brazil. The area is characterized by the phytophysiognomy of Cerradão (14°42'02.6" S e 052° 21'01.5" W), inside the Brazilian Cerrado biome, with plant formations of continuous canopy and tree coverage ranging from 50% to 90% (Silva et al. 2008). The region's climate is tropical rainy (Aw) according to Köppen's classification system (Vianello and Alves 2012), with a dry season from April to September, and a rainy season from October to March (Pirani et al. 2009).

The female specimen of *Rhogeessa hussoni* was captured under the license 18276-1 from IBAMA/SISBIO/MT, and its identification was made based on specialized literature (Vizotto and Taddei 1973; Genoways and Baker 1996; Gardner 2008; Díaz et al. 2011). After the capture, the bat was kept in a cage until the following morning, when cytogenetic procedures were made. After extracting the biological material, the animal was mounted and deposited in the Scientific Collection of Chiroptera of Mato Grosso State University, campus of Nova Xavantina (UNEMAT/NX), registered under the number RM 276.

The chromosome preparations were obtained directly from bone marrow, following Morielle-Versute et al. (1996). The diploid number and the fundamental autosomal number were determined using the Giemsa staining method (2%). The procedure of Howell and Black (1980) was adapted to identify regions of ribosomal DNA (rDNA). The blocks of constitutive heterochromatin were determined using the technique of Summer (1973) with minor modifications: the chromosomal preparations were treated with 2N HCl solutions, barium hydroxide, and 2xSSC at a temperature of 42 ° C, and then stained with Giemsa solution. The karyotype was determined with based on the first cytogenetic description of the species in Suriname. The slides with chromosome preparations for each procedure were photo-documented using an optical microscope with observation at 1000x (Axio vision® -Nikon Digital Sight DS-U3). Next, the analysis and mounting of karyotypes were made using Adobe Photoshop, version 7.0.1.

After the cytogenetic analyses, taxonomic characterizations were made to confirm species identification. External and cranial measurements were taken using a digital pachymeter (precision 0.01 mm) after the taxonomic characterizations. The measured features were: forearm length, total skull length, basal condyle length, canine condyle length, basal length, palatal length, length of upper teeth series, length of interior teeth series, mandible length, width of *cingula* (canine teeth), external width of molar teeth, interorbital width, pos-

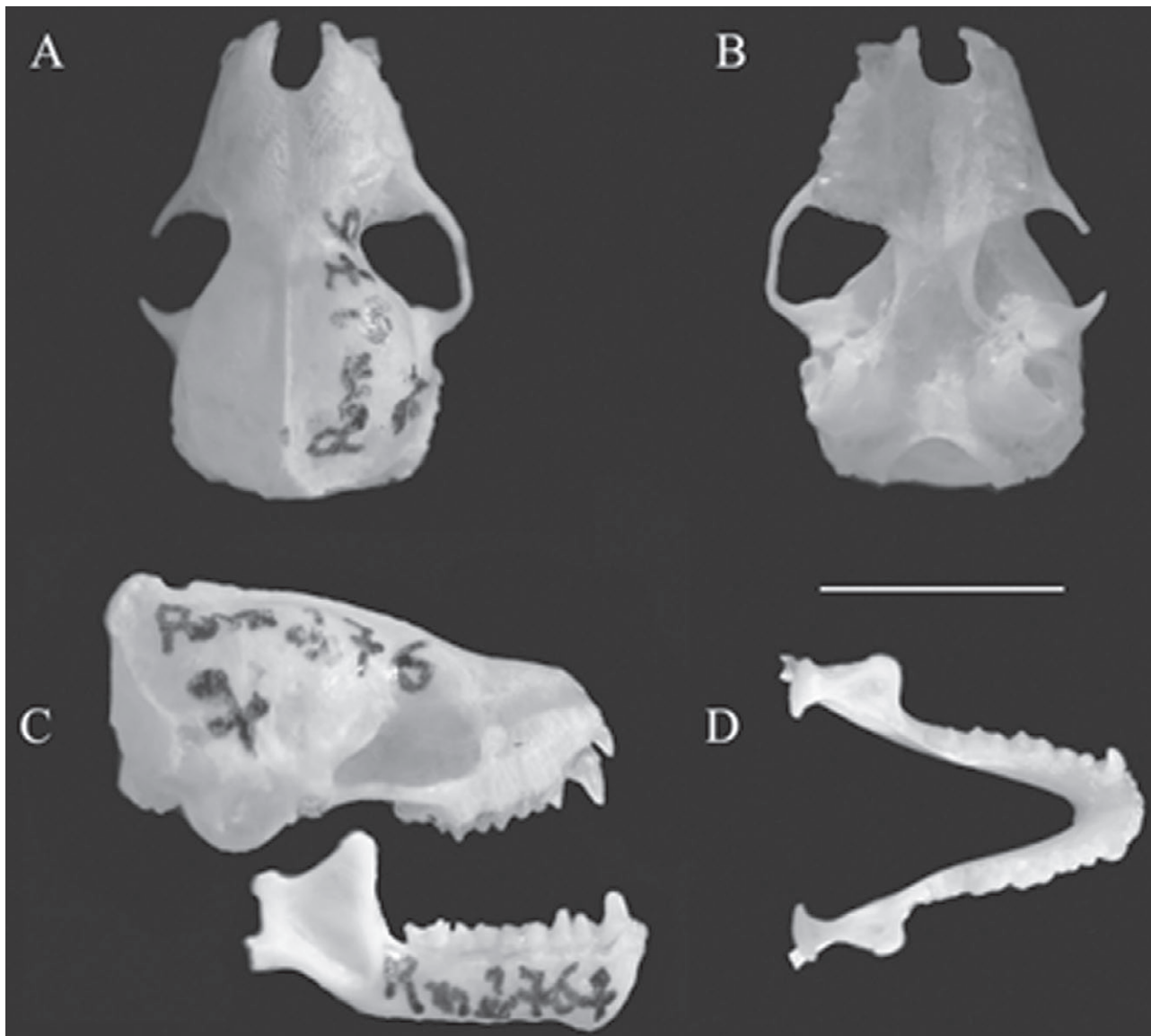


Figure 1. Dorsal view of skull (A), ventral view (B), lateral view (C), and ventral view of mandible (D) (RM 276), specimen of *R. hussoni* (adult female), deposited in the Scientific Collection of Chiroptera/UNEMAT, campus Nova Xavantina, state of Mato Grosso, Brazil (bar scale 6.4mm).

torbital width, zygomatic width, skull width, mastoid width, palatal width, skull height, and occipital height (Fig. 1 and Supplementary Material).

RESULTS

The morphometrical analyses confirmed that the exemplar was an *R. hussoni*. We analyzed more than thirty metaphases to determine the diploid number (2N) and the number of autosomal arms (NF). The speci-

men of *R. hussoni* presented $2n = 52$ and $NF = 54$ (Fig. 2). The set of autosomes is constituted by 23 pairs of acrocentric or subtelocentric chromosomes of large to small dimensions (pairs 1-12, 14-17, 19-25). The sexual set is composed of two X chromosomes, medium size, with submetacentric morphology. The nucleolar organizing regions were evidenced in the pair of autosomal chromosomes number 25 (Fig. 2). With the C banding technique, we showed the formation of heterochromatin blocks in pericentromeric regions of all autosomal chromosomes. Regarding the sexual pair, one of the X chro-

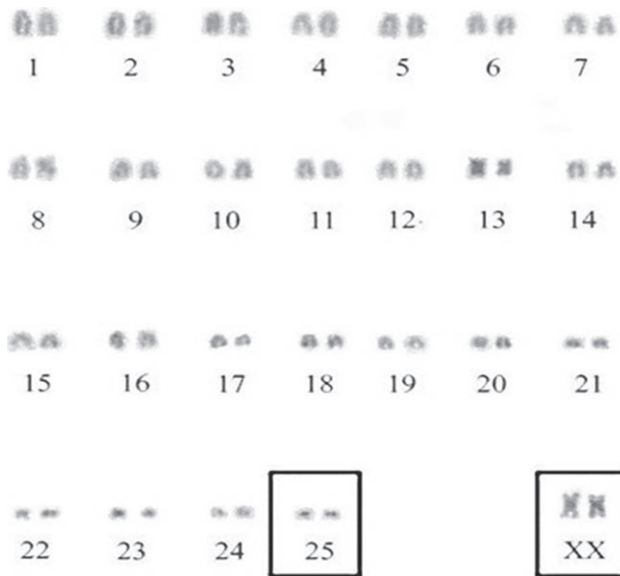


Figure 2. Karyotype of *Rhogeessa hussoni* ($2n = 52$ and $NF = 54$). The highlighted box shows respectively the chromosome pair 25, with rDNA sites, and sex chromosomes.

mosomes has a structure composed almost entirely by heterochromatin regions, whereas a small portion of heterochromatin is located in the pericentromeric region in

the other X chromosome (Fig. 3a). In interphase nuclei, we observed the presence of regions of more condensed heterochromatin, which may suggest the inactivation of one of the X chromosomes (Fig. 3b).

DISCUSSION

For species of the genus *Rhogeessa*, the sole use of morphological features does not allow a clear taxonomic designation, since *Rhogeessa hussoni* and *R. io* show overlap in their forearm size (Gardner 2008). However, cytogenetic information provided fundamental data to designate species of the genus (Genoways and Baker 1996, Gardner 2008). The species *Rhogeessa io* and *R. hussoni* have geographical distribution in Brazilian territory with overlapping areas in the state of Mato Grosso (Gardner 2008; Gurgel-Filho et al. 2015).

The first karyotype description of *R. hussoni* was of a specimen in Suriname (Genoways and Baker 1996), with $2n=52$, whose authors assumed a meta-submetacentric morphology in sex chromosome X, which was followed in the present study. The morphology of the Y chromosome is not mentioned because there are still no cytogenetic descriptions of male specimens.

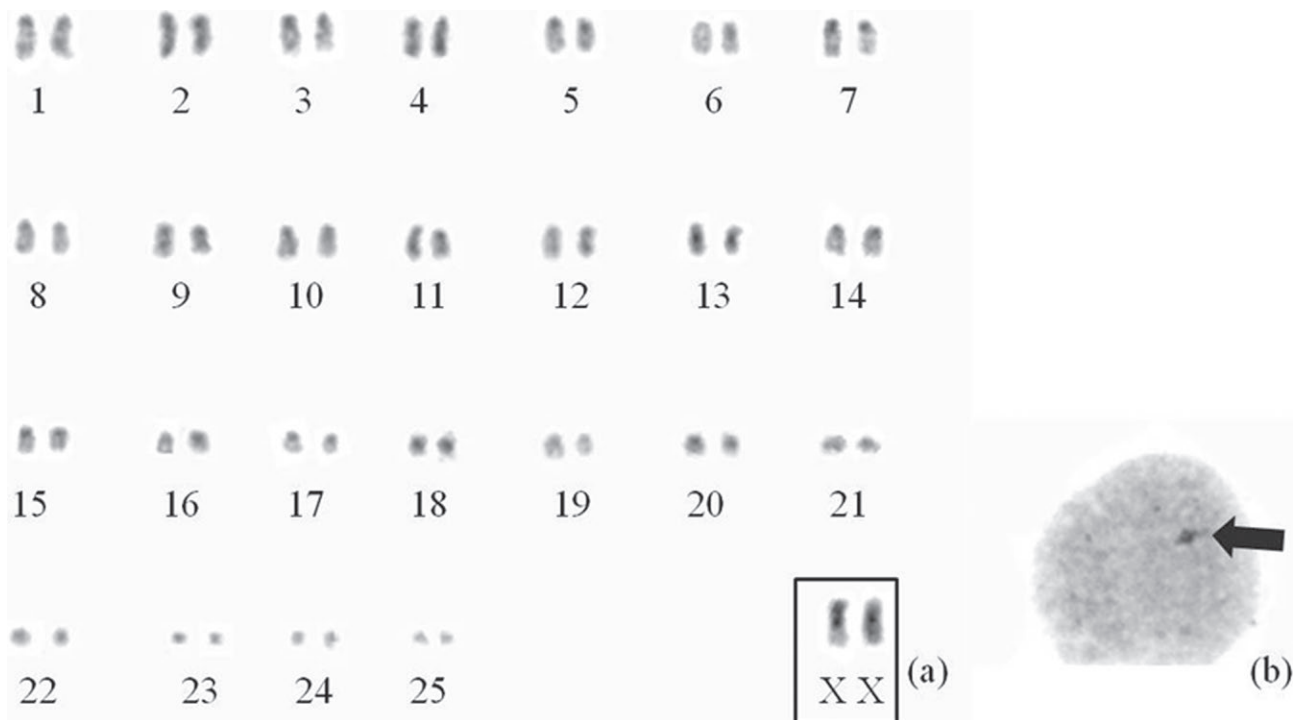


Figure 3. (a) Karyotype of *Rhogeessa hussoni* with C banding, showing blocks of heterochromatin in pericentromeric regions of all chromosomes. The highlighted box shows the pair of sex chromosomes. (b) An interphase nucleus with evidence of Barr corpuscle (indicated by the arrow).

The karyotype of *Myotis* ($2n=44$) has been indicated as the similar ancestral karyotype for the family Vespertilionidae. Some species of the genus *Eptesicus* ($2n=50$, $NF=48$) are proposed as the closest evolutionary kinship of *Rhogeessa* (Bickham 1979). Comparisons of G-bands patterns between chromosomes of *Myotis velifer* and representatives of the genus *Rhogeessa* (*R. parvula*, *R. tumida*, *R. aenus*, and *R. io*) showed homology between the autosomal chromosomes pairs 16/17 and 20/18, with rearrangements of chromosomal fusions shared by species of *Rhogeessa* presenting meta-submetacentric morphology (Bickham 1979; Baker et al. 1985). In the present study, the pair of chromosomes with meta-submetacentric morphology 13 of *R. hussoni* seems to correspond to pair 16/17 of *Myotis* and other species of *Rhogeessa*. Homology between G-bands patterns of pairs 20/18, which corresponds to the morphology of chromosome 18 found in *R. hussoni*, suggest a synapomorphy shared by species of the genus *Rhogeessa* (Bickham and Baker 1977; Baker et al. 1985).

Information about kinship relations has been obtained for some representatives of the family Vespertilionidae through comparative genomic techniques and G-bands patterns (Volleth et al. 2002, 2012; Sotero-Caio et al. 2017). However, there is not a clear knowledge about chromosomal rearrangements in the genus *Rhogeessa* due to lacking data of cytogenetic bandings for some species.

The presence of a pair of ribosomal DNA sites was evidenced in *R. hussoni*. Likewise, studies have shown a single pair of rDNA in *R. tumida* ($2n=34$), *Eptesicus fuscus* ($2n=50$) (Baker et al. 1992), and *Lasiurus cinereus* ($2n=28$) (Marchesin and Morielle-Versute 2004), indicating a maintenance in the number of rDNA sites among species of the genera *Rhogeessa*, *Eptesicus*, and *Lasiurus* within the family Vespertilionidae. Nonetheless, the presence of four chromosome pairs with rDNA sites is acknowledged in *Myotis keaysi* ($2n=44$) (Baker et al. 1992).

The blocks of heterochromatin in *R. hussoni* are ordered in pericentromeric regions in most autosomal chromosomes and in almost all the structure of one of the sex chromosomes X. (Fig. 3a). In *Lasiurus ega*, the presence of constitutive heterochromatin has also been evidenced along all the short arm of the X chromosome (Marchesin and Morielle-Versute 2004), in the same location of regions of heterochromatin in the sex chromosomes of *R. hussoni*. The organization of constitutive heterochromatin is dynamic among species. The existence of a copy of X chromosome, almost totally constituted by heterochromatin, with function in regulating the genic expression and gene dose compensation between complements XX and XY is acknowledged in several mammal

species (Avner and Heard 2001). In *R. hussoni*, the presence of one X chromosome nearly wholly constituted by heterochromatin blocks, with regions of highly condensed interphase nuclei suggest a possible function in regulating gene expression of the X chromosome.

A large amount of information about cytogenetic and molecular comparisons for representative species of *Myotis*, *Eptesicus*, and *Lasiurus* is known (Bickham 1979; Varella-Garcia et al. 1989; Volleth et al. 2002, 2012; Larsen et al. 2012; Seim et al. 2013; Supanuam et al. 2012; Furman et al. 2014). Still, little is known about structural chromosome relations among the species of the genus *Rhogeessa* (Bickham 1979; Baker et al. 1985; Baker et al. 1992), and there are no studies about phylogenetic relationships based on molecular and cytogenetic markers for *R. hussoni* (Baird et al. 2009).

In the present study, we carried out the first cytogenetic characterization with chromosome bandings for *R. hussoni*, broadening knowledge about this species' chromosome composition. Further studies about ecological aspects, geographical distribution, molecular biology, and phylogenetic inferences are necessary to better understand and preserve the species, considering that the kinship relationship within the genus *Rhogeessa* is not clear. Besides, the species is classified as deficient in data from the International Union for Conservation of Nature (IUCN), reinforcing the importance of more data in order to better understand the status of ecological threat (Sampaio et al. 2016).

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SUPPLEMENTARY MATERIAL
CRANIAL MORPHOMETRY OF *RHOGEESSA HUSSONI*

The holotype of *R. hussoni*, female adult, deposited in the Chiroptera Collection of Mato Grosso State University, register number RM 276, has forearm measurements (in millimeters) of 29.04. The craniodental measurements consisted in: total length (12.46); basal condyle length (11.97); canine condyle length (11.83); basal length (10.27); palatal length (6.02); upper teeth series length (4.50); inferior teeth series length (4.85); mandible length (8.59); external width of *cingula* - canines (4.05); external width of molar teeth (6.04); interorbital width (3.89); postorbital width (3.45); zygomatic width (8.49); skull width (6.24); mastoid width (7.25); palatal width (3.43); skull height (4.20); occipital height (5.10) (Fig. 1). The dental formula is constituted by: I: 1/3; C: 1/1; PM: 1/2; M: 3/3.