Trabajo

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Cytogenetic study in the Brazilian semiarid lizard *Tropidurus hispidus* (Squamata, Tropiduridae)

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ABSTRACT

Different classes of repetitive DNA sequences are found in eukaryotes, often composing substantial portions of the genome, associated with their important role in the structural and functional genome organization. In this work, we mapped repetitive DNA sequences (18S rDNA, microsatellites and telomeric motifs) in the karyotype of *Tropidurus hispidus,* a species of lizard from the Brazilian semiarid region. We found a diploid number of 2n = 36 (6 pairs of biarmed macrochromosomes and 12 pairs of microchromosomes). The 18S rDNA clusters were localized at the subterminal region of the long arm of pair 2. The telomeric probes produced signals at terminal, interstitial and centromeric positions of some chromosome pairs, which might indicate the occurrence of chromosomal rearrangements via chromosome fusions. Microsatellite sequences were found in at least two distinct patterns - clustered in the telomeric/pericentromeric regions or observed as scattered signals in the chromosomes. This study represents an initial step to explore the evolutionary dynamics of repetitive sequences in the *Tropidurus* genus and considering the scarcity of data concerning the chromosomal mapping of repetitive sequences in Tropiduridae species, it reinforces the importance of integrating other methodologies, including the isolation and physical mapping of different repetitive DNA sequences, contributing to understanding the patterns of karyotypic evolution in lizards.

Key words: Reptile chromosomes; Microsatellite; 18S rDNA; Telomeric repeats.

Introduction

Tropidurus Wied, 1825 is a genus belonging to the family Tropiduridae, comprising 28 species found in open landscapes in tropical and subtropical South America (Carvalho, 2013; Uetz *et al.*, 2022). Among them, *Tropidurus hispidus* (Spix, 1825) stands out for having a wide distribution, with a continuous and uniform presence in the Caatinga biome and the coastal areas of northeastern Brazil (Carvalho, 2013; Carvalho *et al.*, 2016; Uetz *et al.*, 2022).

Cytogenetic data available for *T. hispidus* show a diploid number of 2n = 36 (Kasahara *et al.*, 1987, 1996), with a typical karyotype of Squamata lizards, exhibiting macrochromosomes (metacentric and submetacentric) and microchromosomes (Veronese

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et al., 2003; Santos *et al.*, 2008; Carvalho *et al.*, 2015; Da Silva *et al.*, 2020a). However, the amount and distribution of constitutive heterochromatin in this species, detected by C-banding, show variation between populations, which could be related to different evolutionary processes such as sex chromosomes differentiation. (Kasahara *et al.*, 1983, 1987, 1996).

An important approach to advancing the cytogenetic knowledge of organisms is to characterize their genome content and organization. In this sense, considering the common occurrence of repetitive DNA sequences in eukaryotic genomes and the wide heterogeneity regarding their distribution and content, the chromosomal mapping of these sequences can provide important pieces of information concerning their role in the evolutionary process (Biscotti *et al.*, 2015; Garrido-Ramos, 2017).

Different classes of repetitive DNA sequences are found in eukaryotes, often composing substantial portions of the genome (Charlesworth *et al.*, 1994; Plohl *et al.*, 2012; López-Flores and Garrido-Ramos, 2012; Garrido-Ramos, 2017). These sequences can be found dispersed (DNA transposons and retrotransposons) or organized *in tandem* (multigenic families, including rDNA and other genes encoding proteins; satellites, and microsatellites) (for review, see López-Flores and Garrido-Ramos, 2012; Biscotti *et al.*, 2015; Garrido-Ramos, 2017).

Repetitive DNA sequences, mainly satellite DNAs, have already been reported at telomeric and centromeric heterochromatic regions, euchromatic regions, as well as at supernumerary and sex chromosomes of plants, insects, fish, amphibians, birds and lizards (Vittorazzi *et al.*, 2011; Carvalho *et al.*, 2016; Gatto *et al.*, 2016, 2019; Milani *et al.*, 2017; Palacios-Gimenez *et al.*, 2017; Silva *et al.*, 2017; Kretschmer *et al.*, 2018; Ruiz-Ruano *et al.*, 2019; Utsunomia *et al.*, 2019; Crepaldi and Parise-Maltempi, 2020; Da Silva *et al.*, 2020b; Ferretti *et al.*, 2020; Cholak *et al.*, 2020). These sequences play important roles in genome organization and often serve as hotspots of genome rearrangements and evolutionary innovations (Garrido-Ramos, 2017).

The available data concerning the distribution of repetitive sequences in lizards are still limited considering the group's diversity known so far. However, the information gathered up to now includes rDNAs, telomeric sequences, transposable elements, COT1, and satDNAs, which helped to understand their genomic dynamics and organization, as well as evolutionary processes they are involved (e.g Bertolotto *et al.*, 2001; Pellegrino *et al.*, 2009; Chaiprasertsri *et al.*, 2013; Rojo *et al.*, 2014; Rovatsos *et al.*, 2015; Carvalho *et al.*, 2015, 2016; Giovannotti *et al.*, 2018, 2020; Da Silva *et al.*, 2020a).

In this sense, considering the scarcity of data concerning the chromosomal mapping of repetitive sequences in Tropiduridae, we analyzed the lizard *T. hispidus* from the Brazilian semiarid, aiming to map some repetitive elements in the chromosomes of this species, adding valuable information to the cytogenetic knowledge of Brazilian reptiles.

Materials y methods

A sample of 10 specimens of *T. hispidus* (6 males and 4 females) was collected at the Picos, state of Piauí, Brazil (6°54'22.9"S 41°33'49.8"W), under a governmental license (number 47710-1/2015) issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). All laboratory procedures were performed at the Biology Laboratory, at the Instituto Federal de Educação, Ciência e Tecnologia do Piauí (IFPI), campus Picos.

Cell suspensions were obtained according to Bertollo *et al.* (1978), using bone marrow extraction from individuals previously treated with 1% colchicine injected intraperitoneally for 24 hours, followed by hypotonic treatment and fixation. Chromosome suspensions were dropped on slides and stained with 10% Giemsa to determine the chromosomal number and morphology.

For fluorescence *in situ* hybridization (FISH) experiments, 18S rDNA probes were obtained from *Hoplias malabaricus* (Pisces: Erythrinidae) genomic DNA (Cioffi *et al.*, 2009). The polymerase chain reaction (PCR) was carried out with primers 18SF (5' CCGAGGACCTCACTAAACCA 3') and 18SR (5' CCGCTTTGGTGACTCTTGAT 3') following Cioffi *et al.* (2009). The telomeric sequence probe was obtained by PCR without DNA templates, using the primers $(TTAGGG)_5$ and $(CCCTAA)_5$ according to Ijdo *et al.* (1991). PCR products of the 18S rDNA were labeled with digoxigenin-11-dUTP by nick translation (Dig-Nick Translation mix; Roche®) following the manufacturer's recommendations and used in FISH experiments according to Pinkel *et al.* (1986). The detection was made using Anti-Digoxigenin Rhodamine (Roche).

FISH experiments with microsatellite probes followed the protocol of Kubat *et al.* (2008), using the oligonucleotides $(CAA)_n$, $(CA)_n$, $(CAC)_n$, $(GAG)_n$, $(GA)_n$, and $(GAA)_n$ directly labeled with Cy5-fluorochrome at the 5' end during synthesis (Sigma-Aldrich). Chromosomes were counterstained with DAPI (2 mg/mL) in VectaShield® mounting medium (Vector).

At least ten metaphases were analyzed for each experiment. Giemsa-stained metaphases were photographed at a Nikon Eclipse microscope coupled with a Thiachron camera and processed using AMscope 3.7® software. The chromosomes were ordered in decreasing size, and the morphology of the chromosomes was determined based on the arm ratio, following Guerra (1986). FISH images were captured using a Zeiss-Axiophot microscope coupled to a fluorescence system and were processed employing the Axiovision software (Zeiss). The metaphase images were later optimized for brightness, contrast and mounting using the Adobe Photoshop CS6 program and Corel Draw X7 software.

Results

Males and females of *T. hispidus* presented 2n = 36, with 12 biarmed macrochromosomes (M) and 24 microchromosomes (m) without observable heteromorphic or sex-related chromosome pair (Fig. 1). No secondary constrictions were observed in conventional staining. Telomeric probes detected the telomeres of chromosomes and, in addition, produced conspicuous signals located at interstitial and centromeric positions of pairs 2 and 4 (Fig. 2 - A). The 18S rDNA probe hybridized at the distal end of the long arm of pair 2, which coincides with the nucleolus organizer region (NOR) (Fig. 2 - B).

Microsatellite probes revealed at least two distinct sequences of repeat motifs: telomeric/pericentromeric regions and scattered signals in the chromosomes. Therefore, $(CA)_n$, $(CAA)_n$, $(GA)_n$, $(CAC)_n$, and $(GAG)_n$ were found in a relatively uniform dispersed distribution in all chromosomes (Fig. 3). Although scattered signals for these arrays were generally observed in *T. hispidus* cells, some of the signals formed band-like patterns in distinct chromosomes and positions, depending on the sequence of the probe. For the motif $(GAA)_n$, signals occurred mainly at telomeric regions of macro and microchromosomes.

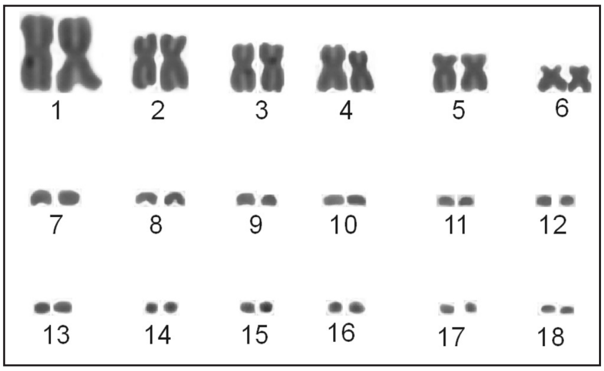

Figure 1. Giemsa-stained karyotype of a female *Tropidurus hispidus* (2n=36, 12M+24m). Pairs 1-6 indicate macrochromosomes (M), while pairs 7-18, microchromosomes (m).

Discussion

In a broad context, chromosomal studies, and particularly those that use *in situ* hybridization, have been able to locate different repetitive DNA sequences in chromosomes and show the distribution of the various classes of this type of intriguing sequences (Biscotti *et al.*, 2015). In this sense, here we mapped repetitive sequences (18S rDNA, microsatellites, telomeric motifs) in the karyotype of *T. hispidus*. In addition, we performed an analysis of the karyotype of *T. hispidus* in conventional staining in Giemsa, to support the repetitive sequence mapping data.

The specimens of *T. hispidus* we investigated in this work showed 12 biarmed macrochromosomes and 24 microchromosomes, as well as found by Kasahara *et al.* (1996) for *T. hispidus* individuals from other localities. In their study, Kasahara and colleagues identified a dot-like Y chromosome in male individuals, thus recognizing an XX:XY sexual differentiation mechanism in *T. hispidus* (Kasahara *et al.*, 1996). For the population analyzed in this work, this differentiated chromosome was not observed, probably due to the quality of the cell material observed and the absence of meiotic analysis.

Microsatellite and satellite DNA accumulation may be associated with various genomic aspects, including differentiation of sex chromosomes, with a significant role in the suppression of recombination, degeneration, and heterochromatinization (Pokorná *et al.*, 2011; Matsubara *et al.*, 2006, 2015; Palacios-Gimenez *et al.*, 2017; Ferretti *et al.*, 2020; Crepaldi and Parise-Maltempi, 2020; Zattera *et al.*, 2020). Among lizards, one example of accumulation of repetitive DNA associated with sex chromosome differentiation is provided by the lacertid *Eremias velox*, in which the sex chromosomes are homomorphic, and the W chromosome is highly heterochromatic (Pokorná *et al.*, 2011). Regarding our data, the analyzed microsatellites were distributed

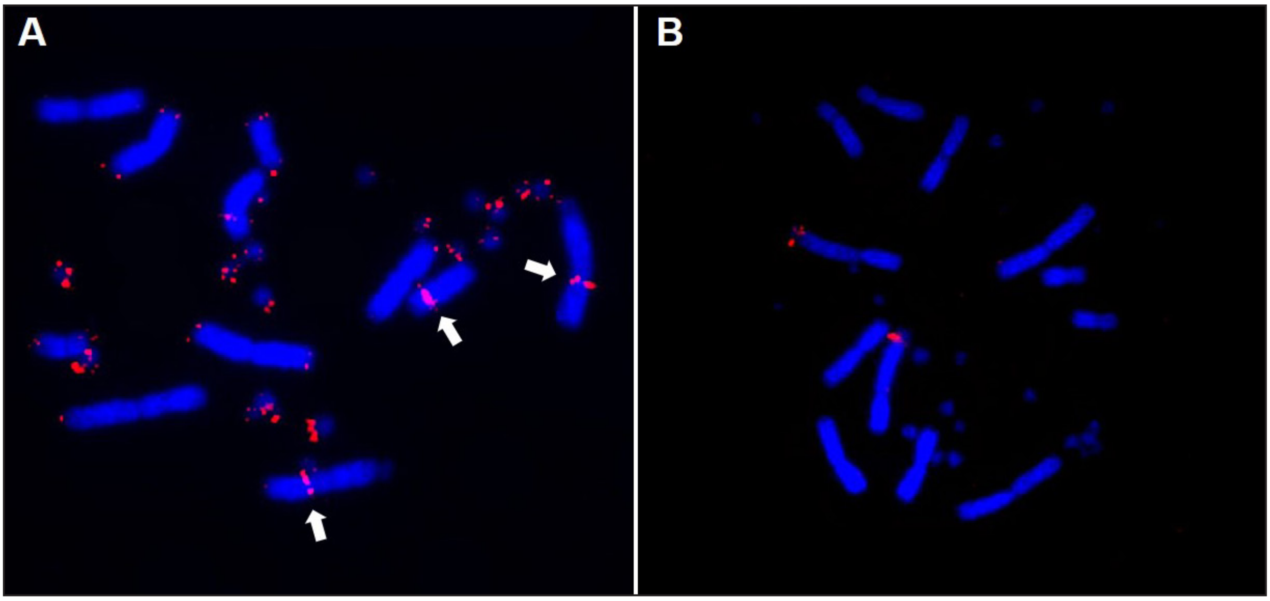

Figure 2. Metaphase chromosomes of *Tropidurus hispidus* hybridized to a telomeric sequence probe (A) and a 18S rDNA probe (B). Arrows in A indicate interstitial telomeric sequences (ITSs).

in different regions of several chromosomes of *T. hispidus*. In addition, male and female individuals analyzed herein did not exhibit differences regarding the distribution of the microsatellite sequences.

In the karyotype of *T. hispidus*, the motifs $(CA)_n$, $(CAA)_n$, $(GA)_n$, $(CAC)_n$, and $(GAG)_n$ were extensively dispersed along the chromosomes, whereas the motif $(GAA)_n$ was accumulated at telomeric and pericentromeric regions of macrochromosomes and some microchromosomes. Similarly, telomeric and pericentromeric blocks of $(GAA)_n$ were previously observed in the lizards *Eremias velox* (Pokorná *et al.*, 2011), *Salvator merianae* (Da Silva *et al.*, 2020a), and *Cyrtodactylus jarujini* (Thongnetr *et al.*, 2021). It has been suggested that repetitive sequences accumulated specifically in the terminal/subterminal region are related to a fundamental role in the chromosomal stabilization and functioning, a common phenomenon in the karyotypes of animals (Oliveira *et al.*, 2017; Cioffi *et al.*, 2011; Xu *et al.*, 2013; Poltronieri *et al.*, 2014; Ruiz-Ruano *et al.*, 2015; Ernetti *et al.*, 2019; Zattera *et al.*, 2020).

Telomeres are regions of repetitive DNA motifs and associated proteins which play a crucial role in maintaining chromosome structure as a single unit, preventing fusions or degeneration, and loss of genetic information during replication events (Blackburn, 2001). Interestingly, despite this remarkable stability, motifs $(TTAGGG)_n$ can also be found at non-terminal positions of chromosomes as interstitial telomeric sequences (ITSs) (Meyne *et al.*, 1990; Lin and Yan, 2008). In addition to usual signals at chromosomal ends, the telomeric probe revealed the presence of ITS at centromeric and pericentromeric regions of chromosome pairs 2 and 4 of *T. hispidus* analyzed herein, possibly due to the occurrence of chromosomal rearrangements, as already described for reptiles (e.g. Pellegrino *et al.*, 2009; Rojo *et al.*, 2014; Rovatsos *et al.*, 2015; Da Silva *et al.*, 2020a; Clemente *et al.*, 2020). This feature is quite interesting and would require further research through more refined genomic analyses to clarify whether the $(TTAGGG)_n$ motif at the centromeres of Tropiduridae lizards has a non-telomeric origin.

In conclusion, it is remarkable that the organization and composition of repetitive sequences in reptiles need clarification not only through physical mapping, which can reveal some aspects of the distribution of these sequences and support their importance in the genome organization and function, but also by refined analyses and studies involving genomic sequencing. To assist in the evolutionary and taxonomic understanding of this group, classical and molecular cytogenetic techniques were used to find potential molecular markers for this group of organisms. Thus, our current data reinforce the importance of increasing the number of chromosomal analyses in the Tropiduridae family, contributing to the understanding of genomic organization and karyotypic evolution in lizards.

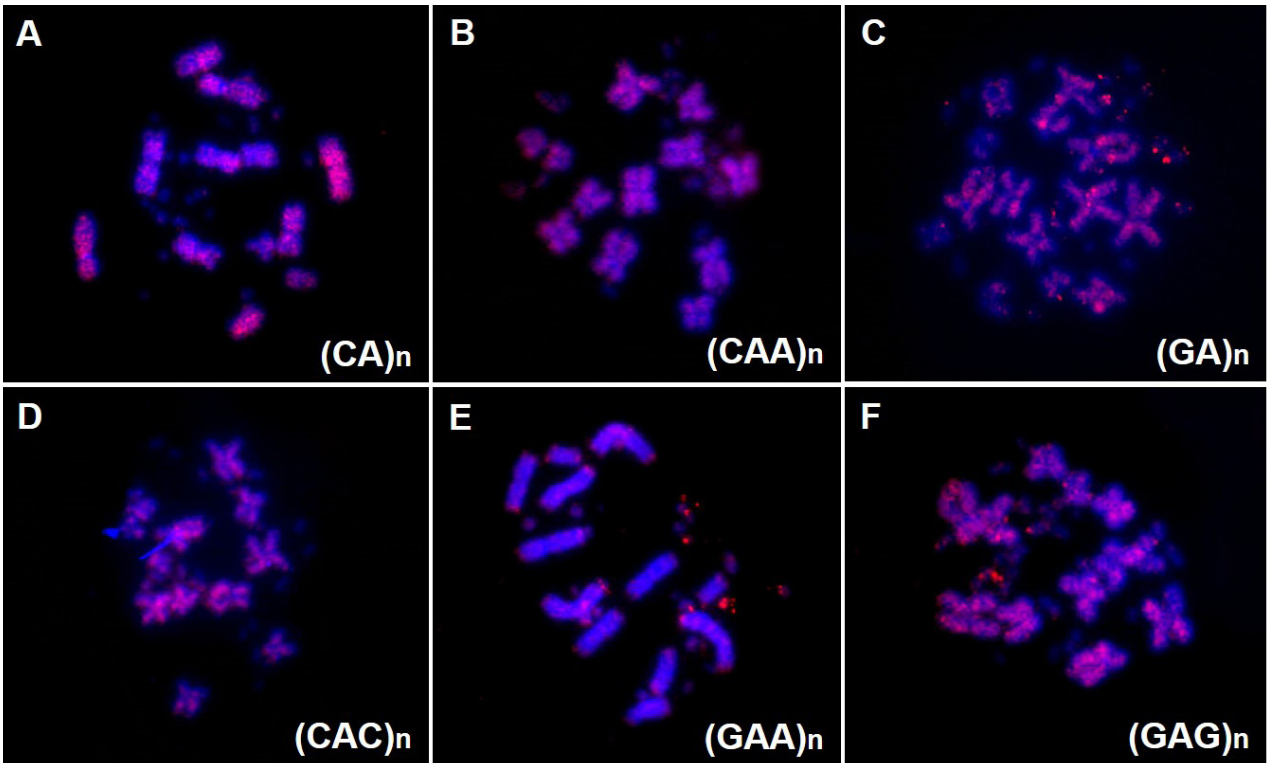

Figure 3. Metaphase chromosomes of *Tropidurus hispidus* after fluorescent *in situ* hybridization with probes for microsatellite motifs.

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