

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

Marcelo João da Silva^{1,2}, Ana Paula de Araújo Vieira², Flávia Manoela Galvão Cipriano², Maria Rita dos Santos Cândido², Tamaris Gimenez Pinheiro², Edivaldo Herculano Correa Oliveira³, Edson Lourenço da Silva⁴

¹ Universidade Estadual Paulista (UNESP), Departamento de Biologia Geral e Aplicada, Instituto de Biociências (IB), Programa de Pós-graduação em Ciências Biológicas (Biologia Celular, Molecular e Microbiologia), Avenida 24A, 1515, Bela Vista, 13506-900, Rio Claro, SP, Brazil.

² Universidade Federal do Piauí (UFPI - CSHNB), Avenida Cícero Eduardo, s/n, Parque de Exposição, 64608-042, Picos, PI, Brazil.

³ Instituto Evandro Chagas (IEC), Rodovia BR-316 km 7, s/n, Levilândia, 67030-000, Ananindeua, PA, Brazil.

⁴ Instituto Federal de Educação, Ciência e Tecnologia do Piauí (IFPI), Campus Picos, Avenida Pedro Marques de Medeiros, s/n, Bairro Pantanal, 64605-500, Picos, PI, Brazil.

Recibido: 20 Abril 2022

Revisado: 19 Agosto 2022

Aceptado: 29 Marzo 2023

Editora Asociada: L. Bolsoni Lourenco

doi: 10.31017/CdH.2023(2022-038)

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

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

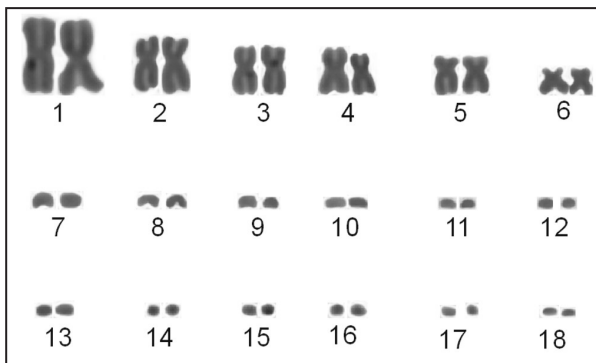


Figure 1. Giemsa-stained karyotype of a female *Tropidurus hispidus* ($2n=36$, 12M+24m). Pairs 1-6 indicate macrochromosomes (M), while pairs 7-18, microchromosomes (m).

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.

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

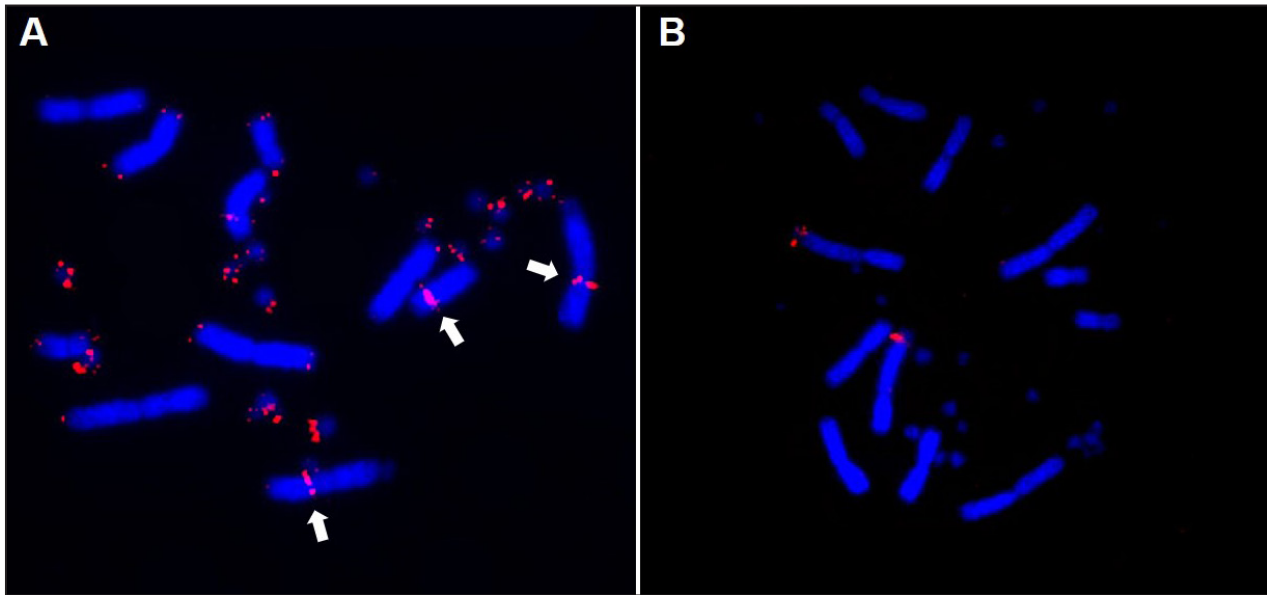


Figure 2. Metaphase chromosomes of *Tropidurus hispidus* hybridized to a telomeric sequence probe (A) and a 18S rDNA probe (B). Arrows in A indicate interstitial telomeric sequences (ITSs).

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.

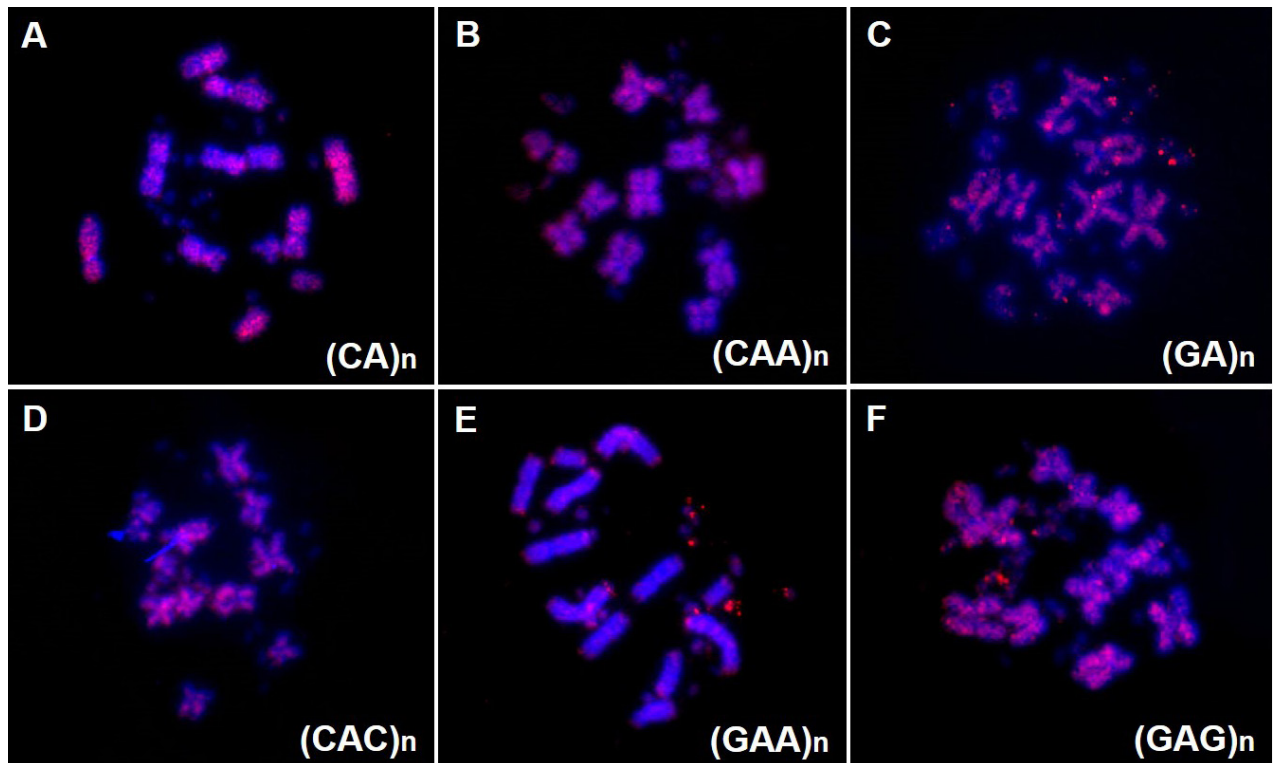


Figure 3. Metaphase chromosomes of *Tropidurus hispidus* after fluorescent *in situ* hybridization with probes for microsatellite motifs.

Acknowledgments

The authors are grateful to Dr. Marcelo de Bello Cioffi (UFSCar) for the donation of the microsatellite probes and to Dra. Ivanete de Oliveira Furo (UFRA) for technical support in FISH experiments. This research was supported by the Program of Support to Scientific and Technological Research (ProAGRUPAR) of the Federal Institute of Education, Science, and Technology of Piauí (IFPI).

Literature cited

- Bertollo, L.A.C.; Takahashi, C.S. & Moreira-Filho O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Brazilian Journal of Genetics* 1: 103-120.
- Biscotti, M.A.; OLMO, E. & HESLOP-HARRISON J.S. 2015. Repetitive DNA in eukaryotic genomes. *Chromosome Research* 23: 415-20.
- Blackburn, E.H. 2001. Switching and signaling at the telomere. *Cell* 106: 661-673.
- Carvalho, A.L.G. 2013. On the distribution and conservation of the South American lizard genus *Tropidurus* Wied Neuwied, 1825 (Squamata: Tropiduridae). *Zootaxa* 3640: 42-56.
- Carvalho, N.D.M.; Arias, F.J.; Da Silva, F.A.; Schneider, C.H. & Gross M.C. 2015. Cytogenetic analyses of five amazon lizard species of the subfamilies Teiinae and Tupinambinae and review of karyotyped diversity in the family Teiidae. *Comparative Cytogenetics* 9: 625-644.
- Carvalho, N.D.M.; Carmo, E.J.; Neves, R.O.; Schneider, C.H. & Gross M.C. 2016. Differential repetitive DNA composition in the centromeric region of chromosomes of Amazonian lizard species in the family Teiidae. *Comparative Cytogenetics* 10: 203-217.
- Carvalho, A.L.G.; Sena, M.A.; Peloso, P.L.V.; Machado, F.A.; Montesinos, R.; Silva, H.R.; Campbell, G. & Rodrigues, M.T. 2016. A New *Tropidurus* (Tropiduridae) from the Semiarid Brazilian Caatinga: evidence for conflicting signal between mitochondrial and nuclear loci affecting the phylogenetic reconstruction of South American Collared Lizards. *American Museum Novitates* 3852: 1-68.
- Chaiprasertsri, N.; Uno, Y.; Peyachoknagul, S.; Prakhongcheep, O.; Baicharoen, S.; Charernsuk, S.; Nishida, C.; Matsuda, Y.; Koga, A. & Srikulnath, K. 2013. Highly species-specific centromeric repetitive DNA sequences in lizards: molecular cytogenetic characterization of a novel family of satellite DNA sequences isolated from the water monitor lizard (*Varanus salvator macromaculatus*, Platynota). *Journal of Heredity* 104: 798-806.
- Charlesworth, B.; Sniegowski, P. & Stephan, W. 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* 371: 215-220.
- Cholak, L.R.; Haddad, C.F.B. & Parise-Maltempi P.P. 2020. Cytogenetic analysis of the genus *Thoropa* Cope, 1865 (Anura-Cycloramphidae) with evolutionary inferences based on repetitive sequences. *Genetics and Molecular Biology* 43: e20190364.
- Cioffi, M.B.; Kejnovsky, E. & Bertollo, L.A.C. 2011. The Chromosomal Distribution of Microsatellite Repeats in the Genome of the Wolf Fish *Hoplias malabaricus*, focusing on the Sex Chromosomes. *Cytogenetics and Genome Research* 132: 289-296.
- Cioffi, M.B.; Martins, C.; Centofante, L.; Jacobina, U. & Bertollo, L.A.C. 2009. Chromosomal variability among allopatric

- populations of Erythrinidae fish *Hoplias malabaricus*: mapping of three classes of repetitive DNAs. *Cytogenetics and Genome Research* 125: 132-141.
- Clemente, L.; Mazzoleni, S.; Pensabene-Bellavia, E.; Augstenová, B.; Auer, M.; Praschag, P.; Protiva, T.; Velenský, P.; Wagner, P.; Fritz, U.; Kratochvíl, L. & Rovatsos, M. 2020. Interstitial telomeric repeats are rare in turtles. *Genes* 11: 657.
- Crepaldi, C. & Parise-Maltempi, P.P. 2020. Heteromorphic sex chromosomes and their DNA content in fish: an insight through satellite DNA accumulation in *Megaleporinus elongatus*. *Cytogenetics and Genome Research* 160: 38-46.
- Da Silva, M.J.; Vieira, A.P.A.; Cipriano, F.M.G.; Santos, M.R.S.; Oliveira, E.H.C.; Pinheiro, T.G. & Silva, E.L. 2020a. The karyotype of *Salvator merianae* (Squamata, Teiidae): analyses by classical and molecular cytogenetic techniques. *Cytogenetics and Genome Research* 160: 94-99.
- Da Silva, M.J.; Destro, R.F.; Gazoni, T.; Narimatsu, H.; Santos, P.S.P.; Haddad, C.F.B. & Parise-Maltempi, P.P. 2020b. Great abundance of satellite DNA in *Proceratophrys* (Anura, Odontophrynidae) revealed by genome sequencing. *Cytogenetics and Genome Research* 160: 141-147.
- Ernetti, J.R.; Gazolla, C.B.; Recco-Pimentel, S.M.; Lucas, E.M. & Bruschi, D.P. 2019. Non-random distribution of microsatellite motifs and (TTAGGG)_n repeats in the monkey frog *Pithecopus rusticus* (Anura, Phyllomedusidae) karyotype. *Genetics and Molecular Biology* 42: 4.
- Ferretti, A.B.S.M.; Milani, D.; Palacios-Gimenez, O.M.; Ruiz-Ruano, F.J. & Cabral-De-Mello, D.C. 2020. High dynamism for neo-sex chromosomes: satellite DNAs reveal complex evolution in a grasshopper. *Heredity* 125: 124-137.
- Garrido-Ramos, M.A. 2017. Satellite DNA: an evolving topic. *Genes* 8:E230.
- Gatto, K.P.; Busin, C.S. & Lourenço, L.B. 2016. Unraveling the sex chromosome heteromorphism of the paradoxical frog *Pseudis tocantins*. *PLoS One* 11: e0156176.
- Gatto, K.P.; Seger, K.R.; Garcia, P.C.A. & Lourenço, L.B. 2019. Satellite DNA Mapping in *Pseudis fusca* (Hylidae, Pseudinae) provides new insights into sex chromosome evolution in Paradoxical Frogs. *Genes* 10: 160.
- Gazoni, T.; Dorigon, N.S.; Da Silva, M.J.; Cholak, L.R.; Haddad, C.F.B. & Parise-Maltempi, P.P. 2021. Chromosome mapping of U2 snDNA in species of *Leptodactylus* (Anura, Leptodactylidae). *Cytogenetics and Genome Research* 161: 63-69.
- Giovannotti, M.; Nisi-Cerioni, P.; Rojo, V.; Olmo, E.; Slimani, T.; et al. 2018. Characterization of a satellite DNA in the genera *Lacerta* and *Timon* (Reptilia, Lacertidae) and its role in the differentiation of the W chromosome. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 330: 83-95.
- Giovannotti, M.; S'khifa, A.; Nisi-Cerioni, P.; Splendiani, A.; Slimani, T.; Fioravanti, T.; Olmo, E. & Caputo-Barucchi, V. 2020. Isolation and characterization of two satellite DNAs in *Atlantolacerta andreanskyi* (Werner, 1929) (Reptilia, Lacertidae). *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 334: 178-191.
- Gonzalez, L.A.; Bonilla, A. & Velasquez, J. 2011. Cariotipo del lagarto *Tropidurus hispidus* (Sauria: Tropiduridae) em el Oriente de Venezuela. *Acta Biológica Colombiana* 16(2): 121-133.
- Guerra, M.S. 1986. Reviewing the chromosome nomenclature of Levan *et al.* *Revista Brasileira de Genética* 9: 741-743.
- Ijdo, J.W.; Wells, R.A.; Baldini, A.; Reeders, S.T. 1991. Improved telomere detection using a telomere repeat probe (TTAGGG)_n generated by PCR. *Nucleic Acids Research* 19: 4780.
- Kasahara S.; Pellegrino, K.C.M.; Rodrigues, M.T. & Yonenaga-Yassuda, Y. 1996. Comparative cytogenetic studies of eleven species of the *Tropidurus torquatus* group (Sauria, Tropiduridae), with banding patterns. *Hereditas* 125: 37-46.
- Kasahara, S.; Yonenaga-Yassuda, Y. & Rodrigues, M.T. 1987. Geographical karyotypic variations and chromosome banding patterns in *Tropidurus hispidus* (Sauria, Iguanidae). *Caryologia* 40: 43-57.
- Kasahara, S.; Yonenaga-Yassuda, Y.; Schincariol, R.A. & L'abbate, M. 1983. Chromosome mechanisms of sex determination, G- and C band patterns, and nucleolus organizer regions in *Tropidurus torquatus* (Sauria: Iguanidae). *Genetica* 60: 151-156.
- Kubat, Z.; Hobza, R.; Vyskot, B. & Kejnovsky, E. 2008. Microsatellite accumulation in the Y chromosome of *Silene latifolia*. *Genome* 51: 350-356.
- Kretschmer, R.; De Oliveira, T.D.; De Oliveira, F.I.; Oliveira Silva, F.A.; Gunski, R.J.; Del Valle Garnerero, A.; Cioffi, M.B.; De Oliveira, E.H.C. & De Freitas, T.R.O. 2018. Repetitive DNAs and shrink genomes: A chromosomal analysis in nine Columbidae species (Aves, Columbiformes). *Genetics and Molecular Biology* 41: 98-106.
- Lin, K.W. & Yan, J. 2008. Endings in the middle: Current knowledge of interstitial telomeric sequences. *Mutation Research* 658: 95-110.
- López-Flores, I. & Garrido-Ramos, M.A. 2012. The repetitive DNA content of eukaryotic genomes. *Genome Dyn* 7: 1-28.
- Matsubara, K.; Tarui, H.; Toriba, M.; Yamada, K.; Nishida-Umehara, C.H.; Agata, K. & Matsuda, Y. 2006. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. *Proceedings of the National Academy of Sciences* 103: 18190-18195.
- Matsubara, K.; Uno, Y.; Srikulnath, K.; Matsuda, Y.; Miller, E. & Olsson, M. 2015. No interstitial telomeres on autosomes but remarkable amplification of telomeric repeats on the W sex chromosome in the sand lizard (*Lacerta agilis*). *Journal of Heredity* 106: 753-757.
- Meyne, J.; Baker, R.J.; Hobart, H.H.; Hsu, T.C.; Ryder, O.A.; Ward, O.G.; Wiley, J.E.; Wurster-Hill, D.H.; Yates, T.L. & Moyzis, R.K. 1990. Distribution of non-telomeric sites of the (TTAGGG)_n telomeric sequence in vertebrate chromosomes. *Chromosoma* 99: 3-10.
- Milani, D.; Ramos, É.; Loreto, V.; Martí, D.A.; Cardoso, A.L.; De Moraes, K.C.M.; Martins, C. & Cabral-De-Mello, D.C. 2017. The satellite DNA AflaSAT-1 in the A and B chromosomes of the grasshopper *Abracris flavolineata*. *BMC Genetics* 18: 81.
- Oliveira, T.D.; Kretschmer, R.; Bertocchi, N.A.; Degrandi, T.M.; Oliveira, E.H.C. & Cioffi, M.B. 2017. Genomic organization of repetitive DNA in Woodpeckers (Aves, Piciformes): implications for karyotype and ZW sex chromosome differentiation. *PLoS One* 12: e169987.
- Palacios-Gimenez, O.M.; Dias, G.B.; Gomes De Lima, L.; Kuhn, G.C.E.S.; Ramos, É.; Martins, C. & Cabral-De-Mello, D.C. 2017. High-throughput analysis of the satelliteome revealed enormous diversity of satellite DNAs in the neo-Y chromosome of the cricket *Eneoptera surinamensis*. *Scientific Reports* 7: 6422.
- Pellegrino, K.C.M.; Yonenaga-Yassuda, Y. & Rodrigues, M.T.

1994. Cytogenetic studies in six species of Tropicuridae (Sauria). *Revista Brasileira de Genética* 17: 401-408.
- Pellegrino, K.C.M.; dos Santos, R.M.L.; Rodrigues, M.T.; Laguna, M.M.; Amaro, R.C. & Yonenaga-Yassuda, Y. 2009. Chromosomal evolution in the Brazilian Geckos of the genus *Gymnodactylus* (Squamata, Phyllodactylidae) from the biomes of Cerrado, Caatinga and Atlantic Rain Forest: evidence of Robertsonian fusion events and supernumerary chromosomes. *Cytogenetics and Genome Research* 127: 191-203.
- Pinkel, D.; Straume, T. & Gray, J.W. 1986. Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences* 83: 2934-2938.
- Pohl, M.; Meštrović, N. & Mravinac, B. 2012. Satellite DNA evolution. *Genome Dynamics* 7: 126-152.
- Pokorná, M.; Kratochvíl, L. & Kejnovský, E. 2011. Microsatellite distribution on sex chromosomes at different stages of heteromorphism and heterochromatinization in two lizard species (Squamata: Eublepharidae: *Coleonyx elegans* and Lacertidae: *Eremias velox*). *BMC Genetics* 12: 90.
- Poltronieri, J.; Marquioni, V.; Bertollo, L.A.C.; Kejnovsky, E.; Molina, W.F.; Liehr, T.; et al. 2014. Comparative chromosomal mapping of microsatellites in *Leporinus* Species (Characiformes, Anostomidae): unequal accumulation on the W chromosomes. *Cytogenetics and Genome Research* 142: 40-45.
- Rojo, V.; Giovannotti, M.; Naveira, H.; Nisi Cerioni, P.; González-Tizón, A.M.; et al. 2014. Karyological characterization of the endemic Iberian rock lizard, *Iberolacerta monticola* (Squamata, Lacertidae): insights into sex chromosome evolution. *Cytogenetics and Genome Research* 142: 28-39.
- Rovatsos, M.; Kratochvíl, L.; Altmanová, M.; Pokorná, M.J. 2015. Interstitial telomeric motifs in squamate reptiles: when the exceptions outnumber the rule. *PLoS ONE* 10: e0134985.
- Ruiz-Ruano, F.J.; Cuadrado, A.; Montiel, E.E.; Camacho, J.P.M. & López-León, M.D. 2015. Next-generation sequencing and FISH reveal uneven and nonrandom microsatellite distribution in two grasshopper genomes. *Chromosoma* 124: 221-234.
- Ruiz-Ruano, F.J.; Navarro-Domínguez, B.; Camacho, J.P.M. & Garrido-Ramos, M.A. 2019. Characterization of the satellitome in lower vascular plants: the case of the endangered fern *Vandenboschia speciosa*. *Annals of Botany* 123: 587-599.
- Santos, R.M.L.; Rodrigues, M.T.; Yonenaga-Yassuda, Y. & Pellegrino, K.C.M. 2008. Differential staining and microchromosomes variation in karyotypes of four Brazilian species of Tupinambinae lizards (Squamata: Teiidae). *Genetica* 134: 261-266.
- Silva, D.M.Z.D.A.; Utsunomia, R.; Ruiz-Ruano, F.J.; Daniel, S.N.; Porto-Foresti, F.; Hashimoto, D.T.; et al. 2017. High-throughput analysis unveils a highly shared satellite DNA library among three species of fish genus *Astyanax*. *Scientific Reports* 7: 12726.
- Thongnetr, W.; Aiumsumang, S.; Kongkaew, R.; Tanomtong, A.; Suwannapoom, C. & Phimphan, S. 2021. Cytogenetic characterisation and chromosomal mapping of microsatellite and telomeric repeats in two gecko species (Reptilia, Gekkonidae) from Thailand. *Comparative Cytogenetics* 15: 41-52.
- Uetz, P.; Freed, P. & Hošek, J. (2022). (eds): The Reptile Database. <http://www.reptile-database.org>, accessed Jan 10, 2022.
- Utsunomia, R.; Silva, D.M.Z.D.A.; Ruiz-Ruano, F.J.; Goes, C.A.G.; Melo, S.; Ramos, L.P.; et al. (2019). Satellitome landscape analysis of *Megaleporinus macrocephalus* (Teleostei, Anostomidae) reveals intense accumulation of satellite sequences on the heteromorphic sex chromosome. *Scientific Reports* 9: 5856.
- Veronese, L.B., Freitas, T.R.O. & Krause, L. 2003. Cytogenetic studies of four Brazilian species of lizards (Squamata, Teiidae). *Caryologia* 56: 107-114.
- Vittorazzi, S.E.; Lourenço, L.B.; Del-Grande, M.L. & Recco-Pimentel, S.M. 2011. Satellite DNA derived from 5S rDNA in *Physalaemus cuvieri* (Anura, Leiuperidae). *Cytogenetics and Genome Research* 134: 101-107.
- Xu, D.; Bao Lou, B.; Bertollo, L.A.C. & Cioffi, M.B. 2013. Chromosomal mapping of microsatellite repeats in the rock bream fish *Oplegnathus fasciatus*, with an emphasis on their distribution in the neo-Y chromosome. *Mol Cytogenet* 6: 12.
- Zattera, M.L.; Gazolla, C.B.; Soares, A.A.; Gazoni, T.; Pollet, N.; Recco-Pimentel, S.M. & Bruschi, D.P. 2020. Evolutionary dynamics of the repetitive DNA in the karyotypes of *Pipa carvalhoi* and *Xenopus tropicalis* (Anura, Pipidae). *Frontiers in Genetics* 11: 637.

