BAG Journal of Basic & Applied Genetics
Vol XXXIII (1): 61-70; July 2022
Journal of the Argentine Society of Genetics

# CYTOGENETIC STUDY IN SAND SPIDERS (SICARIIDAE) FROM THE BRAZILIAN CAATINGA: SEX CHROMOSOME SYSTEM DIVERSITY IN CLOSELY RELATED SPECIES

# ESTUDIO CITOGENÉTICO EN ARAÑAS DE ARENA (SICARIIDAE) DE LA CAATINGA BRASILEÑA: DIVERSIDAD DEL SISTEMA DE CROMOSOMAS SEXUALES EN ESPECIES ESTRECHAMENTE RELACIONADAS

Gimenez-Pinheiro T.[1], Carvalho L.S.[2], Brescovit A.D.[3], Magalhaes I.L.F.[4], Schneider M.C.[5]

[1] Universidade Estadual do Piauí, UESPI, Campus Heróis do Jenipapo, Av. Santo Antônio, s/n, 64280-000, Campo Maior, Piauí, Brasil.

[2] Universidade Federal do Piauí, UFPI, Campus Amílcar Ferreira Sobral, BR 343, km 3.5, 64800-000, Floriano, Piauí, Brasil.

[3] Laboratório de Coleções Zoológicas, Instituto Butantan, Av. Vital Brasil, 1500, 05503-900, São Paulo, São Paulo, Brasil.

[4] División Aracnología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Angel Gallardo 470, C1405DJR, Buenos Aires, Argentina.

[5] Universidade Federal de Mato Grosso, UFMT, Instituto de Biociências, Departamento de Biologia e Zoologia, Av. Fernando Corrêa da Costa, 2367, Bairro Boa Esperança, 78060-900, Cuiabá, Mato Grosso, Brazil.

Corresponding author:
Marielle Cristina Schneider
marielle.mcs@gmail.com
ORCID 0000-0002-2888-6355

**Cite this article as:**
Gimenez-Pinheiro T., Carvalho L.S., Brescovit A.D., Magalhaes I.L.F., Schneider M.C. 2022. CYTOGENETIC STUDY IN SAND SPIDERS (SICARIIDAE) FROM THE BRAZILIAN CAATINGA: SEX CHROMOSOME SYSTEM DIVERSITY IN CLOSELY RELATED SPECIES. BAG. Journal of Basic and Applied Genetics XXXIII (1): 61-70.

Received: 01/02/2022
Revised version received: 02/11/2022
Accepted: 03/15/2022
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.01.05
ISSN online version: 1852-6233

Available online at
www.sag.org.ar/jbag

## ABSTRACT

In this study, we investigated the chromosomes of three species of *Sicarius* spiders from the Brazilian Caatinga, using classical and molecular cytogenetic techniques. Based on the phylogenetic approach, we also discussed about the variation of diploid number, types of sex chromosome system and changes in the localization of ribosomal genes of Scytodoidea. *Sicarius* are Synspermiata spiders that together with the genera *Loxosceles* and *Hexophthalma* constitute the family Sicariidae. In this group, the available cytogenetic data showed a low diploid number range (2n♂=18 to 2n♂=23) and the presence of only multiple sex chromosome systems ($X_1X_2Y$ and $X_1X_20$). Mitotic metaphase cells exhibited 2n♂=16+$X_1X_2Y$ for *Sicarius cariri* and *S. ornatus*, and 2n♂=18+XY for *S. tropicus*. In these species, silver impregnation revealed nucleolar organizer region (Ag-NOR) on the terminal region of pair 1. In *S. ornatus* and *S. tropicus*, the results obtained with fluorescent *in situ* hybridization (FISH) using 18S rDNA probe were similar to Ag-NOR, however in *S. cariri*, the ribosomal sites were localized in the terminal region of the $X_1$ sex chromosome. In this work, we presented the first description of a simple sex chromosome system for Sicariidae, helping to understand how the XY sex chromosome system evolved from the $X_1X_2Y$ system. Additionally, FISH data incongruous with Ag-NOR indicate that the cytogenetic studies in Sicariidae allow investigating the relation between the karyotype evolution and the distribution and the activity of rDNA genes.

Key words: karyotype, mitosis, nucleolar organizer region, rDNA, *Sicarius*

## RESUMEN

En este estudio, investigamos los cromosomas de tres especies de arañas *Sicarius* de la Caatinga brasileña, utilizando técnicas de citogenética clásica y molecular. Usando un enfoque filogenético, también discutimos la variación del número diploide, los tipos de sistema cromosómico sexual y los cambios en la localización de los genes ribosómicos en Scytodoidea. Los *Sicarius* son arañas Synspermiata que, junto con los géneros *Loxosceles* y *Hexophthalma*, constituyen a la familia Sicariidae. En este grupo, los datos citogenéticos disponibles mostraron un rango de número diploide bajo (2n♂=18 a 2n♂=23) y únicamente la presencia de sistemas de cromosomas sexuales múltiples ($X_1X_2Y$ y $X_1X_20$). Las células mitóticas en metafase mostraron 2n♂=16+$X_1X_2Y$ para *Sicarius cariri* y *S. ornatus*, y 2n♂=18+XY para *S. tropicus*. En estas especies, la impregnación de plata reveló la región organizadora nucleolar (Ag-NOR) en la región terminal del par 1. En *S. ornatus* y *S. tropicus*, los resultados obtenidos con la hibridación in situ fluorescente (FISH) utilizando la sonda de ADNr 18S fueron similares a los de Ag-NOR, sin embargo, en *S. cariri* los sitios ribosomales se localizaron en la región terminal del cromosoma sexual $X_1$. En este trabajo, presentamos la primera descripción de un sistema cromosómico sexual simple para Sicariidae, ayudando a entender cómo el sistema cromosómico sexual XY evolucionó a partir del sistema $X_1X_2Y$. Además, los datos de FISH incongruentes con Ag-NOR indican que los estudios citogenéticos en Sicariidae permiten investigar la relación entre la evolución del cariotipo y la distribución y la actividad de los genes de ADNr.

Palabras clave: cariotipo, mitosis, región organizadora nucleolar, ADNr, *Sicarius*

## INTRODUCTION

The spider family Sicariidae is considered of medical importance in the world (Lotz, 2012), including sedentary species, which can be ground-dwelling hunters or web-weavers (Dias *et al.,* 2010). Sicariidae includes 171 species distributed into three genera: *Hexophthalma* composed of eight species, *Sicarius*, with 21 species, and *Loxosceles*, the most diversified genera with 142 representatives (World Spider Catalog, 2021). This latter genus is well known due to the toxicity of its venom, causing skin necrosis, renal failure and haemolysis (Silva *et al.*, 2004; Vetter, 2008). *Hexophthalma* spiders occur only in southern Africa while *Loxosceles* presents widest distribution, with species described in America, Africa, Mediterranean Europe and Asia; however, the largest diversity of species is recorded in the American continent (World Spider Catalog, 2021). *Sicarius* is distributed in South and Central America and is restricted to xeric habitats, mainly deserts and tropical dry forests (Magalhaes *et al.*, 2013).

For many years, in Brazil only one *Sicarius* species was known, *S. tropicus* (Mello-Leitão, 1936). However, recently Magalhaes *et al.* (2013, 2017) described other species from this country, namely *S. boliviensis* Magalhaes, Brescovit & Santos, 2017, *S. cariri* Magalhaes, Brescovit & Santos, 2013, *S. diadorim* Magalhaes, Brescovit & Santos, 2013, *S. jequitinhonha* Magalhaes, Brescovit & Santos, 2017, *S. ornatus* Magalhaes, Brescovit & Santos, 2013, and *S. saci* Magalhaes, Brescovit & Santos, 2017. The monophyly of Sicariidae is well supported by morphological (Platnick *et al.*, 1991; Binford *et al.*, 2008; Labarque y Ramírez, 2012; Magalhaes *et al.*, 2013, 2017) and molecular data (Wheeler *et al.*, 2017 *contra* Binford *et al.*, 2008). Some characteristics considered as synapomorphies for sicariids are modifications in chelicerae setae, tarsal claws, abdominal entapophysis, and the venom protein sphingomyelinase D, which is responsible for the envenomation symptoms (Binford y Wells, 2003; Magalhaes *et al.*, 2017).

Sicariidae belongs to the monophyletic superfamily Scytodoidea composed by (Sicariidae (Drymusidae + Periogopidae) (Ochyroceratidae + Scytodidae))) (Labarque y Ramírez, 2012; Wheeler *et al.*, 2017). In this group, Scytodidae is the most diverse, including a total of five genera and 245 known species (World Spider Catalog, 2021), but only five of them belonging to *Scytodes* were analyzed from the cytogenetic point of view (Araujo *et al.*, 2021). The scytodids present a high variability in diploid number, from 2n♂=13 to 2n♂=31, but a simple and conserved sex chromosome system of the X0 type. The exception is *Scytodes globula* Nicolet, 1849 that revealed an intraspecific variation due to the occurrence of X0 and $X_1X_2$0 systems (Diaz y Saez, 1966; Rodríguez-Gil *et al.*, 2002; Araujo *et al.*, 2008).

Ochyroceratidae possess 168 species described into 10 genera, but only a North American undetermined species of *Ochyrocera* was cytogenetically analysed, exhibiting 2n♂=13 and X0 sex chromosome system (Král *et al.*, 2006). The family Drymusidae includes 17 species with chromosomal data only for *Izithunzi capense* (Simon, 1893), from South Africa, with 2n♂=37+$X_1X_2$Y (Král *et al.*, 2006). Periegopidae is known only by three species from Queensland and New Zealand (World Spider Catalog, 2021), and there are no cytogenetic data for this family.

The family Sicariidae has karyotype information for 15 representatives, showing low diversity in the diploid number (2n♂=18 to 2n♂=23) and the occurrence of only multiple sex chromosome systems of the $X_1X_2$Y and $X_1X_2$0 types (Araujo *et al.*, 2021). *Hexophthalma* only has the diploid number 2n=20 described for females of an undetermined species (Král *et al.*, 2019). The genus *Loxosceles* presents 12 species chromosomally characterized, in which the following diploid numbers were identified: 2n♂=18 in *L. reclusa* Gertsch & Mulaik, 1940; 2n♂=19 in *L. spinulosa* Purcell, 1904; 2n♂=20 in *L. rufipes* (Lucas, 1834); 2n♂=20-21 in *L. rufescens* (Dufour, 1820); 2n♂=23 in *L. amazonica* Gertsch, 1967, *L. gaucho* Gertsch, 1967, *L. hirsuta* Mello-Leitão, 1931, *L. intermedia* Mello-Leitão, 1934, *L. laeta* (Nicolet, 1849), *L. puortoi* Martins, Knysak & Bertani, 2002, *L. similis* Moenkhaus, 1898 and *L. variegata* Simon, 1897. All these species showed $X_1X_2$Y sex chromosomes system, except *L. rufipes* and *L. reclusa* that exhibited $X_1X_2$0 system (Beçak y Beçak, 1960; Diaz y Saez, 1966; Hetzler, 1979; Silva, 1988; Tugmon *et al.*, 1990; Oliveira *et al.*, 1996, 1997; Silva *et al.*, 2002; Král *et al.*, 2006; Kumbiçak, 2014; Araujo *et al.*, 2020).

In the genus *Sicarius*, only two species were investigated, *S. tropicus* (2n♂=19, $X_1X_2$Y) from Brazil and an undetermined species (2n=21, $X_1X_2$Y) from Cusco, Peru (Franco y Andía, 2013; Araujo *et al.*, 2021), more likely to be *Sicarius boliviensis*, owing to the sampling locality (Magalhaes *et al.*, 2017). Nevertheless, the cytogenetic data of *S. tropicus* could be considered preliminary because the karyotype information is restricted to a brief description of diploid number and sex chromosome system (Franco y Andía, 2013).

A cytogenetic analysis of three *Sicarius* species from the Brazilian fauna was accomplished in the present study, using standard staining, silver impregnation to reveal the active nucleolar organizer regions (NORs), and fluorescent *in situ* hybridization (FISH) with 18S rDNA probe to map the number and localization of the major ribosomal genes. Among the 21 Scytodoidea spiders karyotyped, only 10 species were examined regarding to the NOR distribution (Král *et al.*, 2006; Araujo *et al.*, 2008, 2020). Additionally, based on the phylogenetic approach, we discussed about the chromosome evolution of Scytodoidea, focusing in the variation of diploid number, types of sex chromosome system and change in the localization of ribosomal genes.

## MATERIALS AND METHODS

A sample of 35 specimens was analyzed in this work. The data concerning the number of individuals and the collection localities in Brazil are shown in Table 1. The vouchers were deposited in the arachnid collection of the Instituto Butantan, São Paulo, (IBSP; curator A.D. Brescovit); Coleções Taxonômicas of the Universidade Federal de Minas Gerais, Belo Horizonte (UFMG; curator A.J. Santos), and Coleção de História Natural of the Universidade Federal do Piauí, Floriano (CHNUFPI; curator L.S. Carvalho), in Brazil.

The cytological preparations were obtained following the procedures of Araujo *et al.* (2005). The chromosome slides were stained with 3% Giemsa solution (3% commercial Giemsa solution and 3% phosphate buffer pH 6.8, in distilled water), silver-impregnated (Howell y Black, 1980) to detect the NORs and submitted to FISH with 18S rDNA probes to localize the major ribosomal gene. The morphological classification of chromosomes followed the nomenclature proposed by Levan *et al.* (1964).

The 18S rDNA probes were obtained by PCR using the DNA of *Physocyclus globosus* (Taczanowski, 1874) (Pholcidae) and the primers 18S-F 5' CGAGCGCTTTTATTAGACCA and 18S-R 5' GGTTCACCTACGGAAACCTT (Forman *et al.*, 2013). Probes were labeled with 11-dUTP-digoxigenin by PCR. The FISH technique was performed according Pinkel *et al.* (1986). The chromosomal DNA was denatured in 70% formamide for 5 min at 70º C and the hybridization solution was denatured in a termal cycler for 10 min at 95ºC. Probes were detected with anti-digoxigenin antibody conjugated to rhodamine. Chromosome spreads were counterstained with 4'-6-diamidino-2-phenylindole (DAPI) and the slides were mounted with antifading solution. The images were captured using a Zeiss Imager A2 microscope, coupled to a digital camera and the Axio Vision software.

The ancestral condition of diploid number, sex chromosome system and number of rDNA sites was reconstructed in Mesquite (Maddison y Maddison, 2011), using the maximum parsimony approach and the phylogenetic proposal of Wheeler *et al.* (2017). The chromosome data were obtained from the present study and spider cytogenetic database (Araujo *et al.*, 2021).

**Table 1.** *Sicarius* species cytogenetically analyzed in this work, including the number of specimens and collection localities in Brazil. PI=state of Piauí; SE=state of Sergipe; PB=state of Paraíba.

| Species | Number of individuals | Locality |
|---|---|---|
| *Sicarius cariri* | 3♂/1♀ | Parque Nacional da Serra da Capivara (8°49'48.0"S, 42°33'16.0"W), São Raimundo Nonato, PI |
| | 1♀ | (7°9'39.4"S, 41°28'2.5"W), Picos, PI |
| | 2♀ | Horto Florestal (4°16'19.0"S, 41°41'18.9"W), Piripiri, PI |
| | 2♂ | Povoado Saquinho (2°46'2.5"S, 41°48'19.3"W), Ilha Grande do Piauí, PI |
| | 2♂ | Parque Nacional da Serra das Confusões (8°56'16.9"S, 43°51'48.1"W), Cristino Castro, PI |
| | 15♂/1♀ | Parque Municipal Pedra de Castelo (5°12'5.9"S, 41°41'14.0"W), Castelo do Piauí, PI |
| *Sicarius ornatus* | 2♂ | Parque Nacional da Serra de Itabaiana (10°44'57.3"S, 37°20'20.1"W), Itabaiana, SE |
| *Sicarius tropicus* | 3♂ | Reserva Particular de Patrimônio Natural Fazenda Almas (7°23'16.9"S, 36°48'31.8"W), São José dos Cordeiros, PB |
| | 3♂ | Parque Municipal Pedra de Castelo (5°12'5.9"S, 41°41'14.0"W), Castelo do Piauí, PI |

## RESULTS

### *Chromosome characterization*

Mitotic metaphase cells of male and female specimens of *S. cariri* showed the diploid number and sex chromosome system 2n=16+$X_1X_2Y$ and 2n=16+$X_1X_1X_2X_2$, respectively (Fig. 1A-B). All the chromosomes presented metacentric morphology, with exception of submetacentric pair 2. Regarding to the size, the autososomal chromosomes could be classified into three categories: large (pairs 1 and 2), medium (pairs 3 and 4) and small (pairs 5 to 8). In spermatogonial cells, the $X_1$ and Y sex chromosome were easily identified as unpaired elements, which corresponded to the largest and smallest chromosomes of the karyotype. The $X_2$ chromosome showed an intermediary size between the 2nd and 3rd autosomal pairs (Fig. 1A).

*Sicarius ornatus* also presented 2n♂=19. In the karyotype, three unpaired chromosomes were identified, which were similar to the sex chromosomes of *S. cariri*. Thus, *S. ornatus* should also display a $X_1X_2Y$ sex chromosome system. However, in this species, all autosomal pairs revealed metacentric morphology, the $X_1$ and $X_2$ sex chromosomes were submetacentric and the Y was a tiny acrocentric chromosome (Fig. 1C). The autosomal pair 1 exhibited large size, compared to the medium-sized pairs 2 to 5 and the smallest elements of the karyotype, pairs 6 to 8. The $X_1$ chromosome presented a similar size to the pair 1, the $X_2$ chromosome was larger than the pair 2 and the Y was the smallest chromosome of the karyotype (Fig. 1C).

In *S. tropicus*, the mitotic metaphase cells evidenced 2n♂=20, with two unpaired chromosomes, one large and other small-sized. The karyotype comparison with *S. cariri* and *S. ornatus* and the analysis of meiotic cells permitted us interpreted these unpaired elements as X and Y sex chromosomes. In *S. tropicus*, all chromosomes presented metacentric morphology. The pair 1 was

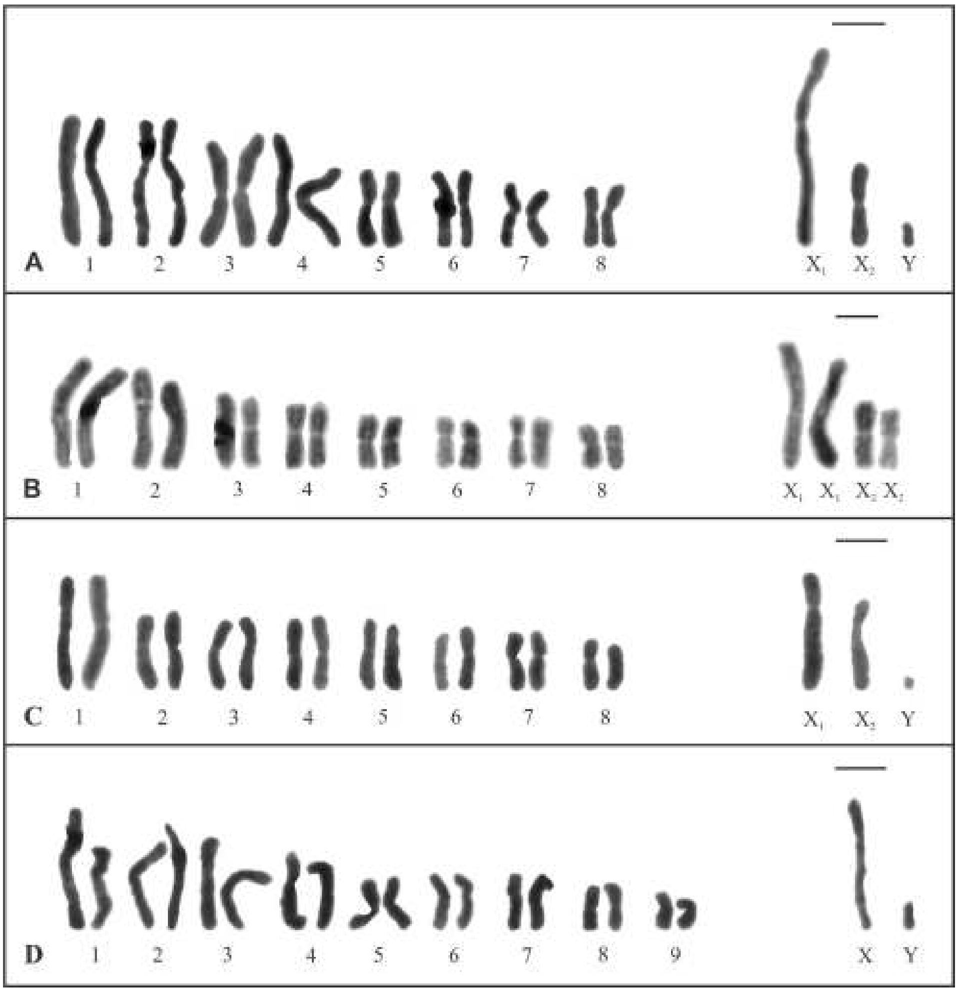


**Figure 1.** Karyotypes of three *Sicarius* species. **A-B**. *Sicarius* cariri with 2n♂=16+$X_1X_2Y$ and 2n♀=16+$X_1X_1X_2X_2$, respectively. **C.** *Sicarius ornatus*, 2n♂=16+$X_1X_2Y$. **D.** *Sicarius tropicus*, 2n♂=18+XY.
In all species, the chromosomes were predominantly metacentrics. Scale bar=5μm.

large-sized, the pairs 2, 3 and 4 medium-sized and the pairs 5 to 9 small-sized. The X and Y sex chromosomes corresponded to the largest and the smallest elements of the karyotype, respectively (Fig. 1D).

The analysis of meiotic cells of *S. cariri* and *S. tropicus* revealed, in the pachytene, autosomal chromosomes completely paired and a single and very small element, interpreted as Y chromosome (Fig. 2A-B). For both species, the X sex chromosomes were not identified in this meiotic substage. Diplotene cells of *S. tropicus* presented 9 autosomal bivalents, with up to two interstitial or terminal chiasmata, and one heteromorphic bivalent, formed by the end-to-end paired XY chromosomes (Fig. 2C). Nuclei in metaphase II of this species showed the haploid sets n=9+X and n=9+Y (Fig. 2D).

Silver-impregnated mitotic metaphase nuclei of the three *Sicarius* species revealed active NORs on the long arm terminal region of pair 1 (Fig. 3A-F). In *S. cariri*, the 18S rDNA sites were located in the long arm terminal region of the $X_1$ sex chromosome (Fig. 4A). In this species, the incongruence between the results of Ag-NOR and FISH were observed among the cells of a same individual as well as in cells of different specimens. In *S. ornatus* and *S. tropicus*, the ribosomal cistrons occurred only in the long arm terminal region of the 1st autosomal pair (Fig. 4B-E), confirming the results of silver impregnation.

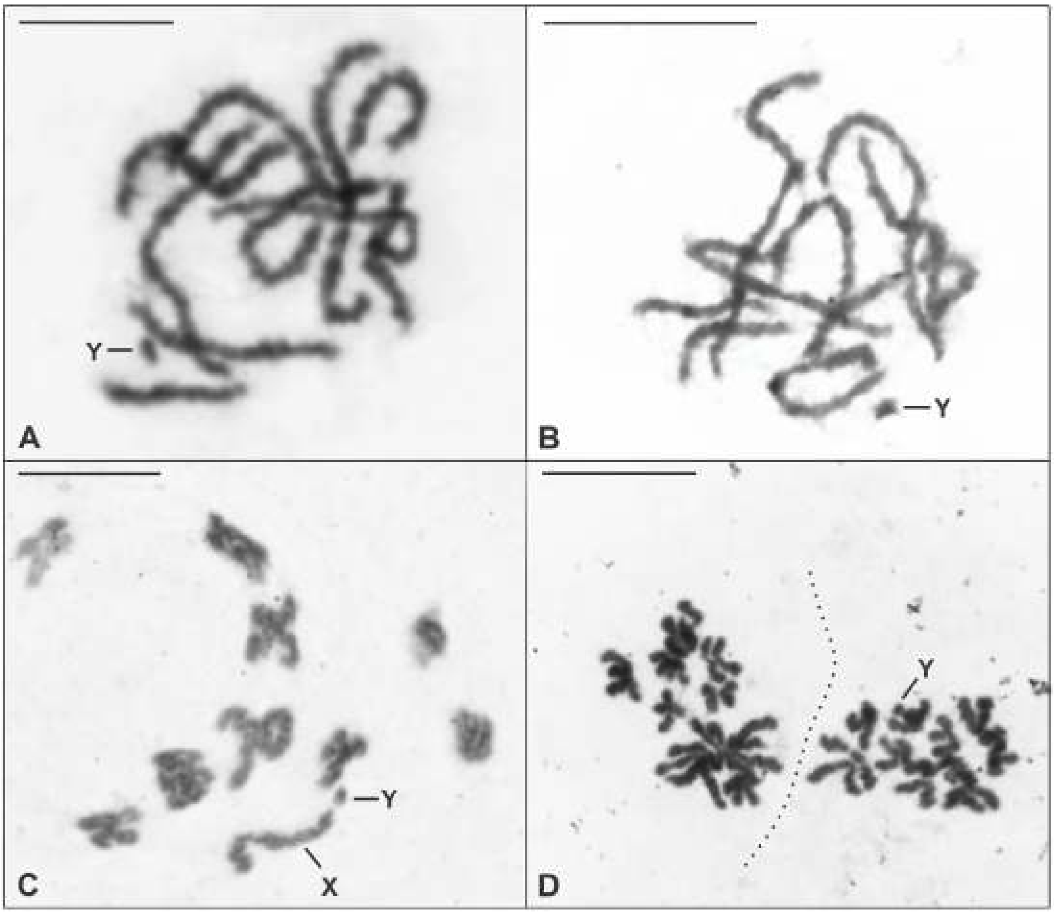


**Figure 2.** Testicular cells of *Sicarius cariri* (A) and *Sicarius tropicus* (B-D) stained with Giemsa. **A-B**. Pachytene nuclei, showing the univalent and very small Y chromosome. **C.** Diplotene with nine autosomal bivalents and one heteromorphic XY bivalent. Note the end-to-end association between the sex chromosomes. **D.** Metaphase II cells, with n=9+X (left) and n=9+Y (right). Scale bar=10µm.

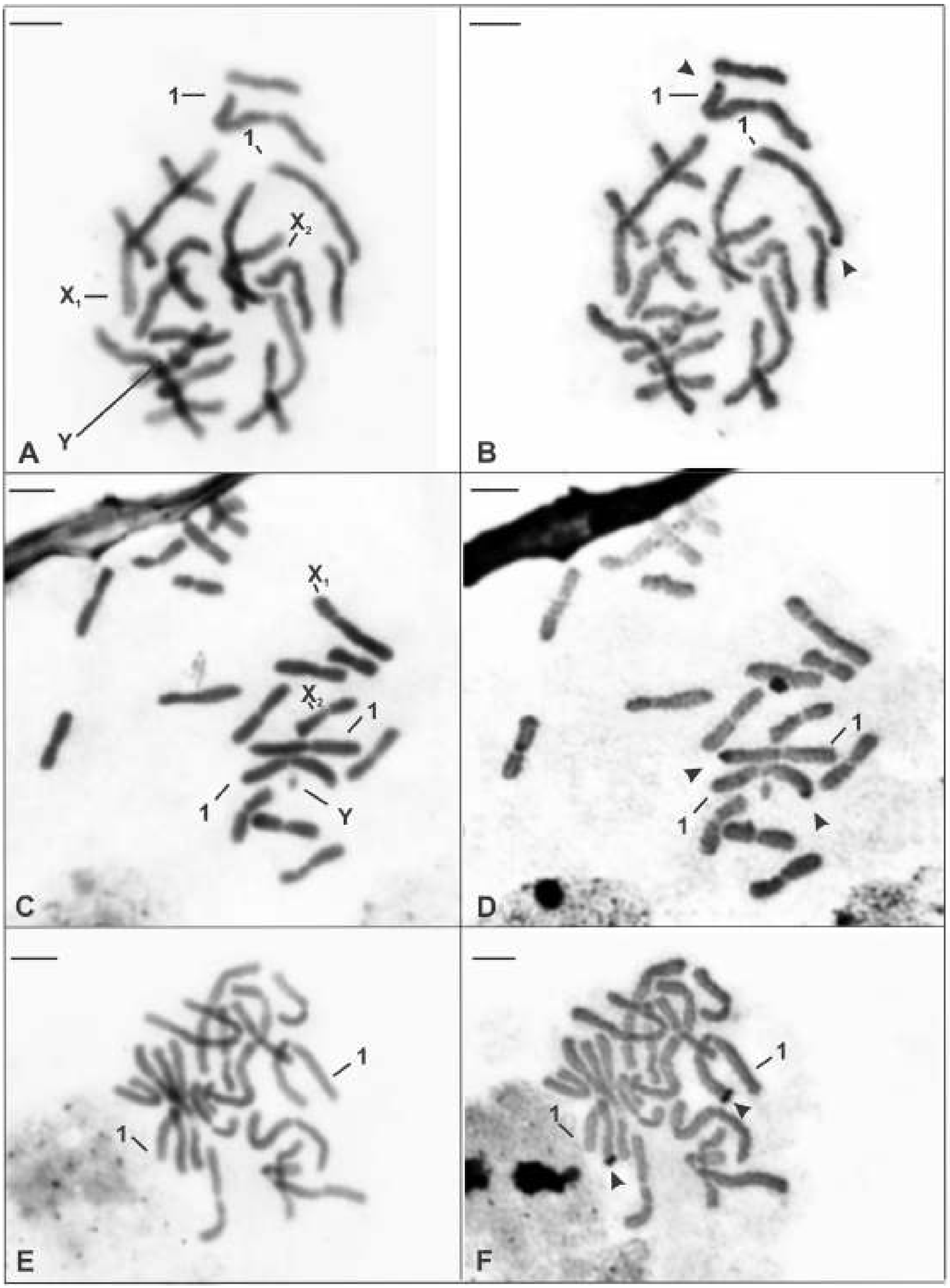


**Figure 3.** Mitotic metaphase cells of *Sicarius* species submitted to Giemsa-staining (A, C, E) and silver impregnation (B, D, F) to reveal the nucleolar organizer regions (arrowhead). **A-B**. *Sicarius cariri*. **C-D.** *Sicarius ornatus*. **E-F**. *Sicarius tropicus*. The cells showed in C and D are with incomplete diploid set. Scale bar=5μm.

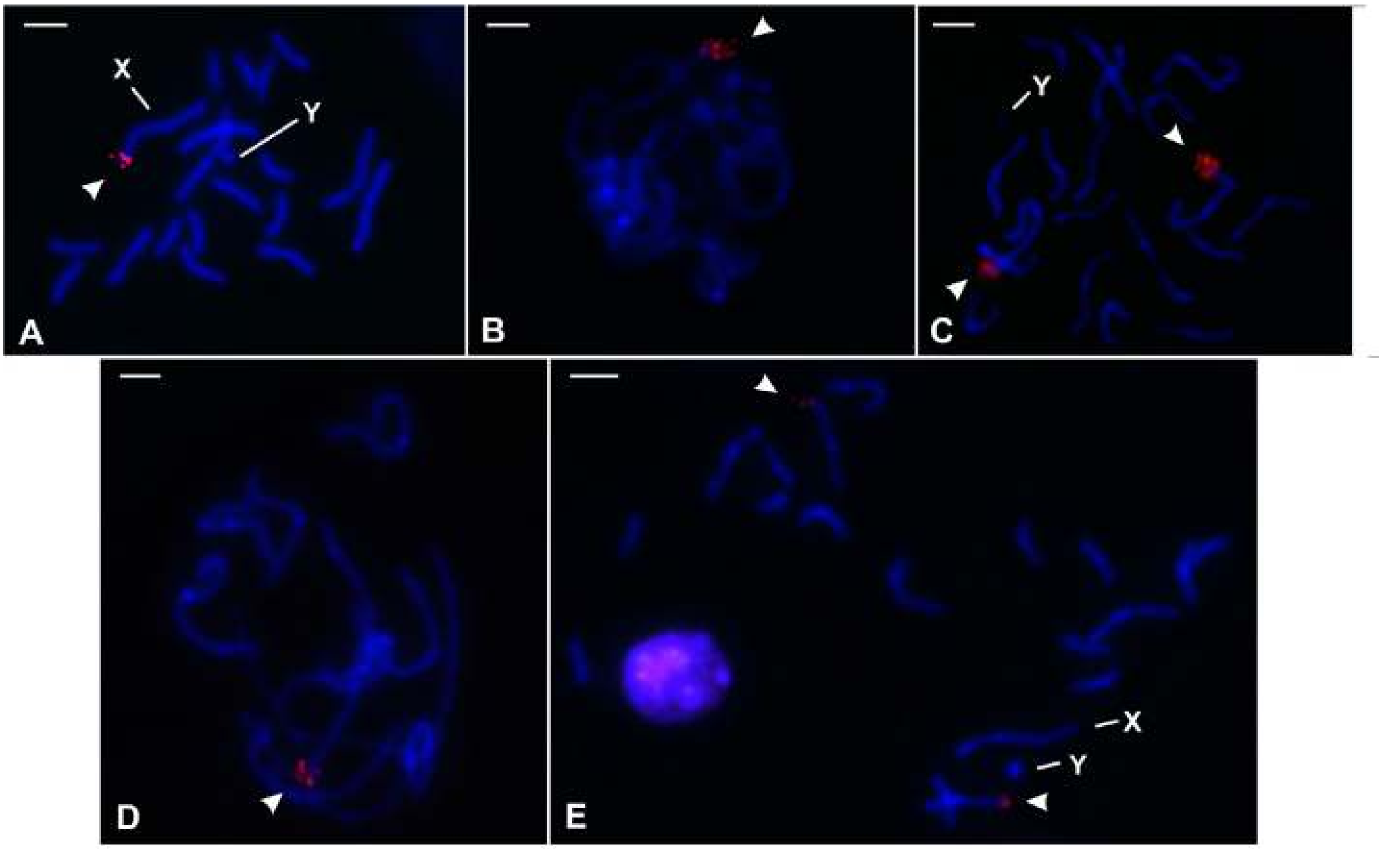


**Figure 4.** Spermatogonial cells of *Sicarius* species after fluorescent in situ hybridization with 18S rDNA probe. **A.** Mitotic metaphase of *Sicarius cariri,* indicating rDNA site (arrowhead) in the $X_1$ chromosome. **B-C.** Pachytene and mitotic metaphase of *Sicarius ornatus*. Note the bright signal (arrowhead) in the terminal region of one bivalent (B) and in pair 1 (C). **D-E.** Pachytene and mitotic metaphase of *Sicarius tropicus* exhibiting rDNA (arrowhead) in the terminal region of one bivalent (D) and in the 1st autosomal pair (E). Scale bar=5µm.

### *Chromosome evolution*

The maximum parsimony analyses revealed 2n=18-22 as the ancestral autosomal number for Scytodoidea and Sicariidae (Fig. 5A). Overall, the karyotype evolution occurred through independent decreased in autosomal number, with the exception of two species of Scytodidae (*Scytodes fusca* - 30 autosomes and *Scytodes* sp. – 26 chromosomes, without description of the sex chromosome system) and Drymusidae (*Izithunzi capense*, 34 autosomes).

The presence of sex chromosome system including two X chromosome and one Y chromosome ($X_1X_2Y$) seems to be the ancestral state for Sicariidae and the clade composed by Sicariidae (Drymusidae + Periogopidae) (Fig. 5B, C). On the other hand, for Ochyroceratidae + Scytodidae, the X0 sex chromosome system is the shared character (Fig. 5B, C). The only exception is an unidentified species of the genus *Scytodes*, in which the sex chromosome system was not described. Within Sicariidae, the only change in the number of X sex chromosome was reported in *S. tropicus* with a XY sex chromosome system. The loss of Y chromosome was recorded only in *L. reclusa* and *L. rufipes*.

Despite the low number of species characterized, the presence of three or four rDNA sites seems to be the ancestral condition for Scytodoidea (Fig. 5D). However, this state was frequently changed during the evolution of this group. In *L. amazonica* and *L. puortoi*, these changes involved the increase of the number of major rDNA cistrons while in *Sicarius* species seems to be occurred a decrease in the number of these sites. The analyses also showed that in Ochyroceratidae + Scytodidae and *Sicarius* species, the ancestral rDNA number is lower than those observed in Scytodoidea.

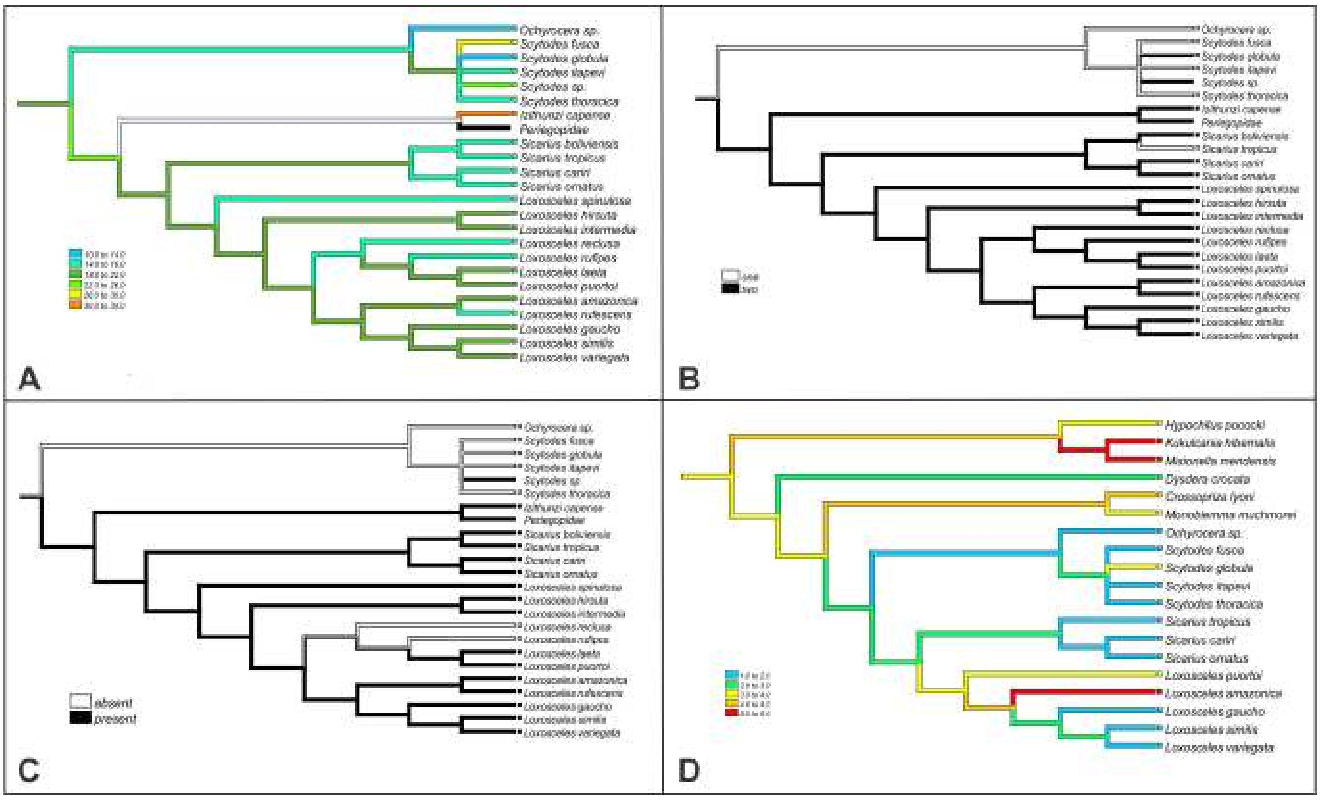


**Figure 5.** Chromosome evolution in Scytodoidea spiders obtained after Mesquite analysis. **A.** Autosomal number. **B.** Number of X sex chromosome. **C.** Presence of sex chromosome system including a Y chromosome. **D.** Number of chromosomes with NOR or rDNA sites.

## DISCUSSION

The diploid number 2n=19, the $X_1X_2Y$ sex chromosome system and the chromosomal morphology predominantly metacentric herein observed in *S. cariri* and *S. ornatus* are similar to those previously described for *S. tropicus* and only one species of the genus *Loxosceles*, *L. spinulosa* (Král *et al.*, 2006; Araujo *et al.*, 2020). Additionally, the $X_1X_2Y$ sex chromosome system verified in *S. cariri* and *S. ornatus* is the most common in Sicariidae, occurring in 12 out of the 15 species cytogenetically characterized so far (Araujo *et al.*, 2020). The tendency of decreasing of the diploid number verified in some Scytodoidea species is the main mechanism of chromosome evolution for spiders and has been reported in many studies accomplished with related species (Stávale *et al.*, 2010; Araujo *et al.*, 2020; Ávila Herrera *et al.*, 2021). In an elegant cytogenetic work with many Pholcidae spiders, in which data of molecular and paleontological studies were discussed, Ávila Herrera *et al.* (2021) suggested that the $X_1X_2Y$ sex chromosome system possesses an ancient origin in spiders and could have arise before the emergence of Araneomorphae lineage.

The karyotype found here for *S. tropicus* (2n=18+XY) differed from that registered for other population of this same species (2n=16+$X_1X_2Y$) (Araujo *et al.*, 2021), and the description of a simple sex chromosome system of the XY type is original for Sicariidae. The high similarity regarding to the size of the Y chromosome among the *Sicarius* species having the $X_1X_2Y$ and XY systems indicates that the evolution of the XY system occurred through rearrangements involving only the X chromosome. The XY system probably had origin from the $X_1X_2Y$ system, in which the ancestral and metacentric $X_1$ and $X_2$ chromosomes were pericentrically inverted, originating subtelo-acrocentric chromosomes, such as those verified in *Sicarius* sp. (Franco y Andía, 2013). In a subsequent event, the $X_1$ and $X_2$ chromosomes were fused, converting the $X_1X_2Y$ into a XY system. This hypothesis regarding XY sex chromosome evolution was proposed by Král *et al.* (2006) and Ávila Herrera *et al.* (2021), analyzing the behavior of the XY sex chromosomes during the meiosis of *Diguetia albolineata* (O. Pickard-Cambridge, 1895) (Diguetidae) and *Wugigarra* sp., (Pholcidae) respectively. In these species as well as in *S. tropicus* analyzed here, the X and Y chromosomes exhibited only one end-to-end association during prophase I, without the presence of chiasma. The present study in *Sicarius* species filled in an important gap in the hypothesis of Král *et al.* (2006)

about the evolution of sex chromosomes systems in basal clades of Araneomorphae, taking into account that the hypothetic $X_1X_2Y$ system with subtelo-acrocentric $X_1$ and $X_2$chromosomes was exclusively observed in *Sicarius* sp. (Franco y Andía, 2013).

The differences related to diploid number and sex chromosome system observed in *S. tropicus* (present study; Araujo *et al.*, 2021) may represent an interpopulational variation, indicating that the karyotype 2n=18+XY is not well established in all populations of this species or it had an independent origin in the populations analyzed by us. Magalhaes *et al.* (2014), performing a phylogeographic study in *S. cariri*, using sequence data of nuclear and mitochondrial genes, revealed highly structured populations, which might be evolving independently. It is possible that *S. tropicus* populations are also strongly structured geographically, which could explain the differences in the karyotypes. Alternatively, the specimens initially described by Araujo *et al.* (2021) as *S. tropicus* could correspond to another species of the genus *Sicarius*, considering that the cytogenetic study accomplished by Araujo preceded the taxonomical and systematic revision of the genus *Sicarius* (Magalhaes *et al.*, 2013, 2017).

The supposed stability of number and localization of NORs in spiders has knocked down with the increase of cytogenetic studies. In an analysis of NORs in 30 Pholcidae spiders, Ávila Herrera *et al.* (2021) revealed a great diversity of number of this site, which can occur in autosomes and/or X sex chromosome. The results obtained herein using FISH with rDNA probe only in three *Sicarius* species revealed the presence of ribosomal cistrons in autosomes (*S. ornatus* and *S. tropicus*) and $X_1$ sex chromosome (*S. cariri*). It is interesting to emphasize that this difference of localization of rDNA in autosome/sex chromosome occurs in species with similar karyotype characteristics, indicating that the changes involving the ribosomal genes can be independent of the differentiation of the sex chromosome system. In *S. cariri*, the localization of active NORs and 18S rDNA showed incongruous data, considering that the silver-impregnated regions were visualized on the terminal sites of the 1st autosomal pair, such as in *S. ornatus* and *S. tropicus*, but the FISH evidenced a bright signal in the terminal region of the $X_1$ sex chromosome. Therefore, in *S. cariri* the silver impregnation might have evidenced false Ag-NORs, taking into account that this technique reveals the NORs indirectly. This occurs due to the affinity of the silver nitrate by acidic proteins associated with the rRNAs or heterochromatic regions (Sanchez *et al.*, 1995; Lorite *et al.*, 1997; Dobigny *et al.*, 2002; Kasahara, 2009; Kavalco *et al.*, 2009; Reis *et al.*, 2012). On the other hand, the impregnation of the terminal region of pair 1 of *S. cariri*, which is certainly carrier of 18S rDNA genes in the two other closely related species, *S. ornatus* and *S. tropicus*, might suggest the presence of cryptic NORs in *S. cariri*, such as those reported by Cabrero y Camacho (2008) in some grasshopper species. The silver impregnation on pair 1 of *S. cariri* can represent a vestigial locus of rDNA gene for this species, which was translocated to the $X_1$ sex chromosome; this vestigial rDNA is very small to be detected by the FISH technique but it retains its transcriptional activity.

In conclusion, the data shown herein expanded the knowledge of the karyotype diversity already registered for sicariid spiders. Moreover, we identified an intriguing variation when the results of Ag-NOR and FISH were compared. Therefore, the Scytodoidea spiders are not only interesting for cytogenetic studies due to the variability in the sex chromosome system, but also because they are suitable for investigating karyotype evolution in spiders and its relationship to the distribution and activity of rDNA genes.

## BIBLIOGRAPHY

Araujo D., Brescovit A.D., Rheims C.A., Cella D.M. (2005) Chromosomal data of two pholcids (Araneae, Haplogynae): a new diploid number and the first cytogenetical record for the new world clade. J. Arachn. 33: 591-596.

Araujo D., Rheims C.A., Brescovit A.D., Cella D.M. (2008) Extreme degree of chromosome number variability in species of the spider genus *Scytodes* (Araneae, Haplogynae, Scytodidae). J. Zool. Syst. Evol. Res. 46: 89-95.

Araujo D., Schneider M.C., Zacaro A.A., Oliveira E.G., Martins R., Brescovit A.D., Knysak I., Cella D.M. (2020) Venomous *Loxosceles* species (Araneae, Haplogynae, Sicariidae) from Brazil: 2n♂ = 23 and $X_1X_2Y$ sex chromosome system as shared characteristics. Zool. Sci. 37: 128-139.

Araujo D., Schneider M.C., Paula-Neto E., Cella D.M. (2021) The spider cytogenetic database. Available in www.arthropodacytogenetics.bio.br/spiderdatabase. Accessed in November 2021.

Ávila Herrera I.M., Král J., Pastuchová M., Forman M., Musilová J., Korinková T., Stalavsky F., Zrzavá M., Nguyen P., Just P., Haddad C.R., Hirman M., Koubová M., Sadilék D., Huber B.A. (2021) Evolutionary pattern of karyotypes and meiosis in pholcid spiders (Araneae: Pholcidae): implications for reconstructing chromosome evolution of araneomorph spiders. BMC Ecol. Evol. 21: 75.

Beçak W., Beçak M.L. (1960) Constituição cromossômica de duas espécies de aranhas do gênero *Loxosceles*. Rev. Brasil. Biol. 20: 425-427.

Binford G.J., Wells M.A. (2003) The phylogenetic distribution of sphingomyelinase D activity in venoms of Haplogyne spiders. Comp. Bioch. Physiol. Part B 135: 25-33.

Binford G.J. et al. (2008) Phylogenetic relationships of *Loxosceles* and *Sicarius* spiders are consistente with Western Gondwanan vicariance. Mol. Phylog. Evol. 49: 538–553.

Cabrero J., Camacho J.P.M. (2008) Location and expression of ribosomal RNA genes in grasshoppers: Abundance of silent and cryptic loci. Chrom. Res.16: 595–607.

Dias S.C., Carvalho L.S., Bonaldo A.B., Brescovit A.D. (2010) Refining the establishment of guilds in Neotropical spiders (Arachnida: Araneae). J. Nat. Hist. 44: 219-239.

Diaz M.O., Saez F.A. (1966) Karyotypes of South-American Araneida. Mem. Inst. Butantan 33: 153-154.

Dobigny G., Ozouf-Costaz C., Bonillo C., Volobouev V. (2002) "Ag-NORs" are not always true NORs: new evidence in mammals. Cytogenet. Genome Res. 98: 75-77.

Forman M., Nguyen P., Hula V., Král J. (2013) Sex Chromosome Pairing and Extensive NOR Polymorphism in *Wadicosa fidelis* (Araneae: Lycosidae). Cytogenet. Genome Res. 141: 43-49.

Franco J.F., Andía J.M. (2013) El cariótipo de *Sicarius* sp. (Araneae: Haplogynae: Sicariidae), y sus relaciones citotaxonómicas. Bioma 1: 4-9.

Hetzler S. (1979) Some studies of spider chromosomes. Am. Arach. 20: 20.

Howell W M., Black D.A. (1980) Controlled silver staining of nucleolus organizer regions with protective colloidal developer: a 1-step method. Experientia 36: 1014-1015.

Kasahara S. (2009) Introdução à pesquisa em citogenética de vertebrados. 1ª ed, Ribeirão Preto: Sociedade Brasileira de Genética. 160p.

Kavalco K.F., Pazza R., Almeida-Toledo L.F. (2009) *Astyanax bockmanni* Vari and Castro, 2007: an ambiguous karyotype in the *Astyanax* genus. Genetica 136: 135-139.

Král J., Musilová J., Stahlavsky F., Rezac M., Akan Z., Edwards R.L., Coyle F.A., Almerje C.R. (2006) Evolution of the karyotype and sex chromosome systems in basal clades of araneomorph spiders (Araneae: Araneomorphae). Chrom. Res. 14: 859-880.

Král J., Forman M., Korinková T., Lerma A.C.R., Haddad C.R., Musilová J., Rezac M., Herrera I.M.A., Thakur S., Dippenaar-Schoeman A.S., Marec F., Horová L., Bures P. (2019) Insights into the karyotype and genome evolution of haplogyne spiders indicate a polyploid origin of lineage with holokinetic chromosomes. Sci. Reports 9: 3001.

Kumbiçak Z. (2014) Cytogenetic characterization of ten araneomorph spiders (Araneae): karyotypes and meiotic features. Biol. (Bratislava) 69: 644-650.

Labarque F., Ramírez M. (2012) The placement of the spider genus *Periegops* and the phylogeny of Scytodoidea (Araneae: Araneomorphae). Zootaxa 3312: 1–44.

Levan A., Fredga K., Sandberg A.A. (1964) Nomenclature for centromeric position on chromosomes. Hereditas **52:** 201-220.

Lorite P., Aranega A.E., Luque F., Palomeque T. (1997) Analysis of the nucleolar organizing regions in the ant *Tapinoma nigerrimum* (Hymenoptera, Formicidae). Heredity 78: 578-582.

Lotz L.N. (2012) Present status of Sicariidae (Arachnida: Araneae) in the Afrotropical region. Zootaxa 3522: 1-41.

Maddison W.P., Maddison D.R. (2011) Mesquite: A modular system for evolutionary analysis. Version 2.75.

Magalhaes I.L.F., Brescovit A.D., Santos A.J. (2013) The six-eyed sand spiders of the genus *Sicarius* (Araneae: Haplogynae: Sicariidae) from the Brazilian Caatinga. Zootaxa 3599: 101-135.

Magalhaes I.L.F., Oliveira U., Santos F., Vidigal T.H.D.A., Brescovit, A.D., Santos A.J. (2014) Strong spatial structure, Pliocene diversification and cryptic diversity in the Neotropical dry forest spider *Sicarius cariri*. Mol. Ecol.: 5323–5336.

Magalhaes I.L.F., Brescovit A.D., Santos A.J. (2017) Phylogeny of Sicariidae spiders (Araneae: Haplogynae), with a monograph on Neotropical *Sicarius*. Zool. J. Linn. Soc. 179: 767-864.

Oliveira E.G., Cella D.M., Brescovit A.D. (1996) The karyotype of *Loxosceles gaucho* and *Ctenusornatus* (Arachnida, Araneae, Sicariidae, Ctenidae). Rev. Bras. Gen. 18: 128.

Oliveira E.G., Cella D.M., Brescovit A.D. (1997) Karyotype of *Loxosceles intermedia* and *Loxosceles laeta* (Arachnida, Araneae, Sicariidae) Neo$X_1$ Neo$X_2$Y sex determination mechanism and NORs. Rev. Bras. Gen. 20: 77.

Pinkel D., Straume T., Gray J.W. (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. USA 83: 2934–2938.

Platnick N.I., Coddington J.A., Forster R.R., Griswold C.E. (1991) Spinneret morphology and the phylogeny of Haplogyne spiders (Araneae, Araneomorphae). Am. Mus. Nat. Hist. 3016: 1-73.

Reis D.A.R., Brandão K.O., Almeida-Toledo L.F., Pazza R., Kavalco K.F. (2012) Localização física dos genes ribossomais 5S e 18S em *Ancistrus* sp. (Loricariidae: Ancistrini) de Angra dos Reis/RJ, Bacia dos Rios Costeiros. Evol. Cons. Biod. 3: 39-44.

Rodríguez-Gil S.G., Mola L.M., Papeschi A.G., Scioscia C.L. (2002) Cytogenetic heterogeneity in common Haplogyne spiders from Argentina (Arachnida, Araneae). J. Arach. 30: 47-56.

Sanchez A., Jimenez R., Burgos M., Stitou S., Zurita F., La Guardia R.D. (1995) Cytogenetic peculiarities in the Algerian hedgehog: silver stains not only NORs but also heterochromatic blocks. Heredity 75: 10-16.

Silva D. (1988) Estudiocariotípico de *Loxosceles laeta* (Araneae: Loxoscelidae). Rev. Per. Entomol. 31: 9-12.

Silva R.W., Klisiowicz D.R., Cella D.M., Mangili O.C., Sbalqueiro I.J. (2002) Differential distribution of constitutive heterochromatin in two species of brown spider: *Loxosceles intermedia* and *L. laeta* (Aranae, Sicariidae), from the metropolitan region of Curitba, PR (Brazil). Acta Biol. Paran. 31: 123-136.

Silva P.H., Silveira R.B., Appel M.H., Mangili O.C., Gremski W., Veiga S.S. (2004) Brown spiders and loxoscelism. Toxicon 44: 693-709.

Stávale L.M., Schneider M.C., Brescovit A.D., Cella D.M. (2010) Chromosomal characteristics and karyotype evolution of Oxyopidae spiders (Araneae, Entelegynae). Gen. Mol. Res. 10: 752-73.

Tugmon C.R., Brown J.D., Horner N.V. (1990) Karyotypes of seventeen USA spiders species (Araneae, Araneidae, Gnaphosidae, Loxoscelidae, Lycosidae, Oxyopidae, Philodromidae, Salticidae and Theridiidae). J. Arach. 18: 41-48.

Vetter R.S. (2008) Spiders of the genus *Loxosceles* (Araneae, Sicariidae): a review of biological, medical and psychological aspects regarding envenomations. J. Arach. 36: 150-163.

Wheeler W.C., Coddington J.A., Crowley L.M., Dimitrov D., Goloboff P.A., Griswold C.E., Hormiga G., Prendini L., Ramírez M.J., Sierwald P., et al. (2017) The spider tree of life: phylogeny of Araneae based on target gene analyses from an extensive taxon sampling. Cladistics: 1-43.

World Spider Catalog (2021) World Spider Catalog. version 22.5. Natural History Museum Bern, online at http://wsc.nmbe.ch. doi: 10.24436/2. Accessed in November 2021.


## ACKNOWLEDGEMENTS


This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (2011/19873-9; 2011/21643-1; 2011/50689-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), as part of the Programa de Pesquisas em Biodiversidade do Semiárido (558317/2009-0; 457471/2012-3), and CNPq grant to ADB (PQ 303903/2019-8) and Fundação Butantan (001.0708.000636/20).