Cytogenetic analysis on geographically distant parthenogenetic populations of *Tityus trivittatus* Kraepelin, 1898 (Scorpiones, Buthidae): karyotype, constitutive heterochromatin and rDNA localization

Renzo Sebastián Adilardi¹, Andrés Alejandro Ojanguren Affilastro², Dardo Andrea Martí³, Liliana María Mola¹

¹ Laboratorio de Citogenética y Evolución - Departamento de Ecología, Genética y Evolución, IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires - Intendente Güiraldes 2160 - C1428EGA CABA, Argentina ² División de Aracnología - Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” - CONICET - Ángel Gallardo 470 - C1405DfR CABA, Argentina ³ Laboratorio de Genética Evolutiva - IBS (CONICET-UNaM) - Félix de Azara 1552 - CP3300 Posadas - Misiones, Argentina

Corresponding author: Dardo Andrea Martí (darmarti@yahoo.com.ar)

Academic editor: V. Gokhman | Received 18 October 2013 | Accepted 9 January 2014 | Published 12 March 2014

Citation: Adilardi RS, Ojanguren Affilastro AA, Martí DA, Mola LM (2014) Cytogenetic analysis on geographically distant parthenogenetic populations of *Tityus trivittatus* Kraepelin, 1898 (Scorpiones, Buthidae): karyotype, constitutive heterochromatin and rDNA localization. Comparative Cytogenetics 8(2): 81–92. doi: 10.3897/CompCytogen.v8i2.6461

Abstract

*Tityus trivittatus* Kraepelin, 1898 is the most medically important scorpion species of Argentina, and parthenogenetic populations are present in the major cities of this country. We performed a detailed cytogenetic analysis of specimens of three synanthropic parthenogenetic populations, all distant about 900 km from each other, using Ag-NOR, C-banding, DAPI/CMA₃ staining and FISH with autologous 28S rDNA probes. The karyotype of females and embryos from the three populations showed 2n=6, with two large and four middle-sized holokinetic chromosomes. Constitutive heterochromatin was found in terminal and interstitial location and its pattern allowed the identification of three chromosome pairs. NORs were found on the terminal heterochromatic region of one pair of middle-sized chromosomes. The use of fluorochromes to characterize heterochromatin showed the absence of GC-rich heterochromatin and a low and variable number of AT-rich heterochromatic regions. We propose that a possible explanation for the lack of karyotypic variation between these geographically distant populations could be a recent colonization of urban areas by human means of synanthropic specimens from a single lineage of northeastern Argentina.
Keywords
Scorpion, holokinetic chromosomes, parthenogenesis, karyotype, FISH, NOR

Introduction

*Tityus* C. L. Koch, 1836 (Buthidae) is the most diversified genus of the order Scorpiiones, with about 200 described species. It occurs from Central America to southern South America, in tropical and temperate areas. Several species of this genus are medically important, and most of the dangerous scorpion species in South America belong to the genus *Tityus* (Salomón and de Roodt 2001, de Roodt et al. 2003). This genus presents holokinetic chromosomes, as well as other genera of the family Buthidae, and a great intra- and interspecific variation of chromosome number, ranging from 2n=5 to 2n=27 (Schneider et al. 2009).

*Tityus trivittatus* Kraepelin, 1898 is one of the most medically important scorpion species of Argentina and it is responsible for several casualties (Maury 1997, de Roodt et al. 2010). It occurs in southern Brazil, Paraguay, and northern and central Argentina. It reaches Buenos Aires and La Plata cities, being the southernmost species of the genus. *Tityus trivittatus* became a synanthropic species in many areas, being present in most of the major cities of Argentina and Paraguay. *Tityus trivittatus*, as many species of the genus, is facultatively parthenogenetic; sexual populations were reported in Paraguay, southern Brazil and northern Argentina, however, Argentinean populations to the south of latitude 28° S are formed exclusively by parthenogenetic females (Maury 1997, Ojanguren Affilastro 2005).

The parthenogenesis in *Tityus* is quite common and besides *T. trivittatus* it has been mentioned to occur in several species, i.e. *T. columbianus* (Thorell, 1876), *T. confluens* Borelli, 1899, *T. metuendus* Pocock, 1897, *T. serrulatus* Lutz & Mello, 1922, *T. stigmurus* (Thorell, 1876) and *T. uruguayensis* Borelli, 1901 (Matthiesen 1962, Zolessi 1985, Toscano-Gadea 2005, Lourenço 2008, Ross 2010, Seiter 2012). Parthenogenesis was confirmed in *T. trivittatus* based on the observation of virgin females which could produce offspring after lifetime isolation in captivity (Toscano-Gadea 2005). Thelytokous parthenogenesis seems to be the principal mode of asexual reproduction in scorpions, except for the claim of arrhenotokous parthenogenesis in *T. metuendus*, which was severely disputed (Lourenço and Cuellar 1999, Francke 2008). Among these species, cytogenetic studies have been performed in parthenogenetic populations of *T. serrulatus* (2n=12) and *T. stigmurus* (2n=16), and in sexual populations of *T. confluens* (2n=13 in males), *T. metuendus* (2n=15 in males and females and 2n=16 in males) and *T. trivittatus* (2n=14 male), all from Brazil (Ptza 1948, 1950, 1952, Schneider and Cella 2010, Mattos et al. 2013). However, we consider that the identity of the specimens of *T. trivittatus* and *T. confluens* from central Brazil analyzed in those studies is doubtful and should be confirmed with a deep taxonomic study of the group, since they have all been collected in areas placed far from the confirmed distribution of these species (Maury 1970, 1974, 1997, Murua et al. 2002, Fernández Campón and Lagos...
Silnik 2009). Records of *T. confluens* from central Brazil mentioned in Bertani et al. (2005) could probably belong to other closely related species.

In this contribution, we have cytogenetically studied specimens from three synanthropic parthenogenetic Argentinean populations of *T. trivittatus*, from Buenos Aires, Posadas, and Catamarca cities, all distant about 900 km from each other. The karyotype, constitutive heterochromatin distribution and composition, and ribosomal DNA localization were characterized.

**Materials and methods**

We have studied females and embryos of *T. trivittatus* collected from urban populations at the cities of Buenos Aires (34°35.66’S, 58°24.68’W) and Posadas (Misiones province) (27°24.99’S, 55°55.96’W), both in Argentina. Seven females and eleven embryos (of four of these females), were collected by the authors in old subterranean tunnels below the children’s Hospital “Dr Ricardo Gutiérrez”, placed in a highly urbanized area of Buenos Aires city. Nine females and seven embryos (of one of these females) were collected by the authors in a backyard of a house in the periphery of Posadas city, Misiones province. Also, two adult females (one of them with six embryos) were provided by the Department of Zoonoses of Catamarca province, Argentina. The exact locality of the specimens of Catamarca is unknown, but these specimens are most likely to have been collected in the city of San Fernando del Valle de Catamarca (28°28.14’S, 65°46.77’W), the biggest city of the province, where the Department of Zoonoses is placed.

All the specimens were carried alive to laboratory and killed by cooling down to -20°C. Their ovaries and embryos were dissected in saline solution (0.154 M NaCl), incubated in hypotonic solution (1:1 saline solution:distilled water) for 30 min, then fixed for 30 min in a freshly prepared Carnoy fixative (ethanol:chloroform:acetic acid, 6:3:1) and stored in fresh fixative. Pieces of ovaries or embryos were placed on slides and dissociated in a drop of 60% acetic acid with tungsten needles. Preparations with a drop of suspension were placed on a heating histological plate at 40–45°C; suspension was spread on the slides using a tungsten needle.

Conventional staining was made with 5% Giemsa solution in distilled water for 12–15 minutes. The C-banding was performed according to the protocol described by Sumner (1972) and stained with Giemsa or DAPI (4’-6-diamidino-2-phenylindole). The study of the nucleolar organizer regions (NORs) was made by silver-staining technique according to Howell and Black (1980). Fluorescent staining with DAPI and CMA_3_ (chromomycin A_3_) was carried out according to Rebagliati et al. (2003).

Ribosomal genes were detected by Fluorescence in situ hybridization (FISH) technique with 28S rDNA probe. Total genomic DNA of *T. trivittatus* was extracted using a DNeasy Tissue Kit (QIAGEN, Hilden, Germany). Unlabelled 28S rDNA probes were generated by PCR using primers 28Sa (5’-GACCCGTCTTTGAACAGCGGA-3’) and 28Sb (5’-TCGGAAGGAACCAGCTACTA-3’) (Whiting et al. 1997). The sequence of the 331bp fragment of the 28S rDNA gene was deposited in the
NCBI database under the accession number KF723293. The probes were labelled by random primed labeling with DIG-11-dUTP using a DIG-High Prime labeling kit. FISH was performed as described by González et al. (2004) with slight modifications, and the probes were detected with Anti-digoxigenin-fluorescein Fab fragments (Roche Applied Science, Mannheim, Germany). The preparations were counterstained with DAPI and mounted in Vectashield (Vector, Burlingame, CA, USA).

To determine the karyotype, chromosome measurements of well-spread prometa-phase cells from specimens of each population were made using Micro-Measure software, version 3.3 (Reeves and Tear 2000). The relative length of each chromosome was calculated as a percentage of total complement length (%TCL). This analysis was based on one female from Buenos Aires (10 cells), four embryos of one female from Posadas (10 cells), and one female (7 cells) and two embryos (10 cells) of another female from Catamarca. These data allowed us to prepare an idiogram.

Results

The study of females and embryos of *Tityus trivittatus* from the parthenogenetic populations of Buenos Aires, Posadas, and Catamarca showed the same chromosome number of 2n=6, with two large and four middle-sized holokinetic chromosomes (Fig. 1a). Each large-sized chromosome presented an average value of 20.72% of the TCL, and the average value of the similar sized medium chromosomes was 14.64% of the TCL (Table 1) (Fig. 2c). The very few cells observed at early anaphase showed parallel arrangement of the sister chromatids, which is characteristic of holokinetic chromosomes (Fig. 1b).

The study of specimens from the three localities revealed a complex pattern of C-bands with terminal, subterminal and interstitial localization, which made it possible to identify three chromosome pairs. This pattern was observed both with Giemsa and DAPI staining, although DAPI allowed a better resolution of smaller C-bands. The two large-sized chromosomes (pair 1) presented terminal and subterminal C-bands at each terminal region and one submedial band. The heterochromatic bands at one of the terminal regions are closer and the submedial band is located near of these bands. A pair of middle-sized chromosomes (pair 2) carried a C-band in one terminal region, a medial C-band and a conspicuous terminal and a subterminal C-band at the other terminal region. The other middle-sized chromosomes (pair 3) carried C-bands at each terminal region and a subterminal C-band (Figs 1c, d, 2c). In the specimens from Buenos Aires one of the terminal bands of pair 3 is more conspicuous and the subterminal band is closer to it.

Silver staining visualized active NORs at a terminal region of two middle-sized chromosomes (Fig. 1e). Cells with sequential C-banding and silver staining showed that NORs are located at the double-banded terminal region of pair 2 (Fig. 1f).

DAPI/CMA$_3$ sequential staining revealed no bright CMA$_3$ bands. Most cells showed chromosomes homogeneously stained with DAPI. Other cells showed some bright DAPI bands that were coincident with C-bands (Fig. 1g). The number of bright
Cytogenetic analysis on geographically distant parthenogenetic populations...

DAPI bands was less than the number of C-bands, and the smaller C-bands were not detected. This technique did not provide reliable results, since the number of DAPI bands was variable between cells with the same degree of chromosome condensation.

DAPI counterstaining in FISH technique revealed a similar pattern of bright bands as C-banding, which allowed for identification of each chromosome pair. Hybridization signals with the autologous 28S rDNA probes were located at the double-banded terminal region of pair 2 (Fig. 2a, c). Late mitotic prophase chromosomes revealed that the rDNA cluster is embedded in the conspicuous terminal C-band of pair 2 (Fig. 2b).

**Figure 1.** Mitotic cells of *Tityus trivittatus* (2n=6). a Giemsa-stained prometaphase b Early anaphase c C-banded prometaphase stained with Giemsa (Buenos Aires city) d C-banded prometaphase stained with DAPI (Buenos Aires city) e Silver-stained metaphase f Sequential C-banding and silver staining on chromosome 2 g DAPI-banded prometaphase after DAPI/CMA<sub>3</sub> staining. The arrows point to the double-banded terminal region of pair 2. Arrowheads point to the NORs. Scale bar= 10 µm.
Discussion

The chromosome number found in the specimens of the Argentinean populations of *Tityus trivittatus* herein studied is one of the lowest in Buthidae, and it is also present in *Tityus martinpaechi* Lourenço, 2001 and some individuals of *Tityus bahiensis* (Perty, 1834) (Piza 1939, Schneider et al. 2009, Mattos et al. 2013).

In other species of *Tityus*, Mattos et al. (2013) described two different patterns of heterochromatin distribution: species with small blocks of constitutive heterochromatin in the terminal regions of some chromosomes and species with more conspicuous blocks of constitutive heterochromatin in the terminal regions of all chromosomes and in the interstitial regions of some or all chromosomes. The specimens of *T. trivittatus* herein studied share the latter pattern of constitutive heterochromatin distribution.

The use of DAPI and CMA₃ fluorochromes to characterize heterochromatin of *T. trivittatus* showed the absence of GC-rich heterochromatin and a low and variable number of AT-rich heterochromatic regions, which were coincident with some of the

**Table 1.** Chromosome measurements of the studied populations of *Tityus trivittatus*. Relative lengths expressed as percentage of total chromosome length (%TCL). Mean values and their standard deviations (SD) are given.

| Chromosome number | Buenos Aires | Catamarca | Posadas |
|-------------------|--------------|-----------|---------|
|                   | %TCL ± SD    | %TCL ± SD | %TCL ± SD |
| 1                 | 21.53 ± 0.99 | 21.54 ± 0.60 | 20.89 ± 0.79 |
| 2                 | 19.96 ± 0.34 | 20.45 ± 0.65 | 19.92 ± 0.93 |
| 3                 | 15.37 ± 0.57 | 15.44 ± 0.51 | 15.87 ± 0.55 |
| 4                 | 14.93 ± 0.34 | 14.82 ± 0.45 | 15.07 ± 0.49 |
| 5                 | 14.35 ± 0.33 | 14.28 ± 0.48 | 14.41 ± 0.63 |
| 6                 | 13.86 ± 0.40 | 13.47 ± 0.55 | 13.84 ± 0.48 |

**Figure 2.** Fluorescence *in situ* hybridization with 28S rDNA probe and idiogram of the karyotype of *Tityus trivittatus*. **a** Mitotic prometaphase with hybridization signals **b** Chromosome 2 at late prophase with hybridization signal; **c.** Idiogram showing distribution of constitutive heterochromatin (black bands) and 28S rDNA clusters (green circles). Chromosomes are counterstained with DAPI (blue). Arrowheads point to hybridization signals (green). Scale bar= 10 µm.
bands revealed by C-banding. In other Buthidae species the number of heterochromatic regions revealed by DAPI/CMA<sub>3</sub> staining was also lower than that visualized by C-banding and these regions were almost exclusively AT-rich (only *T. martinaechei* and *Rhopalurus agamemnon* (C. L. Koch, 1839) show GC-rich terminal regions in one chromosome pair) (Schneider and Cella 2010, Mattos et al. 2013). This difference could be related to the protocol of each technique: C-banding method implies a differential extraction of DNA that leads to a greater contrast between euchromatin and heterochromatin, whereas during direct DAPI/CMA<sub>3</sub> staining there is no DNA extraction and the number of heterochromatic regions observed could be less (Sumner 2003, Barroso-Silva and Guerra 2010). The low number of heterochromatic regions revealed with the latter technique could be also related to the holokinetic nature of the chromosomes, since it has been suggested that this type of chromosomes could be more rigid (Manardioli and Manicardi 2012). A structural difference of the chromatin condensation of buthid mitotic chromosomes could hinder the specific fluorochrome binding to DNA.

The number and terminal location of NORs, as well as their association with constitutive heterochromatin found in the specimens of *T. trivittatus*, are all common features reported in other species of *Tityus* (Schneider et al. 2009, Schneider and Cella 2010, Mattos et al. 2013). Moreover, the terminal location of NORs is found in many other species of invertebrates and plants with holokinetic chromosomes (e.g.: Hemiptera, Lepidoptera, Odonata, Nematoda, Juncaceae, Cyperaceae and *Cuscuta* Linnaeus) (Albertson 1984, Rebagliati et al. 2003, Guerra and García 2004, Mola and Papeschi 2006, Criniti et al. 2009, Nguyen et al. 2010, Heckmann et al. 2011, Sousa et al. 2011, Poggio et al. 2011, Maryńska-Nadachowska et al. 2013), and this location of NORs could be a functional requirement to ensure chromosome stability in this type of chromosomes (Heckmann et al. 2011).

*Tityus trivittatus* is an invasive synanthropic species that easily colonizes urban areas due to its great adaptability, ubiquity and parthenogenetic reproduction. This species was probably introduced into Buenos Aires city during the first half of the twentieth century by anthropogenic means (Maury 1970). Parthenogenetic reproduction may allow the establishment of different karyotypes in isolated synanthropic populations. Nevertheless, all specimens of the three populations herein analyzed show the same karyotype in spite of the fact that the populations are about 900 km apart. The lack of variation between the studied populations could be due to a recent colonization of all these urban areas by specimens from a wild sexual (or even parthenogenetic) population with three pairs of homologous chromosomes. Another possible explanation is that all specimens from these cities belong to a single lineage that originally colonized cities from north-eastern Argentina, where its presence has been recorded long time ago (Werner 1902, Mello-Leitão 1934), and once it became synanthropic, specimens from these populations were easily transported from one city to another by human means. The last hypothesis seems more plausible, and is supported by the recent and fast colonization of all the cities of western Argentina (Murua et al. 2002, Fernández Campón and Lagos Silnik 2009), in areas that are far from the “Wet Chaco”, the original habitat of *T. trivittatus* (Ojanguren-Affilastro 2005). In two distant parthenogenetic popula-
tions of *T. serrulatus* with conserved karyotype, a particular combination of genes was proposed to have been selectively advantageous (Schneider and Cella 2010). This fact could also be related to the establishment of a particular karyotype in *T. trivittatus*.

Taking into account the high incidence of intra- and interpopulation chromosome rearrangements reported in other species of *Tityus*, further cytogenetic studies of unequivocally identified sexual and parthenogenetic populations of *T. trivittatus* are needed to reveal potential chromosome variation within this species.

**Acknowledgments**

This work was supported by grants from the National Council of Scientific and Technological Research (CONICET) (PIP 0342), National University of Buenos Aires (UBA) (Ex 0859) and ANPCyT (PICT 2010-1665) to Drs L. Poggio and L. Mola and ANPCyT (PICT 2010-1764) to Dr A. A. Ojanguren-Affilastro. The authors wish to thank to Dr Carolina Guilleron and Daniel Hermann from Administración Nacional de Laboratorios e Institutos de Salud “Dr Carlos G. Malbrán” for providing the specimens of Catamarca and to Dr María Isabel Duhau, Dr Valeria Malinovsky, Lic. Daniela Rocco, Eng. Fernando Díaz and Dr Adolfo de Roodt for their help in specimen collection at the “Ricardo Gutiérrez” hospital. We are also grateful to Lic. Elio Castillo for his technical advice and to Drs Alexandra Gottlieb and Marcela Rodriguero for their valuable assistance in obtaining FISH probes.

**References**

Albertson DG (1984) Localization of the ribosomal genes in *Caenorhabditis elegans* chromosomes by in situ hybridization using biotin-labeled probes. The EMBO Journal 3: 1227–1234. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC557503/

Barros e Silva AE, Guerra M (2010) The meaning of DAPI bands observed after C-banding and FISH procedures. Biotechnic and Histochemistry 85: 115–125. doi: 10.1080/10520290903149596

Bertani R, Martins R, Carvalho MA (2005) Notes on *Tityus confluens* Borelli, 1899 (Scorpiones: Buthidae) in Brazil. Zootaxa 869: 1–7. http://www.mapress.com/zootaxa/2005/zt00869.pdf

Criniti A, Simonazzi G, Cassanelli S, Ferrari M, Bizzaro D, Manicardi GC (2009) Distribution of heterochromatin and rDNA on the holocentric chromosomes of the aphids *Dysaphis plantaginea* and *Melanaphis pyraria* (Hemiptera, Aphididae). European Journal of Entomology 106: 153–157. doi: 10.14411/eje.2009.018, http://www.eje.cz/pdfarticles/1436/eje_106_2_153_Criniti.pdf

de Roodt AR, García SI, Salomón OD, Segre L, Dolab JA, Funes RF, de Titto H (2003) Epidemiological and clinical aspects of scorpionism by *Tityus trivittatus* in Argentina. Toxicon 41: 971–977. doi: 10.1016/S0041-0101(03)00066-7
de Roodt AR, Coronas FIV, Lago N, González ME, Laskowicz RD, Beltramino JC, Saavedra S, López RA, Reati GJ, Vucharchuk MG, Bazán E, Varni L, Salomón OD, Possani LD (2010) General biochemical and immunological characterization of the venom from the scorpion *Tityus trivittatus* of Argentina. Toxicon 55: 307–319. doi: 10.1016/j.toxicon.2009.08.014

Fernández Campón F, Lagos Silnik S (2009) Primer registro de *Tityus trivittatus* (Scorpiones: Buthidae) en la provincia de Mendoza (Argentina). Revista de la Sociedad Entomológica Argentina 68: 219–221. http://www.scielo.org.ar/pdf/rsea/v68n1-2/v68n1-2a17.pdf

Francke OF (2008) A critical review of reports of parthenogenesis in scorpions (Arachnida). Revista Ibérica de Aracnología 16: 93–104.

Guerra M, García MA (2004) Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). Genome 47: 134–140. doi: 10.1139/g03-098

González G, Confalonieri V, Comas C, Naranjo CA, Poggio L (2004) GISHGenomic in situ hybridization reveals cryptic genetic differences between maize and its putative wild progenitor *Zea mays* subsp. *parviglumis*. Genome 47: 947–953. doi: 10.1139/g04-038

Heckmann S, Schroeder-Reiter E, Kumke K, Ma L, Nagaki K, Murata M, Wanner G, Houben A (2011) Holocentric chromosomes of *Luzula elegans* are characterized by a longitudinal centromere groove, chromosome bending, and a terminal nucleolus organizer region. Cytogenetic and Genome Research 134: 220–228. doi: 10.1159/000327713

Howell WM, Black DA (1980) Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015. doi: 10.1007/BF01953855

Lourenço WR (2008) Parthenogenesis in scorpions: some history—new data. Journal of Venomous Animals and Toxins including Tropical Diseases 14: 19–44. doi: 10.1590/S1678-91992008000100003

Lourenço WR, Cuellar O (1999) A new all-female scorpion and the first probable case of ar-rhenotoky in scorpions. Journal of Arachnology 27: 149–153. http://www.americanarachnology.org/JoA_free/JoA_v27_n1/arac_27_01_0149.pdf

Mandrioli M, Manicardi GC (2012) Unlocking holocentric chromosomes: new perspectives from comparative and functional genomics? Current Genomics 13: 343–349. doi: 10.2174/138920212801619250

Maryańska-Nadachowska A, Kuznetsova VG, Karamysheva TV (2013) Chromosomal location of rDNA clusters and TTAGG telomeric repeats in eight species of the spittlebug genus *Philaenus* (Hemiptera: Auchenorrhyncha: Aphrophoridae). European Journal of Entomology 110: 411–418. doi: 10.14411/eje.2013.055, http://www.eje.cz/pdfs/110/3/411

Maury EA (1970) Redescripción y distribución en la Argentina de *Tityus trivittatus trivittatus* Kraepelin 1898 (Scorpiones, Buthidae) comentarios sobre sus hábitos domiciliarios y su peligrosidad. Physis 29: 405–421.

Maury EA (1974) Escorpiofauna Chaqueña. II. *Tityus confluens* Borelli 1899 (Buthidae). Physis 33: 85–92.

Maury EA (1977) *Tityus trivittatus* en la Argentina, Nuevos datos sobre distribución, partenogénesis, sinantropía y peligrosidad. (Scorpiones, Buthidae). Publicaciones de extensión
cultural y didáctica del Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” 24:1–24.

Matthiesen FA (1962) Parthenogenesis in scorpions. Evolution 16: 255–256. doi:10.2307/2406202

Mattos VF, Cella DM, Carvalho LS, Candido DM, Schneider MC (2013) High chromosome variability and the presence of multivalent associations in buthid scorpions. Chromosome Research 21: 121–136. doi:10.1007/s10577-013-9342-3

Mello-Leitão CD (1934) Estudio Monográfico dos Escoriões da República Argentina. Octava Reunião de la Sociedad Argentina de Patología Regional del Norte. Santiago del Estero 1–97.

Mola LM, Papeshi AG (2006) Holokinetic chromosomes at a glance. Journal of Basic and Applied Genetics 17: 17–33.

Murua F, Acosta LE, Acosta JC, Coria C (2002) Primeros registros de Tityus trivittatus (Scorpiones, Buthidae) en el oeste argentino. Multequina 11: 1–8. http://www.cricyt.edu.ar/multequina/indice/pdf/11/11_6.pdf

Nguyen P, Sahara K, Yoshido A, Marec F (2010) Evolutionary dynamics of rDNA clusters on chromosomes of moths and butterflies (Lepidoptera). Genetica 138: 343–354. doi:10.1007/s10709-009-9424-5

Ojanguren-Affilastro AA (2005) Estudio monográfico de los escorpiones de la República Argentina. Revista Ibérica de Aracnología 11: 75–241.

Piza ST (1939) Comportamento dos cromossomos na primeira divisão do espermatócito do Tityus bachiensis. Scientia Genetica 1: 255–261.

Piza ST (1948) Primeiras observações sobre os cromossômios de Tityus trivittatus Krpkn (Scorpiones – Buthidae). Revista de Agricultura de São Paulo 24: 177–180.

Piza ST (1950) Observações cromossômicas em escorpiões brasileiros. Ciência e Cultura 2: 202–206.

Piza ST (1952) Primeiras observações sôbre os cromossômios do Tityus metuendus Pocock. Scientia Genetica 4: 162–167.

Poggio MG, Bressa MJ, Papeshi AG (2011) Male meiosis, heterochromatin characterization and chromosomal location of rDNA in Microtomus lunifer (Berg, 1900) (Hemiptera: Reduviidae: Hammacerinae). Comparative Cytogenetics 5: 1–22. doi:10.3897/compcytogen.v5i1.1143

Rebagliati PJ, Papeshi AG, Mola LM (2003) Meiosis and fluorescent banding in Edessa meditabunda and E. rufomarginata (Heteroptera: Pentatomidae: Edessinae). European Journal of Entomology 100: 11–18. http://www.eje.cz/pdfarticles/195/eje_100_1_011_Rebag.pdf

Reeves A, Tear J (2000) MicroMeasure for Windows, Version 3.3. http://www.colostate.edu/Depts/Biology/MicroMeasure

Ross LK (2010) Confirmation of parthenogenesis in the medically significant, synanthropic scorpion Tityus stigmurus (Thorell, 1876) (Scorpiones: Buthidae). Revista Ibérica de Aracnología 18: 115–121.

Salomón OD, de Roodt AR (2001) Escorpiones: denuncia espontánea en dos centros de referencia en la ciudad de Buenos Aires, 1997-2000. Medicina (Buenos Aires) 61: 391–396. http://www.medicinabuenosaires.com/revistas/vol61-01/4/v61_n4_p391_396.pdf
Schneider MC, Zacaro AA, Pinto-da-Rocha R, Candido DM, Cella DM (2009) Complex meiotic configuration of the holocentric chromosomes: the intriguing case of the scorpion *Tityus bahiensis*. Chromosome Research 17: 883–898. doi: 10.1007/s10577-009-9076-4

Schneider MC, Cella DM (2010) Karyotype conservation in 2 populations of the parthenogenetic scorpion *Tityus serrulatus* (Buthidae): rDNA and its associated heterochromatin are concentrated on only one chromosome. Journal of Heredity 101:491–496. doi: 10.1093/jhered/esq004

Seiter M (2012) Developmental stages and reproductive biology in *Tityus confluens* Borelli, 1899 and *Tityus ocelote* (Francke & Atockwell, 1987) (Scorpiones, Buthidae). Revista Ibérica de Aracnología 21: 113–118.

Sousa A, Barros e Silva AE, Cuadrado A, Loarce Y, Alves MV, Guerra M (2011) Distribution of 5S and 45S rDNA sites in plants with holokinetic chromosomes and the “chromosome field” hypothesis. Micron 42: 625–631. doi: 10.1016/j.micron.2011.03.002

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. doi: 10.1016/0014-4827(72)90558-7

Sumner AT (2003) Chromosomes: Organization and Function. Blackwell Publishing, Oxford, UK, 287 pp.

Toscano-Gadea C (2005) Confirmation of parthenogenesis in *Tityus trivittatus* Kraepelin, 1898 (Scorpiones, Buthidae). Journal of Arachnology 33: 86–869. doi: 10.1636/S03-21.1, http://www.americanarachnology.org/JoA_free/JoA_v33_n3/arac-033-03-0866.pdf

Werner F (1902) Die Skorpione, Pedipalpen und Solifugen im der zoologisch-vergleichend anatomischen Sammlung der Universität Wien. Scorpiones. Verhandlungen der Kaiserlich-Königlichen Zoologish-Botanischen Gesellschaft in Wien 52: 595–606.

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Systematic Biology 46: 1–68. doi: 10.1093/sysbio/46.1.1

Zolessi LC (1985) La partenogénesis en el escorpión amarillo *Tityus uruguayensis* Borelli, 1901 (Scorpionida, Buthidae). Revista de la Facultad de Humanidades y Ciencias. Tercera Época. Ser. Ciencias Biológicas 1: 26–32.