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Chromosome evolution in lycosoid spiders (Araneomorphae): a scenario based on analysis of seven species of the families Lycosidae, Senoculidae and Trechaleidae

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Abstract. Within the araneomorph lineage Entelegynae, the "Higher lycosoids" ("True Lycosoids" or Lycosoidea s.s.) is one of the major clades varying in its composition based on several conflicting phylogenetic hypotheses. So far, only a few species have been studied cytogenetically. In this work, the chromosomes of a senoculid spider were investigated for the first time, along with a cytogenetic analysis of six species of the families Lycosidae and Trechaleidae. Mitotic metaphase cells of the lycosid species revealed 2n♂ = 18 + X0 in *Hogna sternalis* (Bertkau 1880) and *Lycosa nordenskjoldi* Tullgren 1905, and 2n♂ = 20 + $X_1X_2$0 in *Lycosa erythrognatha* Lucas 1836 and *Lycosa sericovittata* Mello-Leitão 1939. Chromosomal analysis of the trechaleid and senoculid species showed 2n♀ = 22 in *Neoctenus comosus* Simon 1897 (Trechaleidae), and 2n♂ = 26 + $X_1X_2$0 in *Syntrechalea syntrechaloides* (Mello-Leitão 1941) (Trechaleidae) and *Senoculus* sp. (Senoculidae). This latter karyotype is a shared feature in most species of Lycosoidea group. The mitotic and/or meiotic cells of certain individuals of Trechaleidae exhibited one extra chromosome, which could constitute a B chromosome or represent intraspecific variability in the type of sex chromosome system. The results obtained here add new information to the discussion of the main mechanisms of chromosome evolution within this group.

Keywords: Cytogenetics, mitosis, meiosis, sex chromosome system, Lycosoidea

Within Araneomorphae, Entelegynae includes the highest number of species described both taxonomically and cytogenetically. Nevertheless, considering the high diversity of spiders belonging to this group, around 37,100 species (World Spider Catalog 2015), the chromosome information encompasses less than 2% of the entelegynes, with 693 species karyotyped so far (Araujo et al. 2015). Higher lycosoids (true lycosoids or Lycosoidea s.s.) is a clade of variable composition according to phylogenetic hypotheses (Griswold 1993; Silva 2003; Raven & Stumkat 2005; Ramírez 2014), however, the families Psechridae, Oxyopidae, Senoculidae, Lycosidae, Pisauridae, and Trechaleidae (when included) were always recovered within this group. These families contain 3,392 species (World Spider Catalog 2015), corresponding to roughly 9% of the Entelegynae spiders and present a widespread distribution.

Despite conflicting hypotheses on lycosoid phylogeny, Oxyopidae and Senoculidae are always recovered as a monophyletic group, sister to Stiphidiidae (Griswold 1993; Raven & Stumkat 2005), Psechridae (Silva 2003), or Pisauridae (Ramírez 2014) and, in the studies that include Trechaleidae, the families Lycosidae, Pisauridae and Trechaleidae always form a clade, in which Lycosidae is sister to Trechaleidae (Griswold 1993; Raven & Stumkat 2005) or Pisauridae (Silva 2003).

Among the higher or true lycosoids (Griswold 1993; Silva 2003), a total of 159 species belonging to five families were cytogenetically analyzed. Lycosidae and Pisauridae possess 118 and 12 species studied, respectively, in which the karyotype 2n♂ = 26 + $X_1X_2$0 was the most frequently observed. In Trechaleidae, there are karyotypic data for three species that showed 2n♂ = 22 + $X_1X_2$0 or 2n♂ = 26 + $X_1X_2$0. Psechridae contains a single species investigated, which exhibited 2n♂ = 22 + $X_1X_2$0 and Oxyopidae presented more than 60% of its 26 species examined with the karyotypic constitution 2n♂ = 20 + X0 (Araujo et al. 2015).

In species of Lycosidae, Pisauridae and Oxyopidae, intraspecific chromosome variability has sporadically been registered (Araujo et al. 2015). In almost all cases, this intra- or interpopulation variability was related to differences in the diploid number, involving one or two autosomal pairs (Datta & Chatterjee 1983; Parida & Sharma 1987a; Sharma & Parida 1987), with the exception of *Oxyopes salticus* Hentz 1845 (Oxyopidae) that exhibited a high divergence in the number of autosomes and type of sex chromosome system, i.e., 2n♂ = 20 + $X_1X_2$0 (Painter 1914) and 2n♂ = 10 + X0 (Stávale et al. 2011). The presence of supernumerary chromosomes was only reported for *Gladicosa pulchra* (Keyserling 1877) (Lycosidae) and *O. salticus* (Oxyopidae), which exhibited two and one very small extra element, respectively, in some mitotic cells (Montgomery 1905; Stávale et al. 2011).

A variety of cytogenetic information, e.g., diploid number, chromosome morphology, type of sex chromosome system, and pattern of distribution of specific chromosome regions, can provide useful characters to corroborate phylogenetic placements, in addition to other data, such as morphology and molecular biology. Furthermore, the comparison of the cytogenetic data with phylogenetic hypotheses can reveal the trends of chromosome evolution for a taxonomic group. With

[5] *In memoriam*

the aim of increasing the cytogenetic knowledge of lycosoid spiders and discussing the chromosomal evolution that has occurred within this group, we analyzed the mitotic and meiotic chromosomes of seven species of the families Lycosidae, Senoculidae and Trechaleidae.

METHODS

The number of individuals (adults and embryos) and the locality data are listed in Table 1. Collecting permits were provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis – IBAMA and Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (15382-1 and 15157-1). Voucher specimens were deposited in the arachnological collection of the Laboratório Especial de Coleções Zoológicas, Instituto Butantan (IBSP, curator A. D. Brescovit), São Paulo, state of São Paulo, Brazil. The chromosome preparations were accomplished following Araujo et al. (2008) and standard stained with 3% Giemsa solution (3% commercial Giemsa and 3% phosphate buffer, pH 6.8, in distilled water) for 12 min. The chromosomes were measured using LEVAN (Sakamoto & Zacaro 2009), a plugin for Image J (Rasband 1997-2012), designed for classification of chromosomal morphology. In this plugin, the classification follows the nomenclature proposed by Levan et al. (1964).

RESULTS

Lycosidae.—Spermatogonial metaphase cells of lycosids revealed the diploid number and sex chromosome system $2n♂ = 18 + X0$ in *Hogna sternalis* (Bertkau 1880) and *Lycosa nordenskjoldi* Tullgren 1905 (Fig. 1A, B), and $2n♂ = 20 + X_1X_20$ in *Lycosa erythrognatha* Lucas 1836 and *Lycosa sericovittata* Mello-Leitão 1939 (Fig. 1C, D). The karyotypes of the four species were composed of telocentric chromosomes (arm ratio ≥ 7.0 and $\leq \infty$) that gradually decreased in length. The X chromosome of *H. sternalis* and *L. nordenskjoldi* was the smallest element of the complement (Fig. 1A, B). The X_1 and X_2 sex chromosomes of the two other *Lycosa* species exhibited a slight difference in size; in *L. erythrognatha* both the sex chromosomes were the largest elements of the karyotype (Fig. 1C) while in *L. sericovittata*, the X_1 chromosome was the largest element of the diploid complement and the X_2 chromosome was similar in size to the 2nd pair (Fig. 1D). In certain mitotic metaphase cells of *L. erythrognatha* whose chromosomes were less condensed, a secondary constriction in the proximal region of pairs 2 and 6 was observed (Fig. 1C). All oogonial metaphase cells obtained from one individual of *L. sericovittata* demonstrated $2n♀ = 26$ (Fig. 1E), differing from $2n = 24$ that was expected for females of this species. In the karyotype of this individual, the sex chromosomes were not identified because they did not reveal differential features in relation to the autosomes.

In the male meiotic cells of the four Lycosidae species, the sex chromosomes were easily recognized due to their high degree of condensation and positive heteropycnosis (Fig. 2). This recognition is clear even in pachytene, as shown in *L. erythrognatha* (Fig. 2A). The diplotene nuclei revealed nine autosomal bivalents plus one sex univalent ($9II + X0$) in *H. sternalis* and *L. nordenskjoldi* (Fig. 2B, C) and 10 autosomal bivalents plus two sex univalents ($10II + X_1X_20$) in *L. erythrognatha* (Fig. 2D), confirming the diploid number and sex chromosome system established through analysis of male mitotic cells. In this meiotic stage, the autosomal bivalents demonstrated only one interstitial or terminal chiasma. Additionally, in the two *Lycosa* species with X_1X_20 sex chromosome system, the X_1 and X_2 chromosomes were usually disposed side by side (Fig. 2D). The metaphase II

Table 1.——Lycosoids studied herein with their respective samples, karyotype data and collection localities in Brazil. SP = state of São Paulo; MS = state of Mato Grosso do Sul; PR = state of Paraná. E = embryos.

Species	Sample	Diploid number and sex chromosome system	Collection locality
Lycosidae			
Hogna sternalis (Bertkau 1880)	1♂	$2n = 18 + X0$	Miranda (20°14'S, 56°25'W), MS
Lycosa nordenskjoldi Tullgren 1905	1♂	$2n = 18 + X0$	Margem da Lagoa Xambrê, Parque Nacional de Ilha Grande, Altônia (23°52'S, 54°00'W), PR
Lycosa erythrognatha Lucas 1836	5♂	$2n = 20 + X_1X_20$	Rio Claro (22°24'S, 47°34'W), SP
	1♂	$2n = 20 + X_1X_20$	Boituva (23°17'S, 47°40'W), SP
	1♂	$2n = 20 + X_1X_20$	Guarulhos (23°27'S, 46°32'W), SP
Lycosa sericovittata Mello-Leitão 1939	1♀	$2n = 26$	São Roque (23°31'S, 47°08'W), SP
	2♂	$2n = 20 + X_1X_20$	Tietê (23°06'S, 47°43'W), SP
Senoculidae			
Senoculus sp.	1♂	$2n = 26 + X_1X_20$	Rio Claro (22°24'S, 47°34'W), SP
Trechaleidae			
Neoctenus comosus Simon 1897	1♂	$2n = 18 + X_1X_20$	Ilha dos Bandeirantes, Parque Nacional de Ilha Grande, Naviraí (23°01'S, 54°10'W), MS
	1♀	$2n = 22$	
	1♀	$2n = 22$ and 23	Ilha São Francisco, Parque Nacional de Ilha Grande, Guaíra (24°00'S, 54°09'W), PR
	1♂	$2n = 18 + X_1X_20$	Margem da Lagoa Xambrê, Parque Nacional de Ilha Grande, Altônia (23°52'S, 54°00'W), PR
	1♀	$2n = 22$	
Syntrechalea syntrechaleoides (Mello-Leitão 1941)	1♂, 3 E	$2n = 26 + X_1X_20$	Rio Claro (22°24'S, 47°34'W), SP
	3♀, 14 E	$2n = 30$	
	1♂, 3 E	$2n = 29$	

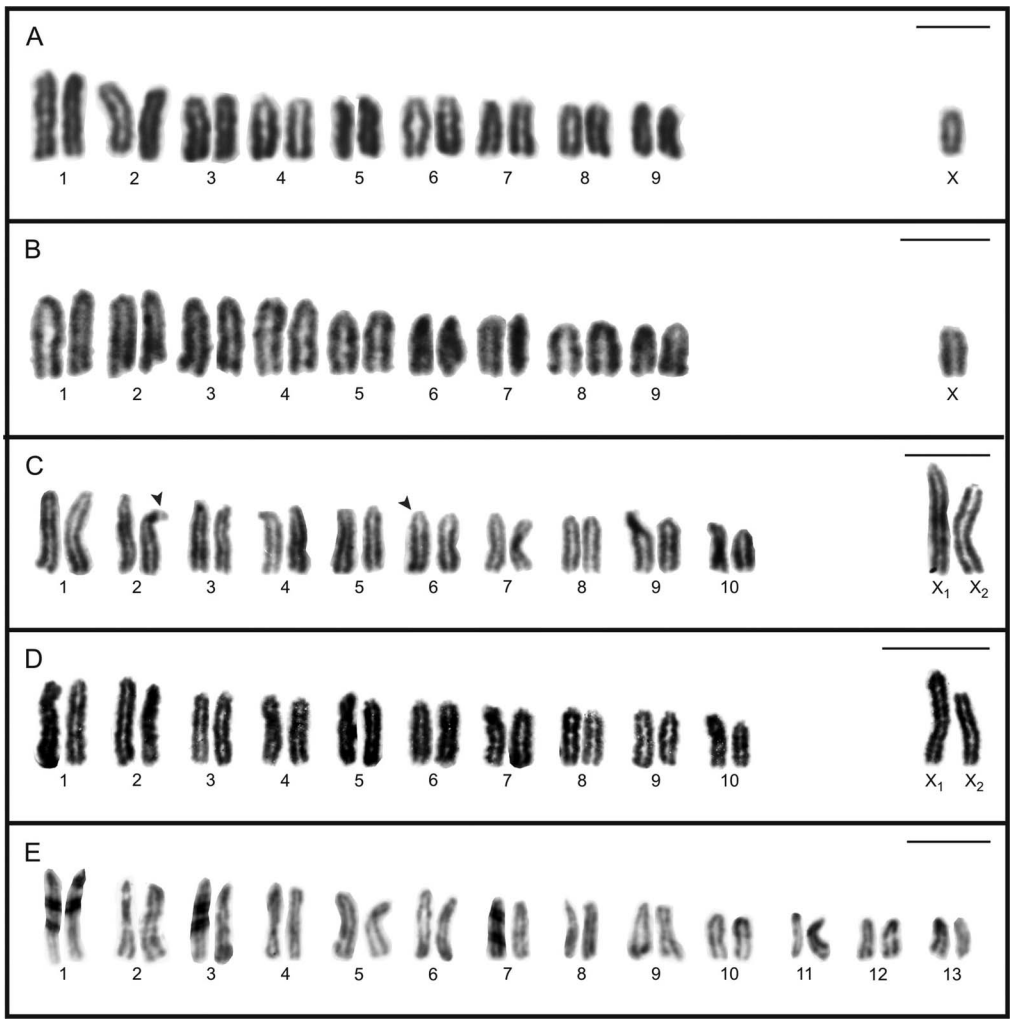

Figure 1.—Karyotypes of Lycosidae species stained with Giemsa. A. *Hogna sternalis*, 2n♂ = 18 + X0. B. *Lycosa nordenskjoldi*, 2n♂ = 18 + X0. C. *Lycosa erythrognatha*, 2n♂ = 20 + X_1X_20. D, E. *Lycosa sericovittata* with 2n♂ = 20 + X_1X_20 and 2n♀ = 26, respectively. The arrowheads in C point to secondary constrictions in the proximal regions of pairs 2 and 6. Scale = 10 μm.

cells showed the haploid sets n = 9 + X and n = 9 in *H. sternalis* (Fig. 2E, F), and n = 10 + X_1X_2 and n = 10 in *L. sericovittata* (Fig. 2G, H), confirming the sex chromosome system in this last species, in which no diplotene cells were found.

Trechaleidae.—The cytogenetic analyses were accomplished in two species, *Neoctenus comosus* Simon 1897 and *Syntrechalea syntrechaleoides* (Mello-Leitão 1941). In *N. comosus*, the mitotic cells of the three females showed 2n♀ = 22 telocentric chromosomes; in these nuclei, the sex chromosomes were not differentiated from the autosomes. Additionally, in one of these female individuals, some metaphase cells revealed 2n♀ = 23; this intraindividual variability of the diploid number occurred due to the presence of one unpaired and small element, which probably corresponds to a supernumerary chromosome (Fig. 3A). The sample of *S. syntrechaleoides* revealed 2n♂ = 28 in one male and three embryos (Fig. 3B), and 2n♀ = 30 in three females and 14 embryos (Fig. 3C). These diploid numbers are consistent with a sex chromosome system of the X_1X_20 type. This species presented chromosomes with telocentric morphology, autosomes that gradually decreased in length, and X_1 and X_2 chromosomes with similar size to the 1st, 2nd, and 13th pairs, respectively (Fig. 3B, C). In some metaphase cells, the proximal region of pairs 3 and 10 exhibited a less condensed and stained chromatin (Fig. 3B). Furthermore, in the sample examined of *S. syntrechaloides*, all cells of one male and three embryos showed 2n♂ = 29 (Fig. 3D).

The analysis of mitotic and meiotic nuclei of these individuals revealed that the diploid number variability occurred due to the presence of one extra chromosome, which possessed an intermediate size between the X_1 and X_2 sex chromosomes (Fig. 3D).

Diplotene cells of male *N. comosus* exhibited 9 autosomal bivalents plus two highly condensed univalents, which were identified as X_1 and X_2 sex chromosomes (Fig. 4A). In the female individual of *N. comosus* that carried the supernumerary chromosome, the pachytene cells revealed 11 bivalents or 11 bivalents plus one extremely less condensed univalent (Fig. 4B), which probably corresponds to the unpaired chromosome observed in some mitotic cells. Testicular cells of *S. syntrechaleoides* with 2n = 28, showed 13 autosomal bivalents with one interstitial or terminal chiasma plus two

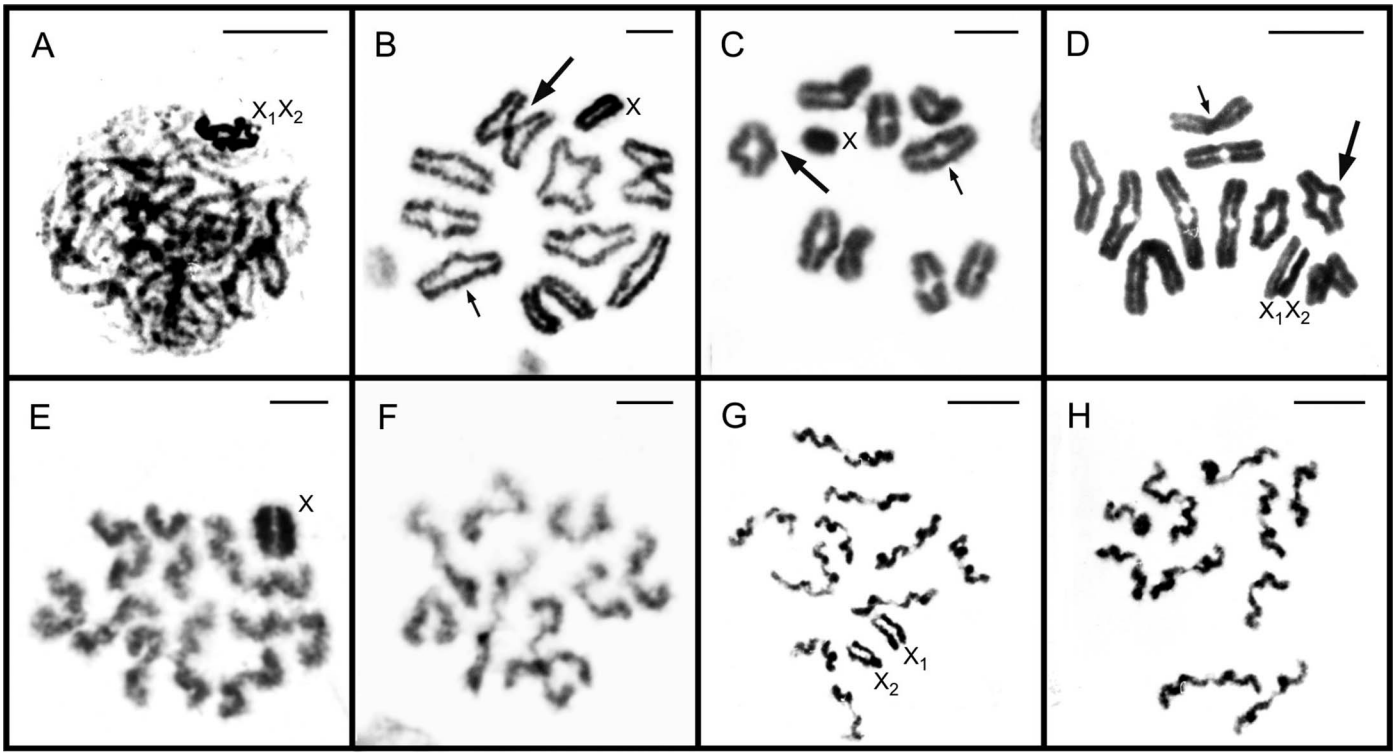

Figure 2.—Standard stained spermatocytes of Lycosidae species. *Lycosa erythrognatha* (A, D), *Hogna sternalis* (B, E, F), *Lycosa nordenskjoldi* (C), and *Lycosa sericovittata* (G, H). A. Pachytene, showing two positively heteropycnotic sex chromosomes, X_1 and X_2. B, C. Diplotene cells, 9II + X0, exhibiting highly condensed X chromosome. D. Diplotene nuclei, 10II + X_1X_20, exhibiting the sex chromosomes X_1 and X_2 disposed side by side. E, F. Metaphase II cells with n = 9 + X and n = 9, respectively. G, H. Metaphase II nuclei with n = 10 + X_1X_2 and n = 10, respectively. Large arrow = interstitial chiasma. Small arrow = terminal chiasma. Scale = 10 μm.

unpaired sex chromosomes in diplotene nuclei (Fig. 4C). Metaphase II spermatocytes showed n = 13 + X_1X_2 and n = 13 (Fig. 4D, E). Prophase I cells of the male with 2n = 29 presented three positively heteropycnotic blocks (Fig. 4F), sometimes very closely grouped (Fig. 4G, H); one of these blocks corresponded to the extra chromosome and the two others to the sex chromosomes.

Senoculidae.—Spermatogonial metaphase nuclei of *Senoculus* sp. showed 2n♂ = 28, with telocentric chromosomes (Fig. 5A). Diplotene nuclei exhibited 13 autosomal bivalents with one terminal or interstitial chiasma, and two positively heteropycnotic X_1 and X_2 univalents (13II + X_1X_20) (Fig. 5B). The meiotic configurations confirm that the male karyotype of *Senoculus* sp. is composed of 2n = 26 + X_1X_20.

DISCUSSION

Chromosome number in Lycosidae.—Among the four Lycosidae species analyzed here, only two populations of *L. erythrognatha* from Argentina (Chemisquy et al. 2008) and Uruguay (Diaz & Saez 1966), and one population of *L. nordenskjoldi* from Uruguay (Diaz & Saez 1966) have previously been described. The diploid number 2n♂ = 22 and the sex chromosome system of the X_1X_20 type observed in *L. erythrognatha* and *L. sericovittata* were similar to those encountered in six identified species of *Lycosa*, including *L. erythrognatha* examined by Diaz & Saez (1966) and Chemisquy et al. (2008), and five other unidentified species of this same genus (Brum-Zorrilla & Postiglioni 1980; Postiglioni & Brum-Zorrilla 1981; Parida & Sharma 1987a,b; Sharma & Parida 1987). The 2n♂ = 18 + X0 found in *L. nordenskjoldi* was similar to that verified in an Uruguayan population of the same species (Diaz & Saez 1966). This is the only species of this genus with the diploid number 2n=19 (Araujo et al. 2015). The karyotype 2n♂ = 18 + X0 verified in *H. sternalis* from Brazil is being reported for the first time for the genus and differed markedly from the 2n♂ = 26 + X_1X_20 registered for *Hogna himalayensis* (Gravely 1924) from India (Mittal 1963). However, only these two *Hogna* Simon 1885 species were karyotyped, and it seems to be premature to hypothesize that this difference in the diploid number is coincident with a geographical pattern.

Among the 23 genera of the Lycosidae karyotyped up to now, the predominant diploid number is 2n♂ = 28. Some interesting exceptions occur in the genera *Lycosa*, *Pirata* Sundevall 1833, and *Schizocosa* Chamberlin 1904. The high karyotype diversity observed in the genus *Lycosa* (Araujo et al. 2015) probably reflects its non-monophyletic nature suggested in the phylogenetic hypotheses of Vink et al. (2002) and Murphy et al. (2006). According to these authors, this genus has been used to assign lycosid spiders that did not fit convincingly in any other genus.

The monophyletic genus *Pirata*, characterized by the predominance of 2n♂ = 26 (Araujo et al. 2015), is sister-group to two non-karyotyped lycosid genera (*Allotrochosina* Roewer 1960 and *Anomalosa* Roewer 1960) and *Venonia* Thorell 1894 (Murphy et al. 2006), collected in India, which

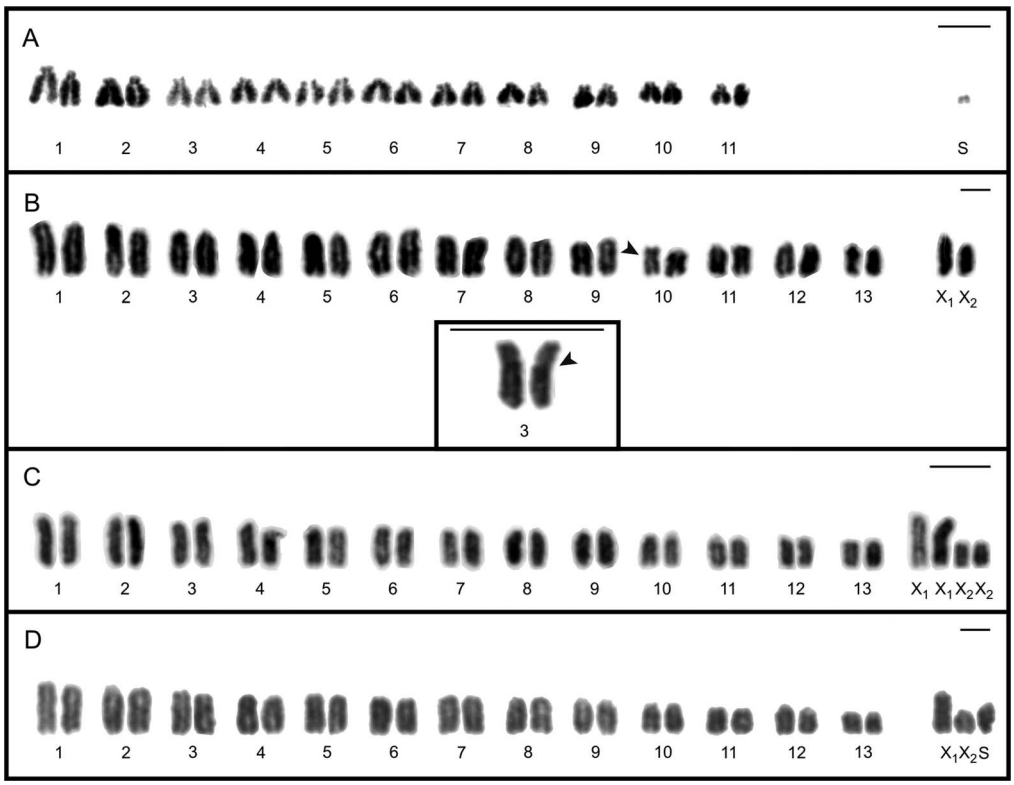

Figure 3.—Karyotypes of Trechaleidae species stained with Giemsa. *Neoctenus comosus* (A) and *Syntrechalea syntrechaleoides* (B–D). A. 2n♀ = 22 + 1S. B, C. 2n♂ = 26 + X_1X_20 and 2n♀ = 26 + $X_1X_1X_2X_2$, respectively. The arrowheads point to secondary constrictions on pairs 3 (detail) and 10. D. Male embryo with 2n = 26 + X_1X_20 + 1S. S = supernumerary chromosome. Scale = 10 μm.

also has 2n♂ = 26 (Mittal 1961, 1963). Only the cytogenetic analysis of *Allotrochosina* and *Anomalosa* can confirm whether the 2n♂ = 26 is a synapomorphy of a clade composed of *Allotrochosina*, *Anomalosa*, *Pirata* and *Venonia* proposed by Murphy et al. (2006). It is noteworthy, however, that the 2n♂ = 26 also occurs in other lycosid clades (Araujo et al. 2015), suggesting several independent reductions from 2n♂ = 28.

All *Schizocosa* species with determined chromosome number, presented 2n♂ = 22 (Araujo et al. 2015), with the exception of two non-identified species with 2n♂ = 28 (Mittal 1960, 1963). Unfortunately, *Schizocosa* representatives were not included in the phylogenetic analyses mentioned above, but it is noticeable that all species with 2n♂ = 22 were collected in the Americas (Argentina, Uruguay, and USA), while species with 2n♂ = 28 were obtained from India. Thus, in addition to the cytogenetic analysis of a high number of Lycosidae species, the taxon sampling should include representatives from different geographic regions necessary to unravel chromosomal differences and address the evolution of karyotypes within this group.

Chromosome number in Trechaleidae and Senoculidae.—Despite the fact that cytogenetic data on Trechaleidae are scarce, the five examined species exhibited variable diploid numbers, i.e., *N. comosus* studied here presented 2n♂ = 18 + X_1X_20, the lowest chromosome number already recorded for the family, *Paratrechalea ornata* (Mello-Leitão 1943) showed 2n♂ = 22 + X_1X_20 (Albo & Postiglioni 2011), and the three other species belonging to distinct genera, *Syntrechalea syntrechaleoides* (present work), *Trechalea bucculenta* (Simon 1898), and *Trechaleoides biocellata* (Mello Leitão 1926) (Albo & Postiglioni 2011) revealed 2n♂ = 26 + X_1X_20. These latter karyotype features corresponded to the most common pattern observed in spiders of closely related groups, such as Lycosidae and Pisauridae, and were also encountered in *Senoculus* sp. (Senoculidae). These data indicate that within true or high lycosoid clade, the diploid number 2n♂ = 28 is a shared feature, occurring in the majority of species of families Trechaleidae, Lycosidae, Pisauridae and Senoculidae. Additionally, the X_1X_20 sex chromosome system was the predominant type in all families of this group, with the exception of Oxyopidae (Araujo et al. 2015).

Chromosome morphology and size of sex chromosomes in Lycosoidea.—The telocentric chromosome morphology and the gradual decrease in the size of the autosomes in the seven species studied in this work constitute a pattern that is shared among Lycosoidea spiders. Our results point to a high morphological karyotype uniformity within the group, which is not exclusive to cytogenetic features, considering that Vink et al. (2002) emphasized the problem of generic limits within this clade due to the morphological uniformity of the species.

The size of the X_1 and X_2 chromosomes seems to differ among certain families, considering that in the Lycosidae species investigated here as well as in the majority of those described in the literature, both sex chromosomes showed a large size (Chemisquy et al. 2008; Dolejs et al. 2011). In contrast, in Ctenidae (Araujo et al. 2014), Trechaleidae (Albo & Postiglioni 2011) and Senoculidae species, the X_1 and X_2 sex chromosomes corresponded in length to the largest and

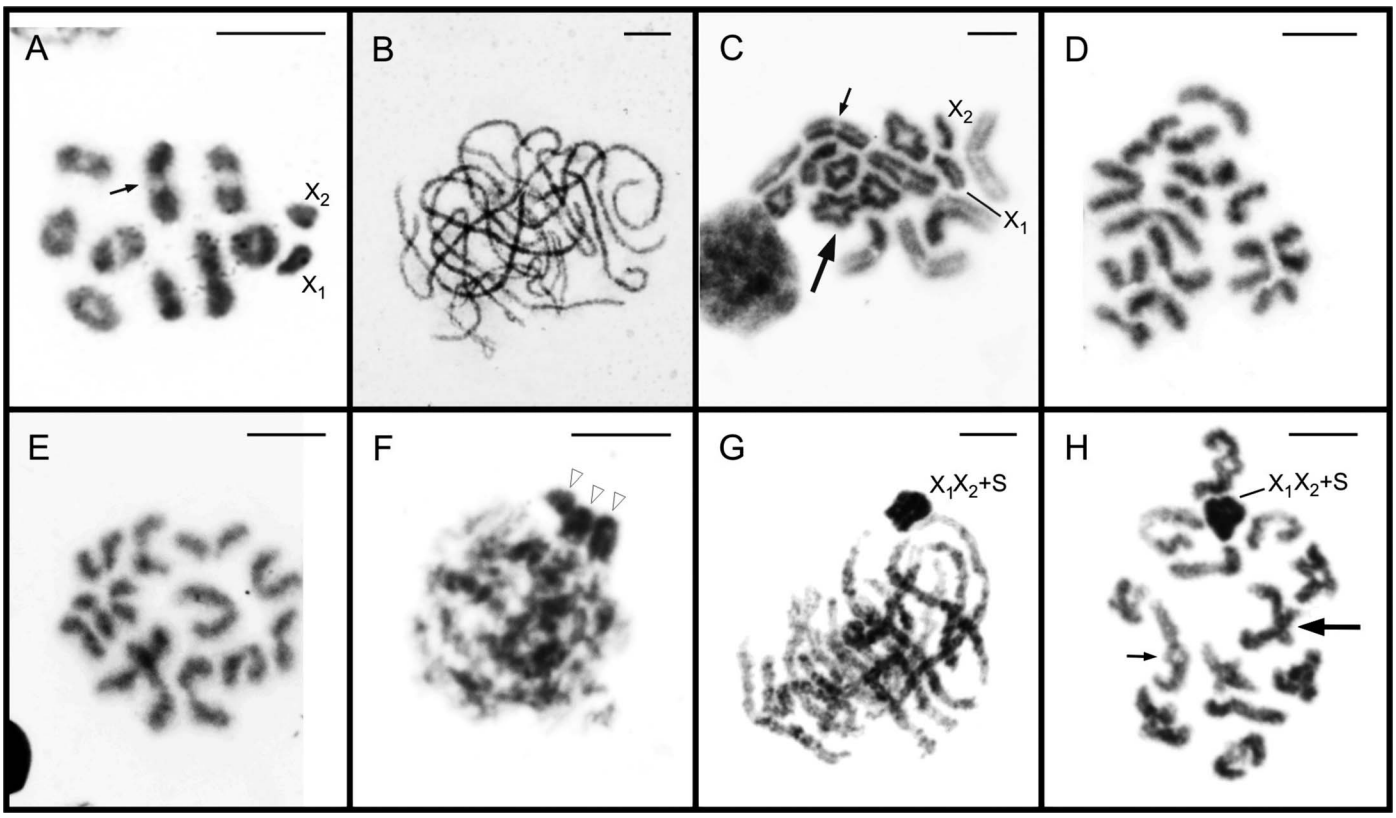

Figure 4.—Meiotic cells of Trechaleidae species. *Neoctenus comosus* (A, B) male and female respectively, and *Syntrechalea syntrechaleoides* with 2n♂ = 28 (C–E) and 2n♂ = 29 (F–H). A. Diplotene, with 9 autosomal bivalents plus two sex univalents (9II + X_1X_20). B. Pachytene with 11 bivalents plus one univalent. Late diplotene, with 13 autosomal bivalents plus two sex univalents (13II + X_1X_20). D, E. Metaphase II cells with n = 13 + X_1X_2 and n = 13, respectively. F. Leptotene, exhibiting three positively heteropycnotic blocks (empty arrows). One of these blocks represents the supernumerary chromosome. G. Pachytene, showing a block composed by the X_1, X_2, and the supernumerary chromosome. H. Diplotene, with 13II + X_1X_20 + S. S = supernumerary chromosome. Large arrow = interstitial chiasma. Small arrow = terminal chiasma. Scale = 10 μm.

smallest autosome pairs, respectively. Except in *Viracucha andicola* (Simon 1906), in which the X_1, X_2 and X_3 chromosomes were regarded as large, medium and small-sized (Araujo et al. 2014), in all other three representatives of lycosoid spiders, included in Lycosidae, Pisauridae and Ctenidae, that showed $X_1X_2X_30$ sex chromosome system, there is no information regarding to the size of the sex chromosomes (Postiglioni & Brum-Zorrilla 1981; Srivastava & Shukla 1986; Chen 1999). The size of the sex chromosome of a lycosid species that possesses an X0 system is being reported for the first time; however, in certain Oxyopidae spiders, the X chromosome presented variable length, from large to small (Bole-Gowda 1950; Barrion et al. 1989; Chen 1999; Stávale et al. 2011). The variability in the length of sex chromosomes, specifically of those belonging to X_1X_20 or X0 system, could be related to alterations in the amount of constitutive heterochromatin by duplications or deletions. Dolejs et al. (2011) analyzing the quantity and distribution of C bands in certain lycosids of the genera *Arctosa* C.L. Koch 1847, and *Xerolycosa* Dahl 1908, suggested that deletions in the sex

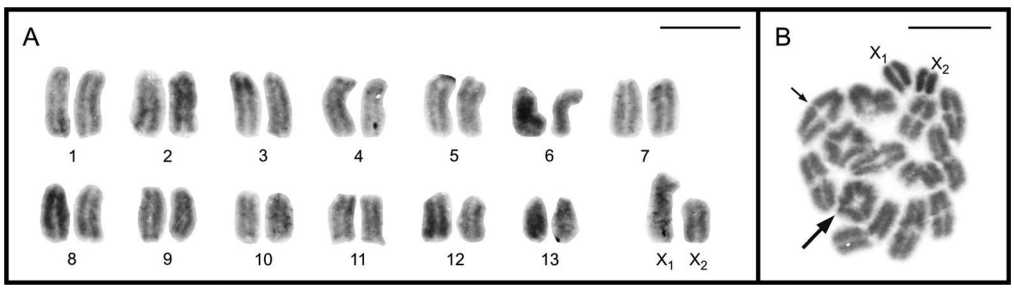

Figure 5.—Testicular cells of *Senoculus* sp. A. Karyotype, 2n = 26 + X_1X_20, with telocentric chromosomes. B. Diplotene, showing 13 autosomal bivalents with one interstitial (large arrow) or terminal (small arrow) chiasma and the unsynapsed X_1 and X_2 sex chromosomes. Scale = 10 μm.

chromosomes or translocations between sex chromosomes and/or sex chromosomes and autosomes could be the mechanism responsible for the differences in the size of the sex chromosomes.

Extra chromosomes in Trechaleidae and Lycosidae.—The presence of extra chromosomal elements, such as verified in *S. syntrechaleoides* has sporadically been registered in spiders (Montgomery 1905; Painter 1914; Avilés & Maddison 1991; Rowell & Main 1992; Zeng et al. 1996; Araujo et al. 2014). In the species studied here, this supernumerary element could resemble a B chromosome, considering that some embryos of a single clutch showed differences in relation to the presence or absence of the extra chromosome. In contrast, this supernumerary element, which showed a pattern of heteropycnosis and condensation similar to the sex chromosomes, could represent an intraspecific variability in the type of sex chromosome system, i.e., X_1X_20 and $X_1X_2X_30$. We excluded the possibility that the extra element has been originated by non-disjunction of the sister-chromatids of the X_1 or X_2 chromosome for two main reasons: 1) it showed a different length in relation to both sex chromosomes; 2) the extra chromosome did not present a synapsis with the sex chromosome or any other chromosome of the complement, confirming the lack of homology. The unexpected diploid number observed in one female of *L. sericovittata* can be explained by the presence of extra chromosomes or inter-populational karyotype variation, taking into account that the male and female individuals were collected in distinct localities, Tietê and São Roque, respectively, both in the state of São Paulo.

General chromosome number and sex chromosomes evolution in Lycosoidea.—The presence of $2n♂ = 28$ in all true or higher lycosoids families with more than one species analyzed (Lycosidae, Oxyopidae, Pisauridae and Trechaleidae) (Araujo et al. 2015), as well as in Senoculidae (present work), reinforces the hypothesis that this condition is ancestral to this group, as already hypothesized by Dolejs et al. (2011). Thus, the karyotype differentiation that has occurred in some species of this group involved a reduction in the number of autosomal pairs. Taking into account the presence of chromosomes with telo/acrocentric morphology in almost all species of the Lycosoidea (Araujo et al. 2015), Dolejs et al. (2011) inferred that a tandem fusion was the main event responsible for the reduction in the chromosome number.

The fact that the X0 and/or $X_1X_2X_30$ sex chromosome systems occur in species of different Lycosoidea lineages (Araujo et al. 2015) suggests that these derived sex chromosome systems evolved independently and repeatedly within some families, such as Lycosidae, Pisauridae and Oxyopidae. Various mechanisms to explain the origin of the X0 and $X_1X_2X_30$ sex chromosome systems have been hypothesized (Araujo et al. 2012; Kořínková & Král 2013), suggesting that several independent origins of the same sex chromosome system is not a rare event, as also showed by Maddison & Leduc-Robert (2013) for the X_1X_2Y and $X_1X_2X_3Y$ systems in salticids.

The cytogenetic investigation presented herein provided karyotype data of one species belonging to Senoculidae for the first time. Despite the fact that certain families are scarcely studied, the main mechanism of karyotype evolution among the Lycosoidea spiders seems to be a decrease in autosomal number without changing the X_1X_20 sex chromosome system and telo/acrocentric chromosome morphology. An exception is present in Oxyopidae, in which the reduction of the diploid number is usually accompanied by alteration in the sex chromosome system from X_1X_20 type to X0 type (Araujo et al. 2015).

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