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Research Article

Cytogenetic studies of three Lycosidae species from Argentina (Arachnida, Araneae)

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Abstract

Cytogenetic studies of the family Lycosidae (Arachnida: Araneae) are scarce. Less than 4% of the described species have been analyzed and the male haploid chromosome numbers ranged from $8+X_1X_2$ to $13+X_1X_2$. Species formerly classified as *Lycosa* were the most studied ones. Our aim in this work was to perform a comparative analysis of the meiosis in "*Lycosa*" *erythrognatha* Lucas, "*Lycosa*" *pampeana* Holmberg and *Schizocosa malitiosa* (Tullgren). We also compared male and female karyotypes and characterized the heterochromatin of "*L.*" *erythrognatha*. The males of the three species had 2n = 22, n = $10+X_1X_2$, all the chromosomes were telocentric and there was generally a single chiasma per bivalent. In "*Lycosa*" *pampeana*, which is described cytogenetically for the first time herein, the bivalents and sex chromosomes showed a clustered arrangement at prometaphase I. The comparison of the male/female karyotypes (2n = 22/24) of "*Lycosa*" *erythrognatha* revealed that the sex chromosomes were the largest of the complement and that the autosomes decreased gradually in size. The analysis of the amount, composition and distribution of heterochromatin with C-banding and staining with DAPI- and CMA_3- showed that "*Lycosa*" *erythrognatha* had little GC-rich heterochromatin in the pericentromeric region of all chromosomes. In addition, the actual occurrence of the genus *Lycosa* in the Southern Hemisphere is discussed.

Key words: meiosis, C-banding, fluorochrome staining, karyotype, spiders, "*Lycosa*" and *Schizocosa*.

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Introduction

Cytogenetic studies of the family Lycosidae (Arachnida, Araneae) are scarce and were performed in less than 4% of the 2324 known species (Platnick, 2008). Most of the analyzed species had only telocentric or acrocentric chromosomes, which ranged from 2n = 18, n = $8+X_1X_2$ (male) to 2n = 28/30 (male/female), n = $13+X_1X_2$ (male). The 2n = 28/30, which is present in 50% of the analyzed species, is probably the modal diploid number of the family. The sex chromosome mechanism $X_1X_2/X_1X_1X_2X_2$ (male/female) occurs in 94% of the lycosid species and is considered as an ancestral trait in spiders. The derived systems are: X0 in *Lycosa barnesi*, *L. nordenskjoldi*, *Wadicosa quadrifera* and an unidentified species of *Schizocosa* (*Schizocosa* sp. 2 in Table 1); $X_1X_2X_3$ in an unidentified species of *Lycosa* (*Lycosa* sp. 8 in Table 1), and a neo-XY system with multiple X chromosomes in *Pardosa morosa* (Král, 2004). Most cytological studies have been performed in species formerly classified as *Lycosa* and the most common male haploid chromosome number was n = $13+X_1X_2$ in the Northern Hemisphere species. The Southern Hemisphere species presented complements with n = $10+X_1X_2$ (males) or derived from it (Table 1).

The content, distribution and composition of the constitutive heterochromatin in spiders have been poorly analyzed (Brum-Zorrilla and Cazenave, 1974; Brum-Zorrilla and Postiglioni, 1980; Rowell, 1985; Datta and Chatterjee, 1988; Rowell, 1991; Gorlova *et al.*, 1997; Silva *et al.*, 2002; Araujo *et al.*, 2005a, 2005b; Rodríguez Gil *et al.*, 2007). The first characterization of heterochromatin in spiders was performed in *Schizocosa malitiosa* using C-banding. All chromosomes showed small pericentromeric heterochro-

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Table 1 - Chromosome data of Lycosidae species.

Specie	2n	n (male)	Origin	References
Allocosa georgicola (Walckenaer 1837)	28	13+X_1X_2	USA	Wise, 1983; Wise, 1984 (sub *Lycosa georgicola*); Wise and Shaw, 1984 (sub *Lycosa georgicola*)
Alopecosa aculeata (Clerck 1757)		13+X_1X_2	Finland	Hackman, 1948 (sub *Tarentula aculeata*)
A. albofasciata (Brullé 1832)		13+X_1X_2	Israel	Gorlova *et al.*, 1997
A. pulverulenta (Clerck 1757)		13+X_1X_2	Finland	Hackman, 1948 (sub *Tarentula pulverulenta*)
Anomalomma sp.	28	13+X_1X_2	India	Sharma *et al.*, 1959
Anomalosa harishi (Dyal 1935)	28	13+X_1X_2	India	Mittal, 1961; Mittal, 1963 (sub *Anomalomma harishi*)
Arctosa alpigena (Doleschall 1852)	26	12+X_1X_2	Finland	Hackman, 1948
A. leopardus (Sundevall 1833)	26	12+X_1X_2	Finland	Hackman, 1948
Arctosa sp.	28 30 f	13+X_1X_2	India	Mittal, 1960, 1963
Crocodilosa leucostigma (Simon 1885)	28	13+X_1X_2	India	Srivastava and Shukla, 1986 (sub *Lycosa leucostigma*)
Evippa praelongipes (O.P.-Cambridge 1870)	26	12+X_1X_2	India Israel	Sharma *et al.*, 1958; Gorlova *et al.*, 1997
Gladicosa pulchra (Keyserling 1877)	28	13+X_1X_2	USA	Montgomery, 1905 (sub *Lycosa insopita*); Gowan, 1985 (sub *Lycosa insopita*) [(1)]
Hippasa agelenoides (Simon 1884)	28 24	13+X_1X_2 11+X_1X_2	India	Bole-Gowda, 1953; Sharma *et al.*, 1958
H. madhuae Tikader and Malhotra 1980	28	13+X_1X_2	India	Parida *et al.*, 1986
H. olivacea (Thorell 1887)	28	13+X_1X_2	India	Parida and Sharma, 1987a; Parida and Sharma, 1987b; Sharma and Parida, 1987
H. pisaurina Pocock 1900	26 28	12+X_1X_2 13+X_1X_2	India India	Mittal 1960, 1963 Srivastava and Shukla, 1986
Hippasa sp.	22	10+X_1X_2	India	Parida and Sharma, 1987b; Sharma and Parida, 1987
Hogna ammophila (Wallace 1942)		11+X_1X_2	USA	Gowan, 1985 (sub *Lycosa ammophila*) [(1)]
H. helluo (Walckneaer 1837)		13+X_1X_2	USA	Gowan, 1985 (sub *Lycosa helluo*) [(1)]
H. himalayensis (Gravely 1924)	28	13+X_1X_2	India	Mittal, 1962, 1963 (sub *Lycosa himalayensis*)
H. lenta (Hentz 1844)		11+X_1X_2	USA	Gowan, 1985 (sub *Lycosa lenta*) [(1)]
Hygrolycosa rubrofasciata (Ohlert 1865)	20 22 f		Russia India	Gorlov *et al.*, 1995
Lycosa barnesi Gravely 1924	27	13+X	India	Srivastava and Shukla, 1986
L. bistriata Gravely 1924	28	13+X_1X_2	India	Bole-Gowda, 1953; Bole-Gowda, 1958
L. carmichaeli Gravely 1924	28 22	13+X_1X_2 10+X_1X_2	India	Mittal, 1961, 1963 Srivastava and Shukla, 1986
L. cf. *praegrandis* C. L. Koch 1878		10+X_1X_2	Israel	Gorlova *et al.*, 1997 (sub *Lycosa* cf. *nordmanni*)
L. chaperi Simon 1885	22	10+X_1X_2	India	Mittal, 1962, 1963
L. coelestis L. Koch 1878	26	12+X_1X_2	Japan	Suzuki, 1954
"*Lycosa*" *erythrognatha* Lucas 1836	22 24 f	10+X_1X_2	Uruguay Brazil India	Díaz and Sáez, 1966a[(2)], 1966b[(2)]; Giroti *et al.*, 2007 This work
L. madani Pocock 1901	24	11+X_1X_2	India	Mittal, 1962, 1963
L. nigrotibialis Simon 1884	28 24	13+X_1X_2 11+X_1X_2	India	Mittal, 1961, 1963 Srivastava and Shukla, 1986
L. nordenskjoldi Tullgren 1905		9+X	Uruguay	Díaz and Sáez, 1966a, 1966b (sub *Lycosa nordenskölii*)
"*Lycosa*" *pampeana* Holmberg 1876		10+X_1X_2	Argentina	This work
L. sericovittata Mello-Leitao 1939	22	10+X_1X_2	Brazil	Giroti *et al.*, 2007
L. thorelli (Keyserling 1877)	22	10+X_1X_2	Uruguay	Brum-Zorrilla and Postiglioni, 1980; Postiglioni and Brum-Zorrilla, 1981
Lycosa sp. 1	28	13+X_1X_2	India	Bole-Gowda, 1953, 1958
Lycosa sp. 2	28	13+X_1X_2	Japan	Suzuki, 1954
Lycosa sp. 3	28	13+X_1X_2	India	Sharma *et al.*, 1958
Lycosa sp. 4		13+X_1X_2		Sokolov, 1960
Lycosa sp. 5		13+X_1X_2	India	Mittal, 1961 (sub *Lycosa* sp. nov.)
Lycosa sp. 6	28	13+X_1X_2	India	Mittal, 1962, 1963

Table 1 (cont.)

Specie	2n	n (male)	Origin	References
Lycosa sp. 7	22 24 f	$10+X_1X_2$	Uruguay	Brum-Zorrilla and Postiglioni, 1980
Lycosa sp. 8	23	$10+X_1X_2X_3$	Uruguay	Postiglioni and Brum-Zorrilla, 1981 (sub *Lycosa* sp.2 (*thorelli* group))
Lycosa sp. 9	22	$10+X_1X_2$	Uruguay	Postiglioni and Brum-Zorrilla, 1981 (sub *Lycosa* sp.3)
Lycosa sp. 10	18	$8+X_1X_2$	India	Srivastava and Shukla, 1986 (sub *Lycosa* sp.I)
Lycosa sp. 11	28	$13+X_1X_2$	India	Srivastava and Shukla, 1986 (sub *Lycosa* sp.II)
Lycosa sp. 12	22	$10+X_1X_2$	India	Sharma and Parida, 1987; Parida and Sharma, 1987a; Parida and Sharma, 1987b (sub *Lycosa* sp.1)
Lycosa sp. 13	28	$13+X_1X_2$	India	Parida and Sharma, 1987b, Sharma and Parida, 1987 (sub *Lycosa* sp.2)
Margonia himalayensis (Gravely 1924)	28	$13+X_1X_2$	India	Mittal, 1961, 1963 (sub *Venonia himalayensis*)
Ocyale kumari Dyal 1935	28	$10+X_1X_2$	India	Sharma *et al.*, 1958
Pardosa agrestis (Westring 1861)	28	$13+X_1X_2$	Russia	Gorlov *et al.*, 1995
P. agricola (Thorell 1856)	28	$13+X_1X_2$ 15(MII) h	Finland	Hackman, 1948 (sub *Lycosa fluviatilis*)
P. amentata (Clerck 1757)	28	$13+X_1X_2$	Finland	Hackman, 1948 (sub *Lycosa paludicola*, sub *Lycosa saccata*); Sokolov, 1960
P. astrigera L. Koch 1878	28 30 f	$13+X_1X_2$	Japan	Suzuki, 1954 (sub *Lycosa cinereofusca*, sub *Lycosa T-insignita*); Matsumoto, 1977 (sub *Pardosa T-insignita*); Kageyama *et al.*, 1978
P. basiri (Dyal 1935)	22	$10+X_1X_2$	India	Mittal, 1960, 1963
P. birmanica Simon 1884	28	$13+X_1X_2$	India	Bole-Gowda, 1953, 1958 (sub *Lycosa birmanica*); Srivastava and Shukla, 1986 (sub *Lycosa birmanica*); Parida and Sharma, 1987a, 1987b; Sharma and Parida, 1987; Datta and Chatterjee, 1989
P. fletcheri (Gravely 1924)	28	$13+X_1X_2$	India	Srivastava and Shukla, 1986 (sub *Lycosa fletcheri*)
P. lahorensis Dyal 1935	28	$13+X_1X_2$	India	Sharma *et al.*, 1958
P. laura Karsch 1879	28 30 f	$13+X_1X_2$	Japan	Kageyama *et al.*, 1978
P. leucopalpis Gravely 1924	28 24	$13+X_1X_2$ $11+X_1X_2$	India India	Bole-Gowda, 1953, 1958; Mittal, 1963, Srivastava and Shukla, 1986
P. lugubris (Walckenaer 1802)	28	$13+X_1X_2$	Russia	Gorlov *et al.*,1995
P. monticola (Clerck 1757)	28	$13+X_1X_2$	Finland	Hackman, 1948 (sub *Lycosa monticola*)
P. mulani (Dyal 1935)	28	$13+X_1X_2$	India	Sharma *et al.*, 1958
P. oakleyi Gravely 1924	26	$12+X_1X_2$	India	Srivastava and Shukla, 1986
P. palustris (Linnaeus 1758)	24 28	$12+X_1X_2$ $13+X_1X_2$	Finland Russia	Hackman, 1948 (sub *Lycosa tarsalis*) Gorlov *et al.*, 1995
P. plumipes (Thorell 1875)	28	$13+X_1X_2$	Russia	Gorlov *et al.*, 1995
P. pseudoannulata (Bösenberg and Strand 1906)	28 30 f	$13+X_1X_2$	Japan India	Suzuki, 1954 (sub *Lycosa pseudoannulatus*); Kageyama *et al.*, 1978 (sub *Lycosa pseudoannulata*) Bole-Gowda, 1953, 1958 (sub *Lycosa annandalei*); Srivastava and Shukla, 1986 (sub *Lycosa annandalei*)
P. pullata (Clerck 1757)	28	$13+X_1X_2$	Finland	Hackman, 1948 (sub *Lycosa pullata*)
P. sumatrana (Thorell 1890)	24	$11+X_1X_2$	India	Sharma, 1961 (sub *Lycosa sumatrana*); Srivastava and Shukla, 1986 (sub *Lycosa sumatrana*)
Pardosa sp. 1		$13+X_1X_2$	India	Bole-Gowda, 1953, 1958
Pardosa sp. 2	28	$13+X_1X_2$	India	Sharma and Gupta, 1956
Pardosa sp. 3	28	$13+X_1X_2$	India	Mittal, 1960
Pirata latitans (Blackwall 1841)	24	$11+X_1X_2$	India	Mittal, 1962, 1963
P. piraticus (Clerck 1757)	26	$12+X_1X_2$	Finland	Hackman, 1948
P. procurvus (Bösenberg and Strand 1906)	26 28 f	$12+X_1X_2$	Japan	Kageyama *et al.*, 1978
P. subpiraticus (Bösenberg and Strand 1906)	26 28 f	$12+X_1X_2$	Japan	Kageyama *et al.*, 1978
P. uliginosus (Thorell 1856)	24	$11+X_1X_2$	Finland	Hackman, 1948
Pirata sp.		$12+X_1X_2$		Sokolov, 1960

Table 1 (cont.)

Specie	2n	n (male)	Origin	References
Rabidosa punctulata (Hentz 1844)		13+X_1X_2	USA	Gowan, 1985[(1)]
R. rabida (Walckenaer 1837)	28 30 f	13+X_1X_2	USA	Wise, 1983, 1984 (sub *Lycosa rabida*); Wise and Shaw, 1984 (sub *Lycosa rabida*); Tugmon *et al.*, 1990 (sub *Lycosa rabida*)
Schizocosa communis (Emerton 1885)	22	10+X_1X_2	USA	Painter, 1914 (sub *Lycosa communis*)
S. crassipes (Walckenaer 1837)	22	10+X_1X_2	USA	Hard, 1939
S. malitiosa (Tullgren 1905)	22 24 f	10+X_1X_2	Uruguay Argentina	Brum-Zorrilla and Cazenave, 1974; Brum-Zorrilla and Postiglioni, 1980 (sub *Lycosa malitiosa*) This work
S. ocreata (Hentz 1844)	22	10+X_1X_2	USA	Stratton, 1997
S. rovneri Uetz and Dondale 1979		10+X_1X_2	USA	Stratton, 1997
S. stridulans Stratton 1984		10+X_1X_2	USA	Stratton, 1997
Schizocosa sp. 1	28	13+X_1X_2	India	Mittal, 1960, 1963
Schizocosa sp. 2	23	11+X	Uruguay	Postiglioni and Brum-Zorrilla, 1981 (sub *Lycosa* sp.1 (*malitiosa* group))
Trochosa punctipes (Gravely 1924)	28	13+X_1X_2	India	Sharma, 1961 (sub *Lycosa punctipes*)
T. ruricola (De Geer 1778)	26 28 f	12+X_1X_2 14(MI)	Finland	Hackman, 1948
T. spinipalpis (F.O.P.-Cambridge 1895)	26	12+X_1X_2	Finland	Hackman, 1948
Venonia sp.	26	12+X_1X_2	India	Mittal, 1963
Wadicosa quadrifera (Gravely 1924)	27	13+X	India	Srivastava and Shukla, 1986 (sub *Lycosa quadrifer*)
Xerolycosa miniata (C.L. Koch 1834)	22	10+X_1X_2	Finland Russia	Hackman, 1948 Gorlov *et al.*, 1995
X. nemoralis (Westring 1861)	26 22	12+X_1X_2	Finland Russia	Hackman, 1948 Gorlov *et al.*, 1995

[(1)]Gowan TD (1985). The life history and reproduction of the wolf spider, *Lycosa lenta* Hentz. PhD. Thesis. University of Florida. 259 pp. [(2)]Díaz and Sáez (1966b) handwrote on each reprint of their paper that the spiders they originally classified as two separate species having n = 10+X_1X_2, actually belonged to "*Lycosa*" *erythrognatha.*

matic bands in this species and in all the other Lycosidae analyzed (Brum-Zorrilla and Cazenave, 1974; Brum-Zorrilla and Postiglioni, 1980; Gorlova *et al.*, 1997).

Morphological and molecular phylogenetic studies questioned the taxonomic position of a number of species currently placed in *Lycosa (sensu lato)* (including *Lycosa erythrognatha*), since they appear not to be closely affiliated with *Lycosa tarantula* (Linnaeus 1758), the type species of the genus (Murphy *et al.*, 2006; Álvares and Brescovit, 2007). In view of this uncertainty, we named the species with its original combination, but with the generic name inside inverted commas.

In this work we analyzed and compared the meiotic behavior of "*Lycosa*" *erythrognatha* Lucas 1836, "*Lycosa*" *pampeana* Holmberg 1876 and *Schizocosa malitiosa* (Tullgren 1905). We used C-banding, DAPI- and CMA_3-staining to analyze the male and female karyotypes and the amount, composition and distribution of the heterochromatin in "*L.*" *erythrognatha*. We compared our results with those reported for other lycosid species. A literature review of the cytogenetics Lycosidae and a discussion on the actual occurrence of the genus *Lycosa* in Southern Hemisphere are also included.

Materials and Methods

We analyzed 21 males and nine females of "*Lycosa*" *erythrognatha* from Buenos Aires City and surroundings (34°48' S - 58°41' W) (17 males, five females), Martín García Island Natural Preserve (34°18' S - 58°24' W, Buenos Aires Province) (two males), Magdalena (35°08' S - 57°51' W, Buenos Aires Province) (one female), Parque Nacional "El Palmar" (24°08' S - 64°58' W, Entre Ríos Province) (one female), Malargüe (35°48' S - 69°59' W, Mendoza Province) (one male, two females) and Posadas (27°40' S - 55°93' W, Misiones Province) (one male); four males of "*Lycosa*" *pampeana* from Buenos Aires City and surroundings; and seven males of *Schizocosa malitiosa* from Martín García Island Natural Preserve (one male), Gualeguaychú (33°04' S - 58°43' W, Entre Ríos Province) (three males, one subadult male), San Juan Poriahú (27°61' S - 56°98' W, Corrientes Province) (one male) and Embalse de Río Tercero (32°17' S - 64°25' W, Córdoba Province) (one male).

Adult males and females were collected in the field and reared at the Arachnology Division of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). Voucher specimens were deposited in the Na-

tional Collection of Arachnology (MACN-Ar, Cristina Scioscia).

For male meiotic analyses, testes were dissected out and kept in 3:1 ethanol:acetic acid at 4 °C. Preparations were obtained by squashing in iron propionic haematoxylin.

Fluorescent staining with 4'-6-diamidino-2-phenylindole (DAPI) and chromomycin A_3 (CMA_3) was carried out on unstained chromosomes. After squashing a piece of testis in 45% acetic acid, the coverslip was removed with the dry-ice method and slides were air-dried. The sequential DAPI-CMA_3 staining was performed according to Rebagliati *et al.* (2003).

For the mitotic analysis specimens of "*Lycosa*" *erythrognatha* were injected with 0.1 mL of a 0.01% colchicine solution. After 1.25 h, several drops of haemolymph were removed from the coxal joints and the gonads together with some digestive tissues were dissected. Each sample was suspended in 2 mL of hypotonic solution (KCl 0.56%) for 15 min, centrifuged at 800 rpm for 5 min, and fixed in 1 mL of 3:1 ethanol:acetic acid. The cell suspension was dropped onto clean slides, air-dried and stained with Giemsa for chromosome counting and karyotyping. C-banding was carried out according to Sumner (1972).

Chromosome measurements were performed in twelve well-spread mitotic metaphases using the MicroMeasure version 3.3 software (Reeves and Tear, 2000). The total haploid complement length (TCL) in females was calculated by adding the mean value of each chromosome pair (in arbitrary units). In males, the TCL was calculated after the analysis of the relative length of all chromosomes, which was used to identify those having no homologues (sex chromosomes). The male and female idiograms were drawn based on the length of each chromosome pair in relation to the TCL. Chromosomes were also measured with a vernier caliper in order to estimate the TCL in microns.

Results

Karyotype of "*Lycosa*" *erythrognatha*

The diploid number in somatic cells was 22 in males and 24 in females. All the chromosomes were telocentric (Figures 1a, b). The X_1 and X_2 were the largest chromosomes of the complement, with 12.83% and 11.69% of the TCL, respectively, whereas the autosomes decreased gradually in size, with the largest and smallest pairs representing 9.54% and 5.47% of the TCL, respectively (Figures 1a, b, d). The total haploid complement length (TCL) was 43.8 µm, the sex chromosomes were 5.03 ± 0.04 µm and 4.78 ± 0.1 µm long and the autosomes ranged between 4.13 ± 0.25 µm and 2.64 ± 0.09 µm. C-banding revealed the

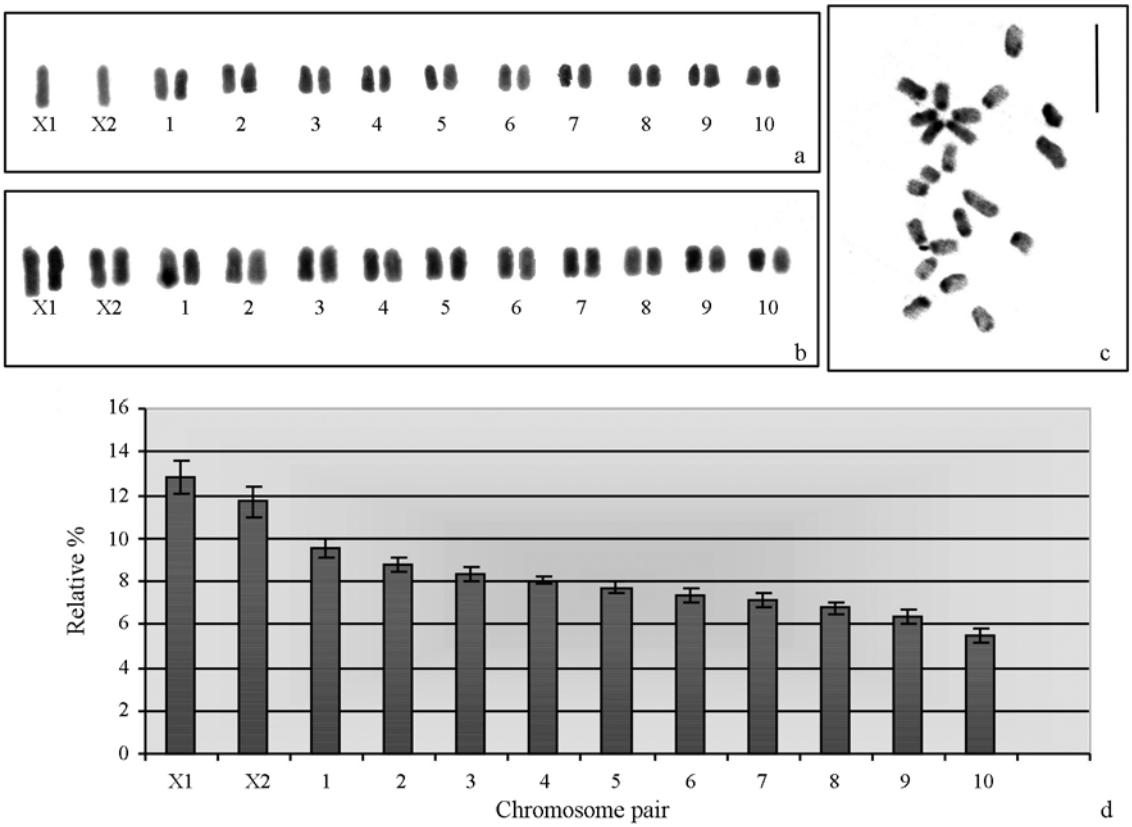

Figure 1 - "*Lycosa*" *erythrognatha* chromosomes (2n = 22): male (a) and female (b) karyograms; C-banded male metaphase (c); relative chromosome sizes in the male (d). Bar = 10 µm.

presence of small positive bands in the pericentromeric region of all chromosomes (Figure 1c).

Male meiosis

"*Lycosa*" *erythrognatha* (2n = 22, n = 10+X_1X_2 and n = 10)

At spermatogonial prometaphases and metaphases the sex chromosomes and the autosomes were isopycnotic (Figure 2a). At prophase I, up to pachytene, the sex chromosomes were positively heteropycnotic and closely associated (Figures 2b, c). The sex chromosomes were usually isopycnotic from diakinesis onwards, but appeared negatively heteropycnotic in some cells; in both cases they remained associated and differed in size (Figures 2d-f). Bivalents had a single proximal or interstitial chiasma (Figures 2d-g; 3e-f), but two chiasmata could occasionally be observed in one of the largest bivalents (Figure 2e). In six in-

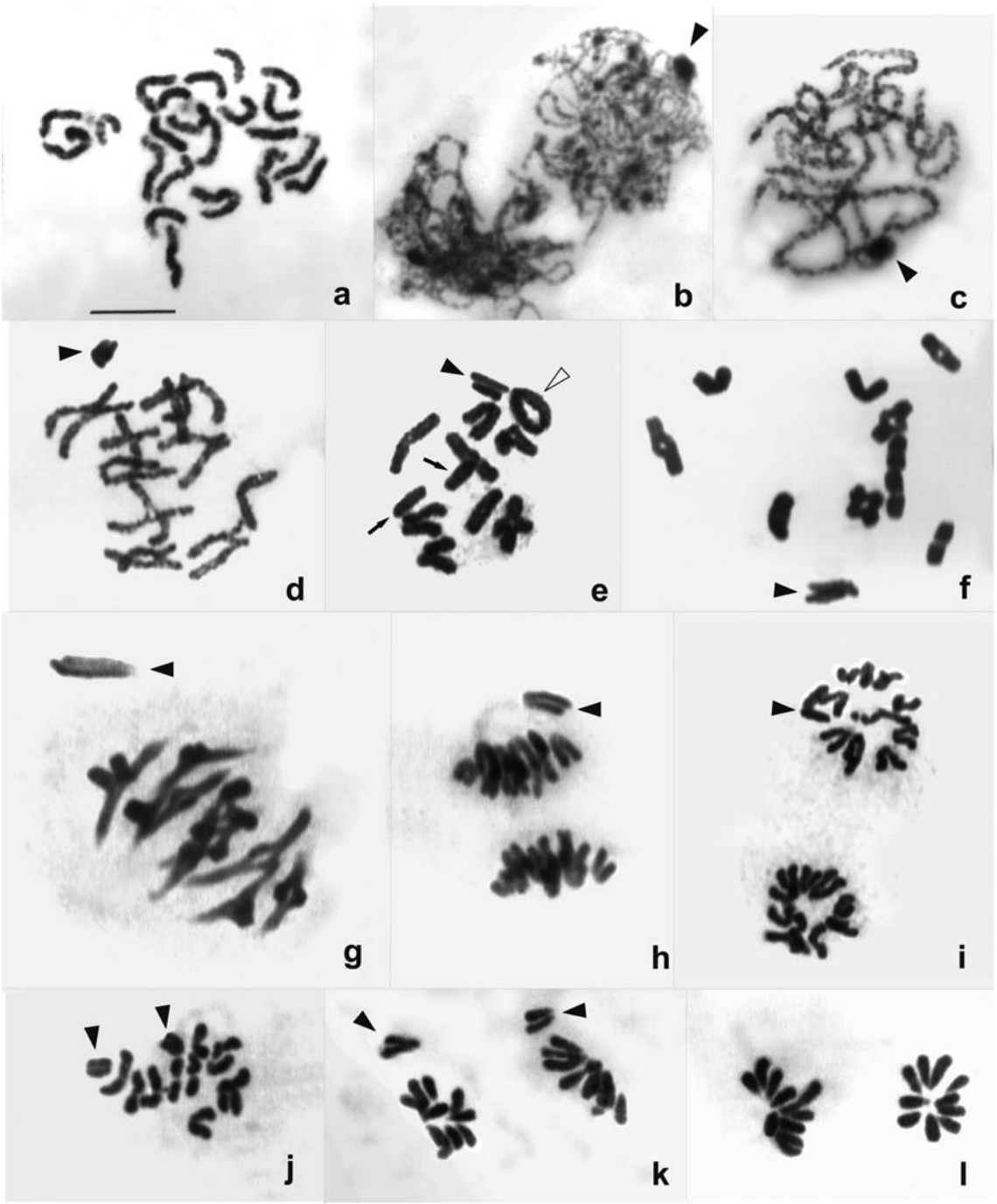

Figure 2 - Meiosis in "*Lycosa*" *erythrognatha* (2n = 22, n = 10+X_1X_2 and n = 10): (a) spermatogonial prometaphase; (b) zygotene; (c) pachytene; (d) early diplotene; (e) late diplotene with two univalents (arrows) and a bivalent with two chiasmata (arrowhead); (f) diakinesis; (g) metaphase I; (h) anaphase I; (i) telophase I; (j) metaphase II with sex chromosomes; (k) anaphase II with sex chromosomes; (l) telophase II without sex chromosomes. The arrowheads point to the sex chromosomes. Bar = 10 μm.

dividuals, a pair of medium-sized autosomal univalents were seen in a low frequency at diakinesis (less than 10% of the cells) and two univalent pairs were seen in a single cell (Figure 2e). The sex chromosomes were located apart from the bivalents at metaphase I (Figures 2g; 3f) and precociously migrated together towards the same pole at anaphase I (Figure 2h-i). This resulted in two types of metaphase II, one with ten autosomes and the other with ten autosomes plus the X_1X_2 chromosomes (Figure 2j). The sister chromatids of each sex chromosome were always closely associated, whereas the autosomal chromatids were only associated by the centromeric region (Figure 2j). The sex chromosomes and autosomes migrated simultaneously and were positioned slightly apart at anaphase II (Figure 2k) resulting in cells with ten autosomes (Figure 2l) and with ten chromosomes plus X_1X_2 in telophase II. An atypical meiosis was observed in some cells of all the males (unpublished data).

Sequential DAPI-CMA_3-staining of spermatogonial prometaphases and metaphases revealed that all the pericentromeric C-positive bands were bright after CMA_3-staining and showed no differential fluorescence with DAPI (Figures 3a-b). The sex chromosomes were brightly fluorescent after DAPI- and CMA_3-staining at early prophase I. The CMA_3-bright bands observed in mitotic chromosomes were composed of several smaller CMA_3-fluorescent bands at pachytene. The same bands were generally dull after DAPI-staining (Figures 3c-d). A single pericentromeric CMA_3-bright band could be observed in the autosomes and sex chromosomes from diplotene onwards (Figures 3e-f).

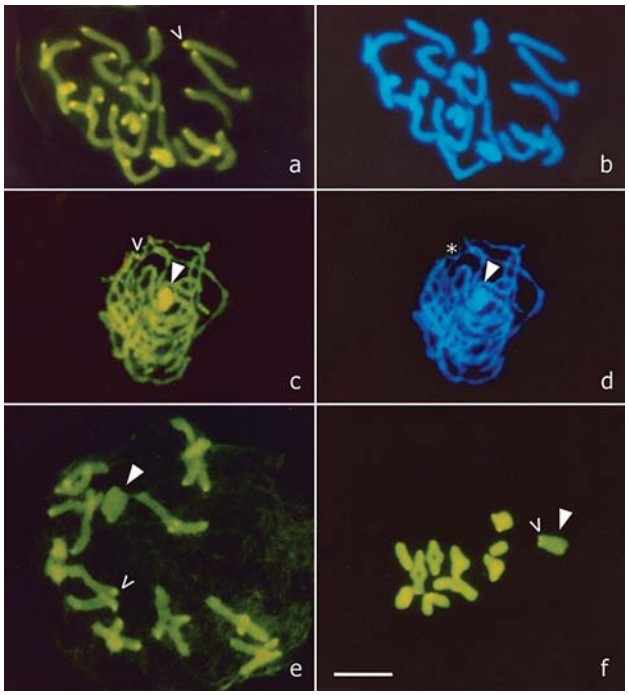

Figure 3 - Testicular cells of "*Lycosa*" *erythrognatha* ($2n = 22 = 20+X_1X_2$) after staining with CMA_3 (a, c, e, f) and with DAPI (b, d): (a-b) spermatogonial prometaphase; (c-d) pachytene; (e) diakinesis; (f) metaphase I. The arrowheads point to the sex chromosomes; the V points to CMA_3 bright bands and the asterisk marks the DAPI dull regions. Bar = 10 µm.

"*Lycosa*" *pampeana* ($2n = 22$, $n = 10+X_1X_2$, $n = 10$)

The sex chromosomes were positively heteropycnotic and closely associated at early prophase I (Figure 4a) and turned isopycnotic at diakinesis, when they remained associated and showed different sizes. All autosomal bivalents presented a single proximal or distal chiasma and bivalents with two chiasmata were never found (Figures 4b-c). Bivalents adopted a particular disposition during prometaphase I, with some of them (from one to four) lining up on the equatorial plate and the others located near the poles. The sex chromosomes were either in the cell equator or at one pole (Figures 4d-f). The bivalents and sex chromosomes lined up on the equatorial plane at metaphase I and the sex chromosomes migrated together to the same pole at anaphase I (Figure 4g). Two types of prometaphases II and metaphases II could be distinguished, one with ten and the other with 12 chromosomes (Figure 4h). Chromosomes with a telocentric morphology were clearly seen at anaphase I and II (Figures 4g, i).

Schizocosa malitiosa ($2n = 22$, $n = 10+X_1X_2$, $n = 10$)

Twenty-two isopycnotic chromosomes were seen in spermatogonial prometaphases and metaphases (Figure 5a). The sex chromosomes were closely associated and positively heteropycnotic at early prophase I (Figure 5b) and turned isopycnotic from diakinesis onwards, when they were close to each other and showed different sizes (Figures 5c-d). Most of the bivalents had a single interstitial or distal chiasma, and less frequently a proximal one, as could be seen at metaphase I (Figure 5e). Some cells also presented one bivalent with two distal chismata (Figure 5c). The sex chromosomes were not lined up on the equatorial plate at metaphase I, but closer to one pole (Figure 5e), and they migrated together to the same pole at anaphase I (Figure 5f). The sex chromosomes remained condensed and positively heteropycnotic at prophase II (Figure 5g) and were similar in size to the largest autosomes at metaphase II. The sister chromatids of the X_1 and X_2 chromosomes were always associated, whereas autosomal chromatids were only associated by their centromeric region (Figures 5h-i). The chromosomes of this species were also telocentric (Figures 5h-j).

Discussion

Only ten species of Lycosidae from South America have been cytogenetically studied. Two of them belonged to the genus *Schizocosa* Chamberlin 1904 and the remaining eight to the genus *Lycosa* Latreille 1804 (Table 1). They were collected in Uruguay, Brazil and Argentina.

In this work, we found a $2n = 22$ ($20+X_1X_2$, male) in *Schizocosa malitiosa*. This species had a karyotype with all telocentric chromosomes, with the sex chromosomes being

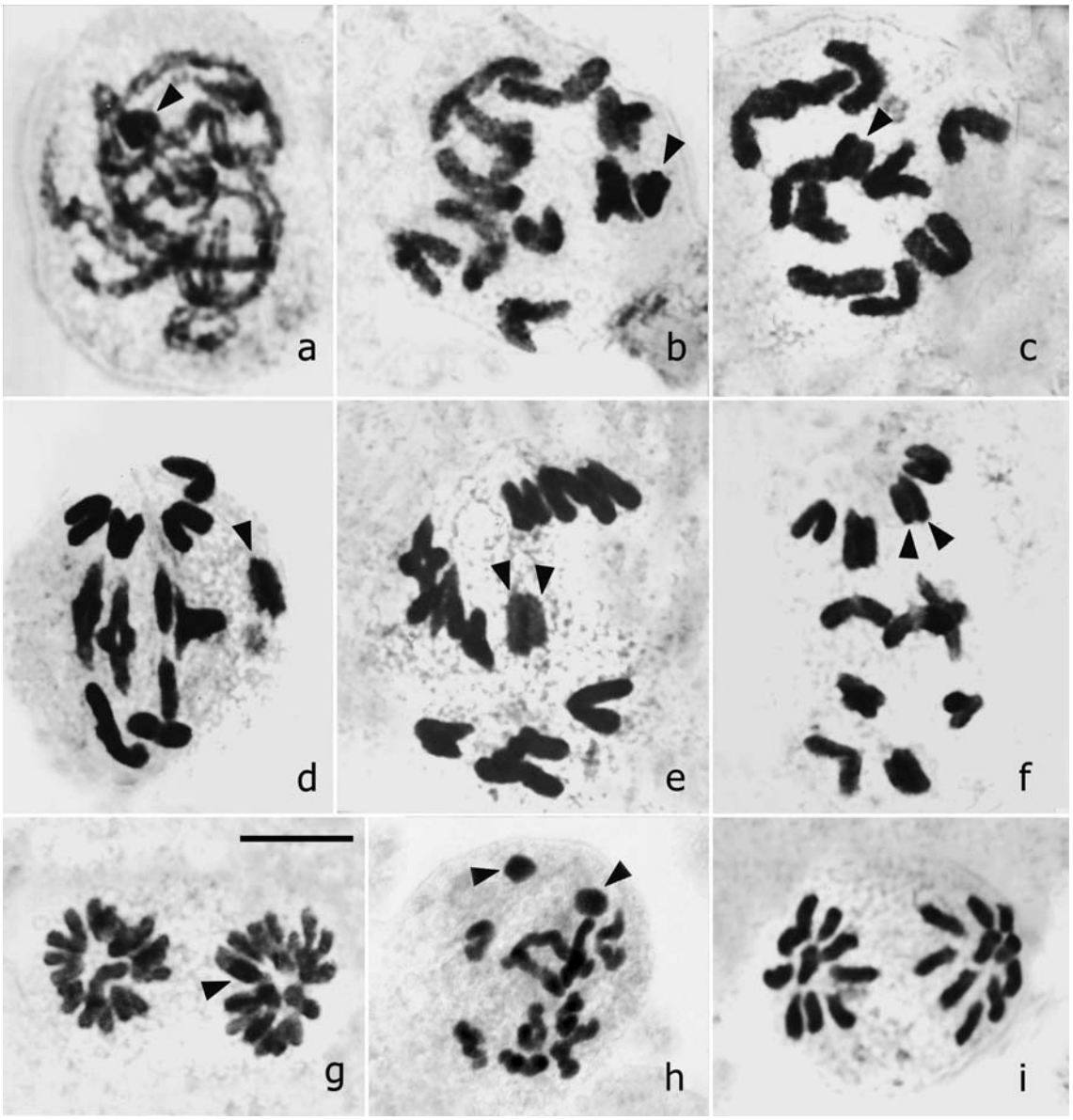

Figure 4 - Meiosis of "*Lycosa*" *pampeana* male (n = 10+X_1X_2 and n = 10): (a) pachytene; (b) diplotene; (c) diakinesis; (d-f) prometaphase I; (g) telophase I; (h) prometaphase II with sex chromosomes; (i) telophase II without sex chromosomes. The arrowheads point to the sex chromosomes. Bar = 10 µm.

the largest of the complement and chiasmata mainly at interstitial or distal positions. In populations of *S. malitiosa* from Uruguay, Brum-Zorrilla and Cazenave (1974) and Brum-Zorrilla and Postiglioni (1980) described 2n = 22 in males and 2n = 24 in females, telocentric chromosomes, sex chromosomes that were the smallest of the complement and bivalents with proximal chiasmata in the males. These results suggest that *S. malitiosa* is polytypic for the size of the sex chromosomes and chiasma position. In the males of an unidentified *Schizocosa* species (*Schizocosa* sp. 2 in Table 1) included within the "*malitiosa* group", Postiglioni and Brum-Zorrilla (1981) found 2n = 23 (22+X), with a metacentric X chromosome probably resulting from the fusion of two telocentric X chromosomes. The five species from the USA already studied (*S. communis*, *S. crassipes*, *S. ocreata*, *S. rovneri*, *S. stridulans*) also had n = 10+X_1X_2, whereas an unidentified species from India (*Schizocosa* sp. 1 in Table 1) had n = 13+X_1X_2 (Painter, 1914; Hard, 1939; Mittal, 1960, 1963; Stratton, 1997). These results allowed us to conclude that the modal chromosome number for the genus is 2n = 22/24 (male/female) and that the sex chromosome determination system is of the X_1X_2/ $X_1X_1X_2X_2$ type.

The present work represents the first cytogenetic study conducted in "*Lycosa*" *pampeana.* This species had 2n = 22 (20+X_1X_2, male), with all telocentric chromosomes. At prometaphase I, the bivalents and the sex chromosomes were peculiarly arranged into three groups, one group being located on the equatorial plane and the remain-

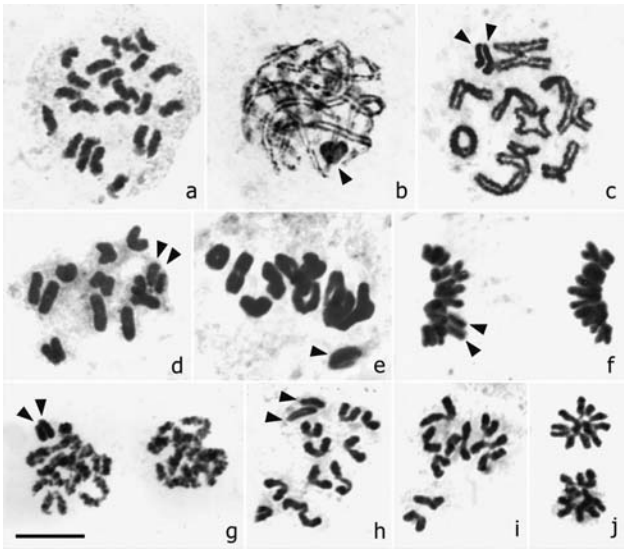

Figure 5 - Meiosis in *Schizocosa malitiosa* (2n = 22, n = 10+X_1X_2 and n = 10): (a) spermatogonial prometaphase; (b) pachytene; (c) diplotene with a bivalent with two chiasmata; (d) diakinesis; (e) metaphase I; (f) anaphase I; (g) telophase I; (h) metaphase II with sex chromosomes; (i) metaphase II without sex chromosomes; (j) telophase II with sex chromosomes. The arrowheads point to the sex chromosomes. Bar = 10 µm.

ing two at the cell poles. This very unusual chromosome disposition in spiders was formerly described for three species of *Tegenaria* (Agelenidae) by Revell (1947), who considered that it resulted from the primary polarization of the bivalents at early prophase I due to the attraction of the heterochromatic regions by the centrosome. When the centrosome began to split, each new centrosome acted as a polarization centre for bivalents, which gradually became aligned on the metaphase plate. This stage, named "transitional metaphase" by Revell (1947), resembles the prometaphases observed in "*Lycosa*" *pampeana*.

"*Lycosa*" *erythrognatha* had 2n = 22/24 (male/female), n = 10+X_1X_2, n = 10 in males, all telocentric chromosomes and the sex chromosomes were the largest of the complement. Bivalents usually had one chiasma, although bivalents with two chiasmata and univalents were occasionally seen and probably resulted from desynapsis. The number and location of the chiasmata during meiosis varied largely among cells. Such variation may be determined not only genetically, but also by environmental factors, both internal and external to the individual (John and Lewis, 1965; Jones, 1987; Appels *et al.*, 1998). The presence of univalents and bivalents with two chiasmata in the same individual and even in the same cell may be a consequence of changes in the mechanisms regulating chiasma frequency and distribution. The chromosome number, some karyotypic features and the meiotic behavior herein observed in "*Lycosa*" *erythrognatha* are consistent with results obtained in specimens of the same species from Uruguay and Brazil (Díaz and Sáez, 1966a, 1966b; Giroti *et al.*, 2007).

"*Lycosa*" *erythrognatha* is characterized by scanty GC-rich heterochromatin located in the pericentromeric region of all chromosomes. The DAPI- and CMA_3-bright fluorescence of the sex chromosomes during early prophase I is consistent with the allocycly of these chromosomes during male meiosis and probably reflects different degrees of chromatin condensation rather than differences in base composition. The heterochromatin content has only been characterized in other four species of the family. C-banding of *Alopecosa albofasciata* showed small blocks of pericentromeric heterochromatin in the autosomes and uniformly heterochromatic sex chromosomes during male meiosis (Gorlova *et al.*, 1997). In *Schizocosa malitiosa, Lycosa thorelli* and in an unidentified species of *Lycosa* (*Lycosa* sp. 7 in Table 1), small AT-rich (Hoechst 33258 positive) C-positive bands were observed in the pericentromeric regions of all chromosomes. A few Hoechst-positive fluorescent bands found in the telomeric regions of some chromosomes of *Lycosa* sp. 7 (Table 1) were not C-positive (Brum-Zorrilla and Postiglioni, 1980). In the male meiosis of *Schizocosa malitiosa*, the sex chromosomes were strongly positively heteropycnotic (Brum-Zorrilla and Cazenave, 1974). The analysis of the heterochromatin content revealed that all the studied species of Lycosidae are characterized by a small amount of tandem repeated DNA sequences. On the other hand, there is some heterogeneity in heterochromatin composition, which can be summarized as follows: a) C-positive, AT-rich pericentromeric heterochromatin; b) C-positive, GC-rich pericentromeric heterochromatin, and c) AT-rich telomeric heterochromatin, undetectable with C-banding.

In a molecular phylogenetic reconstruction of the wolf spiders at the subfamily level, Murphy *et al.* (2006) stated that a number of species currently placed in *Lycosa (sensu lato)* (including "*Lycosa*" *erythrognatha*) do not form a clade and none of them appear to be closely affiliated with *Lycosa tarantula* (Linnaeus 1758), the type species of the genus; in contrast, "*clades within the Lycosinae appear to reflect geographic regions rather than existing recognised morphological parameters*". The authors claimed that Australasian wolf spiders do not possess the defining features of *Lycosa* and that a critical study is necessary to determine their true taxonomic position.

The systematics of South American Lycosidae is poorly known and the taxonomic status of many of the species is far from being resolved. Most of the species originally described under the genus *Lycosa* were transferred to other genera on the basis of poor diagnostic morphological characters. Our karyological study supports previous morphological and behavioral evidence (unpublished data, two thesis works and congress presentations by several authors) indicating that "*Lycosa*" *erythrognatha*, "*Lycosa*" *pampeana* and a group of species from South America should be transferred to another genus (*e.g. Schizocosa*) or that they would belong to a new, still undescribed genus.

Álvares and Brescovit (2007), based exclusively on morphological characters, proposed that these species, as well as *Schizocosa malitiosa*, should be transferred to *Hogna* Simon 1885.

It is outstanding that all the South American species so far analyzed had 2n = 22/24 (male/female) or complements almost certainly derived from it. Even though the number of cytogenetically analyzed *Schizocosa* and *Hogna* species is not representative for the group, all the *Schizocosa* species from the USA already studied presented n = 10+X_1X_2, while *Hogna* species from the USA had n = 13+X_1X_2 and n = 11+X_1X_2 (Table 1).

Cytogenetic analyses of other South American species currently classified within Lycosinae are needed. The information obtained will provide baseline data on the karyotypic evolution within each genus. It will be particularly relevant to formulate a new revision of the taxonomy and phylogeny of the group taking into account morphological, cytogenetical and molecular data. This revision could confirm, as was the case for Australasian wolf spiders (Murphy *et al.*, 2006), that *Lycosa* does not occur in South America.

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