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Cytogenetic studies on five species of spiders from Turkey (Araneae: Gnaphosidae, Lycosidae)

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Abstract. The chromosome diploid number (2n) and the sex chromosome system in males of five species belonging to the families Gnaphosidae and Lycosidae were determined as $2n = 22$ ($20 + X_1X_2$) and $2n = 28$ ($26 + X_1X_2$), respectively. *Nomisia conigera* (Spassky 1941), *Haplodrassus morosus* (O. Pickard-Cambridge 1872) and *Haplodrassus dalmatensis* (L. Koch 1866) have 10 autosomal bivalents and two univalent sex chromosomes, while *Pardosa bifasciata* (C.L. Koch 1834) and *Arctosa cinerea* (Fabricius 1777) have 13 autosomal bivalents and two univalent sex chromosomes during first meiotic stages of prophase I and metaphase I. All species have acrocentric chromosomes and chiasmatic meiosis.

Keywords: Karyotype, meiosis, sex chromosome

Cytogenetic studies of the families Gnaphosidae and Lycosidae (Arachnida: Araneae) are scarce. From the over 4400 species of gnaphosids and lycosids, less than 4% have been cytogenetically analyzed (Chemisquy et al. 2008; Kumbıçak et al. 2009). The diploid number of chromosomes (2n) in males range from 21 to 30 in gnaphosids and 12 to 30 in lycosids (Hackman 1948; Suzuki 1954; Sharma et al. 1958; Kageyama et al. 1978; Srivasta & Shukla 1986; Painter 1914; Gorlov et al. 1995; Akan et al. 2005; Kumbıçak et al. 2009). Most of the analyzed species have only telocentric or acrocentric chromosomes; the sex chromosome system X_1X_2 ♂/ $X_1X_1X_2X_2$ ♀ occurs in 94% of lycosids (Chemisquy et al. 2008) and 99% of gnaphosids analyzed thus far. This paper reports the first results on the karyotypes and spermatogenesis of five common Turkish spider species belonging to the families Gnaphosidae and Lycosidae.

METHODS

Specimens were collected in March–June 2009 (Table 1) and deposited in the collection of Nevşehir University, Science & Art Faculty, Biology Department, Turkey. We made chromosome preparations according to the spreading technique described by Traut (1976) with some modifications. Gonads were dissected out in Ringer's solution and transferred to a hypotonic solution (0.075 KCl) for 20 min. We placed tissues in Carnoy fixative (ethanol: cloroform: glacial acetic acid; 6:3:1) for 35 min. Afterwards, they were macerated in 60% acetic acid on the surface of glass slide (surface temperature 42° C). The suspension was moved by pushing it with a tungsten needle and stained with 5% Giemsa solution in Sörensen phosphate buffer (pH = 6.8) for 30–55 min. We visualized the cells under a Soif XSZ-G microscope and photographed them with an Olympus DP 20-5E Digital Camera by DP2-BSW programme. For karyotyping, we evaluated ten spermatogonial metaphases and calculated relative chromosome lengths (RCL) and centromeric indexes (CI). Chromosome classification was determined according to Levan et al. (1964).

RESULTS

Nomisia conigera (Spassky 1941)

The karyotype of *N. conigera* consists of 22 chromosomes, including the two X chromosomes (Fig. 2a). All autosomal pairs are acrocentric and gradually decrease in size (Fig. 1a). In mitotic metaphase, the length of autosome pairs ranges from 5.38 to 10.15% (Table 2). Spermatocytes show 10 autosomal bivalents and two univalent sex chromosomes in the first meiotic division. During the leptotene and pachytene stages of prophase I, X_1 and X_2 were tightly aligned forming a so-called sex vesicle that is positively heterpycnotic (Fig. 2b). At the diplotene stage of prophase I, diakinesis and metaphase I sex chromosomes were isopycnotic (Fig. 2c).

Haplodrassus morosus (O. Pickard-Cambridge 1872)

The species possesses 22 chromosomes, including two X chromosomes (Fig. 2d). All autosomal pairs and sex chromosomes are acrocentric. Autosomes gradually decreased in size (Fig. 1b). Relative chromosome lengths of autosomal pairs are between 5.99 to 11.47% (Table 2). Relative lengths of sex chromosomes X_1 and X_2 were 10.29 and 8.61%, respectively. There is no significant difference in sex chromosomes in size (Table 2). During the leptotene stage, sex chromosomes were isopycnotic (Fig. 2e). At metaphase I, there were 10 autosomal bivalents and two isopycnotic univalents, the sex chromosomes (Fig. 2f).

Haplodrassus dalmatensis (L. Koch 1866)

The spermatogonial plates consist of 22 chromosomes (Fig. 2g). All autosomal pairs are acrocentric. Autosomes gradually decrease in size (Fig. 1c). Relative lengths of autosomal pairs range from 6.92 to 10.28% (Table 2). The karyotype contains two sex chromosomes, X_1 and X_2 (Fig. 1c). Relative lengths of sex chromosomes X_1 and X_2 are 7.55 and 6.43%. X_2 is the shortest chromosome in the karyotype. During the first meiotic division, sex chromosomes are heavily stained until the end of diakinesis (Fig. 2h). Two types of anaphase II plates were observed with 10 or 12 chromosomes (Fig. 2i).

Pardosa bifasciata (C.L. Koch 1834)

The male karyotype is comprised of 28 acrocentric chromosomes, including two X chromosomes (Fig. 2j). Autosomes gradually decrease in size (Fig. 1d). The relative length of first autosomal pair was 8.63 and the last was 5.09% (Table 2). Relative lengths of sex chromosomes were 7.64 and 6.24%. During pachytene, X_1 and X_2 were tightly aligned forming so-called sex vesicle (Fig. 2k). At diakinesis, there were 13 autosomal bivalents, two positively heteropycnotic univalents the sex chromosomes (Fig. 2l).

Arctosa cinerea (Fabricius 1777)

The karyotype of *A. cinerea* possessed 28 chromosomes, including two X chromosomes (Fig. 2m). All autosomal pairs were acrocentric

Table 1.—Karyotype characteristics, collecting locality and geographical coordinates of the five Gnaphosidae and Lycosidae species cytogenetically analyzed.

Species	2n ♂	n ♂	Sex/stage	Locality and coordinates
Gnaphosidae				
Nomisia conigera	22	10 + XX	3 ♂ (1 subadult, 2 adults), 07 March 2009	Gaziantep/ Sakçagözü 37°11′N, 36°58′E
			1♂ (1 adult), 22 March 2009	Kırşehir/ Çiçekdağ 39°36′N, 34°24′E
			7 ♂ (2 subadults, 5 adults) 28 March 2009, 04 April 2009	Gaziantep/ Nurdağı 37°10′N, 36°44′E
Haplodrassus morosus	22	10 + XX	4 ♂ (adults), 04 April 2009	Gaziantep/ Islahiye 37°01′N, 36°37′E
			2 ♂ (adults), 04 April 2009	Hatay/ Altınözü 36°13′N, 36°09′E
			3 ♂ (1 subadult, 2 adults) 18 April 2009	K.Maraş/ Pazarcık 37°29′N, 37°17′E
Haplodrassus dalmatensis	22	10 + XX	2 ♂ (1 subadult, 1 adult) 14 March 2009	Osmaniye/ Merkez 37°03′N, 36°16′E
			3 ♂ (adult), 25 April 2009	Adıyaman/ Kahta 37°48′N, 38°36′E
			2 ♂ (2 adults), 02 May 2009	Gaziantep/ Merkez 37°06′N, 37°18′E
Lycosidae				
Pardosa bifasciata	28	13 + XX	8 ♂ (2 subadults, 6 adults), 07 March 2009, 28 March 2009, 04 April 2009	Gaziantep/ Nurdağı 37°10′N, 36°42′E
			1♂ (1 adult), 21 March 2009	Kırşehir/ Mucur 39°04′N, 34°22′E
			2♂ (2 adults), 11 April 2009	Adıyaman / Sincik 38°03′N, 38°38′E
			2♂ (2 adults), 18 April 2009	Gaziantep/ Araban 37°24′N, 37°41′E
Arctosa cinerea	28	13 + XX	5♂ (1 subadult, 4 adults) 11April 2009	Adıyaman/ Kahta 37°46′N, 38°38′E
			2♂ (2 adults), 11April 2009	Adıyaman/ Sincik 38°02′N, 38°36′E

and gradually decreased in size (Fig. 1e). In the spermatogonial metaphase, the length of autosome pairs change from 4.85 to 8.26%. Relative lengths of sex chromosomes X_1 and X_2 were 7.38 and 5.73%, respectively. During the first stages of prophase I, X_1 and X_2 were positively heteropycnotic. At the diplotene stage, we observed 13 autosomal bivalents and two univalents (Fig. 2n). Two types of anaphase I plates were found with 13 and 15 chromosomes (Fig. 2o).

DISCUSSION

Cytogenetic studies on the majority of gnaphosid and lycosid spiders reveal similar characteristics: acrocentric or telocentric chromosomes, sex chromosome system in male/female, X_1X_2♂/ $X_1X_1X_2X_2$♀, and chiasmatic meiosis. Chromosomes with metacentric and submetacentric morphology and sex chromosome system of the type X and $X_1X_2X_3$ in males are uncommon (Kumbıçak et al. 2009).

Until now, only one species belonging to the genus *Nomisia* has been studied cytogenetically. *N. riparensis* (O. Pickard-Cambridge 1872) was shown to have 2n = 22 in males and 2n = 24 in females with a X_1X_2 type of sex chromosome system (Table 3). Male *N. conigera* show a diploid chromosome number of 2n = 22 and a X_1X_2 type of sex determining system. Therefore, our results are similar to the *N. riparensis* previously studied (Table 3). The gnaphosid *N. conigera* has significantly different relative lengths of X_1 and X_2. The gnaphosid genera *Callilepis* (Westring 1874) and *Drassodes* (Westring 1851) exhibited dissimilar characteristics in sex chromosomes. In addition, sex chromosomes are the largest elements of *N. conigera* but not of *Callilepis* and *Drassodes* (Painter 1914; Hackman 1948; Suzuki 1954).

The 2n = 22, X_1X_2, acrocentric chromosomes were also described by Hackman (1948) and Gorlov et al. (1997) in *Haplodrassus cognatus* (Westring 1861) and *Haplodrassus signifer* (C.L. Koch 1839), respectively (Table 3). *Haplodrassus morosus* and *H. dalmatensis* have the same diploid number and also the same sex chromosome system. This result, coupled with existing data, supports the hypotheses of a relatively conserved diploid number and sex chromosome system in the family Gnaphosidae. Our results on *H. morosus* and *H. dalmatensis* reveal that there is no significant difference in the relative length of X_1 and X_2 (Table 2).

Ten species of *Arctosa* (C.L. Koch 1847) have been studied so far. With the exception of the results by Akan *et al.* (2005), the diploid chromosome number is 2n = 26 or 28 and the sex chromosome system in males is a X_1X_2 type. The results provided by Akan *et al.* (2005) for *Arctosa perita* (Latreille 1799) were problematic and the reported diploid chromosome number for females (2n = 12) was improbable as the lowest diploid number (Dolejš 2011). The diploid chromosome number and sex chromosome system was determined as 2n = 28 and X_1X_2 for *Arctosa cinerea* (Fabricius 1777) by Dolejš *et al.* (2011), and our karyotype results for *A. cinerea* show similar characteristics (Table 3). Up to now, 25 species belonging to the genus *Pardosa* (C.L. Koch 1847) have been investigated cytogenetically. Most of them have 2n = 28 in males and 2n = 30 in females, but *Pardosa basiri* (Dyal 1935) was found to have 2n♂ = 22, *Pardosa leucopalpis* (Gravely 1924) and *Pardosa sumatrana* (Thorell 1890) 2n♂ = 24 and *Pardosa oakleyi* (Gravely 1924) 2n♂ = 26 (Kumbıçak et al. 2009). Male *P. bifasciata* shows a diploid chromosome number of 2n = 28. Like the previously studied species of *Pardosa*, the sex chromosome system for *P. bifasciata* is a X_1X_2 type (Table 3).

Figure 1.—Karyotypes of gnaphosid and lycosid species analyzed in this study. a. *Nomisia conigera*, b. *Haplodrassus morosus*, c. *Haplodrassus dalmatensis*, d. *Pardosa bifasciata*, e. *Arctosa cinerea* (Bar = 10 μm).

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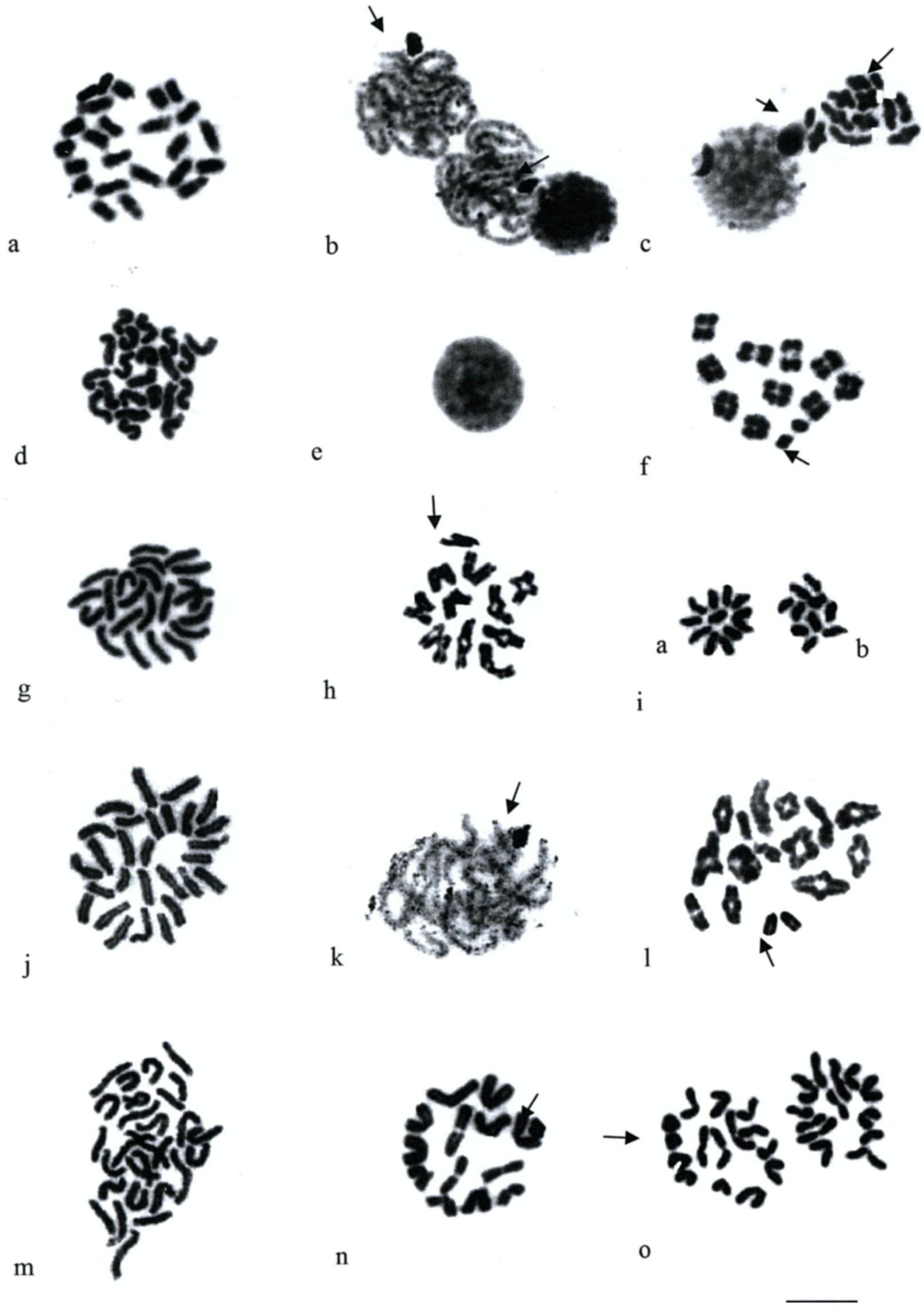

Figure 2.—*Nomisia conigera* a. Spermatogonial metaphase 2n = 22. b. Pacytene nucleus with positively heteropycnotic sex chromosomes (arrows). c. Diplotene with 13 autosomal bivalents and two sex chromosomes (arrows); *Haplodrassus morosus*. d. Spermatogonial metaphase 2n = 22. e. Leptotene nucleus with isopycnotic sex chromosomes. f. First meiotic division metaphase plate with two sex chromosomes (arrow); *Haplodrassus dalmatensis*. g. Spermatogonial metaphase 2n = 22. h. Diplotene with 10 autosomal bivalents and two univalent sex chromosomes (arrow). i. Second meiotic division anaphase plate with 12 (a) and 10 (b) chromosomes; *Pardosa bifasciata*. j. Spermatogonial metaphase 2n = 28. k. Pacytene nucleus with positively heteropycnotic sex vesicle (arrow). l. Diakinesis with 13 autosomal bivalents and two sex chromosomes (arrow); *Arctosa cinerea*. m. Spermatogonial metaphase 2n = 28. n. Diplotene with 13 autosomal bivalents and two associated sex chromosomes (arrow). o. First meiotic division anaphase plate with two sex chromosomes (arrow). (Bar = 10 μm).

Table 2.—Relative length of particular chromosome pairs (RCL) and centromeric indexes (CI) of species studied. Based on spermatogonial metaphases.

	Nomisia conigera		*Haplodrassus morosus*		*Haplodrassus dalmatensis*		*Pardosa bifasciata*		*Arctosa cinerea*	
Pair No	RCL	CI	RCL	CI	RCL	CI	RCL	CI	RCL	CI
1	10.15	10.49	11.47	13.80	10.28	8.09	8.63	7.39	8.26	7.72
2	10.12	8.63	9.06	12.05	9.65	7.42	7.82	7.35	7.75	7.02
3	8.50	7.53	8.61	7.28	9.64	9.71	7.39	13.02	7.46	12.00
4	8.34	9.12	8.46	8.29	9.33	7.38	7.30	7.48	7.35	18.00
5	7.99	9.43	8.14	7.71	8.37	10.81	6.92	9.71	7.26	8.50
6	7.94	19.33	7.99	7.73	8.36	7.64	6.91	20.87	7.16	11.66
7	6.53	9.76	7.84	9.65	8.36	17.05	6.86	9.4	6.76	13.46
8	6.03	9.17	7.23	8.40	7.72	8.67	6.66	9.74	6.57	7.96
9	5.77	12.75	6.31	9.25	7.39	8.50	6.54	18.11	6.50	22.86
10	5.38	10.62	5.99	12.00	6.92	9.43	5.41	7.2	5.93	7.51
11							5.31	18.19	5.84	22.57
12							5.28	13.81	5.20	10.63
13							5.09	13.88	4.85	20.78
X_1	13.01	8.01	10.29	10.16	7.55	8.4	7.64	10.6	7.38	13.03

Table 3.—List of karyotyped species of the genera *Pardosa* and *Arctosa* (Lycosidae) and genera *Nomisia* and *Haplodrassus* (Gnaphosidae). Abbreviations: NMC: Number and morphology of chromosomes in male, SCS: Sex chromosome system, a: acrocentric.

Family/Species	NMC	SCS	References
Lycosidae			
Arctosa alpigena (Doleschall 1852)	26; a	X_1X_2	Hackman 1948
Arctosa leopardus (Sundevall 1833)	26; a	X_1X_2	Hackman 1948
Arctosa sp.	28; a	X_1X_2	Mittal 1960, 1963
Arctosa mulani (Dyal 1935)	28; a	X_1X_2	Sharma et al. 1958
Arctosa alpigena lamperti (Dahl 1908)	28; a	X_1X_2	Dolejš et al. 2011
Arctosa cinerea (Fabricius 1777)	28; a	X_1X_2	Dolejš et al. 2011
Arctosa figurata (Simon 1876)	28; a	X_1X_2	Dolejš et al. 2011
Arctosa maculata (Hahn 1822)	28; a	X_1X_2	Dolejš et al. 2011
Arctosa perita (Latreille 1799)	28; a	X_1X_2	Dolejš et al. 2011
Arctosa renidescens Buchar &Thaler 1995	28; a	X_1X_2	Dolejš et al. 2011
Pardosa agrestis (Westring 1861)	28; a	X_1X_2	Gorlov et al. 1995
Pardosa agricola (Thorell 1856)	28; a	X_1X_2	Hackman 1948
Pardosa amentata (Clerck 1757)	28; a	X_1X_2	Hackman 1948
Pardosa astrigera (L. Koch 1878)	28; a	X_1X_2	Suzuki 1954
Pardosa basiri (Dyal 1935)	22; a	X_1X_2	Mittal 1960, 1963
Pardosa birmanica Simon 1884	28; a	X_1X_2	Bole-Gowda 1958
Pardosa fletcheri (Gravely 1924)	28; a	X_1X_2	Srivastava & Shukla 1986
Pardosa lahorensis Dyal 1935	28; a	X_1X_2	Sharma et al. 1958
Pardosa laura Karsch 1879	28; a	X_1X_2	Kageyama et al.1978
Pardosa leucopalpis Gravely 1924	28; a	X_1X_2	Bole-Gowda 1958
Pardosa leucopalpis Gravely 1924	24; a	X_1X_2	Srivastava & Shukla 1986
Pardosa lugubris (Walckenaer 1802)	28; a	X_1X_2	Gorlov et al. 1995
Pardosa monticola (Clerck 1757)	28; a	X_1X_2	Hackman 1948
Pardosa oakleyi Gravely 1924	26; a	X_1X_2	Srivastava & Shukla 1986
Pardosa palustris (Linnaeus 1758)	28; a	X_1X_2	Hackman 1948
Pardosa pseudoannulata (Bösenberg & Strand 1906)	28; a	X_1X_2	Suzuki 1954
Pardosa pullata (Clerck 1757)	28; a	X_1X_2	Hackman 1948
Pardosa sumatrana (Thorell 1890)	24; a	X_1X_2	Sharma 1961
Pardosa plumipes (Thorell 1875)	28; a	X_1X_2	Gorlov et al. 1995
Pardosa alacris (C.L. Koch 1833)	28; a	X_1X_2	Kumbıçak et al. 2009
Pardosa saltans (Töpfer-Hofman 2000)	28; a	X_1X_2	Kumbıçak et al. 2009
Pardosa sp. 1	28; a	X_1X_2	Bole-Gowda 1953, 1958
Pardosa sp. 2	28; a	X_1X_2	Sharma and Gupta 1956
Pardosa sp. 3	28; a	X_1X_2	Mittal 1960
Gnaphosidae			
Nomisia ripariensis (O. Pickard-Cambridge 1872)	22; a	X_1X_2	Gorlova et al. 1997
Haplodrassus cognatus (Westring 1861)	22; a	X_1X_2	Hackman 1948
Haplodrassus signifer (C.L. Koch 1839)	22; a	X_1X_2	Gorlova et al. 1997