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Karyological data and meiosis of *Drassyllus praeficus* (L. Koch, 1866) (Gnaphosidae) and *Thanatus imbecillus* (L. Koch, 1878) (Philodromidae) from Turkey

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Abstract: In this study, karyotypic and cytogenetic characteristics of *Drassyllus praeficus* (L. Koch, 1866) and *Thanatus imbecillus* (L. Koch, 1878) were investigated for the first time by examining mitotic and meiotic chromosomes obtained from gonad cells. The number and the sex chromosome system of *D. praeficus* and *T. imbecillus* males was 2n = 22 (X_1X_20) and 2n = 28 (X_1X_20), respectively. *D. praeficus* had 10 autosomal bivalents and 2 univalent sex chromosomes, and *T. imbecillus* had 13 autosomal bivalents and 2 univalent sex chromosomes during the first meiotic prophase and metaphase. Both species possessed acrocentric chromosomes in their karyotypes.

Key words: Karyotype, chromosomes, sex chromosome system, Araneae

1. Introduction

Spiders are one of the most diverse groups of animals. Up to now, 42,751 species are known to belong to the order Araneae (Platnick, 2012), yet only about 1.5% have been cytogenetically analyzed (Araujo et al., 2012).

Gnaphosidae is among the largest families of the order Araneae, which includes 2128 species divided into 117 genera (Platnick, 2012). Despite the high number of gnaphosid species, only 35 species (1.6%) have been cytogenetically analyzed (Araujo et al., 2012).

In species whose chromosome morphology has been determined, all chromosomes are acrocentric. Male diploid chromosome numbers range from 22 to 30 and the sex chromosome system is ♂X_1X_2 / ♀$X_1X_1X_2X_2$, which is the so-called X_1X_20 system (Araujo et al., 2012), with the exception of *Urozelotes rusticus* (L. Koch, 1872) and 2 *Drassodes* Westring, 1851 species with 2n♂ = 21 and the sex chromosome system of the X0 type (Srivastava and Shukla, 1986).

There are 29 genera with 535 species that belong to the family Philodromidae (Platnick, 2012). Chromosome characteristics of this family are scarce; the diploid chromosome number varied from 24 to 29 in males and the sex chromosome system is mostly X_1X_20 (Araujo et al., 2012). Derived systems were found only in 3 species. *Philodromus auricomus* L. Koch, 1878 and *Philodromus subaureolus* Bösenberg and Strand, 1906 presented an X0 sex chromosome system (Suzuki, 1952), and *Philodromus* sp. exhibited an $X_1X_2X_30$ system (Mittal and Singh, 1984).

In order to gain a better understanding of the chromosomal characteristics of the families Gnaphosidae and Philodromidae, *Drassyllus praeficus* (L. Koch, 1866) and *Thanatus imbecillus* (L. Koch, 1878) were investigated. Both species investigated have a similar biology. They prefer dry habitats and lawns and were karyotyped here for the first time. Additionally, the results are important cytogenetic data, contributing to the knowledge of the karyotype evolution in the families of Gnaphosidae and Philodromidae.

2. Materials and methods

The sample analyzed in this study includes 12 specimens of *D. praeficus* from Uludağ, 40°03′N, 29°13′E (5 adult males); Kestel, 40°11′N, 29°19′E (3 adult males); and Karamürsel, 40°39′N, 29°39′E (4 adult males); and 12 adult male specimens of *T. imbecillus* from Bursa, 40°09′N, 29°03′E, Turkey. All the individuals were collected between March and June in 2010 and 2011. The specimens were deposited in the collection of Nevşehir University, Arts and Science Faculty, Department of Biology, Nevşehir, Turkey.

Chromosome preparations were made according to the procedure described by Traut (1976), with some

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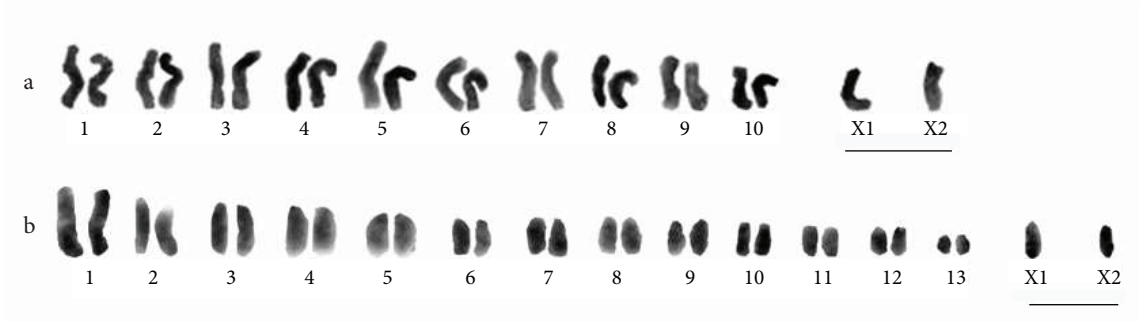

Figure 1. Male karyotypes of (a) *Drassyllus praeficus* (2n = 22, $X_1X_2$0) and (b) *Thanatus imbecillus* (2n = 28, $X_1X_2$0) from Turkey (scale = 10 μm).

modifications. Testes were dissected out in a hypotonic solution (0.075 M KCl) and then transferred into a new hypotonic solution, and they were hypotonized for 20 min in total. Tissues were moved in 2 changes of freshly prepared Carnoy fixative (ethanol, chloroform, and glacial acetic acid, 6:3:1) for 35 min in total. A piece of tissue was suspended in a drop of 60% acetic acid on a slide using a pair of tungsten needles. The slide was dried on a histological plate (surface temperature 42 °C) and stained with a 5% Giemsa solution in Sørensen phosphate buffer

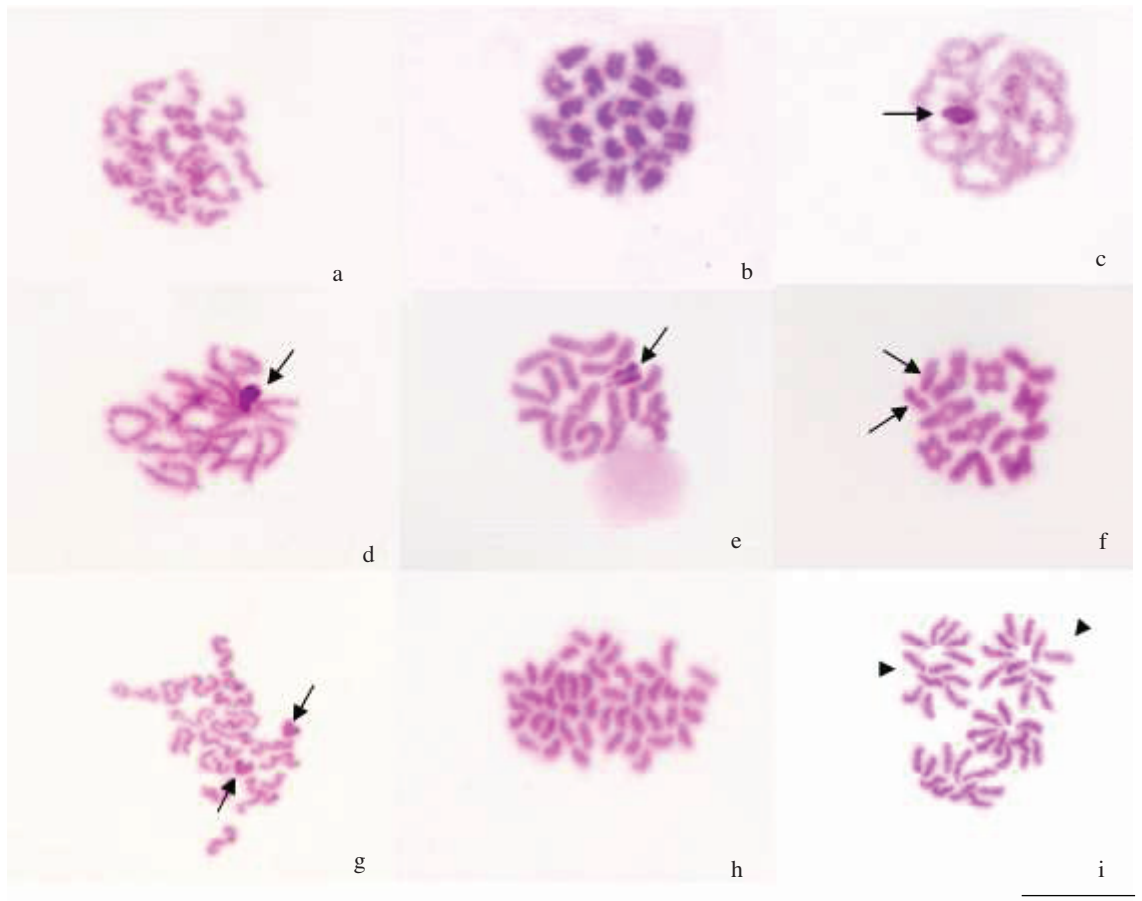

Figure 2. *Drassyllus praeficus* male (2n = 22, $X_1X_2$0): a) spermatogonial prometaphase, b) metaphase, c) pachytene with positively heteropycnotic "sex vesicle" (arrow), d) early diplotene, e) diplotene with 10 autosomal bivalents and 2 sex chromosome univalents (arrow), f) early diakinesis with 10 autosomal bivalents and 2 sex chromosome univalents (arrows), g) prophase II with 2 more condensed sex chromosomes (arrows), h) metaphase II (2 cells together), i) anaphase with 2 types of parts containing n = 10 or n = 12 chromosomes (arrowheads) (scale = 10 μm).

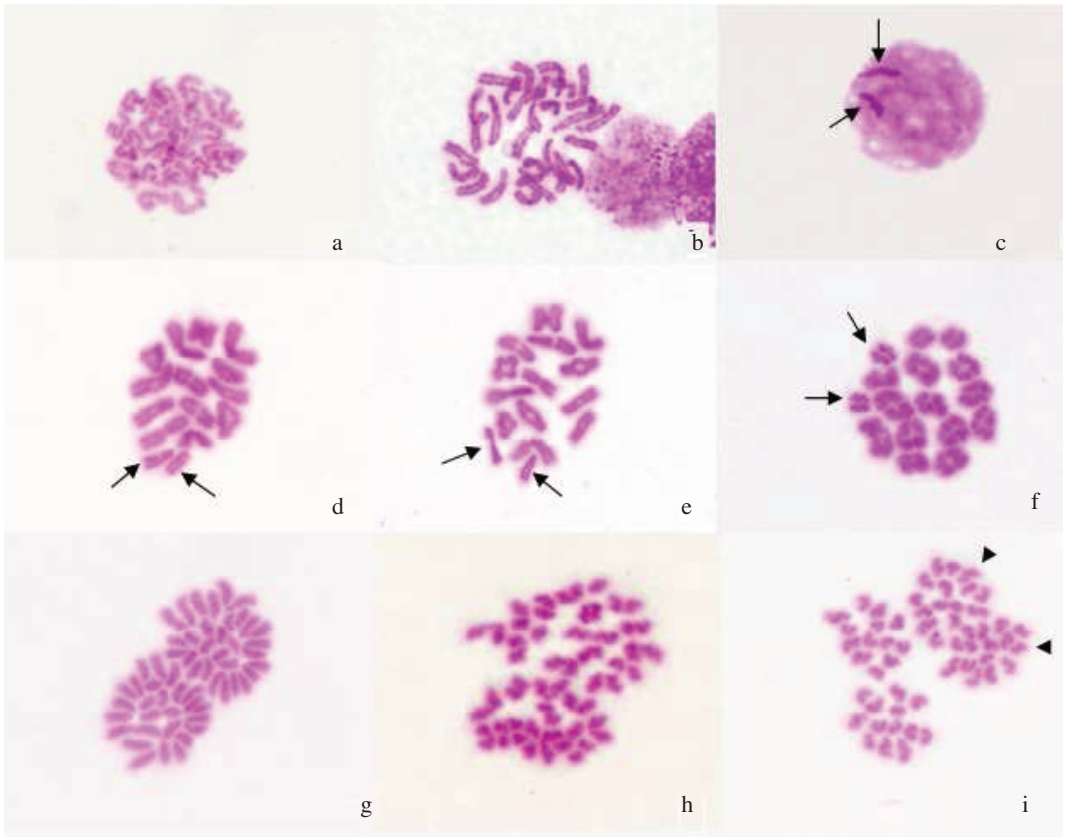

Figure 3. *Thanatus imbecillus* male (2n = 28, X_1X_20): a) spermatogonial prometaphase, b) metaphase, c) early pachytene with positively heteropycnotic sex chromosomes (arrows), d, e) diplotene with 13 autosomal bivalents and 2 univalent sex chromosomes (arrows), f) late metaphase I with 2 sex chromosomes (arrows), g) anaphase I, h) early metaphase II, i) anaphase II with 2 types of parts containing n = 13 or n = 15 chromosomes (arrowheads) (scale = 10 μm).

(pH 6.8) for 27 min. The cells were investigated under an Olympus CX31 microscope and the best mitotic and meiotic figures were photographed with an Olympus DP 25 digital camera and the DP2-BSW program (Olympus). Karyotypes of *D. praeficus* and *T. imbecillus* were constructed by arranging chromosomes in pairs according to size using images of spermatogonial metaphases. Relative chromosome lengths (RCLs) and centromeric indexes (CIs) of each chromosome pair were calculated from 10 metaphase plates obtained from each species. Chromosome morphology was classified according to the method of Levan et al. (1964).

3. Results

The karyotype of *Drassyllus praeficus* is presented in Figure 1a and the karyotype of *Thanatus imbecillus* is presented in Figure 1b.

3.1. *Drassyllus praeficus* (L. Koch, 1866)

The male karyotype consisted of 22 chromosomes (Figures 1a, 2a, and 2b). All autosome pairs were acrocentric. Autosome pairs gradually decreased in size. Relative lengths of autosome pairs ranged from 10.21% to 6.76% (Table 1). The sex chromosome system was of the X_1X_20 type. The X_1 and X_2 sex chromosomes were acrocentric and their relative lengths were 7.69% and 6.93%, respectively.

The sex chromosomes were situated on the periphery of the nucleus during prophase of the first meiotic division and showed positive heteropycnosis until the end of diplotene (Figures 2c, 2d, and 2e). By pachytene, they formed a body (sometimes called the "sex vesicle"). Ten autosomal bivalents and 2 univalent sex chromosomes were observed from diplotene (Figures 2e and 2f) to metaphase I. Prophase II showed 20 superspiralized autosomes and 2 more condensed sex chromosomes (Figure 2g). Sex chromosomes were indistinguishable at metaphase II because of their isopycnotic appearance (Figure 2h). Anaphase II cells were composed either of 10 autosomes or 12 elements (10 autosomes and 2 sex chromosomes) (Figure 2i).

3.2. *Thanatus imbecillus* (L. Koch, 1878)

The male karyotype contained 28 chromosomes including 2 X chromosomes (Figures 1b, 3a, and 3b). The sex

Table 1. Relative length of chromosome pairs (RCL), centromeric index (CI), and chromosome morphology (CM) of *D. praeficus* and *T. imbecillus* based on spermatogonial metaphase cells (a: acrocentric).

	Drassyllus praeficus			*Thanatus imbecillus*		
Pair no.	RCL (%)	CI	CM	RCL (%)	CI	CM
1	10.21	8.10	a	10.75	7.42	a
2	9.68	7.75	a	7.49	11.30	a
3	9.46	9.20	a	7.50	7.62	a
4	9.13	8.21	a	7.28	8.47	a
5	8.47	7.60	a	7.0	9.16	a
6	8.36	11.3	a	6.24	7.58	a
7	7.94	8.76	a	6.10	7.15	a
8	7.78	7.15	a	5.87	12.84	a
9	7.59	7.40	a	5.61	7.31	a
10	6.76	8.30	a	5.35	7.50	a
11				4.67	7.12	a
12				4.31	10.64	a
13				3.05	7.89	a
X_1	7.69	7.63	a	6.17	8.12	a
X_2	6.93	7.06	a	5.84	7.45	a

chromosome system was X_1X_20. All autosomal pairs and sex chromosomes were acrocentric (Figure 1b). Autosomes gradually decreased in size (Table 1). Relative lengths of autosome pairs ranged from 10.75% to 3.05% and relative chromosome lengths of the X_1 and X_2 sex chromosomes were 6.17 and 5.84, respectively (Table 1).

The sex chromosomes were situated on the periphery of the nucleus from pachytene to metaphase I (Figures 3c, 3d, 3e, and 3f). Sex chromosomes manifested positive heteropycnosis only by pachytene (Figure 3c); they remained isopycnotic during the rest of meiotic division (Figures 3g, 3h, and 3i). Anaphase II nuclei contained either n = 13 or n = 15 chromosomes (Figure 3i).

4. Discussion

The diploid chromosome number in spiders ranges from 7 to 110 and in gnaphosids from 21 to 30 (Araujo et al., 2012). Based on our results, the diploid chromosome number of *Drassyllus praeficus* was 22 in males, which agrees with the chromosome number of the other gnaphosids. Currently, there are about 90 species belonging to the genus *Drassyllus* Chamberlin, 1922, spread around the world (Platnick,

Table 2. List of karyotyped species of the genera *Drassyllus* and *Thanatus* (NMC: male diploid chromosome number and morphology of chromosomes; SCS: sex chromosome system; m: male; a: acrocentric).

Family / Genus / Species	NMC	SCS	References
Gnaphosidae, *Drassyllus*			
D. pumilus (C.L. Koch 1839)	22; a	X_1X_20	Kumbıçak et al., 2009
D. praeficus (L. Koch, 1866)	22; a	X_1X_20	This study
Philodromidae, *Thanatus*			
T. formicinus (Clerck, 1757)	28; a	X_1X_20	Hackman, 1948
T. meronensis Levy, 1977	28; a	X_1X_20	Gorlova et al., 1997
T. imbecillus (L. Koch, 1878)	28; a	X_1X_20	This study

2012), but only 1 *Drassyllus* species has been investigated cytogenetically (Table 2). The first chromosomal data of the genus *Drassyllus* were provided by Kumbıçak et al. (2009), who reported 2n♂ = 22 in *Drassyllus pumilus* (C. L. Koch, 1839). Their data are compatible with our results on *D. praeficus*.

In the majority of gnaphosids studied previously, the karyotypes consist of acrocentric chromosomes and the sex chromosome system is X_1X_20. The only exceptions are 2 unidentified *Drassodes* species and *Urozelotes rusticus* reported by Srivastava and Shukla (1986), which have an X0 system. According to our results, *D. praeficus* had acrocentric chromosomes and an X_1X_20 sex chromosome system. It seems that the chromosome number and sex chromosome system are relatively conservative in the family Gnaphosidae.

Despite the high diversity of philodromids, they are poorly known from the cytogenetic point of view. As of today, 15 species have been examined (Araujo et al., 2012). Diploid chromosome numbers in *Thanatus meronensis* Levy, 1977 and *Thanatus formicinus* (Clerck, 1757) are 2n♂ = 28 and the sex chromosome is X_1X_20 (Hackman, 1948; Gorlova et al., 1997). In addition to this, the majority of species belonging to the genus *Philodromus* Walckenaer, 1826 has 2n♂ = 28 (X_1X_20). Our result for the diploid chromosome number of *T. imbecillus* was 2n = 28, which is compatible with the results of both *Thanatus* species studied so far (Hackman, 1948; Gorlova et al., 1997). Moreover, all 3 species exhibit similar karyotypes with the acrocentric chromosomes and the same sex chromosome system. By itself, the current knowledge is not sufficient to make comparisons about the cytogenetic characteristics of the genus *Thanatus*; therefore, new studies are needed.

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