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Karyotype features based on diploid number and sex chromosome system of *Steatoda grossa* (Araneae: Theridiidae) from Turkey

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Abstract

In this study, karyotypic investigation of *Steatoda grossa* belonging to the family Theridiidae was carried out to contribute cytogenetic relationships of the family. A standard air-drying method was used to obtain chromosome slides including three main steps as hypotonisation, fixation and staining. As a result, the diploid number and sex chromosome system was determined as 2n♂=22 (X_1X_2). All chromosomes were telocentric and gradually decreased in size. Sex chromosomes were positively heteropycnotic in the stages of meiosis I, and isopycnotic in meiosis II. The results showed the similarity with the previous investigations obtained from different populations.

Keywords: Araneae, cytogenetics, karyotype, *Steatoda*, Turkey

1. INTRODUCTION

The genus *Steatoda* Sundevall is one of the most familiar genera in the family of Theridiidae that includes over 125 recognised species, distributed around the world [1]. The coloration of this genus is changed by brown to dark brown and the characterisation of an ivory strip on the anterior portion of the abdomen, and fleshy colulus and species are usually known as false widows because of its resemblance with the true widow spiders in the genus *Latrodectus* Walckenaer, 1805, from which can be differentiated by the presence of cheliceral teeth [2]. Eight theridiid spiders of the genus *Steatoda* are distributed in Turkey, namely *S. albomaculata* (De Geer, 1778), S. bipunctata (Linnaeus, 1758), *S. castanea* (Clerck, 1757), *S. dahli* (Nosek, 1905), *S. grossa* (C. L. Koch, 1838), *S. nobilis* (Thorell, 1875), *S. paykulliana* (Walckenaer, 1806), *S. triangulosa* (Walckenaer, 1802) [3]. *S. grossa* is a very common species in Turkey and it is possible to collect them from different populations.

Spiders are divided into three phylogenetic groups namely Mesothelae, Mygalomorphae and Araneaomorphae [4] and the family Theridiidae belongs to the group of Araneomorphae that has the great number of spider species described upto now. Chromosomal studies have been reported for three of the *Steatoda* species; *Steatoda bipunctata* (Linnaeus, 1758), *Steatoda grossa* (C.L. Koch, 1838) and *Steatoda triangulosa* (Walckenaer, 1802) [5]. In these studies chromosomal data have been reported for *S. bipunctata* 2n♂=22 [6]; *Steatoda grossa* 2n♂=22 [7] and *S. triangulosa* 2n♂=22 [8] or 2n♂=26 [9].

This study includes cytogenetical characteristics of *Steatoda grossa* in the point of karyotype structures (i.e. diploid chromosome number and sex chromosome

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system) and basic data of meiotic features for Turkish population.

2. EXPERIMENTAL

A total of 11 male specimens were collected under stones by hand or pitfall traps in various locations during March-May in the year 2016 (Table 1). During the field studies, no application was taken on the spiders and they were transferred directly to the laboratory. Subadult specimens were fed by *Drosophila melanogaster* twice a week until became adult. The used specimens were kept in the collection of Genetic Laboratory, Science and Art Faculty, Nevşehir Hacı Bektaş Veli University.

Table 1. Collection data of *Steatoda grossa* used in this study

Number of specimens	Locality and coordinates	Collection date and museum number
2 ♂♂	Pozantı (Adana) 37º25'3⁰.91"N and 34º51'50.68"E	08.04.2016 TH1605, TH1607
1 ♂	Çamalan (Mersin) 37º11'29.79" N and 34º48'07.05"E	108.04.2016 TH1606
3 ♂♂♂	Pınarbaşı (Kayseri) 38º42'47.64" N and 36º23'26.94"E	12.05.2016 TH1608, TH1609, TH1610
3 ♂♂♂	Gülek (Mersin) 37º15'31.22"N and 34º45'46.98"E	28.03.2016 TH1602, TH1603, TH1604
1 ♂	Alacaşar (Nevşehir) 38º37'15.22" N and 34º35'37.50"E	20.05.2016 TH1611
1 ♂	Göksun (Kahramanmaraş) 38º01'30.67" N and 36º30'46.65"E	21.03.2016 TH1601

Chromosomal preparations were made according to the protocol of Král et al. [10], a standard air drying method includes three main steps as hypotonisation, fixation, and staining, respectively. Gonads were dissected out in a pyhsiological solution for invertebrates under stereomicroscope (Leica EZ4) and transferred into hypotonic solution for 13-15 min and freshly prepared fixative solution (3:1, ethanol: acetic acid) two times (10 min and 20 min) at room temperature (RT). Cell suspensions from gonads was prepared from a piece of tissue in a drop of acetic acid (60 %) on a histological plate (42 ºC) and the drop was moved till to evaporated by a tungsten needle. Slides were stained in Sörensens phosphate buffer (pH 6.8) for 50 min at RT.

Chromosome investigations were made under light microscope (Olympus) and best mitotic cells were photographed using DP26 camera attached to the BX53 microscope (Olympus) with CellSens software. Ten spermatogonial metaphases were used for karyotype analysis. Chromosome lengths were measured micrometric system by CellSens software. Homologues chromosomes were paired and sorted by length order except sex chromosomes that located at the end of the chromosomes. Chromosome morphology was classified according to the nomenclature of Levan et al. [11].

3. RESULTS

The diploid chromosome number of *Steatoda grossa* was consisted of 22 chromosomes including 20 autosomes and two univalent sex chromosomes (Fig. 1, Fig. 2a). Relative lengths of chromosomes were changed between 9.14±0.56 to 6.65±0.36 and chromosome lengths were decreased gradually in size (Table 2). Sex chromosomes were middle sized elements and relative lengths of X_1 and X_2 were 8.22±0.28 and 7.56±0.42, respectively. Both X_1 and X_2 were middle-sized elements in the karyotype. All chromosomes were telocentric.

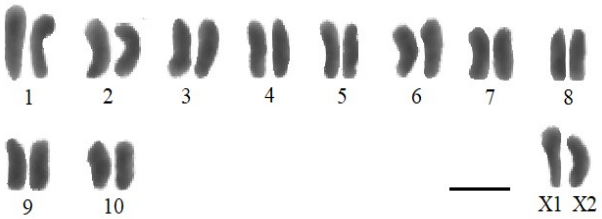

Fig. 1. Karyotype of *Steatoda grossa* from male gonads 2n♂=22 (X_1X_2) (Scale=10 µm)

Leptotene, zygotene and pacythene nuclei showed positively heteropycnotic sex chromosomes as "sex vesicle" located on the periphery of the nucleus (Fig. 2b). Diplotene, diakinesis and metaphase I nuclei obtained 10 autosomal bivalents and two univalent sex chromosomes (Fig. 2c). Autosomal bivalents had usually one chiasma that terminal, interstitial and proximal type. Anaphase I nuclei had two types of

nuclei includes n=10 or n=12 (with sex chromosomes) (Fig. 2d).

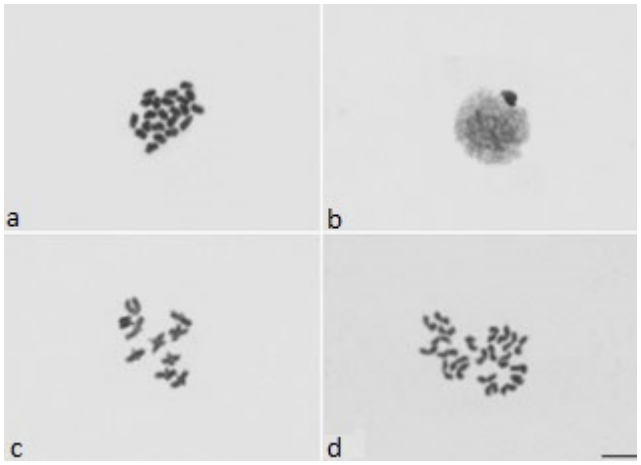

Fig 2. Cell division of *S. grossa* a. mitotic metaphase (2n♂=22), b. leptotene, c. diplotene (10 autosomal bivalents and two univalent sex chromosomes), d. anaphase I (Scale=10 µm)

Table 2. Relative chromosome lengths of haploid set and chromosome morphology of *S. grossa* (±: Standard deviation)

Pair no	Relative Chromosome Length (µm)	Chromosome morphology
1	9.14±0.56	Telocentric
2	8.87±0.12	Telocentric
3	8.60±0.30	Telocentric
4	8.34±0.52	Telocentric
5	8.10±0.67	Telocentric
6	7.79±0.24	Telocentric
7	7.46±0.20	Telocentric
8	7.20±0.47	Telocentric
9	6.92±0.18	Telocentric
10	6.65±0.36	Telocentric
X_1	8.22±0.28	Telocentric
X_2	7.56±0.42	Telocentric

Sex chromosomes were moved together. Prophase II and metaphase II nuclei had isopycnotic sex chromosomes indistinguishable from autosomes by dark staining. Anaphase II nuclei had n=10 or n=12 chromosomes like anaphase I with the exception of chromosome shape which was "V" shaped at anaphase I but "I" shaped at anaphase II.

DISCUSSION

It is known that there are more than 48000 spider species distributed all over the world, In spite of 117 spider families known on the world [1], cytogenetic data have been obtained on 70 families [5], this means that new studies about spider cytogenetics are needed. Thus, all information obtained will contribute to the explanation of topics such as karyotype properties, sex chromosome systems, meiotic division, evolution of chromosomes of spiders. The most studied spider families are the Salticidae and Lycosidae families with 160 and 120 species, respectively. Theridiidae family was studied with 30 species belonging to 15 genera, the most studies genera were listed as *Anelosimus* Simon, 1891 (six species) and *Latrodectus* Walckenaer, 1805 (five species) were the most studied in the family [5]. Although the genus *Steatoda* was represented by 125 species, only three species were investigated by cytogenetically. Previously studies have been showed that the karyotype features were 2n♂=22 (X_1X_20) for both *S. bipunctata* and *S. grossa* and 2n♂=26 (X_1X_20) for *S. triangulosa* [5]. When the karyotypic features of the family are considered, it is observed that the diploid number is mostly 2n♂=22. Except this, samples with diploid number 2n=17 (*Latrodectus geometricus* C.L. Koch, 1841; [12]), 2n=21 (*Argyrodes elevatus* Taczanowski, 1873; [13]), 2n=23 (*Theridion pictum* Walckenaer, 1802; [9]), 2n=24 (*Argyrodes gazingensis* Tikader, 1970; [14], *Chrysso scintillans* Thorell, 1895; [15] and *Nesticodes rufipes* Lucas, 1846; [13]), 2n=26 (*Steatoda triangulosa* Walckenaer, 1802; [9], *Latrodectus hesperus* Chamberlin & Ivie, 1935; [12] and *Latrodectus* gr. *curacaviensis,* [16]) and 2n=29 (*T. pictum*; [9]) were reported. According to [17], a phylogenetic study based on the sequences for two-mitochondrial genes: cytochrome c oxidase subunit I (COI) and ribosomal RNA16S (16S), and three nuclear genes: ribosomal RNAs 18S (18S) and 28S (28S), and histone (H3) on the Theridiidae family, the results of the sequence analysis showed that *Steatoda* and *Latrodectus* were closely related genera. In another study, *Steatoda* and *Latrodectus* species were found to be closed taxa [18]. However, different diploid numbers have been observed in *Steatoda* (2n=22 and 2n=26) and *Latrodectus* (2n=17 and 2n=26) samples,

this indicates an incompatibility on karyotype characteristics among these groups. Studies on other species are needed to explain the karyotype model of the genus *Steatoda*.

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