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A New Diploid Number and Sex Chromosome System for the Family Hersiliidae from the Mediterranean Region of Turkey

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Authors' Contribution
UK collected spiders, helped in laboratory work, made karyotype and analysed the data. ZK helped in laboratory, field trips and wrote and evaluated the manuscript. GH made chromosome slides and arranged the measurements of chromosomes.

Key words
Araneae, Cytogenetics, Hersiliola, Karyotype, Spider.

ABSTRACT

Cytogenetic features, mitotic metaphase chromosomes, karyogram and course of meiosis of *Hersiliola bayrami* were determined based on Giemsa-stained testicular chromosomes for the first time. Results of spermatogonial metaphases showed that the chromosome number and the karyotype formula of the species was $2n♂=35$ ($X_1X_2X_30$) which is the first record for the family Hersiliidae. Both autosomes and sex chromosomes were acrocentric. The lengths of the chromosomes indicated no significant difference between autosomal pairs, however, X_3 was the smallest chromosome but X_1 and X_2 were intermediate in the karyotype.

INTRODUCTION

Hersiliidae Thorell, 1870 is a small family, currently including 181 species belonging to 16 genera (World Spider Catalog, 2017). The hersiliids are conspicuously long-legged, delicate spiders, characterised by extremely elongated posterior lateral spinnerets (Baehr and Baehr, 1987). The majority of hersiliid species are found in tropical and subtropical regions. Recently, they are a subject of several revisions of Australian, Oriental, Neotropical, and Afrotropical faunas. In the Palaearctic, *Hersiliola* Thorell, 1870 is the most species rich genus (Marusik *et al*., 2010).

Cytogenetical data on spiders are important from the perspective of understanding their evolution. Karyological information such as chromosome number and the morphology and size of chromosomes is useful in deducing evolution and interrelationships in the group (Joshi *et al*., 2016). Diploid chromosome numbers of spiders range from 7 (Suzuki, 1954) to 128 (Král *et al*., 2013). Entelegynae spiders (the most derived group) exhibit lower diploid numbers and mostly monoarmed chromosomes when compared with the predominantly high chromosome numbers and biarmed chromosomes of mygalomorphs (Kořínková and Král, 2013; Kumbıçak *et al*., 2014). A multiple sex chromosome system of the X_1X_20 type is the most common in spiders (*i.e.,* Suzuki, 1954) but is rare in other animal groups. In many spider species, sex is not determinated by presence of an Y or W chromosome, as it occurs in most mammals and birds (Araújo *et al*., 2016). Compared with this system, $X_1X_2X_30$ sex chromosome system can rarely be seen in some spider families (*i.e.,* Agelenidae; Ctenidae; Eresidae; Eutichuridae; Linyphiidae; Nemesiidae; Oecobiidae; Selenopidae; Sparassidae; Tetragnathidae) (Araújo *et al*., 2017).

In the present study, we aimed to analyze the chromosomes of an endemic species, *Hersiliola bayrami* Danışman *et al*., 2012 for which chromosomal data were lacking.

MATERIALS AND METHODS

A sample of nine adult males of *H. bayrami* were analyzed. They were collected from the Mediterranean part of Turkey during March-May in 2014 (Table I). The specimens were deposited in the collection of Genetics Laboratory, Science and Art Faculty, Molecular Biology and Genetics Department, Nevşehir.

The chromosomal preparations were made according to the method of Pekár and Král (2001). The gonads were dissected out in a physiolocial solution for invertebrate animals and incubated for 12-15 min in hypotonic solution (0.0075 M KCl); immediately after the gonads were fixed in a freshly prepared methanol (or absolute ethanol): acetic acid (3:1) for 10 and 20 min. The fixed tissues were put in a drop of 60% acetic acid and macerated using a tungsten needle on a histological plate (42°C).

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Table I.- Number of used specimens, collection data including locality, geographical coordinates and dates.

Species	Sample	Collection data	
		Locality (districts) and geographical coordinates	**Date**
Hersiliola bayrami Danışman *et al*., 2012	2♂♂	Alanya, Antalya; 36°32'16.84" N and 31°59'33.90"E	21.04.2014
	1♂	Gözne, Mersin; 37°00'30.47" N and 34°31'50. 09"E	28.03.2014
	2♂♂	Anamur, Mersin, 36°05'59.38" N and 32°49'47.17"E	02.05.2014
	3♂♂	Anamur, Mersin, 36°02'17.48" N and 32°44'00.54"E	04.05.2014
	1♂	Centre, Karaman, 37°08'45.07" N and 33°13'30.92"E	17.04.2014

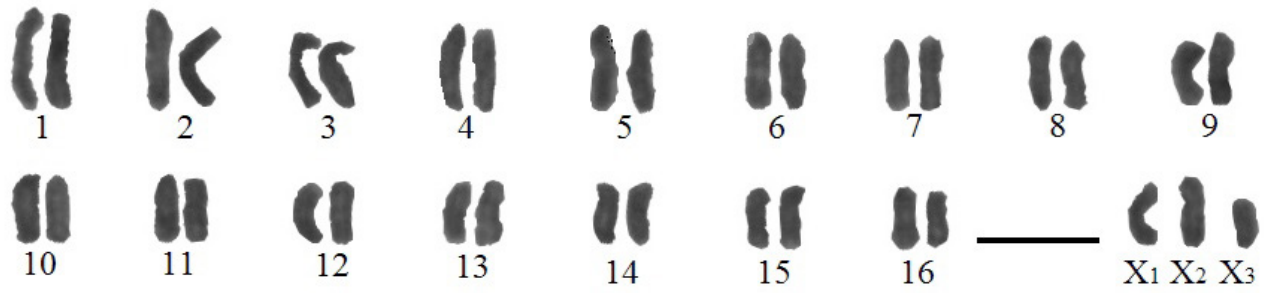

Fig. 1. Karyotype of *Hersiliola bayrami* based on male specimens. Scale bar=10 µm.

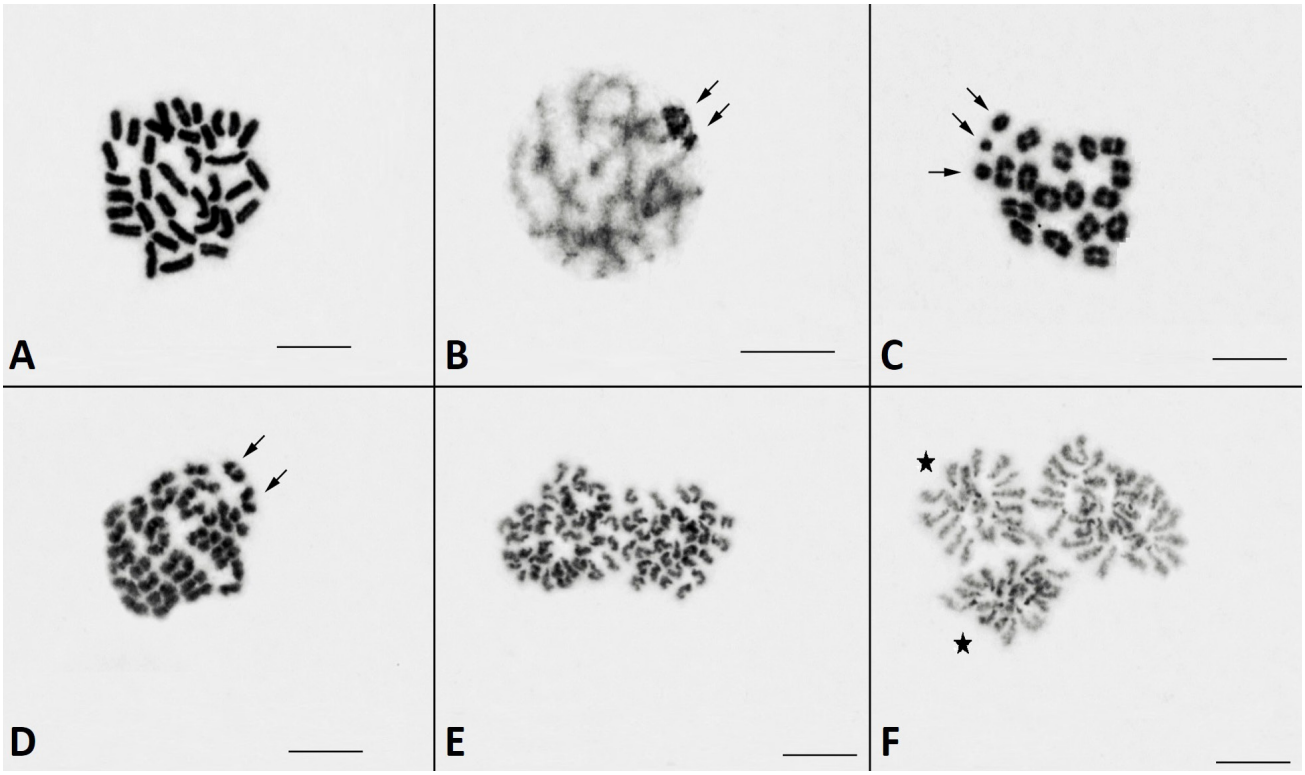

Fig. 2. *Hersiliola bayrami* (2n♂=35, $X_1X_2X_3$). A, Spermatogonial metaphase; B, Pacyhtene; C, Late diakinesis; D, Late metaphase I; E, Metaphase II; F, Anaphase II (arrows sex chromosomes; asterisks, nuclei with sex chromosomes). Scale bars=10 µm.

Slides were investigated under a phase contrast microscope (Leica DM2500) and the slides containing well figures were further analyzed. The slides were stained with 5% Giemsa solution in Sörensen's phosphate buffer (ph=6.8) for 27 min. Mitotic and meiotic stages were photographed by a BX53 light microscope (Olympus) equipped with DP 26 digital camera with CellSens software (Olympus). Karyotypes were constructed by arranging chromosomes in pairs according to size using images of spermatogonial metaphases. Relative chromosome lengths (RCLs) of each chromosome pair were calculated from 10 metaphases. Chromosome morphology was classified according to the nomenclature proposed by Levan *et al.* (1964).

RESULTS

The male karyotype of *H. bayrami* consisted of 2n♂=35 chromosomes (Fig. 2A). All chromosome pairs were acrocentric including the sex chromosomes. The sex chromosome system was ♂$X_1X_2X_3$/♀$X_1X_1X_2X_2X_3X_3$. Values for the RCLs were between 7.94% to 4.23% and gradually decreased in size. RCL of X_1 and X_2 was 6.63% and 5.02%, respectively. The X_3 was the smallest element in the karyotype (Fig. 1).

At the stages of meiotic prophase I (*i.e.*, leptotene, zygotene and pacyhtene), the sex chromosomes were positively heteropycnotic and large X_1 and X_2 associated with each other. However, the small X_3 chromosome was located close to X_1 and X_2 but did not associate with them (Fig. 2B). During diplotene, diakinesis and metaphase I, 16 autosomal bivalents and three univalent sex chromosomes were seen; generally, one chiasma (*i.e.*, interstitial or terminal type) per bivalent was observed (Fig. 2C, D). During the second meiotic stages (*i.e.*, prophase II, metaphase II and anaphase II), sex chromosomes were indistinguishable because they were isopycnotic with the autosomes. Two types of nuclei were observed, either with (n=19) or without (n=16) sex chromosomes (Fig. 2E, F).

DISCUSSION

A karyotype of only one hersiliid species (*Hersilia savignyi* Lucas, 1836) was described up to now. Most of authors reported the diploid number as 2n♂=30 for the species (Bole-Gowda, 1958; Srivastava and Shukla, 1986; Parida and Sharma, 1987; Sharma and Parida, 1987; Prakash and Prakash, 2014), however, Sharma *et al.* (1960) reported also 2n♂=32. The sex chromosome system was always of the ♂X_1X_2/♀$X_1X_1X_2X_2$ type. Karyotype of the genus *Hersiliola* was reported for the first time by this study with the male diploid number 2n♂=35 (32, $X_1X_2X_3$0) which is not so common in spiders. Hersiliidae is closely related with the family Oecobiidae, where the known diploid numbers listed in Araújo *et al.* (2017) are 2n♂=22 in *Oecobius cellariorum* Dugès, 1836 (Youju *et al.*, 1993), 2n♂=25 in *O.putus* O. Pickard-Cambridge, 1876 (Mittal, 1983; Srivastava and Shukla, 1986), 2n♂=39 in *Uroctea lesserti* Schenkel, 1936 (Youju *et al.*, 1993), 2n♂=42 in *U. compactilis* L. Koch, 1878 (Suzuki, 1950, 1954). Based on these results; there is no strong relation on diploid numbers between families, nevertheless, the sex chromosome type $X_1X_2X_3$0 is encountered in both families Hersiliidae and Oecobiidae. To explain the chromosomal differences between these families, additional cytogenetic studies are needed including the species not investigated previously.

The chromosomes in the family studied to date were acrocentric (Araújo *et al.*, 2017), however, Prakash and Prakash (2014) reported also metacentric, submetacentric and subtelocentric chromosomes in the karyotype of *H. savignyi*. In our study, all autosomes and sex chromosomes of *H. bayrami* were acrocentric. This may be due to differences in the techniques used to obtain the figures of chromosomes and measurements of them.

Our study reported the first results of the chromosome number, karyotype and meiotic features for the genus *Hersiliola*. The species in focus, *H. bayrami* was investigated by a classical cytogenetic analysis. This study will promote further studies of the family Hersiliidae.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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