Pakistan J. Zool., vol. 52(2), pp 513-517, 2020. DOI: https://dx.doi.org/10.17582/journal.pjz/20180610190658

# A Cytogenetical Study on Two Ground Spider Species (Gnaphosidae: *Drassodes*) from Nevşehir District

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## ABSTRACT

In this study, we describe a chromosomal analysis of *Drassodes bifidus* and *Drassodes serratichelis* using a classical Giemsa staining method. We collected specimens from different populations in Nevşehir and used totally fourteen male spiders. The two *Drassodes* species had karyotypes comprising 10 pairs of autosomes plus sex chromosomes which were $X_1X_20$ (♂) type. All chromosomes including sex chromosomes were telocentric and relative lengths of autosomal pairs were decreased gradually in size. All male meiosis was chiasmate and during the first meiotic division stages, 10 autosomal bivalents and generally one chiasma per bivalent (rarely two chiasmata) were obtained, the types of chiasmata were proximal, interstitial and terminal. In second meiotic division, two types of nuclei were showed as n=12 and n=10 chromosomes. Considering our results and previously obtained data in other ground spider species, the conventional staining provide important chromosome markers for karyotype evolution in gnaphosids and chromosome structure studies.

**Article Information**
**Received 10 June 2018**
**Revised 11 August 2019**
**Accepted 20 August 2019**
**Available online 17 January 2020**

**Authors' Contribution**
**ZK directed the field and laboratory work and wrote the manuscript. HP helped in field work, manuscript writing, collected spiders, maintained them in the laboratory and prepared chromosome slides and karyotype.**

**Key words**
**Chromosome, *Drassodes*, Karyotype, Meiosis, Nevşehir.**

## INTRODUCTION

Gnaphosid spiders can be found all over the world. The length of them ranges from 1 to 15 mm. These spiders can have two claws, usually with no patterning, and in homogeneous color (*i.e.* black, dark brown or greenish green). Some of them have patterns on the back and abdomen. The arrangement and size of eyes on carapace in gnaphosids are important characters and another that distinguishes gnaphosids from other spider families is; the discrete, cylindrical, and end portions of the front web breech ribs are finished in a blunt form (Chatzaki, 2008). In winter, most of the gnaphosids have limited activity, and they also have an annual biotic cycle with mating to the spring (Chatzaki *et al*., 1998). They live under the stone, leaves and in the dried woods (Logunov and Gromov 2012) and are composed of about 145 species and represented in 30 genera in Turkey (Demir and Seyyar 2017). Among them *Drassodes* (Westring, 1851), *Gnaphosa* (Latreille, 1804), *Haplodrassus* Chamberlin, 1922 and *Zelotes* Gistel, 1848 are included high numbers of species. The genus *Drassodes* is illustrated by 10 species namely *Drassodes bifidus* (Kovblyuk and Seyyar, 2009); *Drassodes cupreus* (Blackwall, 1834); *Drassodes difficilis* (Simon, 1878); *Drassodes lacertosus* (O.P. Cambridge, 1872); *Drassodes lapidosus* (Walckenaer, 1802); *Drassodes lutescens* (C.L. Koch, 1839); *Drassodes pubescens* (Thorell, 1856); *Drassodes serratichelis* (Roewer, 1928); *Drassodes similis* (Nosek, 1905) and *Drassodes villosus* (Thorell, 1856) (Demir and Seyyar, 2017).

Karyological information such as diploid chromosome number, the morphology and size of chromosomes, sex chromosome systems and chromosome behaviours is useful to make inferences about interrelationships in the group (Kumbıçak *et al*., 2018). Only a few species of *Drassodes* have been studied cytogenetically till now; data are available on the species as *D. lapidosus* (2n=22, $X_1X_2$; Hackman, 1948), *D. lutescens* (2n=21, X0; Kumbıçak *et al*. 2014), *D. pubescens* (2n=22, $X_1X_2$; Kumbıçak *et al*., 2009) and *Drassodes* sp. (2n=21, X0; Srivastava and Shukla (1986) and 2n=22, $X_1X_2$; Suzuki (1954)).

In this paper, we report the first knowledges of mitotic and meiotic chromosomes of *Drassodes bifidus* and *Drassodes serratichelis* belonging to the family of Gnaphosidae. The diploid chromosome numbers and sex chromosome systems were determined and compared with the other closely related species and revealed that the basic cytogenetical properties are conservative within the family.

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0030-9923/2020/0002-0513 $ 9.00/0

**Table I.- Data of field studies including number of used specimens, locality and coordinates.**

| Species | No. of used specimens | Locality | Coordinates | Date of collection |
|---|---|---|---|---|
| *Drassodes bifidus* (Kovblyuk and Seyyar, 2009) | 5♂♂ | Mazı, Nevşehir | 38°28'05.71" N; 34°50'56.41" E | 24.03.2014 |
| | 3♂♂ | Göre, Nevşehir | 38°35'48.84" N; 34°43'40.60" E | 18.05.2014 |
| *Drassodes serratichelis* (Roewer, 1928) | 3♂♂ | Göre, Nevşehir | 38°35'48.84" N; 34°43'40.60" E | 09.04.2014 |
| | 1♂ | Acıgöl, Nevşehir | 38°32'22.60" N; 34°32'55.93" E | 13.03.2014 |
| | 2♂♂ | Mazı, Nevşehir | 38°27'56.04" N; 34°50'20.39" E | 27.05.2014 |

## MATERIALS AND METHODS

We investigated two species of ground spiders belonging to the family Gnaphosidae from three different localities of Nevşehir District given in Table I. The specimens were collected by hand under stones during March to May in the year 2014. During the field studies, no treatment was applied to the collected spiders and they were transfered into 5-10 cm sized tubes and taken to the laboratory. Alive spiders were fed twice a week with fruit flies (*Drosophila melanogaster;* Meigen, 1830) until the dissection of gonads. The voucher specimens were kept in the Genetic Laboratory of Nevşehir Hacı Bektaş Veli University.

Due to have lots of dividing cells and meiosis nuclei, male gonads are useful for investigating basic properties of cytogenetics of spiders.

*Preparation of chromosome slides*

Construction of chromosome preparations was performed according to the method of Král *et al*. (2006). The gonads were dissected out in a physiological solution for invertebrates using a stereomicroscope (Leica EZ24). Then three basic steps were applied: (i) hypotonic solution, 0.075 M KCl solution, for 13 min, (ii) fixation, 3:1 methanol:acetic acid, twice for 10 and 20 min and (iii) squashing with 60% acetic acid, on heating plate surface temperature (42°C). The slides were stained with 5% Giemsa solution in Sörensen's phosphate buffer (pH=6.8) for 50 min.

*Analysis of chromosomes*

Chromosome photographs were taken with CellSens (Olympus) software at 100X magnification with a BX53 (Olympus) light microscope equipped with DP 26 digital camera. Relative chromosome lengths (RCL) were measured using CellSens software in micrometrical level (µm). Karyotypes were constructed by arranging chromosomes in pairs according to size using images of mitotic metaphases using Adobe Photoshop CS3 programme. RCL of each chromosome pairs were calculated from 10 metaphases. The nomenclature according to Levan *et al*. (1964) was used to classify the morphology of chromosomes.

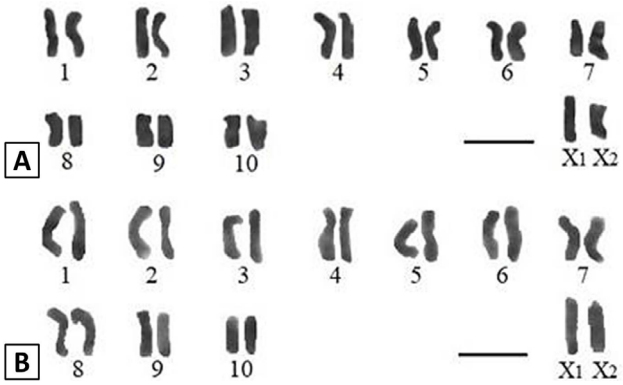


Fig. 1. Karyotypes of male *Drassodes* species from mitotic metaphases; 2n♂=22 ($X_1X_20$). A, *Drassodes bifidus*; B, *Drassodes serratichelis*. Bars=10 µm.

## RESULTS

*Karyotype features and sex chromosome systems*

Drassodes bifidus

The male karyotype consisted of 2n♂=22 chromosomes (Fig. 1A). Sex chromosome system was $X_1X_20$. All chromosomes were telocentric. Relative lengths of autosome pairs were decreased between 9.67±0.12% and 6.40±0.28%. The relative lengths of $X_1$ and $X_2$ were 8.56±0.50% and 7.16±0.16%, respectively (Table II). $X_1$ was the middle element at the karyotype and $X_2$ was longer than three pairs of autosomes.

Drassodes serratichelis

The male karyotype contained 22 chromosomes (Fig. 1B). Sex chromosome system was of $X_1X_20$ type. Both autosomes and gonosomes were telocentric. Relative lengths of autosome pairs were decreased from 9.75±0.36% to 5.56±0.22%. The relative lengths of $X_1$ and $X_2$ were 8.75±0.29% and 7.12±0.14%, respectively (Table II). $X_1$ was longer than the fourth autosomal pair and $X_2$ was the middle element in the karyotype.

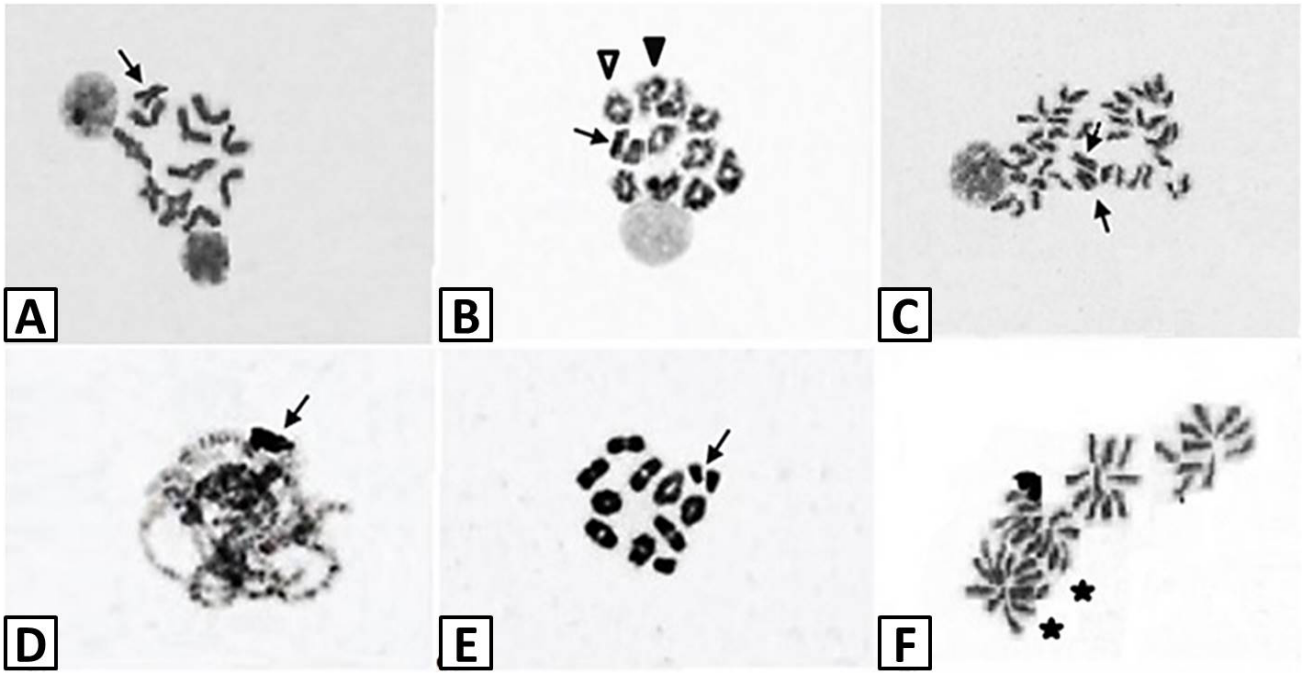


Fig. 2. Meiotic stages of *Drassodes bifidus:* A, diplotene; B, diakinesis; C, anaphase I - *Drassodes serratichelis;* D, pacyhtene; E, diplotene; F, anaphase II (black arrows indicate sex vesicle or sex chromosomes. Black arrowhead indicates chiasma with two chiasmata, white arrowhead indicates ring bivalent and asteriks indicate nuclei with 2n=12 chromosomes). Bar=10 µm.

**Table II.- Chromosome measurements of species studied.**

| Number of chromosomes | *Drassodes bifidus* | | *Drassodes serratichelis* | |
|---|---|---|---|---|
| | RCL (%) | CM | RCL (%) | CM |
| 1 | 9.67±0.12 | T | 9.75±0.36 | T |
| 2 | 9.25±0.28 | T | 9.56±0.14 | T |
| 3 | 9.08±0.05 | T | 9.22±0.05 | T |
| 4 | 8.90±0.36 | T | 8.83±0.18 | T |
| 5 | 8.68±0.22 | T | 8.70±0.32 | T |
| 6 | 8.11±0.17 | T | 8.45±0.10 | T |
| 7 | 7.60±0.08 | T | 8.16±0.19 | T |
| 8 | 6.86±0.25 | T | 7.94±0.07 | T |
| 9 | 6.53±0.50 | T | 7.76±0.36 | T |
| 10 | 6.40±0.28 | T | 5.56±0.22 | T |
| $X_1$ | 8.56±0.50 | T | 8.75±0.29 | T |
| $X_2$ | 7.16±0.16 | T | 7.12±0.14 | T |

RCL, relative chromosome length; CM, chromosome morphology; T, telocentric; ±, standard devition.

*Karyological features in meiotic division stages*

Meiotic properties in two species studied was determined similarly. During the first meiotic stages (*i.e.* leptotene, zygotene, pacyhtene, diplotene and diakinesis) $X_1$ and $X_2$ was positively heteropycnotic and located at the periphery of nucleus (Fig. 2A, B, D, E). Beginning from the pacyhtene to metaphase I, there were 10 autosomal bivalents and two univalent sex chromosomes (Fig. 2A, B, E). Bivalents often had one chiasma, but sometimes two chiasmata and the types of chiasmata were proximal, interstitial or terminal (Fig. 2B, D, E). The bivalents were ring shaped if had two chiasmata (Fig. 2B). At anaphase I, the chromosomes were "V" shaped because of their telocentric morphology (Fig. 2C). During the second meiotic stages, the sex chromosomes were isopycnotic but distinguishable due to their shape and two kinds of nuclei were determined as n=10 or n=12 chromosomes at metaphase II and anaphase II (Fig. 2F).

## DISCUSSION

Karyotypes of 53 gnaphosid spiders from 22 genera are known at present (Araújo *et al*., 2018) and diploid chromosome numbers range from 20 (*Zelotes aeneus,* Simon, 1878; Taşdemir *et al*. (2012)) to 30 (*Scotophaeus domesticus,* Tikader, 1962; Srivastava and Shukla (1986)) in males. However, there are results where the diploid number is 21 (*Drassodes lutescens*, C.L. Koch, 1839, Kumbıçak *et al*. (2014); *Urozelotes rusticus,* L. Koch, 1872, Srivastava and Shukla (1986)), 23 (*Zelotes*

*petrensis,* C.L. Koch, 1839; Taşdemir *et al*. (2012)) and 24 (*Scotophaeus blackwalli,* Thorell, 1871; Mittal (1961) and (1967)). Except these, approximately 90% of previously studied species have 22 chromosomes. Due to encountered frequently in different populations of this taxa suggests that the cytotype of 2n♂=22 is preserved within the family.

Chromosomal studies on the genus *Drassodes* have been carried out using air-drying method previously, and a conservative diploid chromosome number and sex chromosome system reported in males as 2n♂=22, $X_1X_20$ type which can be considered as modal but there is an exception with the karyotype formula in *Drassodes lutescens* which has 21 chromosomes with X0/XX sex chromosome system (Araújo *et al*., 2018). In this karyotype, the large X chromosome maybe originated through the gradual elimination of one X chromosome of $X_1X_2$ type or by reciprocal translocation between $X_1$ and $X_2$ chromosomes that was preceded by distal fission in one sex chromosome and proximal fission in the other X (Maddison and Leduc-Robert, 2013). In our study, we found 2n♂=22, a diploid number and $X_1X_20$, sex chromosome system for both *Drassodes* species which are in agreement with the karyotype features of the Gnaphosidae family. Metacentric, submetacentric, acrocentric and telocentric types of chromosomes are generally found in the Mesothelae and Mygalomorphae groups of the spiders, whereas in Araneomorphae, generally acrocentric or telocentric type chromosomes (Poyraz, 2017). In our study, the chromosomes of telocentric type in all species is compatible with the family characteristics. Moreover, some features such as formation of chiasma during diplotene, diakinesis and metaphase I; generally one chiasma per bivalent; "V" shaped chromosomes in anaphase I and "I" shaped chromosomes in anaphase II; positively heteropycnotic sex chromosomes during meiosis I stages and isopycnotic sex chromosomes during meiosis II stages reflects common structures within the genus.

Cytogenetical analysis may be useful for the evaluation of species, especially in complicated taxonomic groups, as is the case of many spiders but such the conservative characters as diploid number and sex chromosome system are insufficient to distinguish the taxons. Consequently, we suggest that various chromosome banding and molecular techniques will provide more useful data in the classifications of spiders.

## ACKNOWLEDGEMENTS

The authors thank to all members of the Genetic Laboratory from Nevşehir Hacı Bektaş Veli University, Art and Science Faculty for their kindest helps on field trips and feding spiders. The authors would like to thank the anonymous reviewers for their valuable comments and suggestions for the manuscript.

*Satatement of conflict of ınterest*

Authors have declared no conflict of interest.

## REFERENCES

Araújo, D., Schneider, M.C., Paula-Neto, E. and Cella, D.M., 2018. *The spider cytogenetic database*, version 8.0. Available at: www.arthropodacytogenetics.bio.br/spiderdatabase (Accessed on 10 September, 2019).

Chatzaki, M., Trichas, A., Markakis, G. and Mylonas, M., 1998. Seasonal activity of the ground spider fauna in a Mediterranean ecosystem (Mt. Youchtas, Crete, Greece). In: *Proceedings of the 17th European Colloquium of Arachnology, Edinburgh* (ed. P.A. Selden). British Arachnological Society, Bunham Beeches, Bucks, UK, pp. 235-243.

Chatzaki, M., 2008. A critical review of the spider family Gnaphosidae in Greece. In: *Advances in arachnology and developmental biology* (eds. S.E. Makarov and R.N. Dimitrijević). Monographs, 12, Vienna-Belgrade-Sofia, pp. 355-374.

Demir, H. and Seyyar, O., 2017. Annotated checklist of the spiders of Turkey. *Munis Ent. Zool.,* **12**: 433-469.

Hackman, W., 1948. Chromosomenstudien an Araneen mit besonderer berücksichtigung der geschlechtschromosomen. *Acta Zool. Fenn.*, **54**: 1-101.

Král, J., Musilová, J., Št`áhlavský, F., Řezáč, M., Akan, Z., Edwards, R.L., Coyle, F.A. and Almerje, C.R., 2006. Evolution of the karyotype and sex chromosome systems in basal clades of araneomorph spiders (Araneae: Araneomorphae). *Chromosome Res.*, **14**: 859-880. https://doi.org/10.1007/s10577-006-1095-9

Kumbıçak, Z., Ergene, S. and Saygıdeğer, S., 2009. Chromosomal data on six araneomorph spiders belonging to the families Lycosidae and Gnaphosidae (Araneae: Araneomprhae). *Zool. Middle East*, **48**: 89-96. https://doi.org/10.1080/09397140.2009.10638371

Kumbıçak, Z., Ergene, S., Kumbıçak, Ü. and Ekiz, E., 2014. A chromosomal analysis of five spider species (Araneae: Gnaphosidae, Miturgidae and Philodromidae) from Turkey. *Caryologia,* **67**: 155-159. https://doi.org/10.1080/00087114.2014.931637

Kumbıçak, Ü., Kumbıçak, Z. and Huseynli, G., 2018.

A new diploid number and sex chromosome system for the family Hersiliidae from the Mediterranean Region of Turkey. *Pakistan J. Zool*., **50**: 131-134. https://doi.org/10.17582/journal.pjz/2018.50.1.131.134

Levan, A., Feradga, K. and Sandberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas,* **52**: 201-220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Logunov, D.V., Gromov, A.V. and Tımokhanov V.A., 2012. *Spiders of Kazakhstan*. Siri Sci. Press, Manchester, pp. 232.

Maddison, W.P. and Leduc-Robert, G., 2013. Multiple origins of sex chromosome fusions correlated with chiasma localization in *Habronattus* jumping spiders (Araneae: Salticidae). *Evolution,* **67**: 2258-2272. https://doi.org/10.1111/evo.12109

Mittal, O.P., 1961. Chromosome number and sex mechanism in twenty-one species of the Indian spiders. *Res. Bull. (N.S.) Panjab Univ.*, **12**: 271-273.

Mittal, O.P., 1967. Karyological studies on the Indian spiders VII. Mitosis and meiosis in two species belonging to the family Gnaphosidae. *Genetica,* **38**: 516-520. https://doi.org/10.1007/BF01507480

Poyraz, H., 2017. *Cytogenetic ınvestigation on some spider species belonging to Gnaphosidae family*. Master thesis, Nevşehir Hacı Bektaş Veli University, Nevşehir, Turkey.

Srivastava, M.D.L. and Shukla, S., 1986. Chromosome number and sex-determining mechanism in forty-seven species of Indian spiders. *Chromosome Inform. Serv.*, **41**: 23-26.

Suzuki, S., 1954. Cytological studies in spiders. III. Studies on the chromosomes of fifty-seven species of spiders belonging to seventeen families, with general considerations on chromosomal evolution. *J. Sci. Hiroshima Univ. Ser. B: Division 1*, **15**: 23-136.

Taşdemir, B., Varol, I. and Akpınar, A., 2012. Cytotaxonomical studies on six species of spiders (Arachnida: Araneae) from Turkey. *Türkiye Ent. Bült.*, **2**: 55-59.