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RESEARCH ARTICLE

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Karyotypes of Southeastern Turkish Scorpions *Hottentotta saulcyi* and *Buthacus macrocentrus* (Scorpiones: Buthidae)

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ABSTRACT

Objective: The species *Hottentotta saulcyi* is widely distributed from Mardin to Hakkari while the distribution of *Buthacus macrocentrus* is limited to the south-east of Turkey (only Şanlıurfa). The aim of this study is to analyze the cytogenetic structure of *Hottentotta saulcyi* and *Buthacus macrocentrus*.

Materials and Methods: The specimens were collected during the night from Şırnak and Şanlıurfa using a UV lamp. The male *Hottentotta saulcyi* were collected from Şırnak and male and female *Buthacus macrocentrus* from Şanlıurfa. Chromosome preparations were made using cells from the male testes and the female ovariuteri of the species studied. Chromosome preparations were made using the classical spreading method.

Results: The diploid chromosome number for H*ottentotta saulcyi* was 2n=14, and 2n=28 for *Buthacus macrocentrus*.

Conclusion: The karyotypes of *Hottentotta saulcyi* and *Buthacus macrocentrus* have been presented for the first time. Both analyzed species have holocentric chromosomes that gradually decrease in size. Quadrivalent and hexavalent were observed during the first meiotic division in males of *Buthacus macrocentrus*.

Keywords: Karyotype, *Hottentotta saulcyi, Buthacus macrocentrus*

INTRODUCTION

Currently, 213 genera and 2433 scorpion species are classified under 17 families (1). Although scorpions are widely distributed in the tropics and subtropics and all types of terrestrial habitats all over the continents (except Antarctica) (2), our present knowledge of their karyotypes is still scarce. Chromosome data on 155 scorpions belonging to 11 families have thus far been determined. Among them, 91 species of Buthidae have been studied, limited to some geographic regions-especially Brazil and Africa (3). Karyotypes of these scorpions are composed of holocentric chromosomes without a localized centromere region (4).

Cytogenetic studies have been carried out on *Leiurus abdullahbayrami* Yağmur, Koç&Kunt, 2009 and *Compsobuthus matthiesseni* (Birula, 1905) present a karyotype with 2n=22 (5), *Androctonus crassicauda* (Olivier, 1807) has a karyotype of 2n=24 (6), *Aegaeobuthus gibbosus* (Brullé, 1832) shows 2n=28, *Mesobuthus eupeus* (C.L. Koch, 1839) has 2n=20 (Buthidae) and *Euscorpius aladaglarensis* Tropea&Yağmur, 2016 (7) shows 2n=88 (Euscorpiidae), which are distributed in Turkey.

The present knowledge of the cytogenetics of Turkish scorpions is scarce and fragmented. The aim of this paper is to report the first chromosomal data of two species (*Hottentotta saulcyi* and *Buthacus macrocentrus*) from

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Turkey. The genus *Hottentotta* Birula, 1908 is widespread throughout Africa, the Middle East and Asia (8, 9). This genus comprises almost 51 species (10). *H. judaicus* (Simon, 1872) (11), *H. tamulus* (Fabricius, 1798) (12-14) and *H. trilineatus* (Peters, 1861) (15) have been analyzed in cytogenetic studies up to present day. *Hottentotta saulcyi* (Simon, 1880) was firstly recorded in Mardin (10) and then reported in Batman, Şırnak, and Hakkâri in Turkey (9). Meanwhile, the genus *Buthacus* Birula, 1908 is distributed across northern and western Africa, Israel, Palestine, Jordan, Syria, Turkey, the Arabian Peninsula, Iraq, Iran, Afghanistan, and Pakistan. 23 species belonging to *Buthacus* have thus far been described (1, 10, 16-19). In Turkey, *Buthacus macrocentrus* (Ehrenberg, 1828) is known only from Şanlıurfa (10, 18, 20). Cytogenetic analyses have been performed for only one species namely *Buthacus stockmanni* Kovraik, Lowe&Stahlavsky, 2016 having 2n=20 (21).

MATERIALS AND METHODS

The scorpions were collected using a UV lamp during the night from Şırnak and Şanlıurfa respectively (Figure 1). The collected specimens were transferred to the laboratory in individual plastic containers. In total, six specimens of *Hottentotta saulcyi* and seven of *Buthacus macrocentrus* were analyzed (for detailed information, see "Material examined" below).

The gonads were used from both males and females. The specimens were killed by ventral puncture to the prosomal area. Under a stereomicroscope, the gonads were removed by dissection in the presence of physiological salt solution for invertebrates. The gonads were then kept in a hypotonic solution (0.075 M KCl) for 20 min. The gonads were fixed in a freshly prepared fixative (3: 1, methanol: acetic acid) for 20 min. A few drops of 60% acetic acid were then dropped on a slide and then shredded with a tungsten needle. The drop on the slide was placed on the heating plate and spread with tungsten needles (22). The prepared slides were stained with 5% Giemsa in Sörensen's phosphate buffer. The chromosome preparations were analyzed under a Leica DM 500 microscope with a 100x objective. Images were taken with a Leica camera using Leica Application LAZ software. The measurements were analyzed using software ImageJ 1.47 (23) with the plugin Levan (24). The relative length of the chromosomes was calculated as a percentage of the diploid set and it based on seven mitotic metaphases in *Hottentotta saulcyi* and on seven postpachytene in *Buthacus macrocentrus*. The preparations were kept in a slide box and the remains of the specimens were fixed in a solution of 96% alcohol and stored in a refrigerator at 4 °C at the Zoological Museum of Sinop University, Turkey (ZMSU).

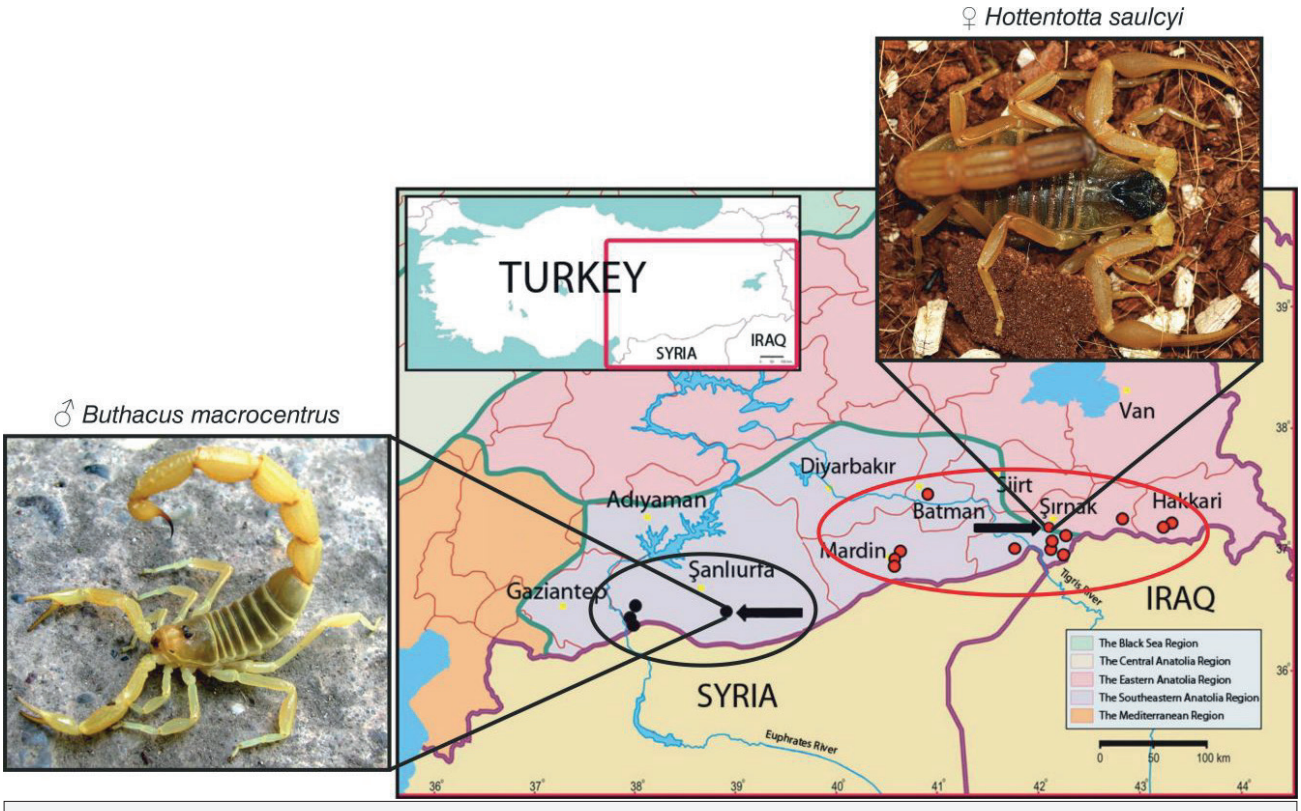

Figure 1. Distribution of *Hottentotta saulcyi* (red circle) and *Buthacus macrocentrus* (black circle) in south-eastern Turkey. Arrows show the localities of examined materials.

RESULTS

Family : Buthidae C.L. Koch, 1837
Genus-1 : *Hottentotta* Birula, 1908

Species-1 : *Hottentotta saulcyi* (Simon, 1828) (Figures 2 and 3)

Material examined: 2♂ 3♀ and 1♂ subadult, Şırnak, 2.5 km SW of Şırnak, 37°29'57.3"N; 42°26'32.7"E, 1024 m, 17.07.2014, leg. E. A. Yağmur (Figure 1).

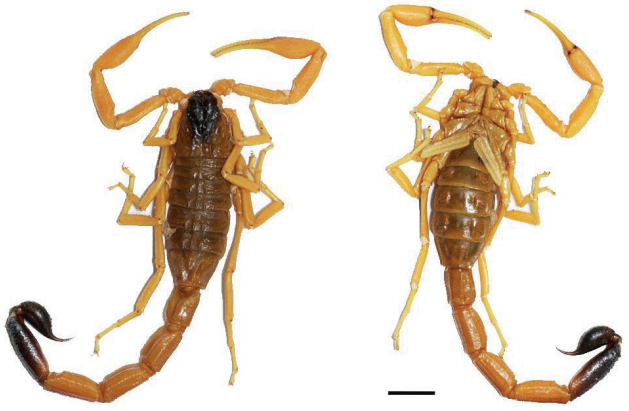

Figure 2. Male *Hottentotta saulcyi*, dorsal and ventral views. Scale=1cm.

Figure 3. Female *Hottentotta saulcyi*, dorsal and ventral views. Scale=1cm.

Karyotype Investigation
The chromosome complement of *Hottentotta saulcyi* consisted of 14 chromosomes (Figure 4). We observed only the mitotic phases for this species. The relative chromosome length gradually decreased from 11.65 to 4.39% of the diploid set (Figure 4a).

Genus-2 : *Buthacus* Birula, 1908

Species-2 : *Buthacus macrocentrus* (Ehrenberg, 1828) (Figures 5 and 6)

Material examined: 4♂, Şanlıurfa, Birecik District, 2 km S of Mezra Village, 36°56'50.1"N; 38°01'20.3"E, 375 m, 08.07.2013, leg. E. A. Yağmur. 3♀, Şanlıurfa, Birecik District, 2 km S of Mezra Village, 36°57'35"N; 38°00'43"E, 387 m, 27.07.2014, leg. E. A. Yağmur (Figure 1).

Figure 5. Male *Buthacus macrocentrus*, dorsal and ventral views. Scale=1cm.

Karyotype Investigation
The number of diploid chromosomes in all male and female *Buthacus macrocentrus* specimens examined was 28 (Figure 7a). The relative chromosome length of the first chromosome (6.31%) was slightly larger than the remaining chromosomes which

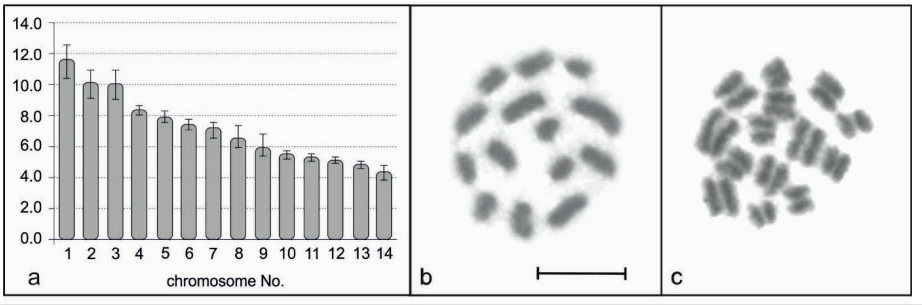

Figure 4. Ideogram and chromosomes of male *Hottentotta saulcyi*, 2n=14. a. ideogram based on mitotic metaphases (y axis - % of the diploid chromosome length, lines indicate min.-max. values); b. mitotic metaphase; c. early mitotic anaphase. Scale=10 µm.

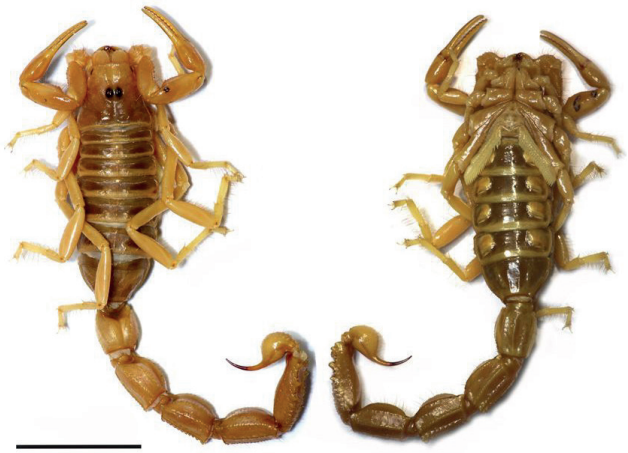

Figure 6. Female *Buthacus macrocentrus*, dorsal and ventral views. Scale=1cm.

gradually decreased from 5.14 to 1.95% of the diploid set (Figure 7a). Achiasmatic bivalents were detected in males during the first meiotic division. In the female, mitotic metaphases were obtained (Figure 7b). A distinct quadrivalent or hexavalent association of chromosomes were found in all individuals and observed in four males during meiosis (Figures 7c and d). The size of the detected multivalent chromosomes gradually reduced. The chromosomes forming quadrivalent or hexavalent were different in size (Figure 7a). During the first meiosis division phase (anaphase, pachytene, postpachytene, metaphase-I), no indication of crossing-over was observed. In the polar view of metaphase I (Figures 7b and c), the majority of these bivalents presented parallel-arranged homologous chromosomes. In pachytene (Figure 7e), bivalents were all strip-shaped.

DISCUSSION

The present study provides the first cytogenetic analysis of the *Hottentotta saulcyi* and *Buthacus macrocentrus*, species of the Buthidae family. The karyotypes of these species consist of 14 and 28 chromosomes (Table 1). 91 species of Buthidae have been cytogenetically studied thus far, and which show the lowest chromosome numbers within scorpions. This family has a diploid chromosome number varying from 2n=5 [*Tityus bahiensis* (Perty, 1833)] to 2n=36 [*Barbaracurus somalicus* (Hirst, 1907) and *Parabuthus mossambicensis* (Peters, 1861)], excluding dubious information (see 3). Buthidae family, as well as the entire order Scorpiones, is characterized by achiasmatic meiosis in males (25). In contrast to other scorpions, the examined species of buthids usually possess a relatively low chromosome number and all of them have holocentric organization (4).

Although there are several faunistic and taxonomic studies on the genus *Hottentotta*, there is a paucity of cytogenetic studies.

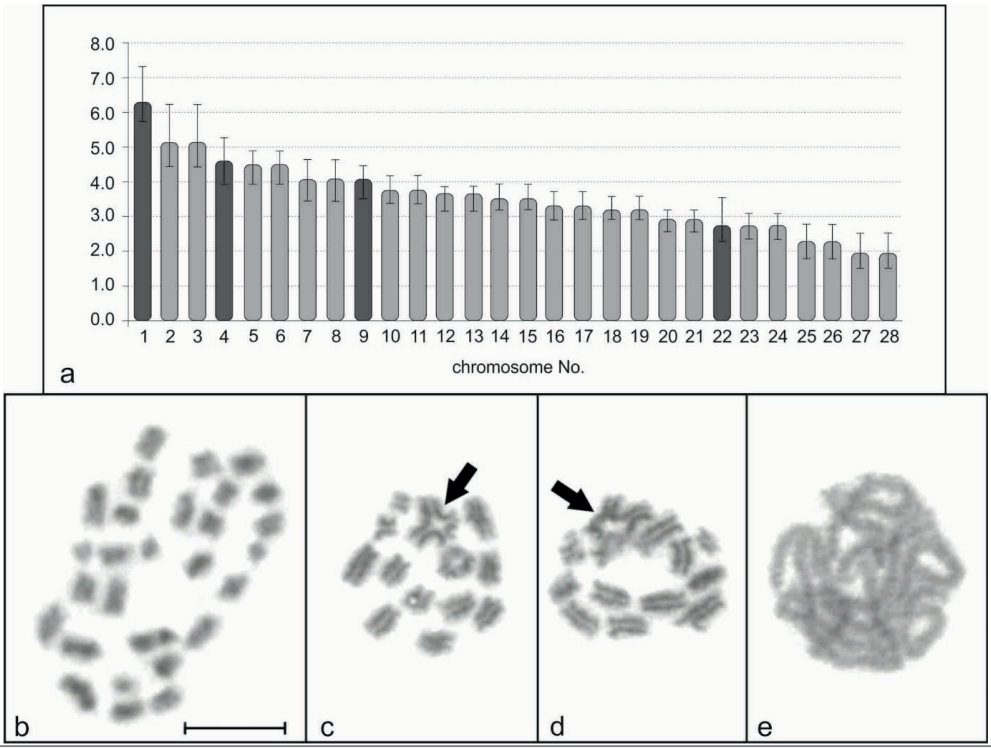

Figure 7. Ideogram (a) and chromosomes of female (b) and male (c-e) *Buthacus macrocentrus*, 2n=28. a. ideogram based on postpachytene with quadrivalent (dark grey) (y axis - % of the diploid chromosome length, lines indicate min.-max. values); b. mitotic metaphase of female; c. metaphase I of male, arrow shows quadrivalent; d. metaphase I of male, arrow shows hexavalent; e. pachytene of male. Scale =10 µm.

Table 1: Number of diploid chromosomes in eight species of scorpions from Turkey.

Taxon	2n	Sampling locality	Reference
Buthidae			
Androctonus crassicauda (Olivier, 1807)	24	Turkey: Şanlıurfa Province	(6)
Buthacus macrocentrus (Ehrenberg, 1828)	28	Turkey: Şanlıurfa Province	the present study
Compsobuthus matthiesseni (Birula, 1905)	22	Turkey: Gaziantep Province	(5)
Hottentotta saulcyi (Simon, 1880)	14	Turkey: Şırnak Province	the present study
Leiurus abdullahbayrami Yağmur, Koç & Kunt, 2009	22	Turkey: Gaziantep Province	(5)
Mesobuthus eupeus (C. L. Koch, 1839)	20	Turkey: Niğde Province	(7)
Aegaeobuthus gibbosus (Schenkel, 1947)	28	Turkey: Niğde Province	(7)
Euscorpiidae			
Euscorpius aladaglarensis Tropea & Yağmur, 2016	88	Turkey: Niğde Province	(7)

Chromosomal data are known for only three of the total of 51 currently recognized species of the genus *Hottentotta* species (10), were included in cytogenetic studies (11-15). *Hottentotta tamulus* species possesses 2n=22, 24 or 20-28 chromosomes forming a continuous series (12-14). But, Venkatanarasimhiah and Rajasekarasetty (13) stated that the chromosome number of same species have 2n=23 stable chromosome number in India. Sharma et al. (12) observed the tetravalent formation which is very common in *H. tamulus*. The diploid number was given as 2n=24 for *Hottentotta trilineatus* by Newlands and Martindale (15). Qumsiyeh et al. (11) reported diploid chromosomes as 2n=16 for *Hottentotta judaicus* specimens from the Palestinian Territories. We obtained diploid chromosomes as 2n=14 for *Hottentotta saulcyi*. Our results supported that genus *Hottentotta* displays interspecific karyotype differences with 2n ranging from 14 (this study) to 24. However, we could not observe multivalent in *H. saulcyi* as previously documented in *H. tamulus*.

The genus *Buthacus* was studied cytogenetically for the first time by Kovařík et al. (21). The karyotype of *B. stockmanni* has 2n=20 chromosomes with holocentric and achiasmatic meiotic complement (21). According to cytological observation of *B. stockmanni*, the first pair of chromosomes are distinctively larger (13.41% of the diploid set) than the other chromosomes that gradually decrease from 5.84 % to 2.69 % of the diploid set. The cytogenetic analyses revealed that *Buthacus macrocentrus* consisted of 2n=28. The chromosomes are holocentric and achiasmatic meiotic complement as Kovařík et al. (21). The ideogram show that chromosomes of 1, 4, 9 and 22 are involving the arrangement of quadrivalent chromosome. These findings confirm the results of Shanahan and Hayman (26) who stated that multivalent formations involve during the achiasmate meiosis of buthid. The first chromosome is significantly longer (6.31%) than the remaining chromosomes that gradually decrease from 5.14% to 1.95% of the diploid set. In our present study, it is interesting to note that the presence of quadrivalents and hexavalents seems to be frequent in *B. macrocentrus* species.

CONCLUSION

Our analysis of karyotype data provides a first step towards understanding the chromosome numbers and the structure of chromosomes in one of the most important and dangerously venomous members of the family Buthidae. In general, our findings support that buthids have a low chromosome number with holocentric chromosomes. In the present study, the karyotype of two buthids, *Hottentotta saulcyi* and *Buthacus macrocentrus*, was identified for the first time as 2n=14 and 2n=28 respectively. The genus *Hottentotta* possess interspecific karyotype differences in a continuous series of the chromosomal number with 2n ranging from 14 to 24. As far as we know, this is the second karyological analysis of the genus *Buthacus*, thus our study is a significant contribution to the description of the chromosomal features of *Buthacus macrocentrus*. Our data highlights that the frequency of the multivalent is very high in this species. The chromosomes were arranged and numbered according to their total length in a gradually decreasing size order and their ideogram was developed (see Fig. 4a and Fig. 7a). Nevertheless, the results show that chromosome numbers are not a useful character in some buthids, and are therefore not effective for taxonomic purposes. Moreover, more detailed analysis of the karyotype of the buthid species is required for comparative cytotaxonomy of the Buthidae.

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Conflict of Interest: The authors declare that they have no conflicts of interest.

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