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Cytogenetics of *Helops glabriventris* Reitter, 1885 (Coleoptera: Tenebrionidae: Helopini)

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Abstract. In this study, the karyotype and chromosomal features of darkling beetle *Helops glabriventris* Reitter, 1885 from Western Anatolia were analyzed using conventional and differential staining. Diploid chromosome number of *H. glabriventris* was determined as $2n = 20$ with $9 + Xy_p$ meioformula. The parachute formation of sex bivalents was clearly observed in both prophase I and metaphase I plates. Both conventionally and differentially stained plates showed that relatively small amounts of heterochromatin are dispersed throughout the whole length of the chromosomes. As a result of silver staining, the existence of a highly impregnated area associated with a small submetacentric chromosome in prophase I, suggests autosomal location of NOR. Although presented karyotype of *H. glabriventris* resemble to those of other members of the tribe Helopini and follows the common patterns of tenebrionid karyotypes, slight differences in chromosome morphologies, NORs and the heterochromatin distribution were detected.

Key words: cytogenetics, Tenebrionidae, Helopini, *Helops glabriventris*, NOR, sex chromosomes, heterochromatin.

Цитогенетика *Helops glabriventris* Reitter, 1885 (Coleoptera: Tenebrionidae: Helopini)

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Резюме. Проанализированы кариотип и хромосомные признаки жука-чернотелки *Helops glabriventris* Reitter, 1885 из Западной Анатолии с использованием обычного и дифференциального окрашивания. Диплоидное число хромосом *H. glabriventris* было определено как $2n = 20$ с мейоформулой $9 + Xy_p$. Половой бивалент формирует ассоциацию «парашют», отчетливо наблюдавшуюся как в пластинках профазы I, так и в пластинках метафазы I. Пластинки как при обычном, так и при дифференциальном окрашивании показали, что относительно небольшое количество гетерохроматина рассеяно по всей длине хромосом. В результате окрашивания серебром наличие сильно импрегнированной области, связанной с небольшой субметацентрической хромосомой в профазе I, предполагает аутосомную локализацию ядрышковых организаторов. Хотя представленный кариотип *H. glabriventris* напоминает кариотип других представителей трибы Helopini и соответствует общим паттернам кариотипов тенебрионид, мы обнаружили небольшие различия в морфологии хромосом, ядрышковых организаторов и распределении гетерохроматина.

Ключевые слова: цитогенетика, Tenebrionidae, Helopini, *Helops glabriventris*, ядрышковые организаторы, половые хромосомы, гетерохроматин.

Introduction

Helops Fabricius, 1775 is the type genus of the tribe Helopini. Species named under this genus are distributed in the Western Palaearctic, the Nearctic and Neotropical regions [Nabozhenko et al., 2016; Nabozhenko, Keskin, 2017]. However, the current genus concept differs between regions and do not cover many phylogenetically distant species. The Palaearctic and West Hemisphere species of *Helops* show important differences in several diagnostic characters including the structure of mentum and male genitalia [Nabozhenko et al., 2016].

Currently, about 68 species are listed in the genus *Helops* s. l. with seven species occurring in the Western Palaearctic [Nabozhenko, 2020] and 61 species in the Nearctic and Neotropical regions [Bousquet et al., 2018]. Although regional faunistic studies and revisions have resulted in several new placements and combinations [Nabozhenko et al., 2016; Nabozhenko, Keskin, 2017; Nabozhenko, Steiner, 2021], the phylogenetic relationships within the genus *Helops* have yet to be described. Therefore, additional datasets on their genome organization may provide valuable information to infer on their phylogenies and establish a valid generic concept that supports monophyly of *Helops* species.

The cytogenetic data among Tenebrionidae have covered only about 1% of the species diversity [Petitpierre et al., 1991; Juan, Petitpierre, 1991a; Holecová et al., 2008; Blackmon, Demuth, 2015; Gregory, 2022]. In general, most of the studied species possess the diploid number, $2n = 20$, but the diploid number ranges from $2n = 14$ to $2n = 38$ in Tenebrionidae [Juan, Petitpierre, 1991a; Pons, 2004; Holecová et al., 2008; Lira-Neto et al., 2012; Blackmon, Demuth, 2015; Gregory, 2022].

Although cytogenetic data for the genus *Helops* are missing, some other members of the tribe Helopini were studied in this respect [Juan, Petitpierre, 1991a; Palmer, Petitpierre, 1997; Şendoğan, Alpagut-Keskin, 2016; Çalışan, 2018; Şendoğan et al., 2019].

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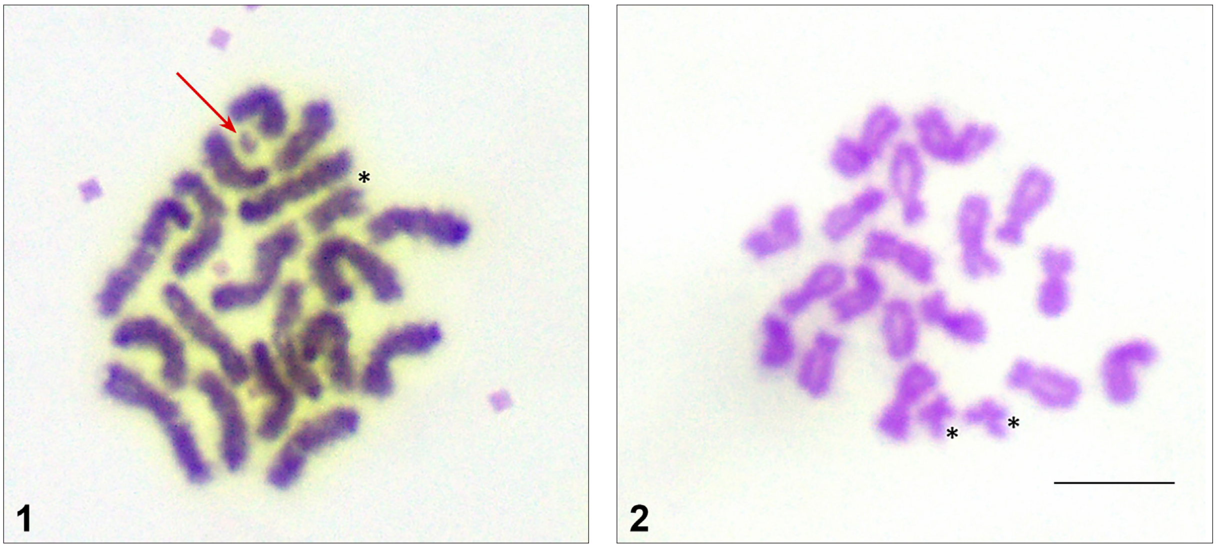


Figs 1–2. Male and female mitotic metaphase plates of *Helops glabriventris*.
1 – AgNOR stained male; 2 – Romanowsky–Giemsa-stained female mitotic metaphase plates. Arrow indicates minute y and asterisks indicate X chromosomes. Scale bar 5 µm.
Рис. 1–2. Митотические метафазные пластинки самца и самки *Helops glabriventris*.
1 – окрашивание нитратом серебра, самец; 2 – окрашивание по Романовскому – Гимзе, самка. Стрелка показывает мельчайшую y, звездочки – X-хромосому. Масштабная линейка 5 µm.

Helops glabriventris is distributed mainly in old coniferous Mediterranean forests in Anatolia, Cyprus and Greece; adult beetles feed on fruticose epiphytic lichens (predominantly Parmeliaceae), and larvae inhabit rotten wood [Nabozhenko et al., 2021]. In this study, with the aim of providing the first cytogenetic information about the genus *Helops*, chromosomal features of *H. glabriventris* specimens from Western Anatolia were analyzed using conventional and differential staining.

Material and methods

Adult specimens of *Helops glabriventris glabriventris* were collected during April and May, from Balçova-İzmir, Turkey. Beetles were found on lichen covered trunks of Calabrian pine (Pinus brutia Tenore, 1815) and Olive trees (Olea europaea L., 1753) after the sunset. Cytogenetic analyses were conducted using the gonads of one female and four male individuals. In order to observe the mitotic and meiotic chromosomes, microspreading [Chandley et al., 1994] and splashing [Murakami, Imai, 1974] methods were applied with some modifications [Şendoğan, Alpagut-Keskin, 2016].

For conventional staining, the slides were stained with 4% Giemsa. In order to determine the position of NORs and the heterochromatin distribution patterns, silver impregnation method [Patkin, Sorokin, 1983] and DAPI staining were used respectively. The mitotic and meiotic plates were photographed with Zeiss Axioscope light microscope using ZEN software. AT-rich chromosomal regions in DAPI stained plates were photographed in Cell Culture and Cell Imaging Laboratory of Ege University Institute of Nuclear Sciences.

The chromosomal measurements were made with Levan plugin [Sakamoto, Zacaro, 2009] of the Image J software [Schneider et al., 2012] and the female karyotype were created.

Results

Cytogenetic analysis conducted with both oogonial and spermatogonial cells of *H. glabriventris*, revealed the diploid number as $2n = 20$ with Xy_p sex determination system (Figs 1–3). In male and female metaphase plates, most of the autosomal pairs showed metacentric or submetacentric morphology, except for the subtelocentric 3rd pair (Table 1).

While in male metaphase plates a heteromorphic pair comprising of a minute telocentric y and a small metacentric X chromosome is apparent (Figs 1–3), no heteromorphism was observed among female metaphase plates (Fig. 2). The largest chromosome of the species was

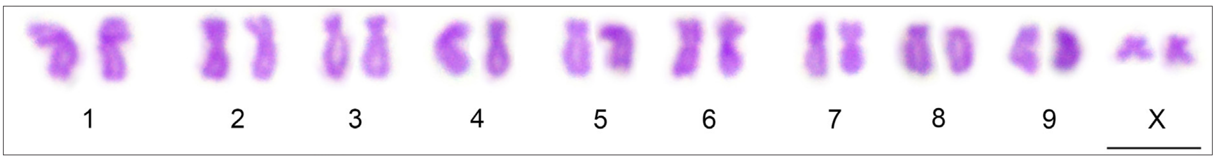


Fig. 3. Female karyotype of *Helops glabriventris*. Scale bar 5 µm.
Рис. 3. Кариотип самки *Helops glabriventris*. Масштабная линейка 5 µm.

Table 1. Chromosome morphologies and measurements of *Helops glabriventris* karyotype.
Таблица 1. Морфология хромосом и измерения кариотипа *Helops glabriventris*.

Chromosome Хромосома	Length (µm) Длина (µm)	CI	%RL	AR	Morphology Морфология
1	4.57	42	14.74	1.40	m
2	3.84	37	12.39	1.67	m
3	3.49	22	11.26	3.52	st
4	3.32	35	10.71	1.85	sm
5	3.27	32	10.55	2.16	sm
6	2.99	46	9.65	1.16	m
7	2.88	42	9.29	1.40	m
8	2.61	31	8.34	2.20	sm
9	2.53	48	8.06	1.07	m
X	1.5	45	4.77	1.22	m

Note. CI – centromere index; RL – relative length; AR – arm ratio; m – metacentric; sm – submetacentric; st – subtelomeric.
Примечание. CI – центромерный индекс; RL – относительная длина; AR – соотношение плеч; m – метацентрический; sm – субметацентрический; st – субтеломерный.

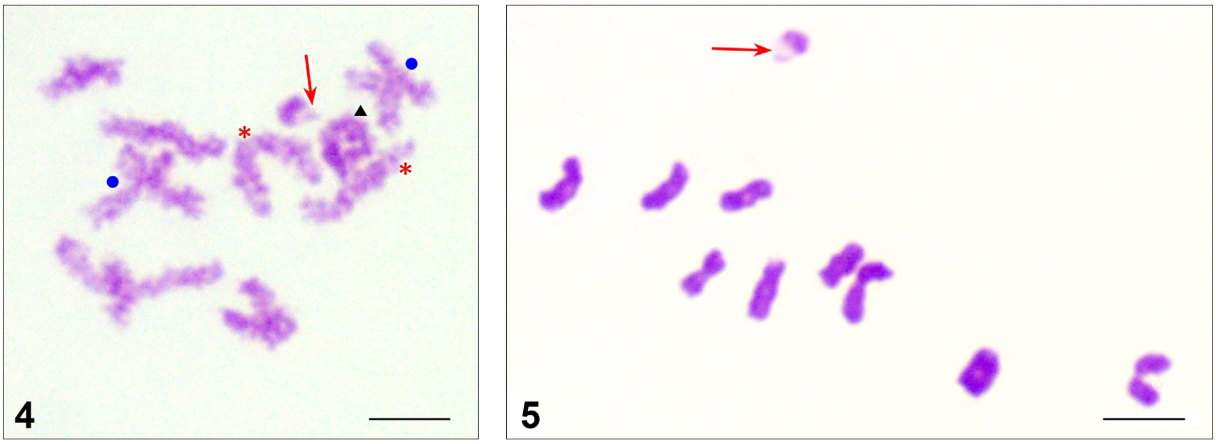
the 1st chromosome with 14.74% relative length and the smallest was y chromosome with 0.78 µm (Table 1).

In diplotene/diakinesis nuclei of *H. glabriventris*, 4–5 rod-shaped (terminal chiasma), 1 ring-shaped (two terminal chiasmata) and 2–3 cross-shaped (interstitial chiasma) bivalents were observed (Fig. 4). The parachute formation of the heteromorphic X and y was apparent in male prophase I and metaphase I plates (Figs 4, 5, 10).

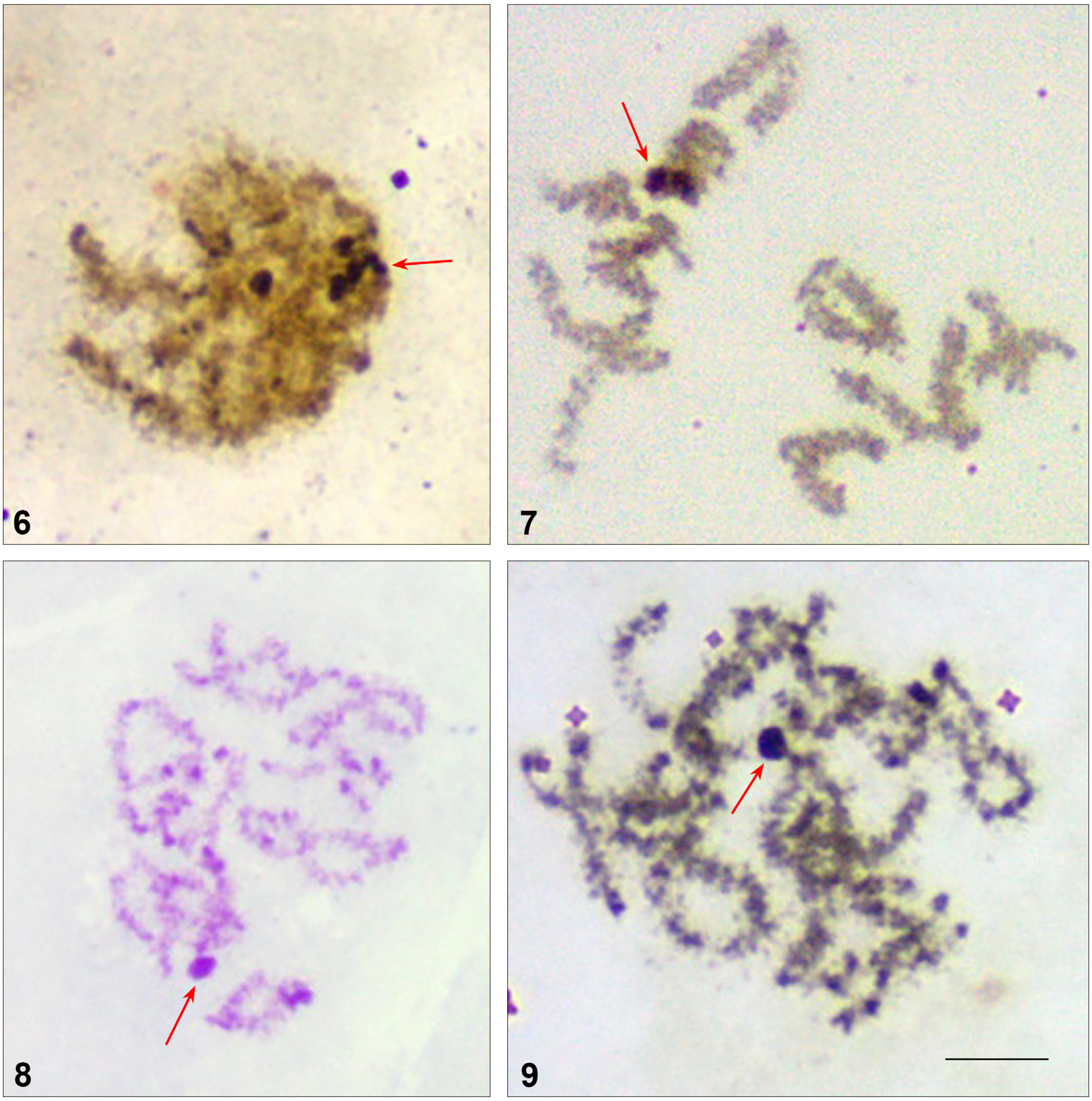
In conventionally stained prophase I nuclei, while most of the chromosomes have relatively small amounts of heterochromatin dispersed throughout the whole length (Fig. 8), a distinctive heterochromatic block was observed for only one chromosome. Silver nitrate staining of the prophase nuclei revealed the existence of a single impregnated mass of nucleolar material (Figs 6, 7, 9). Additionally with silver nitrate (Figs 6, 7, 9) and DAPI staining, small amount of telomeric and interstitial signals (Fig. 10) were observed on the large arms of most chromosomes and on the Xy_p bivalent as well.

Discussion

Tenebrionid karyotypes appear relatively conserved due to the predominant occurrence of the diploid number $2n = 20$ and parachute configuration of sex bivalents in the studied species [Juan, Petitpierre, 1991a; Palmer, Petitpierre, 1997; Pons, 2004]. On the other hand, several karyological variations in diploid number, sex determining systems, chromosome morphology and distribution of heterochromatin were also reported for tenebrionid beetles [Juan, Petitpierre, 1990, 1991a, b; Petitpierre et al., 1991; Juan et al., 1993; Bruvo-Mađarić et al., 2007]. The extent of karyological variations within the family suggests that genomic rearrangements such as inversions, Robertsonian processes or polyploidy are involved in Tenebrionid karyotype divergence [Juan et al., 1990; Juan, Petitpierre, 1991a; Petitpierre et al., 1991; DeAlmeida et al., 2000; Pons, 2004; Holecová et al., 2008; Lira-Neto et al., 2012; Goll et al., 2013].



Figs 4–5. Meiotic plates of *Helops glabriventris*.
4 – diplotene/diakinesis; 5 – metaphase I chromosomes. Arrows indicate Xy_p sex bivalent, circles, asterisks and triangle indicate cross-shaped, rod-shaped and ring-shaped bivalents respectively. Scale bars 5 µm.
Рис. 4–5. Мейотические пластинки *Helops glabriventris*.
4 – диплотена/диакинез; 5 – хромосомы метафазы I. Стрелки указывают на половые биваленты Xy_p, круги, звездочки и треугольник – на крестообразные, палочковидные и кольцеобразные биваленты соответственно. Масштабные линейки 5 µm.



Figs 6–9. Heterochromatin distribution in *Helops glabriventris*.

6, 7, 9 – heterochromatin distribution in prophase I nuclei, AgNOR staining; 8 – the same, Romanowsky–Giemsa staining. Arrows indicate distinctive heterochromatic blocks. Scale bar 5 µm.

Рис. 6–9. Распределение гетерохроматина у *Helops glabriventris*.

6, 7, 9 – распределение гетерохроматина в ядрах профазы I, окрашивание нитратом серебра; 8 – то же, окрашивание по Романовскому – Гимзе. Стрелки указывают на характерные гетерохроматические блоки. Масштабная линейка 5 µm.

Our cytogenetic analysis showed that the karyotype of *Helops glabriventris*, consisting of ten pairs of chromosomes (2*n* = 20, 9 + Xyp), generally resembles that of other tenebrionids. The resemblance in chromosome number is also persistent in the parachute configuration of the sex bivalents. This formula (*n* = 10, Xy_p) was reported for some Helopini genera such as *Nesotes* Allard, 1876 [Juan, Petitpierre, 1986, 1989, 1991a], *Nalassus* Mulsant, 1854 and *Turkonalassus* Keskin, Nabozhenko et Alpagut Keskin, 2017 [Şendoğan, Alpagut-Keskin, 2016], *Accanthopus* Dejean, 1821 [Şendoğan et al., 2019].

Despite this general resemblance, *Helops glabriventris* karyotype consisting of four metacentric, four submetacentric and one subtelocentric autosomal pairs differs from other tenebrionid karyotypes that reported to have mostly metacentric chromosomes [Guenin, 1951a, b, c; Smith, 1952; Juan, Petitpierre, 1988, 1989, 1990; Juan et al., 1989]. These types of differences in chromosome morphologies are also noted for several species from different subfamilies of Tenebrionidae (e.g. *Laena reitteri* Weise, 1877, 2*n* = 18 [Holecová et al., 2008], *Palembus dermestoides* Chevrolat, 1878, 2*n* = 20 [DeAlmeida et al., 2000], *Accanthopus velikensis* (Piller et Mitterpacher, 1783), 2*n* = 20 [Şendoğan et al., 2019]). In addition, the relatively small metacentric X chromosome of *Helops glabriventris*

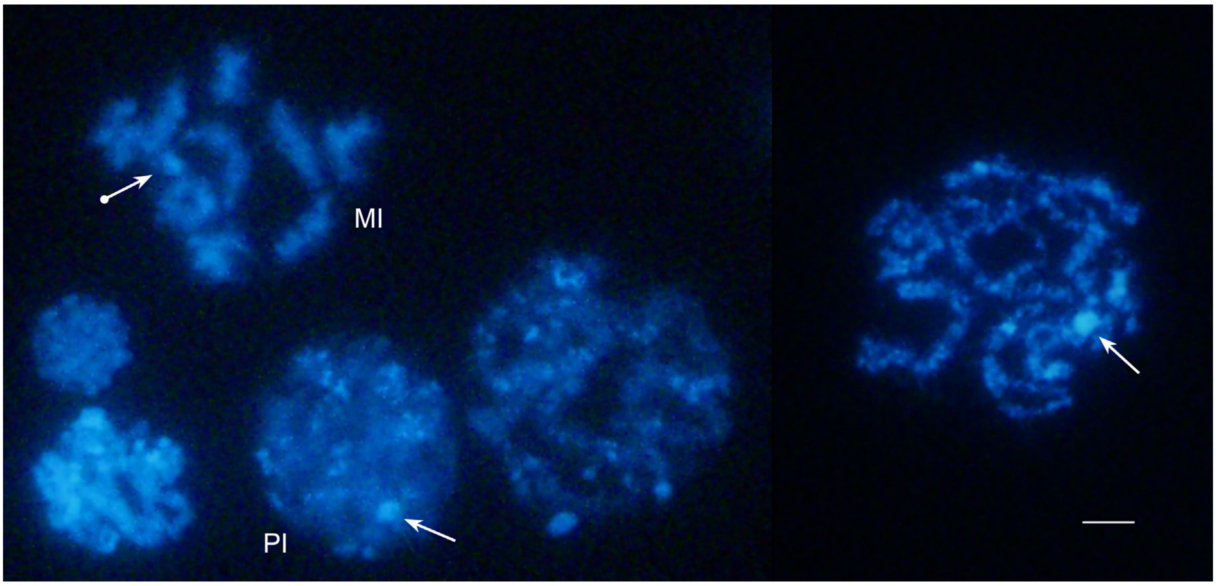


Fig. 10. DAPI staining of metaphase I (MI) and prophase I (PI) nuclei of *Helops glabriventris*. Arrow with circle shows Xy_p sex bivalents and simple arrows indicate AT rich heterochromatic regions. Scale bar 5 µm.

Рис. 10. DAPI-окрашивание ядер метафазы I (MI) и профазы I (PI) *Helips glabriventris*. Стрелка с кружком показывает половые биваленты Xy_p, простые стрелки указывают на богатые AT гетерохроматиновые области. Масштабная линейка 5 µm.

(5.88% RL) is clearly different compared to other Helopini species. Previous studies have shown that the relative length of X chromosome tend to be around 5–6% in Coleoptera [Dutrillaux, Dutrillaux, 2009], but in the tribe Helopini larger submetacentric X (6.55–13.74% RL) was also recorded [Şendoğan, Alpagut-Keskin, 2016; Şendoğan et al., 2019].

Previous studies have revealed that beetle chromosomes can show great variability in both heterochromatin and NOR distribution [Juan, Petitpierre, 1989, 1990; Pons, 2004; Rożek et al., 2004; Cabral-de-Mello et al., 2010; Schneider et al., 2007]. In the majority of studied tenebrionid species, heterochromatic blocks are mainly observed in pericentromeric regions of chromosomes, but interstitial and telomeric blocks were also reported [Juan, Petitpierre, 1989; Juan et al., 1990; DeAlmeida et al., 2000; Moura et al., 2003; Pons, 2004; Goll et al., 2013]. The presence of small amount of interstitial and telomeric signals on *H. glabriventris* chromosomes was demonstrated with both conventionally and differentially stained prophase I nuclei (Figs 6–10). As a result of silver staining, the existence of a highly impregnated area associated with a small submetacentric chromosome in prophase I suggests autosomal location of NOR (Fig. 7). Although, cytogenetic data concerning the location of NORs are only available for a small number of tenebrionid species, both autosomal and sex chromosomal location of nucleolar material was demonstrated within Coleopteran families [Juan et al., 1993; Vitturi et al., 1999; Colomba et al., 2000; Bione et al., 2005; Pons, 2004; Rożek et al., 2004; Schneider et al., 2007; Holecová et al., 2008; Karagyan et al., 2012; Goll et al., 2013; Çalışan, 2018; Şendoğan, Alpagut-Keskin, 2016; Şendoğan et al., 2019]. A similar distinctive heterochromatic block in Romanowsky–Giemsa (Fig. 8), AgNOR (Fig. 9) and DAPI (Fig. 10) stained plates indicate that this single NOR site is associated with AT rich heterochromatin.

In this work, diploid number, chromosome morphology and sex determination system are revealed for the first time for the genus *Helops*. For further studies, comparative molecular cytogenetic and phylogenetic analysis will enhance our understanding in both *Helops* and tenebrionid karyotype evolution.

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References

Bione E., Moura R.C., Carvahlo R., Souza M.J. 2005. Karyotype, C- and fluorescence banding pattern, NOR location and FISH study of five Scarabaeidae (Coleoptera) species. *Genetics and Molecular Biology.* 28(3): 376–381. DOI: 10.1590/S1415-47572005000300006

Blackmon H., Demuth J.P. 2015. Coleoptera Karyotype Database. *The Coleopterists Bulletin.* 69(1): 174–175. DOI: 10.1649/0010-065X-69.1.174

Bousquet Y., Thomas D.B., Bouchard P., Smith A.D., Aalbu R.L., Johnston M.A., Steiner W.E. Jr. 2018. Catalogue of Tenebrionidae (Coleoptera) of North America. *ZooKeys*. 728: 1–455. DOI: 10.3897/zookeys.728.20602

Bruvo-Mađarić B., Plohl M., Ugarković D. 2007. Wide distribution of related satellite DNA families within the genus *Pimelia* (Tenebrionidae). *Genetica*. 130(1): 35–42. DOI: 10.1007/ s10709-006-0017-2

Cabral-de-Mello D.C., Moura R.C., Carvalho R., Souza M.J. 2010. Cytogenetic analysis of two related *Deltochilum* (Coleoptera, Scarabaeidae) species: diploid number reduction, extensive heterochromatin addition and differentiation. *Micron*. 41(2): 112–117. DOI: 10.1016/j.micron.2009.10.005

Çalışan U. 2018. *Turkonalassus quercanus* Keskin, Nabozhenko & Alpagut-Keskin, 2017 (Coleoptera: Tenebrionidae: Helopini) Türünün Sitogenetik Analizi. MSc Thesis. İzmir: Ege Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı. 36 p.

Chandley A.C., Speed R.M., Ma K. 1994. Meiotic Chromosome Preparation. Chromosome Analysis Protocols. *In:* Methods in Molecular Biology. Vol. 29. Totowa, NJ: Humana Press: 27–40. DOI: 10.1385/0-89603-289-2:27

Colomba M.S., Vitturi R., Zunino M. 2000. Chromosome analysis and rDNA FISH in the stag beetle *Dorcus parallelipipedus* L. (Coleoptera: Scarabaeoidea: Lucanidae). *Hereditas.* 133: 249–253.

DeAlmeida M.C., Zacaro A.A., Cella D. 2000. Cytogenetic analysis of *Epicauta atomaria* (Meloidae) and *Palembus dermestoides* (Tenebrionidae) with Xy_p sex determination system using standard staining, C-bands, NOR and synaptonemal complex microspreading techniques. *Hereditas*. 133: 147–157. DOI: 10.1163/22119434-900000220

Dutrillaux A.M., Dutrillaux B. 2009. Sex chromosome rearrangements in Polyphaga beetles. *Sexual Development*. 3(1): 43–54. DOI: 10.1159/000200081

Goll L.G., Artoni R.F., Vicari M.R., Nogaroto V., Petitpierre E., DeAlmeida M.C. 2013. Cytogenetic analysis of *Lagria villosa* (Coleoptera, Tenebrionidae): Emphasis on the mechanism of association of the Xy(p) Sex Chromosomes. *Cytogenetic and Genome Research*. 139(1): 29–35. DOI: 10.1159/000341674

Gregory T.R. 2022. Animal Genome Size Database. Available at: http://www.genomesize.com.

Guenin H.A. 1951a. Chromosomes et Hétérochromosomes de Ténébrionidés. *Genetica*. 25: 157–182. DOI: 10.1007/BF01784829

Guenin H.A. 1951b. La formule chromosomiale de Coleopteres tenebrionides nordafricains, I. Pimeliines et Tentyriines. *Bulletin de la Société Vaudoise des Sciences Naturelles*. 65(278): 7–18.

Guenin H.A. 1951c. La formule chromosomiale de Coleopteres tenebrionides nordafricains, II. Erodiines. *Revue Suisse de Zoologie*. 58(23): 471–475.

Holecová M., Rozek M., Lachowska D. 2008. The first cytogenetic report on *Laena reitteri* Weise, 1877 with notes on karyotypes of darkling beetles. *Folia Biologica (Kraków)*. 56(3–4): 213–217. DOI: 10.3409/fb.56_3-4.213-217

Juan C., Gosalvez J., Petitpierre E. 1990. Improving beetle karyotype analysis: Restriction endonuclease banding of *Tenebrio molitor* chromosomes. *Heredity*. 65: 157–162.

Juan C., Petitpierre E. 1986. Karyological analyses on tenebrionid beetles from Balearic Islands. *Génética Ibérica*. 38(2): 231–244.

Juan C., Petitpierre E. 1988. A chromosome survey of North African and Eastern Mediterranean tenebrionids (Coleoptera). *Cytobios*. 54: 85–94.

Juan C., Petitpierre E. 1989. C-banding and DNA content in seven species of Tenebrionidae (Coleoptera). *Genome*. 32(5): 834–839. DOI: 10.1139/g89-519

Juan C., Petitpierre E. 1990. Karyological differences among Tenebrionidae (Coleoptera). *Genetica*. 80(2): 101–108. DOI: 10.1007/BF00127130

Juan C., Petitpierre E. 1991a. Chromosome numbers and sex determining systems in Tenebrionidae. *In:* Advances in Coleopterology. Barcelona: AEC Press.: 167–176.

Juan C., Petitpierre E. 1991b. Evolution of genome size in darkling beetles. *Genome*. 34(1): 169–173. DOI: 10.1139/g91-026

Juan C., Petitpierre E., Oromi P. 1989. Chromosomal analyses on tenebrionids from Canary Islands. *Cytobios*. 57(1): 33–41.

Juan C., Pons J., Petitpierre E. 1993. Localization of tandemly repeated DNA sequences in beetle chromosomes by fluorescent in situ hybridization. *Chromosome Research*. 1(3): 167–174. DOI: 10.1007/BF00710770

Karagyan G., Lachowska D., Kalashian M. 2012. Karyotype analysis of four jewel-beetle species (Coleoptera, Buprestidae) detected by standard staining, C-banding, AgNOR-banding and CMA3/DAPI staining. *Comparative Cytogenetics.* 6(2): 183–197. DOI: 10.3897/CompCytogen.v6i2.2950

Lira-Neto A.C., Silva G.M., Moura R.C., Souza M.J. 2012. Cytogenetics of the darkling beetles *Zophobas* aff. *confusus* and *Nyctobates gigas* (Coleoptera, Tenebrionidae). *Genetics and Molecular Research*. 11(3): 2432–2440. DOI: 10.4238/2012

Moura R.C., Souza M.J., Melo N.F., Lira-Neto A.C. 2003. Karyotypic characterization of representatives from Melolonthinae (Coleoptera: Scarabaeidae): Karyotypic analysis, banding and fluorescent in situ hybridization (FISH). *Hereditas*. 138: 200–206.

Murakami A., Imai H. 1974. Cytological evidence for holocentric chromosomes of the silkworms, *Bombyx mori* and *B. mandarina*, (Bombycidae, Lepidoptera). *Chromosoma*. 47(2): 167–178. DOI: 10.1007/BF00331804

Nabozhenko M.V. 2020. Tribe Helopini Latreille, 1802. *In:* Catalogue of Palearctic Coleoptera, Volume 5. Tenebrionoidea. Leiden: Brill: 314–339.

Nabozhenko M.V., Keskin B. 2017. Taxonomic review of the genus *Helops* Fabricius, 1775 (Coleoptera: Tenebrionidae) of Turkey. *Caucasian Entomological Bulletin*. 13(1): 41–49. DOI: 10.23885/1814-3326-2017-13-1-41-49

Nabozhenko M.V., Nikitsky N.B., Aalbu R. 2016. Contributions to the knowledge of North American tenebrionids of the subtribe Cylindrinotina (Coleoptera: Tenebrionidae: Helopini). *Zootaxa*. 4136(1): 155–164. DOI: 10.11646/zootaxa.4136.1.7

Nabozhenko M.V., Ntatsopoulos K., Gagarina L.V., Chigray I.A., Lagou L.J., Papadopoulou A. 2021. *Helops glabriventris glabriventris* (Coleoptera: Tenebrionidae), one of the primary consumers of corticolous lichens in the coniferous forests of Cyprus: bionomics, trophic associations and description of larvae. *Annales Zoologici*. 71(4): 767–778. DOI: 10.3161/00034541ANZ2021.71.4.004

Nabozhenko M.V., Steiner W.E. Jr. 2021. *Doyenellus* Nabozhenko and Steiner, a new genus of darkling beetles of the tribe Helopini (Coleoptera: Tenebrionidae) from North America. *Proceedings of the Entomological Society of Washington*. 123(3): 564–588. DOI: 10.4289/0013-8797.123.3.564

Palmer M., Petitpierre E. 1997. New chromosomal findings on Tenebrionidae from Western Mediterranean. *Caryologia*. 50(2): 117–123. DOI: 10.1080/00087114.1997.10797391

Patkin E.L., Sorokin A.V. 1983. Nucleolus-organizing regions chromosomes in early embryogenesis of laboratory mice. *Bulletin of Experimental Biology and Medicine*. 96(2): 1142–1144. DOI: 10.1007/BF00839848

Petitpierre E., Juan C., Alvarez-Fuster A. 1991. Evolution of chromosomes and genome size in Chrysomelidae and Tenebrionidae. *In:* Advances in Coleopterology. Barcelona: AEC Press: 129–144.

Pons J. 2004. Evolution of diploid chromosome number, sex-determining systems and heterochromatin in Western Mediterranean and Canarian species of the genus *Pimelia* (Coleoptera: Tenebrionidae). *Journal of Zoological Systematics and Evolutionary Research*. 42(1): 81–85. DOI: 10.1046/j.1439-0469.2003.00247.x

Rożek M., Lachowska D., Petitpierre E., Holecová M. 2004. C-bands on chromosomes of 32 beetle species (Coleoptera: Elateridae, Cantharidae, Oedemeridae, Ceramcycidae, Anthicidae, Chrysomelidae, Attelabidae and Curculionidae). *Hereditas*. 140: 161–170. DOI: 10.1111/j.1601-5223.2004.01810.x

Sakamoto Y., Zacaro A.A. 2009. LEVAN, an ImageJ plugin for morphological cytogenetic analysis of mitotic and meiotic chromosomes. Initial version. An open-source Java plugin distributed over the Internet from http://rsbweb.nih.gov/ij/.

Schneider C.A., Rasband W.S., Eliceiri K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9(7): 671–675. DOI: 10.1038/nmeth.2089

Schneider M.C., Rosa S.P., DeAlmeida M.C., Costa C., Cella D.M. 2007. Chromosomal similarities and differences among four Neotropical Elateridae (Conoderini and Pyrophorini) and other related species, with comments on the NOR patterns in Coleoptera. *Journal of Zoological Systematics and Evolutionary Research*. 45(4): 308–316. DOI: 10.1111/j.1439-0469.2006.00398.x

Şendoğan D., Alpagut-Keskin N. 2016. Karyotype and sex chromosome differentiation in two *Nalassus* species (Coleoptera, Tenebrionidae). *Comparative Cytogenetics*. 10(3): 371–385. DOI: 10.3897/CompCytogen.v10i3.9504

Şendoğan D., Gündoğan B., Nabozhenko M., Keskin B., Alpagut-Keskin N. 2019. Cytogenetics of *Accanthopus velikensis* (Piller et Mitterpacher, 1783) (Tenebrionidae: Helopini). *Caryologia*. 72(3): 97–103. DOI: 10.13128/caryologia-771

Smith S.G. 1952. The cytology of some tenebrionid beetles (Coleoptera). *Journal of Morphology*. 91(2): 325–364. DOI: 10.1002/jmor.1050910206

Vitturi R., Colomba M.S., Barbieri R., Zunino M. 1999. Ribosomal DNA location in the scarab beetle *Thorectes intermedius* (Costa) (Coleoptera: Geotrupidae) using banding and fluorescent in-situ hybridization. *Chromosome Research*. 7(1): 255–260. DOI: 10.1023/A:1009270613012

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