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New Contribution to the Knowledge on the Chromosome Numbers of Turkish Cerambycidae (Coleoptera)

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Information on the karyotypes of Turkish species of Cerambycidae is scanty. Our study contributes to the knowledge of the karyological data (chromosomal number and mechanism of sex determination) of five Turkish longicorn beetles; karyotypes of four taxa, one endemic, are described for the first time and for the remaining one, *Purpuricenus budensis* (Götz, 1783), the previously published chromosome count is confirmed. The chromosome number of *Purpuricenus desfontainii inhumeralis* Pic, 1891 and *Purpuricenus budensis* (Götz, 1783) (Cerambycinae, Trachyderini) was found to be 2n = 28 (13 + Xy_p); *Clytus rhamni* Germar, 1817 and *Plagionotus floralis* (Pallas, 1773) (Cerambycinae, Clytini) 2n = 20 (9 + Xy_p); and the endemic *Dorcadion triste phrygicum* Peks, 1993 (Lamiinae, Dorcadionini) 2n = 24 (11 + Xy_p). In view of the paucity of data available until now, our study is important for both to improve the poor karyological knowledge of Turkish Cerambycidae and to provide an incentive for other researchers.

Key words: Cerambycidae, *Purpuricenus*, *Clytus*, *Plagionotus*, *Dorcadion*, chromosome.

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For many years Cerambycidae, alias longicorn beetles, have been a group of great interest for cytogenetical studies. They deserve attention since many questions on the systematics and phylogeny of cerambycids are still controversial (SOUZA *et al*. 2020). Answers to some of these questions have been outlined by karyological studies (CESARI *et al*. 2005; DUTRILLAUX & DUTRILLAUX 2014, 2018, 2019; GIANNOULIS *et al*. 2014, 2020). There are many papers that contributed to the knowledge of chromosome number in the family Cerambycidae: EHARA 1956; TEPPNER 1966; TEPPNER 1968; LANIER & RASKE 1970; KUDOH *et al*. 1972; BARAGAÑO GALÁN *et al*. 1981; KIDO & SAITOH 1987; FERREIRA *et al*. 1993; HOLECOVÁ *et al*. 2002; ROŻEK *et al*. 2004; CESARI *et al*. 2005; DUTRILLAUX *et al*. 2007; PING *et al*. 2010; DASCĂLU & FUSU 2012; LI-JUAN *et al*. 2013; TOKHATYAN & KARAGYAN 2013; WEI *et al*. 2013; DUTRILLAUX & DUTRILLAUX 2014, 2018, 2019; GIANNOULIS *et al*. 2014; KARAGYAN & KALASHIAN 2016; YADAV *et al*. 2019. In consequence, the diploid chromosome number of Cerambycidae cover the range between 2n = 10 (NATH *et al*. 1951) and 2n = 36 (SMITH & VIRKKI 1978), while male heterogamety occurs with varying types of sex determining mechanisms such as X0, Xy, XY, Xy_p, and multiple sex chromosomes (KARAGYAN & KALASHIAN 2016). In spite of the great diversity in number of chromosomes, the karyotype 2n = 20 (9 + Xy_p) overwhelmingly predominates in the family, compared to other karyotypes. Interestingly, of all longicorn beetles karyotyped, *Vesperus xatarti* Mulsant 1839 possesses an unusual karyotype as suggested by DUTRILLAUX *et al*. (2007) with a diploid number of 2n = 53♂ - 54♀. This unique chromosome number exclusive to *V. xatarti* requires a revision of its systematic position (DUTRILLAUX *et al*. 2007). That is to say that studies of the karyotype of longicorn beetles have the potential to reveal surprising

data and enhance their importance in taxonomy and systematics.

Turkey is a peninsula and despite its richness in longicorn beetle fauna (OZDIKMEN 2007, 2008a, 2008b), contributions towards cerambycid cytogenetics are scant. This is significant when we realize that the family is broadly distributed in Turkey, often has a high population density, and includes at least 650 described species/subspecies (SABANOGLU & SEN 2016). Pioneering work in this area was initiated by OKUTANER *et al*. (2011a, 2011b, 2011c, 2011d) with the publication of chromosome numbers for certain species, namely *Dorcadion* (*Cribridorcadion*) *anatolicum* Pic, 1900 with n = 11 + Xy_p, *Dorcadion* (*Cribridorcadion*) *scabricolle* (Dalman, 1817) with 2n = 20, *Morimus orientalis* Reitter, 1894 with n = 11 + Xy_p, *Certallum ebulinum* (Linnaeus, 1767) with 2n = 22, and *Vadonia unipunctata* (F. 1787) with 2n = 20. Subsequently, OKUTANER *et al*. (2012) informed that the number of chromosomes of *Pachytodes erraticus* (Dalman 1817) is 2n = 18 and according to OKUTANER and KOCAK (2018), *Ropalopus clavipes* (Fabricius, 1775) has a haploid number of 10 and males have the Xy_p chromosome. Finally, in a more recent study (ASLANTAS & OKUTANER 2019) the chromosome number of *Cortodera flavimana* (Waltl, 1838) was found to be 2n = 20 and that of *Chlorophorus varius* (Müller, 1766) to be n = 9 + Xy_p. Chromosome counts are informative when dealing with taxonomic and phylogenetic issues and would be of great help in taxonomic interpretation and accurate systematic classification (BLACKMAN 1980; SERRANO 1986; ANGUS 1988; GALIÁN *et al*. 1990; PETITPIERRE 1990, 1997, 2011; SERRANO *et al*. 1994; SANTIAGO-BLAY & VIRKKI 1996; GOKHMAN 1997; KANDUL 1997; GALIÁN *et al*. 2002; FUSU 2008; LORITE & PALOMEQUE 2010; ANGUS & TATTON 2011; GAVRILOV-ZIMIN 2011; GEBIOLA *et al*. 2012; DUTRILLAUX *et al*. 2013; GOKHMAN 2015; CORREIA *et al*. 2016). The techniques for karyological research on insects, developed over a long time span, have led to a much more accurate knowledge of cerambycid karyotypes (ROŻEK 1994; DUTRILLAUX *et al*. 2006; GOKHMAN & KUZNETSOVA 2006; KOCAK & OKUTANER 2017).

In the present paper we report the results of chromosomal observations on 5 taxa belonging to three tribes (Trachyderini, Clytini and Dorcadionini) of two subfamilies (Cerambycinae and Lamiinae) to further extend our karyotypic knowledge of Turkish Cerambycidae. New data are presented for four taxa: *Purpuricenus desfontainii inhumeralis*, *Clytus rhamni*, *Plagionotus floralis,* and *Dorcadion triste phrygicum,* while for *Purpuricenus budensis* we confirm the data previously obtained by OKUTANER (2011).

The genus *Purpuricenus* is distributed in all zoogeographical regions except the Neotropical one. To date, about 60 species have been identified in three regions, viz. Palaearctic, Nearctic, and Indomalaya regions, in which the genus is mostly distributed (MACRAE 2000; GHATE *et al*. 2006); 46 of those are from the Palaearctic region and 12 are from Turkey (OZDIKMEN & TEZCAN 2020). Many taxonomic changes have been made in the genus *Purpuricenus*, especially in the Palaearctic region, during the last 50-60 years (KADLEC 2006).

The genus *Clytus* is represented in the world by about 50 species. In the Palaearctic region by 22 species and in Turkey by 12 species, 4 of them being endemic. The genus *Clytus* is a group that probably needs to be separated into new genera and subgenera (OZDIKMEN 2012; OZDIKMEN & TURGUT 2009a). For instance, SAMA (2005) described *Sphegoclytus* as a separate Clytini genus by excluding it from *Clytus*.

The genus *Plagionotus* is represented in the world by some 12 species, in the Palaearctic region by 11 species, and in Turkey by 5 species (OZDIKMEN 2012; OZDIKMEN & TURGUT 2009b; OZDIKMEN 2007). *Clytus* and *Plagionotus* seem to be closely related and subjected to taxonomic revisions. For example, *Clytus latreillei* was described by Laporte and Gory (1836) but later transferred to the genus *Plagionotus* by Aurivillius (1912).

Dorcadion triste phrygicum Peks, 1993 is included in the subgenus *Maculatodorcadion* Breuning, 1943. The genus *Dorcadion* is represented in the world by about 382 species and in Turkey by 192 species, 151 of them are endemic while the subgenus *Maculatodorcadion* is represented both in the world and in Turkey by 4 species, 3 of them endemic (OZDIKMEN & KOCAK 2015). Note that it is not easy to differentiate and identify *Dorcadion* species (ONALP 1991).

Material and Methods

Adult cerambycid males from five taxa of cerambycid beetles were collected from the environs of the Antalya, Eskişehir, and Ankara provinces between March and September between 2015 and 2016. Karyotype determinations were made from acetic acid squashes of testes tissues taken directly from live individuals anaesthetized with ethyl acetate prior to abdomen dissection. The number of specimens were as follows: *Purpuricenus budensis* – 6, *Purpuricenus desfontainii inhumeralis* – 5, *Clytus rhamni* – 8, *Plagionotus floralis* – 11, and *Dorcadion triste phrygicum* – 2. The procedure used in making the chromosome preparation was the squash method developed by ROŻEK (1994) with slight modifications (LACHOWSKA *et al.* 1996; HOLECOVÁ *et al.* 1999; ROŻEK & HOLECOVÁ 2000). Observation of chromosomes was done at 100x magnification, using a Leica DMLB 2 photomicroscope coupled with a Leica DFC320 camera and photographs were taken of the best well-spread metaphase cells.

Results and Discussion

The chromosomal formulae of the five studied taxa and those of other species previously analyzed by different authors and belonging to the same tribes as ours are given in Table 1. The sexual pair in males has a configuration in the shape of a parachute (Xy_p) for all investigated taxa. Additionally, spermatogonial metaphases are illustrated in Fig. 1. Excepting *Purpuricenus budensis*, for *Purpuricenus desfontainii inhumeralis*, *Clytus rhamni*, *Plagionotus floralis*, and *Dorcadion triste phrygicum* this is the first report on chromosome number.

To our knowledge, the chromosome number for *Purpuricenus desfontainii inhumeralis* is reported here for the first time. For *Purpuricenus budensis* this is the second determination of chromosome number. Our figures show *Purpuricenus desfontainii inhumeralis* and *Purpuricenus budensis* males as 2n=28 with a sex determining mechanism of Xy_p. Of the three *Purpuricenus* species previously reported, *Purpuricenus indus* has the same number of autosomal pairs with an identical sex determining mechanism (SMITH & VIRKKI 1978). The haploid chromosome number (n) in *Purpuricenus spectabilis* was found to be 14, in which sex chromosomes have not been identified (EHARA 1956). OKUTANER (2011) counted 2n = 22 in *Purpuricenus budensis* from Turkey and this result is not in agreement with our record. This may be due to a counting error.

In the Clytini, two genera and approximately five species have been karyotyped (EHARA 1956; TEPPNER 1966; SMITH & VIRKKI 1978). *Clytus arietis* (Linnaeus, 1758), *Clytus lama* Mulsant, 1847, *Clytus melaenus* Bates, 1884, and *Plagionotus arcuatus* (Linnaeus, 1758) all have 9 autosomal pairs and a Xy_p sex-determining mechanism with the exception of *Plagionotus pulcher* (Blessig, 1872) which was found to have 10 autosomal pairs with an unshown sex-determining mechanism. *Clytus rhamni* and *Plagionotus floralis* counts agree with those reported by previous workers.

In *Dorcadion triste phrygicum*, chromosome number was 2n=24 (11 + Xy_p) with this being the first report on the chromosomes of this subspecies. The four taxa of the genus *Dorcadion* investigated previously for their chromosomes are *Dorcadion anatolicum* Pic, 1900, *Dorcadion axillare moldavicum* Dascălu &

Table 1

Chromosome numbers and sex chromosome systems in studied and related taxa

Taxa	2n ♂	Male formula	Reference
Subfam. Cerambycinae Latreille, 1802			
Tribe Trachyderini Dupont, 1836			
Purpuricenus desfontainii inhumeralis Pic, 1891	28	13 + Xy_p	Present paper
Purpuricenus budensis (Götz, 1783)	28	13 + Xy_p	Present paper
	22	–	OKUTANER 2011
Purpuricenus indus Semenov, 1908	28	13 + Xy_p	SMITH and VIRKKI 1978
Purpuricenus spectabilis Motschulsky, 1857	–	14	EHARA 1956
Tribe Clytini Mulsant, 1839			
Clytus rhamni Germar, 1817	20	9 + Xy_p	Present paper
Clytus arietis (Linnaeus, 1758)	–	9 + Xy_p	TEPPNER 1966
Clytus lama Mulsant, 1847	–	9 + Xy_p	TEPPNER 1966
Clytus melaenus Bates, 1884	–	9 + Xy	SMITH and VIRKKI 1978
Plagionotus floralis (Pallas, 1773)	20	9 + Xy_p	Present paper
Plagionotus arcuatus (Linnaeus, 1758)	–	9 + Xy_p	TEPPNER 1966
Plagionotus pulcher (Blessig, 1872)	–	10	EHARA 1956
Subfam. Lamiinae Latreille, 1825			
Tribe Dorcadionini Swainson and Shuckard, 1840			
Dorcadion triste phrygicum Peks, 1993	24	11 + Xy_p	Present paper
Dorcadion axillare moldavicum Dascalu & Fusu, 2012	24	11 + Xy_p	DASCÂLU and FUSU 2012
Dorcadion anatolicum Pic, 1900	24	11 + Xy_p	OKUTANER *et al.* 2011c
	24	–	ASLANTAS 2018
Dorcadion scabricolle paphlagonicum Breuning, 1962	20	–	OKUTANER *et al.* 2011c
Dorcadion scabricolle (Dalman, 1817)	20	–	ASLANTAS 2018

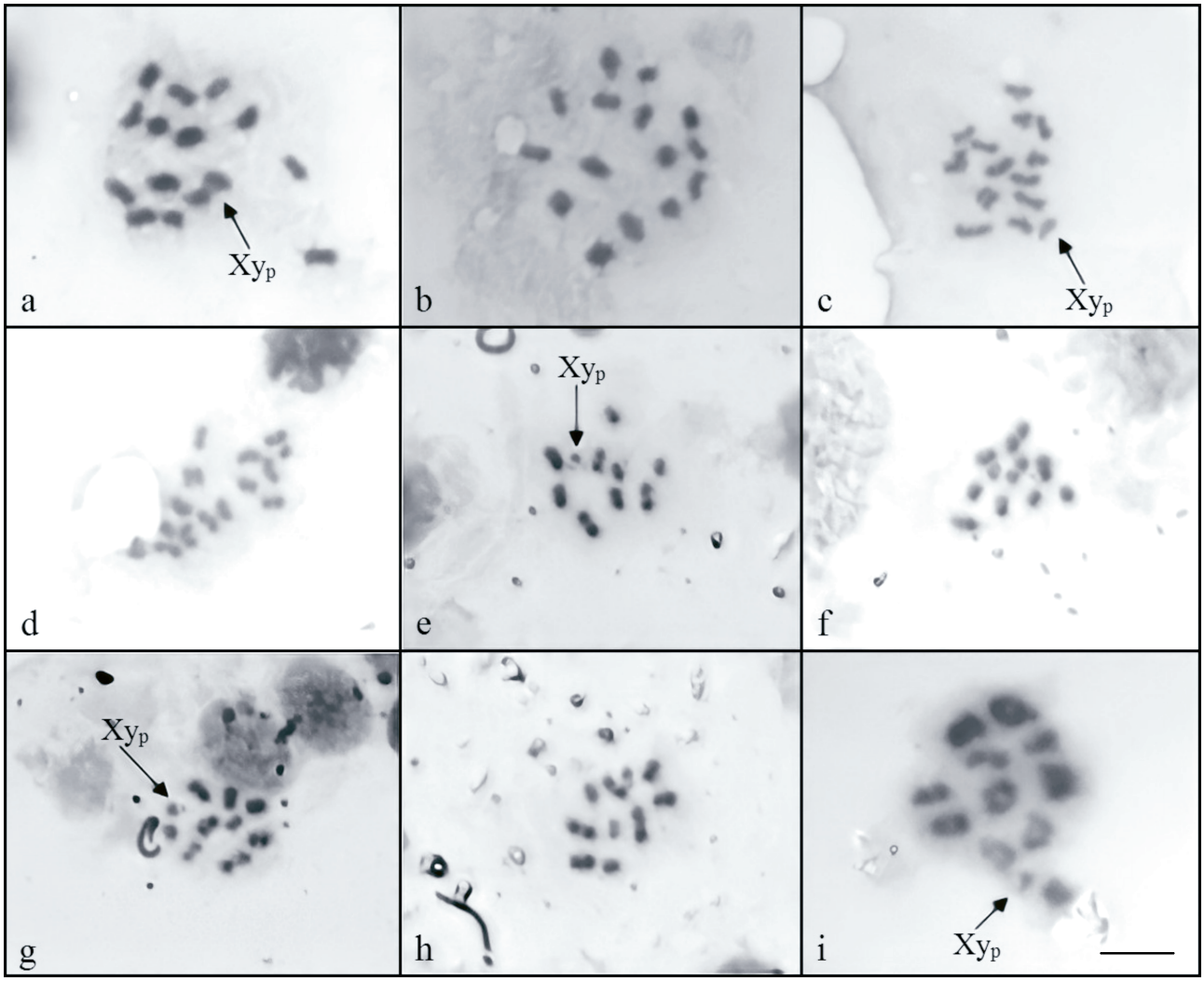

Fig. 1. Chromosome spreads of: a-b. *Purpuricenus desfontainii inhumeralis*, c-d. *Purpuricenus budensis*, e-f. *Clytus rhamni*, g-h. *Plagionotus floralis*, i. *Dorcadion triste phrygicum*. Scale bar = 10 μm.

Fusu, 2012, *Dorcadion scabricolle* (Dalman, 1817), and *Dorcadion scabricolle paphlagonicum* Breuning, 1962. With the exception of *D. scabricolle paphlagonicum* and *D. scabricolle*, with a diploid chromosome number equal to 20 (OKUTANER *et al.* 2011c; ASLANTAS 2018), all the other *Dorcadion* species cytogenetically studied by now have 11 autosomal pairs and a Xy_p sex-determining mechanism; thus our finding for *Dorcadion triste phrygicum* agrees with them (OKUTANER *et al.* 2011c; DASCĂLU & FUSU 2012; ASLANTAS 2018). It is too early to say that there is variation in the diploid chromosome numbers of the species of the genus *Dorcadion* and thus, more chromosomal data may provide useful clues.

Evidently the cerambycids are a large but cytogenetically little-known group of beetles for Turkish fauna. Therefore, more studies are needed to have an exhaustive knowledge of the number and morphology of chromosomes in Turkish species of Cerambycidae.

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Author Contributions

Research concept and design: Y.K.; Collection and/or assembly of data: Y.K., E.Y.; Data analysis and interpretation: Y.K., E.Y.; Writing of article: Y.K.; Critical revision of the article: Y.K.; Final approval of article: Y.K., E.Y.

Conflict of Interest

The authors declare no conflict of interest.

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