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

Yavuz KOÇAK and Elmas YAĞMUR

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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 + Xyp); Clytus rhamni Germar, 1817 and Plagionotus floralis (Pallas, 1773) (Cerambycinae, Clytini) 2n = 20 (9 + Xyp); and the endemic Dorcadion triste phrygicum Pek, 1993 (Lamiinae, Dorcadionini) 2n = 24 (11 + Xyp). 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.

Yavuz KOÇAK, Department of Biology, Faculty of Polatlı Arts and Sciences, Ankara Hacı Bayram Veli University, 06900, Polatlı, Ankara, Turkey.
E-mail: yavuz.kocak@hbv.edu.tr

Elmas YAĞMUR, Department of Biology, Graduate School of Natural and Applied Sciences, Kırşehir Ahi Evran University, 40100, Kırşehir, Turkey.

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: EHLAR 1956; TEPPNER 1966; TEPPNER 1968; LANIER & RASKE 1970; KUDOH et al. 1972; BARAGANO GALÁN et al. 1981; KIDO & SAITO 1987; FERREIRA et al. 1993; HOLECÓVA et al. 2002; ROZEK et al. 2004; CESARI et al. 2005; DUTRILLAUX et al. 2007; PING et al. 2010; DASCÁLU & 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, Xyp and multiple sex chromosomes (KARAGYAN & KALASHIAN 2016). In spite of the great diversity in number of chromosomes, the karyotype 2n = 20 (9 + Xyp) 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
Turkey is a peninsula and despite its richness in longicorn beetle fauna (ÖZDIKMEN 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 \( n = 11 + X_y p, \) **Dorcadion (Cribridorcadion) scabraeole** (Dalman, 1817) with \( 2n = 20, \)** **Morimus orientalis** Reitter, 1894 with \( n = 11 + X_y p, \)** **Ceratium ebulum** (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 O˘EK (1990, 1997, 2011; S. DUTRILLAUX et al. 2011a, 2011b, 2011c, 2011d) with the publication of chromosome numbers for certain species, namely **Dorcadion (Cribridorcadion) anatolicum** Pic, 1900 with \( n = 11 + X_y p, \) **Dorcadion (Cribridorcadion) scabraeole** (Dalman, 1817) with \( 2n = 20, \)** **Morimus orientalis** Reitter, 1894 with \( n = 11 + X_y p, \)** **Ceratium ebulum** (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 \( X_y p \) chromosome. Finally, in a more recent study (ASLANTAS & OKUTANER 2019) the chromosome number of **Cortodera flavimana** (Wallt, 1838) was found to be \( 2n = 20 \) and that of **Chlorophorus varius** (Müller, 1766) to be \( n = 9 + X_y p. \) 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, 2002; FUSU 2008; LÓRTE & PALOMEQUE 2010; ANGUS & TATTON 2011; GAVRILOV-ZIMIN 2011; GEBJOLA 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 Dorcadioni) 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 rhanni**, **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. Palaeartctic, Nearctic, and Indomalaya regions, in which the genus is mostly distributed (MACRAE 2000; GHAŁ & ET AL. 2006); 46 of those are from the Palaeartctic region and 12 are from Turkey (ÖZDIKMEN & TEZCAN 2020). Many taxonomic changes have been made in the genus **Purpuricenus**, especially in the Palaeartctic region, during the last 50-60 years (KADLEC 2006).

The genus **Clytus** is represented in the world by about 50 species. In the Palaeartctic 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 (ÖZDIKMEN 2012; ÖZDIKMEN & 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 Palaeartctic region by 11 species, and in Turkey by 5 species (ÖZDIKMEN 2012; ÖZDIKMEN & TURGUT 2009b; ÖZDIKMEN 2007). **Clytus** and **Plagionotus** seem to be closely related and subjected to taxonomic revisions. For example, **Clytus latreilii** was described by Laporte and Gory (1836) but later transferred to the genus **Plagionotus** by Aurivillus (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 (ÖZDIKMEN & KOCAK 2015). Note that it is not easy to differentiate and identify **Dorcadion** species (ÖNALP 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 tissue samples 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 rhanni** – 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; HOŁECOVÁ et al. 1999; ROŻEK & HOŁECOVÁ 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 (X<sub>y</sub>) 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 X<sub>y</sub>. 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 X<sub>y</sub> 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 + X<sub>y</sub>) 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 moldavicu* 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 + X<sub>y</sub>   | Present paper                        |
| *Purpuricenus budensis* (Götz, 1783)                         | 28   | 13 + X<sub>y</sub>   | Present paper                        |
| *Purpuricenus indus* Semenov, 1908                           | 22   | –                    | OKUTANER 2011                        |
| *Purpuricenus spectabilis* Motschulsky, 1857                  | 28   | 13 + X<sub>y</sub>   | SMITH and VIRKKI 1978               |
| **Tribe Clytini Mulsant, 1839**                              |      |                      |                                      |
| *Clytus rhamni* Germar, 1817                                 | 20   | 9 + X<sub>y</sub>    | Present paper                        |
| *Clytus arietis* (Linnaeus, 1758)                            | –    | 9 + X<sub>y</sub>    | TEPPNER 1966                        |
| *Clytus lama* Mulsant, 1847                                  | –    | 9 + X<sub>y</sub>    | TEPPNER 1966                        |
| *Clytus melaenus* Bates, 1884                               | –    | 9 + X<sub>y</sub>    | SMITH and VIRKKI 1978               |
| *Plagionotus arcuatus* (Linnaeus, 1758)                      | 20   | 9 + X<sub>y</sub>    | Present paper                        |
| *Plagionotus pulcher* (Blessig, 1872)                        | –    | 10                   | EHARA 1956                          |
| **Subfam. Lamiinae Latreille, 1825**                         |      |                      |                                      |
| **Tribe Dorcadionini Swainson and Shuckard, 1840**           |      |                      |                                      |
| *Dorcadion triste phrygicum* Peks, 1993                      | 24   | 11 + X<sub>y</sub>  | Present paper                        |
| *Dorcadion axillare moldavicu* Dascălu & Fusu, 2012          | 24   | 11 + X<sub>y</sub>  | DASCĂLU and FUSU 2012              |
| *Dorcadion anatolicum* Pic, 1900                             | 24   | 11 + X<sub>y</sub>  | OKUTANER et al. 2011               |
|                                                                 | 24   | –                    | ASLANTAS 2018                      |
| *Dorcadion scabricolle paphlagonicu* Breuning, 1962           | 20   | –                    | OKUTANER et al. 2011               |
| *Dorcadion scabricolle* (Dalman, 1817)                       | 20   | –                    | ASLANTAS 2018                      |
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 (ÖKUTANER et al. 2011c; AŞLANTAS 2018), all the other *Dorcadion* species cytogenetically studied by now have 11 autosomal pairs and a *X∗Yp* sex-determining mechanism; thus our finding for *Dorcadion triste phrygicum* agrees with them (ÖKUTANER et al. 2011c; DĂSCĂLU & FUSU 2012; AŞLANTAS 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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