



## First report on the chromosome number of a saproxylic beetle, *Ropalopus clavipes* (Cerambycidae: Cerambycinae: Callidiini)

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**Abstract:** Cerambycidae is poorly known in terms of its cytogenetics. Therefore, longhorn beetles are favorable for intensive chromosome studies. There is little if any chromosomal study in the family for both the tribe Callidiini Kirby, 1837 and its genus *Ropalopus* Mulsant, 1839. The main objective of the present study is to describe the karyotype of the longhorn beetle *Ropalopus clavipes* (Fabricius, 1775) and thus make a contribution to the karyological data of the family. The karyological analysis of testis of *R. clavipes* adults showed a diploid chromosome number of  $2n=22$  ( $n♂=10+Xy_p$ ). The present investigation constitutes the first cytogenetic analysis of *R. clavipes*.

**Keywords:** Chromosome, cerambycidae, callidiini, *ropalopus clavipes*

### Saproksilik kınkanathlı *Ropalopus clavipes* (Cerambycidae: Cerambycinae: Callidiini)'in kromozom sayı hakkında ilk çalışma

**Özet:** Cerambycidae familyası, sitogenetiği açısından iyi bilinmemektedir. Bu nedenle kromozom çalışmaları için uygun bir gruptur. Familyada hem Callidiini Kirby, 1837 tribusu hem de onun genusu *Ropalopus* Mulsant, 1839 için kromozomal çalışmalar yok denecek kadar az seviyededir. Bu çalışmanın asıl amacı *Ropalopus clavipes* (Fabricius, 1775) türünün karyotipini tanımlamak ve böylece familyanın karyolojik verisine katkı sağlamaktır. *R. clavipes* erginlerinin testislerindeki karyolojik inceleme türün diploid kromozom sayısının  $2n=22$  ( $n♂=10+Xy_p$ ) olduğunu göstermiştir. Bu çalışma *R. clavipes* üzerine yapılan ilk sitogenetik incelemedir.

**Anahtar kelimeler:** Kromozom, cerambycidae, callidiini, *ropalopus clavipes*

#### 1. Introduction

In Turkey, we have a better understanding of Cerambycidae fauna and taxonomy than its other biological patterns. The accumulated information about Turkish longhorn beetles remains far from satisfactory due to the lack of planned faunistic studies, and most of taxonomic studies are focused on external morphological characteristics. Nevertheless, the morphological approach coupled with increasing knowledge of geographical distribution is still indispensable to cerambycid studies and continue to serve useful purpose (Alkan and Eroğlu, 2001; Sama and Rejzek, 2002; Tezcan and Rejzek, 2002; Özdikmen and Çağlar, 2004; Özdikmen and Hasbenli, 2004; Özdikmen

and Demirel, 2005; Özdikmen and Okutaner, 2006; Özdikmen and Şahin, 2006; Özdikmen, 2007; Danilevsky, 2010; Yardibi and Tozlu, 2013; Şabanoğlu and Şen, 2016; Şabanoğlu and Sert, 2016; Özdikmen and Cihan, 2016; Danilevsky, 2017; Yıldız, 2017).

Many cerambycid groups, on the other hand, are known to present a complex taxonomy (Gardiner, 1961; Gressitt, 1978; Sama, 1993; Lingafelter, 2008; Wallin et al., 2009; Özdikmen et al., 2009; Dascălu, 2010; Grzymala and Miller, 2013; Santos-Silva et al., 2013; Bjørnstad, 2014; Sláma, 2015; Schapker 2017). Since taxonomic studies were limited to simple morphological characteristics, techniques other than traditional

morphology have been sought to enhance taxonomic diagnoses. Of these, karyotypic features are considered to be of great importance as a taxonomic character in solving taxonomic problems, in the phylogenetic classification and in assessing relationships (Gokhman and Kuznetsova, 2006). There is therefore a good opportunity for a comparison of the morphological and karyological findings in a comparative framework (Jackson, 1971). Unfortunately, despite their taxonomic relevance, chromosome numbers are known for less than 1% of all cerambycids and merely 6 longhorn beetles have hitherto been karyotyped from Turkish Cerambycidae fauna consists of about 650 taxa (Löbl and Smetana, 2010; Okutaner et al., 2011a, 2011b, 2011c, 2011d; Okutaner et al., 2012; Tokhatyan and Karagyan, 2013; Karagyan and Kalashian, 2016).

Turkish Callidiini is composed of 23 species belonging to 8 different genera. Of these, the genus *Ropalopus* Mulsant, 1839 has been represented by 8 species. In this genus, *Ropalopus clavipes* (Fabricius, 1775) has been recorded by different authors from various localities in Turkey (Özdikmen, 2007; Özdikmen, 2008; Cebeci et al., 2011). *R. clavipes* is classified in the IUCN European Red List of Saproxylic Beetles (Nieto and Alexander, 2010; Özdikmen, 2016). Saproxylic cerambycids (dead wood dependent) and other saproxylic beetles are considered to be a useful indicator of forest biodiversity (Pavuk and Wadsworth, 2012). As known, the polyphagan family Cerambycidae consists of phytophagous, especially xylophagous species of agricultural importance. Thereof, those beetles have received increasing attention.

This work is an attempt to throw some light on the phylogenetic relationships of cerambycid beetles by means of chromosome studies. To

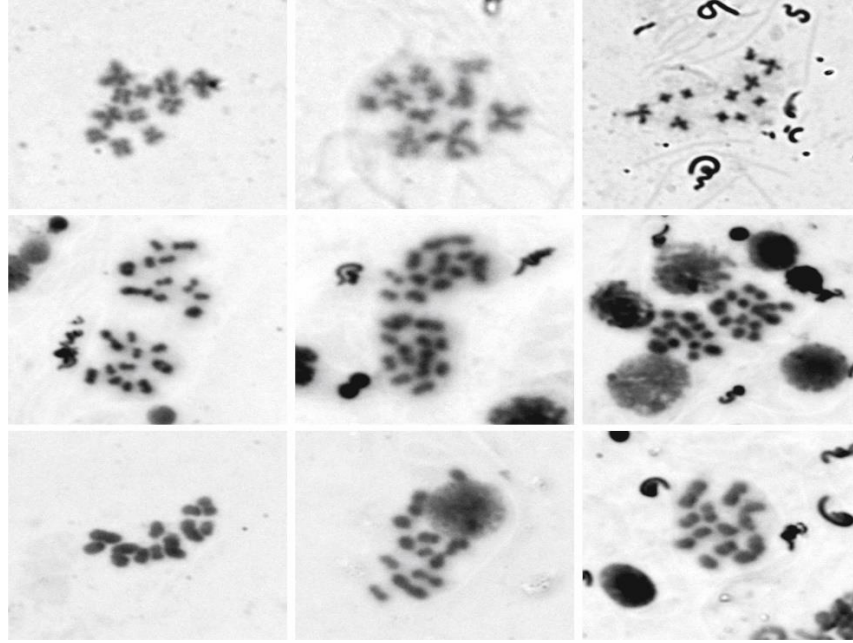
achieve this goal, we describe here for the first time the conventional karyotype of *R. clavipes* and provide comparative cytogenetic analysis of related taxa.

## 2. Materials and Method

Adult male specimens of *R. clavipes* collected from the environs of Çorum province (Turkey) between May and July 2014, formed the material for the present investigations. The individuals were kept in plastic vials and brought alive to the laboratory. Prior to karyological studies, the beetles were anesthetized with ethyl acetate and then their gonads were dissected out of the abdomens under a binocular microscope. Afterwards, the testes were fixed in a freshly prepared solution of ethanol:glacial acetic acid (3:1) and were stored at  $-20^{\circ}\text{C}$ . Chromosome preparations were obtained by using the classical method of testicular follicles squashing described by Rozek (1994) with some modifications and finally stained with Giemsa (4 %, pH 6.8) as usual. The preparations were inspected at 100X magnification, using a Leica DMLB 2 photomicroscope equipped with a Leica DFC320 camera. Well-spread spermatogonial metaphases were selected and photographed for determining the chromosome number.

## 3. Results and Discussions

In the present paper, we described the chromosomes of *R. clavipes* from Turkey. Spermatogonial metaphases revealed 22 chromosomes of various sizes and they are most likely metacentrics and submetacentrics. The male karyotype of *R. clavipes* is constituted by 10 autosomal bivalents and the  $\text{Xy}_p$  sex-chromosome system of parachute type; therefore a meioformula of  $10+\text{Xy}_p$  is assigned to this species (Figure 1).



**Figure 1.** Meiotic chromosomes of *Ropalopus clavipes* [ $2n_{\sigma}^{\delta}=22$  ( $n=10+Xy_p$ )]  
**Şekil 1.** *Ropalopus clavipes*'in mayotik kromozomları [ $2n_{\sigma}^{\delta}=22$  ( $n=10+Xy_p$ )]

To our knowledge, as of yet there have been no published reports describing the karyotype of the *R. clavipes*. Therefore, the current study is thought to be the first report of the chromosomal study of this beetle. Besides, the literature dealing with the chromosomes in the genus *Ropalopus* is very meager and the karyotype of only one species was available. Ehara (1956) reported that *R. signaticollis* has 22 chromosomes. The present diploid count of  $2n=22$  is, thus, in accordance with the previous record for the genus. Comparative karyotype analyses have been severely hampered by paucity of information regarding the

cytogenetics of this genus. Moreover, despite the fact that the tribe Callidiini currently contains 206 species in 41 genera, very few species have been subjected to chromosomal studies, only 5 species of 4 genera (Ehara, 1956; Teppner, 1966; Abe et al., 1971; Smith and Virkki, 1978; Nearn et al., 2018). Chromosomal studies of Callidiini have heretofore been chiefly concerned with the chromosome numbers and sex chromosome mechanisms. These distinguishing karyological characters have been tabulated for all Callidiini species thus far studied cytogenetically (Table 1).

**Table 1.** Chromosomal data of species of Callidiini

**Çizelge 1.** *Callidiini türlerinin kromozomal verileri*

Species	Diploid number	Meioformula	References
<i>Callidium violaceum</i>	22♂	10+Xy	Smith and Virkki 1978
<i>Callidium violaceum</i>	20♀	...	Teppner 1966
<i>Gonocallus collaris</i>	...	6+Xy <sub>p</sub>	Smith and Virkki 1978
<i>Phymatodes maaki</i>	...	9+Xy <sub>p</sub>	Abe et al. 1971
<i>Rhopalopus signaticollis</i>	♂	11 <sub>II</sub>	Ehara 1956

Many efforts that are based on the analysis of few morphological characters of longhorn beetles have yielded taxonomic confusion, since morphological variation in the family Cerambycidae is extreme. Cerambycidae cytogenetics, thus, usually leads to different and/or new approaches and promotes the future taxonomic studies. In spite of many references in which chromosomal data for Cerambycidae are provided, the proportion of species analyzed so far is less than 1 %. While the widespread chromosome number is  $2n=20$ , the range of diploid numbers in this family goes from  $2n=10$  in *Plocaederus obesus* Gahan (Cerambycinae) to  $2n=53-54$  in *Vesperus xatarti* Mulsant (Vesperinae). On the other hand, the “parachute”  $Xy_p$  is the most frequent sex-chromosome system among cerambycids while the others also were recorded (e.g.  $X0$ ,  $Xy$ ,  $XY$ ,  $Xy_p$  and multiple sex chromosomes). It seems that the available results not sufficient to assess relatedness or to reconstruct phylogeny of this group. Therefore, additional karyological works are needed to better understand the diversity, distributional patterns and evolution of the family (Cesari et al., 2005; Dutrillaux et al., 2007; Okutaner et al., 2011a, 2011b, 2011c, 2011d; Okutaner et al., 2012; Tokhatyan and Karagyan, 2013; Dutrillaux and Dutrillaux, 2014; Giannoulis et al., 2014; Karagyan and Kalashian, 2016).

#### 4. Conclusion

Adequately defined species and groups of species or groups of populations are prerequisites for phylogenetic, biogeographical and ecological studies. Endeavors in these fields may provide erroneous knowledge when based on poor taxonomic assessments (Löbl and Smetana, 2013). The use of cytogenetic methods in taxonomy has become a widespread and powerful tool for the delineation and identification of many insects, particularly in beetles (Lachowska et al., 2006). The karyotype is a part of the cytogenetic data required for a better definition of a species along with the other classical characters (Petitpierre, 1997). Undoubtedly, karyotypic findings provide valuable clues for debated taxonomic contexts, bringing data pertaining to a genetic differentiation

between populations/species (Capanna and Civitelli, 1988). Species-specific karyotypes can therefore be deemed a definite element such as any morphological character for taxonomic purposes (Petitpierre, 1997; Lachowska et al., 2006). Since karyological peculiarities are actually morphological and they can be analysed in a way similar to that of the features of external morphology (Gokhman, 1997). Moreover, karyotype structure does not depend on environmental conditions, at least not directly (Baur et al., 2014).

Consequently, the systematic relationships within the family Cerambycidae are much less clear in terms of cytotaxonomic approach. The present paper, thus, sets out to provide a framework for future taxonomic and karyological work on the family and to demonstrate the value of chromosomal studies in its taxonomy.

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