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Notes on the karyology, male genitalia and distribution of *Rhagonycha fulva* (Scopoli, 1763) (Coleoptera: Cantharidae) from Turkey

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Abstract

Several revisions on the cantharid taxonomy have been reported as yet. These revisions have highlighted the need for further studies in the family. Taxonomic tools such as cytogenetics and genitalia morphology may contribute to clarify the relationships among the species. Thus, the present paper deals with the karyotype and the male genitalia of *Rhagonycha fulva* (Scopoli, 1763). The diploid chromosome number was found to be $2n\beta = 13$ (6+X0). The chromosomal finding is essentially identical to the results reported previously for *Rhagonycha fulva*. In addition, the male genital morphology and the distribution map in Turkey are given.

Key words: Cantharidae, Rhagonycha fulva, karyology, male genitalia, distribution

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Türkiye'deki *Rhagonycha fulva* (Scopoli, 1763) (Coleoptera: Cantharidae)'nın karyolojisi, erkek genitali ve yayılışı üzerine notlar

Özet

Cantharid taksonomisi ile ilgili olarak şimdiye kadar çeşitli revizyon çalışmaları yapılmıştır. Bu revizyonlar familya üzerinde daha çok çalışma yapılması gerektiğine dikkat çekmiştir. Sitogenetik ve genital morfolojisi gibi taksonomik araçlar türler arasındaki ilişkilerin açığa çıkarılmasında katkı sağlayabilir. Buradan hareketle, bu çalışma *Rhagonycha fulva* (Scopoli, 1763)'nın karyotip ve erkek genitalini incelemektedir. Diploid kromozom sayısı $2n\delta = 13$ (6+X0) olarak tespit edildi. Kromozomal bulgu *Rhagonycha fulva*'nın daha önce verilen sonuçlarına temelde uygunluk göstermektedir. Ayrıca, türün erkek genital morfolojisi ve Türkiye'deki yayılış haritası da sunulmaktadır.

Anahtar kelimeler: Cantharidae, Rhagonycha fulva, karyoloji, erkek genitali, yayılış

1. Introduction

Cantharids, the soldier or leatherwing beetles, consist of some 150 genera and more than 5.000 described species classified in 9 tribes, 5 subfamilies worldwide (Hernández and Caballero, 2016; Vidal et al., 2016). Many beetles in the family Cantharidae have been heretofore subjected to the revision studies. Traditionally, these taxonomic revisions were mainly based on the morphological characters (Hsiao et al., 2016; Li et al., 2016; Su et al., 2015; Pelletier and Hébert, 2014; Yang et al., 2012; Yang and Yang, 2009; da Silva, 2007; Švihla and Mifsud, 2006; Fender, 1971). However, it is impossible to accurately identify all species and is not easy to resolve the systematic position of some species by only using these features (Su et al., 2015). Since, previously used some characters were complex and confusing that make identification of many species difficult into the family. Moreover, current keys can be used only for identifying males for many genera and are also almost useless for separating most specimens (Pelletier and Hébert, 2014). These difficulties indicate the need for further studies to clarify the cantharid taxonomy either to find newly morphological characters of high diagnostic value or to apply alternative effective methods (Su et al., 2015).

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Chromosomal studies and differences in male genital morphology are two sources of new data which may be useful in helping solve some taxonomic and phylogenetic problems (Maryańska-Nadachowska and Glowacka, 2005).

Comparative chromosomal analyses have quite often proven to be valuable and helpful in elucidating relationships not readily apparent by morphological studies (Saygun and Saygun, 2016; Lachowska-Cierlik et al., 2015; Godoy et al., 2013; Singh and Banerjee, 2004; Lorite et al., 2002; Witten, 1983; Lieppman and Hubbs; 1969). Furthermore, karyological studies in general may bring a new insight on insect taxonomy and classification (Soldán and Putz, 2000). Nevertheless, cytogenetic studies of the cantharid beetles have lagged far behind that of many other insect groups. Out of about 5.000 species, only 33 have been analyzed karyologically so far, corresponding to~0.7% of all nominal cantharid species. The few taxa karyotypically sampled certainly appear scanty to indicate chromosomal diversity in the family (James and Angus, 2007; Rozek et al., 2004; Machado et al., 2001; de Gamberdella and de Vaio, 1978; Yadav, 1973; Virkki, 1963; Smith, 1960). Thus, it is anticipated that further chromosomal studies of soldier beetles would contribute to their taxonomy and systematics.

In many insects, genitalia mostly provide the only way to reliably distinguish species since sexual characters often show particularly great species-specific variation (Özgül-Siemund and Ahrens, 2015). Especially, the male genitalia of insects are very specific in taxa and species-specificity of male genitalia is notably prevalent among insects (Brajković et al., 2010; Song, 2009). Therefore, male genitalia occupy a special place in insect systematics, and their considerable taxonomic value has proven to be enormous in major lineages of insects (Wieczorek et al., 2012; Song, 2009). A perusal of the literature on the Cantharids reveals that male genitalia also played a prominent role in the taxonomic and systematic studies of the family. Many authors adopted the revisions and classifications that put emphasis on the male genitalia rather than other external and internal characters (Fanti and Ghahari, 2016; Hsiao et al., 2016; Li et al., 2016; Su et al., 2015; Pelletier and Hébert, 2014; Yang et al., 2012; Yang and Yang, 2009; da Silva, 2007; Švihla and Mifsud, 2006).

Rhagonycha Eschscholtz, 1830 is one of the richest genera of the soldier beetles with nearly 330 species (da Silva, 2007). The genus *Rhagonycha* has received a good deal of attention by a variety of coleopterists since 1940s (Pelletier and Hébert, 2014; Fender, 1971). *Rhagonycha fulva* has a widespread distribution in Europe and occurs also in Asia and North Africa (Cassar, 2015; Kazantsev and Brancucci, 2007; Kazantsev, 2004; Chobotow, 2002; Drovenik, 2001; Kazantsev, 1995; Kuśka, 1995; Svihla, 1992; Dahlgren, 1975). The purpose of this paper is to present the karyotype and the male genital organ of *Rhagonycha fulva*. Furthermore, distribution map of this species in Turkey is provided.

2. Materials and methods

Adult individuals of *Rhagonycha fulva* were collected from fields in the vicinity of Eskişehir (Turkey) between May and July 2013. Only male adults constituted the materials for the present work and those were investigated through the dissections. Chromosome slides have been obtained from testis cells using the squash technique previously described by Rozek (1994). Slides were examined under light microscope and photographed with a Leica DMLB 2 photomicroscope coupled with Leica DFC320 camera. The distribution map was prepared using the data of material examined in the present and previous studies. The map was prepared by using ArcView GIS Version 3.1. The male genitalia were dissected from the abdomen under a stereoscopic microscope, cleared in 10% KOH solution for several minutes, then placed in a droplet of glycerol prior to investigation (Yang et al., 2012). The male genitalia observed and photographed using a Leica MZ 16A Stereomicroscope with DFC320 imaging system.

3. Results

Familia	: Cantharidae Imhoff, 1856
Subfamilia	: Cantharinae Imhoff, 1856
Tribe	: Cantharini Imhoff, 1856
Genus	: Rhagonycha Eschscholtz, 1830
Species	: Rhagonycha fulva (Scopoli, 1763) (Figure 1)

Rhagonycha fulva can be confused with the wharf borer beetle *Nacerde melanura* (Coleoptera, Oedemeridae) due to similar size and colour pattern. *Rhagonycha fulva* has a totally different shape of pronotum, widest near apical third (Pelletier and Hébert, 2014).



Figure 1. *Rhagonycha fulva*; general habitus (dorsal view). Scale bar = 1 mm.

The meioformula of the species examined was n = 6 + X. A detailed description and comparison of the chromosomal morphology could not be made with confidence because of their very small size (Figure 2).



Figure 2. Meiotic chromosomes from testis of *Rhagonycha fulva* adult (n=6+X)

Records of *Rhagonycha fulva* in Turkey. Adana, Aksaray, Çorum, Erzurum, Eskişehir, Gaziantep, Gümüşhane, Hatay, Isparta, İzmit, Karaman, Kahramanmaraş, Kayseri, Kırklareli, Kocaeli, Konya, Mersin, Nevşehir, Niğde, Osmaniye, Trabzon, Yozgat (Figure 3) (Demirözer and Karaca, 2014; Yıldırım et al., 2011; Dahlgren, 1975).



Figure 3. Distribution of *Rhagonycha fulva* in the provinces of Turkey. The black areas show the provinces in which *Rhagonycha fulva* has been recorded to date.

Range of *Rhagonycha fulva*. Albania, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czechoslovakia, Denmark, Estonia, France, Georgia, Germany, Great Britain, Greece, Holland, Hungary, Iran, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Morocco, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Turkey, Turkmenistan, Ukraine. The male genitalia of *Rhagonycha fulva* are depicted in Figure 4.



Figure 4. *Rhagonycha fulva*; aedeagus (a, dorsal; b, ventral; c, lateral view). Scale bar = 1 mm.

4. Conclusions and discussion

The Cantharidae (Coleoptera) is the most diverse family of soft-bodied elateroids while represents a relatively small family of beetles (Fanti and Ghahari, 2016; Hernández and Caballero, 2016). In spite of the richness of the family, records of chromosome investigation on these beetles are still inadequate. Thus, numerous taxonomic questions and phylogenetic relationships within Cantharidae remain still unresolved. That's why the present study deals with the chromosome number and sex chromosome mechanism in the soldier beetle Rhagonycha fulva. Since, the study of karyotypes can usually help to solve the taxonomic problems (Kandul, 1997). In the current study, the chromosomes were obtained from gonads of adult male specimens. On the other hand, Machado et al. (2001) express that mid-gut cells may be more appropriate for the chromosome analyses as proven to be useful for Cantharidae and Carabidae. For instance, James and Angus (2007) described the karyotypes of 20 cantharid species from only mid-gut cells of adults since the lack of gonial activity in the testes of adult beetles. Similarly, Rozek et al. (2004) also found no mitotic and meiotic metaphases from the gonads of adult males of Rhagonycha fulva. It is probably related to early maturation of the testes and the short lives of the adult cantharids (James and Angus, 2007; Machado et al., 2001). The diploid chromosome number was found here 13 with 6 autosomal pairs and one X chromosome. James and Angus (2007) determined the uniform chromosome number and sex-determining system for Rhagonycha fulva. Some of earlier reports have demonstrated the karyotype of six pairs of autosomes and an X0 sex-determining system for the family (James and Angus, 2007; Machado et al., 2001; de Gamberdella and de Vaio, 1978; Virkki, 1963; Smith, 1960). This putative ancestral karyotype (n=6+X) was proposed to be the standard karyotype to cantharid beetles (Machado et al., 2001; de Gamberdella and de Vaio, 1978). Conversely, remain works on Cantharidae karyology disaccord with this suggestion. Rozek et al. (2004) and Yadav (1973) studied the species of the genus Cantharis and showed that those species have nine pairs of autosomes and an Xy_p sex-determining system. Moreover, James and Angus (2007) observed the chromosomes of the genus *Malthinus* and found the diploid chromosome number $2n\beta = 11$ and $2n\beta = 12$. It is seen obviously that more work is needed on the chromosomes of the Cantharidae.

Male genitalia are widely thought to be of the most variable and divergent of all morphological structures (Simmons, 2014). Indeed, male genitalia are considered one of the most important and useful species-diagnostic characters in insect systematics (Özgül-Siemund and Ahrens, 2015). Observed high stability in various insect taxa makes male genitalia the main criterion for identification of the species in some groups (Brajković et al., 2010). Especially in the family Cantharidae, many taxonomic studies have demonstrated the usefulness of male genitalia in diagnosing species. Fanti and Ghahari (2016) reported that recognition of Cantharis (Cantharis) melaspoides Wittmer, 1971 is only possible by aedeagus investigation. Li et al. (2016) mention the aedeagus as being traditionally the most reliable method to identify the cantharid species. Su et al. (2015) express that the subfamily Cantharinae has traditionally male genitalia-based taxonomy. Yang et al. (2012) presented the redescribed and newly described species of the genus Pseudopodabrus by the aedeagal illustrations. Yang and Yang (2009) suggested that Micropodabrus bicoloriceps (Wittmer, 1989) comb. Nov. should be transferred to the genus Mimopodabrus and it is thus synonymized as Mimopodabrus bicoloriceps Wittmer, 1997 syn. Nov. by investigation of the aedeagus. Da Silva (2007) showed that taxonomically, the study of the morphology of the aedeagus warranted the separation of Rhagonycha galiciana Gougelet & H. Brisout, 1860 and Rhagonycha varians (Rosenhauer, 1856), formerly treated as synonyms, as good species. This separation particularly indicates why male genitalia are so valuable in taxonomic studies of the genus Rhagonycha. In the current work, the male genitalia of Rhagonycha fulva is presented and thus it is expected that it will contribute to the taxonomic knowledge of the genus. Undoubtedly, the further morphological information and finding of male genitalia will be useful for the recognition of genera and species, as well as for systematic and cladistics studies of the family Cantharidae.

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