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Original article

Investigation of aedeagus and spermatheca ultrastructure of *Cryptocephalus turcicus* Suffrian, 1847 (Coleoptera: Chrysomelidae: Cryptocephalinae) from Türkiye by using SEM

Türkiye'den *Cryptocephalus turcicus* Suffrian, 1847'nin (Coleoptera: Chrysomelidae: Cryptocephalinae) aedeagus ve spermatheca ince yapısının SEM kullanılarak araştırılması

Neslihan BAL[a]*, Hüseyin ÖZDİKMEN[a], Damla AMUTKAN MUTLU[a], Zekiye SULUDERE[a], Didem CORAL[b]

[a]*Gazi University, Faculty of Science, Department of Biology, 06500 Ankara, Türkiye*

[b]*Directorate of Plant Protection Central Research Institute, Department of Agricultural Fauna and Microflora, Gayret Mah., Ankara, 06810, Türkiye*

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* Corresponding author: Neslihan BAL

neslihansilkin@gmail.com

ABSTRACT

This paper presents ultrastructures of aedeagus and spermatheca observed for the first time by SEM of a plant pest (Coleoptera: Chrysomelidae: Cryptocephalinae) *Cryptocephalus turcicus* Suffrian, 1847 from Türkiye. In the present study, the morphologies of male and female genitalia of this species belonging to the nominative subgenus *Cryptocephalus* (s.str.) collected from Ankara, Kastamonu and Samsun provinces are examined using Scanning Electron Microscopy (SEM). To date, there has not been a study in Türkiye in this context in which male genitalia (aedeagus) and female genitalia (spermatheca) morphologies of the *Cryptocephalus turcicus* Suffrian, 1847 have been studied together. The genus *Cryptocephalus* is represented by 73 species in Türkiye. Detailed investigations of aedeagus "and spermatheca are very important to obtain new diagnostic characters in the genus *Cryptocephalus*. For this reason, ultrastructural and detailed investigations of aedeagus and spermatheca of *Cryptocephalus turcicus* Suffrian, 1847 from Türkiye were firstly studied with SEM and stereo microscope to obtain new diagnostic characters in the genus *Cryptocephalus*. The new diagnostic characters obtained by examining the ultrastructure of aedeagus and spermatheca belonging to the species will be used in the distinction of species that are especially similar to each other, by determining the differences and important characters among the species in the genus *Cryptocephalus*. Photos in SEM, as well as photos in the stereo microscope, are also given in the text.

INTRODUCTION

Chrysomelids constitute an important group of herbivores in the order Coleoptera (Jolivet 1988). Many species are monophagic and oligophagic with respect to host plants (Strauss 1988). Adults feed on flowers and leaves, while larvae feed on roots and leaves (Matsuda 1988). Adults and larvae of some species are therefore important pests of trees and shrubs (Mirzoeva 2001).

One of the biggest and most numerous subfamilies of the Chrysomelidae is the Cryptocephalinae. One of the most

common tribes of the Cryptocephalini, Cryptocephalus Geoffroy, 1762, has a variety of species throughout the Palearctic. One of the most widespread members of the genus is Cryptocephalus (Schöller 2008). Fauna Cryptocephalinae Türkiye is represented by 107 taxa of the species group (92 species and 15 subspecies) (Lopatin and Dovgailo 2002, Özdikmen and Cihan 2014, Warchalowski 2003). Leaf beetles are nearly all phytophagous, and their success in ecosystems is determined by their ability to occupy many different feeding niches (Jolivet 1988), and by their host specificity to almost all groups of plants (Riley et al. 2002). Most adults feed on living plant parts, such as leaves, polen, flowers, young stems or fruit (Flowers 1996, Jolivet and Verma 2008, Riley et al. 2002, Staines 2002, White 1968, Wilcox 1972). Larvae are found on the surface of leaves or as leaf diggers; others feed on droppings, roots or submerged parts of plants (Jolivet and Verma 2008, Riley et al. 2002, Staines 2002, White 1968). Some larvae feed on eggs and ant waste in their nests (Chamorro Lacayo 2014). Thus, beetles are an important group in the ecosystem as primary consumers, in direct competition with other herbivores (González-Megías and Gómez 2003), and an important in the food web (Basset and Samuelson 1996). *Cryptocephalus turcicus* is generally harmful to *Crataegus monogyna*, *Quercus petraea*, *Quercus rolar* and *Quercus ilex* plants (Gök and Çilbirlioğlu 2005, Selmi 1982).

Cryptocephalus turcicus Suffrian, 1847 is a member of the subgenus *Cryptocephalus* (s.str.) Geoffroy, 1762. This subgenus is well distinguished from other subgenera of *Cryptocephalus* Geoffroy, 1762 by the combination of following characters: Epipleuron of elytra inclined, in side view visible in whole length. Anterior tibiae not explosively broadened and smoothed, elytra without long, erect hairs, occasionally shortly pubescent (Warchałowski 2003). *Cryptocephalus turcicus Suffrian*, 1847 is in the *Cryptocephalus flavipes* species-group.

MATERIALS AND METHODS

The available specimens (a total of 15 specimens) for the present work were collected from Ankara, Kastamonu and Samsun provinces in Türkiye in 1991, 1997 and 2004. The specimens were deposited at Gazi University (Türkiye, Ankara). The aedeagus and spermatheca were dissected from the abdomen, and the remaining tissue was removed with fine tweezers. After cleaning, the samples were placed in 70% ethanol for microscopic examination and examined using an Olympus SZX7 stereo microscope and a Leica Z-16 APO stereo microscope.

For Scanning Electron Microscopy (SEM), the cleaned samples were dehydrated using an increasing series of ethanol (70%-100%) and then dried in air. Afterwards, the samples were mounted on SEM stubs with double-sided adhesive tape, gold-plated using Polaron SC 502 Sputter Coater in Gazi University Prof. Dr. Zekiye Suludere Electron Microscope Center. It was investigated by JEOL JSM 6060 SEM at 10 kV.

RESULTS AND DISCUSSION

Cryptocephalus turcicus Suffrian 1847

Cryptocephalus turcicus Suffrian, 1847 is a Turano-Mediterranean (Turano-Apenninian) species (Figure 1). It is distributed in Europe (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, France, Germany, Italy, Macedonia, Romania, Slovenia, Türkiye, Serbia and Montenegro and Asia (Iranian, Jordan, Syria, Türkiye) (Borowiec and Sekerka 2010, Warchalowski 2010).

Figure 1. The species is widely distributed in Türkiye. It has been recorded from 17 provinces in all Turkish regions. It is reported from Ankara, Antalya, Balıkesir, Bilecik, Bursa, Çankırı, Düzce, Eskişehir, Isparta, İstanbul, İzmir, Kastamonu, Karabük, Samsun, Trabzon, Yalova and Zonguldak provinces in Türkiye (Ekiz et al. 2013, Özdikmen and Kaya 2014).

Materyal examined: Ankara Prov: Kızılcahamam, Soğuksu National Park, 1500m, 21.VI.1991,1 ex; Kızılcahamam, Soğuksu National Park, 21.VI.1991, 1700m, 1 ex; Kızılcahamam, Yukarıçanlı,14.VI.1997, 1540m, 1 ex. Kızılcahamam, Güvem, 26.VI.1997, 1000m, 1 ex; Kastamonu Prov: İnebolu road, 17.V.2004, 3 exs; Doğanyurt 18.VI.2004, 950m, 2 exs; Dadaş driveway, 19.VI.2004,1010m, 1 ex; Samsun Prov: Alaçam, Kapaklık village, 16.IV.2004, 620 m,4 exs. Alaçam, Dürtmen hill skirts, 16.VI.2004, 1460m, 1ex.

Aedeagus and spermatheca of *Cryptocephalus turcicus* were studied with SEM and stereo microscope. Obtaining observations on ultrastructural and detailed morphologies of them are presented as follows:

Aedeagus

In stereo microscope (Figures 1A-1B):

The median lobe is brown.

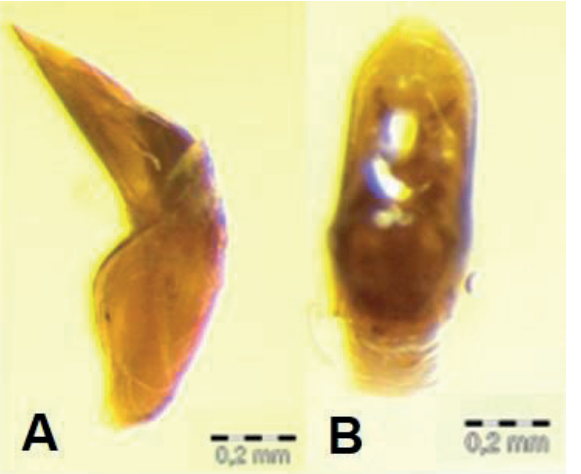

Figure 1. Aedeagus of *Cryptocephalus turcicus* Suffrian, 1847, A. Lateral view, B. Dorsal view

In lateral view, the median lobe gradually narrows slightly from the base to the apical portion. The median lobe is curved at an angle of 120 degrees in the 1/3 part starting from the base. The apex of the median lobe is flat and pointed.

In dorsal view, the median lobe moves parallel to the apex in the 2/3 part while the apex gradually narrows in the 1/3 part. The median lobe is quite narrow and elongated at the apex. The apex part is round in appearance. The median lobe in lateral parts and in the fore part thickened. Thickening in lateral part is distinctly smaller than in the fore parts.

In ventral view, the median lobe moves parallel to the apex in the 2/3 part while the apex gradually narrows in the 1/3 part. The median lobe is quite narrow and elongated at the apex. The apex part is round in appearance.

In SEM (Scanning Electron Microscope) (Figures. 2A-2C, 3A-3C):

SEM images are similar to images from a stereo microscope in general. Only the different characters in the SEM image are shown below. Median lobe especially in the anterior half with scattered, irregular and sparsely ultrastructural pits and sensillae. There are quite a lot of pits and sensillae, especially in the lateral parts and ventral part in the terminal area of the median lobe. Operculum of median lobe without ultrastructural pits and sensillae in dorsal view. The apex of the median lobe gradually narrowed, additionally prolonged.

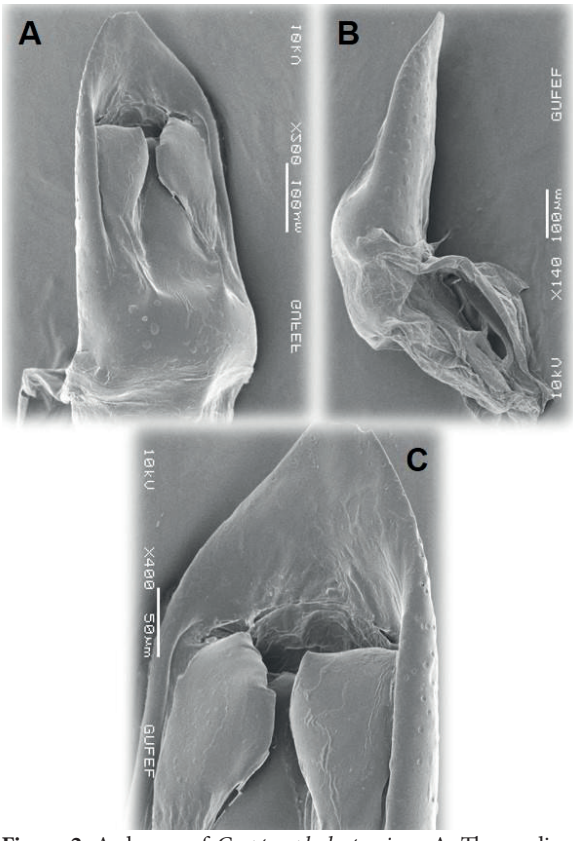

Figure 2. Aedeagus of *Cryptocephalusturcicus*, A. The median tube in dorso-lateral view (SEM), B. The median tube in lateral view (SEM), C. Apical part of the median tube dorsal view (SEM)

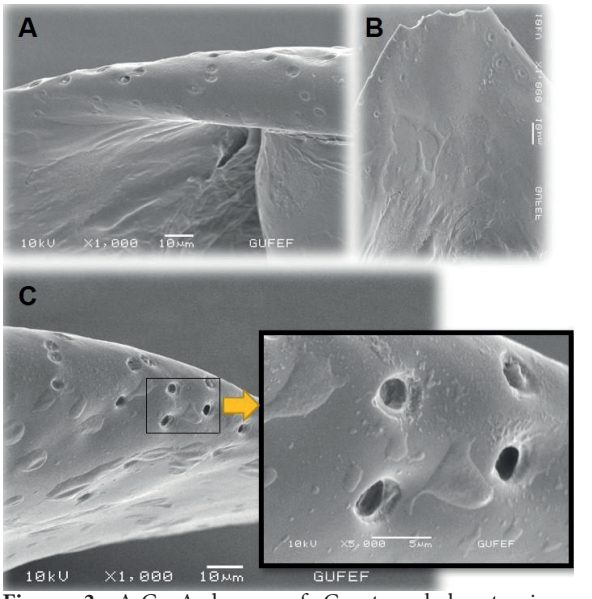

Figure 3. A-C. Aedeagus of Cryptocephalus turcicus, Sensilla on median part of median tube in lateral view (SEM)

Spermatheca

In stereo microscope (Figure 4):

Spermatheca are generally yellow. The general appearance of spermatheca is similar to neutrophil from white blood cells in the blood. The vasculum consists of gnarled partitions. Cornu Ç-shaped. Cornu gradually narrowed towards to apex and the apex of cornu round. Nodulus and cornu are circular in shape and nodulus has a larger structure than cornu. The nodulus is more bulging than the Cornu, and there is a narrowing towards the apex. Ampulla (Collum + Ramus) is not clearly visible. Therefore, spermathecal duct and spermathecal gland outlets cannot be clearly seen in the stereo microscope image.

Figure 4. Spermatheca of *Cryptocephalus turcicus* Suffrian, 1847 in lateral view

In SEM (Scanning Electron Microscope) (Figures 5A-5C, 6A-6D)

SEM images are similar to images from a stereo microscope in general. Only the different characters in the SEM image are shown below. The exit points of the spermathecal gland and spermathecal duct, which cannot be distinguished in stereo microscope pictures, are quite evident in the SEM microscope. The thick one is the spermathecal duct, which is directly connected to the bottom, and the thinner spermathecal gland structure is connected from the side. Nodulus, cornu, proximal tube of ductus spermatheca with scattered, irregular and sparsely ultrastructural pits. The number of pits increases from the cornu to the nodulus.

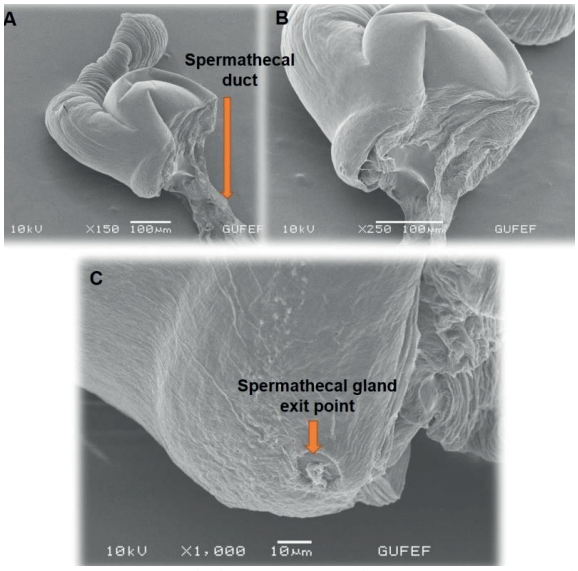

Figure 5. *Cryptocephalus turcicus* Suffrian, 1847, A. General view of spermatheca (SEM), B. Nodulus part (Basal part of spermatheca) of spermatheca (SEM), C. Spermathecal gland exit point of Spermatheca (SEM)

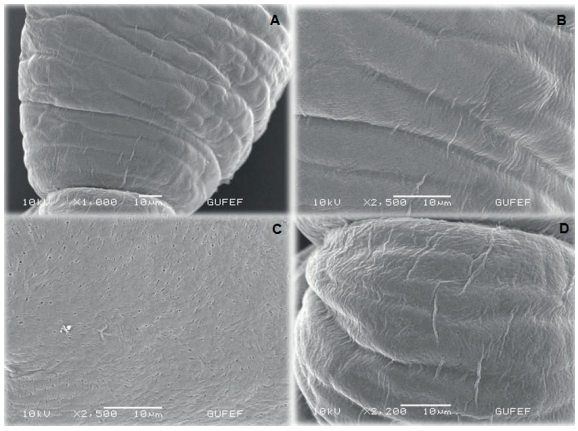

Figure 6. Spermatheca of *Cryptocephalus turcicus* Suffrian, 1847, A- B. Pits on Cornuin lateral view (SEM), C-D. Pits on nodulus in lateral view (SEM)

External morphological features are not sufficient in determining the species diversity in Türkiye and separating the problematic species groups. As a result of the study, the male and female genitals of *Cryptocephalus turcicus* species were identified and sampled using both stereo and Scanning electron microscopy and included in the study. By looking at the genital pictures, it is seen that the species has a very different structure from the other species in the *Cryptocephalus* nominative genus in which it is included. This suggests to us that a taxonomic category arrangement can be made again within this group. The importance of male and female genitalia in this distinction is great. Examining the ultrastructural structure of male and female genitalia will facilitate the identification of the species, the possibility of comparing the male and female genital structures of the species and the taxonomic categories of the species.

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ÖZET

Makale, Türkiye'den bir bitki zararlısının (Coleoptera: Chrysomelidae: Cryptocephalinae) *Cryptocephalus turcicus* Suffrian, 1847'de aedeagus ve spermatecanın SEM tarafından ilk kez gözlemlenen ultrastrüktürlerini sunmaktadır. Bu çalışmada, *Cryptocephalus* (s.str.) nominatif alt cinsine ait bu türün Ankara, Kastamonu ve Samsun illerinden toplanan türün erkek ve dişi üreme organlarının morfolojileri Taramalı Elektron Mikroskobu (Scanning Electron Microscopy-SEM) kullanılarak incelenmiştir. Bugüne kadar Türkiye'de *Cryptocephalus turcicus* Suffrian, 1847'nin erkek genital (aedeagus) ve dişi genital (spermatheca) morfolojilerinin birlikte çalışıldığı bu kapsamda bir çalışmaya rastlanmamıştır. *Cryptocephalus* cinsi Türkiye'de 73 tür ile temsil edilmektedir. Aedeagus ve spermatecanın detaylı araştırılması *Cryptocephalus* cinsinde yeni tanısal karakterler elde etmek için çok önemlidir. Bu nedenle, *Cryptocephalus turcicus* Suffrian, 1847'nin Türkiye'den aedeagus ve spermatecalarının ultrastrüktürel ve detaylı incelemeleri ilk olarak *Cryptocephalus* cinsinde yeni tanısal karakterler elde etmek için SEM ve stereo mikroskop ile incelenmiştir. Türlere ait aedeagus ve spermatecanın ultrastrüktürünün incelenmesiyle elde edilen yeni tanısal karakterler, *Cryptocephalus* cinsindeki türler arasındaki farklar ve önemli karakterler belirlenerek özellikle birbirine benzeyen türlerin ayrımında kullanılacaktır. SEM'deki fotoğraflar ve stereo mikroskoptaki fotoğraflar da metinde verilmiştir.

Anahtar kelimeler: *Cryptocephalus turcicus*, aedeagus, spermatheca, SEM, Türkiye

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