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Morphological Analysis of Male and Female Genital Structures in *Galeruca tanaceti tanaceti* Linnaeus, 1758 (Chrysomelidae: Galerucinae)

Feyza KÖKKAYA [1*] | Neslihan BAL [1]

[1] Gazi University, Science Faculty, Department of Biology, Ankara, Türkiye

Correspondence
Gazi University, Science Faculty, Department of Biology, Ankara, Türkiye
Email: neslihansilkin@gmail.com

This study is our student's research project.

Abstract: The paper presents unknown ultrastructure observed by scanning electron microscope (SEM) and stereo microscope of aedeagus and spermatheca morphologies of Galeruca tanaceti tanaceti Linnaeus, 1758 (Coleoptera: Chrysomelidae: Galerucinae) from Türkiye. This species, which is an important subfamily where the evolution between plants and insects is studied and is recognized as an important biological control agent, was collected in Çankırı in 2015.The genus Galeruca Geoffroy, 1762 includes 62 species in the Palaeartic Region, while it is represented by 13 species in Türkiye. As known, aedeagus and spermatheca morphologies are taxonomically important structures. Before the present study, however, there are no work on these structures of Galeruca tanaceti tanaceti Linnaeus, 1758. For this reason, ultrastructural and detailed investigations of aedeagus morphology of Galeruca tanaceti tanaceti Linnaeus, 1758 from Türkiye were firstly studied with SEM to contain male and female genital descriptions of Galeruca tanaceti tanaceti Linnaeus, 1758. Photos in SEM and stereo microscope are also given in the text.

Keywords: Aedeagus, Galerucinae, Galeruca tanaceti tanaceti, SEM, Türkiye.

INTRODUCTION

Galerucinae, belonging to the Chrysomelidae family (Coleoptera), is among the largest groups of leaf beetles (Yang et al., 2015). Adult Galerucinae are distinguished by their oval to oblong bodies, with visible heads integrated into the prothorax. The front coxal cavities may be either open or closed. Their tarsi are pseudotetramerous, having a bifid third segment and a very small fourth segment nested within the third. The hind femur is slender and lacks a femoral spring. The antennae are composed of eleven segments, with their insertions located close together either in front of or between the eyes. Frontal tubercles are typically well-developed. Generally, the elytral sensilla patch is singular (Samuelson, 1996; Nadein & Bezděk, 2014).

Chrysomelidae, commonly known as leaf beetles, are phytophagous insects that have adapted to consume a diverse array of plant species. Both the adult beetles and their larvae feed on cultivated crops as well as various beneficial wild plants and shrubs (Jolivet et al., 1988). The relationship between the subfamily Galerucinae and their host plants has made them an excellent model for studying the evolution of herbivorous insects, the evolution between insects and plants, and the evolutionary mechanisms driving biodiversity (Mitter and Farrell, 1991; Farrell et al., 1992; Futuyma and McCafferty, 1990). Moreover, many species in this group are used for biological weed control or are important agricultural pests (Booth et al., 1990; Vencl and Morton, 1998; Jolivet and Verma, 2002; Xue et al., 2007; Bunnige et al., 2008; Xue and Yang, 2008; Nie et al., 2012).

When looking at the studies on Galerucinae, it is seen that some species are pests of cultivated plants (Kryzhanovskij 1974). For example, in Latvia, Galerucella tenella (Linnaeus, 1761) and Galeruca tanaceti (Linnaeus, 1758) have been reported as pests of strawberries (Čakstiņa 1962; Priedītis 1971a; Dūks 1976; Petrova et al. 2000, 2006).

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Another study on Galeruca tanaceti was conducted. The effect of temperature on the development and survival of the insect in marginal environmental conditions was studied. Researchers studied the effect of daily exposure to temperatures above the developmental threshold on Galeruca tanaceti. The results of the study showed that average temperatures close to or below the developmental threshold delayed development and in many cases increased mortality. However, They showed that G. tanaceti larvae were able to utilize the daily temperature increase above the developmental threshold in early spring and showed increased developmental rates and survival (Müller & Obermaier, 2012).

Figure 1. A. *Dorsal view of the species Galeruca tanaceti tanaceti Linnaeus, 1758;* ***B.*** *Ventral view of the species Galeruca tanaceti tanaceti Linnaeus, 1758.*

One of the significant genera within the Galerucinae subfamily is Galeruca Geoffroy, 1762. In the Palaearctic region, there are 62 species, with 13 of them found in Türkiye. These species include: Galeruca rufa Germar, 1823; Galeruca armeniaca Weise, 1886; Galeruca circassica Reitter, 1889; Galeruca dahlii dahlii Joannis, 1865; Galeruca impressicollis Pic, 1934; Galeruca interrupta Illiger, 1802; Galeruca jucunda Faldermann, 1837; Galeruca littoralis Fabricius, 1787; Galeruca planiuscula Laboissière, 1937; Galeruca pomonae pomonae Scopoli, 1763; Galeruca spectabilis orientalis Osculati, 1844; Galeruca spectabilis spectabilis Faldermann, 1837; Galeruca tanaceti tanaceti Linnaeus, 1758; and Galeruca melanocephala Ponza, 1805 (Bezdek & Serkerka, 2024). This study will provide detailed images of the aedeagus structure using stereo and SEM microscopes, as well as images of the spermatheca structure using stereo microscopes. The descriptions of the male and female genital organs of these species will be documented for the first time in this research.

MATERIALS and METHODS

The genitalia to be studied in the Gazi University collection were selected from 69 specimens of the Galeruca tanaceti tanaceti Linnaeus, 1758 species collected from Çankırı province in 2015.

The abdomens of the specimens were immersed in hot water containing 10% KOH for 2 to 10 minutes, depending on the size group of the specimens. The external genital structures were cleaned under a microscope. The remaining genital parts were then rinsed with water and preserved in 70% ethyl alcohol. These genital structures were either affixed to a separate small cardboard or placed in a small plastic tube with glycerin (bimcapsule) and pinned beside the specimen. An Olympus SZX7 stereomicroscope was utilized for the dissection of the genitalia.

For Scanning Electron Microscope examinations, the samples fixed in glutaraldehyde will be washed in sodium phosphate buffer. Then, they will be transferred to an increasing ethanol series (70%, 80%, 90% and 100%) for 15 min each and dehydration steps will be performed. After dehydration, they will be dried in the open air and then the samples will be placed on standard aluminum SEM stands to which previously prepared double-sided tapes were glued. Then, they will be coated with gold using Leica ACE 600 model Au/Pd/C coating and finally all the samples will be imaged, identified and photographed in Tescan/ GAIA3+Oxford XMax 150 EDS SEM device at 5-10 kV in Hacettepe University HUNITEK Hacettepe University Advanced Technologies Application and Research Center.

Figure 2. A. *Hacettepe University Advanced Technologies Application and Research Center, from which we provided the SEM images; B. SZX7 Stereo Microscope, from which we removed the sample genitals; C. Tescan Brand GAIA3+Oxford XMax 150 EDS model Electron Microscope, from which we obtained the SEM images.*

RESULTS and DISCUSSION

The genus is represented by 13 species in Türkiye.

***Galeruca tanaceti tanaceti* Linnaeus, 1758 (Figure 1A, B)**

Material examined: **Çankırı prov.:** Ilgaz, Yaylaören, N 40˚ 53'- E 33˚ 30', 29.V.2015, 999m., 3 specimens; Ilgaz, Yaylaören village entrance exit, N 40˚ 52'– E 33˚ 30', 17.VI.2015, 914m., 66 specimens.

The species is found **in Europe**: Albania, Andorra, Austria, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, Finland, France (incl. Corsica, Monaco), Great Britain (incl. Channel Islands), Germany, Greece (incl. Crete), Hungary, Italy (incl. Sardinia, Sicily, San Marino), Crimea, Kazakhstan, Latvia, Liechtenstein, Lithuania, Luxembourg, Moldavia, Montenegro, The Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain (incl. Gibraltar), Sweden, Switzerland, Ukraine; **in North Africa**: Algeria, Morocco (incl. Western Sahara), Tunisia; **in Asia:** Azerbaijan, Armenia, Georgia, Iran, Japan, Kyrgyzstan, Kazakhstan, Syria, Russia, Türkiye (Bezdek & Sekerka, 2024). In Asian Türkiye, it has been recorded in 10 provinces: Ankara, Çankırı, Çorum, Erzurum, Eskişehir, Isparta, Kastamonu, Kars, Ordu and Sinop. The morphology of the aedeagus and spermatheca of *Galeruca tanaceti tanaceti*

Linnaeus, 1758 was examined using SEM and a stereo microscope. Observations on their ultrastructural and detailed morphologies are presented below.

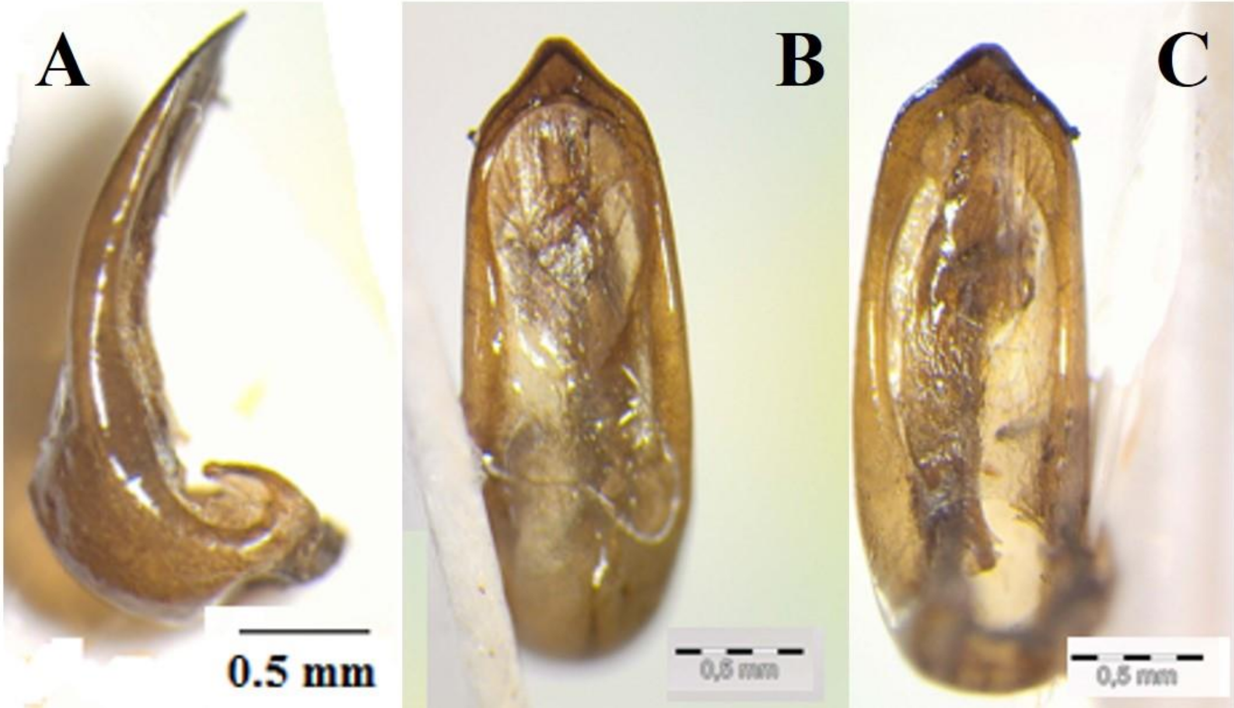

Figure 3. *Aedeagus structure of the species Galeruca tanaceti tanaceti Linnaeus, 1758* ***A.*** *Lateral view in stereo microscope,* ***B.*** *Dorsal view in stereo microscope,* ***C.*** *Ventral view in stereo microscope.*

Aedeagus: In Stereo And Sem (Scannıng Electron Mıcroscope) (Fıgure 3A-C; 4A-C; 5; 6A-D)

In lateral view; the median lobe is brown and gradually narrows from the base to the apex. It is pointed at the apex. In terms of its general shape, the median lobe resembles the claw structure in animals. In dorsal view, the median lobe runs parallel from the base to the top. It narrows at the apex to form a wide-angled triangle. It has a rounded protrusion-shaped tooth structure at the apex. The dorsal wall is seen as a line in ½ of the median lobe. The lateral edges of the median lobe are thin in the middle and thicker at the base and apex. The orifice is wide and long in the shape of an ellipse

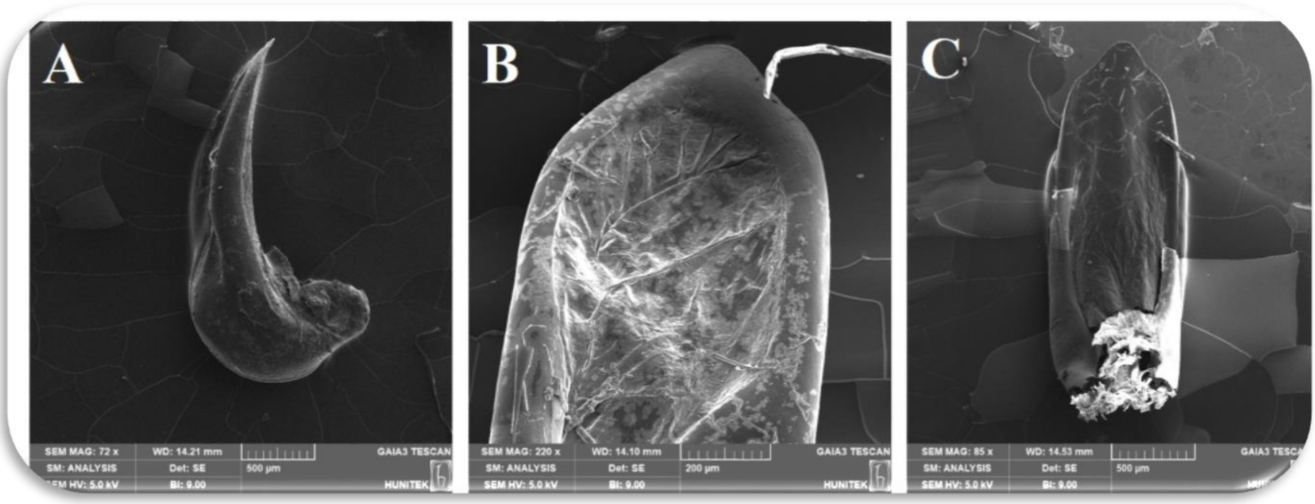

Figure 4. *Aedeagus structure of the species Galeruca tanaceti tanaceti Linnaeus, 1758* ***A.*** *Lateral view in SEM,* ***B.*** *Dorsal view in SEM,* ***C.*** *Ventral view in SEM.*

In the SEM image, unlike the stereo image, no sensilla structure is seen in this species. However, especially in the dorsal view, we noticed small and serial protrusions in the form of buds in the end lateral area (Fig. 5). It may be a distinguishing character within the species, but for confirmation of this, a comparison should be made by conducting a study on another species belonging to the same genus. It may even be a main character seen in the genus. For this reason, more studies of this kind are needed. Sensilla structure is not seen in the median lobe of this species, neither in the ventral nor in the dorsal part (Fig.6).

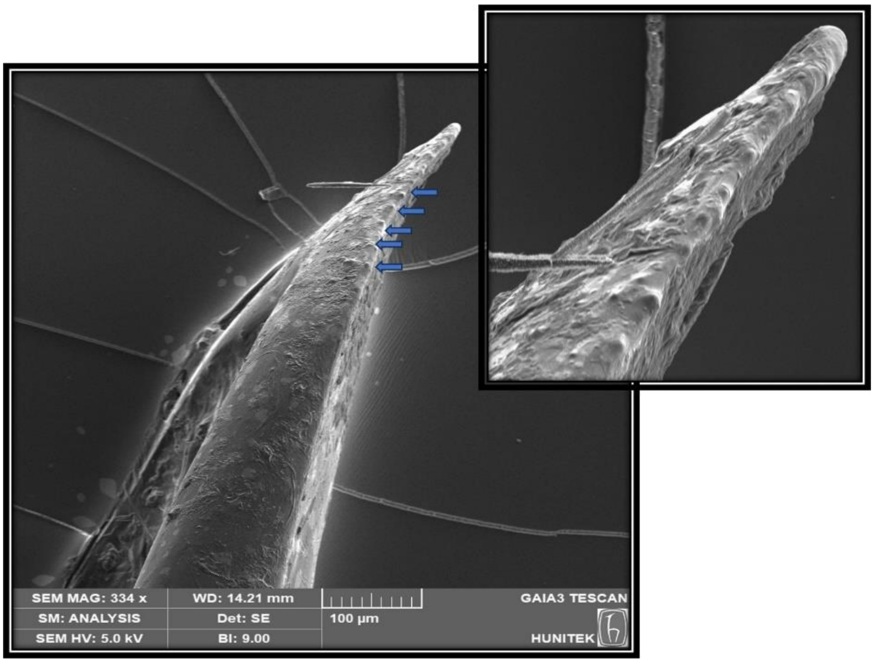

Figure 5. *An important aedegal character that can be used in the distinction of the median lobe located in the aedegal structure of Galeruca tanaceti tanaceti Linnaeus, 1758 in SEM.*

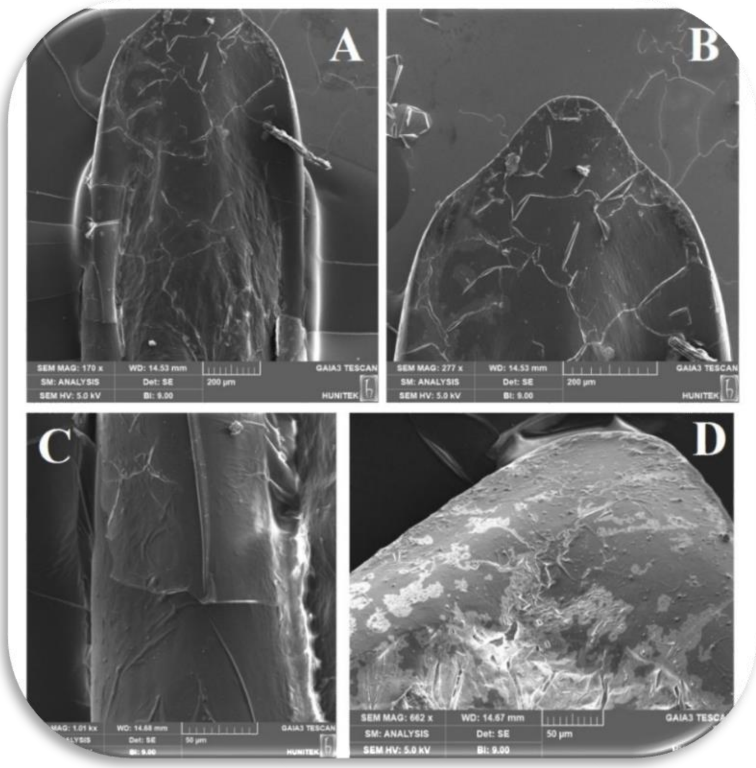

Figure 6. *Galeruca tanaceti tanaceti Linnaeus, 1758;* ***A-B.*** *ventral view of Aedeagus and apical part of the median lobe in ventral in SEM;* ***C.*** *Lateral edges of the median lobe surrounding the orifice in SEM;* ***D.*** *Apical part of the median lobe in dorsal view in SEM.*

SPERMATHECA: IN STEREO MICROSCOPE (Fig. 7)

The female genitalia of Chrysomelidae have not received the same level of attention as male genitalia for diagnostic purposes. Nonetheless, numerous studies have demonstrated the value of various female genital structures for specimen identification (Brivio 1958, 1977; Leonardi 1970, 1972; Bordy and Doguet 1987; Kangas and Rutanen 1993; Doguet 1994; Konstantinov 1998; Bordy 2000; Lingafelter and Konstantinov 2000; Biondi and D'Alessandro 2003; Baselga and Novoa 2005; Baselga 2006). Among these structures, the spermatheca is the most extensively studied due to its interspecific variability, making it a valuable diagnostic tool for taxonomic determination (Baselga, 2007).

The general shape of the spermatheca resembles a hook and is light brown. The cornu forming the vasculum has a rounded end at the apex and is not as wide as the nodulus. The nodulus has expanded almost halfway through the vasculum. The ampulla structure is not very distinct. Therefore, the collum and ramus cannot be distinguished in the stereo microscope image, and the beginning of the spermathecal canal is seen as a straight, thick pipe. Since the entire canal could not be removed, no comment could be made on its length.

Figure 7. *Spermatheca structure of the species Galeruca tanaceti tanaceti Linnaeus, 1758 in stereo microscope.*

CONCLUSION

Over the past twenty-five years, the use of insect genital characters has become increasingly prominent for accurate species identification. This rise in usage is partly due to the limitations of relying on color and other superficial differences, which are influenced by environmental factors and were commonly used in early taxonomic studies. Adult genitalia, which in many insect groups remain entirely inside the body except during copulation and oviposition, are less likely to be affected by environmental conditions and thus provide more reliable characteristics for distinguishing species. Additionally, in the Chrysomelidae family, these structures are typically heavily chitinized, making them less susceptible to modification (Powell, 1941).

In Türkiye, the genus Galeruca Geoffroy, 1762 is represented by 13 species. To date, no descriptive, detailed study has been conducted on the genitalia of this species. The studies conducted are on the development of the insect and the damage it has done, and this study, which will be the first to be conducted, will provide a chance for comparison with species in the group. Perhaps a new diagnostic character may emerge within the genus after the description of another specimen in the same genus. There has been no comprehensive research focused specifically on their reproductive structures. This study presents SEM images of the aedeagus and spermatheca of Galeruca tanaceti tanaceti Linnaeus, 1758 to the scientific community for the first time.

Spermathecal characters used in the identification and differentiation of new species belonging to various genera within the subfamily Galerucinae are very important (Cabrera & Cabrera, 2004; Liang et al., 2023). However, almost no studies have been found on the genitalia of the genus Galeruca that we studied. Each study to be conducted with this genus, which does not have a wide distribution, will allow for the transfer of important information about the genus.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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