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Detailed Morphological Characterization of Male and Female Genital Structures in *Radymna fischeri* Faldermann, 1837

Begüm PARMAKSIZ[1] | **Neslihan BAL**[1*]

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

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

Neslihan BAL, e-mail: neslihansilkin@gmail.com

Abstract: The paper presents unknown ultrastructure observed by scanning electron microscope (SEM) and stereo microscope of aedeagus and spermatheca morphologies of *Radymna fischeri* Faldermann, 1837 (Coleoptera: Chrysomelidae: Galerucinae) from Türkiye. This species, which belongs to one of the genera that is very important in biological control, where it was collected in Çankırı on 2023. The genus *Radymna* Reitter, 1913 includes 9 species in the Palaeartic Region, while it is represented by 5 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 *Radymna fischeri* Faldermann, 1837. For this reason, ultrastructural and detailed investigations of aedeagus morphology of *Radymna fischeri* Faldermann, 1837 from Türkiye were firstly studied with SEM to contain male and female genital descriptions of *Radymna fischeri* Faldermann, 1837. Photos in SEM and stereo microscope are also given in the text.

Keywords: Aedeagus, Galerucinae, *Radymna fischeri*, SEM, Türkiye.

INTRODUCTION

Chrysomelidae or leaf beetles are phytophagous insects adapted to feed on a wide range of plant groups. Both adults and larvae feed on cultivated plants as well as some useful wild plants or shrubs (Jolivet et al., 1988). The close association between the group and the plants is important both ecologically and economically because of their impact on agriculture. Many species are pests and are known to be serious pests of food crops, tree and shrub plantations, medicinal plants and forage crops, but many are useful as biological control agents of weeds (Booth et al., 1990; Jolivet and Verma, 2002).

Species belonging to the family Chrysomelidae can transmit plant pathogenic viruses to the plants they feed on. There are approximately 30 species of Chrysomelidae that carry viruses in this way, and some of these viruses can pose a serious threat to agricultural production. Species belonging to the subfamilies Criocerinae, Chrysomelinae, Alticinae and Galerucinae are potential vector carriers, especially for agricultural crops. Plant groups that are significantly affected by these families are Fabaceae, Cucurbitaceae, Poaceae and Solanaceae (Gillot, 1995).

One of the important genera belonging to the Galerucinae subfamily is *Radymna* Reitter, 1913, and there are 9 species in the Palaearctic region and 5 in Turkey. These are; *Radymna fischeri* Faldermann, 1837 (Figure 1), *Radymna maculicollis* Beenen, 2014, *Radymna nigrifrons* Laboissière, 1914, *Radymna persica* Faldermann, 1837, *Radymna quadrimaculata* L. Redtenbacher, 1849'dır (Bezdek & Serkerka, 2024).

There are very few studies on this breed. As a result of the study conducted by Güven et al 2024b, it was determined that adults and larvae of *R. fischeri* cause damage by gnawing on the vegetative parts (leaves, stem) of *Alhagi maurorum* plant and spend some of its biological stages (adult, egg, larva) on it. In this case, it was evaluated whether the relevant species could be a potential biological control agent on *Alhagi maurorum*. Based on the observations, it is thought that it may be promising in biological control against the weed.

Özdikmen and Silkin (2016) described and photographed the color variations of *Radymna fischeri* (Faldermann, 1837) from Turkey. As can be seen, there are not many studies on the species, but when

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we consider that the species has a narrow distribution area, this situation increases the importance of the species. In this study, the images of the aedeagus structure taken with stereo and SEM microscopes and the images of the spermatheca structure taken with stereo microscopes will be described. The descriptions of the male and female genital organs of the species will be included in a study for the first time.

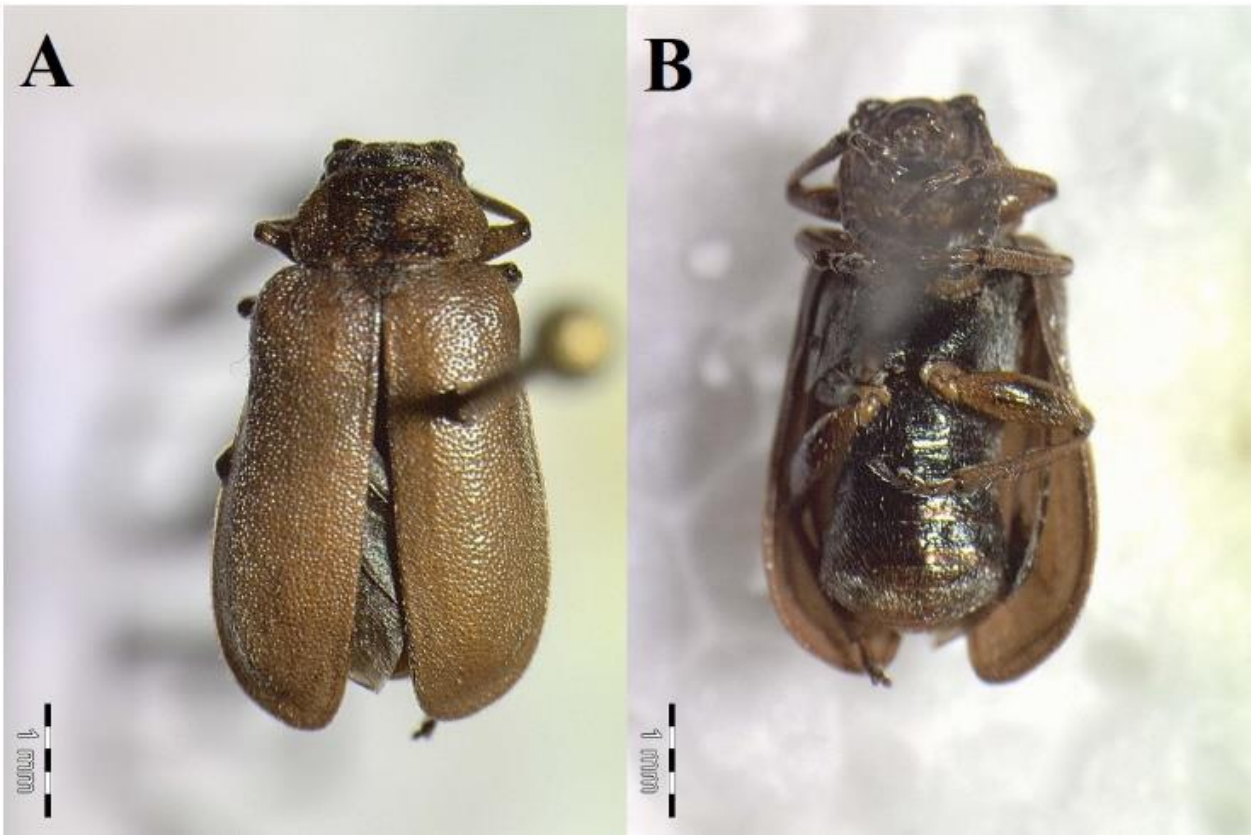

Figure 1. A. *Dorsal view of Radymna fischeri Faldermann, 1837;* ***B.*** *Ventral view of the species Radymna fischeri Faldermann, 1837.*

MATERIALS and METHODS

The genitalia to be studied in the Gazi University collection were selected from 86 specimens of the *Radymna fischeri* species collected from Çankırı province in 2014.

The abdomen of the specimens were left for 2-10 minutes according to the specimen size group in hot water containing 10% KOH. The structure outside the genitalia were cleaned under the microscope. The remaining genital parts were first washed with water and kept in 70% ethyl alcohol. The genital structures were either glued to a separate small card board or put in a small plastic tube with glycerin (bimcapsule) and pinned next to it. An Olympus brand SZX7 stereomicroscope was used for genital removal.

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 5 species in Turkey.

Radymna fischeri (Faldermann, 1837) (Figure 1A, B)

Material examined: **Çankırı prov.:** Kızılırmak, Return to Karallı Village 2nd km, N 40˚ 18'– E 33˚ 56', 25.IV.2014, 606m., 21 specimens; Kızılırmak, Between Karamursel and Boyacıoğlu, N 40˚ 26'– E 34˚ 01', 12.VII.2014, 547m., 36 specimens; Kızılırmak, Karadibek village, N 40˚ 25'- E 34˚ 00', 11.VIII.2014, 601m., 29 specimens.

The species is distributed in Europe (Russia: South European Territory), Crimea and Asia (Azerbaijan, Armenia, Georgia, Iran, Turkmenistan, Türkiye) (Bezdek & Sekerka, 2024). It has been recorded from 13 provinces (Ağrı, Ankara, Çankırı, Erzurum, Gaziantep, Iğdır, Isparta, Kayseri, Kars, Konya, Nevşehir, Niğde, Zonguldak) until now in Asian Türkiye. Aedeagus and spermatheca morphology of *Radymna fischeri* (Faldermann, 1837) were studied with SEM and stereo microscope. Obtaining observations on ultrastructural and detailed morphologies of them are presented as follows.

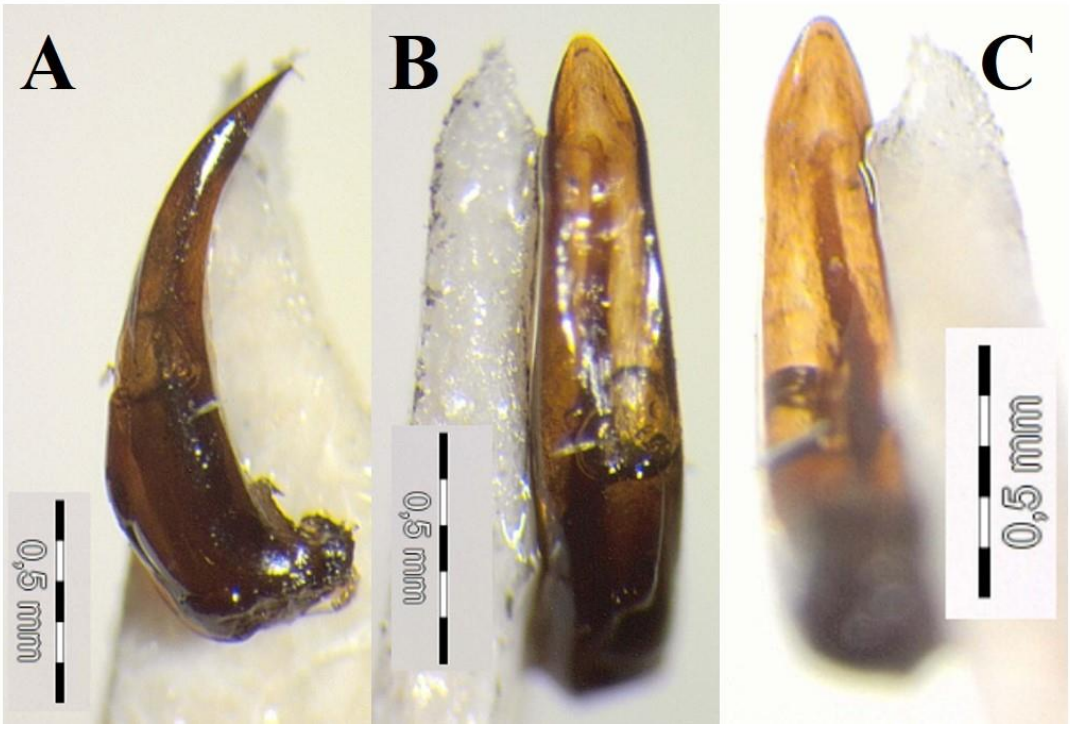

Figure 3. Aedeagus structure of *Radymna fischeri* (Faldermann, 1837) **A.** Lateral view in stereo microscope, **B.** Dorsal view in stereo microscope, **C.** Ventral view in stereo microscope.

Aedeagus: In Stereo And Sem (Scanning Electron Microscope) (Figure 3a-C)

In lateral view; the median lobe is brown and gradually becomes lighter in color from the base to the apex, while it is widest at the base and gradually narrows towards the apex. It is pointed at the apex. It resembles the claw structure in animals. In dorsal view, the median lobe narrows from the base to the apex, the apex has a rounded structure with narrow angles. The median lobe starts to narrow from the base, widens imperceptibly when it reaches the 2/3 part near the tip, and then narrows again. The dorsal wall is not seen. However, the opening from the base of the 1/3 part of the median lobe to the apex is narrow, long and U-shaped.

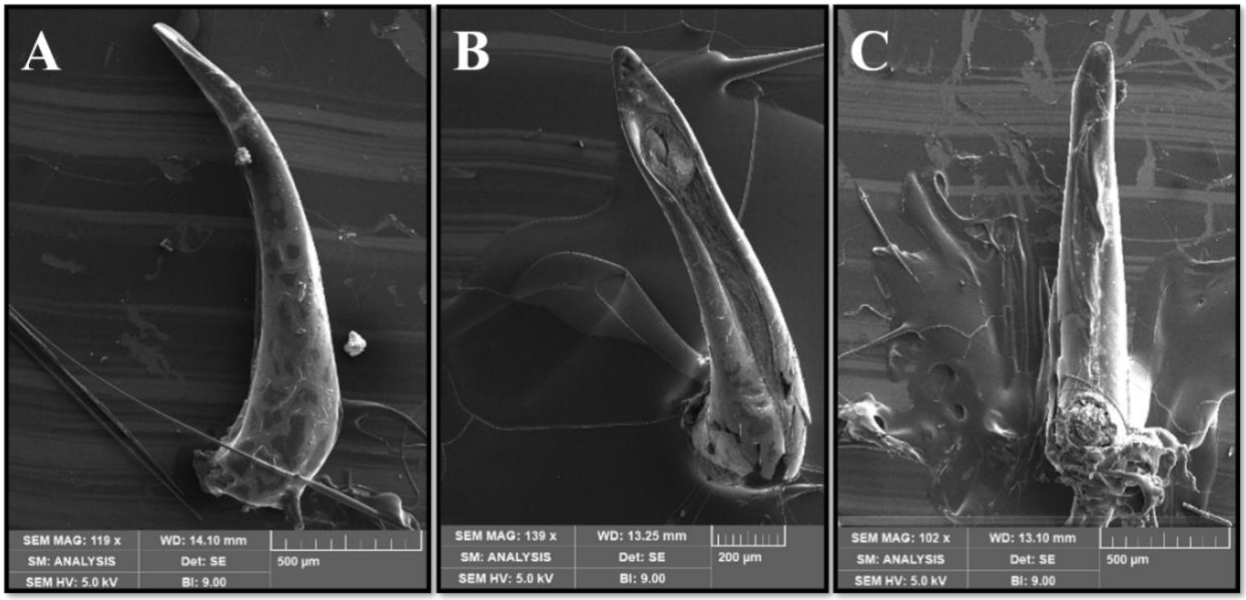

Figure 4. Aedeagus structure of *Radymna fischeri* (Faldermann, 1837) **A.** Lateral view in SEM, **B.** Dorsal view in SEM, **C.** Ventral view in SEM.

In the SEM image, unlike the stereo image, the sensilla also attract our attention in this species. In almost all studies we have conducted in Chrysomelidae to date, sensilla with structures that differ in species are also seen in this species (Ataş et al., 2019a; Ataş et al., 2019b; Ataş et al., 2019c; Bal, 2020; Bal, 2022; Bal et al., 2022a; Bal et al.,2022b; Bal et al.,2022c; Bal et al.,2022d; Özdikmen et al., 2022a; Özdikmen et al., 2022a; Ekmekçi et al., 2023a; Ekmekçi et al., 2023b; Özdikmen et al., 2023a; Özdikmen et al., 2023b; Bal & Mutlu, 2024; The sensilla, which are seen quite densely in the dorsal end of the median lobe, decrease towards the base. In addition, they are almost nonexistent in the ventral part (Fig. 5)

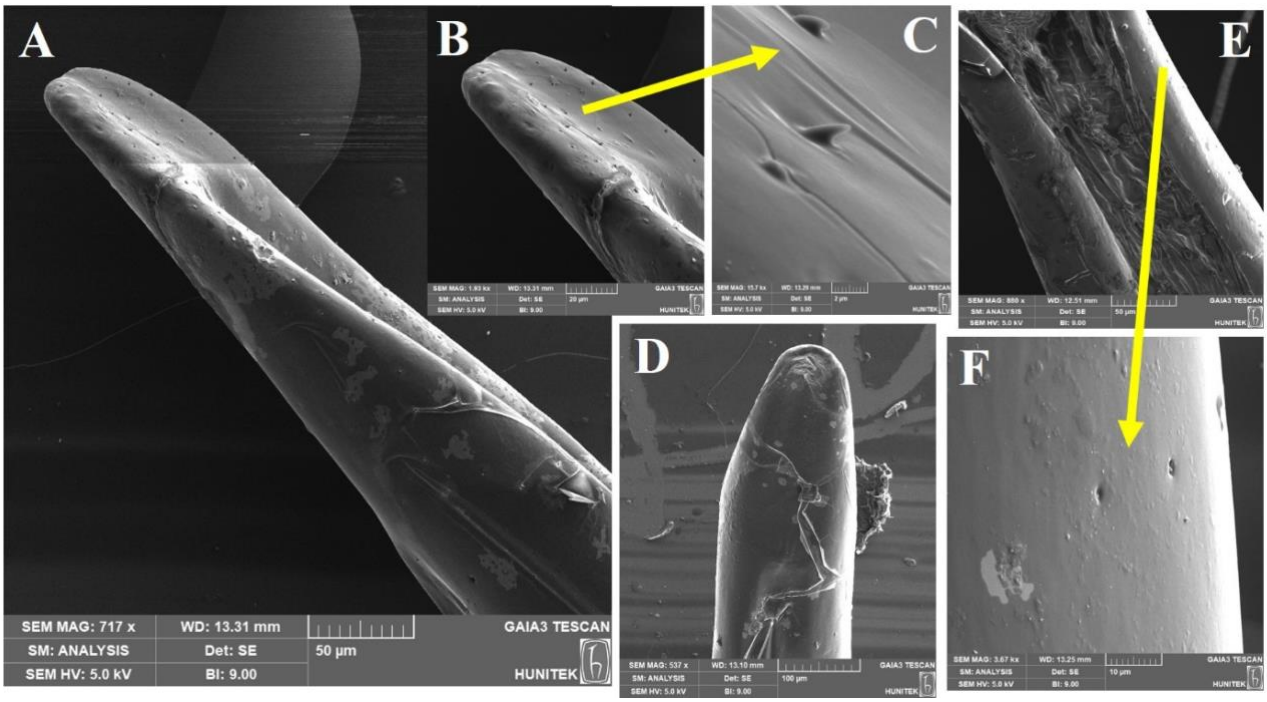

Figure 5. Aedeagus structure of *Radymna fischeri* (Faldermann, 1837) **A.** Lateral view in SEM, **B.** Dorsal view in SEM, **C.** Ventral view in SEM.

Figure 6. spermatheca structure of *Radymna fischeri* (Faldermann, 1837) in Stereo microscope.

Spermatheca: In Stereo Microscope

The female genitalia of Chrysomelidae as diagnostic characters was not taken into account with the same attention as the male genitalia. However, manypapers have shown the usefulness of diverse structures of the female genital armature for the identification of specimens (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 female genitalia, spermatheca is the most studied structure, due to its interspecific variability and thus diagnostic value in determining taxa (Baselga, 2007).

The general shape of the spermatheca resembles a swan's head and is dark brown. The nodulus and cornu that form the vasculum have a swollen structure. The cornu is inclined downwards and ends with a bud-like rounded structure at the apex. The nodulus is quite large, almost twice as wide as the cornu. The connection of the nodulus to the ampulla is not clearly seen. However, the beginning of the spermathecal canal is thin and tubular.

CONCLUSION

In Türkiye, the genus *Radymna* Reitter, 1913 is represented by 5 species. The identification of *Radymna fischeri*, which is found only in Georgia Azerbaijan, Armenia, Georgia, Iran, Turkmenistan in the Asian continent outside of Türkiye, will provide an opportunity for comparison with other *Radymna* species for scientists who are not familiar with this species. However, there is no guiding study especially on their reproductive structure. SEM images of the aedeagus and spermatheca of *Radymna fischeri* are presented to the scientific world for the first time in this study.

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 *Radymna* 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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