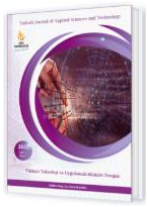
Türkiye Teknoloji ve Uygulamalı Bilimler Dergisi



ISSN: 2528-8652 e-ISSN: 2822-2660
Period Biannually Founded 2017 Publisher Siirt University
https://dergipark.org.tr/tr/pub/tubid

Chaetocnema major (Jacquelin du Val) (Chrysomelidae: Galerucinae: Alticini)'ün aedeagus ve spermateka'sının taramalı elektron mikroskobu ile ince yapısı

Ultrastructure of aedeagus and spermatheca of *Chaetocnema major* (Jacquelin du Val) (Chrysomelidae: Galerucinae: Alticini) by scanning electron microscope

Havva Ekmekçi
Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, TÜRKİYE
https://orcid.org/0000-0002-8122-7914

Hüseyin Özdikmen
Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, TÜRKİYE
https://orcid.org/0000-0002-2960-5172

Neslihan Bal*
Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, TÜRKİYE
https://orcid.org/0000-0001-9568-0093

Damla Amutkan Mutlu
Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, TÜRKİYE
https://orcid.org/0000-0002-4780-8520

Zekiye Suludere
Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, TÜRKİYE
https://orcid.org/0000-0002-1207-5814

Araştırma Makalesi

Geliş Tarihi

27/09/2023

Kabul Tarihi

01/11/2023

DOI

10.5281/zenodo.10117615

Özet

Bu makale, *Chaetocnema major* (Jacquelin du Val) türünün aedeagus ve spermatekasının bilinmeyen ultrastrüktürünü sunmaktadır. *Chaetocnema* (*Majoroides*) Özdikmen alt cinsinin tip türleri ve Türkiye'de bulunan tek türü hem stereo mikroskop hem de Taramalı Elektron Mikroskobu altında incelenmiş ve figüre edilmiştir. Aedeagi ve spermathecae şimdiye kadar sadece *Chaetocnema* Stephens cinsinde stereo mikroskobik çalışmalara dayanarak incelenmiştir. *Chaetocnema* türlerinin erkek ve dişi genital organları üzerinde bugüne kadar SEM ile ultrastrüktürel bir çalışma yapılmadığından, bu çalışma bu amaca yönelik ilk çalışmadır. Buna göre, bu çalışma *Chaetocnema major*'un aedeagus ve spermateka'sının ultrastrüktürel ve detaylı incelemeleri ile yeni diagnostik karakterleri ortaya çıkarmayı ve elde etmeyi amaçlamaktadır. Bilindiği gibi, *Chaetocnema* cinsinin spermatekaları, Alticini kabilesinin çoğu cinsi gibi, kısmen homojendir ve tür düzeyinde iyi teşhis karakterleri sağlamaz. Aedeagus üzerine yapılan çalışmalar, spermatekanın kısmi homojenliğine rağmen, aedeagusun yapısının oldukça geniş bir çeşitlilik gösterdiğini ortaya koymuştur. Öte yandan, *Chaetocnema* cinsi için, alt tür düzeyinde sperm kılıfı morfolojisi, dış morfolojik özelliklerden daha ilgili ve yararlı görünmektedir. Mevcut çalışmadan elde edilen sonuçlar temel olarak bu görüşleri desteklerken, dişi genital organın (spermateka) önemsiz ve tanısal olmadığı, ancak aedeagus tür düzeyinde tanı için önemli olduğu bulunmuştur ve ayrıca, SEM'de incelenerek hem Ultrastrüktürel morfolojiler aedeagus hem de spermathecae için yeni tanısal karakterler ortaya çıkarılmıştır.

Anahtar Kelimeler: Aedeagus, *Chaetocnema major*, spermatheca, SEM, Türkiye, Ultrastructure.

*Sorumlu Yazar: neslihansilkin@gmail.com

Research Article

Received

27/09/2023

Accepted

01/11/2023

DOI

10.5281/zenodo.10117615

Abstract

The paper presents unknown ultrastructure of aedeagus and spermatheca of *Chaetocnema major* (Jacquelin du Val) which is the type species of the subgenus *Chaetocnema* (*Majoroides*) Özdikmen and the single species of the subgenus occurring in Türkiye, are studied and figured under both stereo microscope and Scanning Electron Microscope. Aedeagi and spermathecae have so far only been studied in the genus *Chaetocnema* Stephens based on stereo microscopic studies. Since no ultrastructural studies with SEM have been conducted on male and female genitalia of *Chaetocnema* species so far, this is the first study for this purpose. Accordingly, this study mainly aims to reveal and obtain new diagnostic characters by ultrastructural and detailed examinations of the aedeagus and spermathecae of *Chaetocnema major*. As is known, spermathecae of the genus *Chaetocnema*, like most genera of the tribe Alticini, are partially homogeneous and do not provide good diagnostic characters at the species level. Studies on the aedeagus have revealed that, despite the partial homogeneity of the spermatheca, the structure of the aedeagus shows quite a wide diversity. On the other hand, for the genus *Chaetocnema*, sperm sheath morphology at the subspecies level appears to be more relevant and useful than external morphological features**.** While the results from the present study mainly support these views, the female genitalia (spermathecae) was found to be insignificant and non-diagnostic, but important for diagnosis at the aedeagus species level, and further, investigation revealed new diagnostic characters for both Ultrastructural morphologies aedeagus and spermathecae by examining in SEM.

Keywords: Aedeagus, *Chaetocnema major*, spermatheca, SEM, Türkiye, Ultrastructure.

1. Introduction

Chaetocnema Stephens (1831), member of the Alticini Newman (1835), is a cosmopolitan genus, containing a large number of worldwide distributed species (about 500 species). Mentioned 118 species for Palearctic region of which the boundaries were used larger than traditional sense (1). On the other hand, a total of 75 species were identified, 7 of which were newly described species (2). Because in this study, in contradistinction to Döberl (2010), the boundaries of the Palearctic region were used narrower than the traditional meaning. The genus is represented with a total of 26 species in Türkiye that is in the Palearctic region (3-5). Therefore, Turkish fauna includes approximately 35% of all Palearctic species by Konstantinov et al. while approximately 30% of all Palearctic species by Döberl (1,2).

Subgeneric classification of the genus *Chaetocnema* Stephens was recently reviewed and arranged by Özdikmen (2021). He proposed a total of 13 subgenera for the genus *Chaetocnema* Stephens. The Palearctic subgenus *Chaetocnema* (*Majoroides*) Özdikmen, 2021 is one of the proposed subgenera in his study. Accordingly, *Chaetocnema major* (Jacquelin du Val, 1852) is the type species of this subgenus. Therefore, the Palearctic subgenus *Chaetocnema* (*Majoroides*) Özdikmen, 2021 has three species including the type species as *Chaetocnema major* (Jacquelin du Val, 1852), *Chaetocnema mandschurica* Heikertinger, 1951 and *Chaetocnema schlaeflii* (Stierlin, 1866). In Türkiye, the subgenus is represented only with the type species *Chaetocnema major* (Jacquelin du Val, 1852) (5).

Chaetocnema species are very similar in habit and cannot be separated by external characters alone. Genitalic characters have been used traditionally and are diagnostically important by Konstantinov et al (2). However, works on aedeagi and spermathecae of *Chaetocnema* species so far

were studied on the basis of only stereo microscopic studies (2,6,7). and the others, for example, Ruan et al. hile ultrastructure of aedeagi and spermathecae of *Chaetocnema* species with SEM have not been studies up to now (8,9).

As known, aedeagal morphology in the genus *Chaetocnema* Stephens, 1831, like in most genera of the tribe Alticini, seems to be diagnostic at the species level because of there are many new species described based on aedeagal structure. On the contrary of aedeagal morphology, spermathecal morphology seems to be not diagnostic at the species level because of there is no new species described only based on spermathecal structure. This is probably why the spermathecae of Nearctic species were not studied in White's (1996) revision. The preliminary studies we have done on ultrastructure of aedeagi and spermatheca of the *Cassida* Linnaeus, 1758 and *Hypocassida* Weise, 1893 revealed new diagnostic characters in both species level and subgeneric level (10-19). These positive results obtained in previous studies seem to be valid for the genus *Chaetocnema* and encourage us to carry out the study.

2. Material and Method

The available specimens for the present work were collected in Çankırı and Mersin provinces from Türkiye in April, June and August 2000, 2014 and 2015. The specimens were deposited at Gazi University (Türkiye, Ankara).

The aedeagi and spermathecae were dissected from the abdomen, and the remaining tissue was removed with fine tweezers. For stereo microscopic examination after cleaning, the samples were placed with 70% ethanol and examined with Olympus SZX7 stereomicroscope and Leica Z-16 APO stereo microscope. For scanning electron microscopy (SEM), cleaned samples were dehydrated using an ascending series of ethanol (70%, 80%, 90%, and 100%) and then air dried. After that the specimens were mounted onto SEM stubs using a double-sided adhesive tape, coated with gold using a Polaron SC 502 Sputter Coater, and examined with a JEOL JSM 6060 Scanning Electron Microscope (SEM) at 10 kV in Prof. Dr. Zekiye Suludere Electrone Microscopy Center at Gazi University. The main spermathecal and aedeagal terminologies used in the present study are given below (Figure 1).

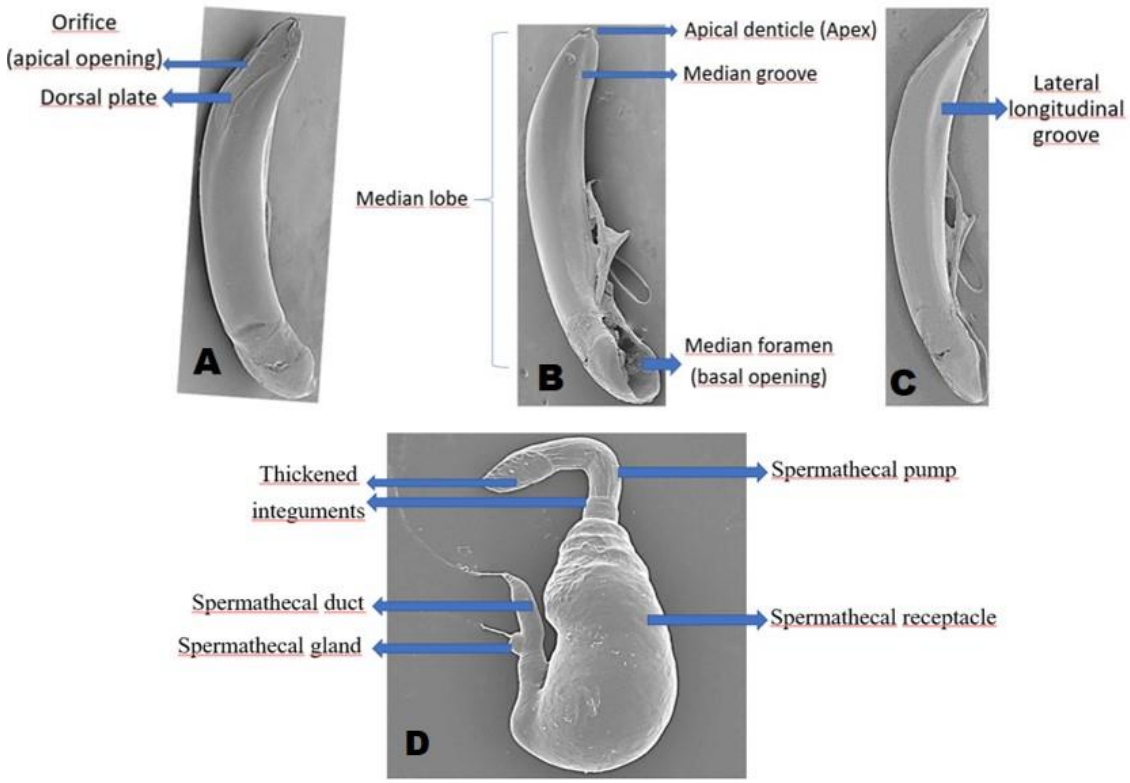


Figure 1. Aedeagal terminologies, **A.** ventro-lateral view, **B.** dorso-lateral view, **C.** lateral view [aedeagus of *Chaetocnema major* (Jacquelin du Val, 1852)], **D.**Spermathecal terminologies [spermatheca of *Chaetocnema major* (Jacquelin du Val, 1852)].

3. Results

Genus *Chaetocnema* Stephens, 1831

(type sp.: *Chrysomela concinna* Marsham, 1802, later designation by Westwood, 1838: 42).

The cosmopolitan genus is represented by a total of 26 species belonging to six subgenera in Türkiye, which is located in the Palearctic region (3-5).According to the number of species included, the subgenus *Chaetocnema* (*Hortensoides*) Özdikmen, 2021 with 13 species (50%) is strikingly prevailing, and the nominate subgenus *Chaetocnema* (*Chaetocnema*) Stephens, 1831 with seven species (about 27%) follows it. Therefore, about 77% of the *Chaetocnema* species in Türkiye belong to these two subgenera. The remaining six species belong to the subgenera *Chaetocnema* (*Plectroscelis*) Dejean, 1836 and *Chaetocnema* (*Pseudochaetocnema*) Özdikmen, 2021 each with two species (about 8%), while to the subgenera *Chaetocnema* (*Majoroides*) Özdikmen, 2021 and *Chaetocnema* (*Udorpes*) Motschulsky, 1845 each with one species (about 4%). All *Chaetocnema* species known to be distributed in Türkiye and their subgenera can present as in following table (Table 1).

Subgenus *Majoroides* Özdikmen, 2021 (type sp.: *Plectroscelis major* Jacquelin du Val, 1852)

The Palearctic subgenusincludes three species. In Türkiye, the subgenus is represented only with the type species *Chaetocnema major* (Jacquelin du Val, 1852) as seen in table below (5).

Table 1. All known *Chaetocnema* species in Türkiye and their subgenera.

***CHAETOCNEMA* SPECIES**	***CHAETOCNEMA* SUBGENERA**
7 species	***Chaetocnema* Stephens, 1831**
C. breviuscula (Faldermann, 1837)	*Chaetocnema* Stephens, 1831
C. concinna (Marsham, 1802)	*Chaetocnema* Stephens, 1831
C. delarouzeei (Brisout, 1884)	*Chaetocnema* Stephens, 1831
C. picipes Stephens, 1831	*Chaetocnema* Stephens, 1831
C. scheffleri (Kutschera, 1864)	*Chaetocnema* Stephens, 1831
C. semicoerulea (Koch, 1803)	*Chaetocnema* Stephens, 1831
C. tibialis (Illiger, 1807)	*Chaetocnema* Stephens, 1831
13 species	***Hortensoides* Özdikmen, 2021**
*C. aerosa (*Letzner, *1847)*	*Hortensoides* Özdikmen, 2021
C. arenacea (Allard, 1860)	*Hortensoides* Özdikmen, 2021
C. arida Foudraas, 1860	*Hortensoides* Özdikmen, 2021
C. aridula (Gyllenhal, 1827)	*Hortensoides* Özdikmen, 2021
C. confusa (Boheman, 1851)	*Hortensoides* Özdikmen, 2021
C.hortensis (Geoffroy, 1785)	*Hortensoides* Özdikmen, 2021
C.igori (Konstantinov *et. Al., 2011)*	*Hortensoides* Özdikmen, 2021
C. imitatrix (Gruev, 1990)	*Hortensoides* Özdikmen, 2021
C. mannerheimii (Gyllenhal, 1827)	*Hortensoides* Özdikmen, 2021
C. montenegrina Heikertinger, 1912	*Hortensoides* Özdikmen, 2021
C. obesa (Boieldieu, 1859)	*Hortensoides* Özdikmen, 2021
C. sahlbergii (Gyllenhal, 1827)	*Hortensoides* Özdikmen, 2021
C. subcoerulea (Kutschera, 1864)	*Hortensoides* Özdikmen, 2021
1 species	***Majoroides* Özdikmen, 2021**
C.major (Jacquelin du val, 1852)	*Majoroides* Özdikmen, 2021
2 species	***Plectroscelis* Dejean, 1836**
C. chlorophana (Duftschmid, 1825)	*Plectroscelis* Dejean, 1836
C. coyei (Allard, 1864)	*Plectroscelis* Dejean, 1836
2 species	***Pseudochaetocnema* Özdikmen, 2021**
C. conducta (Motschulsky, 1838)	*Pseudochaetocnema* Özdikmen, 2021
C. orientalis (Bauduer, 1874)	*Pseudochaetocnema* Özdikmen, 2021
1 species	***Udorpes*, Motshulsky, 1845**
C. procerula (Rosenhauer, 1856)	*Udorpes*, Motshulsky, 1845

Chaetocnema major (Jacquelin du Val, 1852)

Plectroscelis major Jacquelin du Val, 1852: 717

Plectroscelis perrisi Bauduer, 1874: clxi

Material examined: Türkiye: Çankırı prov.: Bayramören, Sazak, 40˚59'14" N 33˚05'11" E, 1408 m, 21.VIII.2014, leg. N. Bal, 16 specimens; Çankırı prov.: Bayramören, between Erenler-Dolaşlar, 40˚56'46''N 33˚8'53''E, 925 m, 24.IV.2015, leg. N. Bal, 1 specimen; Mersin prov.: Mut-Karaman arası, 1140 m, 30.VI.2000, leg. H. Özdikmen, 1 specimen (Figure 3).

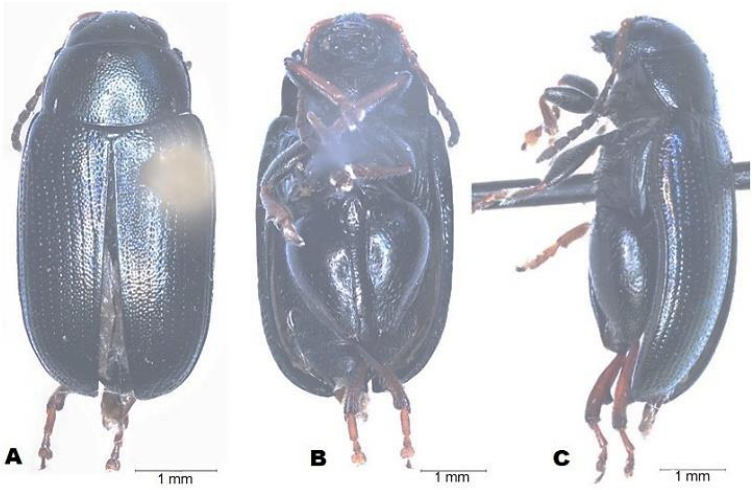


Figure 2. *Chaetocnema* (*Majoroides*) *major* (Jacquelin du Val, 1852) habitus**A.** Dorsal view, **B.** Ventral view, **C.** Lateral view. (in stereo microscope).

The Palearctic species is distributed in Europe (Albania, Austria, Bulgaria, Czeck Republic, European Russia, European Türkiye, France, Greece, Hungary, Moldova, Romania, Serbia, Slovakia, Slovenia, Spain and Ukraine), North Africa (Algeria) and Asia (Armenia, Azerbaijan, Cyprus, Iran, Iraq, Israel, Japan (Honshu), Kazakhstan, Syria, Türkiye (1,2).

The flea beetle is a little known species for Türkiye. It has so far been recorded in nine of Türkiye's 81 provinces and four of seven regions; Adana, Ankara, Burdur, Çanakkale, Çankırı, Eskisehir, Erzurum, Mersin in Anatolia (Asian Türkiye) and Edirne in European Türkiye (Thrace) (Figure 3) (2, 20-29).

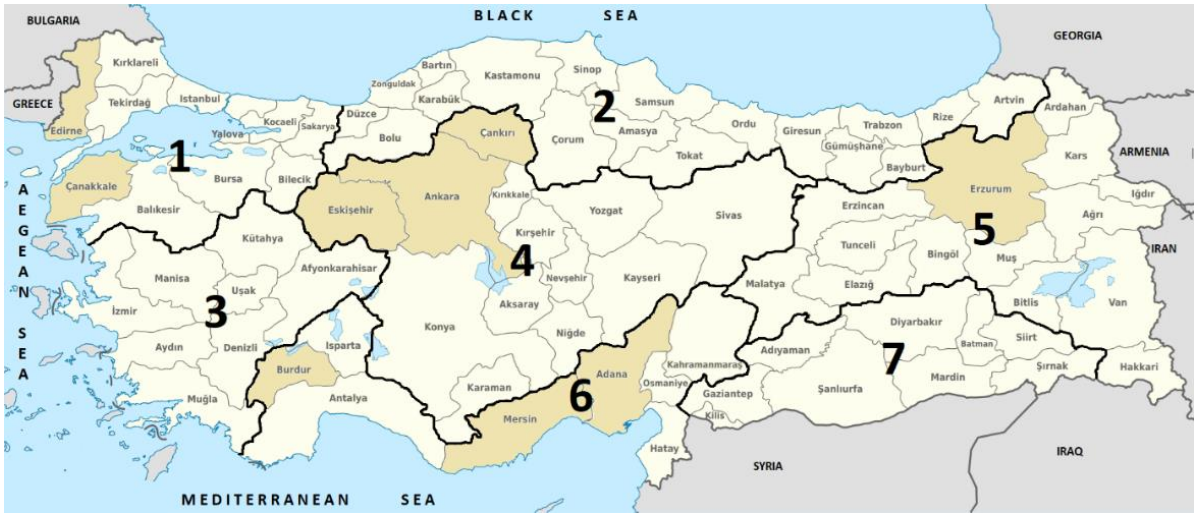


Figure 3. Provincial and regional distribution patterns of *Chaetocnema major* (Jacquelin du Val, 1852) in Türkiye

4. Discussion

Classification is mainly based on external morphological characters, but many of these classifications are constantly changing due to homoplasy and symplesiomorphy within character sets.

For this reason, taxonomists have studied internal and genital morphological traits in addition to external morphological characters to provide a more stable classification (30-32).

As is well known, the structures of the aedeagus and spermateca are taxonomically important. It is therefore important to reveal more diagnostic characters than hitherto known, ultrastructural and detailed examinations of the aedeagus and spermathecae of *Chaetocnema* major (Jacquelin du Val, 1852) in Türkiye were investigated for the first time by scanning electron microscopy and stereo microscopy. The observations obtained are presented below.

Male genitalia organs are commonly used to distinguish species, while female genitalia are used less frequently (33-36). Their variations are useful to distinguish between the species group level and the genus group level (32). There are two types of aedeagus according to the presence of paramers in males of the Chrysomeloidea superfamily. The first type, in which paramers are very well developed, is seen in the families Orsodacnidae, Megalopodidae and Chrysomelidae (Sagriinae and Donaciinae subfamilies). The second type, in which there are no paramers, is seen in all other subfamilies of Chrysomelidae family (37). Therefore, male genitalia does not include paramers in the tribe Alticini and hereby in the genus *Chaetocnema* Stephens, 1831.

Aedeagus (Figures 4-8C)**:**

Apical third of aedeagus light brown colored, remaining parts of aedeagus brown to dark brown or even blackish colored in stereo microscope. Median lobe more or less lanceolate. Aedeagus with long internal flange.

Ventral view:

Median lobe almost completely parallel except for slightly narrowed laterally in the middle part. The apical third of the median lobe is parallel. The width of the aedeagus is almost equal to the width just before the apical slope, distal to the basal opening. The apical part of the median lobe narrows abruptly. Apical denticle or apex of median lobe well-differentiated, distinct, slightly protruded papil-shaped, narrowed, and rounded on top. The surface of the median lobe is convex apically, medially and basally lateral to the median groove. Median groove wide in all parts, more distinct in apical part. Minute transverse wrinkles absent entirely. Median foramen (basal opening) not completely circular. Its upper margin sinuous (recessed towards the median lobe and undulated).

Figure 4. *Chaetocnema* (*Majoroides*) *major* (Jacquelin du Val, 1852) Aedeagus **A.** Lateral view in SEM, **B.** Ventro-lateral view in SEM, **C.** Dorso-lateral view in SEM; Aedeagus, **D.**Ventral view in stereo microscope, **E.** Dorsal viewin stereo microscope, **F.** Ventro-lateral viewin stereo microscope; **G.** Integrated form of aedeagus.

Lateral view:

Median lobe almost evenly and slightly curved towards the ventral side. Median lobe typically with lateral longitudinal grooves along apical part. Apex of median lobe slightly narrowed, not pointed, almost straight or slightly curved dorsally. Maximal curvature situated medially. Median lobe with scattered and sparse ultrastructural sensillae in all parts in SEM. However, the ultrastructural sensillae are denser and more numerous in the apical part than in the otherparts in SEM. Integrated form of aedeagus narrowed sub-oval.

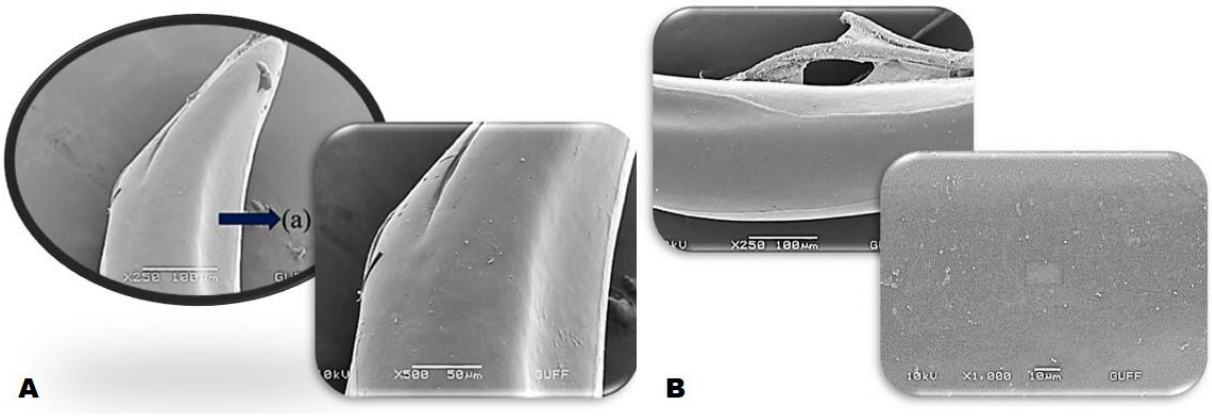


Figure 5.A. Lateral longitudinal groove (a) on apical third of aedeagus in lateral view (SEM); **B.** Sensillae on the middle part of median lobe in lateral view (SEM).

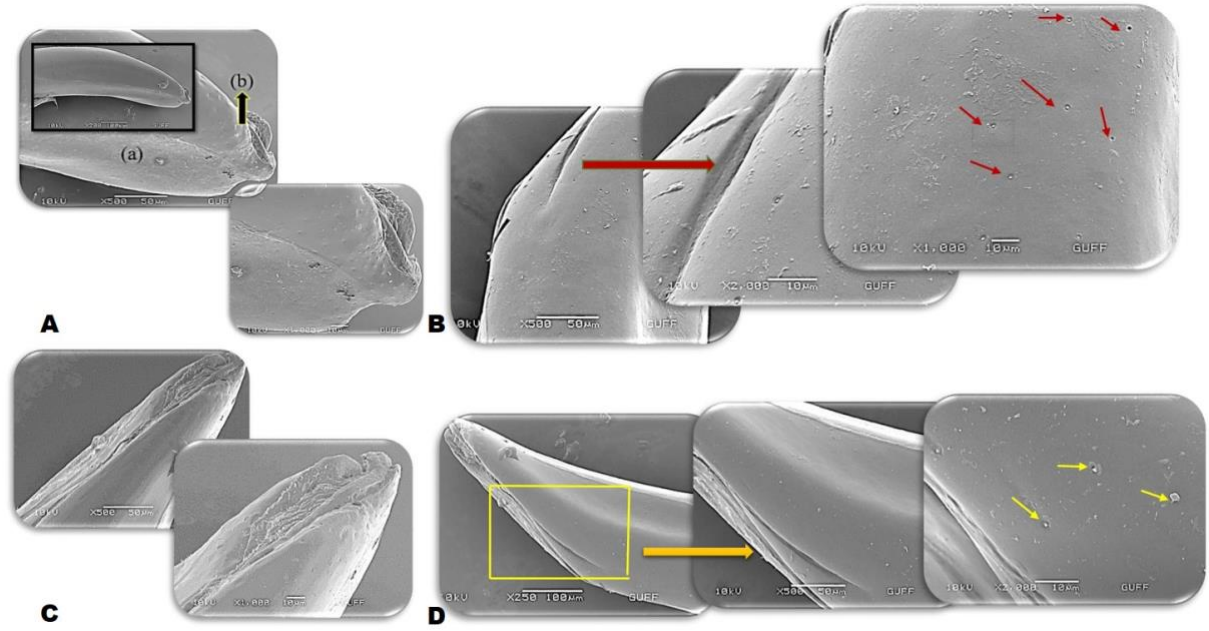


Figure 6. A. Median groove (a), lateral sensillae (b) on apical part in ventro-lateral view (SEM); **B.** Sensillae on apical part of median lobe in lateral view (SEM); **C.** Sensillae and apical denticle on apex of median lobe in latero-dorsal view (SEM); **D.** Sensillae on the apex of median lobe in lateral view (SEM).

Dorsal view:

Median lobe almost evenly convex except for apical part. In the apical part, it depressed towards the ventral side of the median lobe, so median lobe slightly curved towards its ventral side. Dorsal plate narrowed, elongated like a tongue, straight basal to distal, widened distally, so basally narrower than distal. Dorsal plate fused to dorsal surface of median lobe basally and divides the orifice into two halves in the middle. Median lobe with a collapse medially on the bases of apical opening and dorsal plate. Orifice distinct but narrowed. U-shaped or wide-angled V-shaped basally.

The inner reproductive organs of female insects include a pair of ovaries with associated oviducts, the middle ectodermal tube, vagina, bursa copulatrix and spermatheca, an invasion of the eighth abdominal segment whose number and shape depend on the insect group. The spermatheca

maintains and preserves the viability of the sperm until the moment of fertilization and plays an important role in mating and ovulation(32, 38-41).

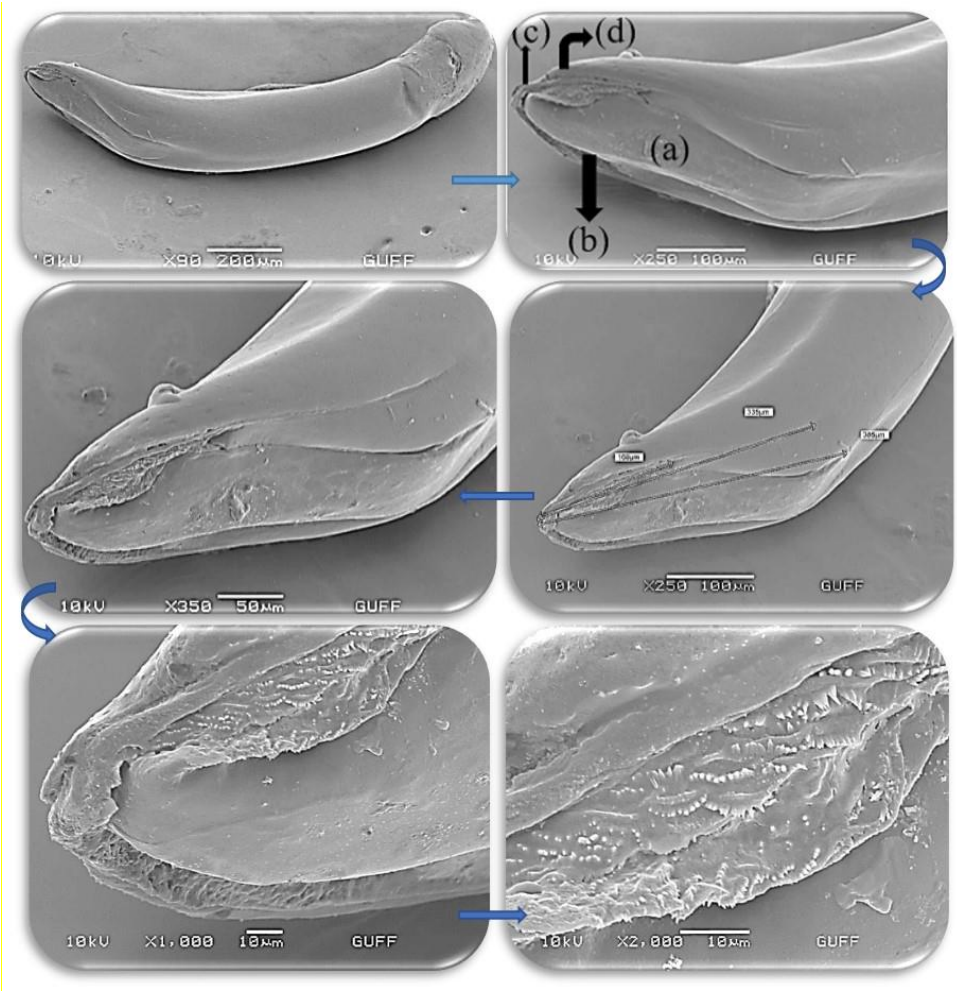


Figure 7. Apical part of median lobe [dorsal plate (a), orifice (b), apical denticle (c) and lateral sensillae (d)] in dorso-lateral view (SEM).

In Coleoptera, five models of spermathecal morphology have been distinguished by De Marzo (2008), with the presence, absence or variations of the spermathecal capsule, duct and gland. The most common model is the presence of only one spermathecal capsule (chamber + pump or velum) connected to the bursa copulatrix by a spermathecal duct and a spermathecal gland (32, 38, 41,42)..

In Chrysomeloidea, the spermatheca has been useful for identifying subfamilies, genera and sometimes species (35, 41, 43-49). According to Rodríguez-Mirón et al. (2017), spermatheca provide characters for the diagnosis of genera and subgenera and also spermatheca have high taxonomic value for the diagnosis of taxa of various orders.

Spermatheca (Figures 1, 8, 9)

Spermathecal pump and spermathecal duct light brown colored, spermathecal receptacle browncolored in stereo microscope. Spermathecal pump long, about as long as spermathecal receptacle or a little shorter, at least always longer than half length of receptacle.

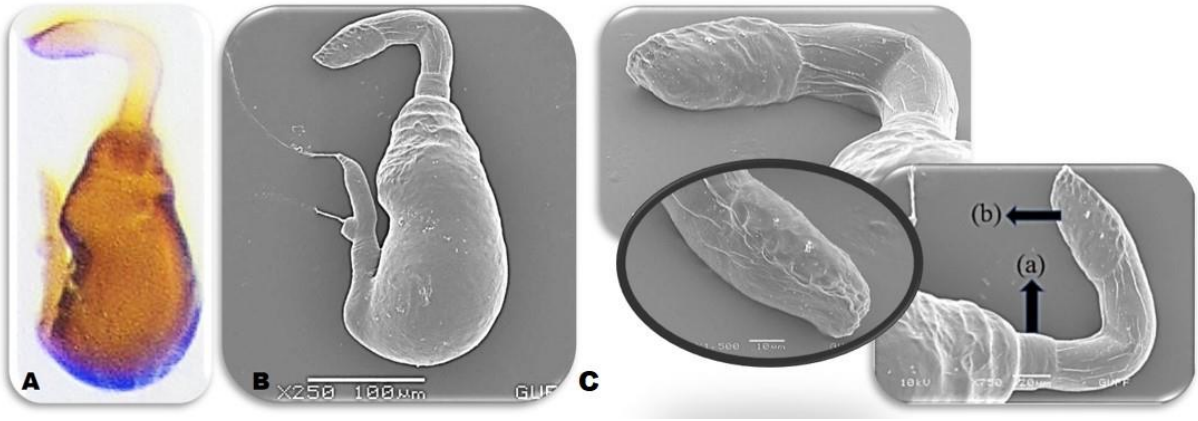


Figure 8. A. Spermatheca in stereo microscope, **B.** Spermatheca in SEM; **C.**Spermathecal pump with basal (a) and apical (b) additional integuments (SEM).

Apex of spermathecal pump more or less cylindrical or rounded. Apical part of spermathecal pump on the underside with an additional thickened integument of which surface distinctly rugose. Therefore, apical part of spermathecal pump seems darker colored in stereo microscopic view. Due to the small protrusion of this additional integument, the apex may present a more or less tapered appearance in SEM. Spermathecal pump in the basal part also with an additional thickened integument of which surface smooth, like a ring encircling the basal part. Spermathecal pump attached to middle of spermathecal receptacle top.

Spermathecal receptacle shortened, widened, more or less pyriform and sinuate ventro-medially. The surface of spermathecal receptacle almost homogeneously rugulose. The maximum width of the spermathecal receptacle is located at the base and gradually narrows towards the tip in the apical part. Therefore, the basal part of the chamber is wider than the apical part and the minimum width of the chamber is located apically.

The spermathecal duct is shortened, shorter than the receptacle. However, the apex of the spermathecal duct is about 4/5 of the chamber and the spermathecal gland is about in the middle of the chamber. The basal and apical parts of the spermathecal duct are more or less flat. The spermathecal duct connects to the ventro-lateral part of the base of the spermathecal receptacle. The general shape of the spermatheca is more or less cylindrical or pyriform.

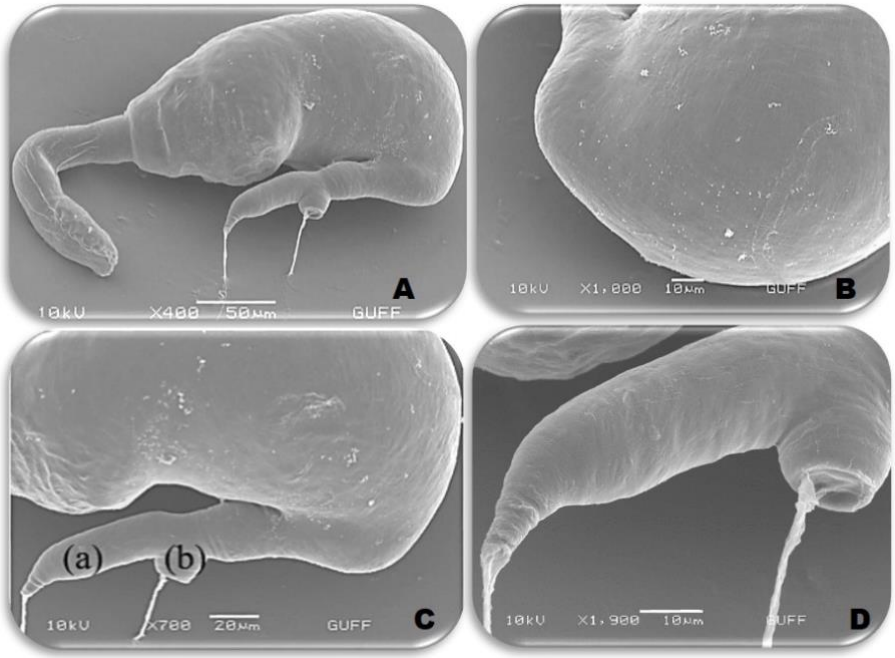


Figure 9. A. Spermathecal receptacle (SEM), ventro-lateral view, **B.** Surface of spermathecal receptacle (SEM), **C.** Spermathecal duct (a) and spermathecal gland (b) (SEM), **D.** Surfaces of spermathecal duct and spermathecal gland (SEM).

5. Conclusion

In Türkiye, the genus *Chaetocnema* Stephens, 1831 is represented by 26 species belonging to 6 subgenera. However, there is no guiding study especially on their reproductive structure. Among these 26 species, the most studied species are *C. coyei* (Allard, 1864), *C. tibialis* (Illiger, 1807), *C. arenacea* (Allard, 1860) and *C. hortensis* (Geoffroy, 1785), and the most information was found in this group. In contrast, *C. aerosa* (Letzner, 1847), *C. delarouzeei* (Brisout, 1884), *C. imitatrix* Gruev, 1990, *C. picipes* Stephens, 1831 and *C. subcoerulea* (Kutschera, 1864) are the least studied species known from only one province in Türkiye. Most of the remaining species are either poorly known or limitedly known in Türkiye. *C. major* (Jacquelin du Val, 1852) is one of the less known species.

At the species level, the adeagal morphology of *Chaetocnema* Stephens, 1831 appears diagnostic, whereas the spermathecal morphology appears non-diagnostic or partially non-diagnostic. According to this view, spermatheca has never before been used for identification and species determination in studies of the genus *Chaetocnema* Stephens (1831). Male genitalia structures (aedeagus) are commonly used, but there are few studies on this subject. Thus, there are many species described using Aedeagal structures, but no species described using only Spermathecal structures. This study supports the above views on species groups. SEM images of the aedeagus and spermatheca of *C. major* (Jacquelin du Val, 1852) are presented to the scientific world for the first time in this study. When identifying specimens belonging to the genus *Chaetocnema* Stephens, 1831, the useful characters in the structure of the aedeagus are mainly the general form of the aedeagus and the apical part of the median lobe. According to Konstantinov et al. (2011), *Chaetocnema major* can be distinguished from other

species by the following characters: The upper middle margin of the metatibia has the same denticle as the upper lateral margin; the aedeagus with lateral longitudinal grooves along the apical part and with a very long inner flange (sometimes exceeding the aedeagal apex in length). As a result of the SEM images obtained in this study, some new diagnostic characters were added to these characters: for aedeagus structure: apical denticle structures, median groove, lateral longitudinal grooves and structure of the dorsal plate in the apical part of the middle lobe, density of sensilla in the middle lobe and chitinization rate of the aedeagus; for spermatheca structure: additional thickened shells of the spermathecal pump, attachment points of the spermathecal pump to the spermathecal receptacle and spermathecal duct, and mainly the chitinization rate of the spermatheca.

Acknowledgments

This study is derived from the first author's doctoral thesis.

References

1. Döberl M. *Alticinae*. In: Löbl, I., Smetana, A. (Eds) Catalogue of Palearctic Coleoptera. Vol. 6. Apollo Books, Stenstrup, 2010; 491-563.
2. Konstantinov AS, Baselga A, Grebennikov VV., Prena J, Lingalfelter SW. Revision of the Palearctic *Chaetocnema* species. (Coleoptera: Chrysomelidae: Galerucinae: Alticini). Pensoft, Sofia, 2011; 363 pp.
3. Ekiz AN, Şen İ, Aslan EG, Gök A. Checklist of leaf beetles (Coleoptera: Chrysomelidae) of Türkiye, excluding Bruchinae. Journal of Natural History, 2013; 47(33-34), 2213-2287.
4. Özdikmen H. Chorotype identification for Turkish Chrysomeloidea (Coleoptera) Part VIII – Chrysomelidae: Alticinae. Munis Entomology & Zoology, 2014; 9(1), 325-375.
5. Özdikmen H. A review: A new subgeneric arrangement of the genus Chaetocnema Stephens (Chrysomelidae: Galerucinae: Alticini) with new subgenera based on spermathecal structures. Munis Entomology & Zoology,2021; 16(1), 41-105.
6. White RE. A revision of the genus *Chaetocnema* of America north of Mexico (Coleoptera: Chrysomelidae). Contributions of the American Entomological Institute, 1996; 29(1), 1-158.
7. Ruan Y, Yang X, Konstantinov AS, Prathapan KD, Zhang M. Revision of the Oriental *Chaetocnema* species (Coleoptera, Chrysomelidae, Galerucinae, Alticini). *Zootaxa*, 2019; 4699(1), 001-206.
8. Ruan Y, Konstantinov AS, Ge S, Yang X. Revision of the *Chaetocnema picipes* species-group (Coleoptera, Chrysomelidae, Galerucinae, Alticini) in China, with descriptions of three new species. Zookeys, 2014a; 387, 11-32.
9. Ruan Y, Konstantinov AS., Ge S, Yang X. Revision of *Chaetocnema semicoerulea* species-group (Coleoptera, Chrysomelidae, Galerucinae, Alticini) in China, with descriptions of three new species. Zookeys, 2014b;463, 57-74.

10. Ataş F, Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. A SEM study on aedeagus and spermatheca of *Cassida seraphina* Ménétries, 1836 (Coleoptera: Chrysomelidae: Cassidinae) from Türkiye. Munis Entomology & Zoology, 2019a; 14(2), 395-411.
11. Ataş F, Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. A SEM study on aedeagus and spermatheca of *Cassida hablitziae* Motschulsky, 1838 (Coleoptera: Chrysomelidae: Cassidinae) from Türkiye. Munis Entomology & Zoology, 2019b; 14(2), 519-529.
12. Bal N, Özdikmen H. Aedeagus structure of *Cassida prasina* Illiger, 1798 (Coleoptera: Chrysomelidae: Cassidinae) in Scanning Electrone Microscope (SEM). KSU Tarım ve Doğa Dergisi - KSU Journal of Agriculture and Nature, 2020; 23: 748-753.
13. Özdikmen H, Bal N. The structure of spermatheca in the subgenus *Cassida* (*Cassida*) Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) and its taxonomic significance. Fresenius Environmental Buletin,2021;30(5), 5270-5291.
14. Özdikmen H, Coral Şahin D, Bal N, A new species of *Cassida* Linnaeus, 1758 from Türkiye (Chrysomelidae: Cassidinae). Microscopy Research and Technique, 2020a; 83, 1171-1177.
15. Özdikmen H, Bal N, Amutkan Mutlu D,Suludere Z. A SEM study on aedeagus and spermatheca of *Cassida nebulosa* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Türkiye. Munis Entomology & Zoology, 2020b;15(1), 252-261.
16. Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. Comparative ultrastructural analysis of six subgenera of *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) based on spermatheca of the type species and its taxonomic significance. Transactions of the American Entomological Society, 2021a; 147(1), 67-99.
17. Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. The structure of spermathecae in the subgenus *Cassida* (*Onychocassis*) Spaeth in Spaeth & Reitter, 1926 (Coleoptera: Chrysomelidae: Cassidinae) and its taxonomic significance. Munis Entomology & Zoology, 2021b; 16(2), 972-984.
18. Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. Ultrasturucture of aedeagus and spermatheca of type species of *Hypocassida* Weise, 1893 (Chrysomelidae: Cassidinae: Cassidini) and their taxonomic significance. International Journal of Tropical Insect Science, 2022a; 42, 2337-2349.
19. Özdikmen H, Bal N, Amutkan Mutlu D, Suludere Z. Comparative ultrastructural analysis to seven subgenera of *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) based on aedeagus of the type species and its taxonomic significance. Transactions of the American Entomological Society, 2022b;148(1), 65-112
20. Sahlberg J. Coleoptera Mediterranea Orientalia, Quae in Aegypto, Palaestina, Syria, Caramania atque in Anatolia Occidentali anno 1904. Öfversigt af Finska Vatenskaps–Societetens Förhandlingar, 1913; 55A(19), 1-281.

21. Tölg F. Feststellungen von Nahrungspflanzen kleinasiatischer Halticinen und Ceuthorrhynchinen. Koleopterologische Rundschau, 1938; 24(6), 234-241.
22. Csiki, E, Heikertinger F. *Chrysomelidae, Halticinae*, in: Junk, W. and Schenkling, S., Coleoterorum catalogus, 's–Gravenshage(1911–1940), *pars 59*, 1-215.
23. Kral J. Wissenschaftlichen Ergebnis der zoologischen Expedition des Nationalmuseums in Prag nach der Türkei, Coleoptera, Alticidae. Acta Entomologica Musei Nationalis Pragae, 1967; 37, 261-268.
24. Medvedev L. A List of Chrysomelidae collected by Dr. W. Wittmer in Türkiye (Coleoptera). Revue suisse de Zoologie,1970; 77, 309-319.
25. Aslan İ, Gruev B, Özbek H. A preliminary review of the subfamily Alticinae (Coleoptera, Chrysomelidae) in Türkiye. Turkish Journal of Zoology, 23(1999), 373-414.
26. Gruev B. Actualized checklist of Alticinae (Coleoptera: Chrysomelidae) in the Balkan Peninsula. *Travaux* Scientifiques de *l'Universite de Plovdiv Animalia*, 2001; 37, 9-28.
27. Aslan EG, Gök A. Host–plant relationships of 65 flea beetles species from Türkiye, with new associations (Coleoptera: Chrysomelidae: Alticinae). Entomological News,2006; 117(3), 297-308.
28. Özdikmen H. A comprehensive contribution for leaf beetles of Türkiye with a zoogeographical evaluation for all Turkish fauna (Coleoptera: Chrysomelidae). Munis Entomology & Zoology, 2011; 6(2), 540-638.
29. Bal N, Özdikmen H, Coral Şahin D. Twenty-six new flea beetles for the fauna of Çankırı province in Türkiye (Chrysomelidae: Galerucinae: Alticini). Munis Entomology & Zoology, 2018; 13(2), 527-537.
30. Kasap H, Crowson RA, The male reproductive organs of Bruchidae and Chrysomelidae (Coleoptera). Turkish Journal of Entomology, 1979; 3, 199-216.
31. Mann, JS, Crowson RA. On the internal male reproductive organs and their taxonomic significance in the Leaf Beetles (Coleoptera: Chrysomelidae). Entomologia Generalis, 1983; 9, 75-99.
32. Suzuki K. Comparative morphology of the internal reproductive system of Chrysomelidae (Coleoptera). In: Jolivet P, Petitpierre E, Hsiao TH (Eds) Biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht, Boston and London, 1988; 317-355.
33. Arnqvist G. The evolution of animal genitalia: distinguishing between hypotheses by single species studies. Biological Journal of the Linnaean Society, 1997; 60, 365-379.
34. Hernández JM, Ortuño VM. Estudio de la genitalia femenina en *Iberodorcadion* Breuning, 1943 y comentarios sobre su valor taxonómico (Coleoptera: Cerambycidae). Graellsia, 1992; 48, 91-97.

35. Hernández JM. La genitalia femenina en el género *Corymbia* Des Gozis, 1886 (Coleóptera, Cerambycidae). Elytron,1993; 7, 99-104.
36. Ferronato EMO. A cápsula da espermateca dos Eumolpinae (Coleoptera: Chrysomelidae) associados a *Theobroma cacao* L. (Sterculiaceae) do Sul da Bahia. Anais da Sociedade Entomológica do Brasil, 2000; 29, 57-63.
37. Kısmalı Ş. İzmir ili ve çevresinde kültür bitkilerinde zarar yapan Chrysomelinae ve Halticinae (Coleoptera: Chrysomelidae) alt familyalarına ait türler, tanınmaları, konukçuları, yayılışları ve kısa biyolojileri üzerinde araştırmalar. Ege Üniversitesi Ziraat Fakültesi Dergisi, Seri A, 1973; 10(2), 341-378.
38. Snodgrass RE. Principles of Insect Morphology. McGraw-Hill Book Co., New York and London, 1935; 667 pp.
39. De Marzo, L. Biodiversita della spermateca nei coleotteri. Atti Accademia Nazionale Italiana di Entomologia Anno, 2008; LVI, 69-96.
40. Pascini TV., Martins GF. The insect spermatheca: an overview. Zoology, 2017; 121, 56-71.
41. Rodríguez-Mirón GM, Zaragoza-Caballero S. Revisión taxonómica del género *Mastostethus* (Coleoptera: Megalopodidae) en México y descripción de dos especies nuevas. Revista Mexicana de Biodiversidad,2017, 88, 312-334.
42. Matsumura Y, Suzuki K. Comparative morphology of internal reproductive systems in leaf beetles of the Donaciinae and Criocerinae (Coleoptera: Chrysomelidae) and its implication for the phylogeny. Zootaxa, 2008; 1845, 1-32.
43. Reid CAM. The Australian species of the tribe Zeugophorini (Coleoptera: Chrysomelidae: Megalopodinae). General and Applied Entomology, 1989; 21, 39-47.
44. Biondi M.Revision of the species of *Chaetocnema* from Madagascar (Coleoptera Chrysomelidae Alticinae). European Journal of Entomology,2001; 98, 233-248.
45. Borowiec L, Świętojańska J. Revision of *Cassida litigiosa* group from southern Africa (Coleoptera: Chrysomelidae: Cassidinae*).* Annales Zoologici, 2001; 51, 153-184.
46. Borowiec L, Skuza M. The structure of spermatheca in the genus *Chelymorpha* Chevrolat, 1837 (Coleoptera: Chrysomelidae: Cassidinae) and its taxonomic significance. Annales Zoologici,2004; 54, 439-451.
47. Borowiec L, Opalinska S. The structure of spermathecae of selected genera of Stolaini and Eugenysini (Coleoptera: Chrysomelidae: Cassidinae) and its taxonomic significance. Annales Zoologici,2007; 57, 463-479.
48. Borowiec L, Pomorska J. The structure of the spermathecae of the genus *Stolas* (Coleoptera: Chrysomelidae: Cassidinae: Mesomphaliini) and its taxonomic significance. Annales Zoologici, 2009; 59, 201-221.

49. Rodríguez-Mirón GM., Zaragoza-Caballero S. Revisión taxonómica del género *Mastostethus* (Coleoptera: Megalopodidae) en México y descripción de dos especies nuevas. Revista Mexicana de Biodiversidad, 2017, 88, 312-334.