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Spermatheca Structure of *Cassida atrata* Fabricius, 1787 (Coleoptera: Chrysomelidae: Cassidinae) in Scanning Electron Microscope (SEM)

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ABSTRACT

It is accepted that male genitalia are not diagnostic, spermatheca are partly diagnostic within the genus *Cassida* Linnaeus, 1758. However, so far, it appears that genital studies are based solely on stereo microscopy. Ultrastructures of genitalia have been not studied except for a few studies. In this study, female genital structure belonging to three *Cassida atrata* Fabricius, 1787 specimens collected from Kayseri and Niğde provinces in 1996 and 2018 from Turkey was examined for the first time in SEM in order to determine whether ultrastructural studies are useful from taxonomic point of view. Thus, new diagnostic characters were obtained and it revealed that it was diagnostic for species in other subgenus. Photos of spermatheca taken by both SEM and stereo microscope are also given.

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Taramalı Elektron Mikroskobunda (SEM) *Cassida atrata* Fabricius, 1787' nın SpermathecaYapısı (Coleoptera: Chrysomelidae: Cassidinae)

ÖZET

Cassida Linnaeus, 1758 cinsi içerisinde genel olarak erkek genitalinin ayırt edici olmadığı, spermateka'nın ise kısmen ayırt edici olduğu kabul edilmektedir. Bununla birlikte şimdiye kadar yapılan genital çalışmalarının sadece stereo mikroskoba dayalı olduğu görülmektedir. Genital yapıların ultrastrüktürel yapısı birkaç çalışma dışında çalışılmamıştır. Ultrastrüktürel çalışmaların taksonomik açıdan kullanışlı olup olmadığını ortaya koymak amacıyla bu çalışmada Türkiye'den 1996 ve 2018 yıllarında Kayseri ve Niğde illerinden toplanan toplam üç *Cassida atrata* Fabricius, 1787 örneğine ait dişi genital yapısı SEM'de ilk defa olarak incelenmiştir. Bu sayede, yeni ve ayırt edici karakterler elde edilmiş ve diğer bir alt cinsteki daha önce çalışmış türlerden ayırt edici olduğu ortaya konulmuştur. Dişi genitalinin hem SEM hem de stereo mikroskop fotoğrafları da çalışma içerisinde sunulmuştur.

Araştırma Makalesi

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INTRODUCTION

The genus *Cassida* Linnaeus, 1758, contains numerous species spread around the world (Palearctic, Neartic, Oriental, Afro-tropical, Madagascar and Australian regions). In the Palearctic and Oriental region, species of the genus *Cassida* appear to be divided into 15 subgenera (Borowiec, 2007; Özdikmen and Bal, 2019).

The Cassidinae fauna of Turkey include 51 species of six genera. The genus *Cassida* has 41 species of 11 subgenera (Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen and Kaya, 2014).

The nominotypical subgenus *Cassida* (*Cassida*) Linnaeus, 1758 includes 46 species distributed around the World (45 Palearctic species including the type species *Cassida nebulosa* Linnaeus, 1758 and one native species from North America). The nominotypical subgenus is represented by 21 species in Turkey. *Cassida atrata* Fabricius, 1787 is one of the species in the nominotypical subgenus.

According to Bordy and Doguet (1987), Borowiec and Świętojańska (2001) and Borowiec (2007), male genitalia are not diagnostic within the genus *Cassida* Linnaeus, 1758. Spermathecae are partly diagnostic. However, studies on genitalia have been based on only stereo microscope up to now. Ultrastructures of genitalia have never been studied except for a few recent works (Ataş et al., 2019a, 2019b).

Hitherto, we think that arrangement of the subgeneric

classification in the genus *Cassida* on the base of aedeagal and especially spermathecal morphologies was overlooked due to this acceptance and approval. However, we believe that ultrastructural and detailed investigations of aedeagi and spermathecae will be very important in the genus *Cassida* with regard to subgeneric classification especially. With this reason, detailed investigations of spermatheca of *Cassida atrata* Fabricius, 1787 from Turkey were studied with stereo microscope. Obtaining observations are presented in below.

The aim of this work is to reveal; detailed morphologies of spermatheca of *Cassida atrata* observed by stereo microscope and SEM.

MATERIALS and METHODS

The available specimens (a total of three specimens) for the present work were collected from Kayseri and Niğde provinces in Turkey in 1996 and 2018. The specimens were deposited at Gazi University (Ankara, Turkey).

The spermatheca were dissected from abdomen, and remaining tissue was removed with fine tweezers. For microscopic examination, the samples were placed in 70% ethanol and examined with Olympus SZX7 stereomicroscope.

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.

RESULTS and DISCUSSION

Cassida atrata Fabricius, 1787(Fig. 1)

Cassida atrata is a European species. Its body length is 5.1-7 mm. It is distributed in Europe (Albania, Austria, Bulgaria, Bosnia, Croatia, Czech Republic, France, Germany, Greece, Herzegovina, Hungary, Italy, Latvia, Macedonia, Moldova, Poland, Romania, Serbia- Montenegro, Slovakia, Slovenia, Turkey, Ukraine) and Asia (Turkey) (Warchalowski, 2010; Borowiec and Sekerka, 2010).

Figure 1. *Cassida atrata* Fabricius, 1787; A. dorsal view, B. ventral view, C. Lateral view. *Scale bars size is 1 mm*
Şekil 1. Cassida atrata Fabricius, 1787; A. dorsal görünüm, B. Ventral görünüm, C. Yandan görünüm. Ölçek çubuk boyutu 1 mm'dir.

The species is widely distributed in Turkey. It has been recorded from nine provinces including Ankara, Bolu, Bursa, Çorum, Eskişehir, Isparta, Mersin and Niğde provinces in Asian Turkey and Edirne province in European Turkey (Ekiz et al., 2013; Özdikmen and Kaya, 2014).

Material examined: Kayseri: Develi, Kocahacılı, 38°11'15" N 35°23'50" E, 07.V.2018, 1092 m, 1 specimen; Niğde: Melendiz, between Hançerli-Küçükköy, 03.VII.1996, 1 specimen; Çamardı, 29.VI.1996, 1 specimen.

Spermatheca of *Cassida atrata* was studied with SEM and stereo microscope. Observations on ultrastructural and detailed morphologies are presented as follows:

Spermatheca: In stereo microscope(Fig. 2): Vasculum (General view of spermatheca) is eggplant-shaped or boxing glove-shaped, distinctly curved, relatively obtuse angled, dissymmetrical C-shaped (apical part of cornu prolonged). It is generally light brown. In vasculum, cornu is much thicker than the nodulus, especially in the apical part. Apical part of cornu is clearly swollen and dark in color. Apex of cornu is not rounded. Nodulus is short, almost parallel, not swollen. Nodulus is darkened on internal surface basally. Ampulla is in the shape of an elongated channel or tubular and connected to the nodulus on the outer surface in the basal part of the nodulus. The ramus is in the form of a long straight and thick

channel or pipe which is slightly enlarged in the middle. The widest part of ramus is slightly thinner than the thickness of the nodulus. The spermatheca gland is attached to the ramus at the outer surface of the ramus. Ductus spermatheca connects straight to the ramus, which is thick and long. It is in the form of a flat tube that folds widely in the proximal part. It is thicker in the next large part, and is regularly curved in a spiral shape. Ductus spermatheca is thinner than ramus.

Figure 2. Spermatheca of *Cassida atrata* Fabricius, 1787. *Scale bars size is 0,5 mm.*

Şekil 2. Cassida atrata Fabricius, 1787, 'nın spermatekası. Ölçek çubuk boyutu 0,5 mm'dir.

In SEM (Scanning Electron Microscope)(Figs. 3-6):

Observations obtained by SEM are similar to those of stereo microscope in general. Therefore, only different characters in photos of SEM are presented below.

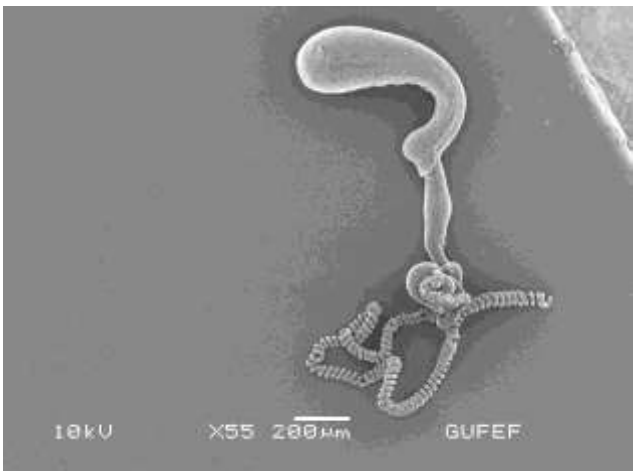

Figure 3.Spermatheca of *Cassida atrata*, lateral view (SEM). *Scale bars size is 200 µm.*

Şekil 3. Cassida atrata'nın spermatekasının yandan görünümü (SEM). Ölçek çubuk boyutu 200 µm.'dir.

Apex of cornu is round. Nodulus has a distinct integument on internal surface basally. The integument behind nodulus fringed that more or less corrugated. Spermathecal gland connected to proximal duct before its first curve. Nodulus, cornu, proximal tube of ductus spermatheca have scattered, irregular and sparsely ultrastructural pits.

Cassida atrata is in the nominotypical subgenus of *Cassida*. According to the results of the present work, spermathecal morphologies of *Cassida atrata* distinctly differ from *Cassida nebulosa* Which is the type species of the genus and nominotypical subgenus.

Many characters in detail are diagnostic. These diagnostic characters can be seen below.

Spermatheca of *Cassida nebulosa* Linnaeus, 1758 in stereo microscope (Fig. 7): General view of spermatheca is in the form of an opened hook or bird beak-shaped, distinctly curved. Nodulus is distinctly swollen and bulbous. Apical part of cornu is clearly sharpened. Apex of cornu is pointed. Cornu has an integument trace in front of the apex. Collum is invisible, and much reduced. Ramus is visible, but small. It is joined to nodulus in latero median part of external surface basally. Ductus spermatheca is very long, rather fine, and regularly spiral almost along its length.

Diagnostic characters of spermatheca for *Cassida nebulosa* and *Cassida atrata* in stereo microscope:

General view of spermatheca is in the form of an opened hook or a bird beak-shaped, distinctly curved. Nodulus is distinctly swollen and bulbous. Nodulus is not darkened on any part. Apical part of cornu is clearly sharpened. Apex of cornu is pointed. Cornu has an integument trace in front of the apex. Ramus visible, but small. It is joined to nodulus in latero median part of external surface basally. Ductus spermatheca is very long, rather fine, regularly spiral almost along its length. *Cassida nebulosa* Linnaeus, 1758

General view of spermatheca is eggplant-shaped or boxing glove-shaped, distinctly curved, relatively obtuse angled, dissymmetrical C-shaped (apical part of cornu prolonged). Nodulus is short, almost parallel, not swollen. Nodulus is darkened on internal surface basally. Apical part of cornu is clearly swollen. Apex of cornu is rounded. Cornu does not have any integument trace in front of the apex. Ramus is invisible, very reduced or absent. Proximal duct is joined to nodulus in latero median part of external surface basally because ramus is invisible. Ductus spermatheca is rather thick, straight tube-shaped at the proximal part, and then this tube is broadly twisted. These parts are probably almost 1/3 of all length of ductus spermatheca. Last part (or last 2/3) of ductus spermatheca is probably rather long, rather thick and regularly spiral. *Cassida atrata* Fabricius, 1787.

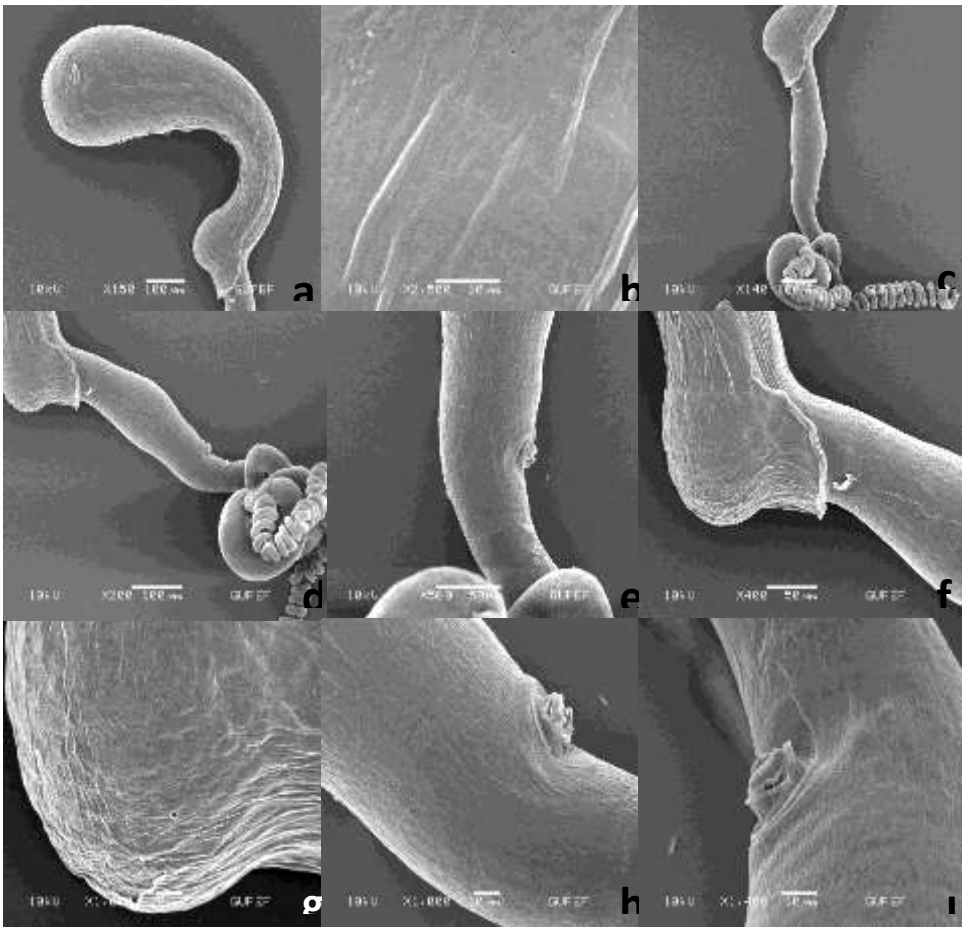

Figure 4. Spermatheca of *Cassida atrata*, **a.** Nodulus, cornu and integument; **b.** Pits on the apical part of cornu; **c and d.** Integument, ampulla, spermathecal gland and ductus spermatheca; **e.** Connection of spermathecal gland on proximal duct; **f.** Integument on basal part of nodulus; **g.** Pits on integument in basal part of nodulus; **h and ı.** spermathecal gland on proximal duct in lateral view (SEM).*Scale bars size are 100 µm, 50 µm, 50 µm, 10 µm, 10 µm, 10 µm, respectively.*

Şekil 4. *Cassida atrata*' nın spermatekası, **a.** Nodulus**,** cornu ve integument; **b.**Cornu'nun apikal kısmındaki çukurlar, **c ve d.** İntegüment, ampulla, spermatekal bez ve spermateka kanalı; **e.** Proksimal kanal üzerindeki spermatekal bağlantı. **f.** Nodulusun kaide kısmı üzerindeki integument**; g.** Nodulusun kaide kısmındaki integümentteki çukurlar; **h ve ı.**Proksimal kanaldaki spermatekal bezin yandan görünümü (SEM). *Ölçek çubuklarının boyutları sırasıyla 100 µm, 50 µm, 50 µm, 10 µm, 10 µm, 10 µm'dir.*

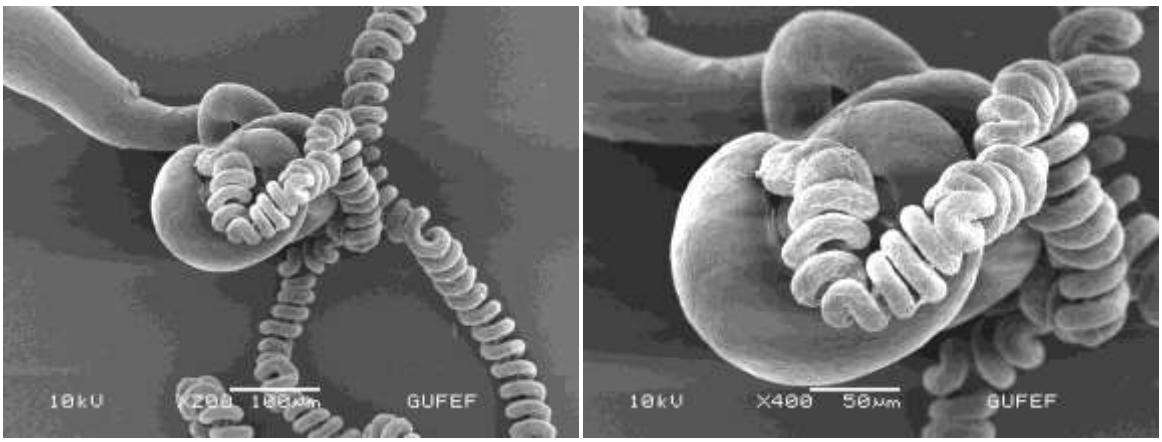

Figure 5.Spermatheca of *Cassida atrata,* ramus, spermathecal gland, proximal duct and ductus spermatheca in lateral view (SEM).*Scale bars size are 100 µm, 50 µm, respectively.*

Şekil 5.*Cassida atrata*' nın spermatekası; ramus, spermatekal bez, proksimal kanal ve spermateka kanalının yandan görünümü (SEM).*Ölçek çubuklarının boyutu sırasıyla 100 µm, 50 µm'dir.*

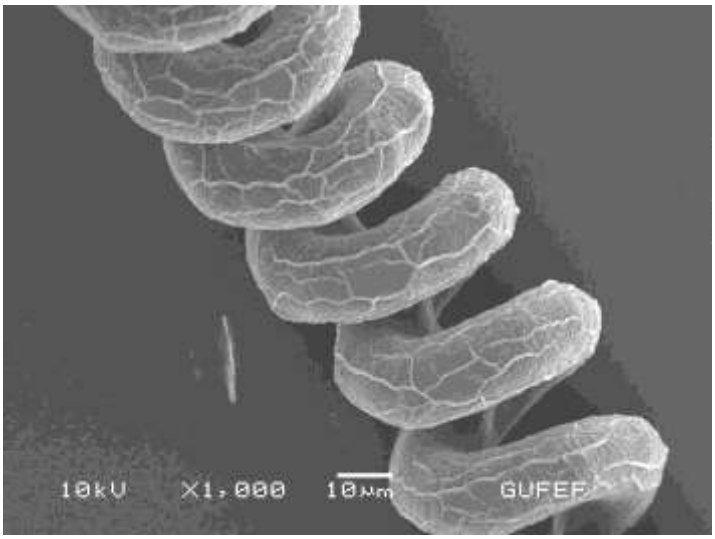

Figure 6.Spermatheca of *Cassida atrata,* spiral part of ductus spermatheca in lateral view (SEM). *Scale bars size is 10 μm.*

Şekil 6.*Cassida atrata*'nın spermatekası, spermateka kanalının spiral kısmı yandan görünümde (SEM).*Ölçek çubuk boyutu10 μm.*

Figure 7.Spermatheca of *Cassida nebulosa* Linnaeus, 1758, lateral view (from Bordy, 2009).

Şekil 7.Cassida nebulosa Linnaeus, 1758 'in spermatekasının yandan görünümü (Bordy, 2009 dan).

CONCLUSION

As mentioned above, nine spermathecal characters for *Cassida nebulosa* are determined. The type species clearly differs from *Cassida atrata* by all determined spermathecal characters.

Accordingly, it can be said that the type species *Cassida nebulosa* and *Cassida atrata* are probably not congeneric.

According to the results of the present study, we believe that ultrastructural and detailed investigations of spermathecae will be very important in the genus *Cassida* with regard to subgeneric classification especially.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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