

**A SEM STUDY ON AEDEAGUS AND SPERMATHECA OF
CASSIDA NEBULOSA LINNAEUS, 1758 (COLEOPTERA:
CHRYSOMELIDAE: CASSIDINAE) FROM TURKEY**

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ABSTRACT: It is accepted male genitalia are not diagnostic, spermathecae are partly diagnostic within the genus *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae). 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 works. The aim of this study is to determine whether the ultrastructural works are efficient. Spermathecae of totally 5 specimens of *Cassida nebulosa* Linnaeus, 1758 for the present work were collected from Konya province in Turkey in 1993 and 2006, which have been examined for the first time. Thus new diagnostic characters were obtained and it revealed that it was diagnostic from species in other subgenus. Photos of aedeagus and spermatheca in SEM as well as in stereo microscope are also given in the text.

KEY WORDS: *Cassida nebulosa*, SEM, ultrastructure, aedeagus, spermatheca, Turkey

The genus *Cassida* Linnaeus, 1758 includes a large number of species distributed around the world (Palearctic, Nearctic, Oriental, Afro-tropical, Madagascar and Australian Regions). The genus is divided into 15 subgenera for the species distributed in Palearctic and Oriental Regions (Borowiec, 2007; Özdikmen and Bal, 2019).

The Cassidinae fauna of Turkey includes 51 species of 6 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 and one native species from North America). The nominotypical subgenus is represented by 21 species in Turkey. *Cassida nebulosa* Linnaeus, 1758 is the type species of the genus *Cassida* Linnaeus, 1758 and so the nominotypical subgenus *Cassida* (*Cassida*) Linnaeus, 1758 (Chrysomelidae: Cassidinae).

According to Bordy & Doguet (1987), Borowiec & 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,b). For this reason, ultrastructural investigations of aedeagi and spermathecae are very important in the genus *Cassida*.

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 nebulosa* Linnaeus, 1758 from Turkey were studied with SEM and stereo microscope. Obtaining observations are presented below.

The aim of this work is to reveal, detailed morphologies observed by SEM and stereo microscope of spermatheca of *Cassida nebulosa* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey.

MATERIAL AND METHODS

The available specimens (a total of 5 specimens) for the present work were collected from Konya province in Turkey in 1993 and 2006. The specimens are deposited in Nazife Tuatay Plant Protection Museum (NTM) (Turkey, Ankara).

The spermathecae and aedeagi were dissected from abdomen, remaining tissue were removed with fine tweezers.

For light microscopic examination after cleaning, the samples were placed 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 5 kV and 10 kV.

RESULTS

Cassida nebulosa Linnaeus, 1758 (Fig. 1)

Cassida nebulosa Linnaeus, 1758 is a Asiatic-European species. It is distributed in most parts of Europe including European Turkey, China, Far East Russia, Kazakhstan, Korea, Mongolia, Siberia, Tadjikistan, Asian Turkey and Uzbekistan (Warchalowski, 2010; Borowiec & Sekerka, 2010; Özdikmen & Kaya, 2014).

The species is rather widely distributed in Turkey. It has been recorded from 14 provinces in 5 of 7 Turkish regions. It is reported from Ankara, Artvin, Balıkesir, Bursa, Çanakkale, Düzce, Giresun, Gümüşhane, İzmir, Kastamonu, Konya, Sakarya, Tokat and Yozgat provinces in Turkey (Ekiz et al., 2013; Özdikmen & Kaya, 2014).

Material examined: Turkey, Konya prov.: Akşehir, Dereçine, 05.VIII.1993, leg. A. Kalkandelen, 2 males and 2 females specimens; Akşehir, Sarayköy, 23.V. 2006, leg. Y. Özdemir, 1 male specimen.

Aedeagus and spermatheca of *Cassida nebulosa* Linnaeus, 1758 were studied with both stereo microscope and SEM for the first time. Obtaining observations on ultrastructures of them are presented as follows:

Aedeagus (Figs. 2-11): In lateral view, median lobe distinctly and regularly curved median foramen to apex in general. Median lobe gradually, but slightly narrowed from the base to the apex. The apex of median lobe almost abruptly sharpened and pointed.

In dorsal view, median lobe barely widened from the median part to the apex, and the apex more or less prolonged and clearly truncated. Upper and lateral

margins of orifice more or less rounded. Dorsal plate distinct and almost covered basal half of orifice. Median lobe in lateral parts and fore part of orifice thickened. Thickening in lateral parts smaller than the fore part. Median lobe behind the orifice joined more or less V-shaped.

Median lobe especially in anterior half with scattered, irregular and sparsely ultrastructural pits. The pits on ventral parts of median lobe much more than on dorsal parts. The pits located only in lateral parts of terminal part of median lobe in dorsal view. Dorsal plate and flattened area behind it without ultrastructural pits in dorsal view. Also the terminal area from upper margin of orifice to aedeagal apex without ultrastructural pits in dorsal view. Apex of median lobe folded to dorsal surface and so appears like a truncated-shaped, but not cut. Apex of median lobe gradually narrowed, not additionally prolonged.

Spermatheca (Figs. 12-18): Vasculum in the form of opened hook or bird beak-shaped, distinctly curved. Apical part of cornu clearly sharpened. Apex of cornu pointed. Apical part of cornu with an integument extended to front of its apex. Nodulus distinctly swollen and bulbous. Nodulus with an integument on internal surface basally. Ampulla bulbous but small. Collum invisible, very reduced. Ramus visible. Ampulla joined to nodulus in external surface basally. Spermathecal gland connected to ampulla in its median part. Spermathecal duct joined to ampulla in its top surface medially. Ductus spermatheca very long, rather fine, regularly spiral almost along its length.

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Figure 1. *Cassida nebulosa* Linnaeus, 1758; dorsal view (left), ventral view (right).

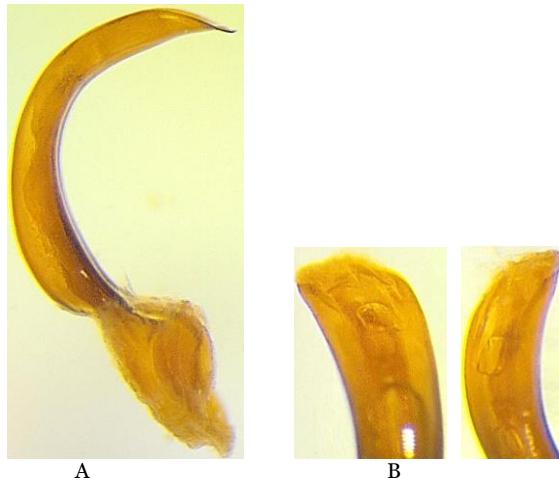


Figure 2. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, A. Lateral view, B, C. Dorsal view.

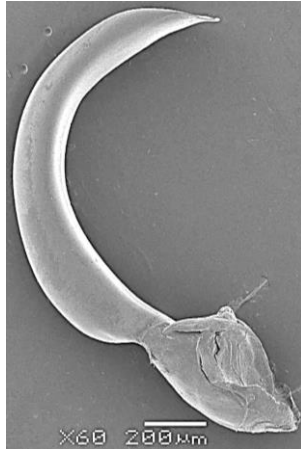


Figure 3. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, lateral view (SEM).



Figure 4. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, dorso-lateral view (SEM).

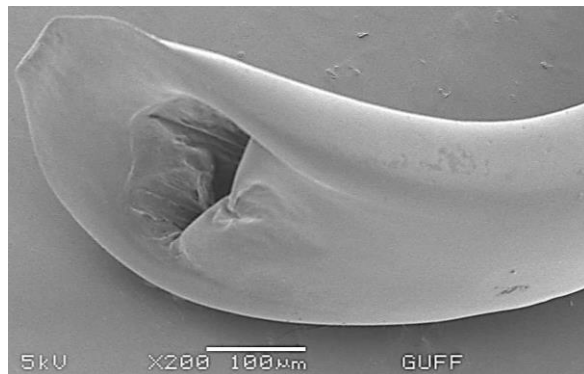


Figure 5. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, apical part of median lobe in dorso-lateral view (SEM).

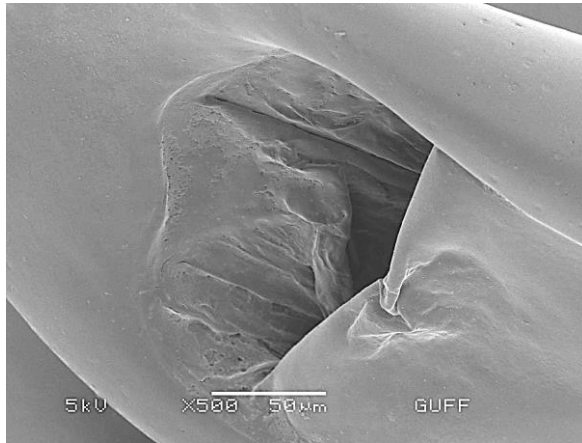


Figure 6. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, dorsal plate (SEM).

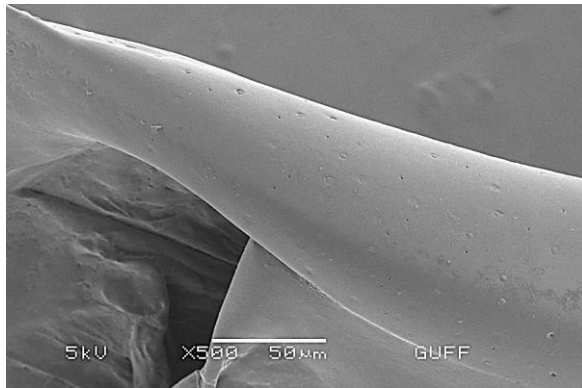


Figure 7. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, ultrastructural pits on lateral part of orifice (SEM).

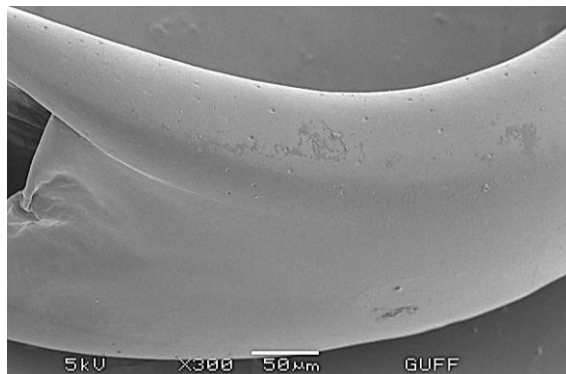


Figure 8. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, ultrastructural pits on lateral part of median lobe (SEM).

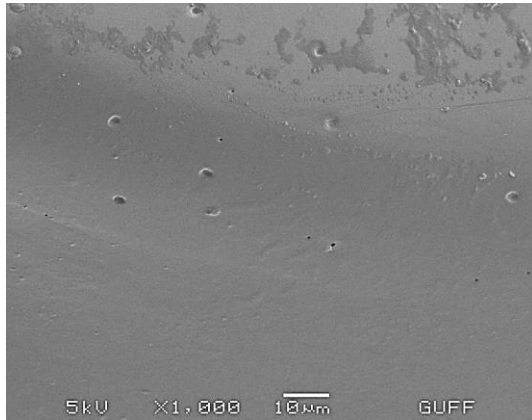


Figure 9. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, ultrastructural pits on lateral part of median lobe (SEM).

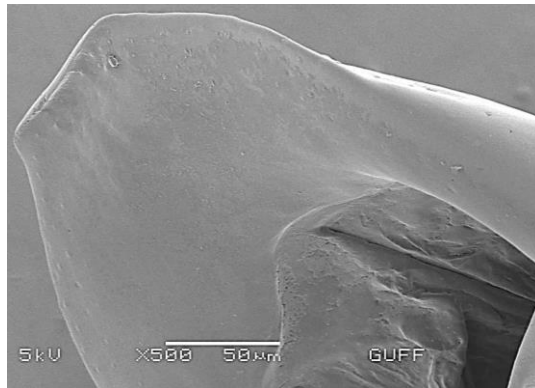


Figure 10. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, folded apex, ultrastructural pits on sides of apical part of median lobe (SEM).

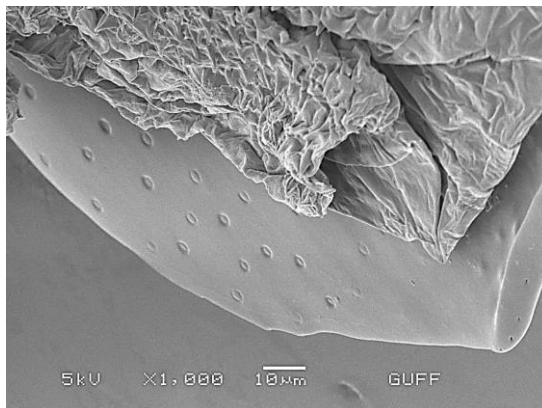


Figure 11. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, folded apex, ultrastructural pits on sides of apical part of median lobe (SEM).



Figure 12. Spermatheca of *Cassida nebulosa* Linnaeus, 1758.



Figure 13. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, lateral view (SEM).

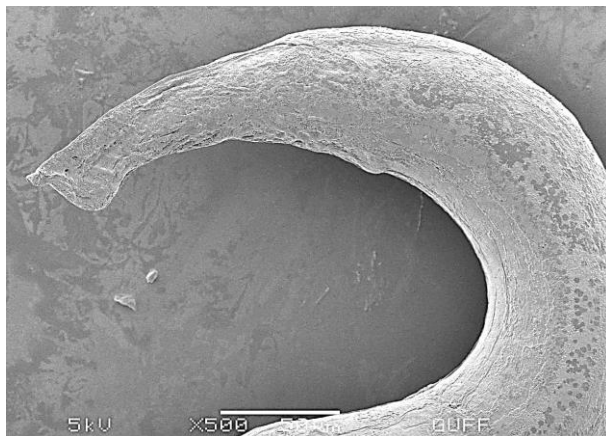


Figure 14. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, apical part of cornu in lateral view (SEM).

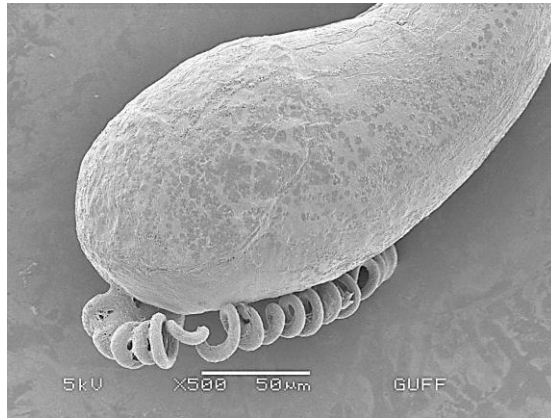


Figure 15. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, the integument on internal surface of nodulus (SEM).

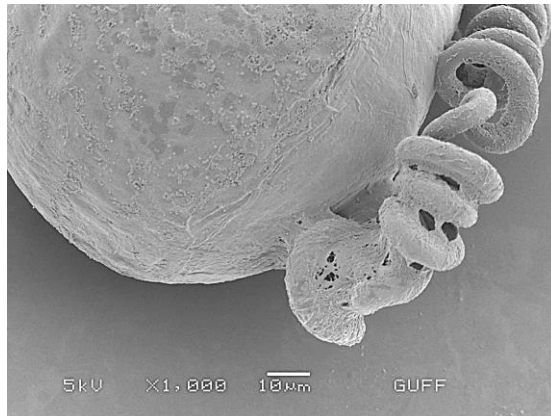


Figure 16. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, basal part of nodulus, ampulla and ductus spermatheca (SEM).

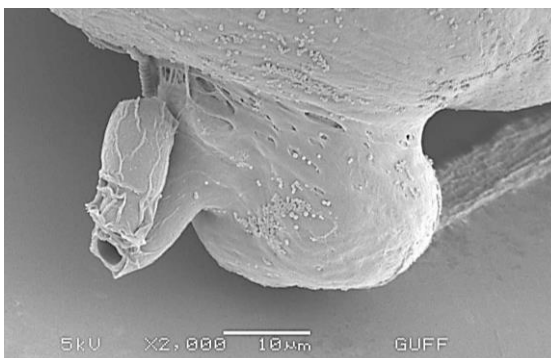


Figure 17. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, ampulla and spermathecal gland (SEM).

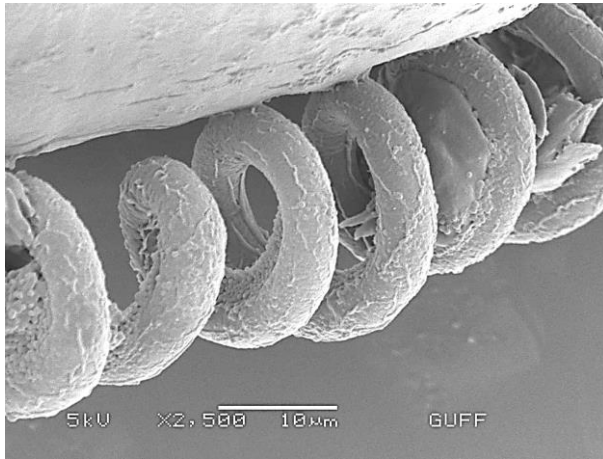


Figure 18. Spermatheca of *Cassida nebulosa* Linnaeus, 1758, ductus spermathecal (SEM).