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Original article (Orijinal araştırma)

A SEM study of the aedeagus and spermatheca of *Cassida viridis* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey

Türkiye'den *Cassida viridis* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae)'in aedeagus ve spermatekaları üzerine bir SEM çalışması

Neslihan BAL[1*]

Abstract

It is accepted male genitalia are not diagnostic, but spermathecae are partly diagnostic within the genus *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae). However, studies on genitalia have been based only on stereomicroscopic examination. The ultrastructure of genitalia has mostly not been studied. The aim of this study was to determine whether this ultrastructures could be diagnostic. Aedeagi and spermathecae of 63 specimens of *Cassida viridis* Linnaeus, 1758 were collected from Çankırı, Hatay, Kayseri, Osmaniye and Zonguldak Provinces in Turkey in 2003, 2007 and 2015, and examined for the first time. Thus, new diagnostic characters were obtained and it revealed that it was diagnostic to either species or subgenus. Photos of aedeagus and spermatheca taken with both SEM and stereomicroscope are given.

Keywords: Aedeagus, *Cassida viridis,* SEM, spermatheca, Turkey

Öz

Cassida Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) cinsi içerisinde genel olarak erkek genitalinin ayırt edici olmadığı, ancak spermatekanın kısmen ayırt edici olduğu kabul edilmektedir. Bununla birlikte şimdiye kadar yapılan genital çalışmalarının sadece stereo mikroskoba dayalı olduğu görülmektedir. Genital yapılarının ince yapısı çoğunlukla çalışılmamıştır. Bu çalışmanın amacı, bu altyapının tanısal olup olmayacağını belirlenmesiydi. Bu çalışmada Çankırı, Hatay, Kayseri, Osmaniye ve Zonguldak (Türkiye) illerinden 2003, 2007 ve 2015 yıllarında toplanan toplam 63 *Cassida viridis* Linnaeus, 1758 örneğine ait aedeagus ve spermateka yapıları ilk defa olarak incelenmiştir. Bu sayede yeni ayırt edici karakterler elde edilmiş ve diğer bir alt cinsteki daha önce çalışmış türlerden ayırt edici olduğu ortaya konulmuştur. Aedeagus ve spermatekanın hem SEM hem de stereo mikroskop fotoğrafları da çalışma içerisinde sunulmuştur.

Anahtar sözcükler: Aedeagus, *Cassida viridis*, SEM, spermateka, Türkiye

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Introduction

Cassida viridis Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) is in the subgenus *Odontionycha* Weise, 1891 of the genus *Cassida* Linnaeus, 1758. The Cassidinae fauna of Turkey includes 51 species of six genera and the genus *Cassida* Linnaeus, 1758 includes 41 species in Turkey (Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen & Kaya, 2014).

The subgenus *Odontionycha* was established by Weise (1891) without designation of type species. *Cassida viridis* Linnaeus, 1758 was designated by Hincks (1952) as type species of the subgenus. According to Borowiec (2007a), *Odontionycha* Weise, 1891 was completely artificial and species included by Weise (1891) in the group spread across five subgenera or genera. The group is coherent morphologically and biologically, and represents a monophyletic group. The subgenus includes six species with the type species *C. viridis* distributed in Palearctic region, *Cassida gansuica* Chen & Zia, 1964, *Cassida inflate* Gressitt, 1952, *Cassida semipunctata* Chen & Zia, 1964, *Cassida tsinlinica* Chen & Zia, 1964 that are distributed only in China and *Cassida innotata* Boheman, 1854 that is distributed only in Afrotropical region (Borowiec, 2007a). Consequently, the subgenus *Odontionycha* is represented only by the type species *C. viridis* in Turkey (Borowiec & Sekerka, 2010; Warchalowski, 2010; Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen & Kaya, 2014).

According to Bordy & Doguet (1987), Borowiec & Świętojańska (2001) and Borowiec (2007a) male genitalia are not diagnostic within the genus *Cassida*. Spermathecae in the genus *Cassida* are partly diagnostic. However, studies on genitalia have been based on only stereomicroscope examination. Ultrastructures of genitalia have mostly not been studied (Ataş et al., 2019a, b). For this reason, ultrastructural investigations of aedeagi and spermathecae are very important in the genus *Cassida.*

The aim of this work was to examine the ultrastructure of aedeagus and spermatheca of *C.* (*Odontionycha*) *viridis* via SEM from Turkey for the first time.

Materials and Methods

The available specimens (63 in total) for the study were collected from Çankırı, Hatay, Kayseri, Osmaniye and Zonguldak Provinces in Turkey in 2003, 2007 and 2015 (Figure 1). The specimens were deposited at Gazi University (Turkey, Ankara). The spermathecae and aedeagi were dissected from abdomen, remaining tissue were removed with fine tweezers. For dissecting 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 by using an ascending series of ethanol (70, 80, 90 and 100%) and then they were air dried. After that, the specimens were mounted onto SEM stubs by using a double-sided adhesive tape, coated with gold by using a Polaron SC 502 Sputter Coater, and examined with a JEOL JSM 6060 SEM at 5 kV and 10 kV.

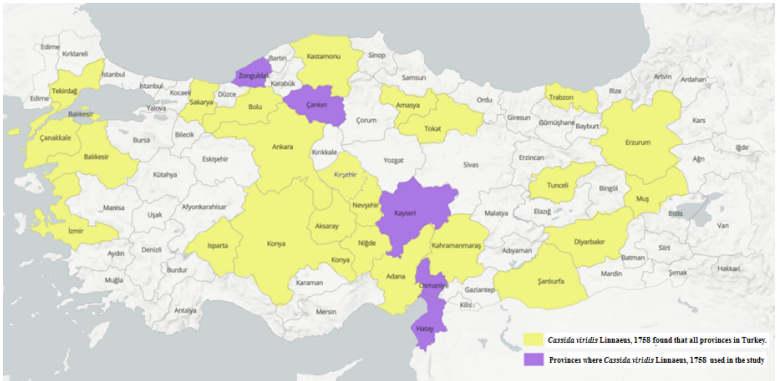

Figure 1. *Cassida viridis* Linnaeus, 1758 in Turkey and the provinces where the samples used in the study were collected.

Results and Discussion

***Cassida viridis* Linnaeus, 1758** (Figure 2)

Cassida viridis Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) has a Palearctic chorotype. It is distributed in most of Europe, Asia (China, eastern Russia, Japan, Kazakhistan, Korea, Siberia, Syria, Turkey, Uzbekistan) and North Africa (Morocco). It is widely distributed in Turkey. It has been recorded by various authors in many provinces from all Turkish regions. It is known from Adana, Aksaray, Amasya, Ankara, Balıkesir, Bolu, Çanakkale, Çankırı, Diyarbakır, Erzurum, Hatay, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Kırşehir, Konya, Muş, Nevşehir, Niğde, Osmaniye, Sakarya, Şanlıurfa, Tokat, Trabzon, Tunceli and Zonguldak Provinces in Asian Turkey (Anatolia); İstanbul and Tekirdağ Provinces in European Turkey (Figure 1) (Günther, 1954; Gül-Zümreoğlu, 1972; Kısmalı & Sassi, 1994; Gök & Çilbiroğlu, 2003; Borowiec, 2007b; Özdikmen, 2011; Ekiz et al., 2013; Özdikmen & Kaya, 2014; Dikmen & Özuluğ, 2018).

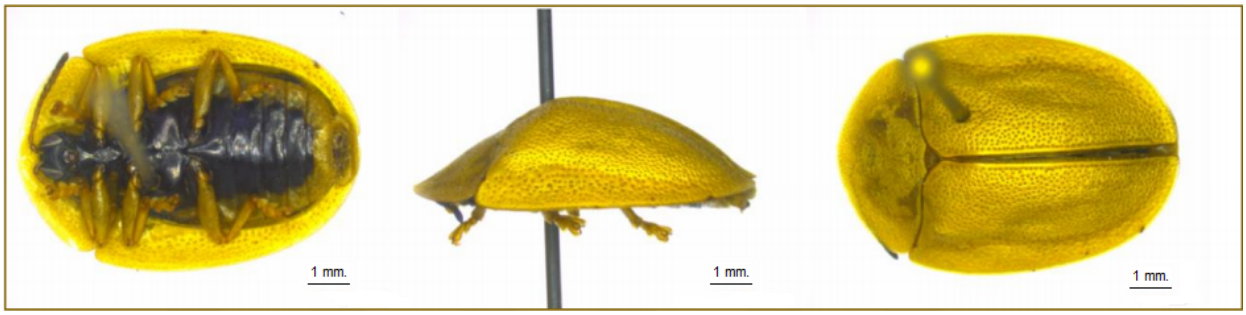

Figure 2. *Cassida viridis* Linnaeus, 1758, ventral view, lateral view and dorsal view in stereomicroscope.

Material examined. Turkey, Çankırı Province. Center, Aşağıçavuş Village, 40°41’ N 33°36’ E, 27.IV.2015, 833 m, 1 specimen; Ilgaz, between Kırşlar-Okçular, 40°56’ N 33°29’ E, 29.V.2015, 1059 m, 1 specimen; Ilgaz, exit of Kırşlar Village, 40°56’ N 33°29’ E, 17.VI.2015, 914 m, 2 specimens; Hatay Province. Serinyol, 36°21'57" N 36°13'02" E, 30.III.2007, 115 m, 19 specimens; between Harbiye-Yayladağı, 36°07'42" N 36°08'41" E, 30.III.2007, 275 m, 1 specimen; Erzin, Gökgül, 36°57'45" N 36°17'22" E, 04.VI.2007, 600 m, 1 specimen; Kayseri Province. Sarız, Yedioluk, 10.VI.2015, 1740 m, 1 specimen; Sarız, Yedioluk, 38°34'24" N 36°25'59" E, 10.VI.2015, 1740 m, 2 specimens; Pınarbaşı, Kerimoğlu, 38°43'11" N 36°24'49" E, 30. V. 2018, 1522 m, 1 specimen; İncesu, Karakoyunlu, 38°37'53" N 35°09'28" E, 28.V.2018, 1114 m, 1 specimen; Pınarbaşı, Uzun Plateau, 38°57'41"N 36°40'15"E, 05.VI.2018, 1623 m, 4 specimens; Pınarbaşı, Aşağıbey Meadow, 38°39'25"N 36°27'01"E, 05.VI.2018, 1622m, 1 specimen; Pınarbaşı, Aşağıbey Meadow, 38°39'29"N 36°27'04"E, 05.VII.2018, 1623 m, 11 specimens; Felahiye, Cumhuriyet, 39°05'11"N 35°33'28"E, 06.VII.2018, 1 specimen; Pınarbaşı, Gümüşgün, 38°44'48"N 36°26'35"E, 31.VII.2018, 1515 m, 1 specimen; Pınarbaşı, Aşağıkızılçevlik village, 38°50'31"N 36°32'24"E, 31.VII.2018, 1622 m, 7 specimens; Osmaniye Province. Issızca, 17.V.2007, 1 specimen; Zorkunfenk Plateau, 36°59'66" N 36°20'64" E, 04.VI.2007, 1049 m, 1 specimen; Zorkun, 5 km to Erzin, 36°58'56" N 36°17'47" E, 04.VI.2007, 989 m, 1 specimen; Zonguldak Province. Yedigöller 35 km to Devraklar, 15.V.2003, 205 m, 5 specimens.

Aedeagus and spermatheca of *C. viridis* were examined with both stereomicroscope and SEM for the first time. There is only one species in Turkey in this subgenus, so it was not possible to compare it with species. Observations on the ultrastructures of the aedeagus and spermatheca were as follows:

Aedeagus

Median lobe completely light brown.

In lateral view, median lobe distinctly and almost regularly (elliptically or semicircularly) curved from median foramen to apex in general. Median lobe gradually, but slightly narrowed from the base to the apex. The apex of median lobe right and pointed with triangular apex (Figures 3, 4).

In dorsal view, lateral margins of the median lobe almost parallel (Figure 4). Apex of median lobe more or less prolonged (Figures 3, 4, 6-10). Upper and lateral margins of orifice more or less rounded (Figures 3, 9, 10). Dorsal plate distinct and covered two-thirds of the orifice (Figures 3, 9, 10). Median lobe in lateral and fore parts of orifice thickened. Thickening in lateral parts smaller than the fore part. Median lobe behind the orifice more or less flattened. Flattened part and basally V-shaped (Figures 3, 8, 9). Median lobe especially in anterior half scattered, irregular and sparsely ultrastructural pits (Figures 6, 7, 9, 10). The pits on ventral parts of median lobe much more than on dorsal parts (Figures 5, 6, 8, 9). The pits located only in lateral parts of terminal part of median lobe (Figures 9, 10). Dorsal plate and flattened area behind it without ultrastructural pits. The terminal area from upper margin of orifice to aedeagal apex also without ultrastructural pits (Figures 9, 10).

In ventral view, lateral margins of the median lobe almost parallel (Figures 6, 7). Apex of median lobe more or less prolonged (Figures 6-10). Ventral surface of median lobe only in apical part with a median, it has a flattened area (Figures 6, 7).

Here, the aedeagi of *C. viridis* have been described from SEM images for the first time. Bordy (2009) gave a stereomicroscopic drawing of aedeagus of this species. The stereomicroscopic results obtained in this study are in agreement with the results of Bordy (2009). Moreover, many new diagnostic characters that were previously unknown based on the ultrastructures of aedeagi were revealed by this SEM investigation.

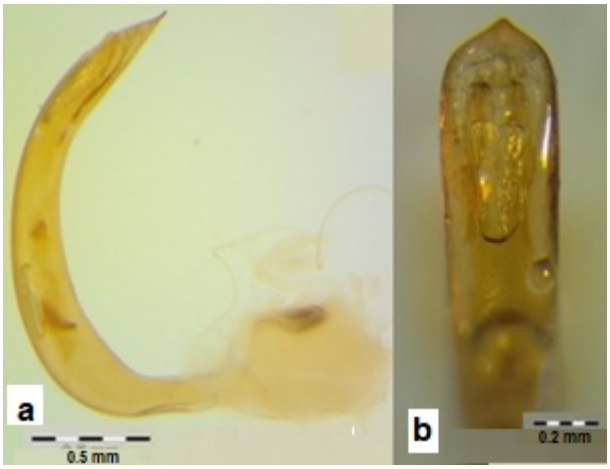

Figure 3. Aedeagus of *Cassida viridis* Linnaeus, 1758, in stereomicroscope, a) lateral view, b) dorsal view.

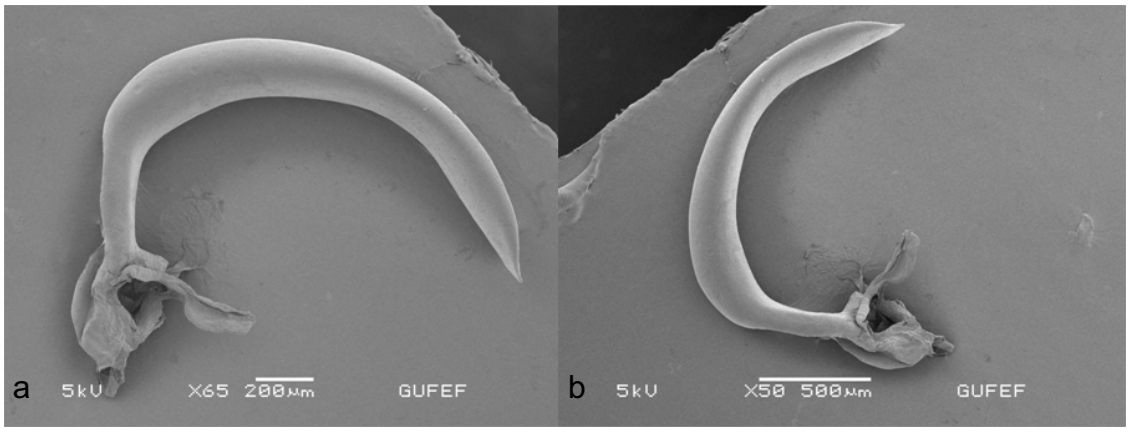

Figure 4. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, lateral view.

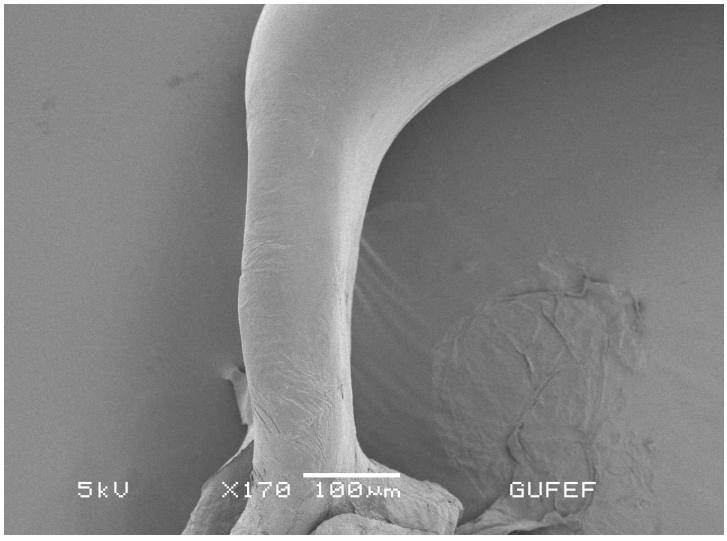

Figure 5. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, median foramen in lateral view.

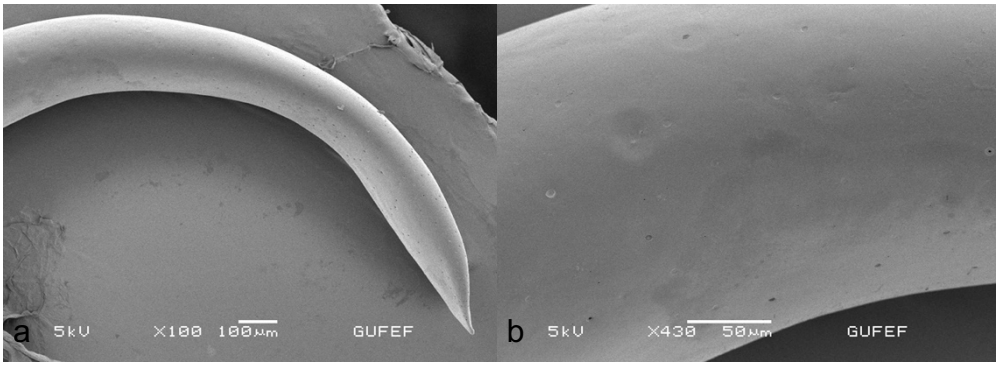

Figure 6. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, a) anterior half of median lobe in lateral view, b) the pits on basal part of median lobe in ventrolateral view.

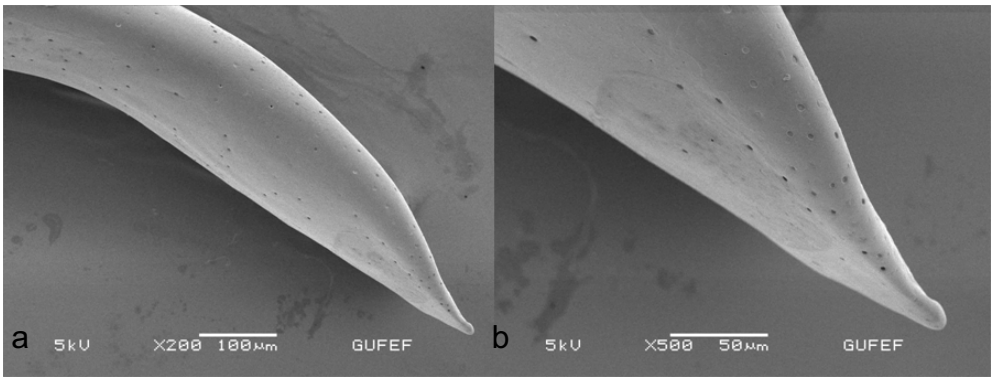

Figure 7. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on anterior half and apical part of median lobe in ventrolateral view.

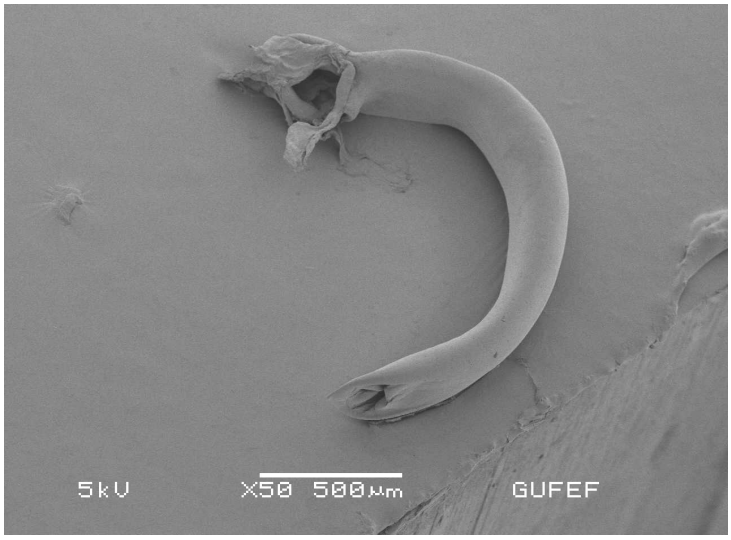

Figure 8. Aedeagus of *Cassida viridis* Linnaeus, 1758 in SEM, dorsolateral view.

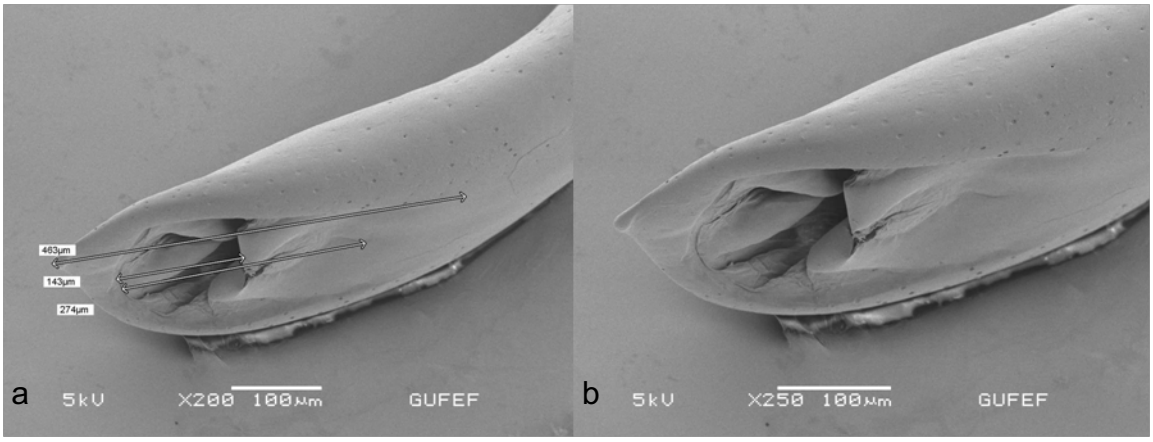

Figure 9. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on terminal part of median lobe in dorsolateral view.

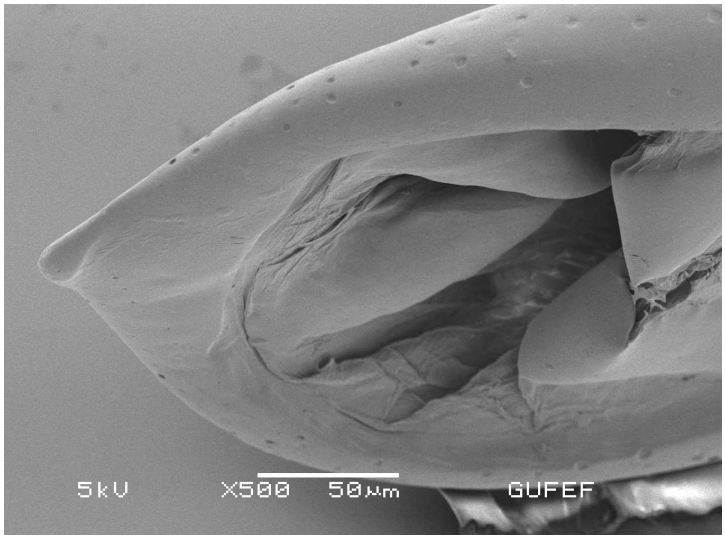

Figure 10. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on apical part of median lobe in dorsal view.

Spermatheca

General view of spermatheca symmetrical C-shaped, distinctly curved (Figures 11, 12). Nodulus almost parallel, not swollen. Nodulus with an integument on internal surface basally (Figures 12, 13). Cornu not swollen. Apex of cornu rounded. Cornu with an appendix (an integument) in front of the apex (Figures 12, 13). Ampulla like a mushroom or doorknob in general. Ampulla joined to external surface of nodulus beyond the basal end of nodulus and so spermathecae symmetrical C-shaped (Figures 11-13). Collum like a peduncle, thinner than the basal part of nodulus (Figures 11-13). Ramus distinctly swollen, like a rounded knob (Figures 11-13). Spermathecal gland broken, joined to ramus just the median part of top surface of ramus (Figures 12-14). Ductus spermatheca joined to ampulla just the area between collum and ramus (Figures 11-14). Ductus spermatheca rather long, thin and distinctly spiral and the diameter of last part almost equal with first part (Figures 11-16). Only collum scattered, irregular and sparsely ultrastructural pits. Ductus spermatheca without ultrastructural pits (Figures 14-16).

Figure 11. Spermatheca of *Cassida viridis* Linnaeus, 1758, in stereomicroscope, lateral view.

Figure 12. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, lateral view.

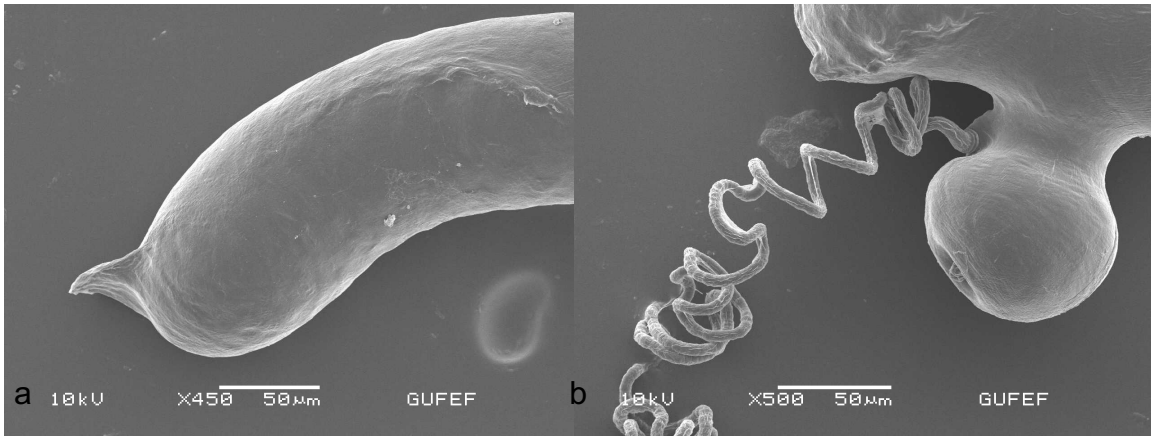

Figure 13. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, a) apex of cornu in lateral view, b) ampulla (collum and ramus) and ductus spermatheca in lateral view.

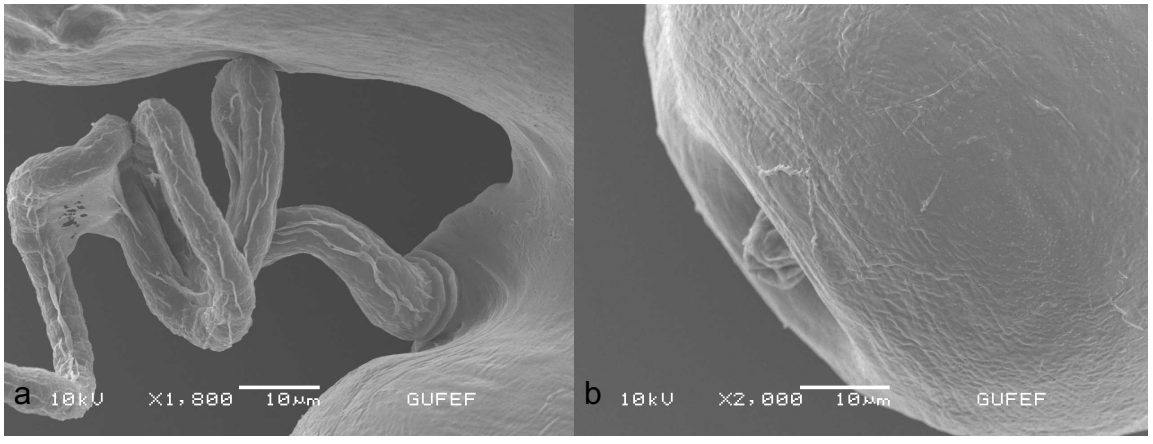

Figure 14. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, a) proximal part of ductus spermatheca in lateral view, b) broken spermathecal gland on top surface of ramus in lateral view.

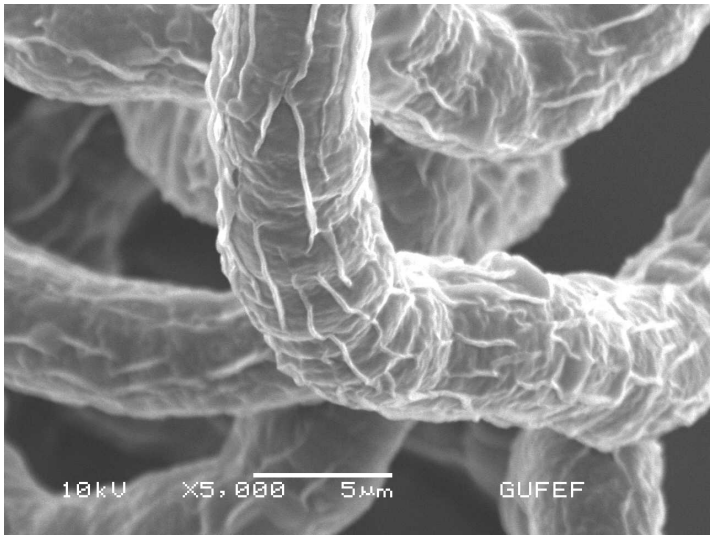

Figure 15. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, ductus spermatheca.

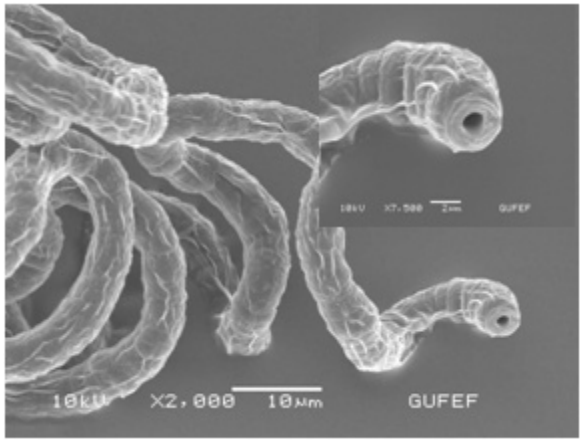

Figure 16. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, basal part of ductus spermatheca.

Here, the spermathecae of *C.viridis* have been described from SEM images for the first time. Bordy (2009) gave a stereomicroscopic drawing of spermathecal of this species. The stereomicroscopic results obtained in this study are in agreement with the results of Bordy (2009). Moreover, many new diagnostic characters that were previously unknown based on the ultrastructures of spermathecae were revealed by this SEM investigation.

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