

Aedeagus Structure of *Cassida prasina* Illiger, 1798 (Coleoptera: Chrysomelidae: Cassidinae) in Scanning Electrone Microscope (SEM)

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ABSTRACT

It is accepted that male genitalia are not diagnostic and 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. Ultra-structures of genitalia have never been studied except for a few works. The aim of this study was to determine whether the ultrastructural works are efficient or not. In the present work, male genitalia from two specimens of *Cassida prasina* Illiger, 1798 collected from Düzce and Karabük provinces in 2001 and 2003, which have been examined by electron microscope for the first time. New diagnostic characters were obtained and it revealed that it was diagnostic from the previously worked species in others subgenus. Photos of aedeagus in both SEM and stereo microscope are also given in the text.

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Taramalı Elektron Mikroskopunda (SEM) *Cassida prasina* Illiger, 1798 (Coleoptera: Chrysomelidae: Cassidinae) 'nın Aedeagus Yapısı

ÖZET

Cassida Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) cinsi içerisinde genel olarak erkek genitalinin ayırt edici olmadığı, spermathekanın ise kısmen ayırt edici olduğu kabul edilmektedir. Bununla birlikte şimdiye kadar yapılan genital çalışmalarının sadece stereo mikroskoba dayalı olduğu görülmektedir. Genital yapıların ince yapısı birkaç çalışma dışında çalışılmamıştır. İnce yapı çalışmalarının verimli olup olmadığını ortaya koymak amacıyla bu çalışmada Türkiye'den 2001 ve 2003 yıllarında Düzce ve Karabük illerinden toplanan toplam iki *Cassida prasina* Illiger, 1798 örneğine ait erkek genital yapısı SEM'de ilk kez incelenmiştir. Bu sayede yeni ayırt edici karakterler elde edilmiş ve diğer bir alt cinsteki daha önce çalışmış türlerden ayırt edici olduğu ortaya konulmuştur. Erkek genitalinin hem SEM hem de stereo mikroskop fotoğrafları da çalışma içerisinde sunulmuştur.

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INTRODUCTION

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

The Cassidinae fauna of Turkey includes 51 species and 6 genera. The genus *Cassida* has 41 species arranged in 11 subgenera (Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen and Kaya, 2014). However, a total of 5 species in 5 subgenera namely; *Cassida*

(*Cassida*) *seladonia* Gyllenhal, 1827, *Cassida* (*Lordiconia*) *canaliculata* Laicharting, 1781, *Cassida* (*Mionycha*) *azurea* Fabricius, 1801, *Cassida* (*Mionychella*) *hemisphaerica* Herbst, 1799 and *Cassida* (*Tylocentra*) *persica* Spaeth, 1926 were reported by Kışmalı&Sassi (1994), Warchalowski (2003, 2010) and Borowiec and Sekerka (2010) from Turkey without any exact locality, but only as Asian Turkey (Anatolia). With this reason, the occurrence of these species in Turkey needs confirmation. Consequently, it can be said that the fauna of Turkish *Cassida* includes 36 species of 9 subgenera on the base of exact localities in Turkey.

The nominotypical subgenus *Cassida* (*Cassida*) Linnaeus, 1758 includes 46 species distributed around the world (45 Palaearctic species including the type species *Cassida nebulosa* Linnaeus, 1758 and one native species from North America) (Borowiec, 2007). The nominotypical subgenus is represented by 21 species in Turkey. *Cassida prasina* Illiger, 1798 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). For this reason, ultrastructural investigations of aedeagi and spermathecae are very important in the genus *Cassida*.

Yet, 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 could be very important in the genus *Cassida* with regard to subgeneric classification. The aim of this study was to present detailed investigations on aedeagus of *C. prasina* Illiger, 1798 from Turkey by using scanning electron microscope. Obtained data are presented below.

MATERIALS and METHODS

The available specimens (a total of 2 specimens) for the present work were collected from Düzce and Karabük provinces in Turkey in 2001 and 2003. The specimens were deposited at Gazi University (Turkey, Ankara).

The aedeagus was dissected from abdomen, remaining tissue were removed with fine tweezers. For microscopic examination after cleaning, the samples were kept 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 5 kV.

RESULTS and DISCUSSION

Cassida prasina Illiger, 1798 (Fig. 1)

Cassida prasina is a Sibero-European species. Its body length is 5-5,9 mm. It is distributed in Europe (Austria, Belgium, Bulgaria, Bosnia and Herzegovina, Croatia,

Denmark, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Macedonia, Poland, Romania, Russia, Serbia, Spain, Slovakia, Slovenia, Sweden, Switzerland -Montenegro, Turkey, Ukraine) and Asia (China, Eastern Siberia, Kazakhstan, Russia: Western Siberia and Turkey) (Warchalowski, 2010; Borowiec and Sekerka (2010).

The species is widely distributed in Turkey. It has been recorded from 25 provinces including Antalya, Aydın, Balıkesir, Bartın, Bilecik, Bolu, Denizli, Düzce, Gaziantep, Giresun, Hatay, İzmir, Kahramanmaraş, Karaman, Kayseri, Kilis, Konya, Karabük, Manisa, Mersin, Sakarya, Tokat, Tunceli and Zonguldak provinces in Asian and Kırklareli province in European Turkey (Ekiz et al., 2013; Özdikmen and Kaya, 2014).

Material examined: **Düzce:** 4 km from Karakaş towards Yedigöller, 12.V.2003, 510 m, 1 specimen; **Karabük:** Exit of Ereğli, opposite Birsca Biscuit Factory, 03.VI.2001, 960 m, 1 specimen.



Figure 1. *Cassida prasina* Illiger, 1798 dorsal view (left), ventral view (right).

Şekil 1. *Cassida prasina* Illiger, 1798; A. dorsal görünüm (sol tarafta), B. Ventral görünüm (sağ tarafta).

Aedeagus: In stereo microscope (Fig. 2):

The median lobe is completely light brown.

In lateral view, median lobe is generally elliptical or semicircular, from the median foramen to the apex, prominent and more or less regularly curved towards the ventral. The median lobe gradually narrows slightly from the base to the apical portion. The apex of the median lobe is thinner in the short section and is flat and pointed.

In dorsal view, the median lobe is slightly enlarged only in the apical portion. The apex is more or less prolonged and clearly truncated. In this section, the apex is curved towards the dorsal, and thus the apex appears to be truncated. The ratio of the truncated portion of the apex to the width of the apical portion of the median lobe is about 26%, more than about 1/4. Upper and lateral margins of orifice are more or less

rounded. Dorsal plate is distinct and almost covered with basal half of orifice. Median lobe in lateral parts and fore part of orifice are thick. Thickening in lateral parts is distinctly smaller than the fore part. Median lobe is more or less U-shaped behind the orifice joined.

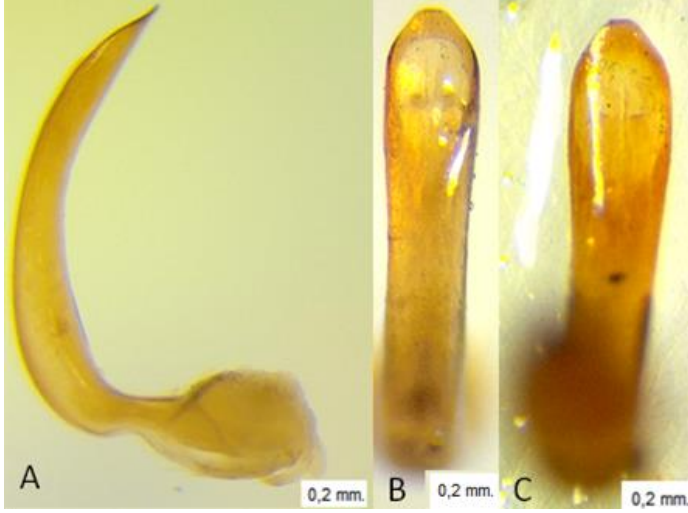


Figure 2. Aedeagus of *Cassida prasina* Illiger, 1798, A. Lateral view, B. Dorsal view, C. Ventral view. Scale bars size are 0,2 mm.

Şekil 2. *Cassida prasina* Illiger, 1798' nin aedeagusunun A. Yandan görünümü, B. Dorsalden görünümü, Ventralden görünümü. Ölçek çubuk boyutu 0,2 mm'dir.

In ventral view, median lobe slightly widened only apical part and the apex more or less prolonged and clearly truncated. Ventral surface of median lobe from median foramen to near apex with a median flattened area that broadly and longitudinally.

Aedeagus : In SEM (Scanning Electron Microscope)
(Figs. 3-7):

Only different characters in photos of SEM are presented below.

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

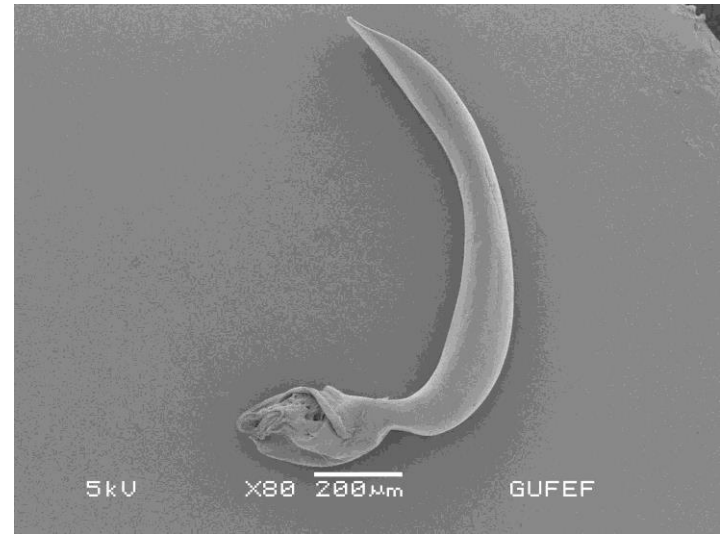


Figure 3. Aedeagus of *Cassida prasina* lateral view (SEM). Scale bars size is 200 µm.

Şekil 3. *Cassida prasina* Illiger, 1798' nin aedeagusunun yandan görünümü (SEM). Ölçek çubuk boyutu 200 µm.'dir.

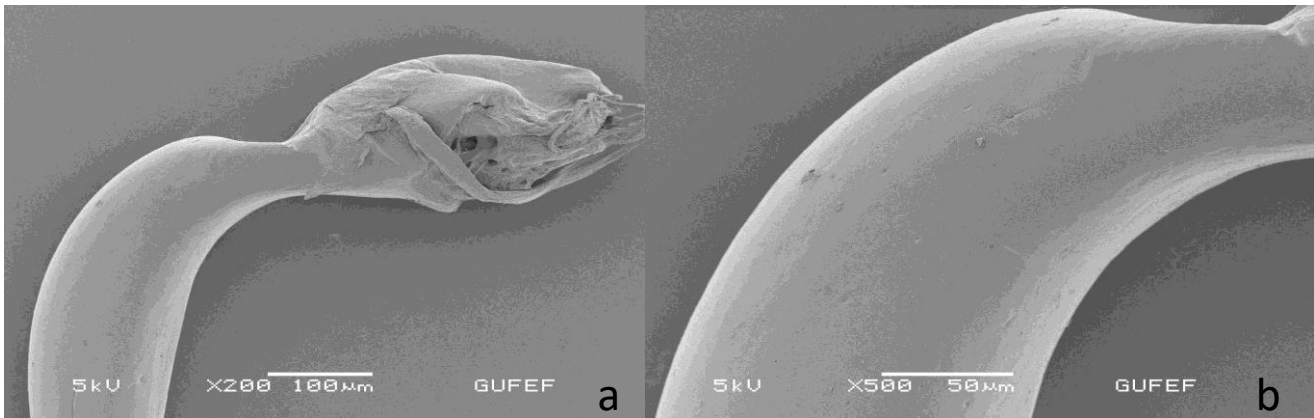


Figure 4. Aedeagus of *Cassida prasina*, a. Median foramen and b. Pits on median part of median tube in lateral view (SEM). a. Scale bars size is 100 µm and b. Scale bars size is 50 µm.

Şekil 4. *Cassida prasina* Illiger, 1798' nin aedeagusu, a. Medyan foramen ve b. Lateral görünümde medyan tüpün orta kısmındaki çukurlar (SEM). a. Ölçek çubuk boyutu 100 µm. ve b. Ölçek çubuk boyutu 50 µm.'dir.

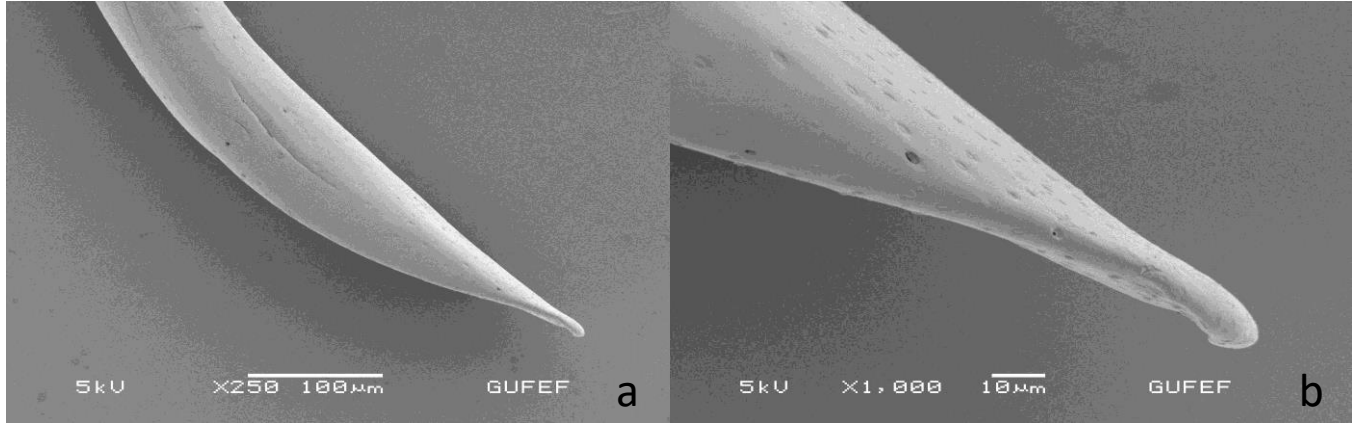


Figure 5. Aedeagus of *Cassida prasina*, a. Folded apex and b. Pits on apical part of median tube in lateral view (SEM). a. Scale bars size is 100 µm and b. Scale bars size is 10 µm.

Şekil 5. *Cassida prasina* Illiger, 1798'nin aedeagusu, a. Kıvrık uç kısım ve b. Yandan görünümde median tüpün uç kısmındaki çukurlar (SEM). a. Ölçek çubuk boyutu 100 µm. ve b. Ölçek çubuk boyutu 10 µm.'dir.

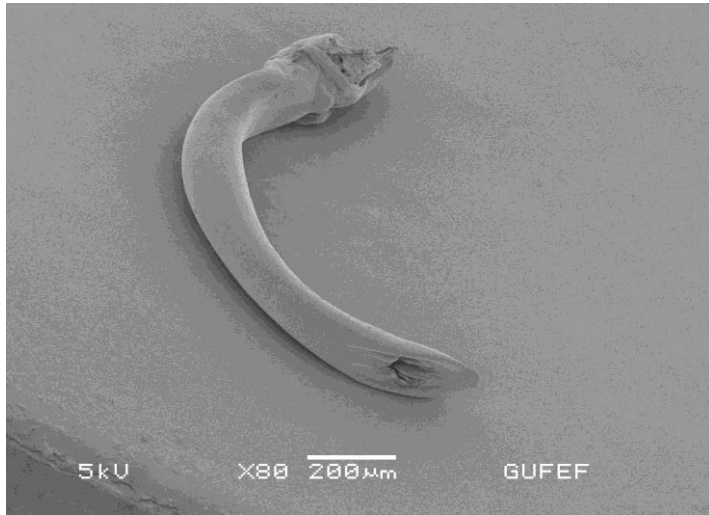


Figure 6. Aedeagus of *Cassida prasina*, apical part of median tube in dorso-lateral view (SEM). Scale bars size is 200 µm.

Şekil 6. *Cassida prasina* Illiger, 1798' nin aedeagusu, dorso-lateral görünümde median tüpün uç kısmı (SEM). Ölçek çubuk boyutu 200 µm.

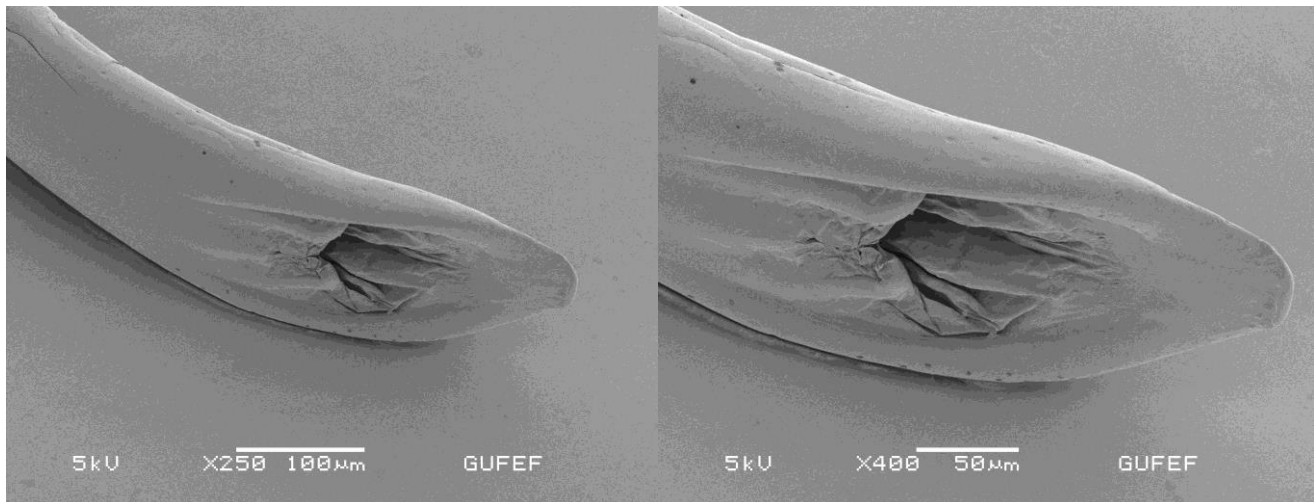


Figure 7. Aedeagus of *Cassida prasina*, folded apex, pits on lateral thickening of apical part of median tube in dorso-lateral view (SEM). Scale bars size are 100 µm and 50 µm.

Şekil 7. *Cassida prasina* Illiger, 1798, Aedeagusu, kıvrık uç, dorso-lateral görünümde median tüpün uç kısmında yan kalınlaşma üzerindeki çukurlar (SEM). Ölçek çubuk boyutu 100 µm. ve 50 µm.

As it is known, *Cassida prasina* is in the nominotypical subgenus *Cassida* (*Cassida*). According to the results of the present work, spermathecal morphologies of *Cassida prasina* distinctly differ from *Cassida nebulosa* Which is the type species of the genus and nominotypical subgenus.

Spermathecal morphologies of *Cassida nebulosa* and *Cassida prasina* are clearly different. Many characters in detail are diagnostic. These diagnostic characters can be seen below.

Aedeagus of *Cassida nebulosa* Linnaeus, 1758 in stereo microscope (Fig. 8): Median lobe is completely brown.

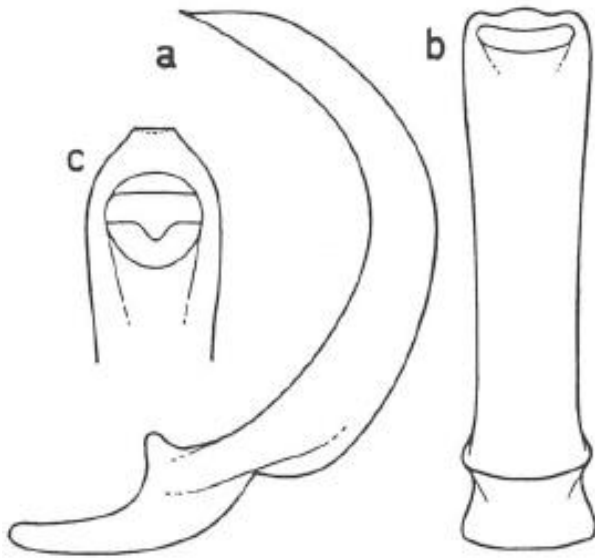


Figure 8. Aedeagus of *Cassida nebulosa* Linnaeus, 1758, a. lateral view, b. dorsal view, c. apex of median tube in dorsal view (from Bordy, 2009).

Şekil 8. *Cassida nebulosa* Linnaeus, 1758' nin aedeagusunun, a. Yandan görünümü, b. Dorsalden görünümü, C.Dorsal görünümde Medyan tüpün uç kısmı (Bordy, 2009'dan).

In lateral view, median lobe is distinctly and regularly curved from median foramen to apex in general. Median lobe is gradually, but slightly narrowed from the base to the apex. The apex of median lobe is almost abruptly sharpened and pointed.

In dorsal view, median lobe is barely widened from the median part to the apex, and the apex is more or less prolonged and clearly truncated. Upper and lateral margins of orifice are more or less rounded. Dorsal plate is distinct and almost covered with basal half of orifice. Median lobe is in lateral parts and the fore part of orifice is thickened. Thickening in lateral parts is smaller than the fore part. Median lobe is more or less V-shaped behind the orifice joined.

Diagnostic characters of aedeagus for *Cassida nebulosa* and *Cassida prasina* in stereo microscope:

- Median lobe is completely brown. In lateral view, median lobe is distinctly and regularly curved from median foramen to apex in general; the apex of median lobe it almost abruptly sharpened and pointed; projection of apex is short and right. In dorsal view, median lobe is barely widened from the median part to the apex.....*Cassida nebulosa* Linnaeus, 1758

- Median lobe varies from dark brown to brown, dark brown in 3/4 of its length basally, apical part (apical fourth) is clearly brown or light brown. In lateral view, median lobe is distinctly and almost regularly (elliptically or semicircularly) curved from median foramen to apex in general; the apex of median lobe gradually narrowed and pointed; the projection of apex is longer and right. In dorsal view, median lobe is slightly widened in only apical part.....*Cassida prasina* Illiger, 1798

CONCLUSION

As mentioned above, 10 aedeagal characters for *Cassida nebulosa*, which is the type species of the subgenus *Cassida* (*Cassida*), were determined. The type species is clearly distinguished from *Cassida prasina* by 4 determined aedeagal characters.

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

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

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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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