

THE STRUCTURE OF SPERMATHECA IN THE SUBGENUS CASSIDA (CASSIDA) LINNAEUS, 1758 (COLEOPTERA: CHRYSOMELIDAE: CASSIDINAE) AND ITS TAXONOMIC SIGNIFICANCE

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ABSTRACT

Spermathecae of 9 species including the type species Cassida nebulosa Linnaeus, 1758 belonging to the subgenus Cassida (Cassida) Linnaeus, 1758 from Turkey have been studied and figured. Spermathecal structures of these species were evaluated under both a stereo microscope and Scanning Electron Microscope (SEM). Vasculum, ampulla, ductus glandula auxiliaris and ductus spermatheca are generally constant in shape within species, but distinct and useful in comparison with other species. Based on the structure of these four parts of spermatheca, six morphological groups have been distinguished and defined in the subgenus Cassida (s.str.). According to the structures of spermathecae, it was revealed that the subgenus Cassida (Cassida) Linnaeus, 1758 is a polymorphic and polyphyletic group, not a monophyletic.In accordance with the results obtained in this study, the problematic taxonomic position of the subgenus Cassida (Cassida) is discussed. Accordingly, three new subgenera are described. The following three names are proposed for the new subgenera of Cassida Linnaeus, 1758; Cassida (Longiampulla) subgen. nov. with the type species Cassida vibex Linnaeus, 1767; Cassida (Diversivascula) subgen, nov. with the type species Cassida sanguinolenta Müller, 1776 and Cassida (Reliquacassida) subgen. nov. with the type species Cassida rubiginosa Müller, 1776.

KEYWORDS:

Coleoptera, Chrysomelidae, Cassidinae, Cassida, morphology, spermatheca

INTRODUCTION

The genus *Cassida* Linnaeus, 1758, a member of the tribe Cassidini Gyllenhal, 1813, has a large number of species spread almostall over the world (Palaearctic, Nearctic, Oriental, Afro-tropical, Madagascar and Australian). In the Palaearctic region

where Turkey is located, this genus comprises at present 167 species [1, 2]. To date, Cassida Linnaeus, 1758 has a total of 15 subgenera proposed for the species spreading in the Palaearctic and Oriental regions. There are no subgenera proposed for species in other regions. Therefore, it can be said that the subgeneric arrangement of this genus is not sufficient and complete [1, 3]. In Turkey, there are 41 species of 11 subgenera belonging to this genus [4-6]. However, 5 species of 5 subgenera as Cassida (Cassida) seladonia Gyllenhal, 1827, C. (Lordiconia) canaliculata Laicharting, 1781, C. (Mionycha) azurea Fabricius, 1801, C. (Mionychella) hemisphaerica Herbst, 1799 and C. (Tylocentra) persica Spaeth, 1926 have been recorded only as "Anatolia" without the actual locality record by Kısmalı & Sassi (1994), Warchalowski (2003, 2010) and Borowiec & Sekerka (2010) [2, 7-9]. Therefore, the presence of these species in Turkey is questionable and must be confirmed. According to the factual records, Cassida fauna of Turkey consists of 36 species belonging to 9 subgenera. The nominative subgenus Cassida (Cassida) Linnaeus, 1758 is represented in the world with 47 species with Cassida inopinata Sassi & Borowiec, 2006 (46 in the Palaearctic region and 1 in the Nearctic region) (Borowiec, 2007). Turkey's fauna consists of 21 species [4-6].

According to Bordy & Doguet (1987), Borowiec & Świętojańska (2001) and Borowiec (2007), aedeagal morphology in the genus Cassida Linnaeus, 1758 is not diagnostic. Spermathecal morphology is partially diagnostic. With this study, the mentioned opinions were supported for the species group. However, so far, genital morphology has been overlooked in the arrangement of the upper categories from species group level, though with the effect of the mentioned opinions. As stated by Borowiec (2007), currently proposed subgenera are based on questionable characters (some only 3-5 adult characters, some partially larval characters and some only pupal characters). Whereas, according to our study, it can be said that the similarities and differences in spermathecal morphology can be easily used in the arrangement of the subgenera [1, 10, 11].



Male genitalia in the genus *Cassida*, like in most genera of the tribe Cassidini, are uniform and do not offer good diagnostic characters. Contrary to the homogenity of male genitalia, studies on spermathecae of a few species of Cassidini suggested quite large diversity of spermathecal structure [12-14]. These positive results stimulated us to study spermathecae of the large and polymorphic genus *Cassida* Linnaeus.

MATERIALS AND METHODS

We studied 179specimens of 9 species of *Cassida* (*Cassida*) collected from various provinces in Turkey in 1991, 1993, 1996, 1997, 2001, 2003, 2004, 2014, 2015, 2018. Most of the specimens were obtained from the private collection of Prof. Dr. Hüseyin Özdikmen at the Department of Biology, Science Faculty of Gazi University (Turkey, Ankara). Some specimens were obtained from Nazife Tuatay Plant Protection Museum (NTM) (Turkey, Ankara). They were identified by Prof. Dr. Hüseyin Özdikmen.

The spermathecae were dissected from the abdomen, and the remaining tissue was removed with fine tweezers. For light microscopic examination after cleaning, the samples were placed 70% ethanol and examined with an 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.In this study, figure 1 was used for the terminology of spermathecal structures.

RESULTS

In this work, structures observed by stereo microscope and SEM of spermathecae of 9 species belonging to the subgenus *Cassida* (*Cassida*) (Coleoptera: Chrysomelidae: Cassidinae) from Turkey are presented. The studied species are given in alphabetical order. Obtaining data are presented as follows:

Genus *Cassida* Linnaeus, 1758 Subgenus *Cassida* Linnaeus, 1758

Cassida atrata Fabricius, 1787 (Figure 2)

Structure of spermatheca. Vasculum is in the form of eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is more proturuded forward). In vasculum, cornu is much thicker than nodulus especially in the apical part. The apical part of cornu is noticeably swollen and dark colored. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is dark colored on the inner surface of the basal part. In SEM, this part carries an integument, the back edge of which is extended backwards in the form of a wavy fringe. Ampulla is in the form of a

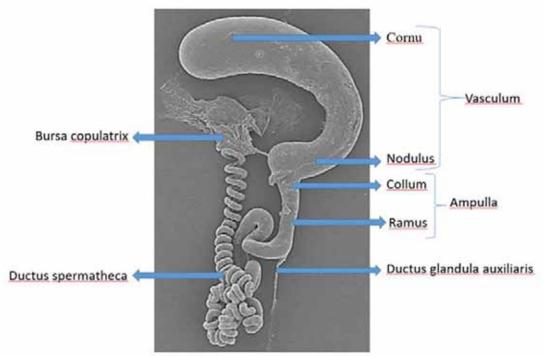


FIGURE1
Terminology of spermathecal structures.



duct or tube, which is very distinct, elongated and is laterally connected to nodulus on the outer surface of its basal part. It is slightly thinner than the thickness of nodulus. Collum is quite short, but distinct. Ramus is very distinct. It is in the form of a long, straight and thick duct or tube, which is slightly enlarged in the middle part. The widest part of ramus is slightly thinner than nodulus. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface at the end of ramus. Ductus spermatheca is connected straightly to ramus, which is thick and long. It is in the form of a flat tube, forming wide folds in the proximal part. It is thinner in the next big part and is spirally curved regularly. Ductus spermatheca is slightly thinner than ramus or approximately equal width with ramus in the proximal part and is much thinner than ramus in the spiral part. Ductus spermatheca is about 4-5 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

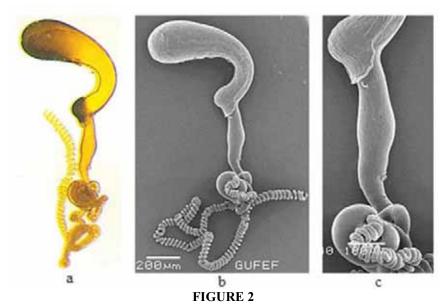
Material examined.Turkey,Kayseri prov.: Develi, Kocahacılı, 38°11'15"N 35°23'50"E, 07.V.2018, 1092 m,1 ex.; Niğde prov.: Melendiz, between Hançerli-Küçükköy, 03.VII.1996, 1 ex; Çamardı, 29.VI.1996, 1 ex.

Cassida fausti Spaeth & Reitter, 1926 (Figure3)

Structure of spermatheca. Vasculum is in the form of an eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is more proturuded forward). In vasculum, cornu is much thicker than nodulus especially in the apical part.

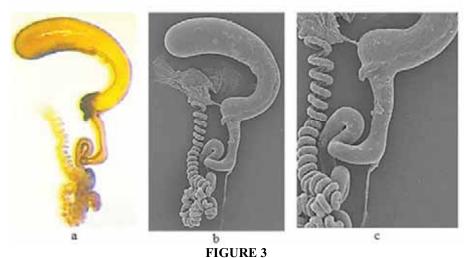
The apical part of cornu is noticeably swollen. Cornu's apex is rounded. Nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is dark colored on the innersurface of the basal part. In SEM, this part carries an integument, the back edge of which is extended backwards in the form of a wavy fringe. Ampulla is in the form of a duct or tube that is very distinct, elongated and is laterally connected to nodulus on the outer surface of its basal part. It is much thinner than nodulus. Collum is quite short, but distinct. Ramus is very distinct. It is in the form of a long, straight and thick duct or tube. Ramus is much thinner than nodulus. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface at the end of ramus. Ductus spermatheca is connected straightly to ramus, which is thick and long. It is in the form of a flat tube, which forms wide folds in the proximal part. It is thinner in the next big part and is spirally curved regularly. Ductus spermatheca is only approximately equal width with ramus in the proximal part and is much thinner than ramus in the spiral part.Ductus spermatheca is about 2-3 times longer than vasculum.In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined.Turkey,Ankara prov.: Çubuk, 1 ex.; Bolu prov.: Bolu-Gerede road, return of Susuz-Kınık villages, exit of Bolu, 17.V.2003, 720 m, 1 ex.; Kastamonu prov.: Kastamonu- Araç road, Kastamonu police forest, exit of Kastamonu 1st km, 16.V.2003, 975 m, 1 ex.; Kayseri prov.: İncesu, Bahçelievler, 38°37'58''N 35°11'48''E, 08.V.2018, 1072 m, 2 exs.; Konya prov.: Kulu, Tavşanlı, 17.V.1997, 1000 m, 1 ex.

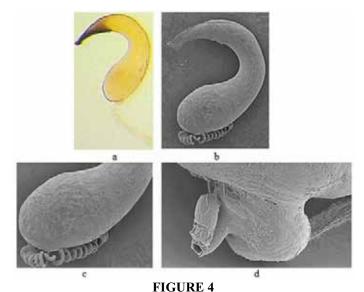


Spermathecae of Cassida atrata, a. view in stereo microscope, b-c. view in SEM.





Spermathecae of *Cassida fausti*, a. view in stereo microscope, b-c. view in SEM.



Spermathecae of *Cassida nebulosa*, a. view in stereo microscope, b-d. view in SEM.

Cassida nebulosa Linnaeus, 1758(Figure 4)

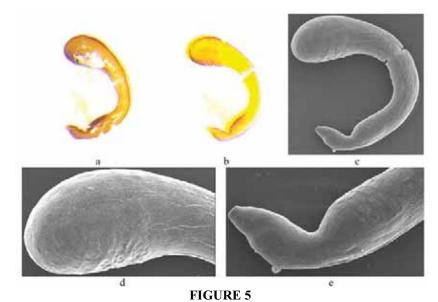
Structure of spermatheca. Vasculum is in the form of an opened hook or bird beak-shaped. It is distinctively curved, relatively wide-angle, symmetrical C-shaped. In vasculum, nodulus is much thicker than cornu especially in the basal part. Apical part of cornu is clearly sharpened and dark colored. Cornu's apex is pointed. Apical part of cornu extended to the front of its apex with an integument. The nodulus is quite long, not parallel, distinctly swollen and bulbous. In SEM, nodulus carriesa wide integument on the inner surface of the basal part. The back edge of the integument isnot extended backwards. Ampulla is very small. It is in the form of a tubercle and is connected to nodulus on the outer surface of its basal part.It is incomparably smaller than nodulus. Collum is invisible, vague or very reduced. Ramus is clearly visible and in the form of a tubercle. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to the end of ramus. Ductus spermatheca is connected to ramus on the outer surface of its middle part. Ductus spermatheca is very long, quite thin, and is spirally curved regularly along almost over its entire length. It is much thinner than ampulla. Ductus spermatheca is about 10 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined: Turkey,Konya prov.: Akşehir, Dereçine, 05.VIII.1993, leg. A. Kalkandelen, 4 exs.

Cassida palaestina Reiche, 1858 (Figure 5)

Structure of spermatheca. Vasculum is in the form of a boomerang or payphone. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is slightly protruded forward). In vasculum, cornu is much thicker than nodulus especially in the apical part. The





Spermathecae of Cassida palaestina, a-b. view in stereo microscope, c-e. view in SEM.

apical part of cornu is noticeably swollen. Cornu's apex is rounded. In SEM, the underside of the apical part of cornu has almost an integument-like thickening. Therefore, this part appears somewhat dark in stereo microscope. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is somewhat dark colored on the inner surface of the basal part. In SEM, this part carries an integument-like thickening. Ampullais distinct. It is tubercular, quite short and is attached flat (parallel) at the bottom to the basal end of nodulus. It is slightly thinner than nodulus. Collum is quite distinct and is in the form of a short stem. Collum is much thinner than nodulus.Ramus is distinct and isin the form of a tuber or cone-like, quite small, swollen and more or less protruding outwardin the middle part.Ramus is slightly thinner than nodulus in its widest part.Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface of its middle part.Ductus spermatheca is connected straightly to ramusin the middle at the end of ramus, but is broken in the examined sample. However, it is understood from the broken pieces that it is probably quite thin and spirally curved over its entire length. Ductus spermateka is much thinner than ramus. Ductus spermatheca is probably about 2-3 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined: Turkey, Aksaray prov.: 5. km after crossing the Aksaray-Ulukışla junction, 29.V.2001, 1275 m, 4 specimens.

Cassida pannonica Suffrian, 1844(Figure6)

Structure of spermatheca.Vasculum is in the form of an eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical

C-shaped (the apical part of cornu is more proturuded forward). In vasculum, cornu is much thicker than nodulus especially in the apical part. The apical part of cornu is noticeably swollen. Cornu's apex is rounded. In SEM, apex has a very small bud-shaped process. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is dark colored on the inner surface of the basal part. In SEM, this part carries an integument, the back edge of which is extended backwards in the form of a wavy fringe. Ampulla is in the form of a duct or tube that is very distinct, elongated and is laterally connected to nodulus on the outer surface of its basal part. It is much thinner than nodulus. Collum is quite short, but distinct. Ramus is very distinct and is in the form of a long, straight and thickish duct or tube. Ramus is much thinner than nodulus. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface at the end of ramus. Ductus spermatheca is connected straightly to ramus, which is thick and long. It is in the form of a flat tube, which forms wide folds in the proximal part. It is thinner in the next big part and is spirally curved regularly. Ductus spermatheca is approximately equal width with ramus in the proximal part and is much thinner than ramus in the spiral part. Ductus spermatheca is about 7-8 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined:Turkey,Adana prov.: ReturnPozantı-Mersin-Çamlıyayla, 20th km, 30.V.2001, 285 m, 2 exs.; Ankara prov.: Kızılcahamam, Aköz village, 28.V.1997, 1150 m, 1 ex.; Bolu prov.: Düzce, Gavurpınarı (Between İğneler-Dibektaş), Yığılca, 1 ex.; Çankırı prov.: Kızılırmak, exit of Karamürsel village, Halimintepe, 40°24'N 34°02'E, 24.IV.2014, 550 m, 1 ex.;

Kızılırmak, entry of Kemalli village, 40°18'N 34°02'E, 24.IV.2014, 686 m, 1 ex.; Kızılırmak, Between Bostanci-Hacilar villages, 40°19'N 33°51'E, 25.IV.2014, 565 m, 1 ex.; Centre, Salt cavern district, 40°31'N 33°45'E, 25.IV.2014, 699 m, 1 ex.; Centre, Between Pehlivanlı-Alaçatı villages, 40°34'N 33°52'E, 26.IV.2014, 925 m, 1 ex.; Centre, Aşağıçavuş-Yukarıçavuş return, 40°40'N 33°35'E, 09.V.2015, 837 m, 1 ex.; Centre, Balıbağı village, 40°34'N 33°46'E, 15.V.2015, 1037 m, 1 ex.; Centre, between Ovacık-Kuzuköy village, 40°32'N 33°53'E, 15.V.2015, 919 m, 1 ex.; Centre entry of Karadayı, 40°24'N 33°45'E, 16.V.2015, 856 m, 2 exs.; Kızılırmak, Yukarıalagöz village, 40°22'N 33°53'E, 16.V.2015, 642 m, 1 ex.; Kızılırmak, Kavaklı, 40°22'N 34°1'E, 16.V.2015, 542 m, 1 ex.; Yapraklı, Bugay village, 40°42'N 33°46'E, 25.V.2015, 897 m, 1 ex.; Yapraklı, entry of Çevrecik, 40°39'N 33°49'E, 25.V.2015, 953 m, 1 ex.; Yapraklı, Kirliakça village, 40°37'N 33°54'E, 26.V.2015, 914 m, 3 exs.; Ilgaz, entry of Yaylaören village, 40°52'N 33°30'E, 17.VI.2015, 914 m, 1 ex.; Ilgaz, entry of Şeyhyunus village, 40°50'N 33°31'E, 18.VI.2015, 1421 m, 1 ex.; Bayramören, Harmancık village road, 41°2'N 33°13'E, 21.VI.2015, 861 m, 1 ex.; Atkaracalar, Budakpınarı village, 40°51'N 33°8'E, 22.VI.2015, 1096 m, 1 ex.; Yapraklı, Entry of Kaymaz village, 40°43'N 33°54'E, 29.VI.2015, 1011 m, 1 ex.; Yapraklı, Yüklü-Çevrecik return, 40°40'N 33°45'E, 29.VI.2015, 926 m, 1 ex.; **Corum prov.:** Göletdere, 5 miles to Karagöl village, 16.VI.2003, 1120 m, 1 ex.; Karabük prov.: Eflani, Yağlıca village, 15.V.2003, 975 m, 1 ex.; Kayseri prov.: Sarız, Cörekdere, 38°28'50"N 36°27'29"E, 6.V.2018, 1637 m. 1 ex.

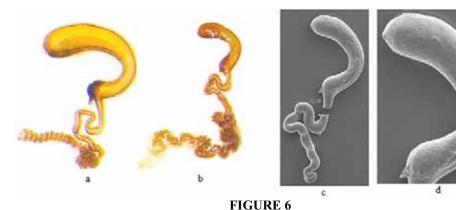
Cassida rubiginosa Müller, 1776(Figure7)

Structure of spermatheca. Vasculum is in the form of a boomerang or payphone. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is slightly protruded forward). In vasculum, cornu is much thicker than

nodulus especially in the apical part. The apical part of cornu is noticeably swollen. Cornu's apex is rounded. The apical part of cornu is dark colored. In SEM, the underside of the apical part of cornu has almost an integument-like thickening. Therefore, this part appears somewhat darker in stereo microscope. The nodulus is quite short, almost parallel, and not swollen. Nodulus is more or less dark colored on the inner surface of the basal part. In SEM, this part carries an integument-like thickening. Ampulla is tubercular, quite short but distinct and is attached flat (parallel) at the bottom to the basal end of nodulus. It is slightly thinner than nodulus. Collum is distinct and it is in the form of a short stem. Collum is much thinner than nodulus. Ramus is distinct and is in the form of a tuber or cone-like, quite small, swollen and more or less protruding outwardin the middle part. Ramus is slightly thinner than nodulus in its widest part. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface in the middle part of ramus. Ductus spermatheca is connected straightly to ramusin the middle at the end of ramus, very thin and spirally curved regularly over its entire length. It is much thinner than ramus. Ductus spermatheca is aproximately equal length with vasculum or about 2 times longer than vasculum.In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

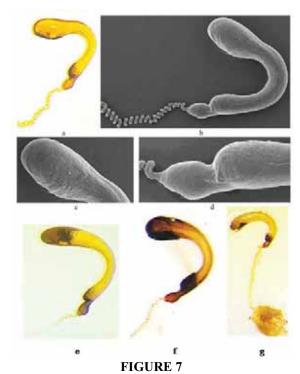
Fresenius Environmental Bulletin

Material examined: Turkey, Adana prov.: Pozantı, Tekir plateau, 23.VI.1997, 1300 m, 1 ex.; Ankara prov.: Kızılcahamam, Aköz village, 28.V.1997, 1150 m, 1 ex.; Bolu prov.: Yedigöller, Between Özbağı-Çıplaklar, Devrek (45 km to Yedigöller), 13.V.2003, 160 m, 1 ex.; İçel prov.: Sertavul pass, 26.VI.2001, 1410 m, 1 ex.; Karabük prov.: Üçevler, 14.VI.2003, 1000 m, 1 ex.; Safranbolu, Bulak village, Hazar district, 14.V.2003, 740 m, 1 ex.; Kayseri prov.: Sarız, Yedioluk, 38°33'27''N 36°27'01''E, 29.V.2018, 1770 m, 20 exs.; Melikgazi, Yeşilyurt, 04.VII.2018, 1090 m, 1 ex.; Sarız, Darıdere, 05.VII.2018, 1555 m, 1 ex.; Sarız,



Spermathecae of Cassida pannonica, a-b. view in stereo microscope, c-d. view in SEM.





Spermathecae of Cassida rubiginosa, a, e-g. view in stereo microscope, b-d. view in SEM.

Yedioluk, 38°33'33''N 36°27'16''E, 05.V.2018, 1812 m, 6 exs.; Pırnarbaşı, Kılıçkışla, 38°39'48''N 36°12'37''E, 05.VII.2018, 1433 m, 24 exs.; Konya prov.: Kulu, Tavşanlı, 31.V.1997, 1000 m, 2 exs.; Nevşehir prov.: Avanos, 20.VII.1992, 1000 m, 1 ex.; Göreme, 19.V.1997, 1260 m, 1 ex.; Niğde prov.: Azatlı Tepeköy road, 21.VIII.1996, 1 ex.; Kayaardı, Niğde vineyard, 17.VI.1997, 6 exs.; Bor, Derbent district, 06.VII.1997, 6 exs.; Tepeköy plateau, Altınhisar-Çiftlik road, 29.IV.1997, 4 exs.

Cassida sanguinolenta Müller, 1776(Figure8)

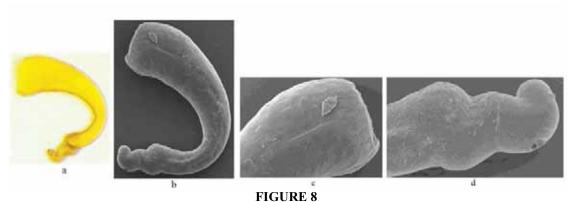
Structure of spermatheca. Vasculum is in the form of an eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is much more protruded forward). In vasculum, cornu is much thicker than nodulus especially in the apical part. The apical part of cornu is very swollen. Cornu's apex is probably rounded. The nodulus is quite short, almost parallel, and not swollen. In SEM, nodulus has almost an integument-like thickening. Therefore, this part appears somewhat dark in stereo microscope.Ampulla is in the form of a wavy duct or tubethat is distinct, thickish, slightly elongated, and is attached flat (parallel) at the bottom to the basal end of the nodulus. The ramus of ampulla is recessed on the outer surface where it connects to the collum. Therefore, ampulla has taken a comma-like wavy shape in general view. Ampulla is slightly thinner than nodulus. Collum is quite short, distinct, and in the form of a wide stem. Ramus is in the form of a wavy duct or tubethat is short but distinct and thickish. Ductus glandula auxiliaris (ductus spermathecal gland) is laterally attached to ramus on the outer surface at the end of ramus. Ductus spermatheca islaterally connected straightly to ramuson the inner surface at the end of ramus. Ductus spermateka is broken. However, it is understood from the remaining fragments that it is probably very thin and spirally curved regularly over its entire length. Ductus spermateka is much thinner than ramus. Ductus spermatheca is about 2 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined: Turkey, Çankırı prov.: Yapraklı, Kirliakça village, 40°37'N 33°54'E, 26.V.2015, 1 ex.; Mersin prov.: Entry of Fındıkpınarı, Municipal board, 31.V.2001, 1 ex.

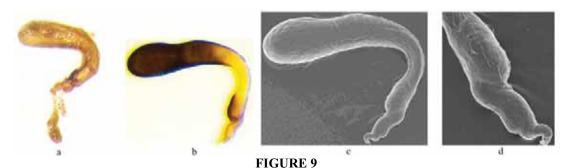
Cassida stigmatica Suffrian, 1844 (Figure 9)

Structure of spermatheca. Vasculum is in the form of an eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is much more protruded forward). In vasculum, cornu is thicker than nodulus especially in the apical part. The apical part of cornu is swollen and dark colored. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is dark colored on the inner surface of the basal part. In SEM, this part carries an integument. Ampulla is in the form of a wavy duct or tubethat is distinct, thickish, slightly elongated, and is attached flat (parallel) at the bottom to the basal





Spermathecae of *Cassida sanguinolenta*, a. view in stereo microscope, b-d. view in SEM.



Spermathecae of Cassida stigmatica, a-b. view in stereo microscope, c-d. view in SEM.

end of the nodulus. The ramus of ampulla is recessed on the outer surfacewhere it connects to the collum. Therefore, ampulla has taken a comma-like wavy shape in general view. Ampulla is slightly thinner than nodulus. Collum is quite short, distinct, in the form of a wide stem. Ramus is in the form of a wavy duct or tubethat is short but distinctand thickish. Ductus glandula auxiliaris (ductus spermathecal gland) is laterally attached to ramus on the outer surface at the end of ramus. Ductus spermatheca islaterally connected straightly to ramuson the inner surface at the end of ramus, short, very thin and spirally curved regularly over its entire length.Ductus spermatheca ismuch thinner than ramus. Ductus spermatheca is approxymately equal length with vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.

Material examined: Turkey, Ankara prov.: Kızılcahamam, Soğuksu National Park, 03.VII.1991, 1400 m, 2 exs.; Kızılcahamam, Güvem village, 28.V.1997, 1100 m, 4 exs.; Kızılcahamam, Aköz village, 30.VIII.1997, 1150 m, 1 ex.; Bolu prov.: Gerede-Bolu road, 8 km to Bolu, 17.V.2003, 710 m, 1 ex.; Pazarköy-Eskipazar road, enter of Kayılar village, 20.V.2004, 1 ex.; Çankırı prov.: Ilgaz, Between Beyköy-Saraycık villages, 40°59'N 33°44'E, 19.VI.2015, 1195 m, 1 ex.; Yapraklı, Yukarıöz, wooded area, 40°51'N 33°44'E, 25.VI.2015, 1380 m, 2 exs.

Cassida vibex Linnaeus, 1767 (Figure 10)

Structure of spermatheca. Vasculum is in the form of an eggplant or boxing gloves. It is distinctively curved, relatively wide-angle, asymmetrical C-shaped (the apical part of cornu is more proturuded forward). In vasculum, cornu is thicker than nodulus especially in the apical part. The apical part of cornu is swollen and dark colored. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is dark colored on the inner surface of the basal part. In SEM, this part carries an integument, the back edge of which is extended backwards in the form of a wavy fringe. Ampulla is in the form of a duct or tube that is very distinct, slightly elongated and is laterally connected to nodulus on the outer surface of its basal part. It is slightly thinner than nodulus. Collum is quite short but distinct. Ramus is very distinct and is in the form of a longish, straight and thick duct or tube. Ramus is slightly thinner than nodulus. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface at the end of ramus. Ductus spermatheca is connected straightly to ramus, thick and longish. It is in the form of a flat tube, which makes wide folds in the proximal part. The next big part is spirally curved regularly. The spirally curved part of ductus spermatheca approximately equal width withits proximal part.Ductus spermatheca is approximately equal width with ramus over its entire length. Ductus spermatheca is about 2 times longer than vasculum. In SEM, spermatheca carries scattered, irregular and sparsely ultrastructural pits on various parts.



Material examined: Turkey, Bolu prov.: Entry of Mengen (Devrek-Mengen road), 18.VI.2003, 650 m, 1 ex.; Çankırı prov.: Atkaracalar, entry of Hüyük village, 40°51'N 33°3'E, 25.IV.2015, 1331 m, 1 ex.; Kızılırmak, between Küçükbahçeli-Büyükbahçeli 40°23'N villages, 33°58'E, 01.V.2015, 583 m,1 ex.; Centre, Aşağıçavuş village, 40°41'N 33°36'E, 09.V.2015, 847 m, 2 exs.; Centre, Alanpınar- Başeğmez villages return, 40°41'N 33°35'E, 09.V.2015, 822 m, 2 exs.; Kurşunlu, Dağören-Sünürlü villages return, 40°48'N 33°16'E, 10.V.2015, 996 m, 1 ex.; Eldivan, Oğlaklı village, 40°32'N 33°33'E, 13.V.2015, 1027 m, 2 exs.; Eldivan, entry of Elmacı village, 40°28'N 33°33'E, 13.V.2015, 937 m, 1 ex.; Eldivan, entry of Çiftlik village, 40°34'N 33°30'E, 14.V.2015, 844 m, 1 ex.; Centre, Tuzlu village-Yapraklı return, 40°35'N 33°40'E, 15.V.2015, 885 m, 1 ex.; Centre, Balıbağı village, 40°34'N 33°46'E, 15.V.2015, 1037 m, 1 ex.; Kızılırmak, Yukarıalagöz village, 40°22'N 33°53'E, 16.V.2015, 642 m, 2 exs.; Ilgaz, exit of Belören village, 40°51'N 33°30'E, 27.V.2015, 903 m, 6 exs.; Ilgaz, Belören village, 40°51'N 33°30'E, 18.VI.2015, 914 m, 4 exs.; Ilgaz, Sazak village, 40°56'N 33°43'E, 18.VI.2015, 1144 m, 1 ex.; Ilgaz, Kırkpınar Highland, 41°00'N 33°41'E, 19.VI.2015, 1230 m, 1 ex.; Yapraklı, Yukarıöz village, 40°51'N 33°44'E, 25.VI.2015, 1380 m, 1 ex.; **Çorum prov.:** Tosya-Kargı road, 2 miles to Akçayazı (Zincirlikuyu env.), 16.VI.2003, 655 m, 1 ex.; Göletdere, 5 miles to Karagöl village, 16.VI.2003, 1120 m, 1 ex.; **İçel prov.:** Fındıkpınarı, exit of Çağlayan, 25.VI.2001, 1070 m, 1 ex.; **Kastamonu prov.:** Exit of Kastamonu 6th km (between Kastamonu-Araç), 16.V.2003, 1000 m, 1 ex.; Kayseri prov.: Tomarza, Toklar, 10.VI.2015, 1425 m, 1 ex.; Niğde prov.: Between Niğde-Bor, Derbent district, 17.VI.1997, 2 exs.; Niğde Mountains, Karyağdı, 17.VI.1997, 1 ex.; Çamardı, Yelatan village, 23.VI.1997, 1280 m, 1 ex.; exit of Ulukışla, Bahçelik, 29.V.2001, 1445 m, 1 ex.

Structure of spermatheca in the subgenus Cassida (Cassida) Linnaeus, 1758. Cassida nebulosa Linnaeus, 1758 is the type species of both the genus Cassida Linnaeus, 1758, and therefore the nominative subgenus Cassida (Cassida) Linnaeus, 1758. Therefore, as a rule, being able to question whether there are other species belonging to this subgenus makes it necessary to firstly define the spermathecal morphology of Cassida nebulosa Linnaeus, 1758. The spermathecal structure of the type species should characterize this subgenus. The structure of spermatheca in Cassida nebulosa Linnaeus, 1758 is given in the text.

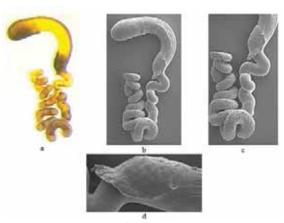


FIGURE 10 Spermathecae of *Cassida vibex*, a. view in stereo microscope, b-d. view in SEM.

Vasculum, ampulla, ductus glandula auxiliaris and ductus spermatheca are generally constant in shape within species, but distinct and useful in comparison with other species. Based on the structure of these four parts of spermatheca we divided the species of the subgenus *Cassida* (s.str.) into six groups:

Group I (Cassida nebulosa group): Vasculum is in the form of an opened hook or bird beak-shaped. It is symmetrical C-shaped. Nodulus is much thicker than cornu especially in the basal part. Apical part of cornu clearly sharpened and dark colored. Cornu's apex is pointed. Apical part of cornu extended to the front of its apex with an integument. The nodulus is quite long, not parallel, distinctly swollen and bulbous. In SEM, nodulus carries a wide integument on the inner surface of the basal part. Ampulla is very small. It is in the form of a tubercle and is connected to nodulus on the outer surface of its basal part. It is incomparably smaller than nodulus. Collum is invisible, vague or very reduced. Ramus is clearly visible and in the form of a tubercle. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus at the end of ramus. Ductus spermatheca is connected to ramus on the outer surface in the middle part of ramus. Ductus spermatheca is very long, quite thin, and is spirally curved regularly almost over its entire length. It is much thinner than ampulla. Ductus spermatheca is at least 6 times longer than vasculum. Here: Cassida nebulosa Linnaeus, 1758 (Figures 4, 12). In addition, Cassida flaveola Thunberg, 1794 (under Bordy, 2009) [15].

Group II (Cassida vibex group): Vasculum is in the form of an eggplant or boxing gloves. It is asymmetrical C-shaped (the apical part of cornu is more protruded forward). Cornu is usually much thicker than nodulus especially in the apical part. The apical part of cornu is usually noticeably swollen. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. In stereo



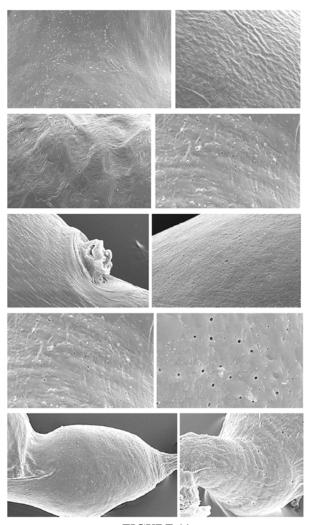


FIGURE 11
The ultrastructural pits on various parts of spermatheca in the subgenus Cassida (Cassida) Linnaeus, 1758 (SEM).

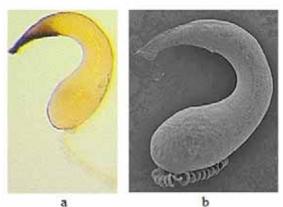
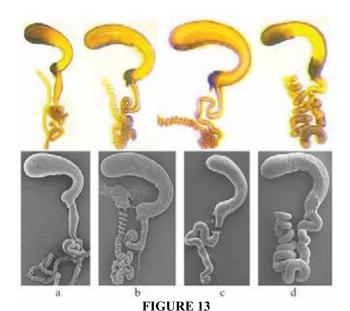


FIGURE 12 Spermathecae of *Cassida nebulosa*, a. view in stereo microscope, b-d. view in SEM.

microscope, nodulus is dark colored on the inner surface of the basal part. In SEM, this part carries an integument, the back edge of which is extended backwards in the form of a wavy fringe. **Ampulla** is in the form of a duct or tube that is very distinct,

elongated and is laterally connected to nodulus on the outer surface of its basal part. It is much or slightly thinner than nodulus. Collum is quite short, but distinct. Ramus is very distinct. It is in the form of a long, straight and thick duct or tube. Ramus is much or slightly thinner than nodulus. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface at the end of ramus. Ductus spermatheca is connected straightlyto ramus, thick or thickish and long or longish. It is in the form of a flat tube, which forms wide folds in the proximal part. It is thinner in the next big part and is spirally curved regularly. Ductus spermatheca is slightly thinner than ramus or approximately equal width with ramus in the proximal part and is usually much thinner than ramus in the spiral part. Ductus spermatheca is at least more than 2 times longer than vasculum. Here: Cassida atrata Fabricius, 1787 (Figures 2, 13), Cassida fausti Spaeth & Reitter, 1926 (Figures 3, 13), Cassida pannonica Suffrian, 1844 (Figures 6, 13), Cassida vibex Linnaeus, 1767 (Figures 10, 13). In addition, Cassida bergeali





Spermathecae in stereo microscope and SEM. a. Cassida atrata, b. Cassida fausti, c. Cassida pannonica, d. Cassida vibex.

Bordy, 1995, Cassida elongata Weise, 1893, Cassida inopinata Sassi & Borowiec, 2006, Cassida major Kraatz, 1874 (under Sassi & Borowiec, 2006), Cassida bergeali Bordy, 1995, Cassida ferruginea Goeze, 1777, Cassida humeralis Kraatz, 1874, Cassida panzeri Weise, 1907 (under Bordy, 2009) and Cassida ferruginea Goeze, 1777, Cassida mongolica Boheman 1854 (under Suenaga, 2013) [15, 16].

Group III (Cassida rubiginosa group): Vascu**lum** is in the form of a boomerang or payphone. It is asymmetrical C-shaped (the apical part of cornu is slightly protruded forward). Cornu is much thicker than nodulus especially in the apical part. The apical part of cornu is noticeably swollen. Cornu's apex is rounded. In SEM, the underside of the apical part of cornu has almost an integument-like thickening. Therefore, this part appears somewhat dark in stereo microscope. The nodulus is quite short, almost parallel, and not swollen. In stereo microscope, nodulus is somewhat dark colored on the inner surface of the basal part. In SEM, this part carries an integument or integument-like thickening. Ampulla is tubercular, quite short but distinct and is attached flat (parallel) at the bottom to the basal end of nodulus. It is slightly thinner than nodulus. Collum is distinct and is in the form of a short stem. Collum is much thinner than nodulus. Ramus is in the form of a tuber or cone-like, quite small but distinct, swollen and more or less protruding outwardin the middle part. Ramus is slightly thinner than nodulus in its widest part. Ductus glandula auxiliaris (ductus spermathecal gland) is attached to ramus on the outer surface in the middle part of ramus. Ductus spermatheca is connected straightly to ramusin the middle at the end of ramus, very thin and spirally curved regularly over its entire length. It is much thinner than ramus. Ductus spermatheca is approximately equal length with vasculum or more than 2 times longer than vasculum. Here: *Cassida palaestina* Reiche, 1858 (Figures 5, 14), *Cassida rubiginosa* Müller, 1776 (Figures 7, 14), In addition, *Cassida deflorata* Suffrian, 1844, *Cassida prasina* Illiger, 1798 and *Cassida rufovirens* Suffrian, 1844 (under Bordy, 2009) [15].

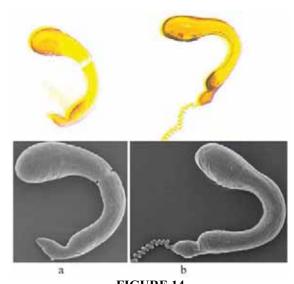
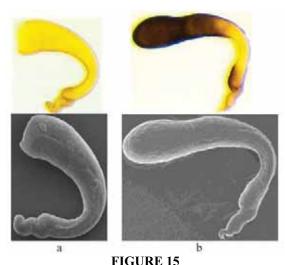


FIGURE 14
Spermathecaein stereo microscope and SEM. a.
Cassida palaestina, b. Cassida rubiginosa.

Group IV (Cassida sanguinolenta group): Vasculumis in the form of an eggplant or boxing gloves. It is asymmetrical C-shaped (the apical part of cornu is much more protruded forward). Cornu is thicker than nodulus especially in the apical part. The apical part of cornu is swollen. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. In SEM, nodulus has almost an integument-like thickening on the inner surface of the basal part. Therefore, this part appears somewhat



dark in stereo microscope. **Ampulla** is in the form of a wavy duct or tubethat is distinct, thickish, slightly elongated, and is attached flat (parallel) at the bottom to the basal end of the nodulus. The ramus of ampulla is recessed on the outer surface where it connects to the collum. Therefore, ampulla has taken a commalike wavy shape in general view. Ampulla is slightly thinner than nodulus. Collum is quite short, distinct, and in the form of a wide stem. Ramus is in the form of a wavy duct or tubethat is short but distinct and thickish. Ductus glandula auxiliaris (ductus spermathecal gland) is laterally attached to ramus on the outer surface at the end of ramus. Ductus spermatheca islaterally connected straightly to ramuson the inner surface at the end of ramus, short, very thin and spirally curved regularly over its length.Ductus spermatheca ismuch thinner than ramus.Ductus spermatheca is approximately equal length with vasculum or about 2-3 times longer than vasculum.Here: Cassida sanguinolenta Müller, 1776 (Figures 8, 15), Cassida stigmatica Suffrian, 1844 (Figures 9, 15). In addition, Cassida coralline Boheman, 1862(under Bordy, 2009) [15].



Spermathecae in stereo microscope and SEM. a. Cassida sanguinolenta, b. Cassida stigmatica.

Group V(Cassida seladonia group): Vasculumis in the form of an eggplant or boxing gloves. It is asymmetrical C-shaped (the apical part of cornu is more protruded forward). Cornu is thicker than nodulus especially in the apical part. The apical part of cornu is swollen. Cornu's apex is rounded. The nodulus is quite short, almost parallel, and not swollen. Nodulus is usually darkened on the inner surface of the basal part. Ampulla is in the form of a more or less wavy duct or tubethat is distinct, thick, elongated, and is attached flat (parallel) at the bottom to the basal end of nodulus. Ampulla is slightly recessed on the outer surface in its middle part where it connects to the ductus glandula auxiliaris. Ampulla is slightly thinner than nodulus. Collum is quite short, distinct, and in the form of a wide stem. Ramus is in the form of a more or less wavy duct or tubethat is

longish, distinct and thick. **Ductus glandula auxiliaris** (ductus spermathecal gland) is laterally attached to ramus on the outer surface in the middle part of ramus. **Ductus spermatheca** is connected straightly to the end of ramus. It is usually in the form of a quite short, thin, flat tube, which forms small folds almost over its entire length. Sometimes it is spirally curved regularlyover its entire length. Ductus spermatheca is much thinner than ramus. Ductus spermatheca is less than vasculum or approximately equal length with vasculum.Here: *Cassida algirica* Lucas, 1849, *Cassida denticollis* Suffrian, 1844 (Figure 16), *Cassida hexastigma* Suffrian, 1844, *Cassida inquinata* Brullé, 1832 and *Cassida seladonia* Gyllenhal, 1827 (under Bordy, 2009) [15].



FIGURE 16
Spermatheca of *Cassida seladonia*in stereo microscope(taken from B. Bordy, 2009) [15].

Group VI (Cassida sanguinosa group): Vas**culum** is in the form of an eggplant or boxing gloves. It is asymmetrical C-shaped (the apical part of cornu is slightly protruded forward). Cornu is thicker than nodulus especially in the apical part. The apical part of cornu is swollen and the underside is darkened. Cornu's apex is rounded. However, apex has a very small bud-shaped process. The nodulus is quite short, slightly swollen, and not parallel. Nodulus is usually darkened on the inner surface of the basal part. Ampulla is in the form of a more or less wavy duct or tube that is distinct, thick, elongated, and is attached flat (parallel) at the bottom to the basal end of nodulus. Ampulla is slightly recessed on the outer surface in its middle part where it connects to the ductus glandula auxiliaris. Ampulla is thinner or slightly thinner than nodulus. Collum is quite short, distinct, and in the form of a wide stem. Ramus is in the form of a more or less wavy duct or tube that is longish, distinct and thick or thickish. Ductus glandula auxiliaris (ductus spermathecal gland) is laterally attached to ramus on the outer surface in the middle part or basal part near the middle of ramus. Ductus spermatheca is connected straightly to the end of ramus. It is usually in the form of a longish, more or less thin, flat tube, which forms small folds almost over its entire length. Sometimes it is spirally curved regularly over its entire length. Ductus spermatheca is much thinner than ramus. Ductus spermatheca is about 1.5-2 times longer than vasculum. Here: Cassida alpine Bremi-Wolf, 1855, Cassida



leucanthemi Bordy, 1995 and Cassida sanguinosa Suffrian, 1844 (Figure 17) (under Bordy, 2009) [15].



FIGURE 17 Spermatheca of *Cassida sanguinosa* in stereo microscope (taken from B. Bordy, 2009) [15].

DISCUSSION

Sassi & Borowiec (2006) stated that most taxonomic studies on Cassidinae are based only on external morphology and feeding preferences, and ignored morphology of genitalia. The structure of both male and female genitalia in many groups of cassids is very uniform and various authors suggested that they are not useful in determining species and in systematic studies. This is a rather old fashioned systematic approach. The taxonomic importance of Cassidinae internal anatomy has been considered by a growing amount of scientific studies[10-14, 16-18]. Therefore, it is expected that the use of these new traits reserves interesting novelties even within species groups. Under these circumstances, the morphology of the spermatheca seems to be more effective in delimiting a natural group.

Studies on the structure of spermatheca so far are based only on stereo microscopic studies. Bordy (2009) is a remarkable work in terms of the structure of spermathecaeof the species belonging to the subgenus *Cassida*(s.str.) in Turkey [15]. As known, *Cassida* (s.str.) includes 46 species in the world. Bordy (2009) studied spermathecae of twenty-four species belonging to *Cassida* (s.str.) as well as other *Cassida* speciesin France only in stereo microscope. Among them, fifteen species occur also in Turkey. A total of 21 speciesin this subgenus is already known from Turkey.

Among them, eight species as *C. atrata*, *C. nebulosa*, *C. pannonica*, *C. prasina*, *C. rubiginosa*, *C. sanguinolenta*, *C. stigmatica* and *C. vibex* were ex-

amined in both stereo microscope and scanning electron microscope (SEM) in this study. These species were also studied by Bordy (2009). The other two species were also examined in this study, however, E-European C. fausti and Turano-E-Mediterranean C. palaestina have not been studied because it is off his topic by Bordy (2009). Moreover, eight species known to be in Turkey but not examined in this study were studied by Bordy (2009). These are C. algirica, C. denticollis, C. ferruginea, C. flaveola, C. inquinata, C. rufovirens, C. sanguinosa and C. seladonia. Thus, structures of spermathecae of totally eighteen species known from Turkey were evaluated by Bordy (2009) and this study [15]. After all, only the remaining 3 species reported from a single locality from Turkeyhave not been studied. These are Cassida elongate Weise, 1893 (just known from Konya province), Cassida lineola Creutzer, 1799 (just known from Kars province) and Cassida reitteri Weise, 1892 (just known from Ankara province). On the other side, most of the species examined in this study were evaluated by SEM for the first

Based on the spermathecal definition of the type species given in the text and the research results given in the literatures (Sassi & Borowiec, 2006; Bordy, 2009; Suenaga, 2013), the similarities and differences in spermathecal morphologies of the studied 10 species belonging to the subgenus *Cassida* (s.str.) were evaluated. Accordingly, no species like the type species were found among the species examined in this study currently accepted in the subgenus *Cassida* (s.str.) [15, 16, 18]. Besides, according to Bordy (2009), only the spermathecal morphology of *Cassida flaveola* Thunberg, 1794is similar to that of the type species [15].

As mentioned, based on the structure of vasculum, ampulla, ductus glandula auxiliaris and ductus spermatheca we divided the species of the subgenus *Cassida* (s.str.) into sixdistinct groups.Based on theresults of this study, relationships among the groups can be evaluated as follows.

According to the structure of vasculum, Group III (Cassida rubiginosa group), Group IV (Cassida sanguinolenta group), Group V (Cassida seladonia group) and Group VI (Cassida sanguinosa group) seem to be more or less similar to each other. Group I (Cassida nebulosa group) and Group II (Cassida vibex group) are different from each other and the other groups. According to the structure of ampulla, Group IV (Cassida sanguinolenta group), Group V (Cassida seladonia group) and Group VI (Cassida sanguinosa group) seem to be more or less similar to each other. Group I (Cassida nebulosa group), Group II (Cassida vibex group) and Group III (Cassida rubiginosa group) are different from each other and the other groups. According to the connection to the ramus of ductus glandula auxiliaris, Group III (Cassida rubiginosa group), Group V (Cassida seladonia group) and Group VI (Cassida sanguinosa



group) seem to be more or less similar to each other. Besides, Group II (*Cassida vibex* group) and Group IV (*Cassida sanguinolenta* group) seem to be also more or less similar to each other. Group I (*Cassida nebulosa* group) is different from the other groups. According to the structure of ductus spermatheca, each group is different from the other.

Based on all these results, it can be said thatGroup I (Cassida nebulosa group) is different from
other groups in terms of all structures. Group II
(Cassida vibex group) also has a very different structure than other groups. It can be said that the closest
relative to this group is Group IV (Cassida sanguinolenta group) compared to other more distant
groups. Group III (Cassida rubiginosa group) also
has a quite different structure than other groups.
Compared to other more distant groups, this group is
close to Group V (Cassida seladonia group) and
Group VI (Cassida sanguinosa group). Group IV
(Cassida sanguinolenta group) has a quite different
structure than other groups, and seems to be more or

less close to Group V (*Cassida seladonia* group) and Group VI (*Cassida sanguinosa* group) compared to other more distant groups. **Group V** (*Cassida seladonia* group) and **Group VI** (*Cassida sanguinosa* group) seem to be closely related to other groups.

This is a proof that the subgenus is not a monophyletic, but a polyphyletic group. Already, Borowiec (2007) stated that this subgenus is not monophyletic, and a few subgroups can be separated in this group according to similar adult morphology and nutritional preferences [1]. Accordingly, similar species of the species examined according to the adult external morphology in the subgenus *Cassida* (s.str) and species for the mentioned six groups accepted in this study according to the structure of spermathecae based on the results of this study and the literature is given in Table 1. Also, at the end of the study, the habitus of adults of the species examined are given in plate I, and a diagnostic key based

TABLE 1 Similar species of the species examined according to the adult external morphology in the subgenus *Cassida* (s.str) and species for the groups accepted according to the sturucture of spermathecaein this study.

Species	Similar species	Species for the groups in this study
Group I (-nebulosa group) C.nebulosa		C.nebulosa - C. flaveola
Group II (-vibex group)		C.atrata - C. bergeali -C. elongata - C.fausti - C. ferruginea - C. humeralis - C. inopinata - C. major -C.mongolica- C.pan- nonica - C. panzeri - C.vibex
C.atrata	C.mongolica	nomea c. panzeri c. rices
C.fausti	C. bergeali - C. elongata - C. inop- inata C. pannonica - C. vibex	
C.pannonica	C. bergeali - C. elongata - C. fausti – C. inopinata - C. vibex	
C.vibex	C. bergeali - C. elongata- C. fausti – C. inopinata - C. pannonica	
Group III (-rubiginosa group)		C. deflorata -C. palaestina-C. prasina -C. rubiginosa-C. rufovirens
C.palaestina	C. algirica - C. alpina - C. deflorata - C. rubiginosa	ruoiginosa e. rujovi ens
C.rubiginosa	C. algirica- C. alpina - C. deflorata - C. palaestina	
Group IV	•	C. corallina -C. sanguinolenta-C. stigmat-
(-sanguinolenta group)		ica
C.sanguinolenta	C. aurora - C. denticollis - C. prasina - C. rufovirens	
C.stigmatica	•	
Group V		C. algirica - C. denticollis - C. hexastigma
(-seladonia group)		- C. inquinata C. seladonia
Group VI		C. alpina - C. leucanthemi - C. sanguinosa
(-sanguinosa group)		



on external and spermathecal morphological characters of adults is also provided.

As clearly seen from the table, the six groups are partly correlated with similar species of the species examinedaccording to the adult external morphological characters. It is understood from this table that the grouping we have made according to the spermathecal structure will be a more accurate approach for grouping this subgenusthan the grouping that can be done according to the external morphological characters. This approach is explicitly supported in Table 2, where the host plants of the species studied in this study are given.

In accordance with our grouping, the host plants of the type species *Cassida nebulosa* are members of Amaranthaceae and Chenopodiaceae, unlike other studied species. The host plants of the other species are members of the Asteraceae family, with the exception of *Cassida atrata* whose host plants are Lamiaceae species. This situation reveals an important

and valuable explanation in terms of the fact that type species *Cassida nebulosa* differs from the other species examined in our study due to the sturucture of spermatheca and that it remains alone in group I. Also host plants of *Cassida flaveola* which regarded a member of group I is members of Caryophyllaceae family according to Bordy (2009). The grouping of other species seems to be partly correlated with the genera of their host plant family given in Table 2.

As another output revealed by the study, is that male and female genital structures in the genus *Cassida* have distinctive importance in different categories and levels in terms of the taxa examined. In general, aedeagal characters are distinctive at the family group level, and spermatecal characters are distinctive at the genus group level. Although spermatecal characters are found to be particularly useful at the species group level, most of the aedeagal characters

The host plants of the species examined in the subgenus Cassida (s.str) [7, 15, 19-35].

Group I (-nebulosa group) C.nebulosa Amaran- Amar thaceae	ranthus Chujô & Kimoto, 1961; Bordy,
(-nebulosa group) C.nebulosa Amaran- Amarathaceae Chenopodi- Atriplex, Beta	3 , , , ,
C.nebulosa Amaran- Amar thaceae Chenopodi- Atriplex, Beta aceae	3 , , , ,
thaceae Chenopodi- Atriplex, Beta aceae	3 , , , ,
aceae	2009
Group II	Kleine, 1916; Chujô & Kimoto, 1961; Kosior, 1975; Brovdij, 1983; Lee & Choo, 2006
(-vibex group)	
. • • • • • • • • • • • • • • • • • • •	Spaeth & Reitter, 1926; Bechyne, 1944; Sekerka, 2008
C.fausti Asteraceae Arctium	n, Cynara Brovdij, 1983; Kismali & Sassi, 1994
1	Bechyne, 1944; Brovdij, 1983; bodium, Jurinea Bechyne, 1944; Brovdij, 1983; Bordy, 1986; Bordy & Doguet, 1987; Kismali & Sassi, 1994; Bordy, 2009
	rduus, Centau- n, Tanacetum Bechyne, 1944; Chujô & Kimoto, 1961; Kosior, 1975; Brovdij, 1983
Group III	.,,, <u>,</u> ,,
(-rubiginosa group)	
, , ,	us, Cirsium, Berti & Rapilly, 1973; Lopatin,
1	vnara, Sylibium 1977; Al-Ali & Abbas, 1981; Borowiec et al., 1997
C.rubiginosa Asteraceae Arctium, Arta	emisia, Centau- Kleine, 1917a,b; Bechyne, 1944;
	m, Carduus, Chujô & Kimoto, 1961; Jolivet,
	a, Onopordum, 1967; Kosior, 1975; Brovdij,
	aussurea, Ser- 1983; Bourdonne & Bordy, 1993;
	um, Tanacetum Majka & Lesage, 2008; Bordy, 2009
Group IV	2007
(-sanguinolenta group)	
	Tanacetum Bechyne, 1944; Brovdij, 1983;
C.sungumotemu Astoraccac Achittea,	Bordy, 2009
C.stigmatica Asteraceae Achillea, Arte	emisia, Tanace- Bechyne, 1944; Brovdij, 1983
t. C.stigmatica Asiciaccae Acimica, Arie	• • • • • • • • • • • • • • • • • • • •



are similar among species and therefore unusable. However, it cannot be concluded that genital morphology should not be used at the species group level.It is quite clear that evaluations with SEM, in particular, will be more efficient in this respect and that it can provide diagnostic characters that cannot be evaluated under a stereo microscope.

TAXONOMY

The taxonomic position of the subgenus is under discussion. In accordance with the results obtained in this study, Borowiec (2007) stated that the group is not monophyletic becauseat least the type species *Cassida nebulosa* and *Cassida reitteri* Weise, 1892 are probably not congeneric with most of other species. Also the position of *Cassida lineola* Creutzer, 1799 and *Cassida flaveola* Thunberg, 1794 is doubtful. The four problematic species have some details about adult morphology or immaturestages unlike other taxa. On the other hand, within *Cassida* s. str.we can create several subgroups with similar morphology and feeding preferences.

Steinhausen (2002) proposed the name *Betacassida* for the wide-spread Palaearctic *Cassida nebulosa* Linnaeus, 1758 based on the structure of the pupa [36]. *Betacassida* is an objective synonym in terms of *Cassida nebulosa* is the type species of *Cassida* Linnaeus. Steinhausen (2002) also proposed the name *Pseudocassis* for the wide-spread Palaearctic *Cassida flaveola* Thunberg, 1794 based on the structure of the pupa. According to Borowiec (2007), its position within *Cassida* is still unclear. This situation, proposalof *Betacassida* and *Pseudocassis*, supported the mentioned opinions of Borowiec (2007) [1, 36].

Despite these opinions, Borowiec (2007) also stated other Palaearctic species belonging to the nominotypical subgenus except for the four problematic species form a coherent group. Consequently, he did not propose any subgeneric name for the remaining species in the nominotypical subgenus [1].

Based on all these, it is necessary that severalgroups found to be distinctly different in terms of spermathecal structures are described as new subgenera. We do not refrain from this responsibility, and we propose the new subgenera based on the groups given in the text. In accordance with the results obtained in this study, the proposed names of the new subgenera and the recommended species of them are presented.

Genus Cassida Linnaeus, 1758

Subgenus *Cassida*Linnaeus, 1758

Cassida Linnaeus, 1758: 362 (type species: Cassida nebulosa Linnaeus, 1758, designated by Spaeth, 1914: 92) [37].

Description of the spermathecal structure is given as group I (*Cassida nebulosa* group).

Here: Cassida nebulosa Linnaeus, 1758 and Cassida flaveola Thunberg, 1794 [38] (Tables 3, 4).

Host plants: The members of the Amaranthaceae, Chenopodiaceae, Caryophyllaceae families.

Note: Although there is the name *Pseudocassis* proposed by Steinhausen (2002) based on the structure of the pupa for *Cassida flaveola* Thunberg, 1794 whosehost plants are members of the Caryophyllaceae family, its spermathecal sturucture is more or less similar to *Cassida nebulosa* Linnaeus, 1758.On the other side, I agree with Steinhausen (2002) that this is not close to other members of Palaearctic *Cassida* (s.str.). Its position within *Cassida* is stillunclear [36].

Subgenus Longiampulla subgen. nov.

Longiampulla subgen. nov. (type species: Cassida vibex Linnaeus, 1767).

Description of the spermathecal sturucture is given as group II (*Cassida vibex*group).

Here: Cassida atrata Fabricius, 1787; Cassida bergeali Bordy, 1995; Cassida elongate Weise, 1893; Cassida fausti Spaeth & Reitter, 1926; Cassida ferruginea Goeze, 1777; Cassida humeralis-Kraatz, 1874; Cassida inopinata Sassi & Borowiec, 2006; Cassida major Kraatz, 1874; Cassida mongolica Boheman 1854; Cassida pannonica Suffrian, 1844; Cassida panzeri Weise, 1907; Cassida vibex Linnaeus, 1767 (Tables 3,4).

Host plants: The members of the Asteraceae family.

Etymology: The new subgenus is named after combining "longus" in Latin (meaning long in English) and the spermathecal sturucture "ampulla" words.

Subgenus Diversivascula subgen. nov.

Diversivascula subgen. nov.(type species: Cassida sanguinolenta Müller, 1776).

Description of the spermathecal sturucture is given as group IV (*Cassida sanguinolenta* group).

Here: Cassida corallina Boheman, 1862; Cassida sanguinolenta Müller, 1776; Cassida stigmatica Suffrian, 1844 (Tables 3,4).

Host plants: The members of the Asteraceae family.

Etymology: The new subgenus is named after combining "diversus" in Latin (meaning different in English) and the spermathecal sturucture "vasculum" words.

Subgenus Reliquacassida subgen. nov.

Reliquacassida subgen. nov.(type species: Cassida rubiginosa Müller, 1776).

Description of the spermathecal sturucture is given as group III (*Cassida rubiginosa* group), group



V (*Cassida seladonia* group) and group VI (*Cassida sanguinosa* group).

Here: Cassida algirica Lucas, 1849; Cassida alpine Bremi-Wolf, 1855; Cassida deflorata Suffrian, 1844; Cassida denticollis Suffrian, 1844; Cassida hexastigma Suffrian, 1844; Cassida inquinata Brullé, 1832; Cassida leucanthemi Bordy, 1995; Cassida palaestina Reiche, 1858; Cassida prasina Illiger, 1798; Cassida rubiginosa Müller, 1776; Cassida rufovirens Suffrian, 1844; Cassida sanguinosa Suffrian, 1844; Cassida seladonia Gyllenhal, 1827(Tables 3,4).

Host plants: The members of the Asteraceae family.

Etymology: The new subgenus is named after "reliqua" in Latin (meaning remainder in English).

As known, the nominative subgenus *Cassida* (s.str.) includes forty-seven species with *Cassida inopinata* Sassi & Borowiec, 2006 (forty-six in the Palaearctic region and one in the Nearctic region) in the world [18]. After all, thirty species currently regarded in *Cassida* (s.str.) are recommended to four subgenera of the genus *Cassida* with this work.

Cassida reitteri Weise, 1892 and Cassida lineola Creutzer, 1799, two of the remaining seventeen species, are problematic species as stated. As Borowiec (2007) mentioned, Cassida reitteri Weise, 1892is not congeneric at least with Cassida nebulosa Linnaeus, 1758. Also the position of Cassida lineola Creutzer, 1799 is doubtful. According to external morphological characters, Cassida reitteri Weise, 1892 is unique, and is more or less similar to Chiridula semenoviWeise, 1889 which is the type species of the monotypic genus Chiridula Weise, 1889. Also Cassida lineola Creutzer, 1799 is unique morphologically, and its position is still doubtful and unclear [1]. These species need to be confirmed and are therefore not recommended for any subgeneramentioned here. The remaining fifteen species that have not been recommended in any subgenerain this study, need further confirmation.

Fifteen species uncertain position, however, are discussed as follows:

The W Mediterranean species *Cassida angusti-frons* Weise, 1891is unique morphologically. Its position is still uncertain.

The C and E European *Cassida aurora*Weise, 1907 is more or less similar to *Cassida rufovirens*-Suffrian, 1844 morphologically. Its host plants are the members of Asteraceae family. Therefore, it may belong to *Cassida* (*Reliquacassida*) subgen. nov.

The Caucasian species *Cassida circassi-ca*Medvedev, 1962is unique morphologically. However, Borowiec & Świętojańska (2018) noted that its paratype is conspecific with *Cassida palaestina* ab. *nigrofemorata*. Therefore,it may belong to *Cassida (Reliquacassida)* subgen. nov. [39].

The position of S African species *Cassida distinguenda*Spaeth, 1928 is uncertain as most of the other African species.

The E Asiatic species Cassida fuscorufa Motschulsky, 1866and Cassida jacobsoniSpaeth, 1914is more or less similar to each other morphologically. The host plants of Cassida fuscorufa the members of the Asteraceae family. Under this circumstance, it may belong to Cassida (Longiampulla) subgen. nov. or to a new subgenus that can be proposed with Cassida jacobsoni Spaeth, 1914, Cassida japana Baly, 1874, Cassida piperata Hope, 1842 and Cassida pallidicollis Boheman, 1856 according to external morphologically.

The Spanish endemic species *Cassida hyalina* Weise, 1891is more or less similar to *Cassida corallina* Boheman, 1862 morphologically. Therefore, it may belong to *Cassida (Diversivascula)* subgen. nov.

The Tanzanian endemic *Cassida impompalis* Spaeth, 1924is uncertain as most of the other African species.

The E Asiatic species *Cassida mandli* Spaeth, 1921is more or less similar to the recommended species in *Cassida* (*Reliquacassida*) subgen. nov. morphologically. Its host plants are the members of the Asteraceae family. Therefore, it may belong to *Cassida* (*Reliquacassida*) subgen. nov.

The Far Eastern species *Cassida japana* Baly, 1874 and *Cassida piperata*Hope, 1842is more or less similar to each other morphologically. The host plants of *Cassida piperata* Hope, 1842 are the members of the Amaranthaceae and Chenopodiaceae families. Under this circumstance, it may belong to *Cassida (Longiampulla)* subgen. nov. or to a new subgenus that can be proposed with *Cassida fuscorufa* Motschulsky, 1866, *Cassida jacobsoni* Spaeth, 1914 and *Cassida pallidicollis* Boheman, 1856 according to external morphologically.

The E Palaearctic species Cassida pallidicollis Boheman, 1856 is more or less similar to Cassida mongolica Boheman 1854. Its host plants are the members of Chenopodiaceae family. Under this circumstance, it may belong to Cassida (Longiampulla) subgen. nov. or to a new subgenus that can be proposed with Cassida fuscorufa Motschulsky, 1866, Cassida jacobsoni Spaeth, 1914, Cassida japana Baly, 1874 and Cassida piperata Hope, 1842 according to external morphologically.

The American species *Cassida relicta* Spaeth, 1927 is more or less similar to *Cassida ferruginea* Goeze, 1777 and *Cassida panzeri* Weise, 1907 morphologically. Therefore, it may belong to *Cassida (Longiampulla)* subgen. nov.

The E European-C Asiatic *Cassida sareptana* Kraatz, 1873 is more or less similar to the recommended species in *Cassida* (*Reliquacassida*) subgen. nov. morphologically. Its host plants are the members of the Asteraceae family. Therefore, it may belong to *Cassida* (*Reliquacassida*) subgen. nov.



TABLE 3 Proposed subgenera and their species according to this study

Subgenus Cassida (Cassida)

Cassida nebulosa Linnaeus, 1758

Uncertain position to Cassida (Cassida)

Cassida angustifronsWeise, 1891 Cassida impompalisSpaeth, 1924 Cassida reitteriWeise, 1892

Cassida flaveola Thunberg, 1794

Cassida distinguenda Spaeth, 1928 Cassida lineola Creutzer, 1799

Subgenus Cassida (Longiampulla) subgen. nov.

Cassida vibex Linnaeus, 1767 Cassida bergeali Bordy, 1995 Cassida fausti Spaeth & Reitter, 1926 Cassida humeralis Kraatz, 1874 Cassida major Kraatz, 1874 Cassida pannonica Suffrian, 1844

Cassida ferruginea Goeze, 1777 Cassida inopinata Sassi & Borowiec, 2006 Cassida mongolica Boheman 1854 Cassida panzeri Weise, 1907

Cassida atrata Fabricius, 1787

Cassida elongata Weise, 1893

Uncertain position to Cassida (Longiampulla)

?Cassida fuscorufaMotschulsky, 1866 ?Cassida japanaBaly, 1874 ?Cassida piperataHope, 1842

?Cassida jacobsoniSpaeth, 1914 ?Cassida pallidicollisBoheman, 1856 Cassida relictaSpaeth, 1927

Subgenus Cassida (Diversivascula) subgen. nov.

Cassida sanguinolenta Müller, 1776 Cassida stigmatica Suffrian, 1844

Cassida corallina Boheman, 1862

Uncertain position to Cassida (Diversivascula)

Cassida hyalinaWeise, 1891

?Cassida spaethiWeise, 1900

Subgenus Cassida (Reliquacassida) subgen. nov.

Cassida rubiginosa Müller, 1776 Cassida alpina Bremi-Wolf, 1855 Cassida denticollis Suffrian, 1844 Cassida inquinata Brullé, 1832 Cassida palaestina Reiche, 1858 Cassida rufovirens Suffrian, 1844 Cassida seladonia Gyllenhal, 1827

Cassida algirica Lucas, 1849 Cassida deflorata Suffrian, 1844 Cassida hexastigmaSuffrian, 1844 Cassida leucanthemi Bordy, 1995 Cassida prasina Illiger, 1798 Cassida sanguinosa Suffrian, 1844

Uncertain positionto Cassida (Reliquacassida)

Cassida auroraWeise, 1907 Cassida mandliSpaeth, 1921 Cassida circassica Medvedev, 1962 Cassida sareptanaKraatz, 1873

TABLE 4 Turkish species according to the subgenera proposed in this study

Subgenus Cassida (Cassida)

Cassida nebulosa Linnaeus, 1758

Uncertain position to Cassida (Cassida)

Cassida lineola Creutzer, 1799

Subgenus Cassida (Longiampulla) subgen. nov.

Cassida vibex Linnaeus, 1767 Cassida elongata Weise, 1893 Cassida ferruginea Goeze, 1777 Cassida flaveola Thunberg, 1794

Cassida reitteriWeise, 1892

Subgenus Cassida (Diversivascula) subgen. nov.

Cassida sanguinolenta Müller, 1776

Cassida atrata Fabricius, 1787 Cassida fausti Spaeth & Reitter, 1926 Cassida pannonica Suffrian, 1844

Cassida stigmatica Suffrian, 1844

Subgenus Cassida (Reliquacassida) subgen. nov.

Cassida rubiginosa Müller, 1776 Cassida denticollis Suffrian, 1844 Cassida palaestina Reiche, 1858 Cassida rufovirens Suffrian, 1844 Cassida seladonia Gyllenhal, 1827

Cassida algirica Lucas, 1849 Cassida inquinata Brullé, 1832 Cassida prasina Illiger, 1798 Cassida sanguinosa Suffrian, 1844



The E Palaearctic species *Cassida spaethi* Weise, 1900 is more or less similar to the recommended species in *Cassida* (*Diversivascula*) subgen. nov. morphologically. Its host plants are the members of the Asteraceae family. Therefore, it may belong to *Cassida* (*Diversivascula*) subgen. nov.

The species recommended for the subgenera proposed in this study with the species uncertain position are given in Table 3.

Consequently, the taxonomic position of the subgenus is still under discussion. It is hoped that the result of this study will contribute to the solution of this problem. It is hoped that this work will create a reference for future discussions, comments and new arrangements that can be made about the taxonomic position of the subgenus.

A key to the species examined.

......Cassida (Cassida) nebulosa Linnaeus, 1758

-. Elytra not regularly seriate-punctate, at least with some extra punctures between rows 3 and 4. Pronotum on both sides laterally more or less narrowed. Upper side black, on anterior margin of pronotum two pale fused spots. Underside mostly black, but borders of abdomen pale. Legs completely black. In spermatheca, vasculum asymmetrical C-shaped, cornu much thicker than nodulus, apex of cornu rounded. Ductus spermatheca about 4-5 times longer than vasculum. Length 5.1-7.0 mm. C- and E-European species. Habitus in Figure 18b.......

... Cassida (Longiampulla) atrata Fabricius, 1787

- 3. On elytra intervals covered by very short, white hairs. Ampulla laterally connected to nodulus on the outer surface of its basal part. In spermatheca, ductus spermatheca in the form of a flat tube which makes wide folds in the proximal part, spirally curved regularly in its next big part distally......
- **4.** Elytra with a triangular darkened area (not sharply limited) on sides and behind the scutellum

basally. Femora at least in basal half black. In spermatheca, ductus spermatheca is about 2-3 times longer than vasculum, its spiral part thin.Length 5.7-6.4 mm. E-European species. Habitus in Figure 18 c.....

Cassida (Longiampulla) fausti Spaeth & Reitter, 1926

- 5. Femora not or feebly darkened. In spermatheca, ductus spermatheca about 7-8 times longer than vasculum. It approximately equal width with ramus in its proximal part and much thinner than ramus in its spiral part.Length 6.5-8.2 mm. Sibero-European species. Habitus in Figure 18d........Cassida (Longiampulla) pannonica Suffrian, 1844
- -. Femora at least in basal half black. In spermatheca, ductus spermatheca is about 2 times longer than vasculum. It approximately equal width with ramus over its entire length.Length 5.6-7.0 mm. Asiatic-European species. Habitus in Figure 18e.....

......Cassida (Longiampulla) vibex Linnaeus, 1767

- 7. Clypeus shining, smooth, rather scarcely punctate. Femora usually entirely pale or only basal third black. In spermatheca, nodulus of vasculum relatively thinner basally. Length 6.5 mm. Turano-E-Mediterranean species. Habitus in Figure 18 f.....

Cassida (Reliquacassida) palaestina Reiche, 1858

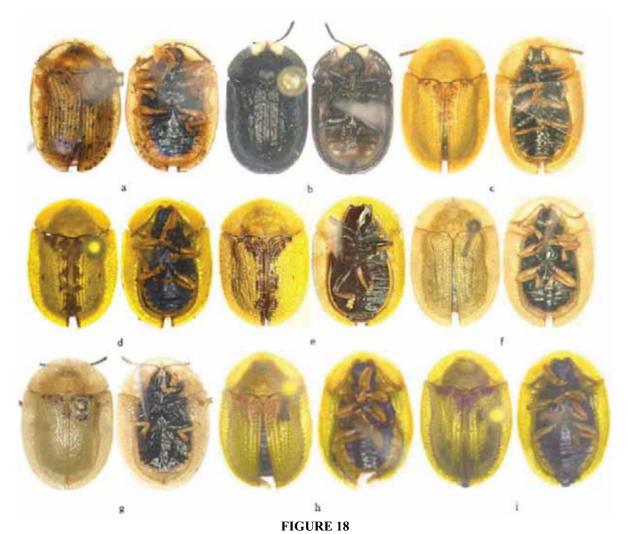
-. Clypeus matt, reticulate, more densely punctate. Femora mostly black, at most only apical third yellowish. Elytra with a dark dot behind the scutellum and a few darkened spots on both sides of scutellum. In spermatheca, nodulus of vasculum relatively thicker basally. Length 6.5-7.5 mm. Holarctic species. Habitus in Figure 18g

Cassida (Reliquacassida) rubiginosa Müller, 1776

8. Pronotum as broad as elytra.In spermatheca, cornu of vasculum relatively thicker apically. Ductus spermatheca is about 2 times longer than vasculum. Length 5.0-5.9 mm. Sibero-European species. Habitus in Figure 18i

Cassida (Diversivascula) sanguinolenta Müller, 1776





Habitus of, a. Cassida nebulosaLinnaeus, b. Casida atrata Fabricius, c. Cassida fausti Spaeth & Reitter, d. Cassida pannonica Suffrian, e. Cassida vibex Linnaeus, f. Cassida palaestina Reihe, g. Cassida rubiginosa Müller, h. Cassida sanguinolenta Müller, i. Cassida stigmatica Suffrian.

.Cassida (Diversivascula) stigmatica Suffrian, 1844

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