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Ultrastructure of the aedeagus and spermatheca of *Chaetocnema conducta* (Motschulsky), 1838 (Chrysomelidae: Galerucinae: Alticini) by Scanning Electron Microscope

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Özdikmen H. *et al.* 2023: Ultrastructure of the aedeagus and spermatheca of *Chaetocnema conducta* (Motschulsky), 1838 (Chrysomelidae: Galerucinae: Alticini) by Scanning Electron Microscope. *Acta Musei Moraviae, Scientie biologicae* **108(1–2):** 43–56. – The paper presents unknown ultrastructure of aedeagus and spermatheca of *Chaetocnema conducta* (Motschulsky), 1838, studied and figured under both stereo microscope and Scanning Electron Microscope (SEM). This is the type species of the subgenus *Pseudochaetocnema* Özdikmen, 2021 and one of two species of the subgenus occurring in Türkiye. Aedeagi and spermathecae so far have been studied on the basis of only stereo microscopic studies within the genus *Chaetocnema* Stephens, 1831. Until now, there is only one ultrastructural study with SEM on male and female genitalia of *Chaetocnema* species, therefore, the present study is the second in existence. In accordance with this, this study primarily aims to reveal and obtain new diagnostic characters with ultrastructural and detailed investigations of aedeagus and spermatheca of the species *Chaetocnema conducta*. As known, spermathecae in the genus *Chaetocnema*, like in most genera of the tribe Alticini, are partially uniform and do not offer good diagnostic characters at the species level. Contrary to the partial homogeneity of spermathecae, studies on aedeagi suggested quite large diversity of aedeagal structure. While the results from the present study basically support these opinions, the female spermatheca is found less significant but aedeagus is found important for diagnosis at the species level. In addition, new diagnostic characters for both the aedeagus and spermatheca have been revealed by investigations of ultrastructural morphologies using SEM especially.

Keywords. Ultrastructure, aedeagus, spermatheca, SEM, *Chaetocnema conducta*, Coleoptera, Chrysomelidae, Galerucinae, Alticini, Türkiye

Introduction

Chaetocnema Stephens, 1831, a member of the tribe Alticini Newman, 1835, is a cosmopolitan genus which includes a large number of species (about 500) spreading all over the world (Palaearctic, Nearctic, Neotropical, Oriental, Afrotropical, Madagascan and Australian regions) (Döberl (2010), Konstantinov *et al.* (2011), Özdikmen, 2021, Zhang *et al.*, 2023). Döberl (2010) mentioned 118 species for the Palaearctic region, whose delineated boundaries were larger than those traditionally used. On the other side, there were a total of 75 species in Konstantinov *et al.* (2011), seven of which are newly described species. This is due to the fact that contrary to Döberl (2010), the boundaries of Palaearctic region used in this study were narrower than the traditional ones. The genus is represented with a total of 26 species in Türkiye which is in the Palaearctic region (Ekiz *et al.*, 2013; Özdikmen, 2014, 2021). Therefore, Turkish fauna includes approximately 35% of all Palaearctic species according to Konstantinov *et al.* (2011), while approximately 30% of all Palaearctic species according to Döberl (2010).

Subgeneric classification of the genus *Chaetocnema* Stephens was recently reviewed and arranged by Özdikmen (2021). He proposed a total of 13 subgenera for the genus *Chaetocnema* Stephens, 1831. *Pseudochaetocnema* Özdikmen, 2021 is one of the proposed subgenera in his study. According to Özdikmen (2021), the subgenus is represented in the Palaearctic (5 species and 1 possible species), Nearctic (possible 8 species), Oriental (12 species and 7 possible species), Afrotropical (5 species) and Madagascan (1 species) regions, with the exception of the Neotropical and Australian regions. In Türkiye, the subgenus is represented with only two species as the type species *C. conducta* (Motschulsky, 1838) and *C. orientalis* (Bauduér, 1874) (Özdikmen, 2021).

According to Konstantinov *et al.* (2011), *Chaetocnema* species are habitually very similar and cannot be separated only with external characters. Genitalic characters have been used traditionally and are diagnostically important. However, works on aedeagi and spermathecae of *Chaetocnema* species so far were studied on the basis of only stereo microscopic studies, as shown by revisional studies of White (1996), Konstantinov *et al.* (2011), Ruan *et al.* (2019) and others, for example, Ruan *et al.* (2014a,b), while ultrastructure of aedeagi and spermathecae of *Chaetocnema* species with SEM have not been studies until now, except for a study of *Chaetocnema concinna* (Marsham, 1802) (Ekmekçi *et al.*, 2023).

As known, aedeagal morphology in the genus *Chaetocnema* Stephens, 1831, like in most genera of the tribe Alticini, seems to be diagnostic at the species level because there are many new species described based on aedeagal structure. Unlike the aedeagal morphology, spermathecal morphology seems to be less characteristic at the species level.

The preliminary studies we have done on the ultrastructure of aedeagi and spermatheca of *Cassida* Linnaeus, 1758 and *Hypocassida* Weise, 1893 revealed new diagnostic characters at both the species and subgeneric level (Ataş *et al.*, 2019a,b; Bal & Özdikmen, 2020; Özdikmen & Bal, 2021; Özdikmen *et al.*, 2020a,b, 2021a,b, 2022a,b). These positive results obtained in previous studies appear to be applicable also for the genus *Chaetocnema* and they encouraged us to carry out the present study.

Materials and Methods

The available specimens for the present work were collected in Çankırı and Kayseri provinces from Türkiye in late May, June, August and early September 2013, 2016, 2017 and 2018. The specimens were deposited at Gazi University (Türkiye, Ankara) and Nazife Tuatay Plant Protection Museum (Türkiye, Ankara).

The aedeagi and spermathecae were dissected from the abdomen, and the remaining tissue was removed with fine tweezers. For stereo microscopic examination after cleaning, the samples were placed in 70% ethanol and examined with Olympus SZX7 stereomicroscope and Leica Z-16 APO stereo microscope. For scanning electron microscopy (SEM), cleaned samples were dehydrated using an ascending series of ethanol (70%, 80%, 90%, and 100%) and then air dried. Following this, the specimens were mounted onto SEM stubs using a double-sided adhesive tape, coated with gold

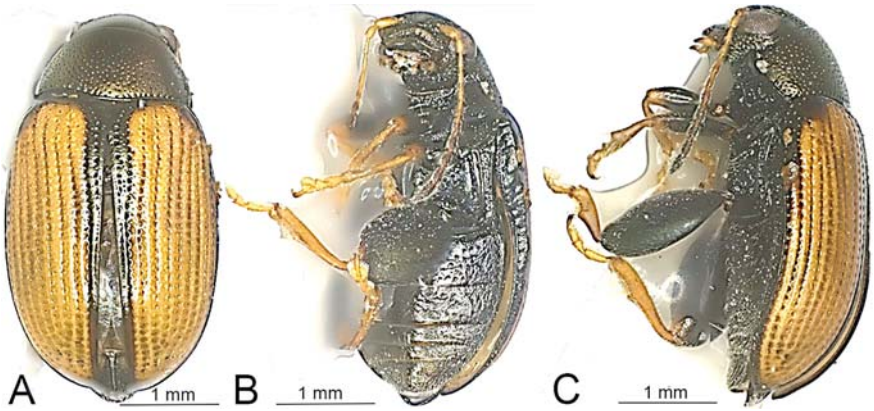

Fig. 1. Habitus of *Chaetocnema conducta* (Motschulsky): A – dorsal view, B – semiventral view, C – lateral view.

using a Polaron SC 502 Sputter Coater, and examined with a JEOL JSM 6060 Scanning Electron Microscope (SEM) at 10 kV in Prof. Dr. Zekiye Suludere Electrone Microscopy Center at Gazi University. The principal spermathecal and aedeagal terms used in the present study are given below (Figs 1, 2).

Results and Discussion

Genus *Chaetocnema* Stephens, 1831

Type species: *Chrysomela concinna* Marsham, 1802, subsequent designation by Westwood, 1838: 42

The cosmopolitan genus is represented with a total of 26 species of six subgenera in Türkiye that is in Palaearctic region (Ekiz *et al.*, 2013; Özdikmen, 2014, 2021). According to the number of species included, the subgenus *Hortensoides* Özdikmen, 2021 with 13 species (50%) is strikingly prevailing, and the nominate subgenus *Chaetocnema* Stephens, 1831 with seven species (about 27%) follows it. Therefore, about 77% of the *Chaetocnema* species in Türkiye belong to these two subgenera. The remaining six species belong to the subgenera *Plectroscelis* Chevrolat, 1836 and *Pseudochaetocnema* Özdikmen, 2021, each with two species (about 8%), while the subgenera *Majoroides* Özdikmen, 2021 and *Udorpes* Motschulsky, 1845 are represented by one species each (about 4%).

Subgenus *Pseudochaetocnema* Özdikmen, 2021

Type species: *Haltica conducta* Motschulsky, 1838)

According to Özdikmen (2021), the subgenus includes a total of 39 species (23 species and possible 16 species) worldwide. In Türkiye, the subgenus is represented only with

Fig. 2. Provincial and regional distribution patterns of *Chaetocnema conducta* (Motschulsky) in Türkiye: (1) Marmara region, (2) Black Sea region, (3) Aegean region, (4) Central Anatolian region, (5) Eastern Anatolian region, (6) Mediterranean region, (7) South-Eastern Anatolian region.

two species as the type species *C. conducta* (Motschulsky, 1838) and *C. orientalis* (Bauduér, 1874) (Özdikmen, 2021).

Chaetocnema conducta (Motschulsky, 1838)

Haltica conducta Motschulsky, 1838: 180
Plectroscelis foudrasi Bauduér, 1874: 163
Chaetocnema ahngeri Jacobson, 1901: 139

Material examined: Türkiye: Çankırı prov.: Çerkeş, between Kabak-Çaylı villages, 40°54′34″N 32°56′20″E, 1236 m, 25.VIII.2013, 1 ex.; Kayseri prov.: Develi, Kızık, 38°24′0″N 35°27′50″E, 08.VI.2016, 1321 m, 1 ex.; İncesu, Örenşehir, 38°42′21″N 35°15′27″E, 20.VI.2016, 1043 m, 1 ex.; İncesu, Örenşehir, 38°42′21″N 35°15′27″E, 20.VI.2016, 1043 m, 1 ex.; Yeşilhisar, Çöl Gölü, 38°27′4″N 35°11′8″E, 20.VI.2016, 1078 m, 2 exs.; Yeşilhisar, Çöl Gölü, 38°27′4″N 35°11′8″E, 20.VI.2016, 1078 m, 2 exs.; Yahyalı, Dikme, 38°1′14″N 35°31′56″E, 14.IX.2017, 1418 m, 2 exs.; Yahyalı, Dikme, 38°1′14″N 35°31′56″E, 14.IX.2017, 1418 m, 2 exs.; Akkışla, Alevkışla, 39°0′27″N 36°5′51″E, 30.V.2018, 1262 m, 1 ex. (Fig. 3).

The Centralasiatic-European + Afrotropico-Mediterranean species are distributed throughout Europe (Albania, Austria, Bosnia & Herzegovina, Bulgaria, Croatia, Czechia, UK: England, European Russia, European Türkiye, France, Germany, Greece, Hungary, Italy, Macedonia, Moldova, Montenegro, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland and Ukraine), North Africa (Algeria, Egypt, Morocco and Tunisia), Asia (Armenia, Iran, Israel, Kazakhstan, Kirgizia, Syria, Tajikistan, Türkiye and Turkmenistan), and the Afrotropical region (Ethiopia, Kenya, Sudan and Tanzania) (Döberl, 2010; Konstantinov *et al.*, 2011).

The flea beetle is a little-known species for Türkiye. However, it has sporadically been recorded by various authors from 13 out of 81 provinces and five of seven regions in Türkiye until now: Adana, Aksaray, Ankara, Burdur, Çankırı, Erzurum, Eskişehir,

Isparta, İstanbul, İzmir and Kayseri provinces in Asian Türkiye (Anatolia) and Edirne and Kırklareli provinces in European Türkiye (Thrace) (Ekiz *et al.*, 2013; Özdikmen, 2014; Şen & Gök, 2014; Aslan *et al.*, 2015; Bal *et al.*, 2018; Özdikmen *et al.*, 2020; Özdikmen & Coral Şahin, 2022).

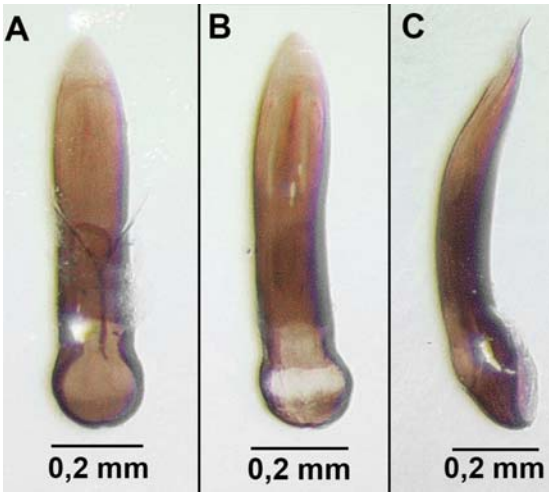

Fig. 3. Aedeagus of *Chaetocnema conducta* (Motschulsky) in stereo microscope: A – ventral view, B – dorsal view, C – ventro-lateral view.

Classification systems are primarily based on the external morphological characters, however, most of these classifications change constantly because of homoplasy and symplesiomorphy within these character sets. To provide a more stable classification, therefore, taxonomists have studied internal and genital morphological features in combination with the external morphological characters (e.g. Kasap & Crowson, 1979; Mann & Crowson, 1983; Suzuki, 1988).

As known, the structures of aedeagus and spermatheca are taxonomically important. For this reason, to reveal more diagnostic characters than hitherto known, ultrastructural and detailed investigations of aedeagus and spermatheca of *Chaetocnema conducta* (Motschulsky, 1838) from Türkiye were firstly conducted using scanning electron microscope (SEM) and stereo microscope. The observations obtained are presented below.

Male genitalia have been widely used to differentiate species, while the female internal reproductive organs have been used less frequently (e.g. Arnqvist, 1997; Hernández & Ortuño, 1992; Hernández, 1993; Ferronato, 2000). Their variations are useful in distinguishing the species group level and even genus group level (Suzuki, 1988). There are two types of aedeagus according to the presence of paramers in males of the Chrysomeloidea superfamily. The first type, in which paramers are very well developed, is seen in the families Orsodacnidae, Megalopodidae and Chrysomelidae (Sagriinae and Donaciinae subfamilies). The second type, in which there are no paramers, is seen in all other subfamilies of the Chrysomelidae family (Kismali, 1973). Therefore, male genital does not include paramers in the tribe Alticini and hereby in the genus *Chaetocnema* Stephens, 1831.

Aedeagus (Figs 3–5): Apical third of aedeagus light brown colored, remaining parts of aedeagus brown to dark brown or even blackish colored in stereo microscope. Median lobe more or less lanceolate.

Ventral view: Median lobe almost parallel except for apical part. Apical third of median lobe narrowed, lanceolate. Width of aedeagus, distal to basal opening almost

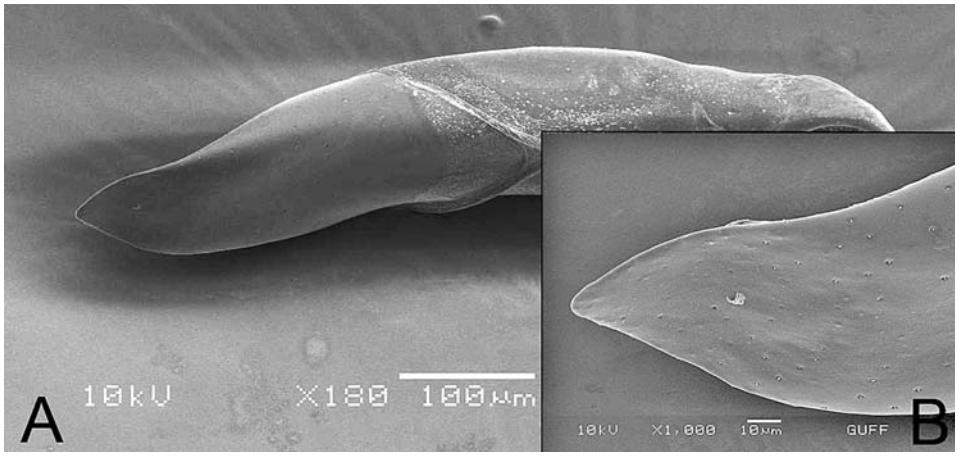

Fig. 4. *Chaetocnema conducta* (Motschulsky), aedeagus: A – median lobe in ventral view, B – detail of sensillae in apical third. (SEM)

subequal to width just before apical declivity. Apical part of median lobe gradually narrowed regularly towards the apex laterally, and broadly prominently depressed. Thus, apex without an apical denticle but roundedly pointed. Surface of median lobe, lateral to median convex apically, medially and basally. Ventral longitudinal groove and transverse wrinkles absent. Median lobe with a short, barely noticeable median groove basally, and also with a depression just before apical declivity. Median foramen (basal opening) not completely circular, resembling a flower vase. Its upper margin straight. Median lobe with scattered and sparse ultrastructural sensillae in apical third in SEM.

Lateral view: Median lobe almost evenly and slightly curved towards the ventral side. Maximal curvature of median lobe situated medially. Median lobe sinusoidal near apex. Apex of median lobe distinctly narrowed and pointed, slightly curved ventrally. Median lobe with scattered and sparse ultrastructural sensillae in apical third in SEM. Integrated form of aedeagus narrowed headed ovaloid.

Dorsal view: Median lobe almost evenly convex except for apical part. In the apical part, median lobe depressed towards the ventral side firstly, then re-directed towards dorsal side at and near the apex. Dorsal plate is divided into three parts, a wider and longer median plate, and two much narrower and shorter lateral plates. The plates are distinctly separated from each other by straight incisions.The incisions separating the median plate from the others extend straight backwards well past the base of the orifice to the beginning of the apical third of the median lobe.The external incisions of the lateral plates extend only to the basal part of the orifice. All plates of dorsal plate straight in their apical margin. Dorsal plate fused to dorsal surface of median lobe basally. Orifice distinct.

Besides, internal reproductive organs of female insects include a pair of ovaries with their respective oviducts, a median ectodermal tube, a vagina, a bursa copulatrix and the

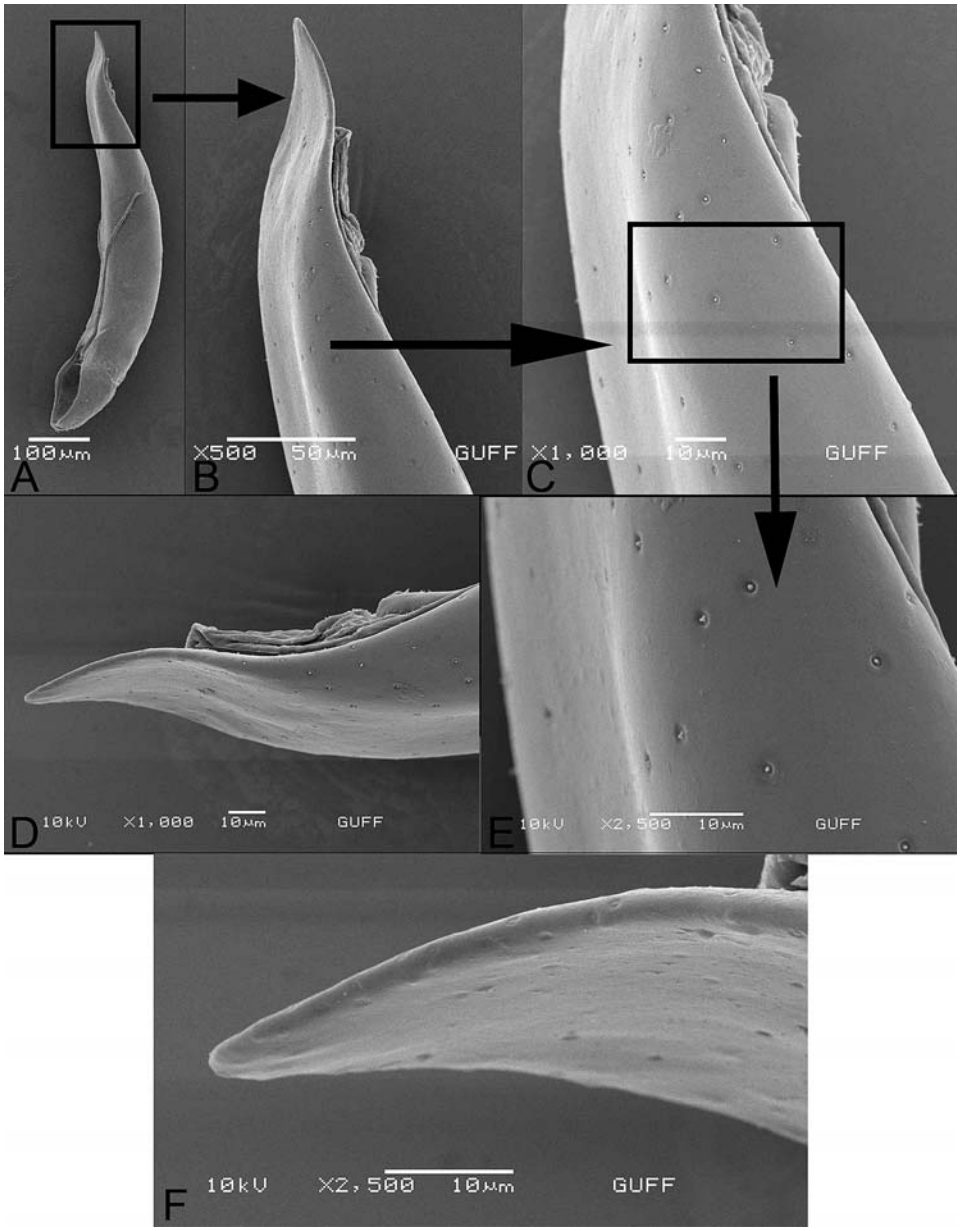

Fig. 5. *Chaetocnema conducta* (Motschulsky), aedeagus: A – median lobe in lateral view, B–F – various details of apex and sensillae. (SEM)

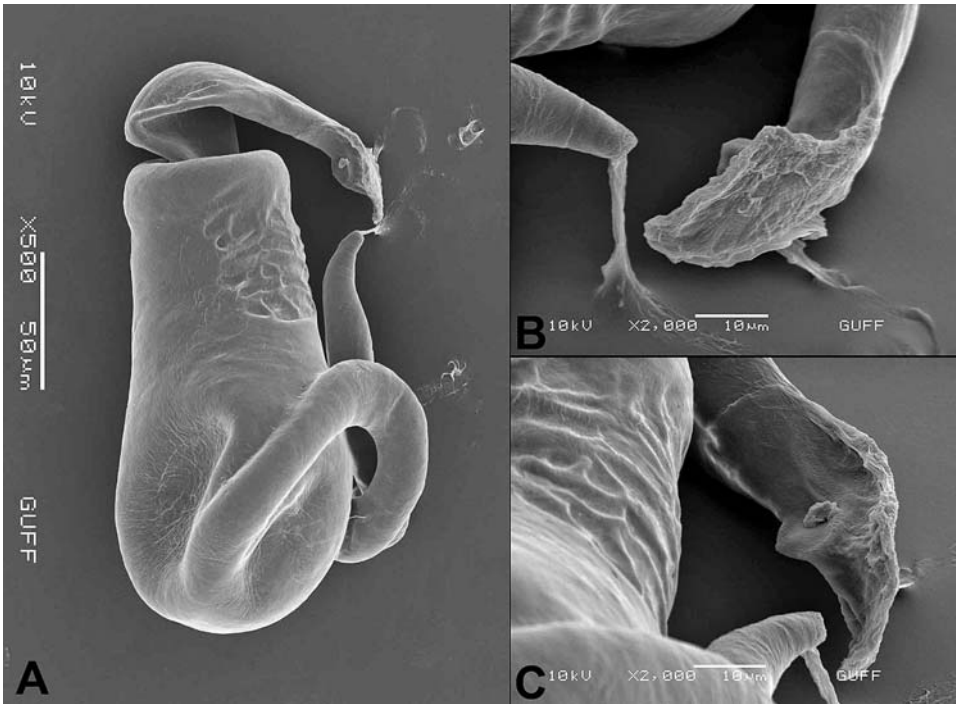

Fig. 6. *Chaetocnema conducta* (Motschulsky), spermatheca: A – general appearance of spermatheca, B – spermathecal pump with apical additional integument, C – spermatheca and the apex of the spermathecal duct. (SEM)

spermatheca which is an invagination of the eighth abdominal segment and its number and shape depend on the group of insects. The spermatheca stores and maintains viability of sperm until fertilization and has an important role in copulation and oviposition (Snodgrass, 1935; Suzuki, 1988; De Marzo, 2008; Pascini & Martins, 2017; Rodríguez-Mirón *et al.*, 2017).

In Coleoptera, five patterns of spermathecal morphology were distinguished by De Marzo (2008) with the presence, absence or variations of spermathecal capsule, spermathecal duct, and spermathecal gland. The most widespread pattern is to have only one spermathecal capsule (receptacle + pump or velum) connected with the bursa copulatrix by one spermathecal duct, and one spermathecal gland (Suzuki, 1988; Matsumura & Suzuki, 2008; De Marzo, 2008; Rodríguez-Mirón *et al.*, 2017).

In Chrysomeloidea, the spermatheca has been useful to define subfamilies, genera and sometimes species (Reid, 1989; Hernández, 1993; Biondi, 2001; Borowiec & Świętojańska, 2001, Borowiec & Skuza, 2004; Borowiec & Opalinska, 2007; Borowiec & Pomorska, 2009; Rodríguez-Mirón & Zaragoza-Caballero, 2017; Rodríguez-Mirón *et al.*, 2017). According to Rodríguez-Mirón *et al.* (2017), the

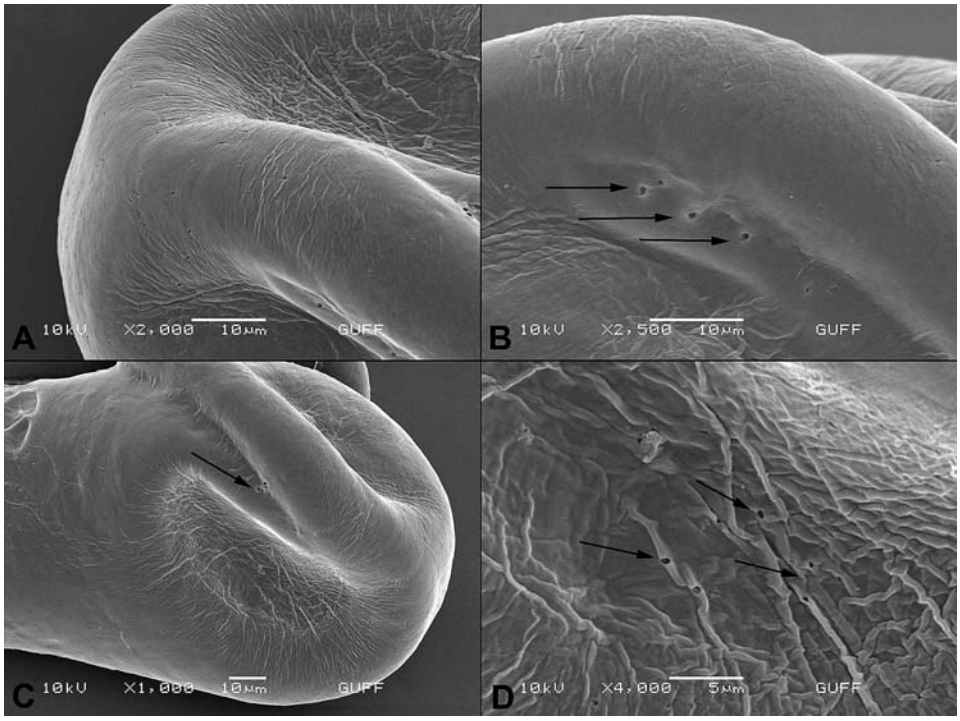

Fig. 7. *Chaetocnema conducta* (Motschulsky), spermatheca: A – exit point of spermathecal duct from spermathecal receptacle, B – spermathecal receptacle with micro pores on surface, C – regular micro pores where spermathecal chamber meets spermathecal duct, D – irregular micropores on spermathecal receptacle. (SEM)

spermatheca provides characters to diagnose genera and subgenera. They also observed that the spermatheca has a high taxonomic value for diagnosing taxa at various ranks.

Spermatheca (Figs 6–8): Spermathecal pump and distal part of spermathecal duct light brown colored, spermathecal receptacle and proximal part of spermathecal duct brown-colored in stereo microscope. Spermathecal pump much shorter than receptacle.

Spermathecal pump straight basally, bent apically. Apex of spermathecal pump flattened and so pointed. Apical part of spermathecal pump on the underside with an additional thickened integument. Therefore, apical part of spermathecal pump seems darker colored in stereo microscopic view. Spermathecal pump without an additional thickened integument in the basal part. Spermathecal pump attached to middle of spermathecal receptacle top.

Spermathecal receptacle relatively shortened, more or less widened in the basal part, pyriform or cylindrical, but not sinuate. The surface of spermathecal receptacle almost homogeneously smooth but with some scattered and sparsely micro pores especially basally on the surface, however, a few micro pores placed on both sides of the basal part

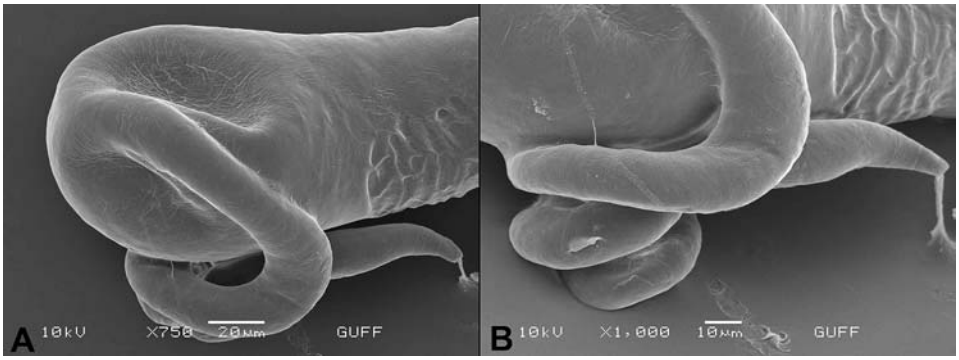

Fig. 8. *Chaetocnema conducta* (Motschulsky), spermatheca: A – exit point of spermathecal duct, B – spermathecal duct and spermathecal gland structure. (SEM)

of spermathecal duct, conspicuously wider than the others. Maximum width of receptacle situated basally. In the apical part, spermathecal receptacle slightly narrowed towards the tip. Therefore, basal part of receptacle wider than apical part, and minimum width of receptacle situated apically.

Spermathecal duct shorter than receptacle but straight basally, then more or less corrugated, and finally straight again in the apical part after the spermathecal gland. Apex of spermathecal duct up to 2/3 or 3/4 of receptacle but spermathecal gland situated near middle in basal part of receptacle. Spermathecal duct attached to ventro-lateral part of spermathecal receptacle base. Spermathecal duct with only a few micro pores sporadically on the surface. General shape of spermatheca more or less cylindrical or pyriform.

Conclusion

In Türkiye, 26 species of 6 subgenera are represented in the genus *Chaetocnema* Stephens, 1831. Of these 26 species, the most studied species are *C. coyei* (Allard, 1864), *C. tibialis* (Illiger, 1807), *C. arenacea* (Allard, 1860) and *C. hortensis* (Geoffroy, 1785) respectively, and the most information on this group has been found in these species. On the contrary, the least studied species known only from one provincial record in Türkiye are *C. aerosa* (Letzner, 1847), *C. delarouzeei* (Brisout, 1884), *C. imitatrix* Gruev, 1990, *C. picipes* Stephens, 1831 and *C. subcoerulea* (Kutschera, 1864). Most of the remaining species are known either little or limitedly in Türkiye. *C. conducta* (Motschulsky, 1838) is one of little-known species. At the species level, aedeagal morphology in the genus *Chaetocnema* Stephens, 1831 seems to be more diagnostic, while spermathecal morphology seems to be diagnostic. Generally, the male genital (aedeagus) structure has been used, however, there are few studies on this subject. For this reason, although there are many species described within the genus, only few species are based on aedeagal structure., With this study, the above-mentioned opinions were supported for the species

group. SEM images of aedeagus and spermatheca of *C. conducta* (Motschulsky, 1838) are presented to the scientific world with the present study for the first time. When diagnosing specimens belonging to the genus *Chaetocnema* Stephens, 1831, the useful characters in the structure of the aedeagus include primarily the general form of aedeagus and apical part of the median lobe. As a result of the SEM images obtained with the present study, some new characters have been added: for aedeagus: the structures of apex of median lobe in all views; the structure of dorsal plate of the median lobe; the structure, placement and density of the sensillae on the median lobe and chitinization rate of aedeagus; for spermatheca: additional apical thickened integuments of spermathecal pump; attaching points to spermathecal receptacle of spermathecal pump and spermathecal duct; the structure, placement and density of the micro pores on the surface of spermathecal receptacle and chitinizationrate of spermatheca mainly. The results obtained from this study are compatible with the results obtained from the *Chaetocnema concinna* species in Ekmekçi *et al.*, 2023, but are different from them to a normal extent, as seen in the text.

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