

## Histomorphological Structure of Male Reproductive System in *Capnodis tenebrionis* (Linnaeus, 1761) (Coleoptera: Buprestidae)

Üzeyir ÇAĞLAR<sup>1</sup>, Nurcan ÖZYURT KOÇAKOĞLU\*<sup>2</sup>, Selami CANDAN<sup>2</sup>

<sup>1</sup>Gazi University, Vocational School of Health Services, Gölbaşı, Ankara, Turkey

<sup>2</sup>Gazi University, Science Faculty, Department of Biology, Ankara, Turkey

ORCID ID: Üzeyir ÇAĞLAR: <https://orcid.org/0000-0002-8401-0155>; Nurcan ÖZYURT KOÇAKOĞLU: <https://orcid.org/0000-0001-7137-8631>;  
Selami Candan: <https://orcid.org/0000-0002-7402-1360>

Received: 21.09.2020

Accepted: 23.12.2020

Published online: 26.12.2020

Issue published: 31.12.2020

**Abstract:** In this study, anatomy and histology of the male reproductive system in adults of *Capnodis tenebrionis*, which are called the Mediterranean flat-headed root-borer, are described and illustrated by using light and scanning electron microscopies. Data from the male gonad of this species provide more extended and precise knowledge regarding to histoanatomical structure of the testis in Buprestidae. In this study, male reproductive system of *C. tenebrionis* consisted of two testes, two vas deferens and two accessory glands which are long tube-like, a pair of spermatophoral glands, an ejaculatory duct, and an aedeagus. Each testis was subdivided into a lot of tubular testicular follicles. Vas deferens was a tubular structure and located just below the testis. Its wall was surrounded by a monolayer cylindrical epithelium and a thin muscle layer. In its lumen, it was observed that mature sperm stacks were stored. Vas deferentia were immediately opened ejaculatory duct. It was surrounded by a single layered epithelium and a very thick muscle layer. Long, tubular, convoluted a pair of accessory glands were seen which were connected to the ejaculatory duct. In the accessory glands, it was seen that the wall was surrounded by monolayer cubic epithelium and sheath. In addition, it was seen that oval, sheet-shaped, paired spermatophoral glands were opened into the ejaculator canal. Its histological examinations revealed that the wall was surrounded by multilayered epithelial cells and muscle layer. The ejaculatory canal was connected to the aedeagus with very dense striated muscle bundles. The aim of the present study is to contribute information on the histomorphology of the male reproductive system of the insects.

**Keywords:** Spermatogenesis, Spermatozoa, Testes, accessory glands, electron microscope.

### *Capnodis tenebrionis* (Linnaeus, 1761) (Coleoptera: Buprestidae)'te Erkek Üreme Sisteminin Histomorfolojik Yapısı

**Öz:** Bu çalışmada, Akdeniz düz başlı kök kurdu ergin *Capnodis tenebrionis*'in erkek üreme sisteminin morfolojisi ve histolojisi, ışık ve taramalı elektron mikroskopları kullanılarak tanımlanmış ve gösterilmiştir. Bu türün erkek gonadından elde edilen veriler, Buprestidae'deki testisin histomorfolojik yapısına ilişkin daha kapsamlı ve kesin bilgi sağlamaktadır. Bu çalışmada, *C. tenebrionis*'in erkek üreme sistemi, bir çift testis, bir çift vas deferens, uzun tüp benzeri bir çift yardımcı bez, bir çift spermatoforal bez, bir ejakülatuar kanal ve aedeagustan oluşmaktadır. Her testis, çok sayıda tübüler testis foliküllerine bölünmüştür. Vas deferens tübüleri bir yapıdadır ve testisin hemen altında bulunmaktadır. Vas deferensin duvarı tek katmanlı silindirik epitel ve ince bir kas tabakası ile çevrelenmiştir. Lümeninde olgun sperm yığınlarının depolandığı görülmüştür. Vas deferens, devamında ejakülatör kanala açılmaktadır. Ejakülatör kanal tek tabakalı epitel ve çok kalın bir kas tabakası ile çevrelenmiştir. Ejakülatör kanalla birleşen uzun, tübüleri, kıvrımlı bir çift yardımcı bez görülmüştür. Yardımcı bezlerin tek tabakalı kübik epitel ve kılıfla çevrili olduğu görülmüştür. Ayrıca oval, yaprak şeklinde, bir çift spermatoforal bez ejakülatör kanala açıldığı görülmüştür. Histolojik incelemeler, spermatoforal bezin çok tabakalı epitel hücreleri ve kas tabakası ile çevrili olduğunu ortaya çıkarmıştır. Ejakülatör kanal oldukça yoğun çizgili kas demetlerine sahip aedeagus ile birleşmiştir. Bu çalışmanın amacı, böceklerin erkek üreme sisteminin histomorfolojisi ile ilgili katkı sağlamaktır.

**Anahtar kelimeler:** Spermatogenez, Spermatozoa, Testis, yardımcı bezler, elektron mikroskobu.

### 1. Introduction

The Coleoptera order accounts for almost 25% of all life forms and with approximately 400,000 species identified, 40% of the identified insect species showing the richest species diversity in the insect class (Hammond, 1992). The Buprestidae family comprises of 15,000 species and is one of the largest families of the Coleoptera species in terms of species richness (Sezer, 2018).

*Capnodis tenebrionis* (Buprestidae), which is Mediterranean flatheaded peachborer, has been reported as a common phytophagous beetle of several cultivated and wild species of tree and shrub belonging to the Rosaceae family (such as apricot, peach, plum, nectarine,

cherry, and etc.) for some countries of the Mediterranean sub-regions. Invasions to orchards have economic effects and its heavy attacks can often result in death of the plants as a result of larval tunneling into the roots (Ben-Yehuda, Assal, & Mendel, 2000; Vit, 2004; Marannino & de Lillo, 2007). Adult species feed on the buds and petioles and bark of shoots and usually prefers diseased trees (Bonsignore & Bellamy, 2007).

In the male reproductive system of Coleoptera, a testis generally consists of a single long tube, coiled into a tight ball surrounded in a membrane. This type of testis is very unusual in other insects. As well as the testis, the male reproductive system in Coleoptera generally includes a pair of vasa deferentia, which may have a convoluted or

\*Corresponding authors: nurcanozyurt@gazi.edu.tr

thickened and specialized area (vesicula seminalis). They join with the ectodermal ejaculatory duct. It receives the openings of the accessory glands, which are typically two pairs but occasionally with three, one or no pairs. The pairs of accessory glands frequently differ considerably from each other in terms of form, appearance and sometimes color; probably because one is mesodermal origin, the other is ectodermal origin (Crowson, 1981). In Buprestidae, the testis is joined with its base to the vas deferens. It unites with the accessory gland of that side before inserting on the ventral surface of the ejaculatory duct at it is connected with spermatophoral glands. Buprestidae has only a pair of accessory and the spermatophoral glands. The tubular accessory glands are thin-walled, lie along the abdomen, and the accessory glands vary among species. The thick walled, club-shaped, paired, spermatophoral glands are uniform. Spermatophores from the spermatophoral glands are similar in form and its shape conforms closely to the walls of the spermatophoral gland (Gardner, 1986).

As a consequence, many of the male reproductive system parts of the Coleoptera order differ from each other in terms of appearance, shape, and structure among different species and these structures are systematically important. Therefore, the knowledge of anatomical and histological structure of the male reproductive system in *C. tenebrionis* will be beneficial to the Coleoptera order and other orders systematically. Furthermore, it will contribute to the agricultural struggle with *C. tenebrionis* which is a harmful species for many plants.

## 2. Material and Methods

### 2.1. Biological samples

Adult of *C. tenebrionis* were collected from the the infested apricot twigs located in Konya, Turkey in May 2019. 20 samples were placed in the laboratory of Zoology for light and SEM examination.

### 2.2. Light microscope (LM) examinations

Ten samples were anesthetized and their male reproductive organs were dissected in sodium phosphate buffer (0.1 M, pH 7.2) removed under a dissecting stereomicroscope (SM) (Olympus SZX7). Then, photos of the general views were taken. To prepare thin sections for histological study, samples were fixed in 10% neutral formalin liquid for 24 hours. Then, tissues were washed with tap water (24 hours) and the male reproductive organs were dehydrated from 70% to 100% ethanol for an hour each. The samples were left in the mixture of Xylene-Paraffin three times for fifteen minutes each. After being saturated with paraffin for 24 hours, it was embedded in paraffin wax (65°C). Paraffin blocks were sectioned (6-7 µm) by using HM310 microtome. They were made in transverse and longitudinal planes. The slides containing tissues were stained by using Mallory's trichrome staining and Hematoxylin-Eosin (H & E). Stained sections were examined under Olympus BX51 light microscope.

### 2.3. Scanning electron microscope (SEM) examinations

For scanning electron microscopy, adult samples were fixed with 2.5% Glutaraldehyde (pH 7.2, Sodium phosphate buffer) after they were rinsed three times in sodium phosphate buffer, dehydrated with from 50 to

100% alcohol series. After the dehydration, the organs were dried with Hexamethyldisilazane (HMDS). Finally, they were mounted on aluminium SEM stubs. The stubs were covered with gold by using a sputter coater (Polaron SC 502) and samples were examined and photographed with a JEOL JSM 6060 LV SEM operating between 5-10 kV.

## 3. Results

According to the obtained results, it is seen that the reproductive system of male *Capnodis tenebrionis* consists of two testes, two vas deferens, two accessory glands, two spermatophoral glands, an ejaculatory duct, and an aedeagus (Fig. 1a). Each testis is oval-shaped and the anterior and posterior parts of the testis are narrower and the median region is wider than the other parts (Fig. 1b). Each testis is surrounded by a yellowish sheath (Fig. 1a). On the surface of the sheath, very thick, tubular shaped trachea and tracheoles in the form of thinner channels formed by the trachea into thin arms are clearly distinguished (Fig. 1b). Very thin, long, tubular, numerous testicular follicles are seen just below the sheath (Fig. 1c). Trachea and tracheoles are also found among these follicles (Fig. 1d). In the histological sections of the testicular follicles, transverse and longitudinal sections come from the follicles and different developmental stages of the sperm are observed within these follicles (Fig. 1e, f). These differentiation stages in testicular follicles are composed of 3 different parts: growth zone, maturation zone, and differentiation zone (Fig. 1e). Numerous spermatocytes are clustered in the follicle close to the anterior region of the testis (Fig. 2a, b). This stage is the growth zone where the spermatocyte heads are clearly aggregated together (Fig. 2a). In the maturation zone, spermatocytes differentiate into spermatids by passing meiosis. In the near of median region of the testis, it is observed that the tail regions are markedly differentiated as thin, long strands in the spermatids (Fig. 2c, d). In the differentiation zone, the spermatids are differentiated to spermatozoa, which are the last stage of sperm development (Fig. 2e, f). The differentiation zone covers the region close to the posterior part of the testis and the mature sperm is passed after this stage to the vas deferens (Fig. 3c). In the longitudinal sections of the follicles, it is observed that the sperm tails cluster together forming regular bundles (Fig. 3a-d).

Vas deferens is a thin, long, tubular structure that is located just below the testis. Histological and anatomical examination of vas deferens reveals that its wall is surrounded by a monolayer cylindrical epithelium and a thin muscle layer (Fig. 4a, c). When the lumen is examined, it is observed that mature sperm stacks are stored. Sperm tails in the lumen are seen as quite long and filamentous structures (Fig. 4a-d). Then, vas deferens is connected with the ejaculatory duct.

A long, tubular, convoluted pair of accessory glands are seen which are connected to the ejaculatory duct at the same site. When the accessory glands are examined anatomically, trachea nets and balloon-shaped structure of the trachea on its surface are found (Fig. 5a). The accessory glands exhibit a simple cubic epithelium and sheath (Fig. 5b-d). Their lumen is very large and filled with secretory substances (Fig. 5b, c).



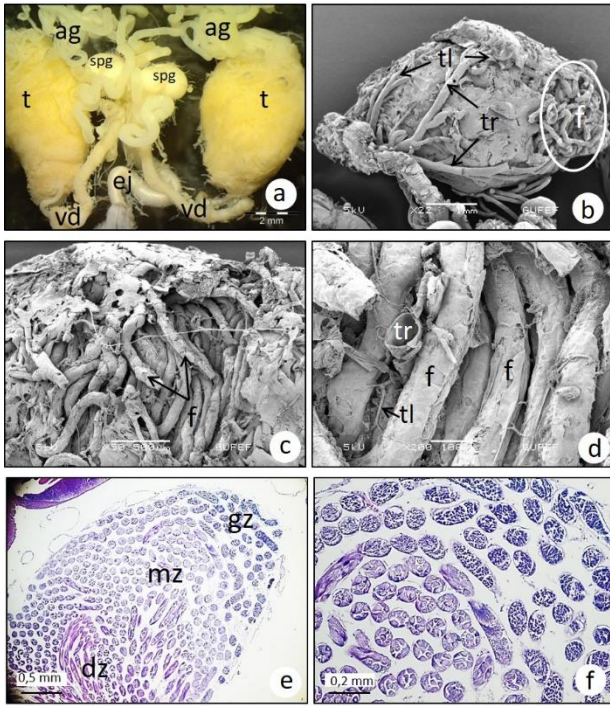


Figure 1. General structure of dissected male reproductive organs and testis: a. Male reproductive organs in *C. tenebrionis* (SM). b. The surface morphology of testis (SEM). c. The view of testicular follicles under the sheath (SEM). d. Detailed view of follicles. e, f The histological section of testis (LM) (H&E). ae-aedeagus; ag-accessory gland; dz-differentiation zone; ej-ejaculatory duct; f-follicule; gz-growth zone; mz-maturation zone; spg-spermatophoral gland; t-testis; tr-trachea; tl-tracheol; vd-vas deferens.

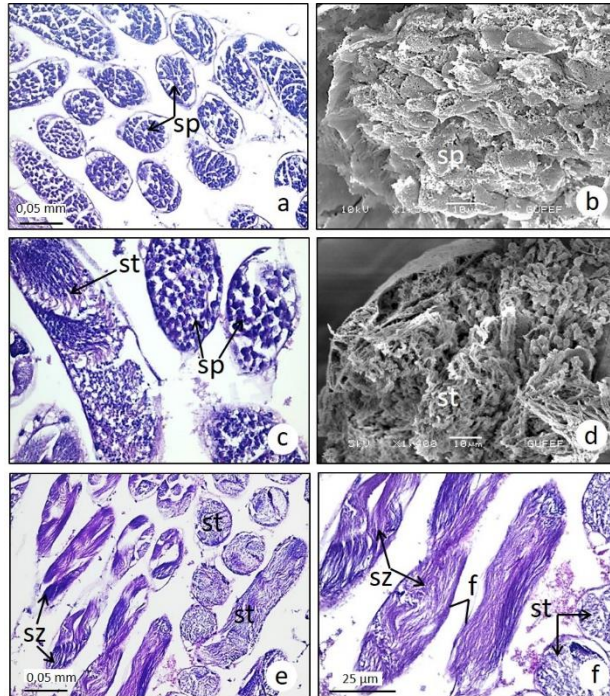


Figure 2. The detailed view of developmental zones in follicles. a. Spermatocytes in the growth zone (H&E) (LM). b. The detailed view of spermatocytes (SEM). c. The spermatocytes and spermatids in the maturation zone (H&E) (LM). d. The detailed view of spermatids (SEM). e, f. Spermatids and spermatozoa in the differentiation zone (H&E) (LM). f-follicule; sp-spermatocytes; st-spermatids; sz-spermatozoa.

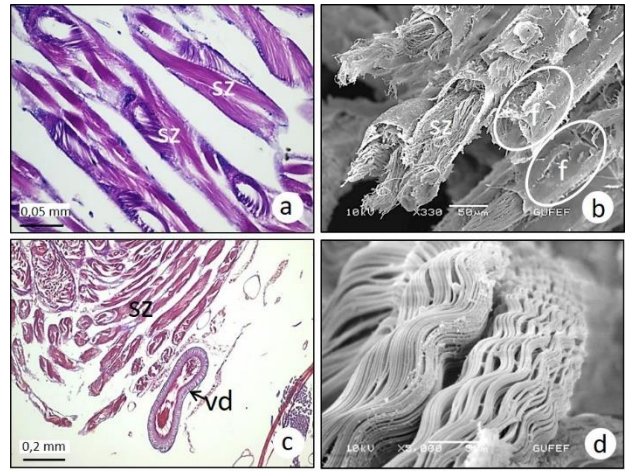


Figure 3. The detailed view spermatozoon groups. a, b. Spermatozoon bundles in testicular follicles (H&E) (LM, SEM). c. View of spermatozoa near of vas deferens (Mallory) (LM). d. The detailed view of spermatozoon bundles (SEM). f-follicule, vd-vas deferens, sz-spermatozoa.

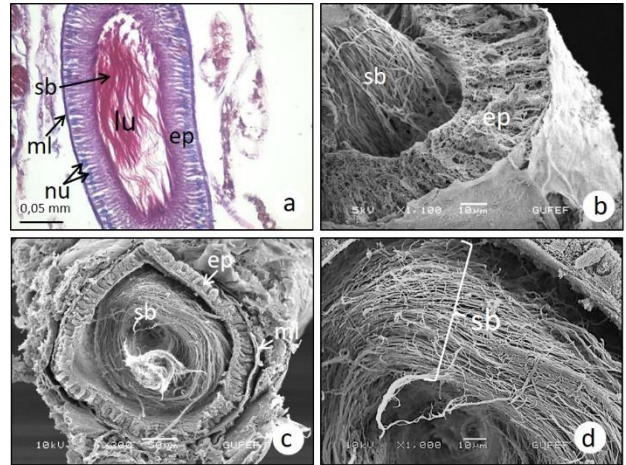


Figure 4. The detailed view of the vas deferens. a. Longitudinal section of the vas deferens (H&E) (LM). b, c. The morphological structure of vas deferens (SEM). d. The sperm bundles in vas deferens lumen (SEM). ep-epithelium; lu-lumen; nu-nucleus, ml-muscle layer, sb-sperm bundles.

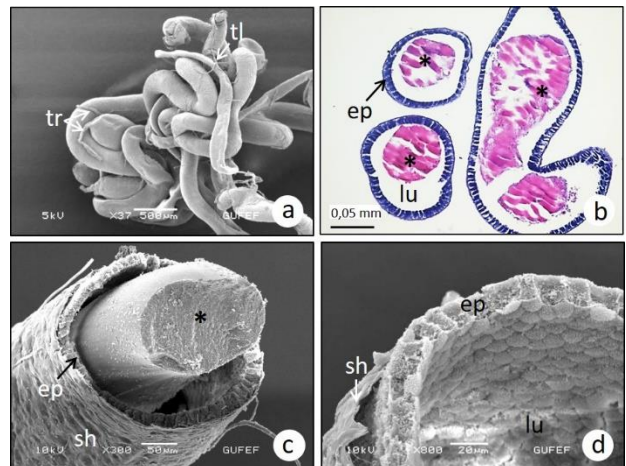


Figure 5. The detailed view of the accessory glands. a. The morphological view of accessory glands (SEM). b. Longitudinal and cross section of the accessory glands (H&E) (LM). c, d. Lumen, sheath, and epithelium view in the accessory glands (SEM). ep-epithelium; lu-lumen; sh-sheath; tr-trachea; tl-tracheol, asterisk-secretory substance.



Another structure, which is attached to the ejaculatory duct, is an oval, sac-shaped, spermatophoral gland (Fig. 6a). In the anatomical examination of the spermatophoral gland, it is surrounded by a flat sheath, and trachea and tracheol are observed on the sheath surface (Fig. 6b). Its histological examination reveals that the wall is surrounded by multilayered epithelial cells and muscle layer (Fig. 6c, d). Right on the point where this gland is attached to the ejaculatory duct, the thickness of the muscle layer increases (Fig. 6c). The lumen of this gland is oval shaped and the contents are filled with secretory material. The spermatophoral glands are associated with spermatophore production.

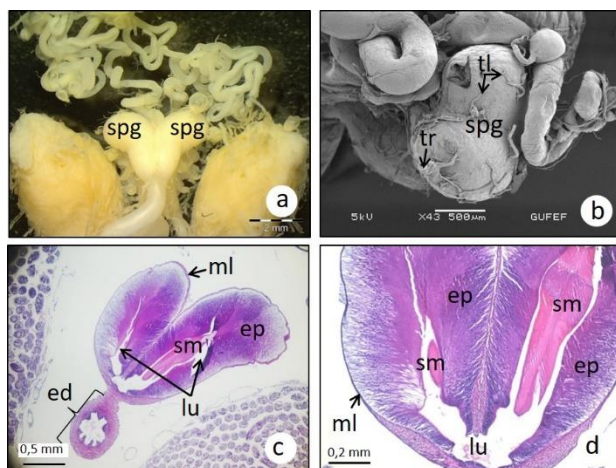


Figure 6. The detailed view of the spermatophoral glands. a. The general view of spermatophoral glands (SM). b. The morphological view of the spermatophoral glands (SEM). c, d. The longitudinal section of lumen, muscle layer, and epithelium in the spermatophoral glands (H&E) (LM). ed-ejaculatory duct; ep-epithelium; lu-lumen; ml-muscle layer; spg-spermatophoral gland; tr-trachea; tl-tracheol, sm- secretory substance.

Histologically and anatomically, the ejaculatory duct is surrounded by a single layered epithelium and a very thick muscle layer (Fig. 7a, b). The epithelium is covered with a thin cuticle called intima internally (Fig. 7a). When its muscle structure is examined in detail, streaks, banding and a large number of nucleus attract attention (Fig. 7c, d). Expansion or contraction of lumen shows variety depending on the contraction and relaxation of the sac (Fig. 7a, b). On the surface of this duct, a large number of trachea and thacheoles are found in the form of nets and hand (Fig. 7e, f).

The ejaculatory canal is connected to the aedeagus with very dense muscle bundles (Fig. 8a, b). When the muscle structure is examined in detail, the striations can be clearly distinguished (Fig. 8c, d).

#### 4. Discussion

The male reproductive tract has two with sperm tubes or follicles in *C. tenebrionis*, as most Coleopteran species (Alzahrani, Abdelsalam, Elmenshawy, & Abdel-Moneim, 2013; Aslam, 1961; Barker, 1989; Goldson & Emberson 1981; Hoffman & Raffa 1992; Ress & Cardé, 2009; Wu et al., 2017). This structure is similar to the reproductive structures of *Dendroctonus monticolae* Hopk. (Scolytidae) (Cerezke, 1964), *Phytalus sanctipauli* Blanchard, 1850 (Scarabaeidae) (Diefenbach, Redaelli, & Gassen, 1998), *Spasalus silvarum* Kuwert (Passalidae) (Salazar, Dias, Boucher, Lino-Neto, & Serrão, 2016), *Rhynchophorus*

*ferrugineus* Oliv. (Dryophthoridae) (Paoli et al., 2014), and *Tanymecus dilaticollis* Gyll. (Curculionidae) (Özyurt Koçakoğlu, Candan, & Güllü, 2019).

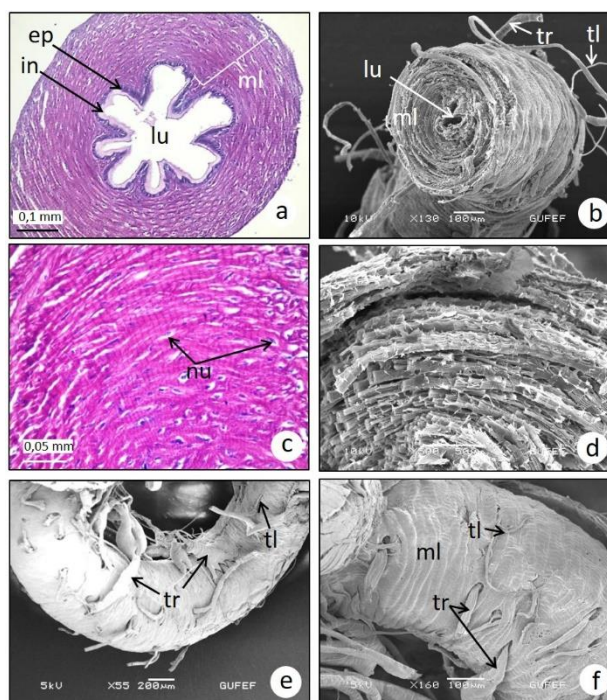


Figure 7. The detailed view of the ejaculatory duct. A. The cross section of ejaculatory duct (X200) (H&E) (LM). b. The morphological view of the ejaculatory duct (SEM). c. The cross section of muscle layer in ejaculatory duct (H&E) (LM). d. The morphological view of ejaculatory duct (SEM). e, f. The surface view of ejaculatory duct (SEM). ep-epithelium; lu-lumen; in-intima; ml-striated muscle layer; nu-nucleus; tr-trachea; tl-tracheol.

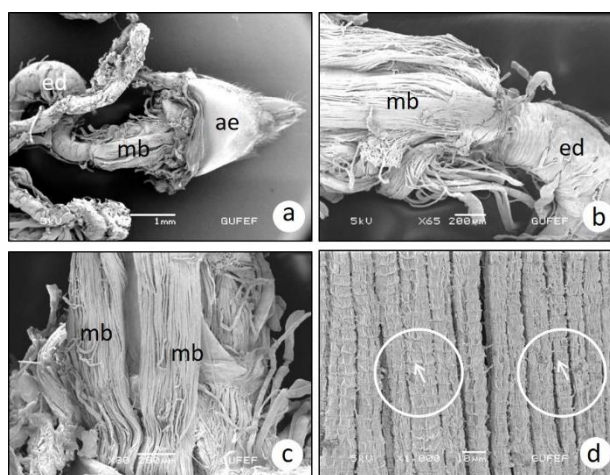


Figure 8. The detailed view of muscle bundles. a. The connection of the ejaculatory duct to the aedeagus with the muscles (SEM). b. The morphological structure of muscle fibrils and ejaculatory duct (SEM). c. The muscle bundles around aedeagus (SEM). d. The streaks, banding in muscle bundles (SEM). ae-aedeagus; ed-ejaculatory duct; mb- striated muscle bundles; arrow- striae.

However, there are remarkable anatomical varieties in male reproductive system of Coleoptera. The general appearance, color and follicular tube number of the testis vary in different insect species. In Buprestidae, including *C. tenebrionis*, each testis has spirally twisted into a conical and oval view (Gardner 1986). The testis in *D. monticolae* (Scolytidae) (Cerezke, 1964) and *T. dilaticollis* (Curculionidae) (Özyurt Koçakoğlu et al., 2019) is bean

shaped; however, it is discoid shape in *R. ferrugineus* (Dryophthoridae) (Paoli et al., 2014). The testis in species belonging to the Buprestidae family has generally a bright orange sheath in some species but in others it is pale (Gardner, 1986). The testis is seen yellowish color in *C. tenebrionis*. These differences (general appearance, color and follicular tube number of the testis) will provide important information about the systematic and reproductive biology of these insects.

Each testis has a group of testicular follicles. In the follicular tubule of each testis found in Coleoptera, as in other insect orders, the number, form, and modes of insertion of the each follicle differ greatly (Barker, 1989; Crowson, 1981). The number of follicles may be reduced to two or even one, or increased to scores or even hundreds. These differences in testis may have a considerably systematic value (Crowson, 1981). In *C. tenebrionis*, as in other species of the Buprestidae family, each testis consists of many follicular tubules (Gardner 1986). Each testis in *T. dilaticollis* (Özyurt Koçakoğlu et al., 2019) and *Hylobius pales* (Herbst) (Curculionidae) have two follicles (Hoffman & Raffa, 1992); on the other hand, they are divided into six follicles in *D. monticolae* (Scolytidae) and *P. sanctipauli* (Coleoptera: Scarabaeidae) (Cerezke, 1964; Diefenbach et al., 1998). As well as there are 8-10 follicles in each testis of *Hyperodes bonariensis* Kuschel (Curculionidae) and *Listronotus bonariensis* (Kuschel) (Curculionidae) (Barker, 1989; Goldson & Emberson, 1981). However, each testis has about 50-60 follicles in *R. ferrugineus* (Dryophthoridae). In *Dendroctonus armandi* Tsai and Li (Curculionidae) and *Tentyria cypria* Kraatz, 1865 (Tenebrionidae), each testis has 20 testicular follicles (İzzetoğlu & Gülmez, 2018; Paoli et al., 2014; Wu et al., 2017).

The histological and anatomical stages of spermatogenesis, which has the development of the germinative cells in *C. tenebrionis* take place within follicles like in most insects. The testes have different types of germ cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and nearly mature sperms (Phillips, 1970; Cerezke, 1964; Suzuki, 1988; Barker, 1989; Alzahrani et al., 2013; Özyurt, Candan, & Suludere, 2015; Wu et al. 2017; Candan, Özyurt Koçakoğlu, & Suludere, 2018; İzzetoğlu & Gülmez, 2018; Özyurt Koçakoğlu et al., 2019).

Apart from the testis, the male reproductive system of *C. tenebrionis* includes two vasa deferentia, two accessory glands, two spermatophoral glands, an ejaculatory duct, and aedeagus. Their anatomical characteristics have been considered important in the phylogenetic reconstructions of different taxa of insects (Dallai, 2014; Salazar et al., 2016).

There is a seminal vesicle where mature sperms are stored at the center of each testis in *T. dilaticollis* (Curculionidae) (Özyurt Koçakoğlu et al., 2019) and *R. ferrugineus* (Dryophthoridae) (Barker, 1989; Paoli et al., 2014) but in *L. bonariensis* (Curculionidae) the seminal vesicle is located at the basal end of the testis (Goldson & Emberson, 1981). Moreover, the testis posterior end of *C. tenebrionis* is connected with vas deferens. Sperm are stored along a region of the vas deferens, that region corresponds to the seminal vesicle.

Anatomy and number of the male accessory glands

vary among Coleopterans (Barker, 1989; Wu et al., 2017). There are two types of glands in *C. tenebrionis*, a pair of accessory glands, which are long, tubular structure, and spermatophoral glands which are sac-shaped. In *P. sanctipauli* (Scarabaeidae), there is a single pair of tubular coiled accessory glands (Diefenbach et al. 1998). *H. bonariensis* (Curculionidae), *L. bonariensis* (Curculionidae) and *T. dilaticollis* (Curculionidae) has paired blind-ended tubules accessory glands and paired multilobed prostate glands (Barker, 1989; Goldson & Emberson, 1981; Özyurt Koçakoğlu et al., 2019). *R. ferrugineus* (Dryophthoridae) has three types of glands as a long-tube accessory, a prostate, and a small accessory gland (Paoli et al., 2014). In *D. armandi* (Curculionidae), the accessory glands have paired curled glands and strandshaped glands with long and short branches (Wu et al., 2017). The accessory glands are responsible for sperm fluid and spermatophore formations (Gillott, 2005; Goldson & Emberson, 1981; Klowden, 2008; Senhal, 1985).

As a consequence of this study, the histological structures of male reproductive organs in male *C. tenebrionis* are generally similar to those in other Coleoptera species despite their many anatomical differences. This study intends to contribute to the male reproductive biology studies in Coleoptera and other insect orders species and may be useful for future studies in the taxonomy studies of insect histoanatomy. Furthermore, it will contribute to the agricultural struggle studies with *C. tenebrionis* which is a harmful species for many plants.

**Acknowledgements:** For the proof-reading of this paper, the authors are grateful to Gazi University Academic Writing Center.

## References

- Alzahrani, A.M., Abdelsalam, S.A., Elmenshawy O.M., & Abdel-Moneim, A.M. (2013) Ultrastructural characteristics of spermiogenesis in *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Florida Entomologist*, 96(4), 1463-1469.
- Aslam, N.A. (1961). An assessment of some internal characters in the higher classification of the Curculionidae. *Transactions of the Entomological Society of London*, 113, 417-480.
- Barker, G.M. (1989). Functional anatomy of the reproductive system of *Listronotus bonariensis* (Kuschel). *New Zealand Entomologist*, 12(1), 34-42.
- Ben-Yehuda, S., Assal, A., & Mendel, Z. (2000). Improved chemical control of *Capnodis tenebrionis* and *C. carbonaria* in stone-fruit plantations in Israel. *Phytoparasitica*, 28(1), 27-41.
- Bonsignore, C.P. & Bellamy, C. (2007). Daily activity and flight behaviour of adults of *Capnodis tenebrionis* (Coleoptera: Buprestidae). *European Journal of Entomology*, 104, 425-431. <https://doi.org/10.14411/eje.2007.062>
- Candan, S., Özyurt Koçakoğlu, N., & Suludere, Z. (2018). Morphological and histological structure of the male reproductive system of the water strider *Gerris lacustris* (Linnaeus 1758) (Gerridae, Heteroptera). *Microscopy Research and Technique*, 81(7), 770-780. <https://doi.org/10.1002/jemt.23035>
- Cerezke, H.F. (1964). The morphology and functions of the reproductive systems of *Dendroctonus monticolae* Hopk. (Coleoptera: Scolytidae). *The Canadian Entomologist*, 96(3), 477-500.
- Crowson, R.A. (1981). *The Biology of the Coleoptera*. Academic Press, London, 814 pp.
- Dallai, R. (2014). Overview on spermatogenesis and sperm structure of Hexapoda. *Arthropod Structure & Development*, 43, 257-290.
- Diefenbach, L.M.G., Redaelli, L.R., & Gassen, D.N. (1998). Characterization of the internal reproductive organs and their state as diapause indicator in *Phytalus sanctipauli* Blanchard, 1850 (Coleoptera, Scarabaeidae). *Revista Brasileira de Biologia*, 58(3), 541-546.
- Gardner, J.A. (1986). *Revision of the higher categories of Stigmoderini (Coleoptera: Buprestidae)*, (PhD Thesis), The University of Adelaide, South Australia.

- Gillott, C. (2005). *Entomology*, Dordrecht, The Netherlands, Springer, 832 pp.
- Goldson, S.L. & Emberson, R.M. (1981). Reproductive morphology of the Argentine stem weevil, *Hyperodes bonariensis* (Coleoptera: Curculionidae). *New Zealand Journal of Zoology*, 8(1), 67-77.
- Hammond, P. (1992). *Global Biodiversity*, Springer, Netherlands, 585 pp.
- Hoffman, G.D. & Raffa K.F. (1992). Maturation of the male pales weevil (Coleoptera: Curculionidae) reproductive system and its effect on male response to females. *Annals of the Entomological Society of America*, 85(5), 571-577.
- Klowden, M.J. (2008). *Physiological Systems in Insects*. Beijing, Science Press, 696 pp.
- İzzetoglu, G.T. & Gülmez, M. (2018). Macroscopic and histological structures of testes in three different *Tentyria* species. *Kahramanmaraş Sutcu Imam University Journal of Natural Sciences*, 21(3), 433-437.
- Marannino, P. & de Lillo, E. (2007). *Capnodis tenebrionis* (L. 1758) (Coleoptera: Buprestidae): morphology and behaviour of the neonate larvae, and soil humidity effects on the egg eclosion. In *Annales de la Société entomologique de France* 43(2), 145-154. <https://doi.org/10.1080/00379271.2007.10697504>
- Özyurt, N., Candan, S., & Suludere, Z. (2015). Ultrastructure of male reproductive system of *Eurydema ventrale* Kolenati 1846 (Heteroptera: Pentatomidae). *Microscopy Research and Technique*, 78, 643-653. <https://doi.org/10.1002/jemt.22514>
- Özyurt Koçakoğlu, N., Candan, S., & Güllü, M. (2019). The histomorphological structure of the male reproductive system of maize leaf weevil *Tanymericus dilaticollis* Gyllenhal, 1834 (Coleoptera: Curculionidae). *Microscopy Research and Technique*, 82, 1345-1352. <https://doi.org/10.1002/jemt.23286>
- Paoli, F., Dallai, R., Cristofaro, M., Arnone, S., Francardi, V., & Roversi, P.F. (2014). Morphology of the male reproductive system, sperm ultrastructure and  $\gamma$ -irradiation of the red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae). *Tissue & Cell*, 46(4), 274-285. <https://doi.org/10.1016/j.tice.2014.06.003>
- Phillips, D.M. (1970). Insect sperm: their structure and morphogenesis. *Journal of Cell Biology*, 44, 243-277.
- Ress, V.H. & Cardé, R.T. (2009). *Encyclopedia of Insects*. London, UK, Academic Press, 1168 pp.
- Salazar, K., Dias, G., Boucher, S., Lino-Neto, J., & Serrão, J.E. (2016). Morpho-anatomy of the male reproductive tract and spermatogenesis of the South American *Spasalus silvarum* Kuwert (Coleoptera: Passalidae). *Zoomorphology*, 135(4), 487-497. <https://doi.org/10.1007/s00435-016-0321-z>
- Senhal, F. (1985). Morphology of insect development. *Annual Review of Entomology*, 30, 89-109.
- Sezer, D. (2018). *Gökçeada ve Bozcaada Coleoptera takımına ait türler üzerinde faunistik çalışmalar* (Master's thesis), Fen Bilimleri Enstitüsü, Hacettepe Üniversitesi, Ankara, Türkiye.
- Suzuki, K. (1988). Comparative morphology of the internal reproductive system of the Chrysomelidae (Coleoptera). In: Jolivet P, Petitpierre E, Hsiao TH (eds), *Biology of Chrysomelidae*, Series Entomologica, Dordrecht, Springer. 42, 317-355.
- Vit, K. (2004). *Fauna Europaea: Buprestidae*. Fauna Europaea, Coleoptera. Fauna Europaea version 1.1. Retrieved from <http://www.faunaeur.org>
- Wu, Y.F., Wei, L.S., Anthony Torres, M., Zhang, X., Wu, S.P., & Chen, H. (2017) Morphology of the male reproductive system and spermiogenesis of *Dendroctonus armandi* Tsai and Li (Coleoptera: Curculionidae: Scolytinae). *Journal of Insect Science*, 17(1), 1-9. <https://doi.org/10.1016/j.micron.2009.02.003>