J. Entomol. Res. Soc., 16(1): 101-110, 2014 *ISSN:1302-0250*

Structure of the Male Reproductive System and Spermatogenesis of *Codophila varia* (Fabricius, 1787) (Heteroptera: Pentatomidae) by Light and Scanning Electron Microscopy

Nurcan ÖZYURT Selami CANDAN Zekiye SULUDERE

Department of Biology, Faculty of Science, Gazi University, 06500 Teknikokullar, Ankara, TURKEY, e-mails: nurcanozyurt@gazi.edu.tr, scandan@gazi.edu.tr, zekiyes@gazi.edu.tr

ABSTRACT

Structural features of the male reproductive system in *Codophila varia* (Fabricius, 1787) were investigated utilizing both light and scanning electron microscopes. Microscope examinations showed that the male reproductive system in *C. varia* consists of two testis, two vas deferentia, two seminal vesicles, two accessory glands, two ectodermal sacs, ejaculatory bulb, ejaculatory duct and aedeagus. The male reproductive tract, showed each testes with intense red coloration in slightly oval structure, with seven follicles. The vas deferens and seminal vesicle were similar in color to the testes and formed long filaments. Vas deferens and seminal vesicle insert on the anterior medial portion of the ejaculatory bulb. The ejaculatory duct extends from the base of the ejaculatory bulb to the aedeagus. Also, structural similarities in the male reproductive systems of *C. varia* and some other Hemipteran species are discussed.

Key words: Testes, vas deferens, seminal vesicle, ejaculatory bulb, SEM (Scanning Electron Microscope).

INTRODUCTION

Codophila varia (Fabricius, 1787) (Insecta: Heteroptera: Pentatomidae) is present in most of Europe and in Turkey where it feeds on *Carthamus* sp., *Centaurea* sp., *Circium* sp., *Daucus* sp*., Echium plantagineum, Medicago sativa*, *Onopordum* sp., *Sinapis* sp., *Verbascum* sp., umbelliferous and graminaeous plants and also on weeds (Lodos *et al*., 1998). While most pentatomid species are plant feeders, a few species have been determined to have biocontrol potential. Despite the economic importance of Pentatomidae on various crops, few reports exist detailing the internal morphology of the reproductive tract of these organisms, especially with a reference to the male reproductive system. The male reproductive morphology of *Nezara viridula* (Linnaeus, 1758) (Pendergrast, 1956; Ramamurty, 1969), *Perillus bioculatus* (Fabricius) (Adams, 2001), *Podisus nigrispinus* (Dallas) (Lemos *et al.*, 2005) (Rodrigues *et al.*, 2008), *Dolycoris baccarum* (Özyurt *et al*., 2013) have been studied.

Morphologically, the male reproductive tract of heteropteran insects typically consists of a pair of testes and a pair of vas deferentia that end in a medium ejaculatory duct (Pendergrast, 1956; Davey and Krieger, 1985; Happ, 1992; Nijhout, 1998; Chapman, 1998; Adams, 2001; Lemos *et al.*, 2005).

Histologically, each testes contains multiple testicular follicles (in which spermatozoa are produced), of varying lengths among genera and species (Gonçalves *et al.*, 1987; Chapman, 1998; Nijhout, 1998; Lemos *et al.*, 2005; Freitas *et al.*, 2007, 2010). Each follicle has its own germarium, analogous to the ovarioles of females, except that the testicular follicles are generally by enveloped in a common sheath, making the testis a fairly compact body (Nijhout, 1998; Lemos *et al.*, 2005).

The male reproductive organs of the Pentatomidae are in general, more complex than those of other families of Heteroptera. This complexity is apparent in the structure of the bulbus ejaculatorius and in the presence of often elaborate ectodermal accessory glands in addition to mesodermal accessory glands (Pendergrast, 1956). Most insects also have a number of accessory glands, which open into the vas deferens or the ductus ejaculatorius (Davis, 1956; Matsuda, 1976; Happ, 1992; Chapman, 1998; Adams, 2001; Lemos *et al.*, 2005). Both types of glands serve to produce the seminal fluid (Davis, 1956; Pendergrast,1956; Davey and Krieger,1985; Chapman, 1998; Nijhout, 1998).

There is not any study about *C. varia* species on the investigation of male reproductive system of Heteroptera. The aim of present study is to investigate the reproductive morphology and histology of the male reproductive system of *C. varia*. This research is provide key information for additional research into the reproductive biology and ecology of Heteroptera.

MATERIALS AND METHODS

Gross anatomy

Adult males of *C.* varia were collected from in July 2011 in Ankara, Turkey. Adult males were anesthesia with ethyl acetate fumes and dissected in 70% ethyl alcohol under a stereomicroscope. For the morphological analysis, the dorsal cuticle was first removed from the prothorax to the last full-sized abdominal segments. Subsequently, the epidermis and the digestive system were removed. Two entomological pins were inserted laterally through the last full-sized sternite to spread apart the abdomen and to expose the base of the reproductive system attached to the genital segments. The gross morphology of the the reproductive systems of the males were examined and photographed with a Leica EZ4D stereomicroscope.

Light microscopy

For the histological analysis, the reproductive systems of ten males were fixed in Bouin's for 24h. Thereafter, the tissues were washed, dehyrated in a grade series of ethanol solutions (70%,% 80,% 90, 100%) and finally embedded in paraffin. Paraffin sections were cut into 6-7 µm thick slides and stained with Hematoxylin-Eosin and Mallory's triple stain for light microscopic examination. The sections were viewed and photographed using an Olympus BX51 microscope.

Scanning electron microscopy

For additional analysis a scanning electron microscopy was used. Cleaned and dried male specimens (Polaron CPD 7501 Critical Point Dryer) were mounted using

double sided tape on SEM stubs, coated with gold in a Polaron SC 502 sputter coater, and examined with a JEOL JSM 6060 LV scanning electron microscope at accelerating voltage 10 kV. Photos were taken.

RESULTS

The male reproductive system of *C. varia* consists of two testes, two vas deferentia, two seminal vesicles, two ectodermal sacs, one ejaculatory bulb, one ejaculatory duct and two accessory glands (Fig. 1). These structures are located ventrally in the abdominal cavity.

Testes

Paired testes lie on either side of the digestive tract. The testes are lined with the tunica propria and peritoneal sheath with embedded tracheoles (Figs. 3, 5). The red pigmented testis are roughly oval and consist of seven testes tubules which enter the vas deferens (Figs. 1, 2). Three development zones were noted within the testes tubules (growth zone, maturation zone and differentiation zone) (Fig. 2); the growth zone, where groups of spermatogonia (in cysts) may undergo several mitotic divisions before they finally become spermatocytes (in cysts) (Figs. 3-7); the maturation zone, where two meiotic divisions occur and these cells become bundles of spermatids (Figs. 8-10), the differentiation zone, where spermatids differentiate and become spermatozoa (Figs. 11,12). When the spermatozoa are completely mature, the cysts break open. The released bundles of spermatozoa remain together, held by extracellular material that surrounds the anterior portion of their heads (Figs. 13,14). The spermatozoa migrate to the vas deferens, pass through the vas deferens, and are transferred to the seminal vesicle.

Vas deferens and Seminal vesicle

Vas deferens are also red pigmented, and consist of very narrow ducts which extend posteriorly from the testes to the seminal vesicle. There is no outstanding difference between the histology and morphology of the vas deferens and the seminal vesicle (Figs. 15,16). The walls of these ducts consist of an inner layer of simple epithelium which is surrounded by a network of muscle fibers extending in various directions (Fig. 15). The seminal vesicle receives and stores the spermatozoa (Fig. 15).

Ejaculatory bulb

Vas deferens and seminal vesicle are inserted on the anterior medial portion of the ejaculatory bulb, which is complex. It is partly covered by an epithelium here termed the investing epithelium. The walls of ejaculatory bulb are formed by muscles running concentrically to the lumen of the ducts. It is continuous with the ejaculatory duct (Figs. 17,18).

Ejaculatory duct

The ejaculatory duct consists of an epithelium of cells which progressively cuboidal posteriorly and a chitinous tube carrying the sperm and secretions (Figs. 17,18). Ejaculatory duct extends from the base of the ejaculatory bulb to the aedeagus.

Ectodermal sacs

The ectodermal sac, located near the ejaculatory bulb, is two lobed and spherical (Figs. 17,18). It is continuous with the aedeagus and covers the cuticle.

Accessory glands

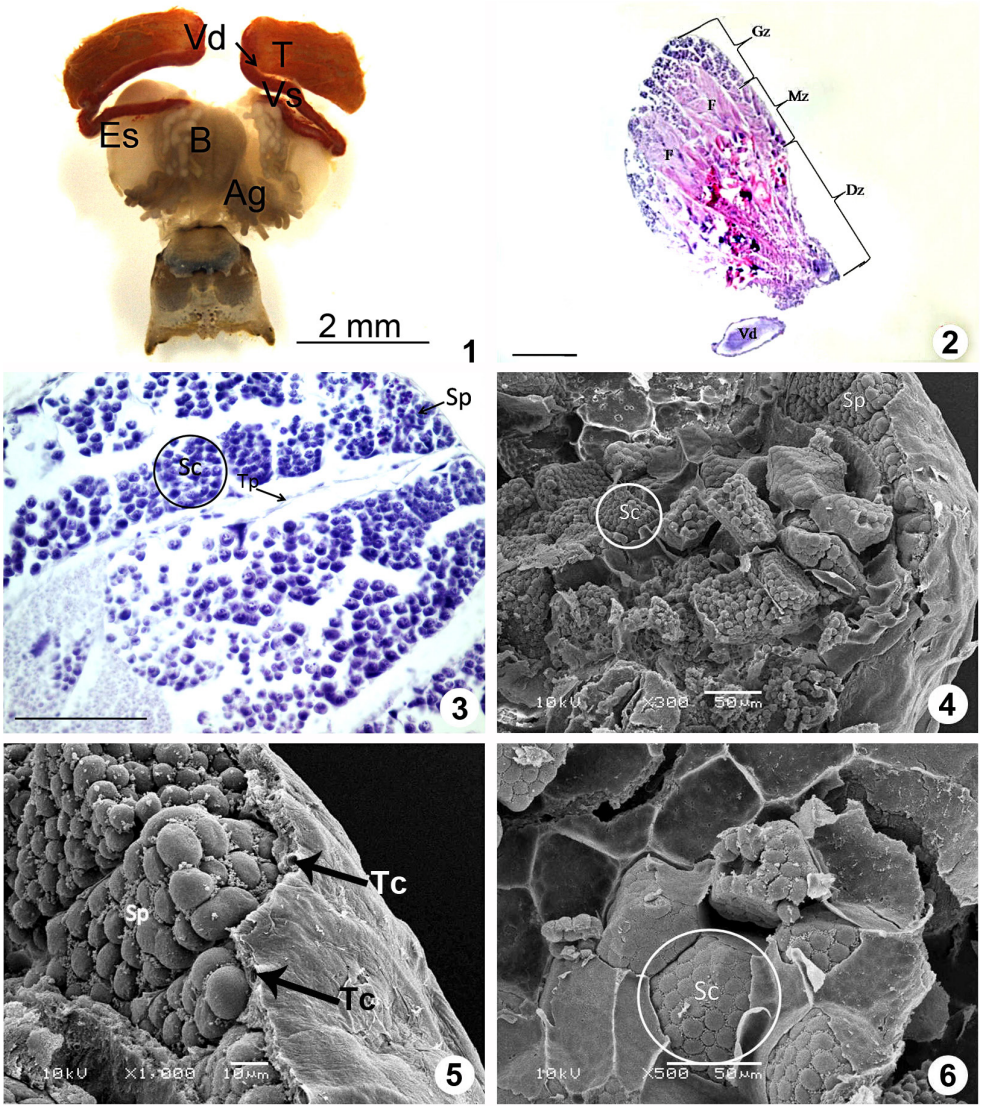
There are multiple accessory glands in *C. varia* which are situated in the posteroventral region of the male, milk white in color and sack-shaped (Figs. 18-20). The accessory glands consist of three different parts: the muscle layers or muscularis (Mcl), the secretory epithelium and the lumen. The lumen is filled with granular secretions (Figs. 17,19).

DISCUSSION

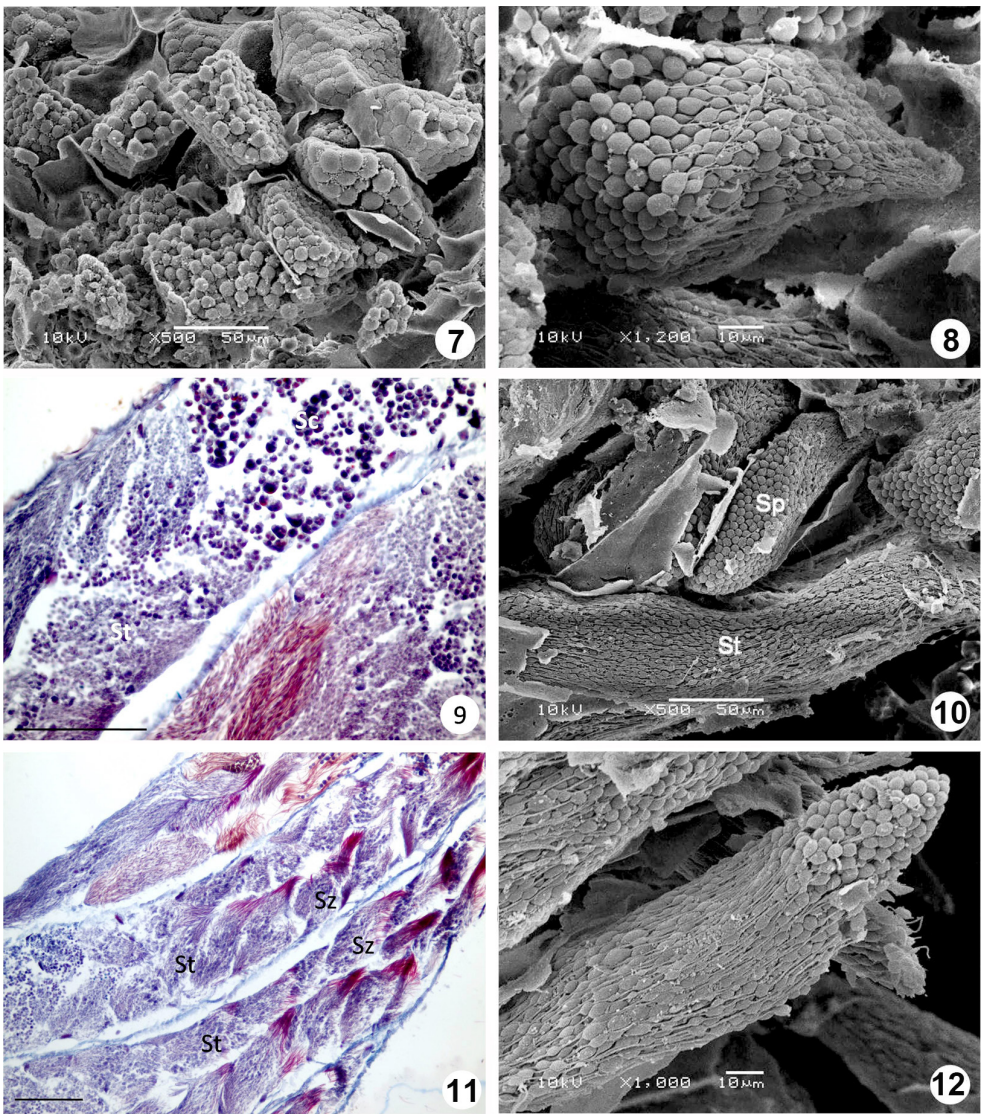
In this study, the male reproductive morphology and histology of *C. varia* is described. The male reproductive organs of *C. varia* are consist of paired testis, a pair of vas deferentia, a pair of seminal vesicles, a ejaculatory bulb, a ejaculatory duct, a pair of ectodermal sacs and accessory glands. The results support the existence of differences as regards location, size, shape and colour of *C. varia* male reproductive system when compared to other Heteroptera. However, despite such differences, that general morphological and histological pattern is very similar for the entire Pentatomidae (Bonhag *et al.*, 1953; Davis, 1956; Pendergrast, 1956; Matsuda, 1976; Grasse, 1982; Chapman, 1998; Adams, 2001; Lemos *et al.*, 2005; Bushrow *et al.*, 2006; Pires *et al.*, 2007; Rodrigues *et al.*, 2008; Freitas *et al.*, 2010; Karakaya *et al.*, 2012; Özyurt *et al.*, 2013). Morphological studies of the male reproductive system have been made for several Heteroptera species (Pendergrast, 1956; Davey and Krieger, 1985; Happ, 1992; Nijhout, 1998; Chapman, 1998; Adams, 2001; Lemos *et al.*, 2005; Rodrigues *et al.*, 2008; Özyurt *et al.*, 2013).

The number of testes follicles varies widely between species and presents taxonomic importance (Davis, 1956; Pendergrast, 1956; Gonçalves *et al.*, 1987; Adams, 2001; Lemos *et al.*, 2005; Rodrigues *et al.*, 2008; Freitas *et al.*, 2010). The male reproductive system of *C. varia* consists of paired testes that consisting of 14 sperm tubes. In in *Nezara viridula*, *Podisus nigrispinus, Dolycoris baccarum* (Heteroptera: Pentatomidae) and *Aphelocheirus aestivalis* (Heteroptera: Aphelocheiridae) observed 4-6 sperm tubes (Pendergrast, 1956; Lemos *et al.*, 2005; Papáček and Soldán, 2008; Özyurt *et al.*, 2013).

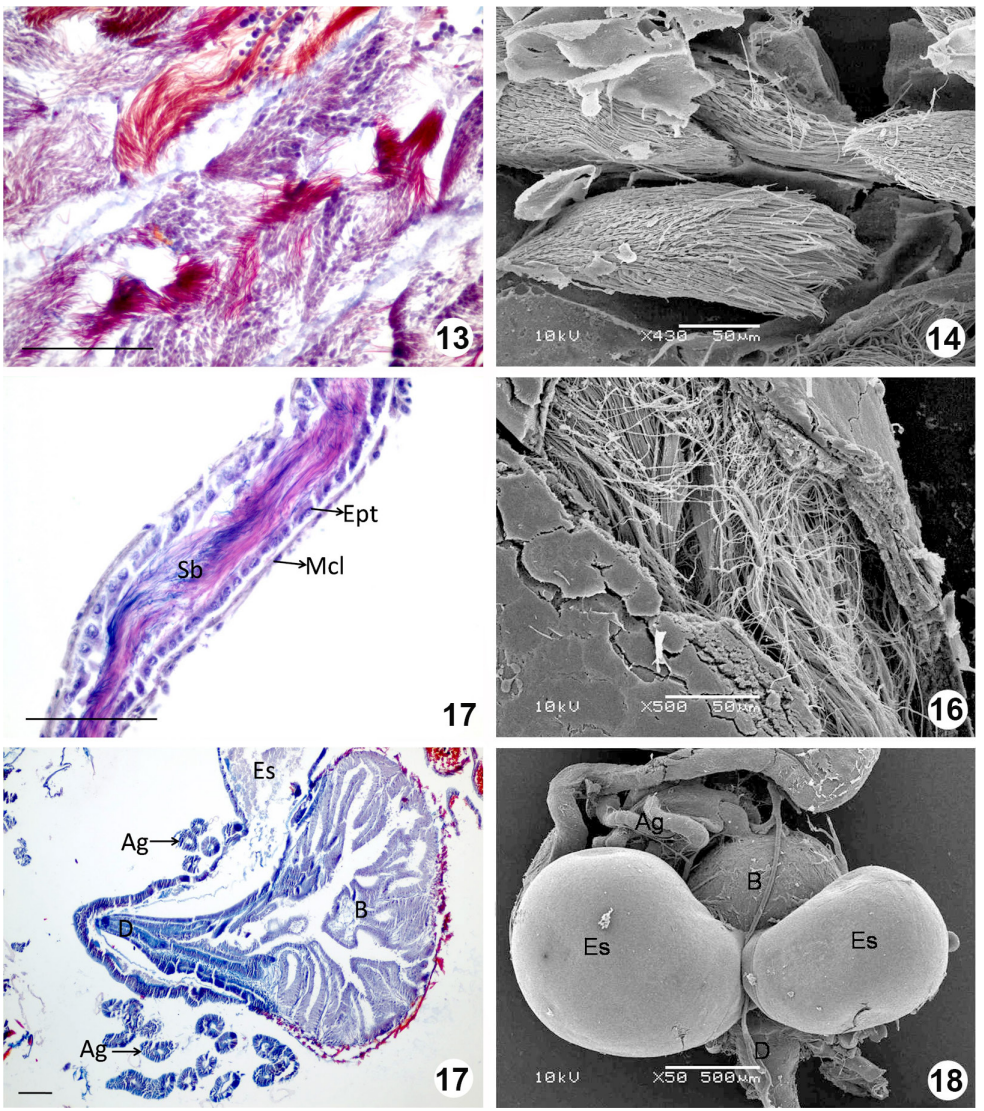
Like other Heteropterans, the testes in *C. varia* consists of a peritoneal sheath enclosing the testes tubules. A basement membrane is formed on the outer surface of the peritoneal epithelium enclosing the testis and on the inner surface of the testicular tubules (Davis, 1956; Chapman,1998; Pires *et al.*, 2007; Rodrigues *et al.*, 2008). The various zones of development described in the testicular tubules of *C. varia* have not been as clearly distinguishable in the other Heteropterans (Davis, 1956). The testes contain sperm at different stages of spermatogenesis (spermatogonia, spermatocytes, spermatids, spermatozoa) along the length of the sperm tubes.



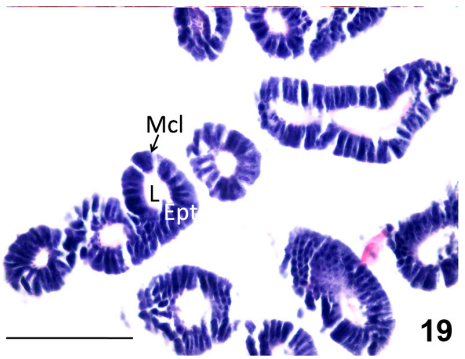
Figs. 1-6. Male reproductive system of *C. varia*. Fig.1. General view, T: Testis, Vd: Vas deferens, Vs: Seminal vesicle, B: Ejaculatory bulb, D: Ejaculatory duct, Ag: Accessory glands, A: Aedeagus. Fig.2. Longitudinal section of a testes follicle (F) showing growth zone (Gz), maturation zone (Mz), differentiation zone (Dz), vas deferens (Vd) (Bar 500 µm). Fig. 3. Histological section of germarium region, the beginning of the differentiation of the spermatogonia (Sp) and spermatocytes forming cysts (Sc),and the tunica propria (Tp).Fig. 4. SEM photo of germarium region, the beginning of the differentiation of the spermatogonia (Sp) and spermatocytes forming cysts (Sc). Fig. 5. Spermatogonia in cysts, trachea (Tc) in peritoneal sheath (Ps). Fig. 6. Spermatocytes in cysts (Bar 100 µm).

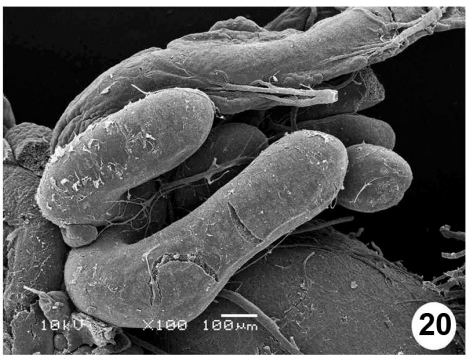


Figs. 7-12. Developmental stages of spermatocytes. Fig. 7. SEM photo of spermatocytes in cysts. Fig. 8. spherical spermatids undergoing differentiation. Fig. 9. Histological section of spermatocyte (Sc) developmental stages and differentiated spermatids (St) in the maturation zone. Fig. 10. Developmental stages and differentiated spermatids. Fig. 11. Histological section of spermatids (St) at different developmental stages and differentiated spermatozoa (Sz). Fig.12. Differentiation of spermatids and spermatozoa (Bar 100 µm).



Figs. 13-18. Spermatozoa bundles, vas deferens, ejaculatory bulb, ejaculatory duct, accessory glands, ectodermal sac. Fig.13. Spermatozoa bundles. Fig. 14. SEM photo of large numbers of spermatozoa bundles. Fig. 15. Longitudinal section of the vas deferens with numerous spermatozoa bundles (Sb), epithelium (Ept), muscle layers (Mcl). Fig. 16. Vas deferens with numerous spermatozoa bundles. Fig. 17. Histological section of ejaculatory bulb (B), ejaculatory duct (D), ectodermal sacs (Es) and accessory glands (Ag). Fig. 18. Ejaculatory bulb (B), ejaculatory duct (D), ectodermal sacs (Es), accessory glands (Ag) (Bar 100 μm).





Figs. 19-20. Light and SEM photos of accessory glands. 19. Histological section of an accessory gland illustrating the muscularis (Mcl), the well-developed secretory epithelium (Ept) and the lumen (L). Fig. 20. SEM photo of accessory glands (Bar 100 µm).

Mechanisms of spermatogenesis in *C. varia*, including sperm differentiation, are rather similar to those found in other Heteroptera (Bowen, 1922; Davis, 1956; Engelmann, 1970; Chapman, 1998; Lemos *et al.*, 2005; Pires *et al.*, 2007; Rodrigues *et al.,* 2008; Karakaya *et al.*, 2012; Özyurt *et al.*, 2013). The number of spermatogonial generations varies from species to species (Engelmann, 1970; Lemos *et al.*, 2005). Once fully developed, the sperm travel from the testes to the seminal vesicle via the vas deferens. In *C. varia*, as in other heteropterans, a portion of each vas deferens is dilated to form a tubular chamber. The seminal vesicle receives and stores the spermatozoa (Davis, 1956).

As with other Heteroptera, the seminal vesicles of *C. varia* connects to the ejaculatory bulb is balloon shaped and covered by irregularly shaped accessory glands (Özyurt *et al.*, 2013). The ejaculatory bulb joins to form the common ejaculatory duct (Bushrow *et al.*, 2006). The terminal portion of the ejaculatory duct may be sclerotized to form the intromittent organ, the aedeagus. It differentiates from a pair of ectodermal lobes associated with the ninth abdominal segment and is often concealed within a genital chamber (Klowden, 2007).

Among male insects, the accessory glands notably vary in size, shape, and number (Adiyodi and Adiyodi, 1975; Leopold, 1976; Grasse, 1982; Chapman, 1998; Freitas *et al.*, 2007). One of the functions of the secretions of these glands is a contribution to the seminal fluid and activation of the spermatozoa (Leopold 1976; Chen, 1984; Davey and Krieger, 1985).

As a result of present study, these goals were acheived: an extension of our knowledge of the male reproductive system of Pentatomidae and addition of important information for future research into the reproductive biology of the Heteroptera.

REFERENCES

Adams, T. S., 2001, Morphology of the internal reproductive system of the male and female two-spotted stink bug, *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae) and the transfer of products during mating. *Intervertebrate Reproduction and Development*, 39(1): 45-53.

Adiyodi, K. G., Adiyodi, R. G., 1975, Morphology and cytology of the accessory sex glands in invertebrates. *International Review of Cytology*, 43: 355-398.

Bonhag, P. F., Wick, J. R.,1953, The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *Journal of Morphology*, 93: 177-284.

Bowen, R. H.,1922, Studies on insect spermatogenesis II. The components of the spermatid and their role in the formation of sperm in Hemiptera. *Journal of Morphology*, 37: 79-193.

Bushrow, E. S., Fuller, C. L., Cowan, D. P., Byrd, C. A., 2006, Anatomy of the male reproductive system and sperm morphology in the caterpillar-hunting wasp *Ancistrocerus antilope* (Hymenoptera, Vespidae). *Invertebrate Biology,*125 (4): 354-362.

Chapman, R. F., 1998, *The Insects: Structure and Function. The Reproductive System*, Cambridge University Press, Cambridge, UK, 319-377.

Chen, P. S., 1984, The functional morphology and biochemistry of insect male accessory glands and their secretion. *Annual Review of Entomology*, 29: 233-255.

Davey, K. G., Krieger, F. L., 1985, Variations during the gonotropic cycle in the titer of the myotrophic ovulation hormone and the response of the ovarian muscles in *Rhodnius prolixus*. *General and Comparative Endocrinology*, 59: 452-457.

Davis, N. T., 1956, The morphology and functional anatomy of the male and female reproductive systems of *Cimex lectularius* L. (Heteroptera, Cimicidae). *Annals of the Entomological Society of America*, 49: 466-493.

Engelmann, F., 1970, The *Physiology* of *Insect Reproduction*. Pergamon Press, *New York*. *307* pp.

Freitas, S. P. C., Gonçalves, T. C. M., Serrao, J. E., Santos-Mallet, J. R., 2007, Fine Structure of the male accessory glands of *Triatoma rubrofasciata* (Hemiptera: Triatominae). *Microscopy Research and Technique*, 70: 355-360.

Freitas, S. P. C., Gonçalves, T. C. M., Serrao, J. E., Costa, J., Santos-Mallet, J. R., 2010, Male reproductive system structure and accessory glands ultrastructure of two species of *Triatoma* (Hemiptera, Reduviidae, Triatominae). *Micron*, 41: 518-525.

Gonçalves, T. C. M., Lent, H., Almeida, J. R., 1987, Estudo anatômico e morfométrico dos foliculos testiculares de algumas espécies de Triatominae (Hemiptera: Reduviidae). *Memórias do Instituto Oswaldo Cruz*, 82: 543-550.

Grasse, P. P., 1982, *Termitologia: Anatomie, Physiologie, Reproduction des Termites*, Masson, Paris, 676.

Happ, G. M., 1992, Maturation of the male reproductive system and its endocrine regulation. *Annual Review of Entomology*, 37: 303-320.

Karakaya, G., Özyurt, N., Candan, S., Suludere, Z., 2012, Structure of the male reproductive system in *Coreus marginatus* (Linneaus, 1758) (Hemiptera: Coreidae).*Turkish Journal of Entomology*, 36(2): 193-204.

Klowden M. J., 2007, *Physiological Systems in Insects.* Academic Press, San Diego, California, 415.

Lemos, W. P., Serrao, J. E., Ramalho, F. S., Cola Zanuncio, J. C., Lacerda, M. C., 2005, Effect of diet on male reproductive tract of *Podisus* (Heteroptera: Pentatomidae). *Brazilian Journal of Biology*, 65(1): 91-96.

Leopold, R. A., 1976, The role of male accessory glands in insect reproduction. *Annual Review of Entomology*, 21: 199-221.

Lodos, N., Onder, F. Pehlivan, E., Atalay, R., Erkin, E., Karsavuran, Y., Tezcan, S., Aksoy,S., 1998, *Faunistic Studies on Pentatomoidea (Plataspidae, Acanthosomatidae, Cydnidae, Scutelleridae, Pentatomidae) of Western Black Sea, Central Anatolia and Mediterranean Regions of Turkey*. Ege Üniversitesi Ziraat Fakültesi Yayınları, İzmir, 75.

Matsuda, R., 1976, *Morphology and Evolution of the Insect Abdomen*,Pergamon Press, Oxford, 534.

Nijhout, H. F., 1998, *Insect Hormones*. Princeton University Press, USA, 267.

Özyurt, N., Candan, S., Suludere, Z., 2013, The morphology and histology of the male reproductive system in *Dolycoris baccarum* Linnaeus 1758 (Heteroptera: Pentatomidae). Light and scanning electron micoscope studies. *Micron*, 44: 101-106.

Papáček M., Soldán T., 2008, Structure and development of the reproductive system in *Aphelocheirus aestivalis* (Hemiptera: Heteroptera: Nepomorpha: Aphelocheiridae). *Acta Entomologica Musei Nationalis Pragae,* 48(2): 299-318.

Pendergrast, J. G., 1956, The male reproductive organs of *Nezara viridula* with a preliminary account of theirdevelopment (Heteroptera: Pentatomidae). *Transactions of the Royal Society of New Zealand*, 84(1): 139-146.

Pires, E. M., Ferreira, P. S .F., Guedes, R. N. C., Serrao, J. E., 2007, Morphology of the phytophagous bug *Platyscytus decempunctatus* (Carvalho) (Heteroptera: Miridae). *Neotropical Entomology*, 36(4): 510-513.

Ramamurty, P. S., 1969, Histological studies of the internal organs of reproduction in *Nezara viridula* Fabr. Pentatomidae: Heteroptera, Insecta. *Zoologischer Anzeiger*, 183: 119-139.

Rodrigues, A. R., Serrao, J. E., Teixeira, V. W., Torres, J. B., Teixeira, A. A., 2008, Spermatogenesis, changes in reproductive structures, and time constraint associated with insemination in *Podisus nigrispinus*. *Journal of Insect Physiology*, 54(1): 1543-1551.

Received: May 10, 2013 *Accepted: January 28, 2014*