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Orijinal araştırma (Original article)

Structure of the male reproductive system in *Coreus marginatus* (L.) (Hemiptera: Coreidae)

Coreus marginatus (L.) (Hemiptera: Coreidae)'un erkek üreme sisteminin yapısı

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Summary

Using light and scanning electron microscopy, histology and morphology of the male reproductive system of *Coreus marginatus* (Linnaeus, 1758) are identified and described. Reproductive system consist of two testes, in which different stages of spermatogenesis are observed, two tubular vas deferens, two vesicula seminalis, accessory glands (mesadenia, ectadenia), bulbus ejaculatorius, a pair of ectodermal sacs, and a single muscular ejaculatory duct, which opens via the aedeagus. Knowledge of basic reproductive morphology and histology can be used to infer function and so provide key information for future research into reproductive behavior, evolution and physiology in the Heteroptera (Hemiptera).

Key words: Male reproductive system, Coreidae, Heteroptera, light microscope, scanning electron microscope

Özet

Bu çalışmada, *Coreus marginatus* (Linnaeus, 1758)'un erkek üreme sisteminin histolojisi ve morfolojisi ışık ve taramalı elektron mikroskobu (SEM) ile detaylı olarak incelenmiştir. *Coreus marginatus'* un erkek üreme sistemi, farklı spermatogenez safhalarının gözlendiği 2 testis, tüpsü 2 vas deferens, 2 vesicula seminalis, yardımcı bezler (mesedenia ve ektedenia), bulbus ejakulator, bir çift ektodermal kese, kaslı bir ejekulator kanal ve kanalın açıldığı aedeagus'tan oluşmaktadır. Üreme sisteminin temel morfolojisi ve histolojisinin bilinmesi, heteropterlerin üreme fonksiyonunun anlaşılmasında ve daha sonra yapılacak olan Hetroptera (Hemiptera) üreme davranışları, evrim ve fizyoloji araştırmalarında anahtar bilgi olarak kullanılabilir.

Anahtar sözcükler: Erkek üreme sistemi, Coreidae, Heteroptera, ışık mikroskobu, taramalı elektron mikroskobu

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Introduction

The internal morphology of reproductive systems of Heteroptera has been reviewed by Matsuda (1976). Detailed morphological descriptions of reproductive structures have been used primarily as taxonomic tools (Davis, 1955; Kumar, 1969a, 1969b, 1970; Rider & Chapin, 1991; Schuh & Slater, 1995; Ahmad & McPherson, 1998; Papacek & Soldan, 2008; Pluot-Sigwalt & Lis, 2008). The male reproductive tract of heteropteran insects typically consists of a pair of testes, a pair of vas deferens, a pair of vesicula seminalis, a complex bulbus ejaculatorius, a pair of ectodermal sacs, mesodermal (mesadenia) and ectodermal (ectadenia), accessory glands and an ejaculatory duct, which opens via the aedeagus.

Morphologically, the testes have seven testicular follicles in which spermatozoa are produced, with different lengths among genera and species (Gonçalves et al., 1987; Freitas et al., 2007, 2010). Testicular follicles open separately into the mesodermally derived sperm duct or vas deferens. This usually expands posteriorly to form a sperm storage organ or vesicula seminalis which opens into the ejaculatory duct.

In most insects, the ejaculatory duct is single, located medially and has a cuticular cover, demonstrating its ectodermic origin. Typically, tubular, paired accessory glands are formed as diverticula of the vas deferens, but sometimes the vas deferens themselves are glandular and fulfill the functions of accessory glands. The paired vas deferens unite where they lead into the ectodermally derived ejaculatory duct, the tube that transports the semen or the sperm package to the gonopore. In a few insects, particularly certain flies, the accessory glands consist of elongated glandular parts of the ejaculatory duct. The accessory gland of male insects can be classified as two types, according to mesodermal or ectodermal derivation (Matsuda, 1976; Adams, 2001). Almost all are mesodermal in origin and those that appear to be ectodermal have been poorly studied. Secretions of the male accessory glands form the spermatophore and contribute to the seminal fluid which nourishes the spermatozoa during transport to the female. These secretions are also involved in the activation of spermatozoa and may alter female behavior (Pendergrast, 1956; Davey & Krieger, 1985; Chapman, 1998; Nijhout, 1998).

The male reproductive organs of the Pentatomidae are in general, more complex than those of other families of the 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). This study is the first to describe the male reproductive morphology and histology of *Coreus marginatus* (Linnaeus, 1758), by means of light and scanning electron microscopy (SEM).

Material and Methods

Adult males of *C. marginatus* were collected in July 2010 in Kazan, Ankara, Turkey. The adults were killed with ethyl acetate fumes and dissected in 70% ethyl alcohol under a steromicroscope. For the morphological analysis, the dorsal cuticle was first removed from the prothorax to the last full-sized abdominal segment. Subsequently, the epidermis and the digestive system were removed. Two insect 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 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 10% formalin containing a phosphate buffer for 24 h; additional samples were fixed in Bouin's for 12 h. Thereafter, the tissues were washed, dehyrated in a grade series of ethanol solution (70%, 80%, 90% and 100%) and finally embedded in paraffin. Paraffin sections then were cut into 4-7 μm thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed by Olympus BX51 light microscope.

Scanning electron microscopy

For the scanning electron microscopy, samples were cleaned, dehydrated with ethyl alcohol and dried with Critical Point Drying (Polaron, CPD 7501, Critical Point Dryer); then the samples 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 scanning electron microscope at accelerating voltages of 10-15 kV. Photos were taken subsequently.

Results

Morphology

The male reproductive organs of *C. marginatus* are comprised of paired testes, a pair of vas deferens, a pair of vesicula seminalis, a complex bulbus and a ejaculatory duct, a pair of ectodermal sacs, and both mesodermal and ectodermal accessory glands (Figures 1, 2). Its testes are elongate, leaf-like, folded and orange in colour, and lie on either side of the digestive tract (Figures 1-5). The paired testes are connected to the vesicula seminalis by the vas deferens (Figures 2, 3, 6). The testes have seven sperm tubes (Figures 3, 4). The vas deferens, through which the sperm travels, are long, tubular and white. Once fully developed, the sperm travel from the testes to the vesicula seminalis via the vas deferens. The vesicula seminalis, where the sperms is stored prior to mating, are globular, white, and connects to the bulbus ejaculatorius which is balloon shaped (Figure 7). The male accessory glands (mesadenia and ectadenia) are sack-shaped and milk white in color and open into the vas deferens at the distal end of the ejaculatory duct (Figure 7).

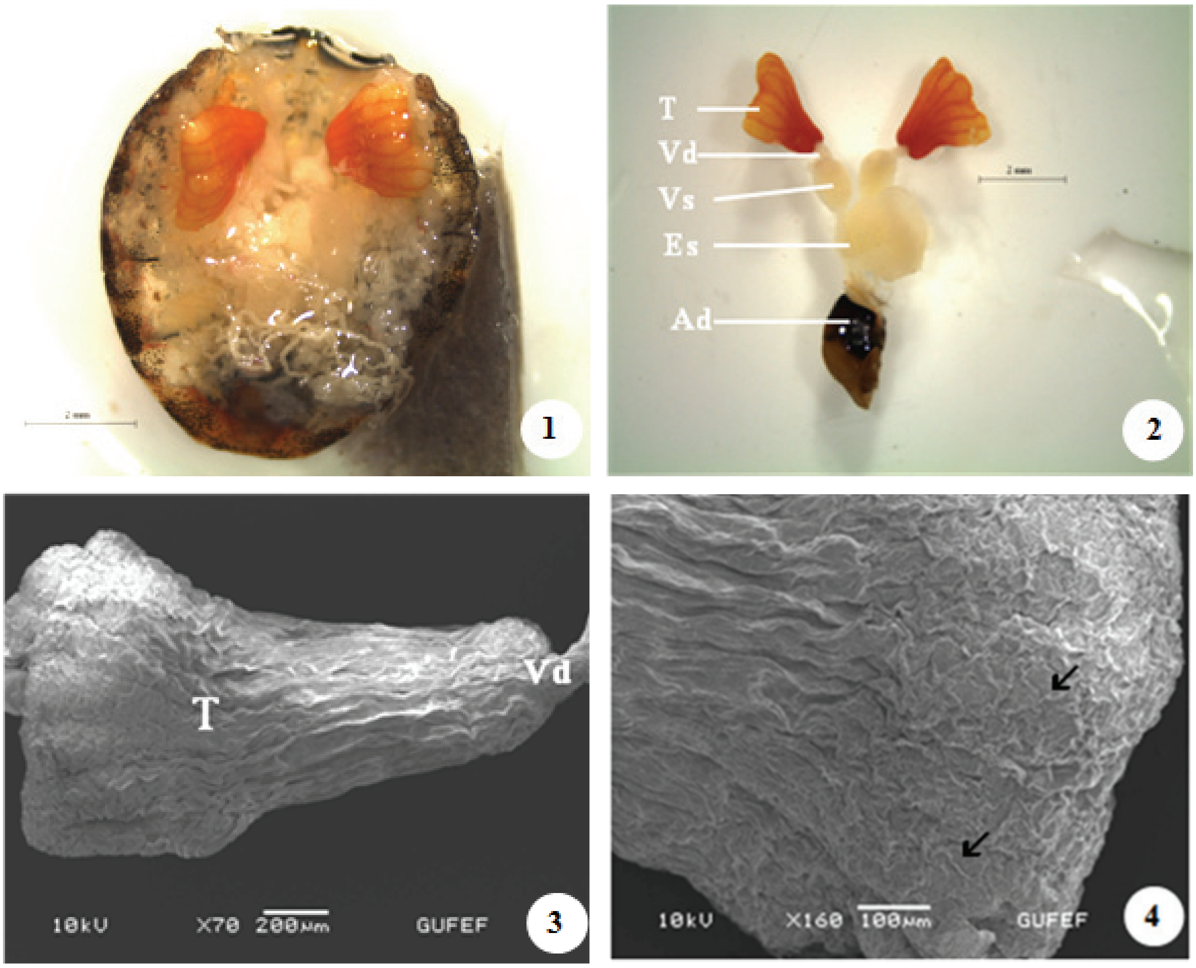

Figure 1-4. Light microscope and SEM photos of the male reproductive system of *Coreus marginatus*. 1-2. General view of the reproductive system in *C. marginatus*. Testes (T), vesicula seminalis (Vs), ectodermal sac (Es), aedeagus (Ad). 3. Vas deferens (Vd) and testes (T). 4. Sperm tubes in testes (➔).

The bulbus ejaculatorius is continuous with the ejaculatory duct which connects with the aedeagus, is single, located medially and has cuticular cover, demonstrating its ectodermic origin. The ejaculatory duct begins at the base of the accessory glands. The terminal portion of the ejaculatory duct may be sclerotized to form the intromittent organ, the aedeagus. It is differentiated from a pair of ectodermal lobes associated with the ninth abdominal segment and is often concealed within a genital chamber (Figures 7, 8).

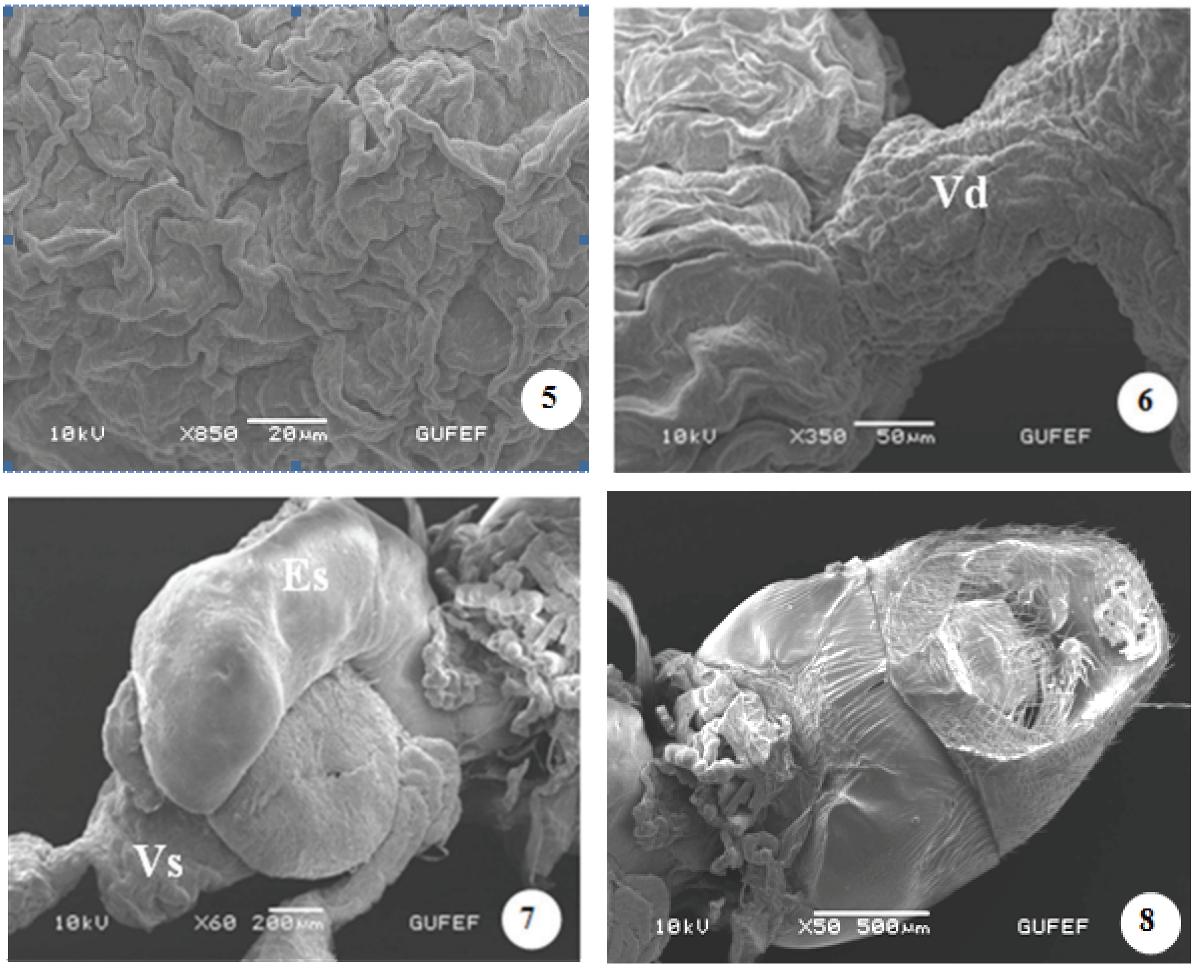

Figure 5-8. SEM photos of the male reproductive system of *Coreus marginatus*. 5. Texture surface of the testes. 6. Connection of the testes with the vesicula seminalis (Vs), the vas deferens (Vd). 7. Vesicula seminalis (Vs) and ectodermal sac (Es). 8. Aedeagus.

Histology

The testes are composed of seven sperm tubes that are surrounded by connective tissue with embedded tracheoles. The tubes are made up of a peritoneal sheath, separated from an epithelial sheath by the basal lamina. Spermatogenesis occurs within the lumen of the sperm tubes; different stages of spermatogenesis can be observed along the lengths of the sperm tubes. Within the testicular tubules of *C. marginatus* are three development zones (the growth zone, the maturation zone, the differentiation zone) (Figure 9).The growth zone is where groups of spermatogonia become separated from the germarium and form into spherical clusters. These groups of cells become enclosed by several cells which form the wall of a cyst (Figures 10, 11). Spermatogonia increase and allowing the occurrence of mitosis and differentiation into spermatocytes (Figures 12, 13).The maturation zone is where two meiotic divisions occur and these cells became spermatids (Figures 14, 15). The differentiation zone, where spermatids enlarge and change shape, thus forming spermatozoa (Figures 16-19). The spermatozoa are contained in cysts which are grouped together in bundles (Figures 18,19), which are approaching the vas deferens. Spermatozoa can be observed in the lumen of the vas deferens of virgin males (Figures 20-23). During this period, the heads of the sperm at the apex of the cyst are directed toward the germarium. The vas deferens are the ducts leading from the testes to the vesicula seminalis. These tubes have a thick epithelium. The entire structure is surrounded by the peritoneal sheath. Within the centre of this simple tube, spermatozoa are being transported to the seminal vesicles. In *C. marginatus*, the heads of the spermatozoa are embedded in the epithelial lining of the vesicula seminalis and their tails extend posteriorly in a spiral into the lumen (Figures 22, 23).

The vesicula seminalis is continuous with the bulbus ejaculatorius. The bulbus ejaculatorius is elliptical, fusiform and has a very complex construction. It has an inner and outer epithelium, with a band of striated muscle between that is continuous with that of the ejaculatory duct (Figures 24, 25). The ejaculatory duct transports sperm and secretions of the mesadenes. It is continuous with the aedeagus, is ectodermal in origin and is covered with cuticle. The ectodermal sac located near the bulbus ejaculatorius is two lobed and spherical. The male accessory glands of *C. marginatus* are multi-lobed, tubular structures that lie adjacent to the seminal vesicles (Figures 26, 27). The accessory glands are responsible for spermatophore production and produce some active peptides that stimulate ovary activation and thus decrease female receptivity following mating. Functions of these secretions are to facilitate sperm transfer, to further insemination, and to alter the behaviour of the female.

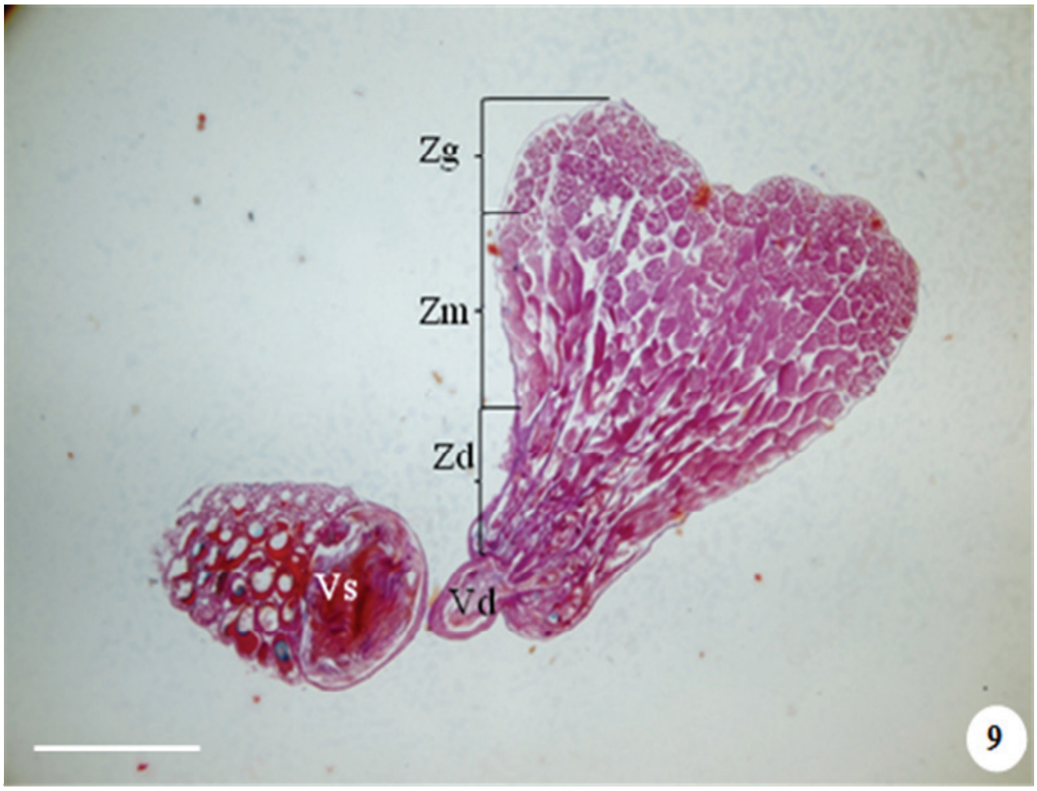

Figure 9. Longitudinal section of the testes tubules of *Coreus marginatus.* Testes in transverse section showing the areas and development stages of the spermatozoa: growth zone (Zg), maturation zone (Zm), differentiation zone (Zd), follicles (F), vas deferens (Vd), vesicula seminalis (Vs) (Bar =100 µm).

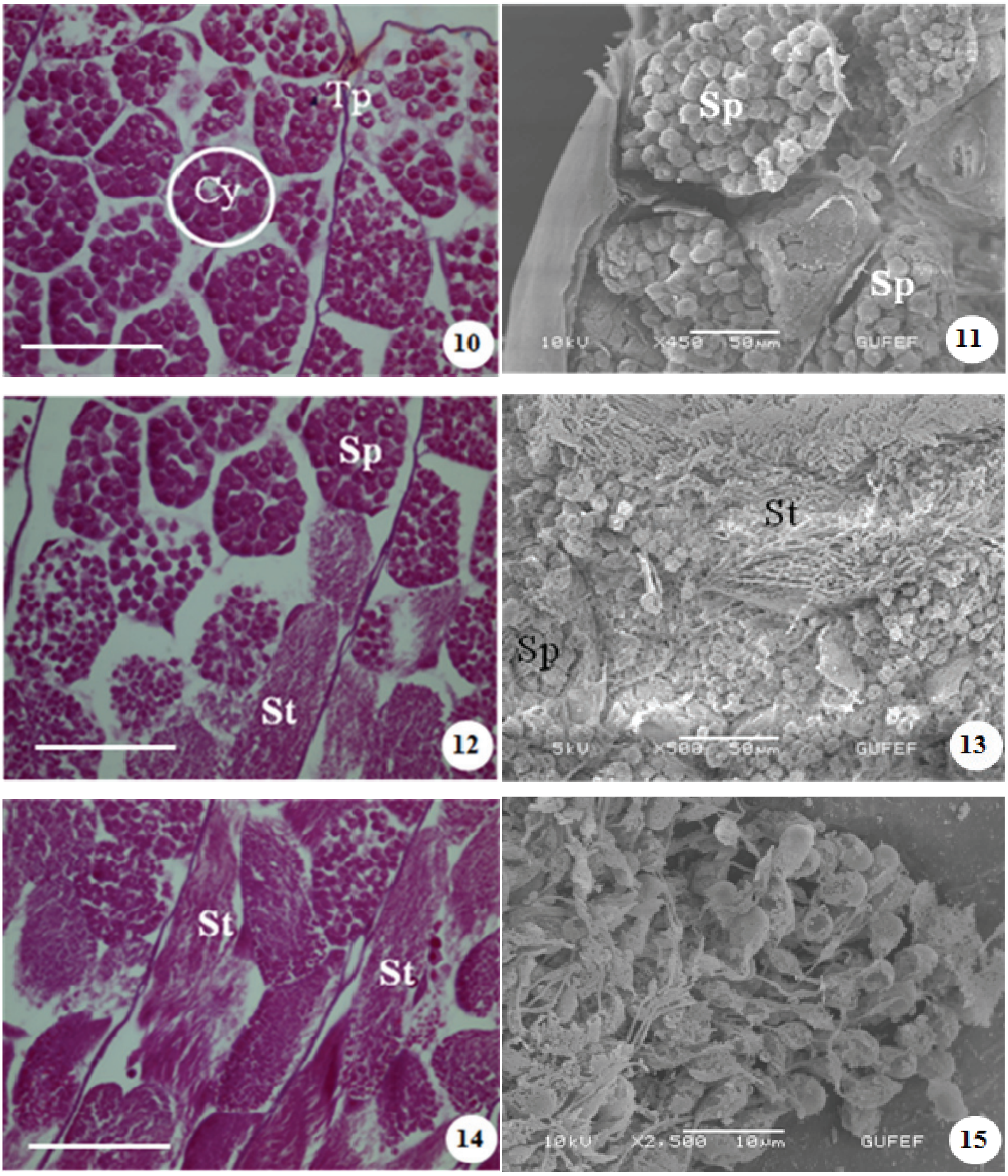

Figure 10-15. Light microscope and SEM photos illustrating the areas of growth and maturation zones in the testes of *Coreus marginatus*. 10-11. Different magnifications of testes tubes, showing the tunica propria (Tp), Germarium region showing the beginning of the differentiation of the spermatocytes into cysts (Cy). 12-13.The presence of spermatocytes (Sp) at different developmental stages and differentiated spermatids (St) (Bar= 100µm) 14-15. The presence of spermatids in the maturation zone (Bar =100 µm).

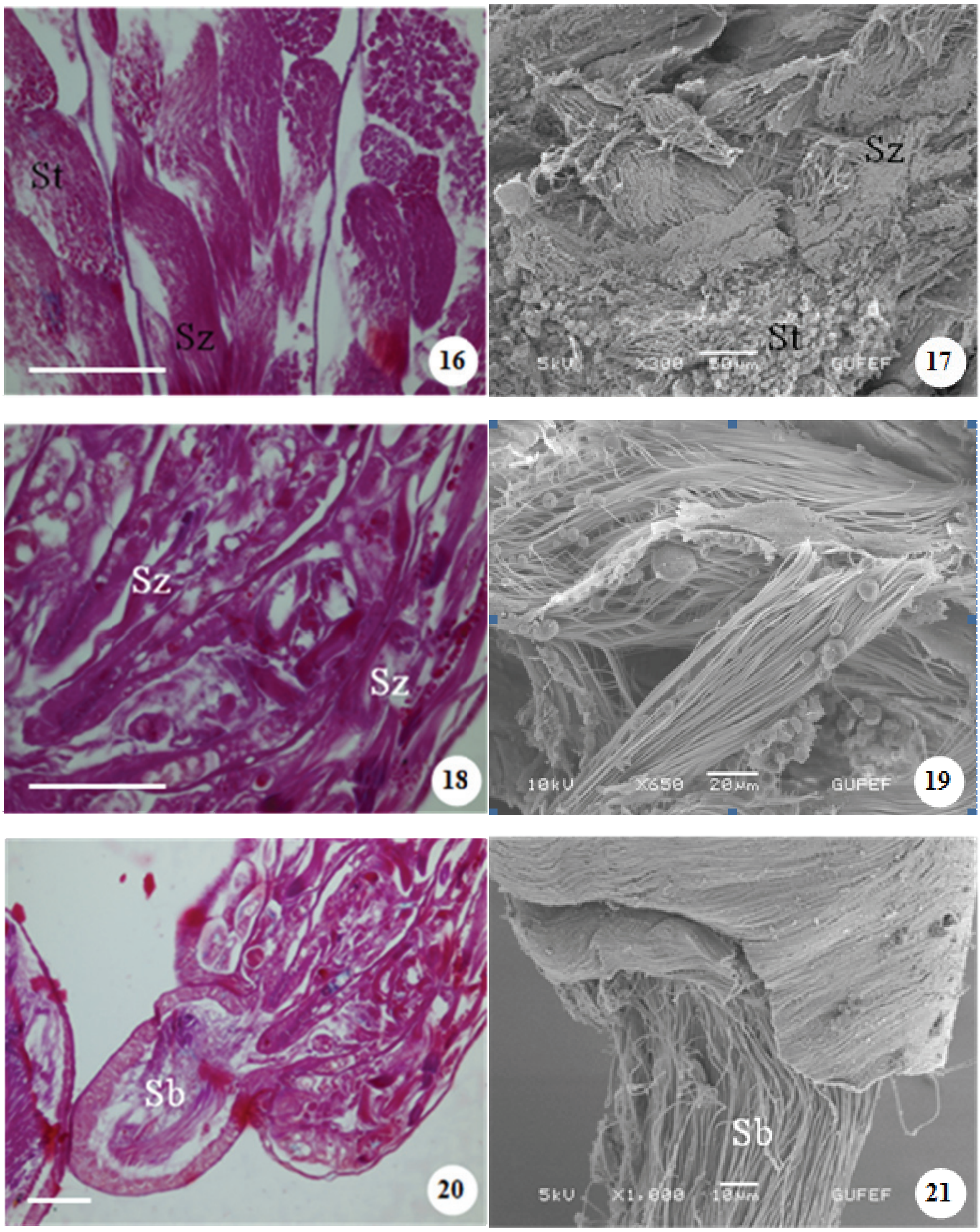

Figure 16-21. Light microscope and SEM photos showing differentiation zones in the testes of *Coreus marginatus*. 16-17. The presence of spermatids (St) at different developmental stages and differentiated spermatozoa (Sz). 18-19. Differentiation zone with large numbers of spermatozoa bundles. 20-21. General view of lumen of the vas deferens with large numbers of spermatozoa bundles (Sb) (Bar =100 µm).

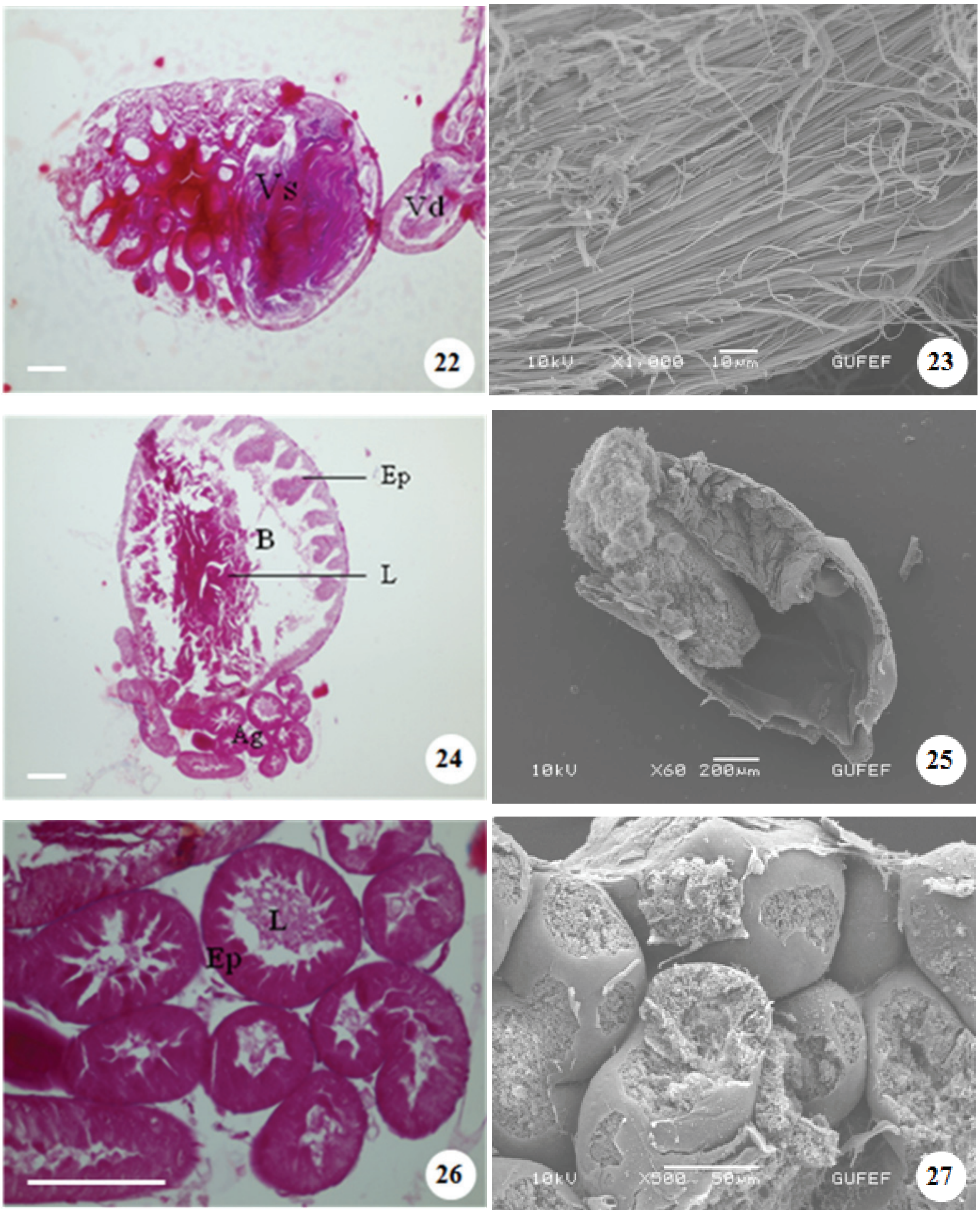

Figure 22-27. Light microscope and SEM photos showing the vas deferens, vesicula seminalis, bulbus ejaculatorius, accessory glands of *Coreus marginatus*. 22-23. Spermatozoa bundles (Sb) in vas deferens (Vd) and vesicula seminalis (Vs). 24-25. Bulbus ejaculatorius (B), epithelium (Ep), lumen (L). 26-27. Accessory glands (Ag) in *C. marginatus*, epithelium, lumen (Bar =100 µm).

Discussion

Morphological character studies of the male reproductive system have been made in several insect species, resulting in considerable progress with histological, ultrastructural and cytochemical studies of the structures that make up this organ (Forbes & Do-Van-Quy, 1965; Bairati, 1968; Louis & Kumar, 1971; Bahadur, 1975; Wheeler & Krutzsch, 1992; Ferreira et al., 2004; Mikheyev, 2004; Lemos et al., 2005; Freitas et al., 2007, 2010). The morphology of the adult male reproductive system of *C. marginatus* is similar to that observed in other Heteroptera, in being associated with the third abdominal segment, with a pair of testes, two vas deferens, two vesicula seminalis, a pair of accessory glands, a complex bulbus and an ejaculatory duct, a pair of ectodermal sacs (Bonhag & Wick, 1953; Davis, 1955; Nijhout, 1998; Chapman, 1998; Adams, 2001; Lemos et al., 2005; Rodrigues et al., 2008; Freitas et al., 2010). However, the testes of some species of heteroptera may be fused into a single median mass testes (Nijhout, 1998). The male reproductive morphology of *Nezara viridula* (Linnaeus, 1758) (Pendergrast, 1956; Ramamurty, 1969), *Chrysocoris stolli* Wolf (Singh, 1968), *Perillus bioculatus* (F.) (Adams, 2001), *Oebalus poecilus* (Dallas, 1851), (Santos et al., 2003), *Podisus nigrispinus* (Dallas) (Lemos et al., 2005) and (Rodrigues et al., 2008), *Triatoma brasiliensis* Neiva, 1911 and *T. melanica* Neiva & Lent, 1941 (Freitas et al., 2010), have been the subject of detailed studies, with an emphasis function.

The sperm tubes in the testes of *C. marginatus* are held together by connective tissue, which has an orange pigmentation, are elongate, usually located near the back of the abdomen; the vas deferens and vesicula seminalis are cylindirical and white; and the testes are paired, while the testes, vas deferens and vesicula seminalis in a closely related species, *Oebalus poecilus* (Hemiptera: Pentatomidae), are long and orange (Santos et al., 2003). The number of testicular follicles in the testes of *C. marginatus* is seven and is different from those found in other Heteroptera (Pendergrast, 1956; Adams, 2001; Lemos et al., 2005; Freitas et al., 2010). Like other Heteroptera, each follicle of *C. marginatus* has a germarium in the apical region. The testicular follicles are lined with a layer of cells (peritoneal sheath), an epithelial sheath, and a non-cellular layer (tunica propria) (Davis, 1955; Chapman, 1998; Pires et al., 2007; Rodrigues et al., 2008).

The testicular tubules of *C. marginatus* have three development zones. The growth zone is where groups of spermatogonia become separated from the germarium and form into spherical clusters. Spermatogonia numbers increase through mitosis and then differentiate into spermatocytes. The maturation zone is where two meiotic divisions occur and these cells became spermatids. In the differentiation zone, spermatids transform into spermatozoa. Mechanisms of spermatogenesis, including sperm formation, are similar to those found in other Coreidae (Bowen, 1922; Davis, 1955; Engelmann, 1970; Chapman, 1998; Pires et al., 2007; Rodrigues et al., 2008). The number of spermatids/spermatozoa per cyst, which is determined by the number of cell divisions, is constant for each species, but may vary from species to species. Thus, this number has been used as additional information in the systematics of Hymenoptera (Zama et al., 2007; Lino-Neto et al., 2008).

Once fully developed, the sperm travel from the testes to the vesicula seminalis via the vas deferens. The epithelial layer of the vas deferens is relatively thick in *C. marginatus*. As in many other Coreidae, the heads of the spermatozoa are embedded in the epithelial lining of the vesicula seminalis and their tails extend posteriorly in a spiral into the lumen (Davis, 1955).

The accessory glands of male insects can be classified into two types, according to their mesodermal or ectodermal derivation. If the acessory glands have a mesodermal origin they are termed Mesadenia and open into the vas deferens. Those of ectodermal origin are known as ectadenia and open into the ejaculatory duct. In *Nezara viridula* (Heteroptera: Pentatomidae), as in other Pentatomidae where the reproductive system has been described, including *C. marginatus*, both ectadenia and mesadenia are present (Pendergrast, 1956; Chapman, 1998). However, in the Acanthosominae, usually regarded as a sub-family of the Pentatomidae, the ectadenia is missing. Consequently, we believe as does Leston that the Acanthosominae should be removed from the Pentatomidae and accorded family status (Leston, 1953). Among male insects, the accessory glands vary notably in size, shape, number and embryological origin (Adiyodi & Adiyodi, 1975; Leopold, 1976; Grasse, 1982; Chapman, 1998). Among the functions of the secretions of these glands is a contribution to the seminal fluid and activation of the spermatozoa (Chen, 1984; Davey & Krieger, 1985). The accessory glands in heteropterans are responsible for spermatophore production and produce some active peptides that stimulate ovary activation after mating and decrease female receptivity for mating. The functions of these secretions are to facilitate sperm transfer, to further insemination, and to alter the behaviour of the female (Pendergrast, 1956; Leopold, 1976; Adams, 2001; Kubli, 2003; Freitas et al., 2007, 2010).

As with other Coreidae, the ejaculatory duct of *C. marginatus* is single, located medially and has a cuticular cover demonstrating its ectodermic origin. It begins at the base of the accessory glands and later 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). The ejaculatory duct of *Oncopeltus* (Heteroptera) is also extremely complex, being specialized for the erection of the penis (Chapman, 1998).

In conclusion, the present study provides fundamental information on the male reproductive morphology and histology of *C. marginatus*. These findings will be important for future work on the diversity of reproductive morphology within the Heteroptera.

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