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Detailed Morphological Study of the Male Reproductive Organ in *Mylabris cernyi* Pan and Bologna, 2014 (Coleoptera: Meloidae)

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ABSTRACT

This study provides a detailed analysis of the male reproductive system of *Mylabris cernyi*, contributing to a broader understanding of the morphology of the blister beetle. The male reproductive system of *M. cernyi* consists of an aedeagus, 2 seminal vesicles, 2 vasa deferentia, an ejaculatory duct, 3 pairs of accessory glands, and 2 testes. We examined the structure of these organs using optical and electron microscopy. The testes contain 14–15 radially arranged follicles, with distinct maturation, growth, and differentiation zones progressing from the periphery to the center. The testes give rise to the vas efferens, which subsequently join to form the seminal vesicle and vas deferens. The lumens of the vesicles contain mature sperm masses. There are 3 pairs of accessory glands (Ag1, Ag2, and Ag3). Ag1 is a short, tubular, light yellow gland with columnar epithelium and a muscular layer that secretes basophilic materials. Ag2 is long, coiled, and whitish, containing both basophilic and fibrous secretions, with a squamous epithelial layer. Ag3 is a long, coiled gland that produces eosinophilic chemicals. These glands open into the ejaculatory duct, which is lined by an epithelium, thick muscle layer, and intima. The inner surface of the intima is covered with spines. The aedeagus is the external opening of the ejaculatory duct. This study represents the first morphological description of the male reproductive system of *M. cernyi* and provides valuable insights for future research in this field.

Keywords: Spermatozoa, Testicular follicles, Accessory glands, Light microscopy, Electron microscopy

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Introduction

The Meloidae family, also known as blister or oil beetles, includes 177 species in Turkey and over 3000 species globally. These insects are known to cause significant economic losses by feeding on the leaves and shoots of various crops, including vegetables and fruits [1-3]. Extensive research has been conducted on the taxonomy, biology, systematics, and fauna of Meloidae, attracting considerable scientific interest [2]. Despite this, there have been relatively few studies focused on the reproductive systems of these beetles [4].

In this study, we employed light microscopy (LM) and scanning electron microscopy (SEM) to explore the male reproductive system of *M. cernyi*, a species within the Meloidae family. The male reproductive system of Meloidae typically includes 1 pair of testes, 1 pair of vasa deferentia, 3 pairs of accessory glands, an ejaculatory duct, and 1 aedeagus [4]. The testes contain numerous follicles, with the number varying across species, where sperm maturation occurs. The accessory glands play a crucial role in forming the spermatophore, facilitating sperm transport, nourishment, and secure delivery to the female. Key characteristics such as the color of the testicular peritoneal sheath, the shape of the testes, the number of testicular follicles, and the shape and number of accessory

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glands vary among species [4-9]. This study aims to provide a detailed examination of the male reproductive system of *M. cernyi* to enhance our understanding of blister beetles in general.

Materials and Methods

In July 2019, *M. cernyi* specimens (n = 20) were taken from Keklikdere, Bingöl, Turkey. The adult beetles were first sedated using ethyl acetate vapor before their internal organs were carefully dissected under a stereo microscope (SM). After dissection, the organs were transferred to formalin for preservation, and some were embedded in paraffin for further examination. For light microscopy (LM) analysis, tissue sections measuring 5–6 µm in thickness were prepared and stained with hematoxylin and eosin (H&E). Other organs were treated with Hexamethyldisilazane (HMDS) and air-dried. These dried specimens were then analyzed using a scanning electron microscope (JEOL JSM 6060 LV).

Results and Discussion

The male reproductive system of *M. cernyi* consists of several key structures: a pair of testes (Te), vasa deferentia, seminal vesicles (Sv), three pairs of accessory glands (Ag1, Ag2, Ag3), an ejaculatory duct (Ed), and an aedeagus (Ae), which is consistent with other blister beetle species [4].

Testis

The testis of *M. cernyi* is characterized by a globular shape and a light yellow color **(Figure 1a)**. Some areas of the testis surface contain tracheal structures (Tr) **(Figure 1c)**. The testis is spherical **(Figures 1a-1c)** and contains testicular follicles (Tf) arranged radially. The size and the number of these follicles can vary between species. For *M. cernyi*, the testis is approximately 12 mm in size and contains between 14 and 15 follicles **(Figure 1d)**. In comparison, the testis of Meloe proscarabaeus contains 100 to 120 follicles per organ [4].

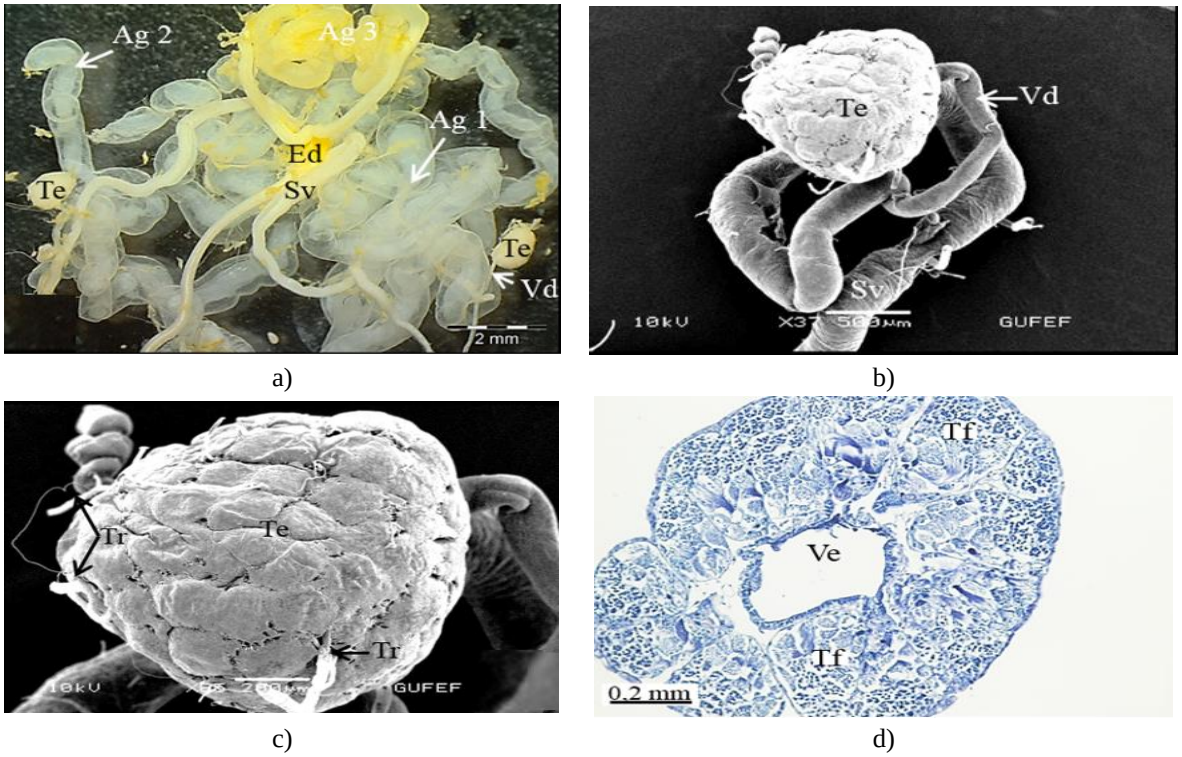


Figure 1. a) the male reproductive system of *M. cernyi* viewed under a stereo microscope (SM); b) the testis, vas deferens, and seminal vesicle observed under scanning electron microscopy (SEM); c) SEM image showing the testis with its globular-like structure; and d) cross-sectional view of the testis under light microscopy (LM).

In the testicular follicles of *M. cernyi*, three distinct regions—growth, maturation, and differentiation—can be identified, progressing from the outer edge to the center of the testis, similar to observations in *Chrysolina herbacea* (Duftschmid, 1825) and *Chrysomela populi* Linnaeus, 1758 (Chrysomelidae) [8, 9]. Both light and scanning electron microscopy images of the testis reveal that it is lined by a single layer of epithelial cells (Ep) and surrounded by a peritoneal sheath (Ps).

At the peripheral area of the testis, the growth zone can be recognized, where spermatocytes (Sg) are found within cysts (Cy). The nuclei of these spermatocytes exhibit basophilic staining, with the nucleus covering almost the entire sperm head **(Figure 2c)**.

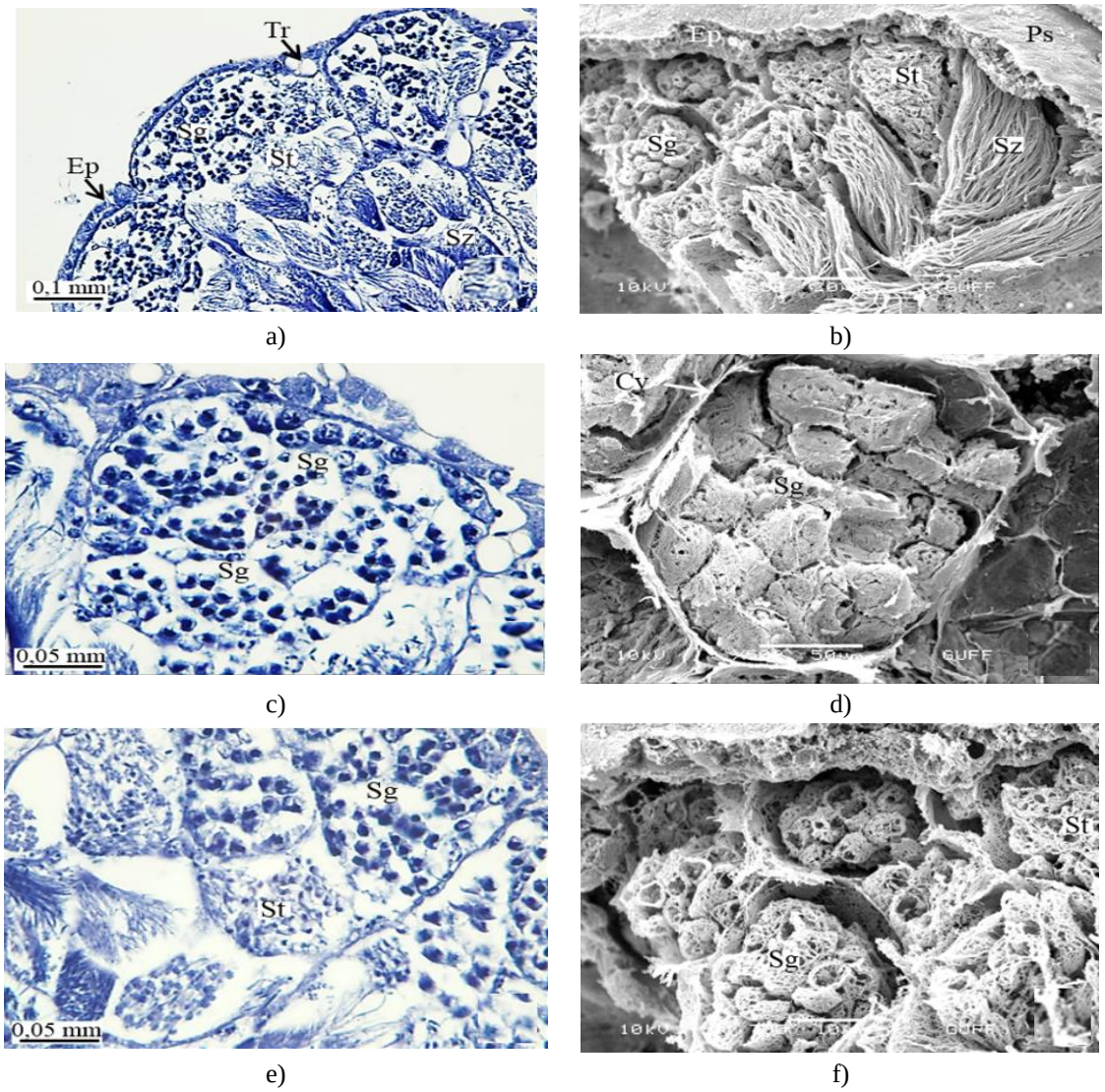


Figure 2. a, b) light and scanning electron microscope images showing spermatocytes, spermatids, and spermatozoa within the testicular follicles; c, d) light and scanning electron microscope images of spermatocytes within cysts in the growth zone; and e, f) light and scanning electron microscope images of the differentiation of spermatocytes into spermatids in the maturation zone.

In the maturation region of the testis, spermatocytes undergo meiosis, resulting in a complete transformation into spermatids (St) **(Figures 2e and 2f)**. During this stage, both the head and the flagellum (Fl) of the spermatids become distinct, with the flagellum consisting of long, slender filaments, though their arrangement lacks a clear pattern **(Figures 3a and 3b)**.

As the testis approaches the vas deferens, spermatids begin to differentiate into spermatozoa (Sz) within the differentiation zone **(Figures 3a and 3b)**. The spermatozoa are found in well-organized bundles **(Figures 3a-3f)**.

Examination under both light and scanning electron microscopy reveals that the sperm heads (Hd) are thin and have a hooked shape **(Figures 3e and 3f)**.

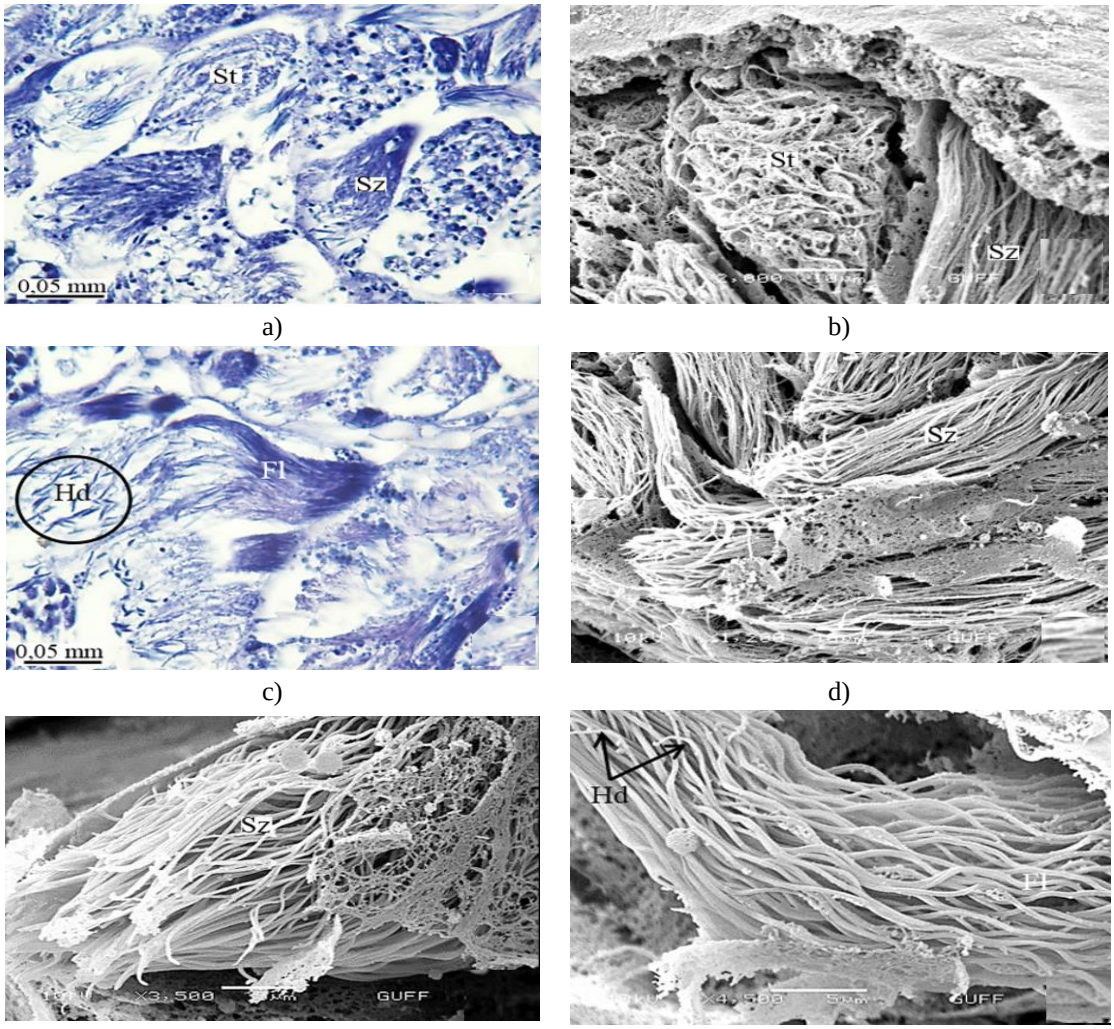
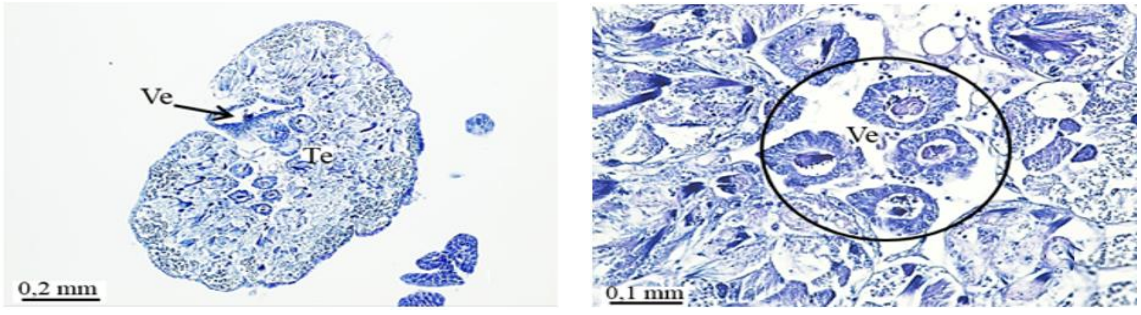


Figure 3. a, b) LM and SEM photographs of the transformation of spermatids into spermatozoa in the differentiation zone (LM, SEM); and c-f) The regular spermatozoa bundles (LM, SEM).

The Vas Efferens, Vas Deferens, and Seminal Vesicle

The testis gives rise to the vas efferens (Ve) **(Figures 4a and 4b)**, which eventually merge to form the vasa deferentia. In both LM and SEM images, mature sperm (Sp) masses are visible within the lumen (Lu) of the vas efferens **(Figures 4b-4d)**. The wall of the vas efferens is lined with a single layer of epithelial cells, which contain round, basophilic nuclei (Nu) **(Figure 4c)**.



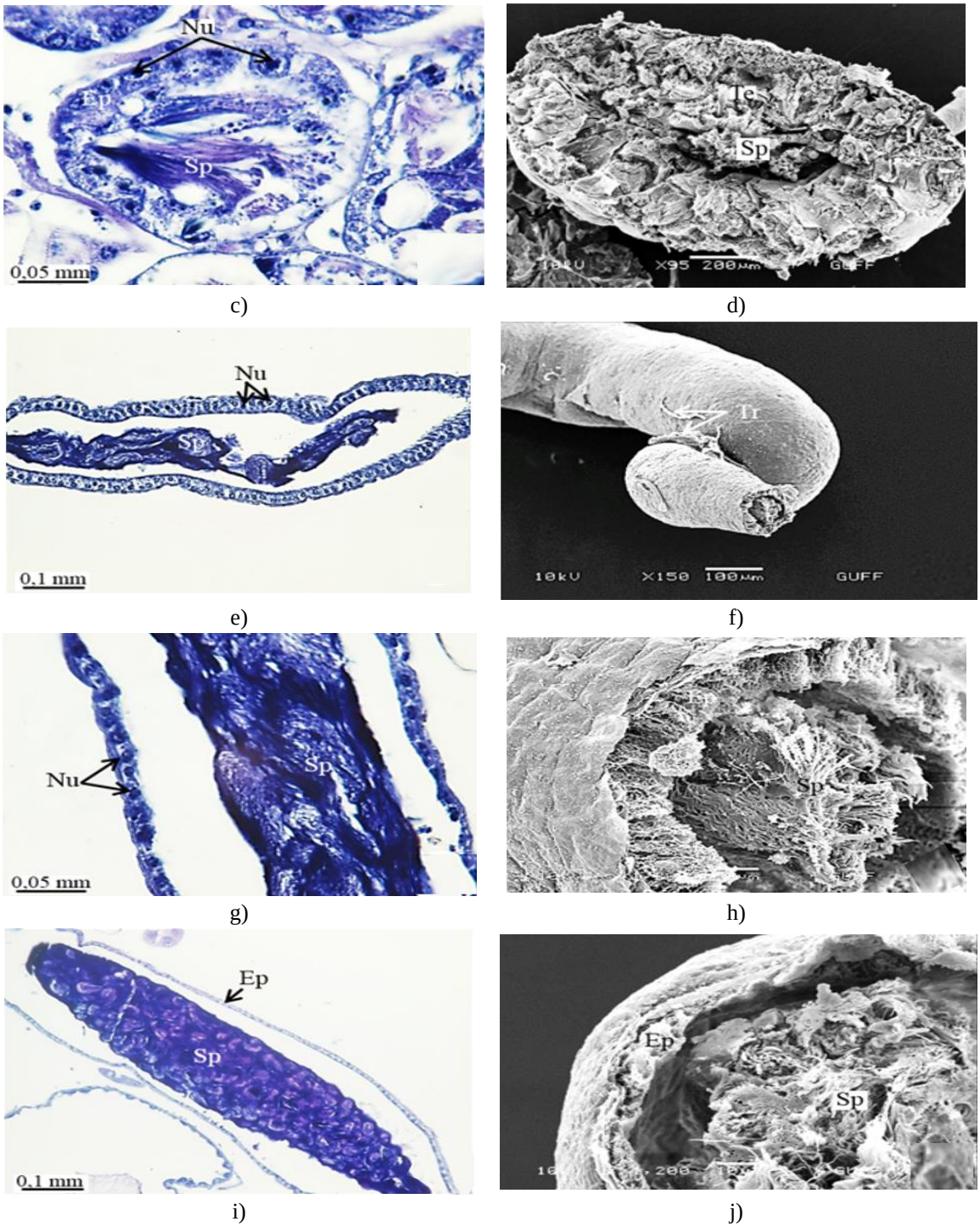


Figure 4. a, b) histological images showing the testis and vas efferens; c, d) light and scanning electron microscope images of the vas efferens; e-h) light and scanning electron microscope images of the vas deferens; and i, j) light and scanning electron microscope images of the seminal vesicle.

In *M. cernyi*, similar to species such as *C. herbacea* and *C. populi* (Chrysomelidae), the vas efferens are responsible for transporting mature sperm from the testes to the vas deferens [8, 9]. Close to the testes, the vas deferens remain narrow, and the seminal vesicle appears similarly constricted **(Figure 1b)**. Both the vas deferens and the seminal vesicle are encased by a simple cuboidal epithelium **(Figures 4e-4j)**, a feature that is also present in *Pimelia subglobosa* (Pallas, 1781) (Tenebrionidae) [10]. However, in *Capnodis tenebrionis* (Linnaeus, 1761) (Buprestidae), the wall of the vas deferens is lined with simple cylindrical epithelium [5].

In *M. cernyi*, the nuclei of epithelial cells in the seminal vesicle and vas deferens are round and centrally located **(Figures 4g and 4i)**. Both structures' lumens contain mature sperm arranged in bundles **(Figures 4e-4j)**, a

characteristic shared with *C. herbacea* (Chrysomelidae), *C. populi* (Chrysomelidae), and *C. tenebrionis* (Buprestidae) [5, 8, 9]. The vas deferens connect to the ejaculatory duct via the seminal vesicle.

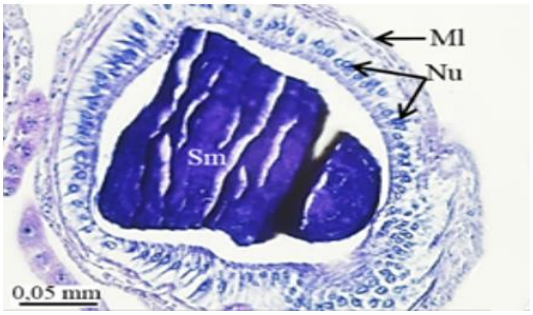
Accessory glands

M. cernyi has 3 pairs of accessory glands **(Figure 1a)**, a characteristic shared with *M. proscarabaeus* (Meloidae) [4]. The number and structure of these glands vary across species. For instance, *C. herbacea* (Chrysomelidae) contains 2 tubular accessory glands [8], while *Tanymecus dilaticollis* (Gyllenhal, 1834) (Curculionidae) has 2 blind-ended accessory glands and two multilobed prostate glands [7].

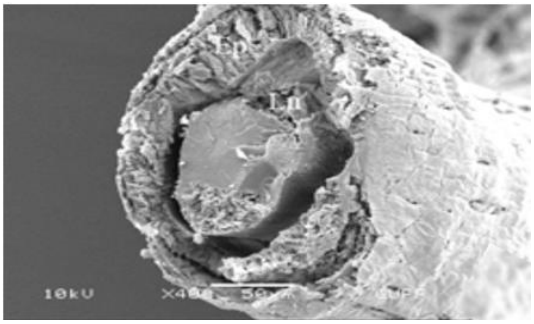
These glands empty into the ejaculatory duct **(Figure 1a)**. The 1st pair in *M. cernyi* is short, tubular, and yellowish **(Figure 1a)**. The glands consist of columnar epithelial cells, surrounded by a muscle layer (Ml) **(Figures 5a-5d)**. Their lumen contains basophilic secretions **(Figures 5a and 5c)**.

The 2nd pair of glands are long, coiled, and tubular, with a pale white appearance **(Figure 1a)**. Their lumen is filled with basophilic and fibrous secretions (Sm) **(Figures 5e and 5f)**. These glands are surrounded by a single layer of squamous epithelium **(Figures 5e, 5f, and 5h)**. SEM images show tracheal structures on parts of their surface **(Figure 5g)**.

The third pair of accessory glands in *M. cernyi* also have a coiled, long, and tubular structure **(Figures 1a and 5l)**, with their lumen containing eosinophilic secretions **(Figures 5i and 5j)**. Tracheal structures are visible on their surface in SEM images **(Figure 5k)**. The gland walls are composed of pseudostratified epithelium with oval-shaped nuclei, surrounded by muscle tissue. The epithelium is recessed into the lumen **(Figures 5i, 5j, and 5l)**.



a)



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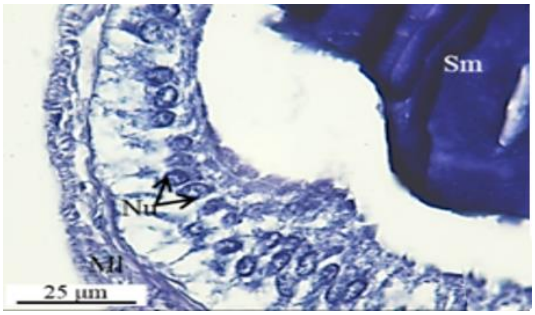

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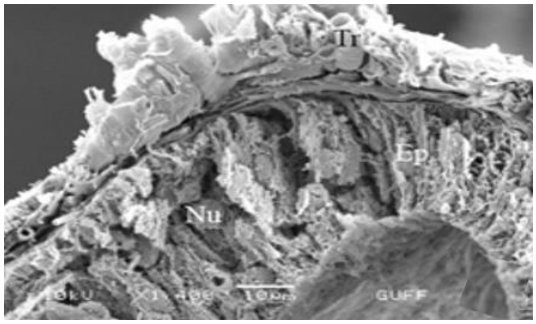
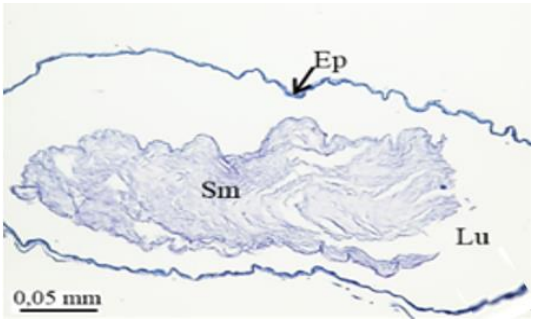
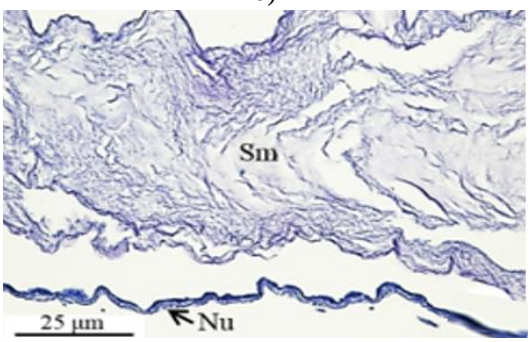


d)



e)



f)

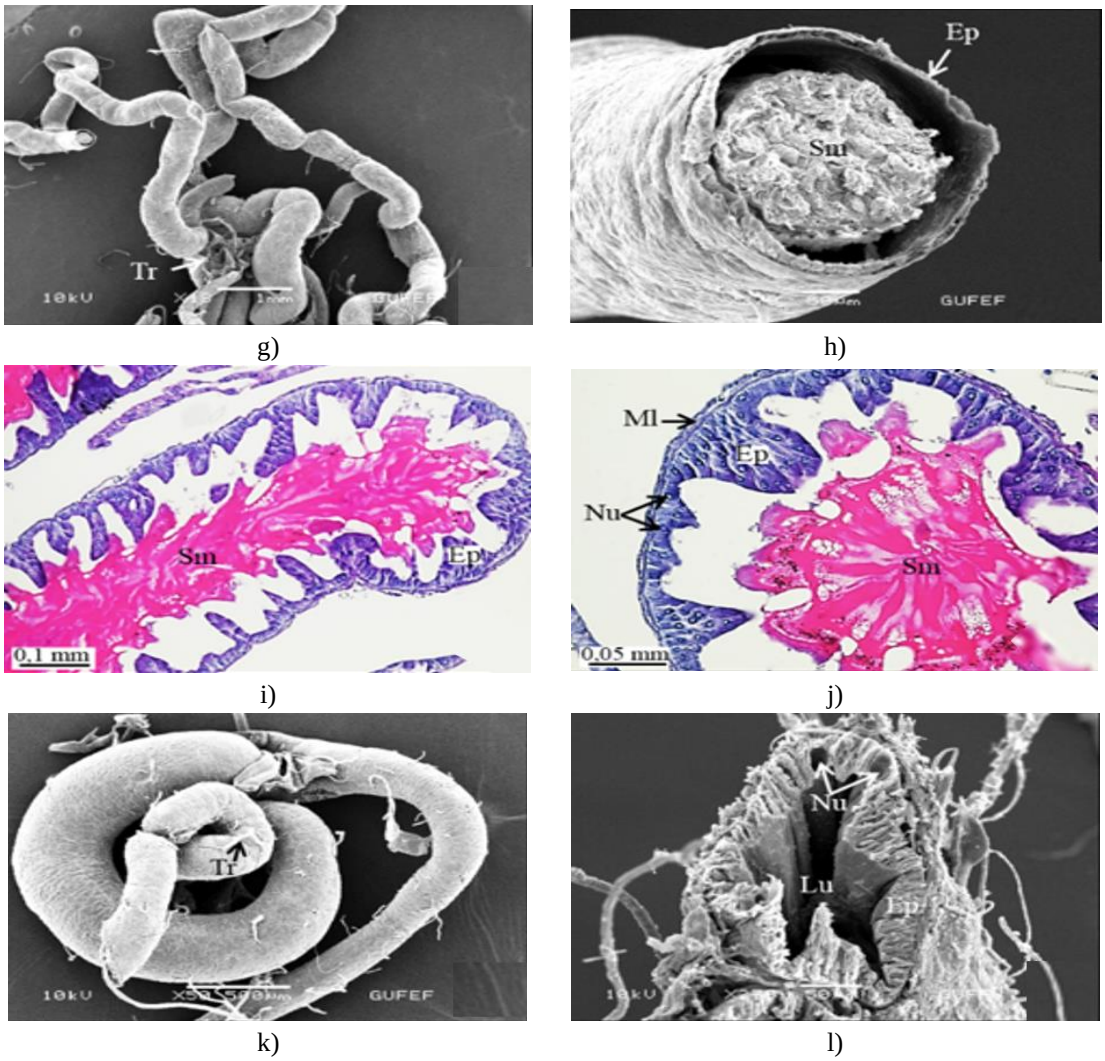


Figure 5. a-d) light microscopy (LM) and scanning electron microscopy (SEM) images of a cross-section of the first pair of accessory glands at varying magnifications; e, f) longitudinal sections of the second pair of accessory glands; g) SEM images of the second pair of accessory glands; h) SEM images of the cross-section of the second pair of accessory glands; i, j) longitudinal and cross-sectional views of the third pair of accessory glands (LM); k) SEM image of the third pair of accessory glands; and l) SEM image of a cross-section of the third pair of accessory glands.

The ejaculatory duct

The ejaculatory duct's wall consists of several layers: the intima (In), epithelium, and a substantial muscle layer, a structure common in other species [5-7, 10]. The epithelium forms deep recesses that extend toward the duct's lumen. On the side of the lumen, facing the intima layer, spines (Spi) are present. Histological examinations reveal the presence of multinucleated striated muscle bundles (Mb) located between the ejaculatory duct and the aedeagus **(Figures 6 and 7)**. The ejaculatory duct merges with the aedeagus structure. SEM imagery reveals that the aedeagus surface is flat **(Figure 7d)**.

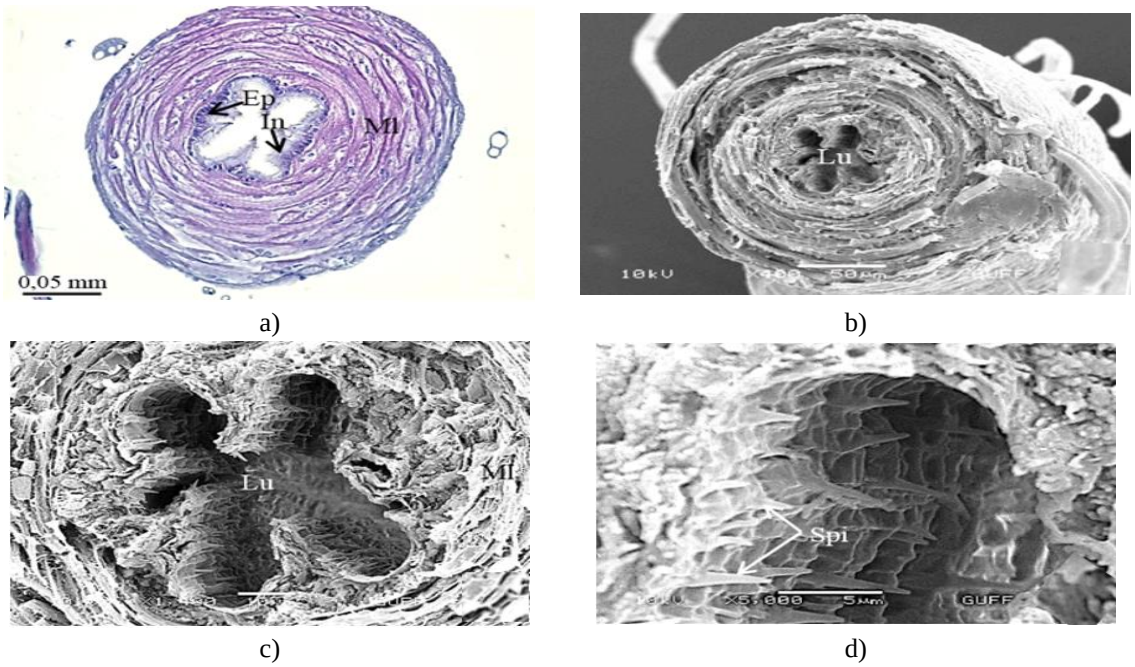


Figure 6. a, b) light microscopy (LM) and scanning electron microscopy (SEM) images of a cross-section of the ejaculatory duct; and c, d) SEM images showing spines extending from the intima of the ejaculatory duct.

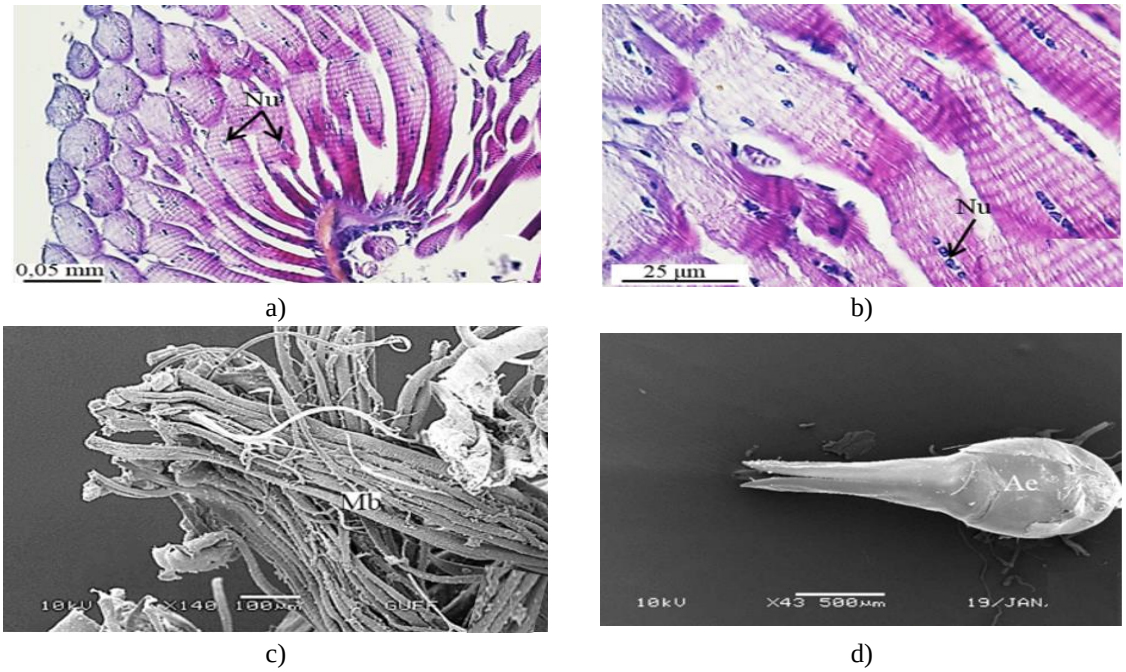


Figure 7. a, b) histological sections of striated muscle between the ejaculatory duct and aedeagus (LM); c) SEM image of muscle bundles between the ejaculatory duct and aedeagus; and d) SEM image of the aedeagus.

Conclusion

This study provides a detailed description of the male reproductive system morphology in *M. cernyi*. It contributes to enhancing our findings of the reproductive organs within the Coleoptera order, particularly in the Meloidae family. Furthermore, variations in testis color, shape, follicle count, and the number and shape of accessory glands across species are highlighted, making this research valuable for advancing systematic studies.

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Conflict of Interest: None

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Ethics Statement: None

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