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RESEARCH

# Light and scanning electron microscopies characterizations on the male reproductive system of adult *Agapanthia kirbyi* (Gyllenhal, 1817) (Coleoptera: Cerambycidae)

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**Abstract**

*Agapanthia kirbyi* has potential as a biological control agent against certain weed species. In such studies, understanding reproductive anatomy is essential; however, no previous research exists on the reproductive system of *A. kirbyi*. This study investigated the male reproductive morphology using light and electron microscopy. The male reproductive system comprises a pair of testes, vas deferentia, seminal vesicles, accessory glands, and an aedeagus. Each testis includes two yellowish, oval lobes, each containing 40 follicles where spermatogenesis progresses from the periphery toward the center. The lobes connect via the vas efferentia to the vas deferens and then to the seminal vesicle. Spermatogenesis occurs in three zones: growth, maturation, and differentiation. All stages from spermatogonia to spermatozoa were observed. Spermatozoa are arranged in bundles with spindle-shaped heads and long flagella. The accessory glands, located at the end of the seminal vesicle and opening into the ejaculatory duct, are tubular and wavy, lined by a single layer of granular columnar epithelium. The ejaculatory duct is composed of cuboidal epithelial cells with chromatin-dense, oval nuclei and is surrounded by a muscle layer. These findings provide crucial baseline data for understanding *A. kirbyi*'s reproductive biology and support future studies in biological control and taxonomy.

**Keywords** Spermatozoa · Testis follicle · Accessory glands · Light microscopy · Scanning electron microscopy

## Introduction

The family Cerambycidae, belonging to the Coleoptera, is distinguished by its members' notably elongated antennae, often exceeding body length. This family is commonly referred to by various vernacular names, including capricorns, roundheaded borers, timber beetles, and goat beetles (Halperin and Holzschuh 1993; Kariyanna et al. 2017). Comprising approximately 36,000 described species, Cerambycidae is globally distributed, except Antarctica (Gillott 2005; Haack 2017). Within Türkiye, 21 species of the genus *Agapanthia* have been documented, of which eight are endemic to the region (Danilevsky 2020). Among these, *Agapanthia kirbyi* stands out as a more frequently encountered species (Miroğlu et al. 2019).

The primary host plants of *A. kirbyi* are species of *Verbascum* (Halperin and Holzschuh 1993). Additionally, *A. kirbyi* feeds on the stems of particular weed species (such as *Centaurea iberica*, *C. solstitialis*, *Silybum marianum*, *Onopordum*, and *Carduus* species), causing the plants to dry out and die before producing seeds. For this reason, *A. kirbyi* has been considered of interest for weed suppression. Therefore, understanding the morphology of the male reproductive system of this species contributes to a better understanding of its reproductive biology, which may provide a useful basis for future studies and potential applications in various research and management contexts (Miroğlu et al. 2019).

In Cerambycidae, the male reproductive system consists of a pair of testes, vas deferentia, seminal vesicles, an ejaculatory

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duct, accessory glands, and an aedeagus. The shape, color, and number of these parts vary among species. Different stages of sperm development (spermatogonia, spermatocytes, spermatids, spermatozoa) can be observed in the testes, with the development direction varying by species. Mature sperm pass into the vas deferens and, together with secretions from the accessory glands, are transferred through the ejaculatory duct and aedeagus to the female (Gaikwad and Patil 2017; Özyurt Koçakoğlu et al. 2023).

Studies on the morphology of the male reproductive system in the Cerambycidae family are quite limited (Gaikwad and Patil 2017; Özyurt Koçakoğlu et al. 2023), and despite its biological importance, there are no studies on this subject for the genus *Agapanthia*. Therefore, in this study, the male reproductive system of *A. kirbyi*, which could be a potential biological control agent, was examined anatomically and histologically using stereo, light, and electron microscopy.

## Materials and methods

### Insect and stereomicroscope (SM)

Adults of *Agapanthia kirbyi* were collected from Üçbaş locality (40.427900°N, 32.668888°E; altitude: 1140 m), Kızılcahamam district, Ankara, Türkiye, in June 2025 (Fig. 1). The collected specimens were stored in plastic containers at ambient temperature. Adult individuals were anesthetised with ethyl acetate, and organs were examined using an Olympus SZX7 stereo microscope.

## Light microscope (LM)

The 5 adult male organs were initially excised and preserved in 10% neutral buffered formalin for 24 h for histological analysis. After fixation, the organs were thoroughly washed under running tap water and dehydrated through increasing ethanol concentrations ranging from 50% to 99%. The specimens were then cleared in xylene with two successive 15-minute immersions. After the clearing step, the tissues were progressively infiltrated with molten paraffin by transitioning from xylene. Embedding was carried out at 65 °C, and the paraffin blocks were left to harden at ambient temperature. Thin sections of approximately 5–6 µm were cut using a Microm HM 310 rotary microtome. These sections were stained with hematoxylin and eosin (H&E) to allow for histological examination. Microscopic images were taken using an Olympus BX51 light microscope.

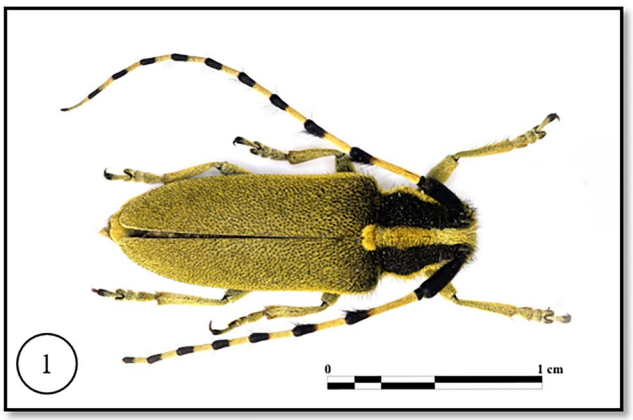



**Fig. 1** The general view of the adult *Agapanthia kirbyi* male

## Scanning electron microscope (SEM)

The 5 adult male organs were carefully dissected in sodium phosphate buffer (pH 7.2) and then fixed in 2.5% glutaraldehyde prepared using the same buffer solution. After fixation, the samples were washed three times with sodium phosphate buffer (pH 7.2) to remove residual fixative. Dehydration was performed using a graded ethanol series ranging from 70% to 99%. Following dehydration, the samples were treated with hexamethyldisilazane (HMDS) and allowed to air-dry. After drying, the samples were mounted on SEM stubs, coated with a thin layer of gold, and analyzed under a JEOL JSM 6060 LV scanning electron microscope at the Gazi University, Faculty of Science for detailed imaging and surface examination.

## Result

The male reproductive system of *A. kirbyi* consists of a pair of testes, vas deferentia, seminal vesicles, accessory glands, and an aedeagus (Fig. 2). Each light yellow testis comprises a pair of oval-shaped testis lobes (width: 1.25 mm; length: 1.56 mm). Each lobe connects to a thin, long, whitish vas efferens, opening into the vas deferens—a thicker (approximately three times the diameter of the vas efferens), yellowish duct. The vas deferens is highly convoluted, and its distal end expands to form the seminal vesicle (Fig. 2).

Immediately below the seminal vesicles lies the accessory glands, which are whitish in color and displays a wavy, tubular structure (Fig. 2). In Fig. 3a, tracheal networks can be observed on the sheath surface of the testis lobe. Each testis lobe contains 40 testicular follicles, and spermatogenesis progresses from the periphery toward the center. Figure 3b shows a longitudinal section of the testis follicles

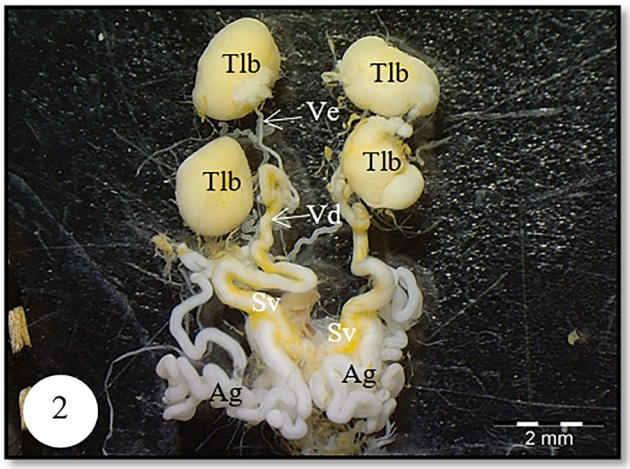



**Fig. 2** General view of the male reproductive system in *A. kirbyi* (SM).(Ag-accessory glands, Sv-seminal vesicles, Tlb-testis lobe, Vd-vas deferens, Ve-vas efferens)

within the lobe. In Fig. 4a, the different developmental zones of sperm growth, maturation, and differentiation zones are visible, along with the respective stages: spermatogonia, spermatocytes, spermatids, and spermatozoa.

In the growth zone near the periphery of the testis lobe, spermatogonia undergo mitotic divisions and differentiate into spermatocytes (Figs. 4a, b). The nucleus of the spermatogonium is densely heterochromatic. In contrast, the spermatocyte nucleus is smaller and basophilic (Fig. 4a). In the maturation zone, the spermatocytes undergo meiotic divisions and form spermatids s with distinct morphological changes—spherical heads with basophilic nuclei and thread-like flagella (Fig. 5a). In the differentiation zone, located toward the middle of the testis lobe, spermatids are observed differentiating into spermatozoa (Fig. 5b), forming well-organized bundles (Fig. 6a). The spermatozoa possess needle-shaped basophilic heads, while their flagella are arranged in long, orderly bundles (Figs. 6a, b).

The testis lobe connects via the vas efferens to the vas deferens and subsequently to the seminal vesicle (Fig. 7). Mature spermatozoa are found in large aggregates within the lumens of these structures. The walls of these organs are lined by a simple cuboidal epithelium with basophilic oval nuclei and a muscle layer (Fig. 7).

Accessory glands, which open into the ejaculatory duct at the end of the seminal vesicle, exhibit a wavy appearance and contain prominent tracheal networks between them (Fig. 8a). Histological sections of the accessory glands reveal a simple columnar epithelium with lightly chromatin-dense oval nuclei and basophilic, granular cytoplasm (Figs. 8b and 9a). Short apical microvilli are evident on epithelial cells, and the lumen contains eosinophilic secretory material (Fig. 9a). In Fig. 9b, the lumen of the accessory glands is seen filled with numerous small secretory granules.

In histological sections of the ejaculatory duct—into which both the seminal vesicle and accessory glands discharge—the lumen is lined with a simple cuboidal

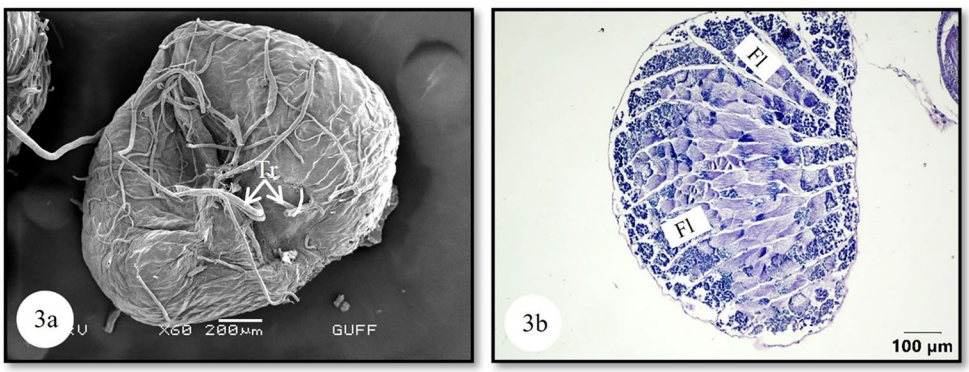



**Fig. 3** **a**. General view of the surface of the testis lobe (SEM). **b**. Histological section of the testis follicle (LM). (Fl-testis follicle, Tr-trachea

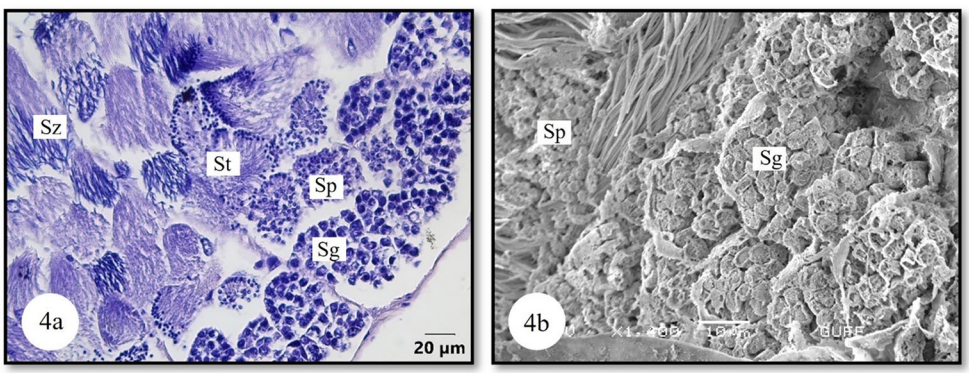



**Fig. 4** **a**. Spermatocytes, spermatids, and spermatozoa in different zones of the testis follicle (LM). **b**. Spermatogonia and spermatocytes in the growth zone of the testis follicle (SEM) (Sg-spermatogonia, Sp-spermatocytes, St-spermatids, Sz-Spermatozoa)

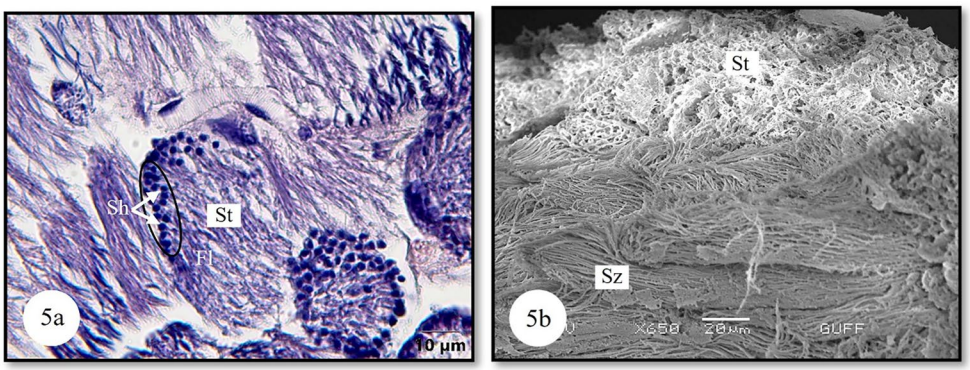



**Fig. 5** **a.** Spermatids in the maturation zone of the testis follicle (LM). b. SEM image of spermatids and spermatozoa (SEM) (Fl-flagellum, Sh-sperm head, St-spermatids, Sz-Spermatozoa)

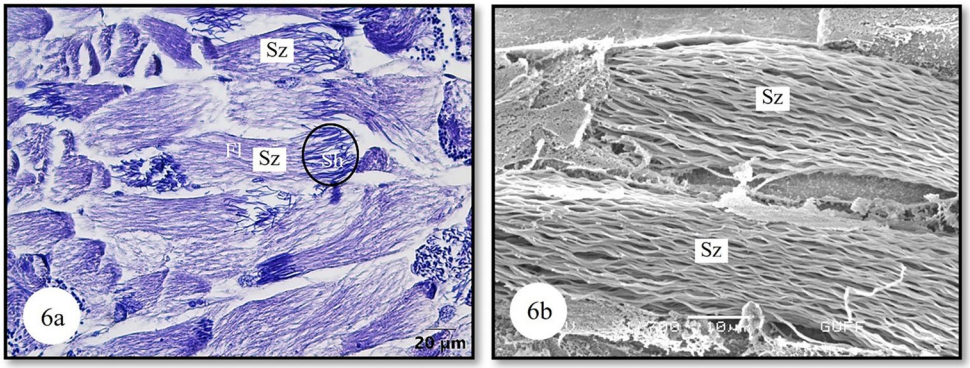



**Fig. 6** **a, b.** Spermatozoa in the differentiation zone of the testis follicle (LM, SEM) (Fl-flagellum, Sh-sperm head, Sz-Spermatozoa)

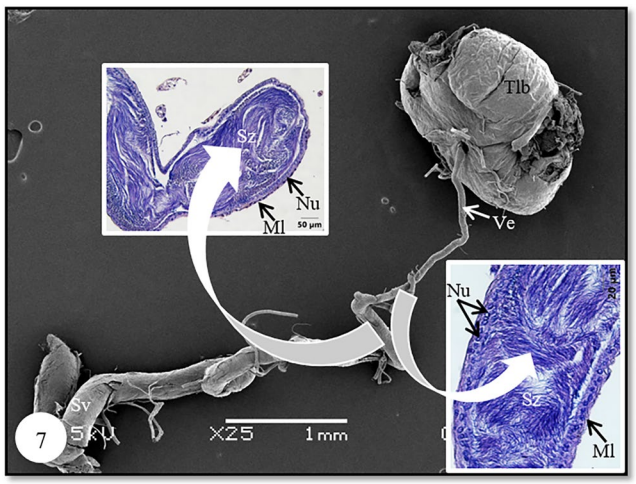



**Fig. 7** SEM and LM image of the connection between the testicular lobule and the vas deferens via the vas efferens, and histological sections of these structures (Ml-muscle layer, Nu-nucleus, Sv-seminal vesicles, Sz-Spermatozoa, Tlb-testis lobe,Ve-vas efferens)

epithelium containing deeply invaginated, chromatin-dense oval nuclei. This epithelium is surrounded by a thick muscle layer arranged in 6 rows (Fig. 10a). Muscle bundles and interconnections are visible between the ejaculatory duct and the aedeagus (Figs. 10b and 11a). The final part of the male reproductive system, the aedeagus, which is connected to the ejaculatory duct, is distinguished in Fig. 11b.

## Discussion

The male reproductive system of *A. kirbyi* consists of a pair of testes, each having two lobes, as seen in other beetle species such as *Chrysolina herbacea* (Chrysomelidae) *Chrysomela populi* (Chrysomelidae), and *Isotomus speciosus* (Cerambycidae), (Özyurt Koçakoğlu et al. 2021; Özyurt Koçakoğlu 2022; Özyurt Koçakoğlu et al. 2023). Testis coloration may vary among species. Each testis of *A. kirbyi* is pale yellow and oval-shaped. Likewise, the testicular lobes of *I. speciosus* (Cerambycidae) are also characterized by a light yellow and oval morphology (Özyurt Koçakoğlu et al. 2023). Conversely, the testes of *Aeolesthes holosericea* (Cerambycidae) are whitish in hue (Gaikwad and Patil 2017), while those of *C. populi* Linnaeus, 1758 (Chrysomelidae) exhibit a light orange coloration (Özyurt Koçakoğlu 2022).

Testis dimensions may vary among species. In *A. kirbyi*, each testicular lobe measures roughly 1.25 mm in width and 1.56 mm in length. The testis diameter of *Alphitobius diaperinus* (Tenebrionidae) is approximately 1.7 mm (Hopkins et al. 1993). In *C. populi* (Chrysomelidae), each testis lobe is about 0.5 mm wide and 0.9 mm long (Özyurt Koçakoğlu 2022), whereas in *I. speciosus* (Cerambycidae), the testis lobe is around 0.8 mm wide and 1.2 mm long (Özyurt Koçakoğlu et al. 2023).

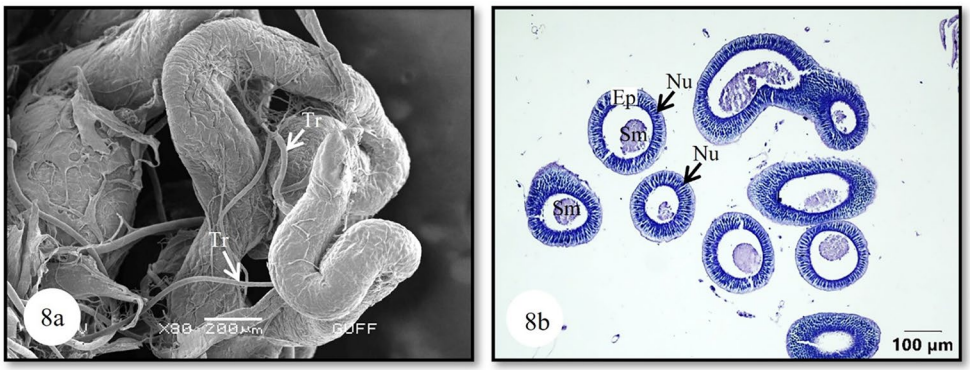



**Fig. 8** **a.** General view of the surface of the accessory glands (SEM). **b**. Histological section of the accessory glands (LM) (Ep-epithelium, Nu-nucleus, Sm-secretory material, Tr-trachea)

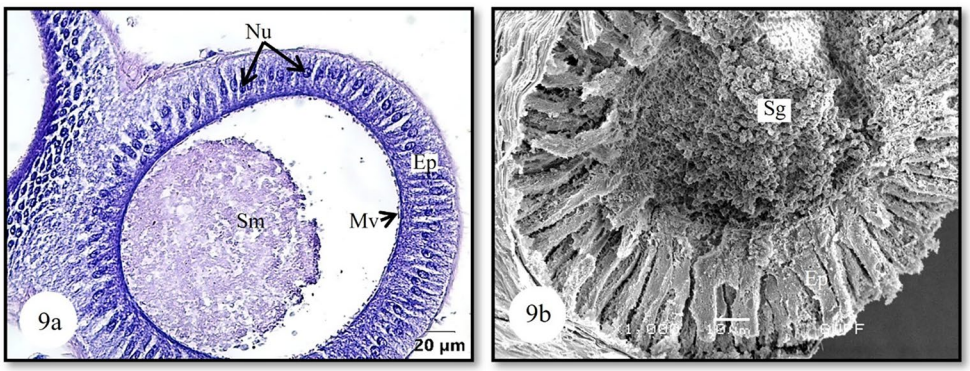



**Fig. 9** **a, b.** LM and SEM images of the cylindrical epithelium surrounding the accessory glands and the granules in the lumen (Ep-epithelium, Mv- microvilli, Nu-nucleus, Sg-secretion granules, Sm-secretory material)

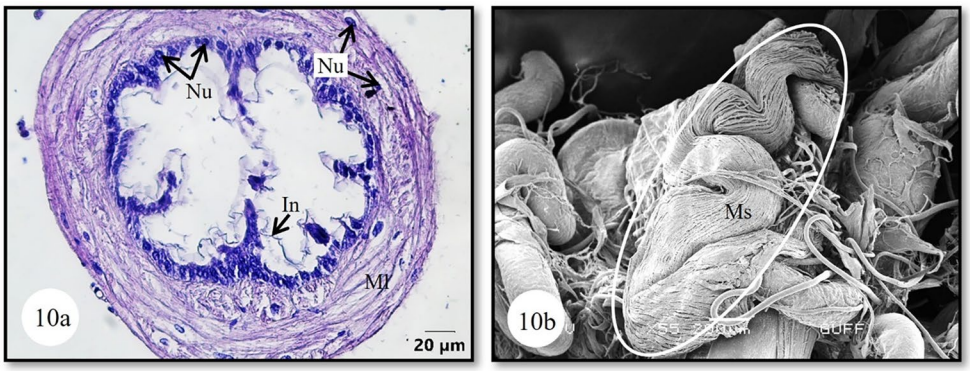



**Fig. 10** **a**. Intima surrounding the wall of the ejaculatory duct, single-layered cuboidal epithelium with basophilic nuclei, and the muscle layer arranged in 6 rows (LM). **b**. SEM image of the muscle bundles near the ejaculatory duct (In-intima, Ml-muscle layer, Ms-muscle bundles, Nu-nucleus)

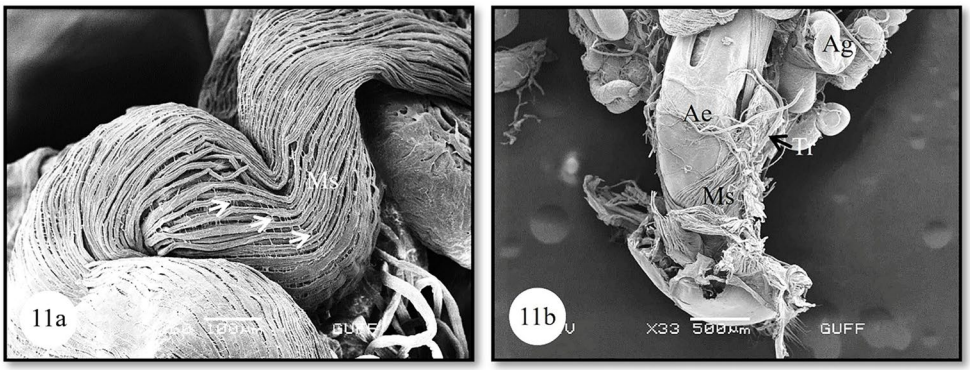



**Fig. 11** **a**. The connections (→) between muscle bundles (SEM). b. General view of the aedeagus (SEM). (Ae-aedeagus, Ag-accessory glands, Ms-muscle bundles, Tr-trachea)

In conclusion, notable differences in testis dimensions were observed among the examined species. These differences indicate that testis size in the order Coleoptera exhibits interspecific variation. Such variation in testis dimensions reflects the adaptive diversity of male reproductive system morphology.

Each testis of *A. kirbyi* consists of a pair of testis lobes, as seen in other beetle species such as *C. herbacea* (Chrysomelidae) *C. populi* (Chrysomelidae) and *I. speciosus* (Cerambycidae), (Özyurt Koçakoğlu et al. 2021; Özyurt Koçakoğlu 2022; Özyurt Koçakoğlu et al. 2023). Each testis shows significant differences among species in terms of the number, shape and arrangement of follicles (Barker 1989; Crowson 1981). The number of follicles can be as low as one or two or as high as tens or even hundreds. (Crowson 1981).

The number of follicles per testis varies among species. For example, in *A. kirbyi*, each testicular lobe has approximately 40 follicles. In *Prionoplus reticularis* (Cerambycidae), the testis is composed of 12 to 15 follicles (Edwards 1961). In *I. speciosus* (Cerambycidae), this number reaches approximately 65 follicles (Özyurt Koçakoğlu et al. 2023). In contrast, *C. populi* (Chrysomelidae) has only 10 follicles in each testicular lobe (Özyurt Koçakoğlu 2022), while *C. herbacea* (Chrysomelidae) has 20 (Özyurt Koçakoğlu et al. 2021). *Meloe proscarabaeus* (Meloidae) has a more complex follicular structure with approximately 100–120 follicles (Muzzi et al. 2020), while *Popillia japonica* (Scarabaeidae) has only six follicles (Paoli et al. 2020). In conclusion, the number of testicular follicles per testis shows considerable variation among Coleoptera species. These marked interspecific differences indicate that follicle number is a species-specific characteristic and represents an important morphological parameter of the male reproductive system. Such variation in follicle number likely reflects adaptive differences related to reproductive strategies and sperm production capacity.

These interspecific differences in testicular characteristics, including colour, size, and follicle number, are not only morphological details, but may also provide valuable clues about the taxonomic distinctiveness, phylogenetic relationships, and reproductive biology of species in the order Coleoptera. Therefore, comparative studies of testis morphology and follicle organisation can significantly increase our knowledge of insect evolution and reproductive adaptations.

Similar to *I. speciosus* (Cerambycidae), *A. kirbyi*, has a vas efferens in the centre of each testis lobe. These lobes are connected to the vas deferens by a thin, duct-like vas efferens (Özyurt Koçakoğlu et al. 2023). A similar structure was observed in *Dendroctonus monticolae* Hopkins, 1905 (Scolytidae), *Spasalus silvarum* Kuwert, 1898 (Passalidae), and *Cyclocephala barrerai* Martínez, 1969 (Melolonthidae) (Cerezke 1964; Salazar et al. 2016; Sanchez-Cruz et al. 2022).

The histological structures of the vas deferens and vas efferens in different Coleoptera indicate differentiated functions related to sperm transport and storage. In *A. kirbyi*, the presence of mature spermatozoa in the vas deferens lumen and the epithelial structure consisting of a single row of basophilic oval nucleated cuboidal cells suggests an active transport and temporary storage function. In contrast, in *C. populi* (Chrysomelidae), the vas efferens is surrounded by simple flat epithelium (Özyurt Koçakoğlu 2022). In *I. speciosus* (Cerambycidae), both the presence of mature sperm in the vas efferens and the fact that the vas deferens is covered with a single layer of cuboidal epithelium indicate that these two structures complement each other in sperm transport (Özyurt Koçakoğlu et al. 2023). In conclusion, the differences observed in the histological structures of the vas deferens and vas efferens among the examined Coleoptera species reveal species-specific functional adaptations related to sperm transport and storage. The presence, distribution, and epithelial organization of mature spermatozoa within these ducts indicate a range of functional roles, from active transport and temporary storage to functioning primarily as a passage duct.

In *A. kirbyi*, the male reproductive system exhibits well-organized muscle layer surrounding each duct, which play a fundamental role in sperm transport, temporary storage, and ejaculation. The vas efferens emerging from the testicular lobes is surrounded by a thin muscle layer, facilitating the transport of sperm from the testis to the vas deferens through peristaltic movements. This arrangement allows for the continuous and controlled transfer of sperm within the reproductive tract (Chapman 1998; Gillott 2003). The vas deferens is encased in a thicker muscle layer and contributes both to sperm transport and temporary storage (Snodgrass 1935; Gillott 2003). The seminal vesicles are surrounded by well-developed muscle layer, which facilitate the discharge of mature spermatozoa into the ejaculatory duct. Contraction of these muscles ensures a dense, controlled release of sperm, thereby enhancing transfer efficiency (Chapman 1998).

In species belonging to the order Coleoptera, accessory glands vary in number and structure (Kaulenas 1992; Schubert et al. 2017). *A. kirbyi* has a tubular, wavy appearance and a pair of accessory glands. *A. holosericea* (Cerambycidae) has three pairs of accessory glands (A, B and C). Accessory glands A is a cylindrical and curved tube. Accessory glands B and C are shorter and simpler. These structures differ in location and shape (Gaikwad and Patil 2017). *M. proscarabaeus* (Meloidae) has three pairs of accessory glands with different forms, such as C-shaped tubular structures, tubular structures only, and curved, irregularly shaped structures (Muzzi et al. 2020). In *Blaps polychresta* (Tenebrionidae), two pairs of accessory reproductive glands were found, one bean-shaped and the other long tubular (El-Zoheiry, 2021). The male reproductive system of *I. speciosus* (Cerambycidae) has three pairs of tubular accessory glands called A, B, and C (Özyurt Koçakoğlu et al. 2023). *Lasioderma serricorne* (Ptinidae) has five pairs of accessory

glands, four of which are tubular and one (glands 4) is oval (Reis et al. 2021). This shows that the number and structure of accessory glands vary from species to species.

In *A. kirbyi*, the accessory glands are surrounded by a simple columnar epithelial layer, and the gland lumen is filled with eosinophilic secretion material and numerous small secretory granules. Similarly, in *I. speciosus* (Cerambycidae), the accessory glands are lined with simple columnar epithelial cells; however, their lumens contain dark-colored, basophilic, filamentous secretions (Özyurt Koçakoğlu et al. 2023). The accessory glands in *Capnodis tenebrionis (*Buprestidae) have a simple cuboidal epithelium (Çağlar et al. 2020). In *Pimelia subglobosa* (Tenebrionidae), the first pair of accessory glands is surrounded by a stratified epithelium and muscle layer. In contrast, the second pair consists of simple cuboidal epithelial cells and a muscle layer (Özyurt Koçakoğlu and Candan 2022). These findings reveal that the morphological structure and number of layers of the epithelial cells surrounding the accessory glands vary among species.In conclusion, the number and structural organization of accessory glands vary markedly among species within the order Coleoptera. Differences in the number, shape, and arrangement of these glands represent species-specific characteristics and reflect the morphological diversity of the male reproductive system. Such variation suggests functional adaptations of accessory glands in relation to species-specific reproductive strategies and contributes to a better understanding of reproductive system specialization in Coleoptera.

In *A. kirbyi*, the epithelial cells of the accessory glands exhibit short apical microvilli, which increase the surface area for secretion and absorption. These microvilli facilitate the efficient release of glandular secretions into the ejaculatory duct during copulation. The accessory gland secretions observed in this species likely play multiple crucial roles, consistent with reports in other insects: they enhance sperm viability, provide a nutritive medium, protect sperm from oxidative and microbial damage, and assist in sperm transport through the female reproductive tract (Gillott 2003; Pitnick et al. 1999). The presence of secretory granules in the gland lumen and the abundant microvilli suggest that these secretions are actively produced and are essential for ensuring sperm survival and successful fertilization in *A. kirbyi*.

The accessory glands are surrounded by muscle layer that facilitate the transfer of their secretions into the ejaculatory duct. The muscle layer ensures that the secretions accumulated in the gland lumen are expelled together with sperm during ejaculation. Accessory gland secretions have been widely reported to enhance sperm viability and to assist in protecting and transporting sperm within the female reproductive tract (Gillott 2003).

As *I. speciosus* (Cerambycidae), the wall of the ejaculatory duct of *A. kirbyi* consists of a layer of intima covered with spines, a layer of single-layered epithelial cells, and a muscle layer underneath. At the junction between the ejaculatory duct and aedeagus, prominent muscle bundles are clearly distinguished (Özyurt Koçakoğlu et al. 2023).

The ejaculatory duct is one of the structures in the system with the thickest and most well-organized muscle layer. Its thick musculature facilitates the propulsion of sperm and accessory gland secretions toward the aedeagus under high pressure (Snodgrass 1935; Chapman 1998).

In this study, the histological and anatomical features of the male reproductive system of *A. kirbyi* were presented in detail. The data obtained provide important contributions to the literature on the morphology of the male reproductive system in Coleoptera, especially in the family Cerambycidae. In addition, the comparative evaluation of *A. kirbyi* with related taxa provides new information regarding biodiversity and provides guidance in determining its suitability for use in potential

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**Data availability** The data supporting this study's findings are available in this article's supplementary material.

## Declarations

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Competing interests** The authors declare no competing interests.

**Additional information** This article was initially submitted by the authors in English and was first published here.

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