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THE VENOM GLAND OF THE SCORPION SPECIES *Euscorpius mingrelicus* (SCORPIONES: EUSCORPIIDAE): MORPHOLOGICAL AND ULTRASTRUCTURAL CHARACTERIZATION

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ABSTRACT: The histology and ultrastructure of venom glands in the scorpion *Euscorpius mingrelicus* (Kessler, 1874) are described and illustrated in the current study for the first time by employing light microscopy and transmission electron microscopy (TEM). The venom apparatus is composed of a pair of venom glands and a stinger, both situated in the last segment of the metasoma. The venom glands are completely separate but similar. The two glands are segregated within the telson by striated muscle bundles, and their outer surfaces are surrounded by a cuticle. An internal layer constitutes the secretory epithelium. This epithelium is made up of simple columnar cells. The nucleus and organelles involved in cellular synthetic activity are situated basally. In the apical portion, near the gland lumen, there are many secretory granules of different sizes, shapes and electron densities.

KEY WORDS: *Euscorpius mingrelicus,* scorpion, telson, venom gland, histology, ultrastructure.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Scorpions have long been of interest to humans, primarily because of their ability to give painful and sometimes life-threatening stings. These animals are also an important and beneficial component of many ecosystems and they are one of the oldest known terrestrial arthropods. Scorpions present an elongated body. The abdomen consists of 12 distinct segments and the last five segments of abdomen form the metasoma that is commonly called as a tail. The telson, situated at the end of the metasoma, is a bulb-shaped structure that contains the venom glands and a sharp, curved stinger to deliver venom. The scorpion venom is used for both prey capture and defense. It is a complex mixture of neurotoxins and other substances; each species has a unique composition. Despite their intimidating reputation, about 30 species worldwide have venom sufficiently potent to be considered dangerous to humans (1).

Scorpion venoms are rich sources of bioactive peptides but they contain only some biogenic amines (21). Most scorpion toxins, which are ligand peptides, are described in some literatures. They can be recognized and can interact specifically with ion channels (Na^+, K^+, Cl^- and Ca^{2+}) of excitable or non-excitable cells (1, 6, 16, 20). In scorpion venom, there are also many toxins that present antimicrobial activity against both gram-negative and gram-positive bacteria (3, 13, 19).

Venoms or whole tails of scorpions have been used as a traditional Chinese medicine to treat some neurological diseases such as stroke, rheumatism and tetanus for more than 2,000 years. Therefore, it is very interesting to identify the pharmacologically effective components, and gain further insight into their functions and action mechanisms. Up to now, numerous disulfide-bridged toxins have been identified and characterized from several scorpion species. However, there are many compounds yet to be discovered in venom (22).

For the aforementioned reasons, there are many studies on scorpion venoms of various species. Little is known about the histology and the ultrastructure of venom glands, although the histology has been investigated by several researchers (7, 9-11, 17). Nevertheless, these studies were limited to a few scorpion species whose stings are recognized for being fatal to humans (12, 18). In our study, we describe the histology and ultrastructure of *Euscorpius mingrelicus* (Kessler, 1874) venom gland.

While the venom is not sufficiently potent to harm humans, it presents a mixture that can affect prey.

E. mingrelicus belongs to the Euscorpiidae family which is widespread in central and southern Europe, and also found in Africa (Mediterranean coast), North America (Mexico), Central America (Guatemala), South America (Brazil, Peru, and Venezuela), and Asia (west, central, south and southeast). One species has become established in some parts of southern England. The countries where *E. mingrelicus* can be found are: Georgia, Syria, Turkey, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Italy, Romania, Russia, Slovenia and Yugoslavia (4). In Turkey, the scorpion is widely distributed throughout the following regions: Marmara, Mediterranean, Aegean and Black Sea (5).

MATERIALS AND METHODS

Scorpions

In the present study, 12 adult *E. mingrelicus* scorpions were used. They were collected from the Çamlıdere – Çamkoru Forest (33°E, 40°N, Ankara, Turkey), having been found under stones in September, 2005. The animals were identified, reared in special cages and fed insects and grasshoppers at the Biology Department of Kirikkale University. Their telsons were removed, and the venom glands were employed as microscopic specimens under a stereo microscope (SMZ800®, Nikon Instech Co. Ltd., Japan).

Transmission Electron Microscopy (TEM)

The telson was fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) for 2 hours at 4°C and then washed four times with the same buffer; after that, it was postfixed in 1% osmium tetroxide for an hour at room temperature. To remove osmium tetroxide, the samples were washed four times in sodium phosphate buffer. They were then dehydrated in a graded ethanol series. After dehydration, the telson was embedded in Araldite® CY 212 (Agar Scientific Ltd., UK). From the blocks thereby prepared, thin sections (60 to 70-nm-thick) were cut with glass knifes on RMC MT-X® ultramicrotome (Boeckeler Instruments, USA) and mounted on copper grids (8). Thin sections were stained with uranylacetate, followed by lead citrate and examined under a Jeol JEM 100 SX® TEM (Jeol Ltd., Japan) at 80 kV.

Light Microscopy

The araldite blocks prepared for TEM were also used to obtain semithin sections for light microscopic examination that were mounted on slides. Then they were stained with 1% toluidine blue and examined under a Leica-DM LS2® light microscope (Leica Microsystems, Germany).

RESULTS

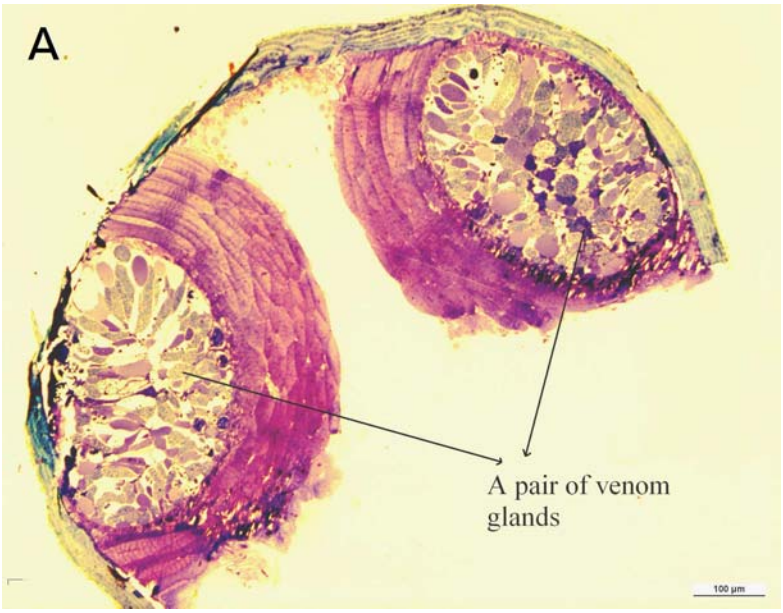
The venom apparatus of *E. mingrelicus* is composed of a pair of venom glands and a stinger. The venom glands that produce venom fill the telson and are a pair, with equal size and shape (Figure 1A); they are completely separate but similar to each other. The two glands were segregated within the telson by striated muscle bundles; their outer surfaces are surrounded by a cuticle (Figure 1B). In the cross sections, the telson is covered by a cuticle as well as all the body. The telson cuticle is composed of two layers: the exocuticle and the endocuticle. Telson endocuticle consists of lamellar layers of chitin; while the exocuticle is a homogeneous layer. There are some hemolymph vessels and cuticle canals in the endocuticle (Figure 1C).

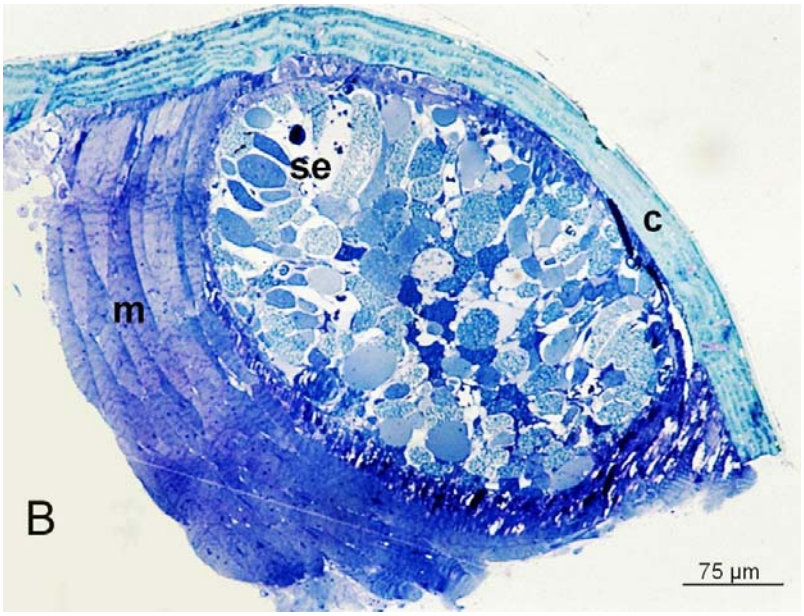
The venom glands are covered by the innermost muscle layer and by cuticle outside. The muscle layer terminates at the cuticle, where the glandular epithelium is attached by two layers of cuboidal cells. In other words, a muscle layer is not present on the side of the glandular epithelium facing the cuticle. And there are two layers of cuboidal cells between cuticle and glandular epithelium. These cells form a continuous layer under the telson cuticle and are attached to the basal lamina of secretory epithelium. Their nuclei are round and centrally located (Figure 1C). In the thin sections, two layers of the cuboidal cells can be seen easily by TEM (Figure 1D). Muscle bundles surrounding the secretory epithelium adhere tightly to the telson cuticle (Figure 2). In this region of connection, myosins that are found in muscle bundles terminate with thick tendons. The attachment of muscle bundles to the telson cuticle is mediated by dense intercalated tendons that firmly attach the muscle bundles to the cuticle (Figures 2A, 2B and 2C).

The venom quickly goes through ducts by the contraction of the muscle bundles that surround secretory glands. Ultrastructural examination of muscle tissue reveals that the Z lines of the muscle bundles have irregular structures. In the muscle, M lines

and H bands are indistinguishable (Figure 3). The basal lamina separates muscle layer from glandular epithelium (Figure 4).

An internal layer constitutes secretory epithelium that is made up of simple columnar cells. Along with a lack of epithelial folding, there is a narrow lumen in the center of the venom gland. The secretory cells present a nucleus placed close to their basal portion. There are many secretory granules of different sizes, shapes and electron densities in the apical portion of the cells near the gland lumen (Figure 5A). Venomous secretory materials produced within the venom gland were subdivided into several types according to their locations and structures. These granules vary in their reactions to the same stain. One type is coarse-grained electron-dense granules. Another type of granule has a spongy structure and large electron-dense granules in secretory cells. Still another one is electron lucent with transparent granules (Figures 5B and 5C). That the secretory granules demonstrate such variety indicates that the venom secretion is a complex mixture.

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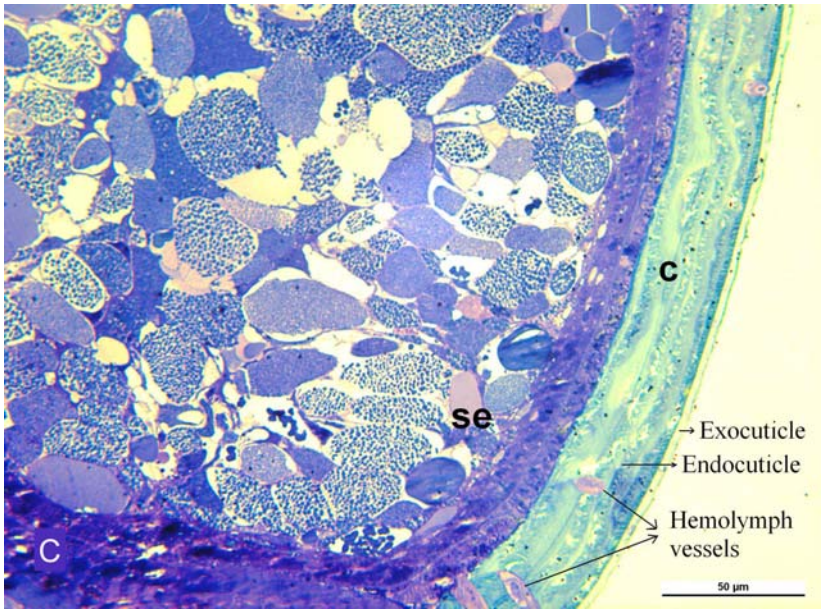
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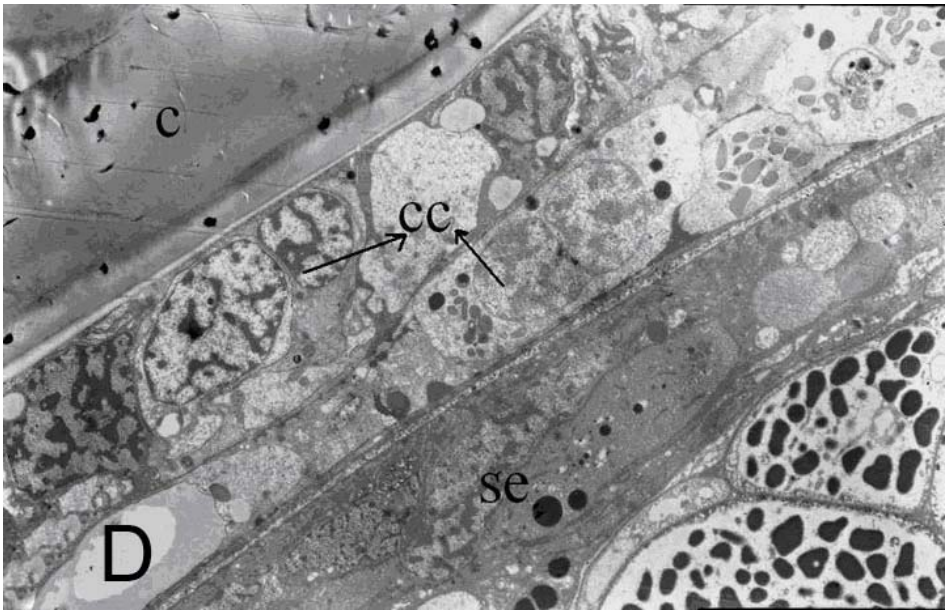
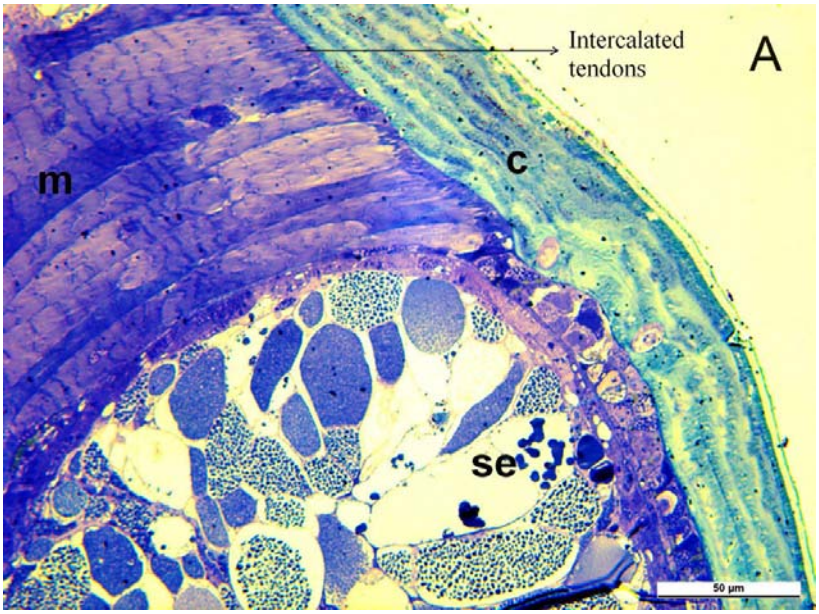


Figure 1. (**A**) General view of transverse section of *E. mingrelicus*'s telson consisting of a pair of venom glands. The two glands were segregated within the telson by gross striated muscle bundles; the outer surfaces of venom glands are covered by cuticle. (**B**) One of the venom glands. Muscle bundles converge to regions where the venom glands contact telson cuticle. Cuticle (c), muscle tissue (m), secretory epithelium (se) and secretion granules are visible (167x). (**C**) The telson cuticle consists of exocuticle that is a homogeneous layer and endocuticle composed of lamellar layers. There are some hemolymph vessels and cuticle canals in the endocuticle. The cytoplasm of glandular epithelium is filled with many secretory granules of different sizes, shapes and densities. (**D**) In the region where cuticle and gland merge, there are two layers of cuboidal cells (cc) between cuticle (c) and glandular epithelium (se) (2,763x).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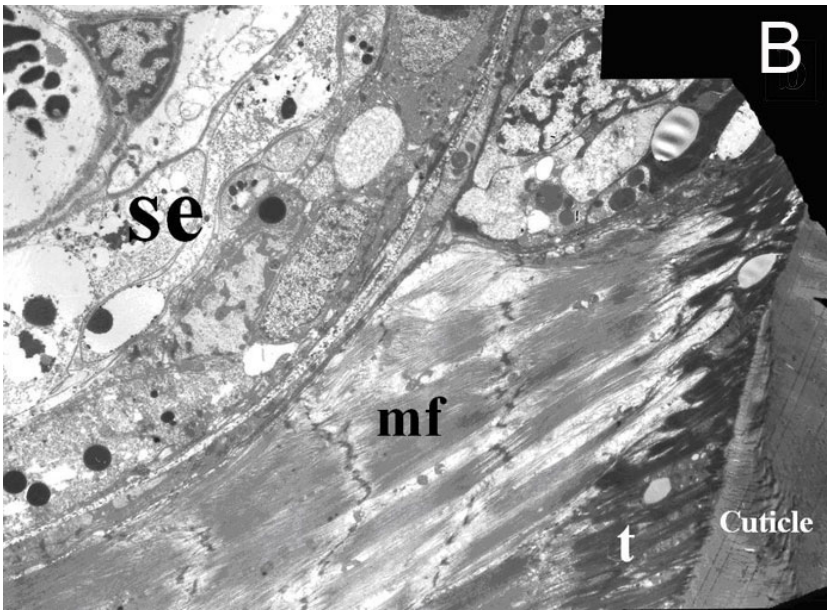
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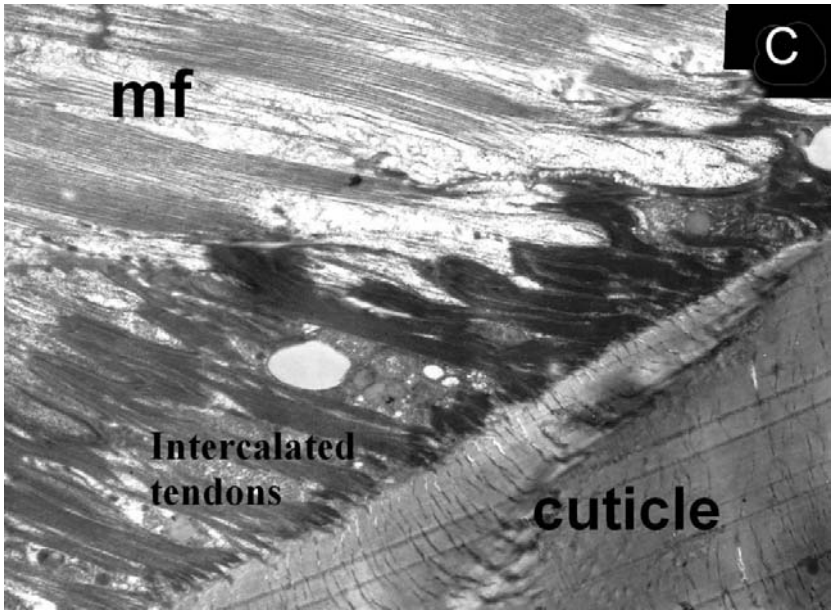


Figure 2. Attachment region of muscle bundles and telson cuticle. (**A**) Muscle bundles joined to telson cuticle (c). (**B**) The attachment is mediated by intercalated tendons (t) (2,652x). (**C**) Intercalated tendons, muscle myofibrils (mf) and cuticle at higher magnification (7,200x).

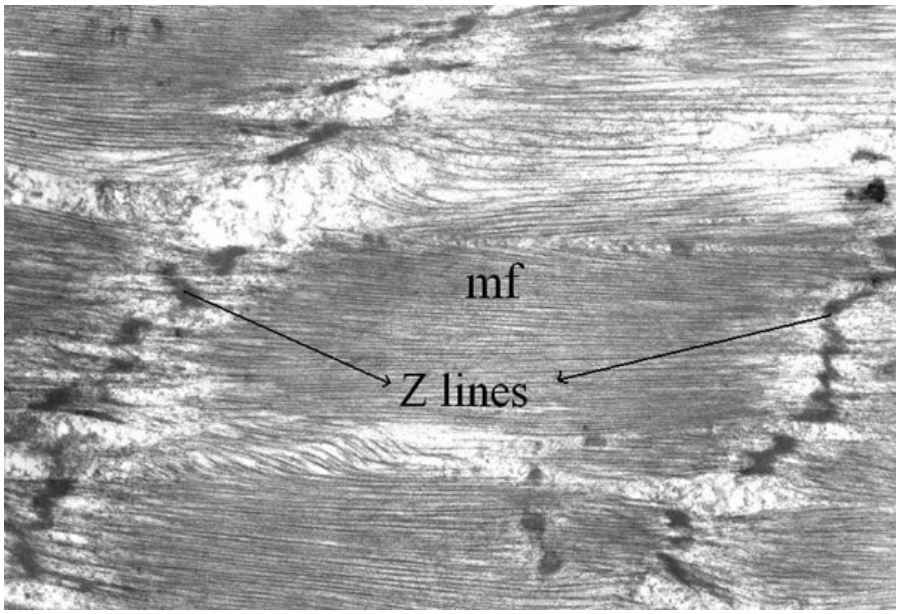


Figure 3. Two Z lines and myofibrils (mf) are shown in muscle layer in a longitudinal section. The muscle layer Z lines present irregular structure (12,738x).

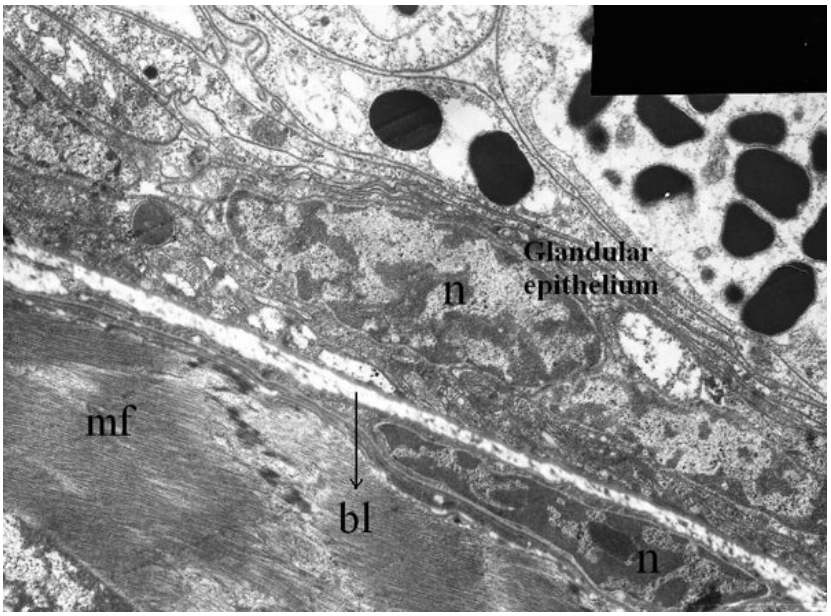
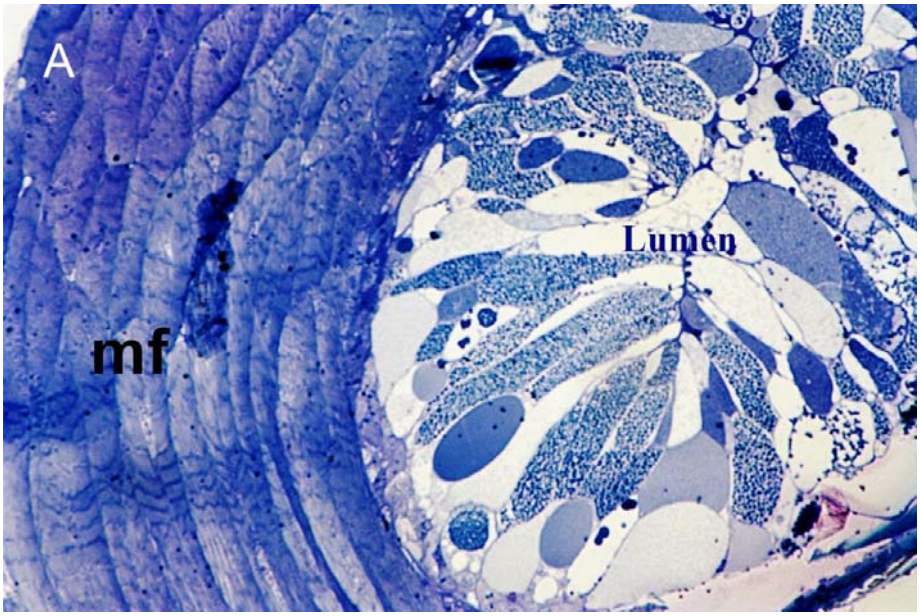


Figure 4. The muscle layer and glandular epithelium are separated by basal lamina (bl). Secretory cells present a nucleus (n) placed close to their basal portion, while the nucleus of the muscle cells are peripheral (7,922x).



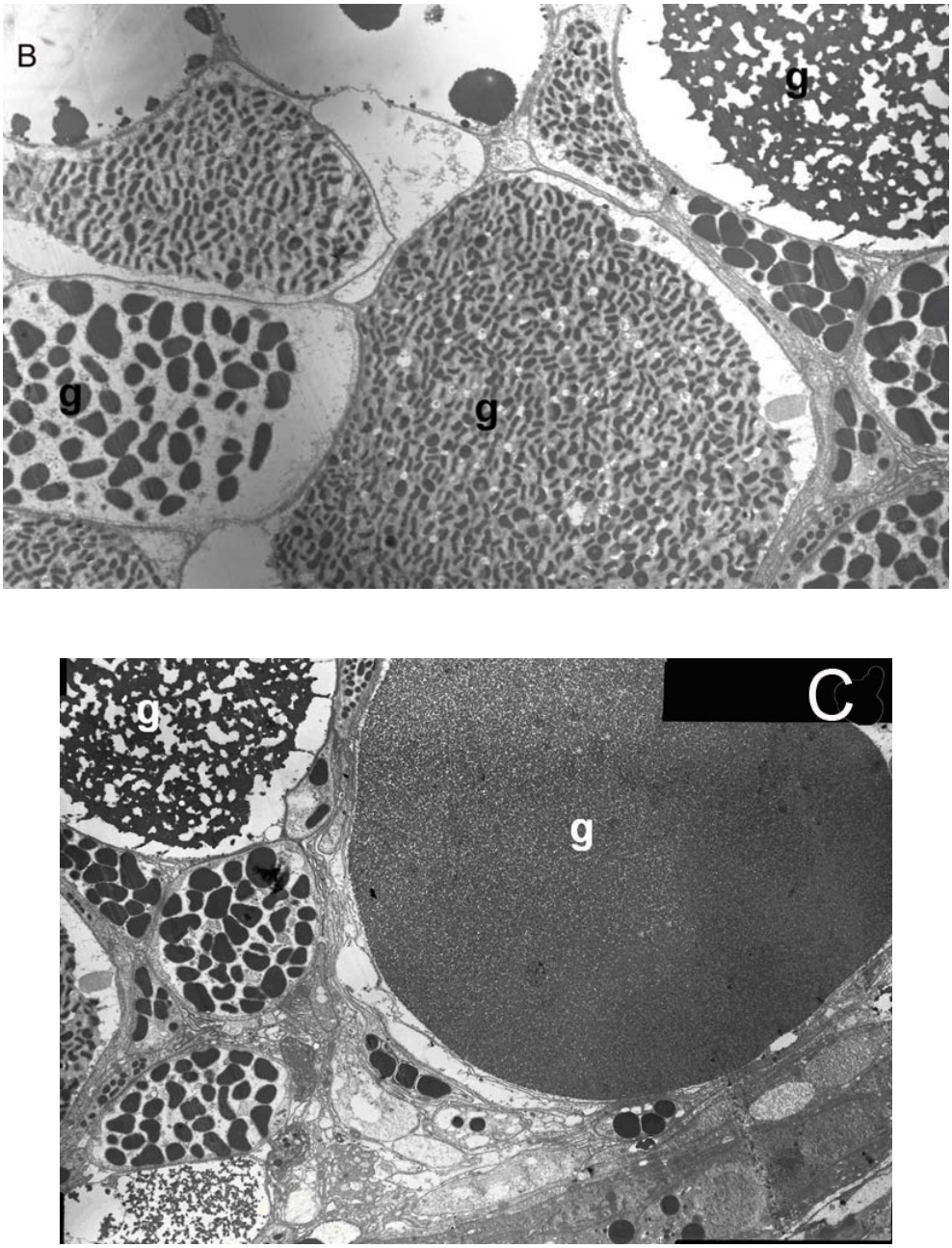


Figure 5. (**A**) Thick muscle layer, glandular epithelium, beginning of lumen, glandular epithelium made up of simple columnar secretory cells and their cytoplasm filled with many secretory granules of different sizes, shapes and densities under light microscope (397x). (**B**, **C**) The different granules (g) in secretory cells under TEM (**B**: 3,040x; **C**: 2,828x).

DISCUSSION

The venom apparatus of *E. mingrelicus* is composed of a pair of venom glands producing the venom that is, in turn, injected by stingers. The venom apparatus of *Leiurus quinquestriatus* (Hemprich et Ehrenberg, 1827) is composed of two completely separate but similar glands, each with its own canal (18). The differentiation of the venom glands into lobes was observed by Kanwar *et al*. (10) in *Buthus tamulus* gland where the glands were divided longitudinally into parts by a septum. Quiroga *et al*. (17) studied the venom gland histology in *Tityus caripitensis* (Quiroga, 1988) adult females. The venom gland of *T. caripitensis* consists of two ovoid lobes that fill the vesicle except for a small cavity where the venom accumulates; the cavity continues distally into an excretory duct. Similarly, our histological results showed that *E. mingrelicus* presents two distinct venom glands that were completely separate but similar to each other, and each venom gland opened separately into its own venom duct.

The scorpion needs to quickly eject the poison produced in its venom glands or inject it into its victim. For this, each venom gland is surrounded by a striated muscle layer. Striated muscle fibers vary in their biochemistry, ultrastructure and manner of contraction. Some undergo a slow, continuous contraction (type I) and are fatigue-resistant, while others undergo a fast contraction (type II) for a short period of time and fatigue quickly. According to this classification, type I is the red muscle tissue and type II is the white muscle tissue. Type I has ultrastructurally regular Z lines and abundant mitochondria. Type II has irregular Z lines, M lines and H bands that cannot be distinguished and has few mitochondria (2). In our study, as clearly visible under light and electron microscopy, the striated muscles surrounding the venom gland of *E. mingrelicus* have irregular Z lines and M lines, whereas H bands cannot be distinguished. Besides, this muscle tissue is white. When these characteristics are taken into consideration, the muscle tissue of the venom gland is included in type II. Given their short and rapid contraction characteristics, type II muscles offer an advantage to scorpions because they can inject their venom into their victims as soon as they catch them.

The secretory epithelium of *E. mingrelicus* is a simple glandular epithelial type (type I in agreement with Pawlowsky’s classification) because there is no folding and a

narrow lumen in the center of the venom gland. Pawlowsky (14, 15) reported on six of seven known families and found that the morphology followed a generalized scheme, with the major differences consisting of the presence versus the absence of folds in the secretory epithelium. Two main types were listed: type I (primitive gland) possesses a smooth and indented epithelium and type II (complex gland) presents true folds.

Quiroga *et al*. (17) determined that *T. caripitensis's* venom glands are made of a simple, pseudostratified epithelium. The epithelium contains secretory cells that have either coarse-grained or thin granules, basal cells or nonsecretory cells. Taib and Jarrar (18) reported that the secretory epithelium of *L. quinquestriatus* venom glands is made up of three cell types: venom-producing cells, mucous cells and supporting cells. The venom apparatus of *Centruroides sculpturatus* (Ewing) was studied under light and electron microscopy by Mazurkiewicz and Bertke (12). Each of the paired glands is lined by secretory epithelium made up of a single layer of columnar cells. The secretory products were observed in single epithelium cells and in the gland lumen. Similarly, we found that *E. mingrelicus* venom gland is composed of different cell types. One type is the venom-producing cells that have several granules of different sizes, shapes and electron densities. Another type, the supporting cells, is found between glandular epithelium and cuticle or between glandular epithelium and muscle bundles. Still another type is goblet cells, found among secretory epithelium cells, whose function is to secrete mucus. To summarize, it is possible to mention three types of cells in *E. mingrelicus* venom glands.

Taib and Jarrar (18) studied *L. quinquestriatus* venom glands using histochemical methods and at least five types of granular secretory products were determined inside the cells. As it can clearly be seen by light and electron microscopy, there are granules with different morphologies and different locations in the secretory cells. This is a plain proof that the venom secretion which has a complex structure is the combination of many secretion products.

In the present study, *E. mingrelicus* venom glands were studied in terms of their histology and ultrastructure. The venom gland structural aspects registered by light and electron transmission microscopy will constitute a basis for future research on this species.

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