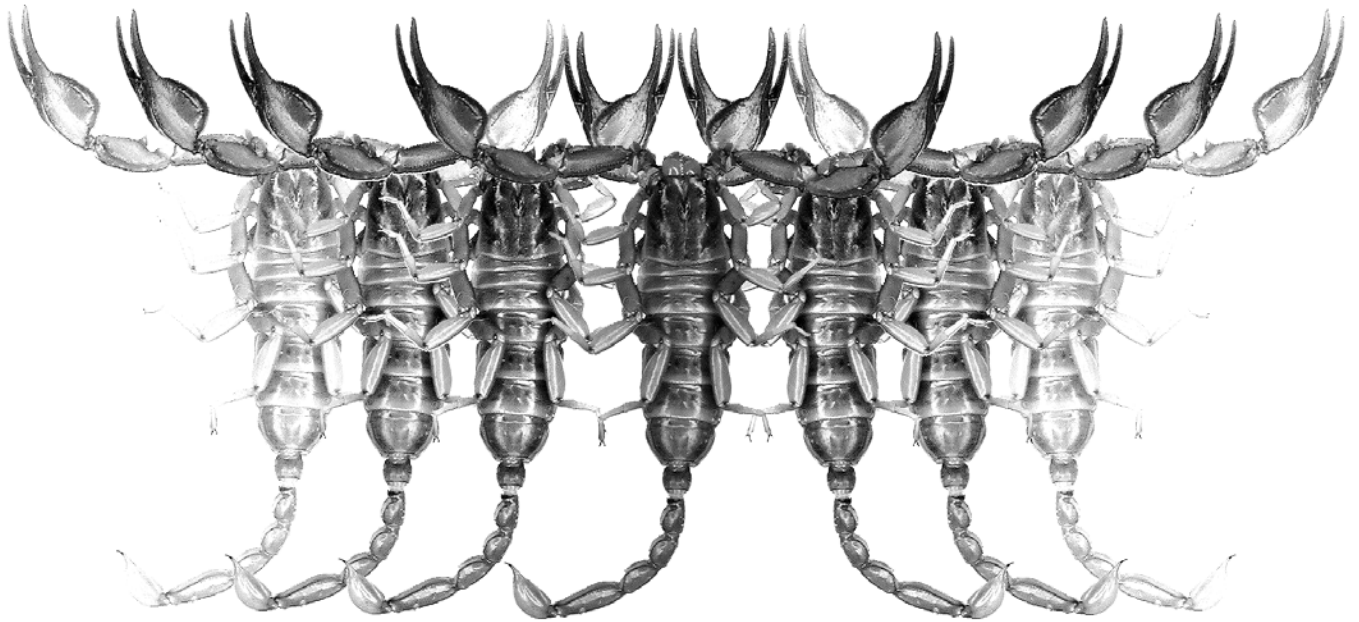


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Occasional Publications in Scorpiology



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The sting of *Mesobuthus gibbosus* (Scorpiones: Buthidae): morphological and ultrastructural characterization

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Summary

The objective of our study is to characterize the morphological and ultrastructural features of the sting of scorpion species *Mesobuthus gibbosus* (Brullé, 1832) (Buthidae) by using light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The venom is delivered by venom ducts, passing through the sting and exiting from the venom pores. Each venom gland has its own venom duct and pore. The venom pores are situated on both sides of the tip of sting. Both venom ducts and the cells that support the venom ducts are identified in the transverse discussion in this study.

Introduction

Despite their bad reputation and the great number of scorpion species, only a few, mostly in the family Buthidae, are dangerous to humans. About 30 out of more than 1,300 described species worldwide have venom potent enough to be considered dangerous to human beings (Fet et al., 2000; Brownell & Polis, 2001). Among the most dangerous genera are *Leiurus*, *Parabuthus*, *Tityus*, *Centruroides*, and *Androctonus* (Lucas & Meier, 1995). *Mesobuthus gibbosus*, whose venom is not that powerful, has venom that can be fatal only to children and the elderly.

In this study, *Mesobuthus gibbosus* (Brullé, 1832) (Scorpiones: Buthidae), widespread in the Eastern Mediterranean region, were studied. *M. gibbosus* is known to occur in Albania, Bulgaria, Montenegro, Former Yugoslav Republic of Macedonia, Greece, and Turkey (Fet et al., 2000; Fet & Soleglad, 2007). The species is distributed throughout Anatolia (Turkey), except for the Black Sea coast in northern Turkey, and is the most abundant scorpion in this region. In Turkey, *M. gibbosus* is widespread and is reported to be commonly found in houses in rural or urban areas; and they present an epidemiological problem, especially among children (Taş, 2004). In the present study, the morphology and ultrastructure of *M. gibbosus* sting are examined.

Materials & Methods

Specimens. Ten adult *Mesobuthus gibbosus* of both sexes were collected from under stones in Yahsihan (Kirikkale, Turkey, 39°50' N, 33°31' E) in September

2005. Specimens were kept alive in the lab until needed. Telsons were removed by cutting, and stings were studied under a stereo microscope (Nikon SMZ800).

Scanning Electron Microscopy. The stings were fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) for two hours and then washed four times in sodium phosphate buffer, and postfixed in 1 % osmium tetroxide (OsO₄) in the same buffer for 2 hours at +4 °C. Stings were then washed four times in sodium phosphate buffer and dehydrated in a graded ethanol series (40%–100% ethanol). The last stages of dehydration were performed with propylene oxide. The specimens were dried and coated with a thin layer of gold with a Polaron SC 500 sputter coater. The materials were examined at an accelerating voltage of 10–20 kV with a Jeol JSM 5800 Scanning Electron Microscope and the electron micrographs were recorded following the procedures in Hayat (1981).

Transmission Electron Microscopy. The fixation and dehydration processes for TEM specimens were conducted by use of the same method employed in the SEM preparation. After fixation and dehydration, the stings were embedded in Araldite CY 212 (Hayat, 1981). Thin sections (60–70 nm) were cut with glass knives on RMC MT-X ultra-microtome and mounted on 100-mesh grids. These sections were stained with uranylacetate, followed by lead citrate and examined under a Jeol JEM 100 SX TEM at 80 kV.

Light Microscopy. The same araldite blocks as prepared for TEM were also used to obtain semi-thin

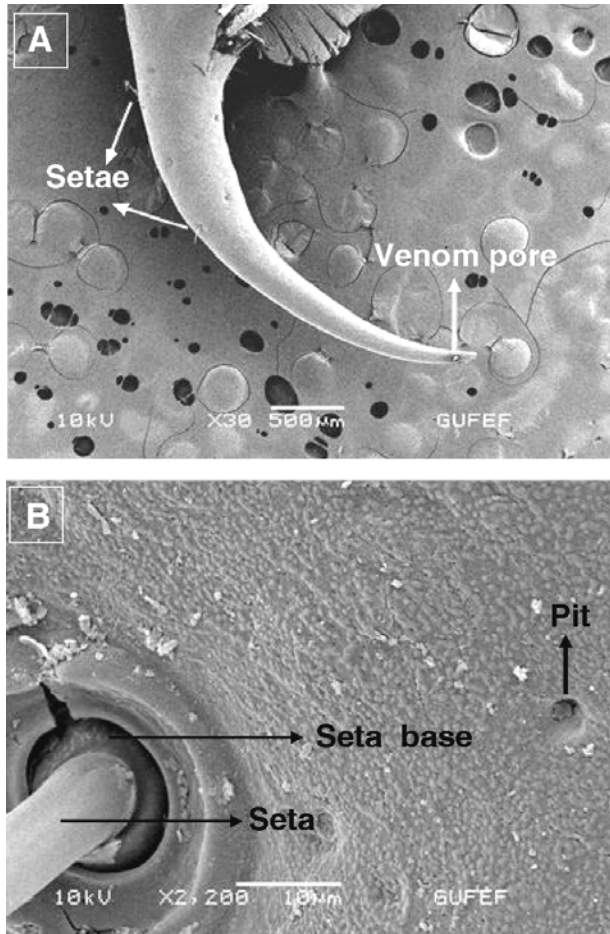


Figure 1: A. Lateral view of *M. gibbosus* sting. The venom pore located at the tip of the sting, and several setae situated more basally are visible, x30. B. A cuticular seta, seta base, and a cuticular pit on the sting at higher magnification, x2,200

sections (0.5–1.5 μm) for light microscopic examination. Semi-thin sections were cut off and mounted on slides. The sections were stained with 1 % toluidine blue and examined with a Leica-DMLS2 light microscope.

Results & Discussion

The sting, or aculeus, which is a part of the scorpion venom apparatus, is situated on the final segment of the metasoma called the telson. The telson, as well as the entire body, is covered by cuticle, which has various types of sensory setae and pits on its surface (Brownell & Polis, 2001). In our study, we observed numerous cuticular setae and pore canals over the sting cuticle of *M. gibbosus* by SEM.

Cuticular sensory organs are common in all arachnids. In scorpions, short, curved chemosensory setae are scattered all over the body. Fet et al. (2003) studied the metasoma of *Orthochirus* (Scorpiones: Buthidae) and found a peculiar array of over 1,000

cuticular pits both ventrally and laterally on the posterior segments of the metasoma and telson. SEM shows those pits adorned with variable size setae, which exhibit microanatomical features characteristic of chemoreceptors. In another recent SEM study (Fet et al., 2006), a new structure found in a majority of the Vaejovidae family species was described for the first time. This new structure located on the laterobasal aspect of the telson aculeus was named *laterobasal aculear serrations* (LAS). This structure provided a new synapomorphy for this New World family. These discoveries show that further investigation of microanatomical features of insufficiently studied scorpion body structures such as the sting is of great importance.

The slender, sturdy, and flexible metasoma enables the sting to penetrate hard tissue. The general appearance of the sting of *M. gibbosus* is similar to other scorpion stings. The tip of the curved sting is pointed and sharp. The venom is carried by two venom ducts; it passes through the sting and exits from the venom pores. Two venom pores are situated on each side of the tip of sting and are used for injecting venom. Each gland has its own duct at the base of the sting. The venom produced by each venom gland is carried through its own venom duct and exits from its own venom pore (Fig. 1A–B).

As in all arachnids, the bodies of scorpions are covered by cuticle. The cuticle covering the telson is composed of three main layers: an outermost epicuticle, which is a waxy-like layer; an exocuticle, the homogenous middle layer; and the innermost endocuticle, which is the thickest of the three layers. The endocuticle is constructed of alternating layers of chitin (Mazurkiewicz & Bertke, 1972).

This three-layer cuticle structure can easily be distinguished in the sting cuticle of *M. gibbosus*. In the transverse section of the sting, both venom ducts and the cuticle can be seen easily under a light microscope (Fig. 2A). The cuticle of the sting consists of three main layers (epicuticle, exocuticle, endocuticle) as follows: outermost epicuticle, a thin transparent layer with an amorphous appearance; exocuticle, a thick homogenous middle layer; and innermost endocuticle, a lamellar layer thicker than the epicuticle. Numerous chitin and hemolymph channels located in the exocuticle and endocuticle are also observed (Fig. 2B).

The cuticle can be further divided into different parts within three main layers (Hjelle, 1990). In our study of the sting, such internal layers were not observed. The epicuticle layer, which is the outermost and the thinnest part, was observed as a homogeneous and a transparent layer. Unlike in the telson cuticle, exocuticle in the sting constitutes the thickest layer of cuticle, and has a hyaline structure. Endocuticle, found in the innermost part, has a lamellar structure as in the telson cuticle but is thinner (Fig. 2B).

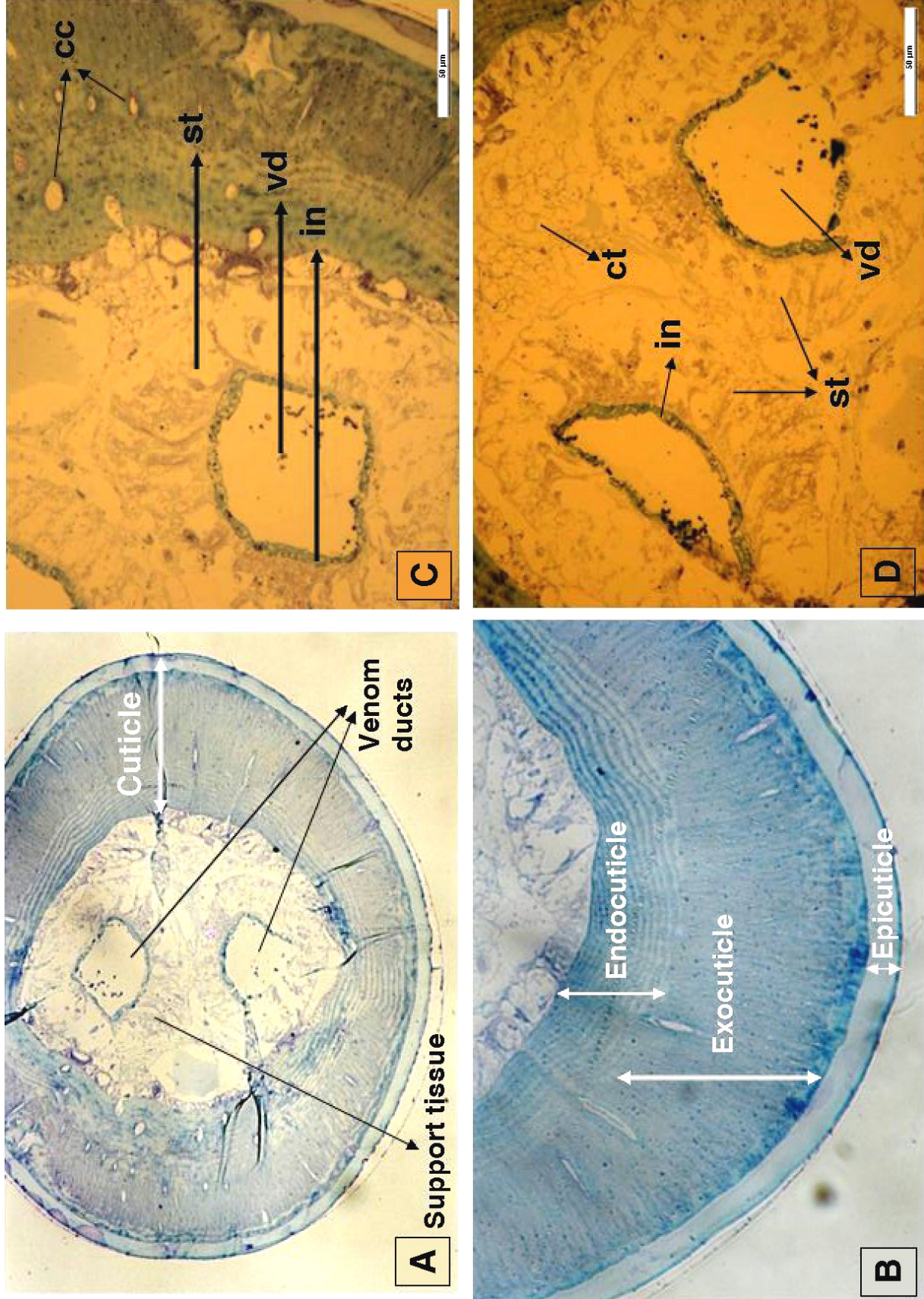


Figure 2: The transverse section of the sting, light microscopy. **A.** Two venom ducts and support tissue surrounded by thick cuticle, x50. **B.** The cuticle of the sting consists of three main layers: epicuticle, exocuticle, endocuticle, x200. **C.** Visible inside the cuticle: numerous hemolymph and chitin channels; a single row of support cuboidal cell layer below the cuticle; chitinous intima covering the venom ducts; and a single row of support tissue cells lying on the intima, x400. **D.** Both venom ducts and support tissue cells lying on their intima, x400.

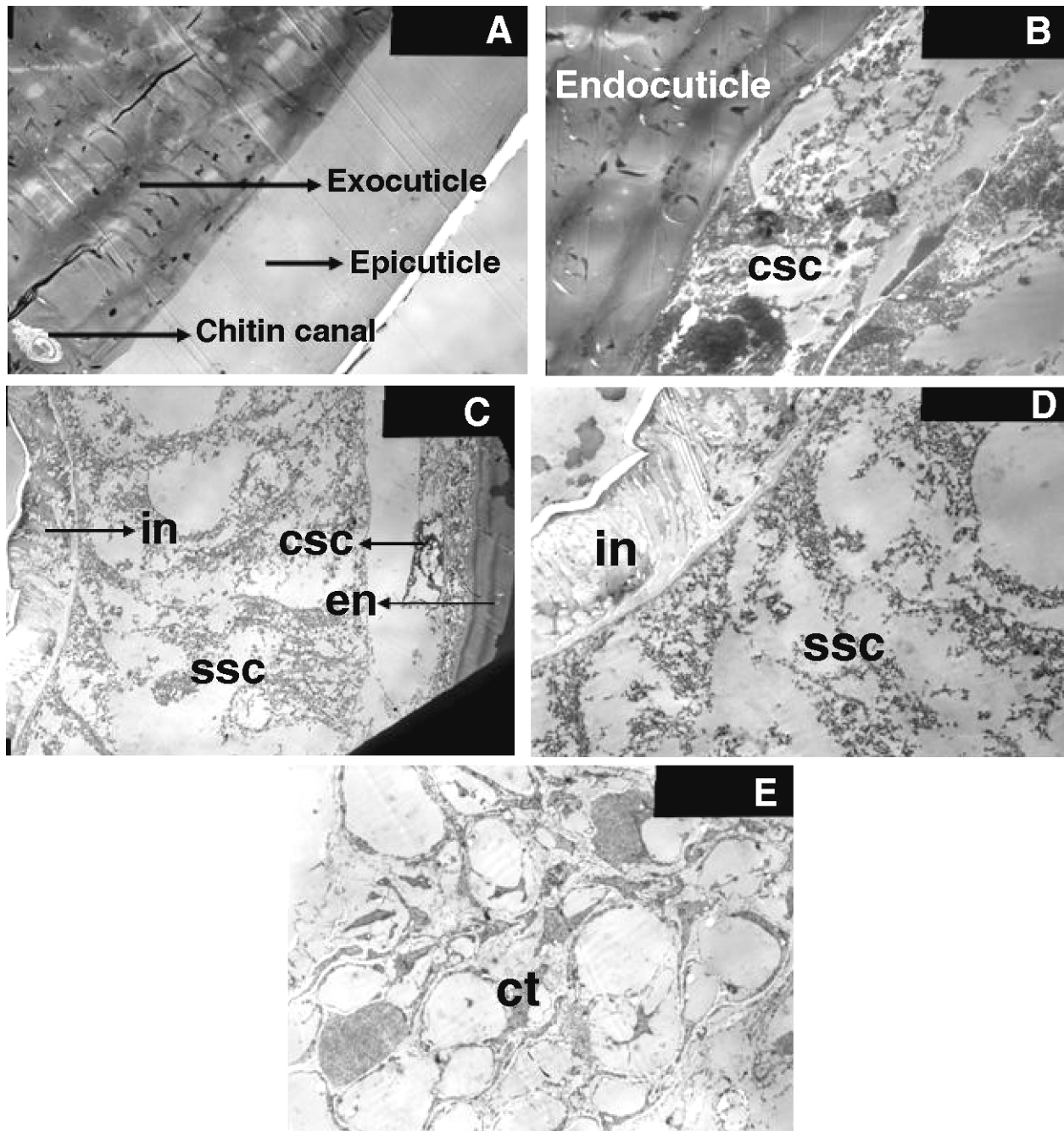


Figure 3: TEM micrographs of transverse sections of sting. **A.** Epicuticle and exocuticle of the three-layer sting cuticle; a chitin channel in the exocuticle, x1,900. **B.** Lamellar endocuticle layer and the underlying single row of cuboidal support cells (csc), x3,600. **C.** Intima, cylindrical support cells, endocuticle, and cuboidal support cells, x1,400. **D.** Intima and cylindrical support cells covering the intima, x2,900. **E.** Connective tissue cells filling the gaps within the sting, x2,900.

Below the thick three-layer cuticle lies a row of cuboidal cell layers, which supports the cuticle and connects it to the underlying tissues. In the internal part of the sting, which has a sparse tissue structure, two venom ducts and cylindrical epithelial support cells that circularly cover these ducts are observed. As seen in both light and TEM micrographs, the support tissue cells

have abundant cytoplasm and scarce organelles. In the sting, three types of cells that support the venom ducts are observed. The first type includes cylindrical cells that cover the intima, an amorphous layer with chitinous material in the internal layer of the venom ducts lumen. The second type includes the cuboidal epithelial cells below the cuticle. The third type is the loose connective

tissue cells that fill the gaps within the sting (Fig. 2C–D, Fig. 3).

The present study reveals that the inner surface of the venom ducts, which are covered by intima, has a cuticular structure. In general, intima is defined as the innermost layer of an organ (especially the inner lining blood or lymphatic vessels, or gland ducts). In the scorpion sting, intima, which has chitinous structure, reinforces the venom ducts because of the absence of a muscle layer in ducts (Figs. 2–3).

The general histology and fine structure of venom glands of several scorpion species have been investigated by several researchers (Pawłowsky, 1913; Samano Bishop & Gomez de Ferriz, 1964; Mazurkiewicz & Bertke, 1972; Kanwar et al., 1981; Taib & Jarrar, 1993; Quiroga et al., 1998); however, the fine structure of venom ducts was not characterized. Thus the further study of scorpion sting morphology and ultrastructure is warranted.

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