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Fine structural analysis of the stinger in venom apparatus of the scorpion *Euscorpius mingrelicus* (Scorpiones: Euscorpiidae)

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ABSTRACT: In this study, the morphology, histology and fine structure of the stinger, a part of the venom apparatus of *Euscorpius mingrelicus* (Kessler, 1874) (Scorpiones: Euscorpiidae) were studied by light microscopy and transmission electron microscopy (TEM). The stinger, located at the end section of the telson, is sickle-shaped. The venom is ejected through a pair of venom pores on its subterminal portion. Both venom ducts extend along the stinger without contact with each other since they are separated by connective tissue cells. The stinger cuticle is composed of two layers. Additionally, there are many pore canals and some hemolymph vessels in the cuticle. This work constitutes the first histological and fine structure study on *Euscorpius mingrelicus* stinger.

KEY WORDS: scorpion, *Euscorpius mingrelicus,* telson, stinger, fine structure, transmission electron microscopy.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

The body of a scorpion can be divided into two separate parts: the cephalothorax (head and thorax) and the abdomen. The abdomen, in turn, can be divided into 12 distinct segments, with the last five segments forming the metasoma, most often referred to as the tail. The tail usually curves upwards and towards the body, and contains venom glands. When a scorpion captures its prey in its claws, it strikes the prey with its tail. At the end of the metasoma is the telson, which is a bulb-shaped structure containing the venom gland and a sharp, curved stinger, which is also known as the aculeus to deliver the venom. The venom of scorpions is used for both prey capture and defense.

Worldwide, scorpion stings are the most important cause of arachnid envenoming and are responsible for significant morbidity and pediatric mortality in many parts of Central and South America, the Middle East, Asia, and both northern and southern Africa (1-7).

Human deaths from scorpion bites normally occur in the young, elderly, or infirm; scorpions are generally unable to deliver enough venom to kill healthy adults. Some people, however, may be allergic to the venom of some species. Although all scorpions are venomous, the most diverse and widespread family, Buthidae, includes the majority of medically significant scorpion species. Despite their fearsome reputation, about 30 species worldwide have venom sufficiently potent to be considered dangerous to human beings (8). The other scorpion species, however, are only venomous enough to affect small vertebrate animals and insects that are their own prey.

In our study, we attempt to describe the histology and fine structure of the stinger of the scorpion species *E. mingrelicus*. While *E. mingrelicus* does not possess venom that is potent enough to be considered dangerous for human health, it has a venom mixture that can affect its prey.

E. mingrelicus is a species belonging to 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) (9, 10). One species has become established in some parts of southern England. *E. mingrelicus* (Kessler 1874) is distributed among many parts of Asia (Georgia, Syria, Turkey, Bosnia, Herzegovina, Bulgaria, Croatia, Greece, Italy, Romania, Russia, Slovenia and

Yugoslavia); within Turkey, this species is widely distributed throughout the Black Sea, Marmara, Mediterranean and Aegean regions (11, 12).

MATERIALS AND METHODS

Scorpions

In the present study, 12 adult *E. mingrelicus* scorpions were used. They were collected from under stones in the Camlidere-Camkoru forest (33ºE, 40ºN, Ankara, Turkey) in September 2005. The scorpions were identified and then reared in special cages where they were fed insects and grasshoppers at the Biology Department of Kirikkale University. The microscopic specimens of the telson, which is the last portion of the metasoma, were removed and studied under a stereomicroscope (SMZ800®, Nikon Instech Co. Ltd., Japan).

Transmission Electron Microscopy (TEM)

The stingers were fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for two hours and then washed four times with buffer, and post fixed in 1% osmium tetroxide (OsO_4) for two hours at +4ºC and then washed four times with buffer. They were then dehydrated in a graded ethanol series (40-100% ethanol). The last stages of dehydration were performed with propylene oxide. After dehydration, the samples were embedded in Araldite® CY 212 (Agar Scientific Ltd., UK) and the araldite blocks were made. Thin sections (60-70 nm) were cut from blocks with glass knives on RMC MT-X® ultramicrotome (Boeckeler Instruments, USA) and mounted on 100-mesh grids. 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 (13).

Light Microscopy

The same araldite blocks prepared for TEM were also used to obtain semi-thin sections (0.5-1.5 µm) for light microscopic examination. Semi-thin sections were cut with glass knives and mounted on slides. The sections were stained with 1% toluidine blue and examined under Leica-DM LS2® light microscope (Leica Microsystems, Germany).

RESULTS AND DISCUSSION

The venom apparatus of *Euscorpius mingrelicus* is situated in the telson and is composed of a pair of venom glands and a stinger. Furthermore, the telson has numerous sensory hairs (Figure 1). Each venom gland has its own venom duct, and these ducts, which carry the venom produced in the venom gland, lie along the stinger starting from the base of the stinger. The stinger, located on the end section of the telson, is in the shape of a sickle. The venom is ejected through a pair of venom pores on the subterminal of the stinger (Figure 1).

In the transverse section of the stinger, both venom ducts can be easily seen by light microscope (Figure 2 – a). The internal layer of the lumen of the venom ducts contains an amorphous layer with chitinous materials called intima. The duct is supported by connective tissue cells and surrounded by a thick cuticle. The gap between the two venom ducts is filled by cells similar to those found in the connective tissue. These cells have a round nucleus and several inclusions. The two venom ducts, separated from each other by connective tissue cells, extend along the stinger without contacting each other (Figure 2 – b, c). The cells arranged on the intima have basal membrane folds and copious mitochondria (Figure 3 – a, b).

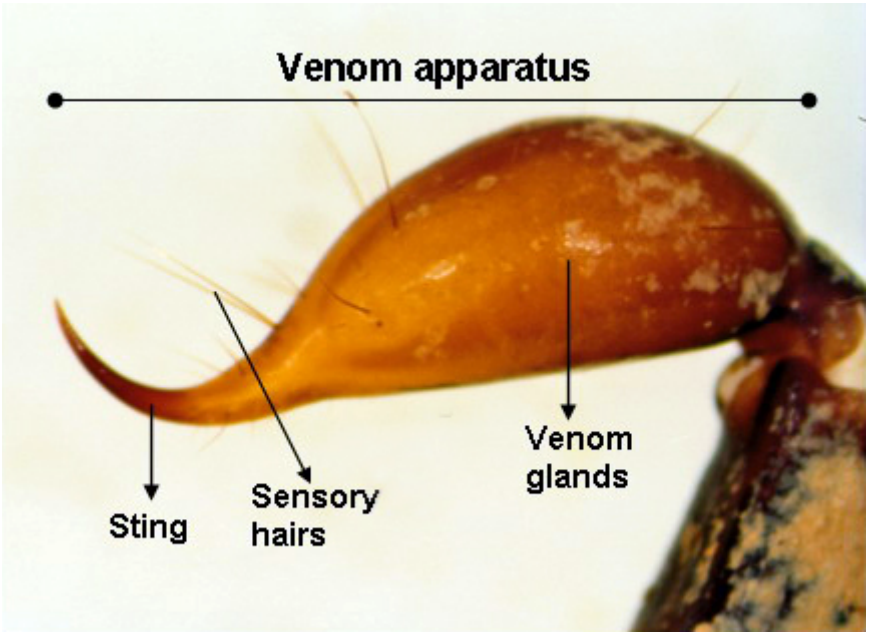

Figure 1. Overall lateral view of the telson of *E. mingrelicus* (taken with a dissecting stereo microscope, 133x).

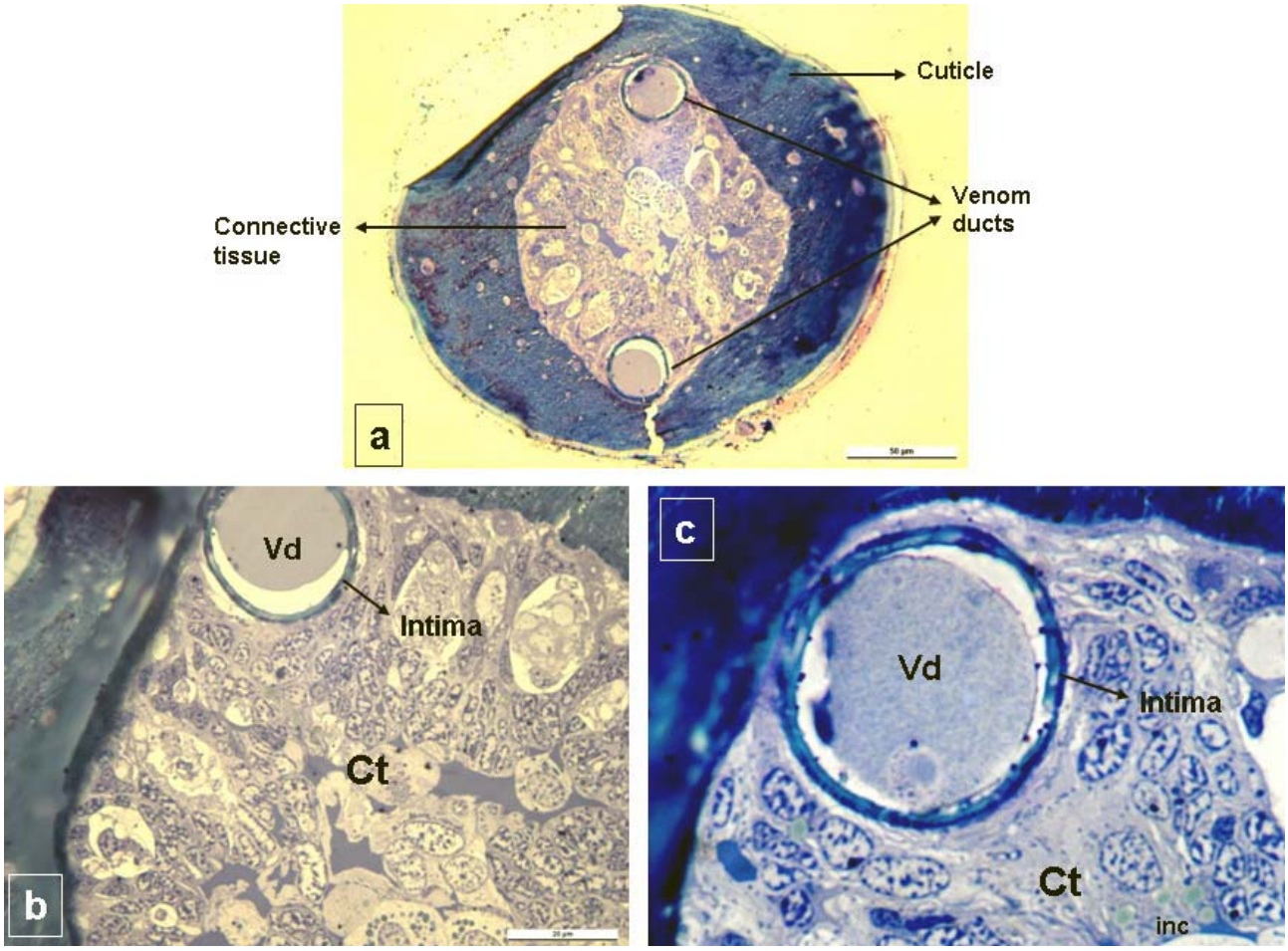

Figure 2. (**a**) The semi-thin transverse section of an *E. mingrelicus* stinger. (**b, c**) Only one venom duct (Vd); the intima covered the venom duct and connective tissue (Ct) cells at two different magnifications. Furthermore, these connective tissue cells contain numerous inclusions (inc) (Figure 2 – c, 10.668x).

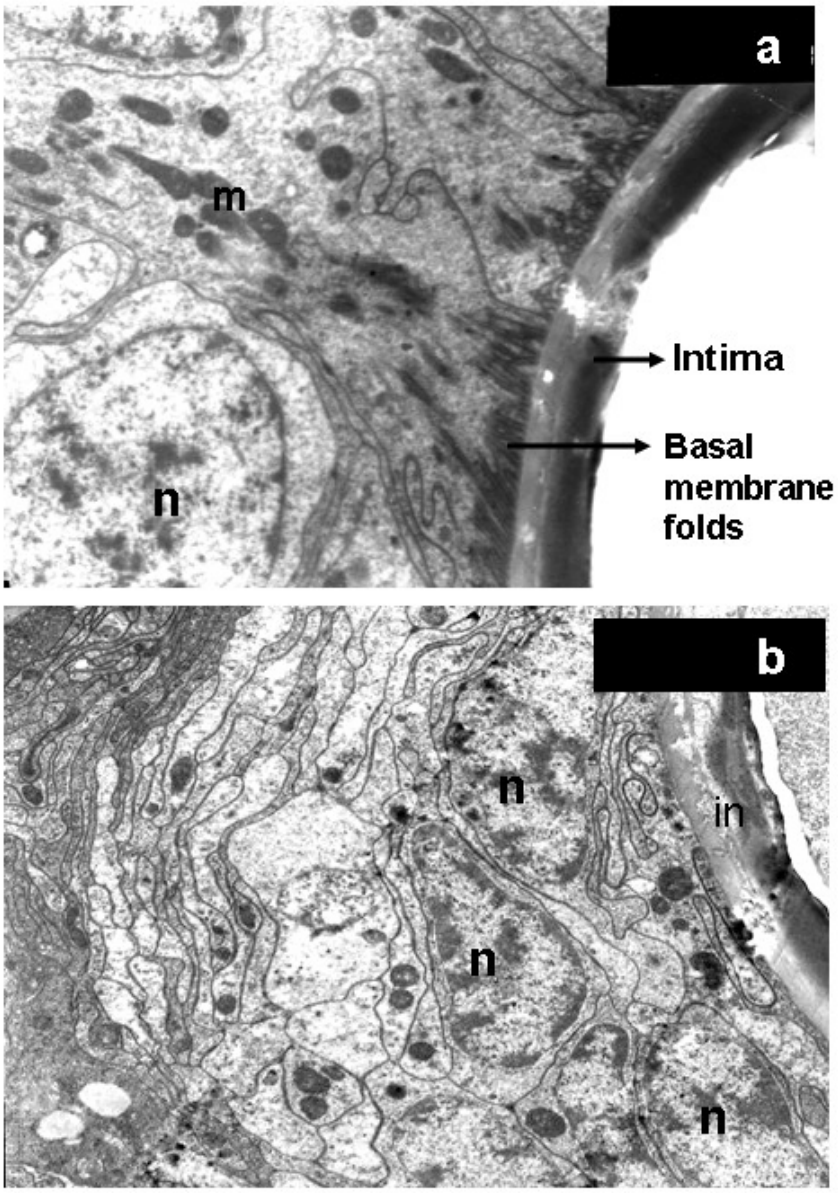

Figure 3. (**a**) The cells are arranged on the intima (in), while their basal membrane folds, nucleus (n) and mitochondria (m) are clearly visible (7.731x). (**b**) The cells surround the venom ducts and intima with chitinous structure, seen at a higher magnification (6.518x). The cells have rich lateral membrane folding and mitochondria.

The stinger cuticle is composed of exocuticle and endocuticle, although the endocuticle of the stinger is thicker than that of the telson. The exocuticle has homogenous structure; the stinger endocuticle is constructed of alternating layers. There are numerous cuticle pore holes that traverse the cuticle layers vertically and terminate at the surface (Figure 4 – a).

Chitin is secreted by epithelial cells then transported to the cuticle surface by chitin channels. Also, there are some hemolymph vessels in the cuticle.

The inner surface of hemolymph vessels is covered with a layer of epithelial cells known as endothelium. These epithelial cells have several microvilli on the parts of the apical surface, which face hemolymph vessel lumen (Figure 4 – b, c).

This region presented defense cells including lymphocytes and macrophages. This cell has undulated cell membrane, pseudopods and groups of microtubules in its cytoplasm (Figure 4 – b, d). Given its structure and morphology, it may be said that this cell is mobile and probably defensive.

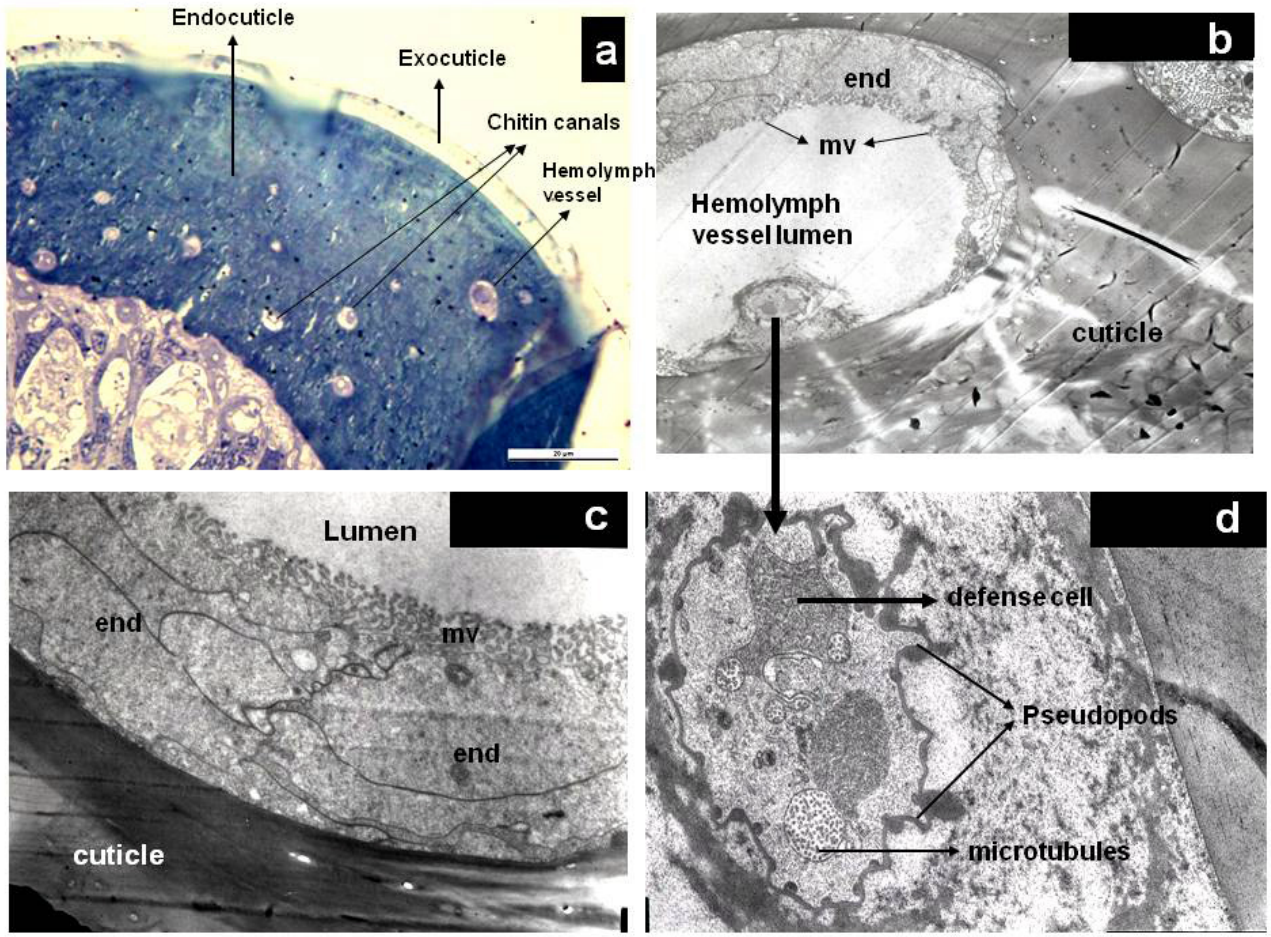

Figure 4. (**a**) The thick stinger cuticle is composed of exocuticle and endocuticle layers. Many pore holes and hemolymph vessels are easily visible in the cuticle. (**b**) A hemolymph vessel in the stinger cuticle, the endothelial cells (end) of hemolymph vessels and numerous microvilli (mv) are arranged on the apical portion of these endothelial cells (3.865x). (**c**) The endothelial cells cover the inner surface of hemolymph vessel situated in chitin while endothelial cells present abundant microvilli (9.818x). (**d**) The vessel lumen presents a type of defense cell. This cell has undulated cell membrane, several pseudopods and the groups of microtubules (2.470x).

Many articles on scorpion venoms, venom chemistry and their medical use are available in the literature (14-19). However, in order to be able to understand the chemistry and physiology of the venom, one should first know the anatomy, histology and fine structure of the venom gland and the venom stinger. The histology and fine structure of the venom gland in several scorpion species have been investigated by several researchers, but the fine structure of venom ducts were not analyzed (20-26).

In our study, the stinger, a part of the venom apparatus of *E. mingrelicus* (Scorpiones: Euscorpiidae), which is a scorpion species belonging to Euscorpiidae family, was studied in relation to its morphology, histology and fine structure. This study is the first work to examine the fine structure of the stringer and will constitute the foundation for future works.

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