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Original Article

Comparative Histological Characterization of the Venom Apparatus in Five Iranian Scorpion Species

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Abstract

Background: Scorpion envenomation represents a significant public health concern worldwide. The telson is located at the distal part of the metasoma, where venom is secreted from a pair of venom glands. The curved stinger is responsible for venom delivery.

Methods: This study conducted a histological examination of five scorpion species, including *Hottentotta juliae*, *Hottentotta zagrosensis*, *Odontobuthus kermanus*, and *Scorpio maurus*, which were gathered from Fars Province, as well as *Hemiscorpius lepturus*, collected from Khuzestan Province. The telson histology was assessed using the hematoxylin-eosin method.

Results: The exocuticle, endocuticle, and secretory epithelium, which consists of secretory and supporting cells, were identified in the tissues of all species. Cuticle pores were distributed throughout the telson. Each venom gland possesses a central lumen, where secreted venom accumulates within a venom sac. The intercalated tendon is located beneath the basal membrane and connects the glandular epithelium to the cuticle. The sizes and shapes of the secretory epithelial cells vary depending on the species.

Conclusion: While histology elucidates the structural organization of scorpion venom glands, integrating histological findings with proteomic and histochemical approaches would provide a more comprehensive understanding of venom composition and interspecific variation.

Keywords: Scorpion; Telson histology; Venom gland; Fars; Khuzestan

Introduction

Annually, millions of scorpion envenomation cases are recorded worldwide (1). Scorpion envenomation can have serious medical consequences for humans, making it a significant public health problem in tropical, subtropical, and developing countries (2). The annual direct non-medical costs incurred by patients suffering from injuries from scorpion stings and snake bites are estimated to be $ 130 Purchasing Power Parity (PPP) and $150 PPP, respectively (3).

According to the latest report, 2868 scorpion species have been identified globally and are classified into 24 families (4). Scorpions inhabit a wide range of terrestrial ecosystems, except Antarctica (5). Due to its diverse climate, Iran is home to a variety of scorpion species, including *Odontobuthus kermanus*, *Mesobuthus eupeus*, *Androctonus crassicauda*, *Compsobuthus matthiesseni* and *Hottentotta juliae* (6).

The arthropod cuticle is a complex and vital body structure indispensable for survival. The cuticle is rigid due to sclerotized proteins and chitin (7). It acts as a protective barrier, shielding soft-bodied insects from dehydration, preventing the invasion of pathogens and parasites and enabling the exchange of gases nec-

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essary for various biochemical reactions essential for proper bodily function (8). The venom glands in the telson are covered by a cuticle, which features several types of pits and sensory setae on its surface (9). The telson at the end of the metasoma (commonly called the tail) contains a pair of venom glands and a curved stinger used for venom delivery. The secreted venom is crucial for prey capture and defense (5). A layer of connective tissue surrounds venom glands. Two types of secretory epithelial cells have been identified in the venom glands: columnar and goblet (10).

As a fundamental area of science, histology provides insights into the microscopic structure of venom apparatuses. The importance of scorpion venom gland histology is the understanding of venom composition and glandular structure. Examining the histology of venom glands enhances our understanding of the synthesis, storage and secretion of these components among species (11).

To date, the histology of venom glands in several scorpion species has been investigated, including *Tityus caripitensis* (12), *Hottentotta tamulus* (13), *Centruroides sculpturatus* (14), *Androctonus crassicauda* (15), *Urodacus novaehollandiae* (10), *H. lepturus*, *S. maurus townsendi* and *O. kermanus* (16). Additionally, several studies have investigated the histology and ultrastructure of scorpion venom glands, including those of *Scorpio maurus kruglovi* (17), *A. crassicauda* (15), *Euscorpius mingrelicus* (18), *Leiurus quinquestriatus* (19), *E. mingrelicus* (20), *Androctonus amoreuxi* (11) and *Mesobuthus gibbosus* (21). In the current study, we conducted a histological investigation of the venom glands of five Iranian scorpions, including *H. juliae*, *H. zagrosensis*, *O. kermanus*, *S. maurus* and *Hemiscorpius lepturus.*

Materials and Methods

Scorpion collection and identification

In July 2020, scorpions were collected from Fars Province (Sepidan and Shiraz County) and Khuzestan Province (Baghmalek County). The species were gathered using diurnal probing from their hiding places, such as under stones, and at night using ultraviolet (UV) black light. All collected specimens were transported alive to the Medical Entomology Laboratory at Shiraz University of Medical Sciences, where they were identified using valid identification keys (22).

Histological preparation

The telson was dissected from each specimen using a sharp blade and immersed for five days in 10% neutral buffered formalin (NBF) (pH 7.4) and 2% calcium acetate (11). The samples were then removed from the formalin solution, thoroughly washed with water, dehydrated, cleared and embedded in paraffin wax (23). The tissues were sectioned with a microtome (MICROM HM 325) at a thickness of 5 µm (micrometers). Finally, the sections were stained using hematoxylin and eosin (H and E) staining methods (24) and they were examined for their general features under a light microscope (Nikon E200 Eclipse) connected to a Sony video camera. The histological structure of the venom glands was carefully examined to identify and describe their main anatomical and cellular components. The observed structures included the cuticle, lumen, secretory epithelium, muscle bundles, cuticle pores, intercalated tendons, exocuticle, outer cuticle, endocuticle, inner cuticle, simple columnar cells, secretory granules, nucleus, nucleus of muscle fibers and basal lamina.

Results

The analysis of histological cross-sections of the telsons from five dominant scorpion species illustrated the spatial organization of their venom systems. The cellular structure investigation revealed that in all studied species, the venom glands were surrounded by a cuticle composed of two layers: the exocuticle (also known as the outer cuticle) and the endocuti-

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cle (also known as the inner cuticle). The telson endocuticle consists of lamellar layers of chitin, while the exocuticle is a homogeneous and transparent layer, as observed in all investigated species (Fig. 1A–E).

A basal lamina, comprising two layers of cuboidal cells with round nuclei, was clearly visible between the cuticle and the secretory epithelium in *H. zagrosensis* and *H. juliae* (Fig. 1B, D). Although presumed to be present in all species, the basal lamina was not distinctly observable in the sections of *O. kermanus*, *S. maurus* and *H. lepturus*.

Muscle fibers are organized into muscle bundles, with the nuclei of the muscle fibers clearly visible in the cross-section of *H. lepturus* (Fig. 1C). In the cross-sections of the other species, muscle bundles are present but not clearly discernible.

Cuticle pores pass vertically through the endocuticle layer, terminating at the endocuticle–exocuticle junction in *H. zagrosensis*, *S. maurus* and *O. kermanus* (Fig. 2A–C). Cuticle pores are generally present in the endocuticle layer; however, in our study, they were not distinctly visible in the histological sections of *H. juliae* and *H. lepturus*.

The two venom glands, symmetrically located on both sides of the telson, have similar shapes and sizes. A lumen is at the center of each venom gland and thick muscle bundles separate the glands. The structure of the venom glands, lumen location, and muscle bundles are shown in the cross-sections of *O. kermanus*, *H. zagrosensis*, *H. lepturus* and *S. maurus* (Fig. 3A–D).

The muscle bundles surrounding the secretory epithelium are strongly connected to the telson cuticle by intercalated tendons, as shown in the cross-sections of *H. lepturus*, *H. zagrosensis*, *O. kermanus*, *S. maurus* and *H. juliae* species (Fig. 4A–E).

The glandular epithelium consists of two types of cells: secretory cells and non-secretory supporting cells, the latter of which are folded on the inner side, as illustrated in the cross-section of *H. zagrosensis* (Fig. 5). The secretory cells are of the apocrine type, simple columnar, and filled with granules, as characterized in the cross-sections of *H. zagrosensis* (Fig. 5) and *H. juliae* (Fig. 6B). Numerous secretory granules of varying sizes and shapes were observed in the apical part of the cells near the lumen in the cross-sections of *O. kermanus*, *H. juliae* and *S. maurus* species (Fig. 6A–D).

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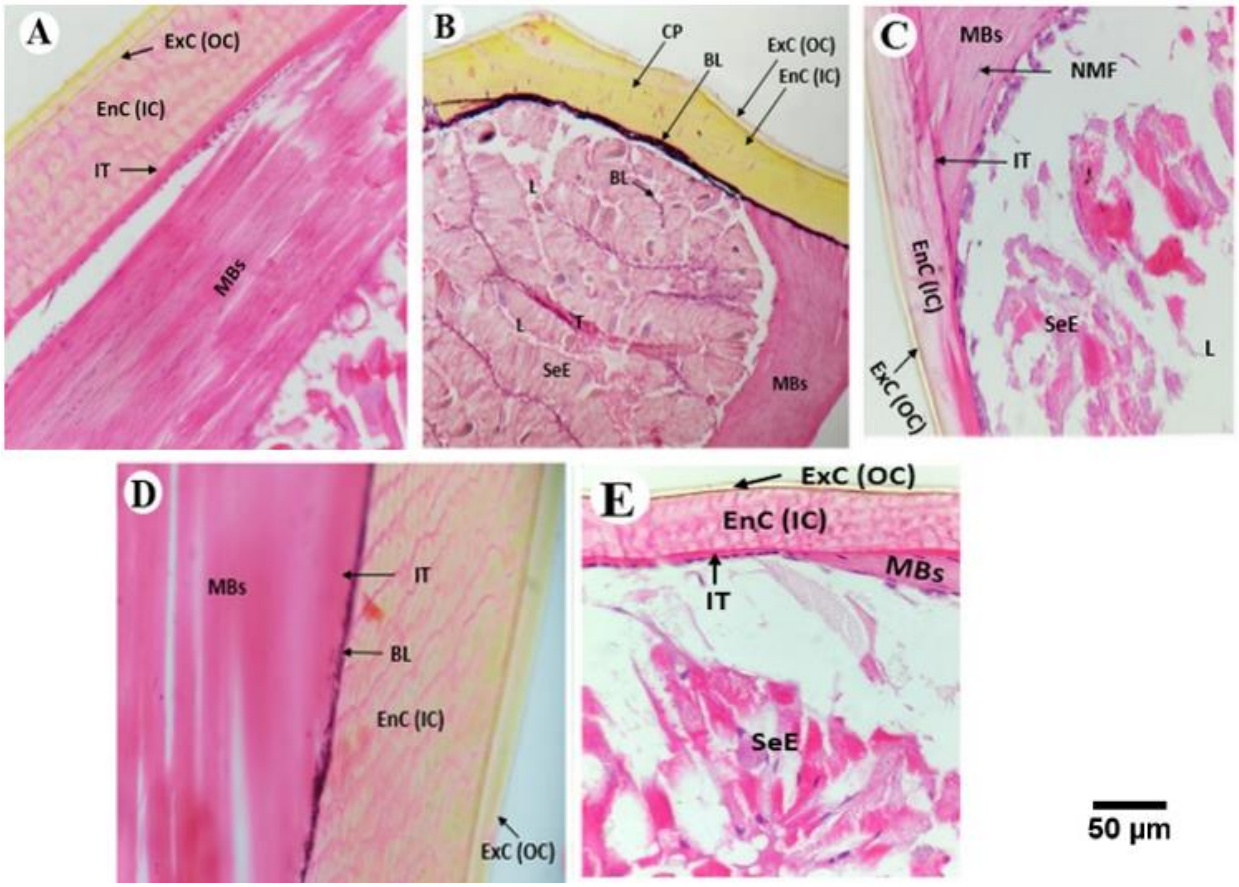

Fig. 1. The venom gland apparatus Endocuticle (EnC), Exocuticle (ExC) structure, and the basal lamina (BL) between the cuticle and the secretory epithelium (SeE), intercalated tendon (IT) and basal lamina (BL) which attach the secretory epithelium (SeE) to the cuticle (C), and nucleus of muscle fiber (NMf) of muscle bundles (MBs) in cross-section of five species (A) *Odontobuthus kermanus*, (B) *Hottentotta zagrosensis*, (C) *Hemiscorpius lepturus*, (D) *Hottentotta juliae*, and (E) *Scorpio maurus*. (Magnifications ×40 and ×100)

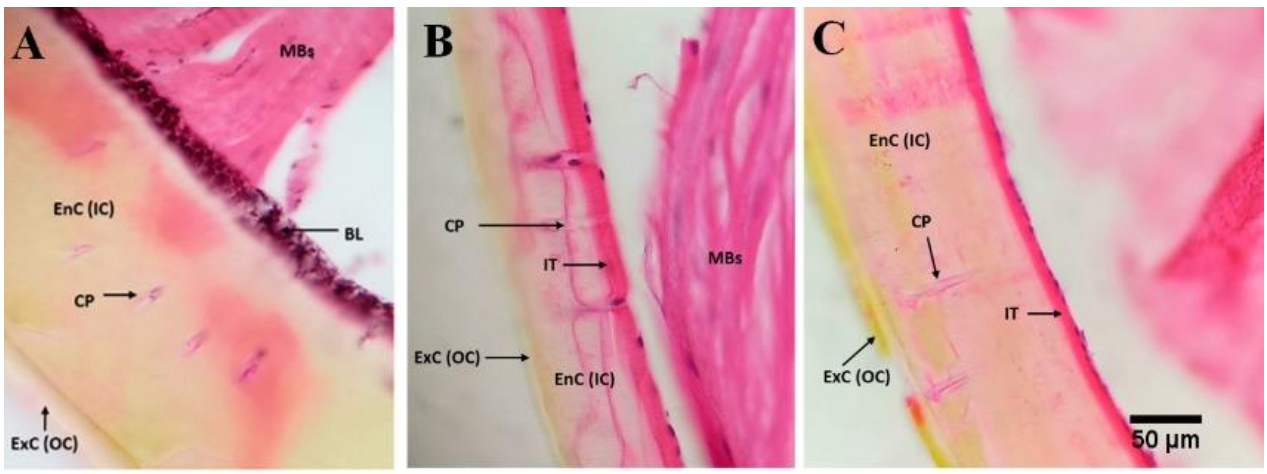

Fig. 2. The cuticle pores (CP) that overpass the cuticle (C) layers vertically and end at the Endocuticle (EnC) –Exocuticle (ExC) jointing in the cross-section of (A) *Hottentotta zagrosensis*, (B) *Scorpio maurus*, and (C) *Odontobuthus kermanus.* (Magnifications ×40 and ×100)

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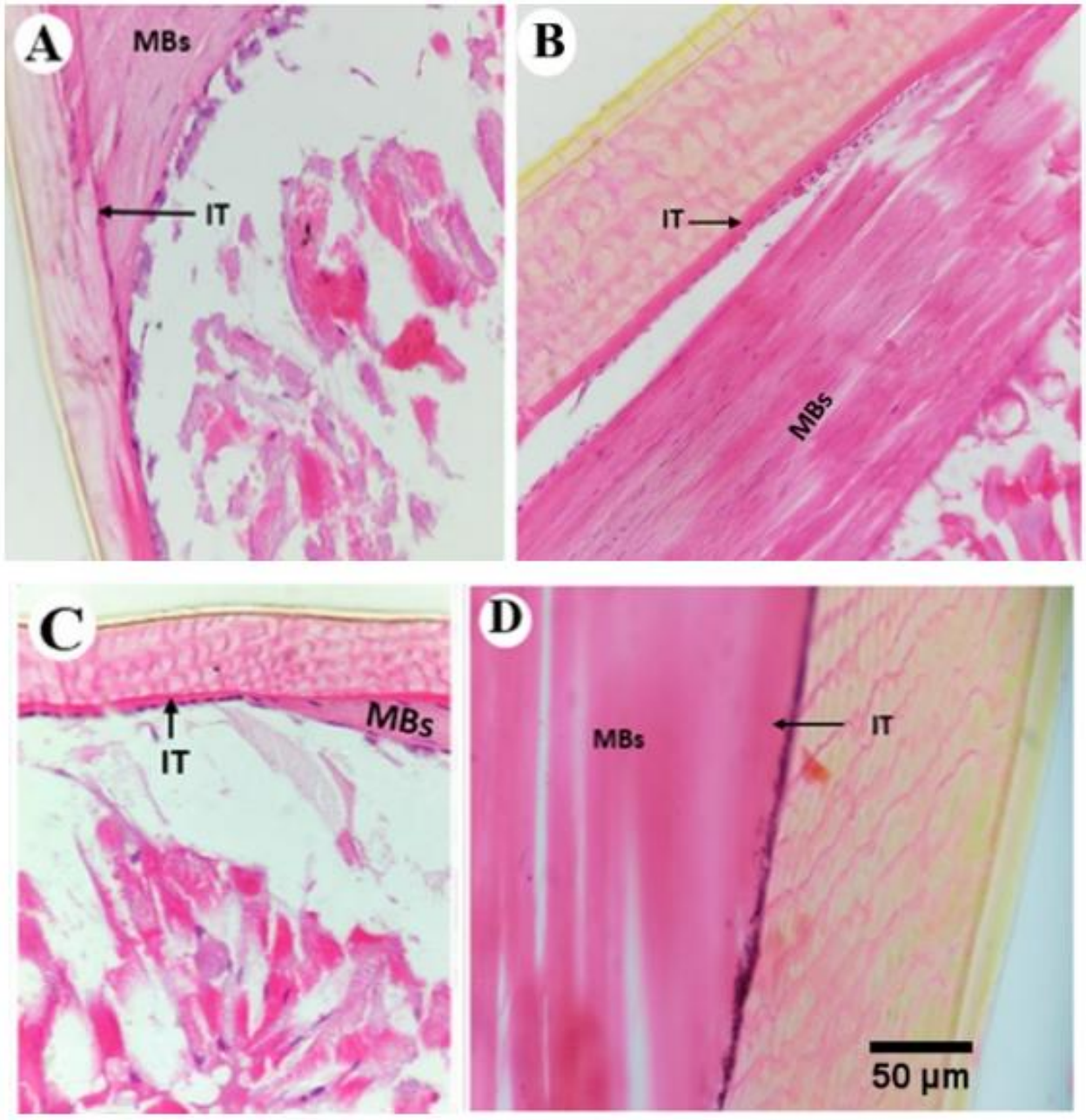

Fig. 3. The position of venom glands, which are located symmetrically on both sides of the telson, lumen (L) at the center of each venom gland, and muscle bundles (MBs) which separate two venom glands in cross-section of (A) *Odontobuthus kermanus*, (B) *Hottentotta zagrosensis*, (C) *Hemiscorpius lepturus*, and (D) *Scorpio maurus*. (Magnifications ×40 and ×100)

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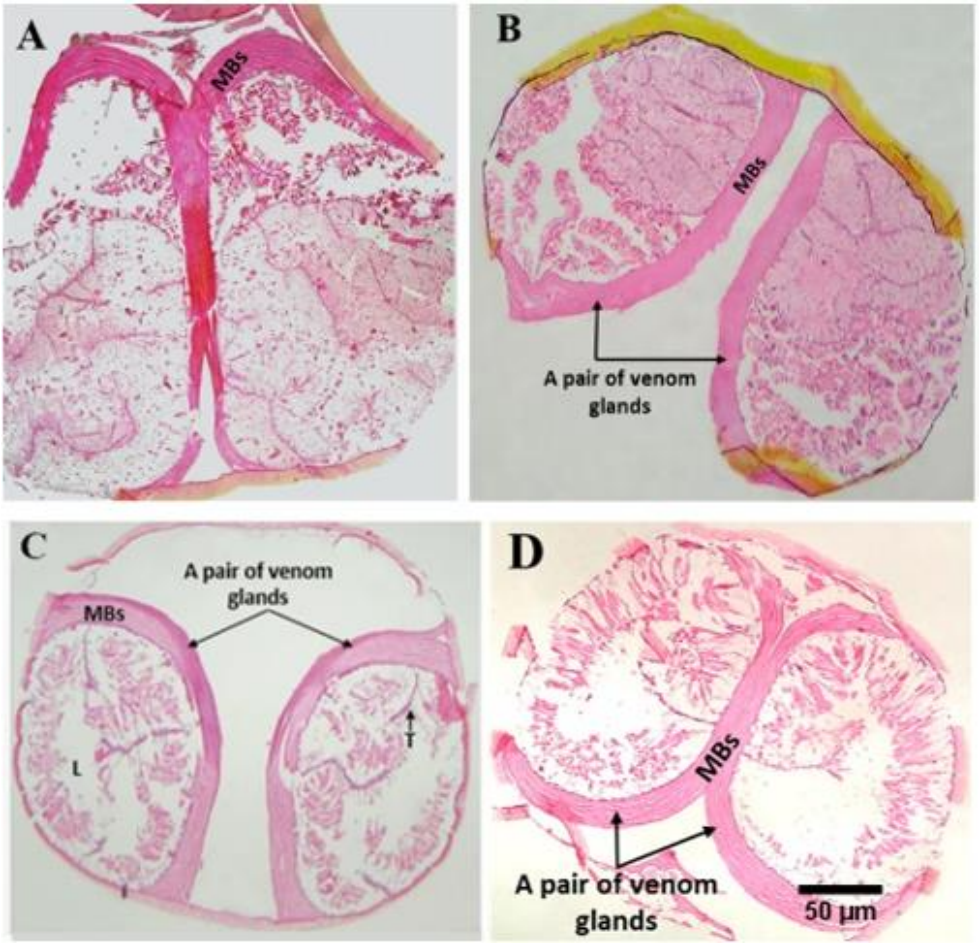

Fig. 4. The muscle bundles (MBs) all over the secretory epithelium (SeE) are strongly connected to the cuticle (C) of the telson by an intercalated tendon (IT) in the cross-section of (A) *Hemiscorpius lepturus*, (B) *Odontobuthus kermanus*, (C) *Scorpio maurus* and (D) *Hottentotta juliae*. (Magnifications ×40 and ×100)

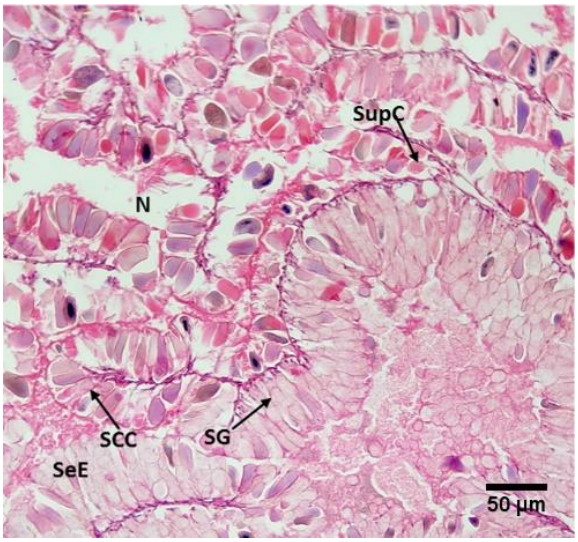

Fig. 5. Simple columnar cell (SCC) and non-secretory supporting cells (SupC), which make secretory epithelium (SeE) in the cross-section of *Hottentotta zagrosensis*. (Magnifications ×40 and ×100)

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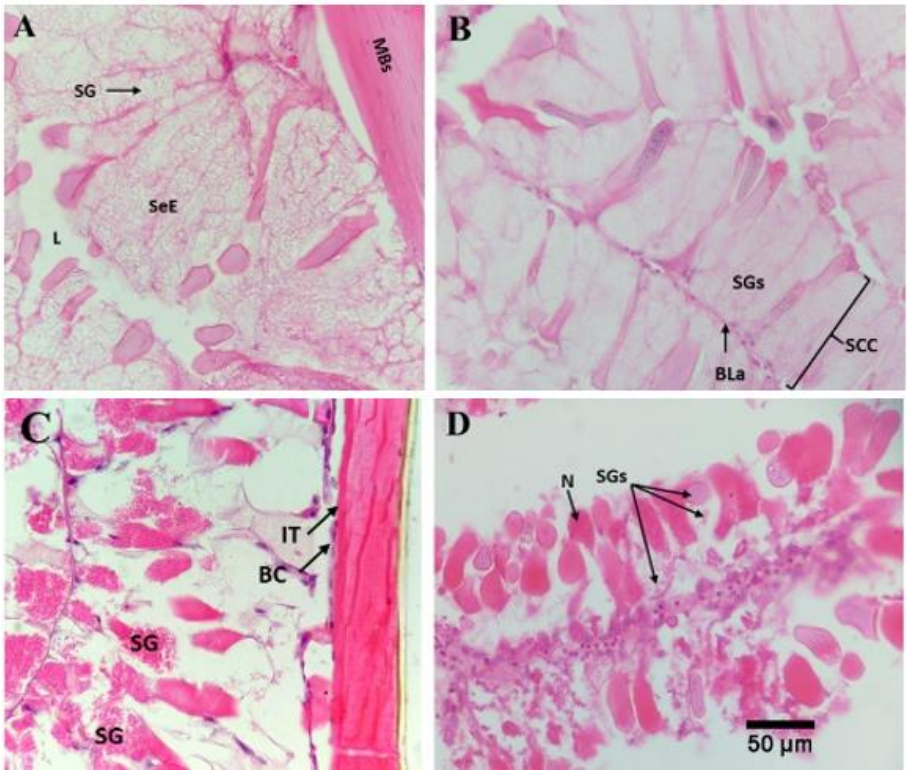

Fig. 6. Secretory granules (SG) in different sizes and shapes in cross-section of (A) *Odontobuthus kermanus*, (B) *Hottentotta juliae* and (C and D) *Scorpio maurus*. (Magnifications ×40 and ×100)

Discussion

In the current study, *H. lepturus* and *S. maurus* were collected from different regions compared to the previous survey of Navidpour et al. in 2018 (16). The histology of *H. zagrosensis*, *O. kermanus* and *H. juliae* was characterized for the first time. In this study, the structure of the cuticle layers was consistent with the findings from histological studies on *S. maurus kruglovi* in Latifawa (Erbil City/ Iraq) (17) and *C. sculpturatus* (14).

The cuticle pores were observed in *H. zagrosensis*, *S. maurus* and *O. kermanus*. In *H. juliae* and *H. lepturus*, however, pores were not detected, potentially due to technical limitations. The visibility of pores depends on section orientation and histological processing; sections not perpendicular to the surface may obscure them and fixation or staining can cause pores to collapse, shrink, or stain poorly, making them difficult to detect. Previously, cuticle pores have been detected using a scanning electron microscope in *Buthus martensii* (25), *B. quinquestriatus* (26) and *E. mingrelicus* (20) scorpion stingers. The pore canals, connecting the apical plasma membrane of epidermal cells to the cuticle surface, facilitate the modification and transport of lipids, primarily to the cuticle. Moreover, the canal walls exhibit esterase activity, which likely contributes to wax synthesis (27).

As we illustrated in *H. lepturus*, *H. zagrosensis*, *O. kermanus*, *S. maurus* and *H. juliae*, the intercalated tendon and basal lamina serve to anchor the glandular epithelium to the cuticle; a similar structure of venom glands has been reported in *Urodacus novaehollandiae* (12), *A. amoreuxi* (11) and *E. mingrelicus* (18). The intercalated tendon and basal lamina secure the glandular epithelium to the cuticle, providing structural support and maintaining tissue integrity. The muscle fibers of the muscle

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bundles in scorpions play a crucial role in the motor system and the movement of the telson muscles for delivering a defensive sting (28).

As shown in the cross-section of *H. zagrosensis*, both non-secretory supporting cells and secretory cells were present. But in other species studied, supporting cells were not clearly visible. Likewise, two types of epithelial cells have been reported in *Pandinus imperator* (29), *U. novaehollandiae* (30), *Centruroides vittatus*, *A. crassicauda* (15) and *Centruroides limpidus* (31). However, some researchers believe that only one type of secretory cell exists in scorpions' venom glands (32). On the other hand, three types of secretory epithelium cells were characterized in *B. martensii* (31) and *L. quinquestriatus* (19). According to Taib and Jarrar (21), glandular epithelial cells can be divided into at least five types based on their contents. As we showed, Ahmad also characterized the simple columnar cell in the cross-section of *H. lepturus* in *S. maurus kruglovi* in 2015 (17). These differences likely reflect species-specific functional adaptations in venom production and secretion mechanisms.

Numerous secretory granules of varying sizes and shapes were observed in all five species studied, indicating that scorpion venom comprises a mixture of diverse compounds. However, *O. kermanus* exhibited a higher number of secretory cells within the glandular epithelium, along with a more complex cellular organization and extensive folding compared to the other species. These structural features increase the gland's surface area, enhance secretory capacity and facilitate efficient venom storage and release. Overall, the pronounced structural complexity of the venom gland in *O. kermanus* suggests a highly developed and efficient venom-secreting system relative to the other examined scorpions.

Conclusion

Histological examination provides valuable information about the structural organization and cellular composition of scorpion venom glands, but has limitations in revealing the molecular diversity of venom components. Therefore, integrating histological analysis with proteomic approaches is useful for a comprehensive understanding of venom synthesis, composition, and functional diversity among scorpion species. On the other hand, differences in venom gland histological structure and secretory cells among scorpion species may be better revealed using other histological and chemo-histological techniques.

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Ethical consideration

No human or animal data or tissues were utilized in this research. All experiments adhered to ethical principles as well as national regulations and standards for conducting medical research in Iran. The study received approval from the Iranian National Committee for Ethics in Biomedical Research (Approval ID: IR. SUMS. REC.1400.029).

Conflicts of interest statement

The authors declare there is no conflict of interest.

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