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Determining with SEM, structure of the venom apparatus in the tube web spider, *Segestria florentina* (Araneae: Segestriidae)

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ABSTRACT

The objective of the present study was to describe functional morphological features of venom apparatus in the tube web spider, *Segestria florentina* (Rossi, 1790) (Araneae: Segestriidae) by using scanning electron microscope (SEM). The venom apparatus is situated in the anterior part of the prosoma, and is composed of a pair of venom glands and chelicerae. The chelicera of *S. florentina* has two parts: basal segment and a movable articulated apical segment (fang). The cheliceral fang rests in a groove on the basal segment of chelicerae. A venom hole is located on the subterminal part of each fang. A pair of venom glands is completely separate but similar to each other within the prosoma. Each venom gland is surrounded by striated muscle bundles, such as with the capsules. The venom, produced in the venom glands, is carried by venom ducts passing throughout the chelicerae. Each venom gland has its own venom duct, chelicera and fang. The venom is excreted from the venom pore on the subterminal part of the fang by means of muscular contractions covering the venom gland.

Keywords: Spider, *Segestria florentina*, Venom gland apparatus, Morphology.

1. Introduction

With the exception of some groups of spiders, all spiders have a venom apparatus and produce venom. Their venom is toxic to insects which are usually their prey. Most spider venom does not threaten human health, except for children and elderly people. Spiders are predators, and that is why the primary purpose of spider venom is to kill or paralyze prey. The venom targets the nervous system. The major targets of these toxins are ion channels on the cell membranes [1]. The venoms of spiders are made up of complex mixtures of biologically active and inactive substances, similar to the venoms of snakes and scorpions. The major constituents of spider venom are protein, polypeptide, polyamine neurotoxins, enzymes, nucleic acids, free amino acids, monoamines and inorganic salts [2]. Proteases, hyaluronidases, sphingomyelinases, phospholipases and isomerases have been reported in spider venoms [3,4,5]. Spider venoms can be divided into two major groups according to their effect being either neurotoxins or necrotoxins. Neurotoxins, affecting both insect and mammalian nerve systems, are a constituent of spider venom. Necrotoxins are defined as toxins which induce tissue necrosis at the site of envenomization. The venom apparatus contains venom glands which produce the venom and structures which inject venom into the prey [6,7,8]. The venom apparatus of spiders consists of a pair of chelicerae and venom glands. The shape and position of the venom gland is different in various species. In large tarantulas (mygalomorphs) the venom glands are quite small and lie inside the chelicerae. In contrast, most labidognath spiders have relatively large venom glands that may extend out of the chelicerae and reach far into the prosoma [6].

There are many studies on venoms of various spiders. In recent years, some studies have reported that spider venom has been used in medical treatment of some diseases and also in pest control [9,10,11,12,13]. There is a little knowledge on functional morphology of venom apparatus, however. The objective of the present study is to describe the functional morphology of the venom apparatus of *Segestria florentina* that is placed on the list of venomous spiders and is known as toxic and dangerous to human beings in Turkey. *S. florentina* bites are sharp and painful; they cause some epidemiological problems. The bites may be fatal especially for the children and elderly people. *S. florentina* is widely distributed throughout Europe to Georgia [14]. Some common names for this spider are tube web spider or cellar spider.

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2. Materials and Methods

Ten adult females of *Segestria florentina* were collected from the cavities of almond trees in Muğla (a city in the Aegean Region from Turkey = 37° 13.2'N; 28° 21.0'E) in September 2006. In order to dissect, spiders were narcotized with ether. The carapax was gently removed, and the venom glands and chelicerae were taken as specimens for electron microscopic scanning under a stereo microscope (Nikon SMZ800).

These specimens were fixed in 3% glutaraldehyde for two hours and then washed four times in sodium phosphate buffer (pH 7.2), post fixed in 1 % osmium tetroxide (OsO₄) for 2 hours at a temperature of +4 °C, and then washed four times in sodium phosphate buffer. They were dehydrated in a graded ethanol series (from 40% to 100% ethanol) [15]. The specimens were dried and coated with a thin layer of gold by Polaron SC 502 sputter coater. The materials were examined at an accelerating voltage of 10-20 kV with a Jeol JSM 6060.

3. Results

The organization of the venom apparatus of *Segestria florentina* follows the general architecture of venom apparatus of the other venomous spiders. The venom apparatus is situated in the anterior part of the prosoma, and is composed of a pair of venom glands and chelicerae. Each chelicera consists of two parts: basal segment

(Paturon) and a movable articulated apical segment (fang). The whole body is covered with hairs but, the basal segments of the chelicerae are very sturdy and strong hairs. Chelicerae of the dorsal region have long hair whereas Chelicerae of the ventral region have hair short and sparse as a local. In addition, there are four rows of hairs which are located to both sides of the outer side of the marginal teeth and parallel to marginal teeth. There is groove between the cheliceral marginal teeth. Fang closes over the groove. Fang is also connected with joints to basal segment. 2 marginal teeth are on one side of the groove, while the other side has 3 marginal teeth. These teeth are used for holding the prey (Fig. 1). Spider venom is discharged through the fang. The fang tip narrows and becomes sharp to serve the purpose of the ejection function. The fang has been formed specifically to allow penetration. The cuticle fang tip has a corrugated structure and there are saw teeth on one side of the fang. The remaining cuticle extending to one joint region is homogeneous, and has a flat structure. The sting of the corrugated cuticle penetrates the prey. The saw Tooth helps penetration process to prey, so better and easier to be able to penetrate in prey (Fig. 2). A venom pore is located on the subterminal part of each fang through which the venom is ejected (Fig 1, 2).

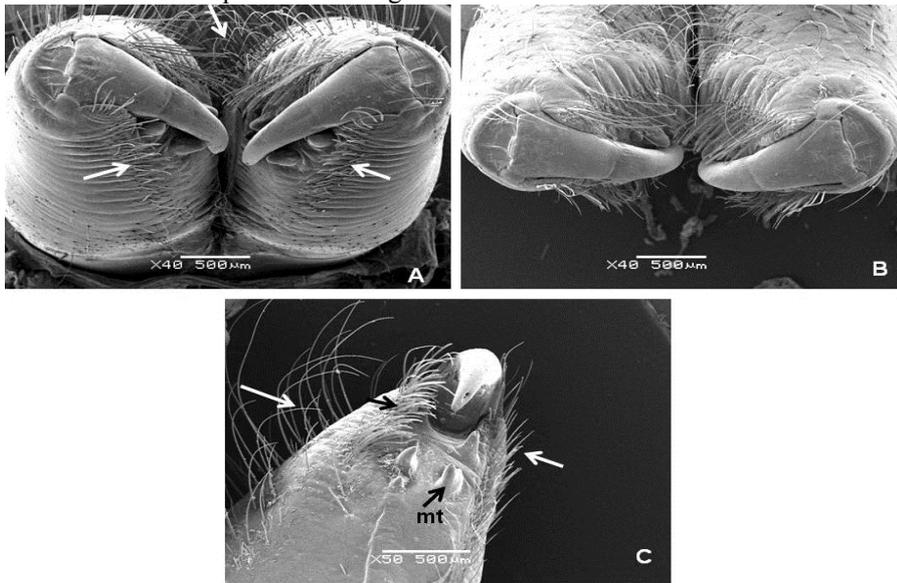


Figure 1: A) A pair of spider chelicerae, Long hairs are locally arranged on one side of chelicerae, Short and sparse hairs are arranged on the other side of chelicerae. Four rows of hairs are seen. The hairs are on the outside of the marginal teeth, and extend in parallel with the marginal teeth. B) EM image of a pair of spider's chelicerae was taken from a different perspective. C) Fang open state; the marginal teeth are in the edge of chelicerae groove (one side of the marginal teeth (m) 2 and 3 on the other side), cheliceral groove is smooth cuticular structure and hairs are arranged as parallel to the cheliceral groove.

Each pair of venom glands is completely separate but similar to each other within the prosoma. The venom glands are equal in size, and they are bulb-shaped. Each gland is surrounded by a bulky muscular layer (Fig 3). These gross muscle bundles cover the venom gland spirally. Venom is produced in the venom glands, carried by venom ducts passing through the chelicerae, and exits from the venom pore during muscular contractions.

Spiders have to move fast to catch and discharge their poison into prey. Therefore each of the venom glands needs to eject the venom quickly and this is accomplished by the sudden contraction

capability of the surrounding muscle tissue. The muscle tissue is abundant in nerve fibrils, making sudden voluntary contractions possible. Muscle contractions are rapid and consistent as a result, and the prey is neutralized quickly by the spider.

The venom glands were also examined with SEM by cutting along the longitudinal axis. A wide lumen was found at the center of the venom glands. There is a row of secretory epithelial cells around the lumen, and on outside the venom gland is surrounded by a layer of striated muscle. More detailed analysis of secretory cells has shown that these cells have been distinguished as having different

morphologies than secretory granules. Some secretory granules were shown as small granules, but some granules were formed by a combination of small granules that were dense and were formed into macro granules. The presence of these secretory granules of

heterogeneous structure of spider venom is an indication of a heterogeneous mixture (Fig 4).

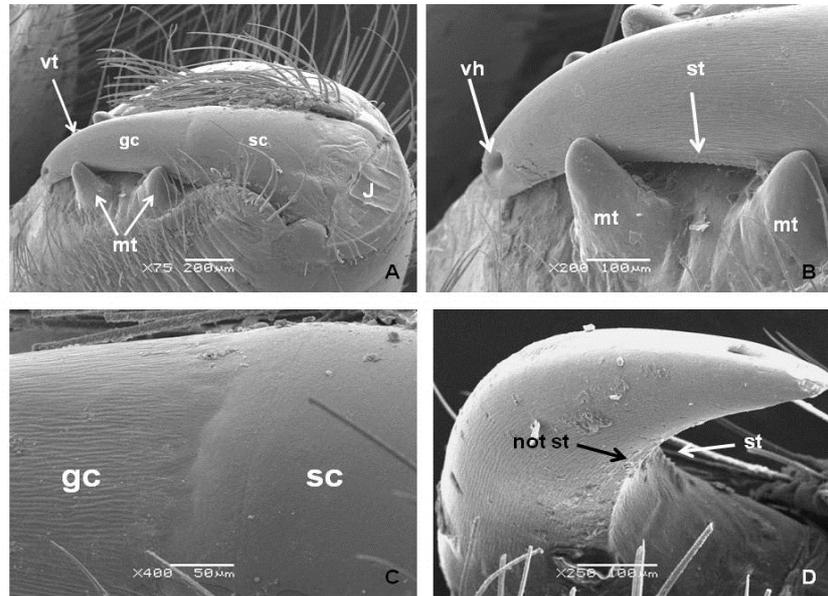


Figure 2: A) Fang, on the closed state cheliceral groove, venom teeth (vt) are connected with joints (J) to basal segment, tapering towards fang-to-end, the groove cuticle (gc) on the fang extends to the end of the saw teeth, between the saw tooth and joint are cuticle smooth (sc) B) Between two marginal tooth (mt) are seen the saw tooth (st) and there is venom hole (vh) at the end of the fang C) Region of junction on the fang groove and smooth cuticle D) The side of fang is not a saw tooth (not st) .

4. Discussion

All spiders except for the families Uloboridae and Holarchaea possess venom glands situated in the prosoma. The positions of the fangs on chelicerae in spiders allow spiders to be divided into two suborders: Orthognatha and Labidognatha. It has been noted that Orthognath and Labidognath spiders move their chelicerae in quite different manners. Orthognatha fangs are parallel to each other and

to the longitudinal axis of the body. Labidognatha fangs inject venom perpendicular to the longitudinal axis of the body [16]. We have determined that *S. florentina* is a labidognath spider. The morphology of the venom apparatus of *S. florentina* is basically similar to those of the other labidognath spiders [16,17,18].

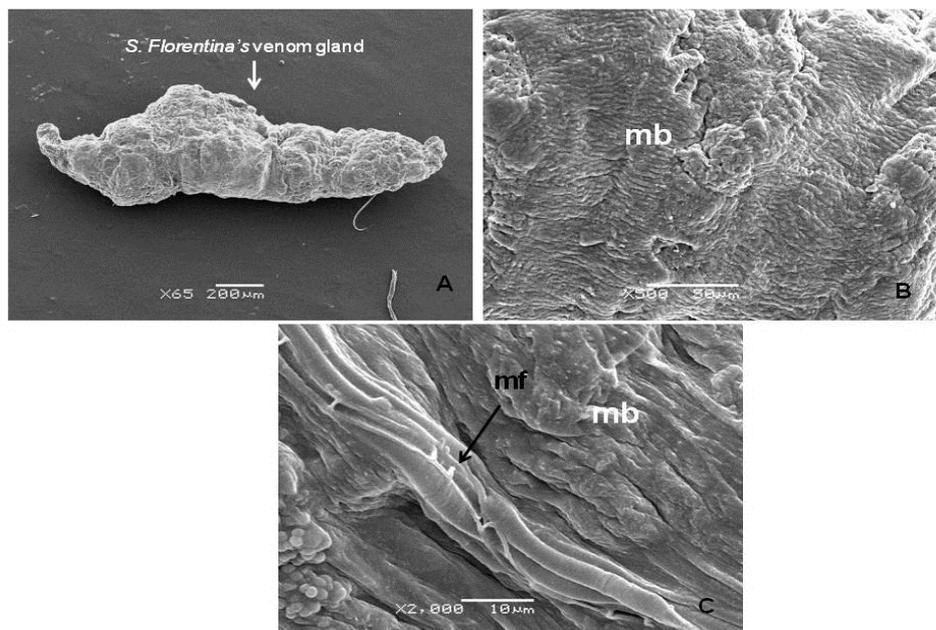


Figure 3: A) The venom gland of *S. florentina* is shaped like a bulb and covered by a bulky muscle layer B) Distinctive muscle bundles (mb) at higher magnification C) The nerve fibers (nf) among the muscle bundles.

The chelicerae of spiders are connected to venom glands, and are used to inject venom into prey. Venom is generally injected into prey via a duct in the fangs, which in large spiders can be strong enough to pierce a human fingernail ^[19]. Fang-morphology is remarkable and facilitates the injection of venom. As a result of a detailed examination by SEM, we found that *S. florentina*'s fang

narrowed at the end, becoming sharp and pointed. There is a groove structure located on the tip of the fang cuticle. In addition, saw teeth are located on one side of the fang, and serve a cutting function during penetration of prey. In addition, the groove structure of the fang reduces the friction force, making penetration easier.

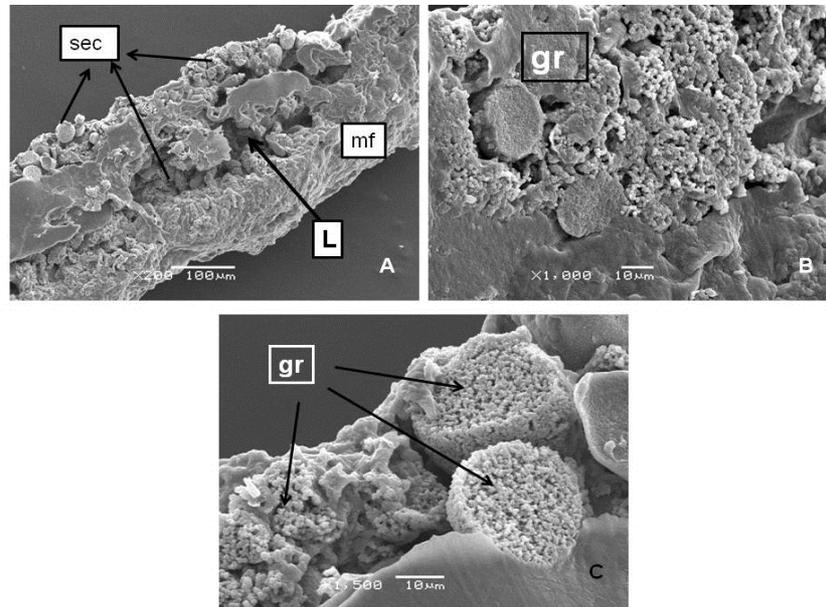


Figure 4: A) Image of venom glands is prepared by cutting along the longitudinal axis. A wide lumen (L) in the center of the gland, secretory epithelial cells (sec) around the lumen and outermost layer of striated muscle B-C) the secretory granules (gr) of venom have different sizes and images.

As the chelicerae of spiders are hinged where each chelicera meets the cephalothorax and also where the fang joins the basal segment, the fangs of spiders can be directed easily into a flat surface, such as the palm of a human hand ^[19]. When Fang of *S. florentina* is examined we see that it is connected with a joint to the basal segment. With this structure fang closes on groove between the cheliceral marginal teeth as a closed pocket knife blade. In addition, while on one side of cheliceral groove has two marginal teeth; on the other side has a 3 marginal teeth.

These teeth are used for holding the prey. As most spider species have their own pattern of cheliceral teeth ^[20,21], the number and shape of these cheliceral teeth plays an important role as a taxonomic character ^[6,17].

In our study, fang removed and examined. Fang at the bottom of the cuticle and was observed as a flat structure.

Yet another type of spider *Nephila clavata* has many small protrusions on the surface of the cheliceral groove ^[19].

The venom glands of *S. florentina* are paired structures located in the prosoma that communicate with the outside through two ducts that lead into the fangs. Most labidognath spiders including *S. florentina* have relatively large venom glands that extend out of the chelicerae and reach the middle of the prosoma. Kovoov and Munoz-Cuevas ^[22] described the structure and histochemistry of the spiders poison glands in *Lycosa tarentula* (Lycosidae), four *Peucetia* species and *Oxyopes lineatus* (Oxyopidae). All these species show two voluminous gland sacs situated dorsally in the prosoma, over the nervous system. The shape of the venom glands is different in various species of spiders: bulbous in *Loxosceles*

intermedia ^[2], carrot-like in *Plesiophrictus collinus* (Theraphosidae), and sac-like or cylindrical and consisting of two lobes in *Heteropoda venatoria* (Heteropodidae) and *Lycosa indagatrix* (Lycosidae) ^[24], whereas the venom glands of *S. florentina* are bulb in shape.

The venom glands are equal size and surrounded by bulky muscular layer. In *L. intermedia*, the external muscular bundles are web-like ^[23]. However, in *S. florentina* and other studied spiders' species such as *A. labyrinthica* ^[17] and *L. ixobulus* ^[18] the spiral blocks of muscle bundles has surrounded the glands like a capsule. This is very noticeable the muscle bundles. In our study, we found that its venom gland is surrounded by a tight muscle bundle. Spiders capture to their prey with a rapid movement, and must inject quickly venom into the prey. Also, in parallel, the venom, produced venom glands must give out of the poison quickly. For this purpose, around venom gland needs the ability of a sudden contraction of muscle tissue. Because of these reasons, venom gland, which is surrounded by a thick layer of muscle is very tight muscles and has a strong structure.

The contraction of the relatively strong muscular layer must be controlled by the strong nerve system. When any stimulate is received by the spider, a stimulant by means of nerve cells is transmitted to venom glands. In this way, the produced venom mature is given out to venom gland lumen. Then, venom is carried by venom ducts passing throughout the chelicera by the contraction of gland muscles. Consequently, venom is given out of the venom pores.

The venoms of spiders are made up of complex mixtures of biologically active and inactive substances [2,4,5]. Our studies have shown that within the venom gland secretory cells have complex structures that have granules of different size and appearance. These heterogeneous secretory granules show that the spider's venom is a complex mixture.

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