New Vision for Structure of Posterior Salivary Gland of *Octopus vulgaris* from the Red Sea, Egypt

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ABSTRACT

The posterior salivary gland of *Octopus vulgaris* is apocrine and has a discrete character in the animal kingdom. In general, studying the evolution of any toxic organism typically starts with an examination of its defense system, including the minute structures with similar organisms across various phyla in the animal kingdom. The posterior salivary gland of *Octopus vulgaris* consists of a thick gland wall with a contraction function for pushing the toxin through the gland duct and into the pass tube, which opens in a buccal mass. The posterior salivary gland of *Octopus vulgaris* secretes toxic saliva, which is thermostable liquid resistant to heat and has a darkened blue appearance when stained with bromophenol blue, more pronounced than the venom. The present gland consists of nine types of cells associated with a canal that transports the toxin from the posterior to anterior salivary glands. These glands are located in the middle of the gland and consist of a mixture of circular and longitudinal muscle fibers, respectively. The secretion of the present gland is considered analogous to the evolutionary progression from octopus to the cobra snake. In the cobra snake, there are two types of cells with thick walls that aid in the contraction of the gland to push the toxin outside the mouth. In octopuses, two small cells represent an early evolutionary stage observed in glands across the animal kingdom, as recorded in the earliest records.

INTRODUCTION

Octopus vulgaris defense system is the first qualified and complete system in the animal's kingdom, appearing in the shape of cells and structure of glands which is never unclear for qualified researchers for toxicology. The best study for toxicology and evolution process in toxic animals starts with a study of the structure of the defense system through all toxic animals' phyla **(Scheffer, 1991; Savitzky *et al*., 2012; Brady *et al*., 2017; Oziolor *et al*., 2020; Cerda, 2023)**. The octopus is renowned for its comprehensive defense system, which includes remarkable swimming abilities. Despite lacking a traditional skeleton, its hydrostatic structure enables agile and fast movement, distinguishing it within the animal kingdom. Toxicologists try to find the relation between the defense system and the evolution process, as documented in the literature, which may sometimes appear reversed. The recent literature indicates that toxicity in the same phylum has no different types or species between the same individual in the same

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phylum (**Ueda *et al*., 2006; Kiriake *et al*., 2013; Kiriake *et al*., 2014; Saggiomo *et al*., 2021; Brighton Ndandala *et al*., 2023**). The posterior salivary gland consists of a parenchymatic wall and muscle medulla, all composed of connective tissue to facilitate the contraction of gland secretion outside the gland. Moreover, there are nine types of cells, all of which secrete the toxin. Some types of cells are also used to store the toxic saliva, composed of tetradotoxin. The gland is composed of thick parenchyma and is similar to the anterior salivary gland, with small secretion granules scattered inside. This feature is common in *Octopus vulagis* (**Martin, 1971; Matus, 1971; Barlow, 1972; Ebd Elrheem, 2022**). The secretion of this gland and toxicity among toxic animals was defined about 125 years ago. However, there is a common misconception that only *Hapalochlaena maculosa*, a type of octopus, possesses toxicity. All feeding and environment are similar and the escaping of predators requires good adaptation, including shape, toxicity, inking, colorations, and finally coordination of the cartilaginous skeletal system with movement (**Ebd Elrheem, 2022, 2023a, 2023b**). The secretion of present gland is unique among animals, as it contains both anterior salivary glands that secret venom, while the posterior salivary gland secrets tetradotoxin in all members of the Octopoda class within the phylum Mollusca. The different types of secretion make animals more suitable for their environment. The fast acting secretion of the venomous anterior salivary gland is used for quickly incapacitating prey, while the secretion of the posterior gland provides protection against large predators. The cell types and shapes look resemble those found in toxic animals across the animal kingdom. This suggests a low level of differentiation, indicating a shared evolutionary process driven by adaptation to diverse environments. The secretions of the posterior salivary gland can immobilize prey for an extended period, providing protection against large-sized attackers. However, during feeding, the same secretions are used to quickly incapacitate prey (**Matus, 1971; Martin *et al*, 1972; Oziolor *et al*., 2020**). The amino acids composition inside the saliva indicate the presence of tetradotoxin in all types of octopuses, as revealed by sequence investigation, thus all previous secretions contain tetradotoxin. Staining with different types of stain types is very useful for the demonstration of all secretion types of these glands, and for understanding the natural structure of the cells. Younger octopuses exhibit lower toxicity (allometric toxicity) than adults, indicating a maturation process corresponding to secretion. This suggests that as animals progress in the feeding process, they become increasingly self-reliant and better suited to their environments (**Gibbs *et al*, 1978; Raimundo *et al*., 2008; Moniz, 2022; Liu *et al*., 2023**). This work aimed to demonstrate of secretion of this gland and provide evidence of its structure, particularly focusing on previously undescribed structure.

MATERIALS AND METHODS

Sampling

Samples of *Octopus vulgaris* were collected in March from one site on the Western coast of the Red Sea. This site is located 17km south of Safaga City (latitude 26° 38' N longitude 33° 59' E). The collection site is on rocky shores and samples were collected from the intertidal zone at the time of low tide. The collection was done by hand, and the samples were packed up. Each

specimen was collected and put in plastic containers containing seawater. The specimens were narcotized by adding menthol crystals (El-Naser Chemicals Company in Egypt) to the water surface of the jar and allowing them to relax. Specimens dissected in the field to get the studied organs were fixed and put in Bouin's solution for 24 hours for histological preparations.

Histological studies

Organs sectioned were cut off from the body and placed into Bouin's solution in seawater for 24 hours. Fixed parts were then passed to the graded series of alcohol from 30 to 100%. They were cleared in toluene three times each for 5 minutes then embedded in paraffin wax. Sectioning was made by microtome at 5- 7μm thickness. Moreover, sections were stained with the following stains:

- Harris hematoxylin and eosin combination (H&E) **(Harris, 1900)**.
- Mercuric bromophenol blue for demonstration of general proteins **(Mazia *et al.,* 1953)**.
- Periodic acid Sheaf's reaction (PAS) for the demonstration of polysaccharides in various cells and tissues **(McManus, 1948)**.

The slides were dehydrated through an ascending series of ethanol after staining. They were then passed through xylene to mounting medium and covered with coverslips.

RESULTS

The shape of the *Octopus vulgaris* in the Red Sea is typical of the species and exhibits the same characteristics as the models shown in Fig. (1).
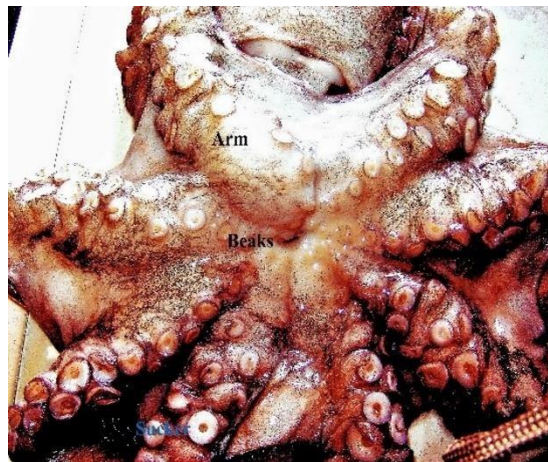

Fig. 1. The body plan of a marine *Octopus vulgaris* (Cuvier, 1797). (Ventral view)

Octopus vulgaris has a complete defense system including both the anterior salivary gland and posterior salivary gland. Both have a good contact point above the first stomach and below the buccal mass with direct contact by a pair of ducts connected the two glands. The gland consists of a wide, thick wall similar to previous animals and is composed of connective tissue

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parenchyma. It contains gland pressure cells (GPC), which are present in the beginning stages and are more advanced compared to those found in cobra snakes. Also, type A (T.A) cells secrete the mucus (Mu) to facilitate the passing of toxic saliva through paired ducts of the glands. The gland is supplied with blood sinus to provide adequate blood flow, enabling gland enlargement (Fig. 2A, B, C, D, G, H, I, J). The second type of cells (T.B) are commonly observed as free-form cells and their function may involve supporting the parenchyma or having a free function (Fig. 2C). The fourth type of cells (T.C) exhibit a mixed shape, ranging from rounded to flattened, and serve multiple functions based on their structure as observed. The primary function is the secretion of the mucus (Mu), while the secondary function is the secretion of the saliva (Fig. 2D). The fifth type (T.E) exhibits a structure similar to the anterior salivary gland structure in *Octopus vulgaris*. These cells are commonly scattered throughout the gland parenchyma and secrete saliva. They are differentiated and stained by bromophenol blue stain (Fig 2D, I). The sixth type of cell T.F) appears as irregular in shape and performs mixed functions, including transporting saliva and secreting mucus (Mu) exclusively. These cells are stained by basic stains as H&E, but slightly. This indicates low acidophilic granules, as shown in Fig. (2E). The seventh type (T.G) appears completely rounded and is involved in transporting saliva to facilitate rapid defense. Similar to the previous type, it is lightly stained by basic stains (Fig. 2F). The eighth type (T.H) is also for transporting, but it is characterized by thick connective tissue within the cell. This type is commonly found in glands to supports the gland's transport function (Fig. 2G). The pair of ducts also make contact with both the anterior and posterior salivary glands, with the contact site referred to as the gland duct (GD) in this study. This duct is responsible for transporting the entire gland secretion from the posterior salivary gland to the anterior (Fig. 2H). The nine types of cells (T.I) are rounded and scattered with the parenchyma, but they are positioned deep. They secret saliva (S) and appear rounded and fan-shaped. These cells are deeply stained blue at the sites of salvia secretion by basic stains such as H&E (Fig. 2H). Saliva cells (S) are visible in the section and are completely stained by basic stains (H&E) and bromophenol blue stain. Additionally, mucus secretion is observed in this gland, as shown in all previous figures (Figs. 2H, I, J).
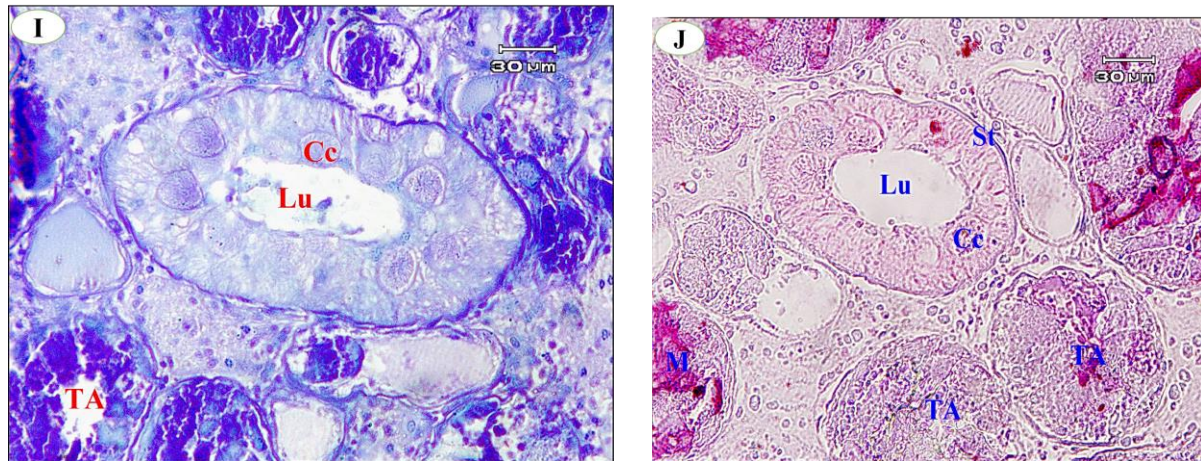
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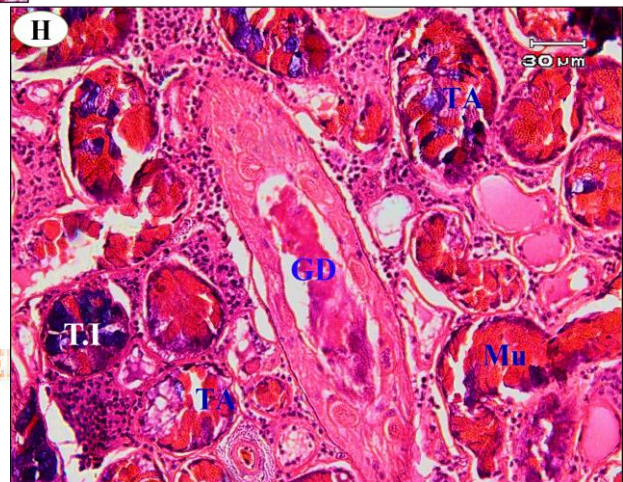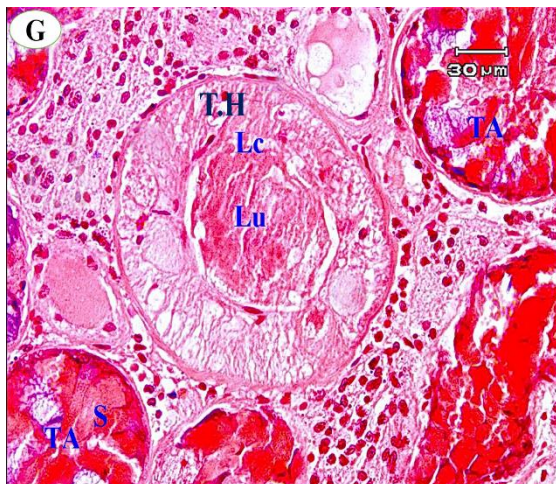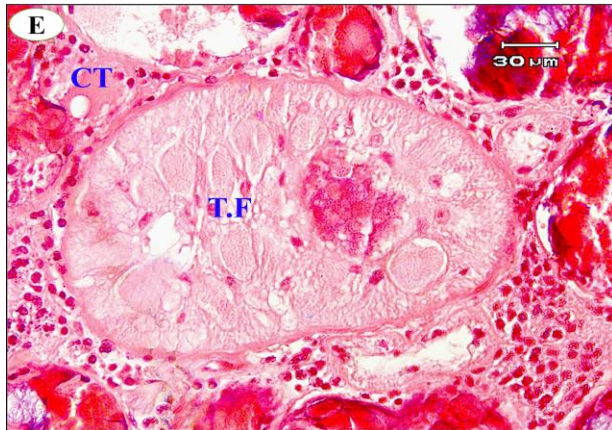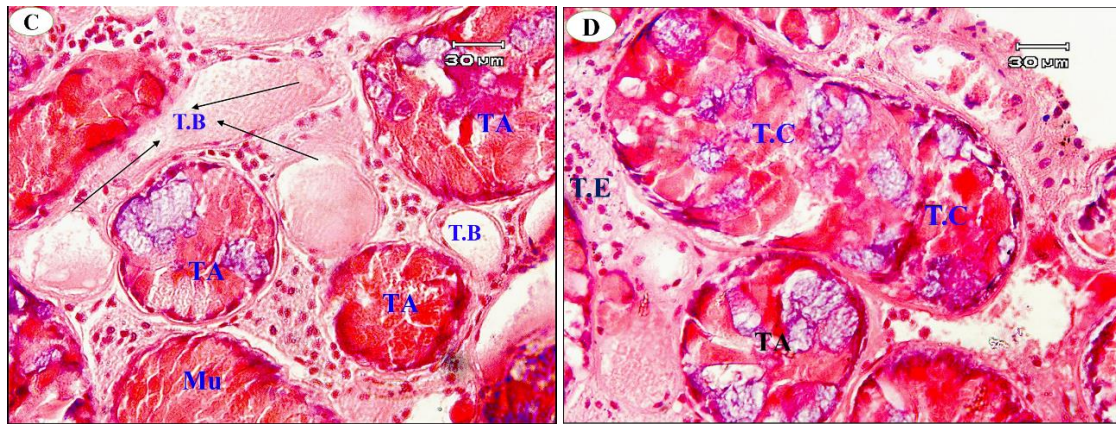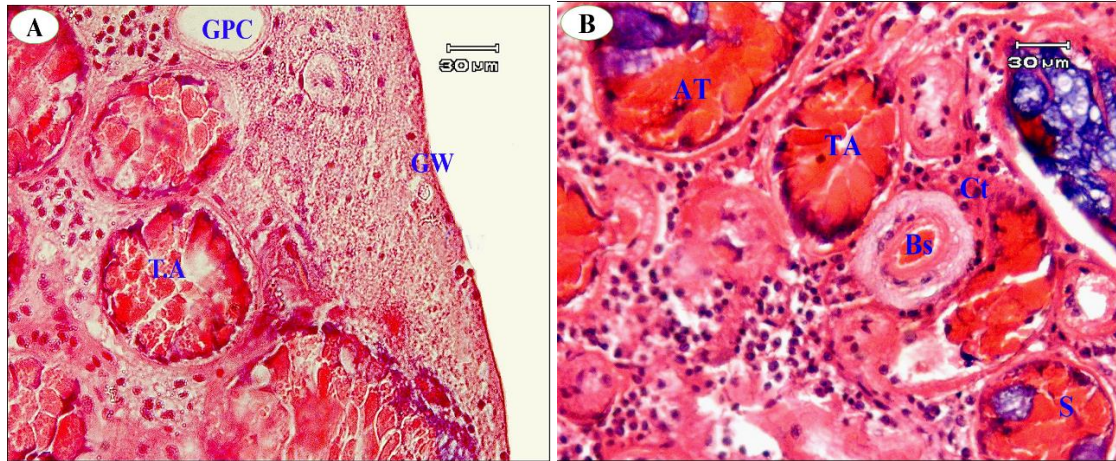
Fig. 2A- J. All images are taken at high magnification (30- 100μ) and depict the structure of the posterior salivary gland of *Octopus vulgaris*. **A- H** stained with H&E show various types of cells including T.A, T.B, T.C, T.E, T.F, T.G, T.H, and T.I, as well as features such as Bs (blood sinus), GW (gland wall), C (connective tissue between cells), GB (gland parenchyma), GD (gland duct), and S (saliva). **(I)** stained by protein stain (Bromophenol blue stain) shows both types T.A and T.E, which secrete proteins. **(J)** stained by periodic acid chief (Carbohydrates stain) highlights mucus secretion sites in T.A cells

DISCUSSION

The posterior salivary gland of *Octopus vulgaris* exhibits a very advanced structure, which aids in reducing predation from larger prey animals. The structure of this gland is mentioned in those literatures, such as **Matus (1971)**, **Martin *et al*. (1972)**, **Moustafa and Awaad (2016)** and **Elrheem (2022)**. The types of gland cells and the structure of their parenchyma are similar to the structure of the anterior salivary gland of *Octopus vulgaris* (**Abd Elrheem, 2022**). **Pagella *et al*. (2014)** discussed the structure and functions of different cells similar to those found in glands. The stain with bromophenol blue is positive in some cells, indicating their contents and structural nature. The mucus is positive for periodic acid stain, while the protein secretion gives a negative reaction by the same stain, in accordance with the basic science principle. The allometric phonemon is common in the animal kingdom for giving the relations between two changing parameters (**Gibbs & Greenaway, 1978; Raimundo *et al*., 2008; Moniz, 2022; Liu *et al*., 2023**). The development of animals in defense system is opposite to the development of animals during evolution, leading to a relationship that is commonly reversed (**Ueda *et al*., 2006; Kiriake *et al*., 2013; Kiriake *et al*., 2014; Saggiomo *et al*., 2021; Brighton Ndandala *et al*., 2023**). The evolution of the defense system through the animal kingdom is differentiated by position in the evolution site, thus the octopu*s* occupies an advanced position in the systematic hierarchy by their defense system.

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CONCLUSION

The aim of this study is to accurately describe the structure of the posterior salivary gland and to elucidate its features using various staining techniques, with the objective of identifying any differences between our findings and those of previous studies. This study elucidates the common cells that have not been previously described, providing evidence for all previously identified cells using various staining techniques.

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