ISSN: 2319-5967
ISO 9001:2008 Certified
International Journal of Engineering Science and Innovative Technology (IJESIT)
Volume 2, Issue 5, September 2013

Ultrastructure of the salivary gland of second in star larvae of Cephalopina titillator (Diptera:Oesteridae)

1Afaf Abdel-Meguid,2Duaa Musleh 3*Nancy Taha, ,
1Entomology, Faculty of Science, Cairo University, Cairo, Egypt.
2Entomology, Faculty of Science, Cairo University, Cairo, Egypt.
3Department of Zoology and Entomology, Faculty of Science, Helwan University

Abstract: ***Cephalopina titillator is an obligate parasite that cause nasopharyngeal myiasis in camels. In the present study, the salivary gland consists of a pair of simple long translucent tubules situated laterally in the fore gut region which lies ventrally in the body cavity. A thin short and narrow efferent duct emerges from the anterior of each gland and then unites together to form a translucent single median common deferent duct, which is inserted in the dorsal cephalopharyngeal skeleton. Their apical part projecting into the lumen of the gland, the nucleus is oval and lies basally in the cell. The lumen is narrow usually full of secretary vacuoles and secreted material. Large secretary granules are observed within cells., the cells of the salivary gland possess well-developed long compact microvilli,thick well-developed basement membrane with basal lamina, Rough endoplasmic reticulum is well developed has parallel cisternae mostly near the nucleus and is dilated at the basal and apical region of the cell. Many secretary vacuoles are visible near the base of the microvilli and in the basal part of the cell.***

Key words: **Ultra structure, Salivary gland, nasalbotfly, diptera.**

I. INTRODUCTION

Nasopharyngeal myiasis of camels is caused by the larvae of Cephalopina titillator, an obligate parasite of the Oestridea family that attacks only camels [1]. The adult fly is widely distributed in areas where camels are found [1]. During part of its life-cycle, the female fly darts towards the nostrils and deposits its larvae directly into the nasal cavity. From there the larvae crawl up to the nasopharynx and sometimes the paranasal sinuses and molt twice while attached to the naso-pharyngeal and par nasal mucous membranes and cause extensive irritation and tissue damage. They remain attached to the mucous membrane of these organs for up to 11 months; during which time they feed and cause extensive irritation and tissue damage [2]. The mature white or 30 -grey third stage larvae grow up to 35 mm and up to 15 mm in the second stage, but the L1 stage is only about 0.7 mm long. These infestations impair animals' welfare, reduce host physiological functions, destroy host tissues and cause significant economic losses to livestock through abortion, reduction of milk production and losses in terms of weight gain, fertility and hide quality [3]. Infested camels lose their appetite, show difficulty in breathing, snort, sneeze, expel the larvae from their nostrils and may show abnormal behavior resembling cranial coenuriasis and they often become restless and may even stop feeding [4]. They infrequently may finally die from meningitis caused by secondary bacterial or viral infections [5]. The intensity of clinical signs depends on the amounts of damage by migrating larvae. The aim of this paper is to study the structural differences in the cells of salivary gland of second in star of Cephalopina titilator in order to clarify their role in the invasion of host.

II. MATERIALS AND METHODS

A. Collection of larvae

The slaughterhouse was visited weekly. The heads of slaughtered camels were separated from the rest of the body and the skull was incised sagittally. The cavities of the skull were inspected carefully for the presence of second instars of *C. titillator,* and the recovered larvae from each camel were collected in a plastic container. The larvae were immediately transferred to the laboratory for differentiation of second instars according to Zumpt [4].

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ISO 9001:2008 Certified
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B. Dissection of organs

Salivary glands of second instar larvae of C.titilattor were dissected using fine entomological needles under a stereoscopic microscope at 4X magnification in phosphate-buffered saline [PBS;10 mM Na2SO4, 145 mM NaCl (pH 7.2)] and transferred to a micro centrifuge tube with a small volume of PBS.

C. Histological preparations

Tissue sections (5um) were cut and stained with hematoxylin and eosin, toluidine blue, observed under light microscopy, and photographed with a digital camera.

D. Transmission electron microscope preparations

Approximately 20 specimens of 2-day-old larvae were removed from the rearing box and individually dissected in phosphate buffer pH of 7.4under a binocular dissecting microscope (Olympus®,Japan). The salivary gland was separated from alimentary canal. The dissected salivary glands were transferred from the phosphate buffer and prefixed with 2.5% glutaraldehyde in phosphate buffer solution at a pH of 7.4 at 4°C for 24 h to accomplish primary fixation. Then rinsed twice with phosphate buffer solution at 10-min intervals. Rinsed specimens were treated with 1% osmium tetroxide at room temperature for 30 minutes for post fixation. Post-fixation was followed by rinsing twice with phosphate buffer solution and dehydrating with alcohol. To replace the water in the specimens with alcohol, they were subjected to ascending series of alcohol. After that, organ specimens were placed in acetone for 2 h before transferring into ratios of resin to acetone of 1:3 for 24 h, 1:1 for 24 h, and 3:1 for 24 h, sequentially. This was followed by treatment with pure resin twice for 3 h. Each sample was then embedded in Epon resin by placing them into a plastic block and by incubating at 70°C for 24 h. Semithin section (0.5 ìm) of each sample was made with a glass knife on an Ultra microtome (Boeckeler®, USA). This was followed by staining with 1% methylene blue mixed with 1% Azure II (1:1) to view under a light microscope (Olympus®, Japan). The ultrathin sections (90 nm) were stained with uranyl acetate and lead citrate then examined with the ZEISS EM 10 electron microscope (Germany).

III. RESULTS

A. Results of anatomy

Observation of the whole excised alimentary canal of *Cephalopina titillator* second instar larvae under light microscope demonstrated that the salivary gland is almost one third the alimentary canal .The salivary gland consists of a pair of simple long translucent tubules situated laterally in the fore gut region which lies ventrally in the body cavity . A thin short and narrow efferent duct emerges from the anterior of each gland and then unites together to form a translucent single median common deferent duct, which is inserted in the dorsal cephalopharyngeal skeleton (Fig.1). The gland is bathed in haemolymph , surrounded by adipose tissue and invaded by tracheae. Both efferent and deferent ducts are structurally similar, flexible and not associated with connective tissue (Fig.1).

B. Results of histology

The semi-thin sections from the middle region of the gland revealed that the epithelial cells are a monolayer of striated epithelial cells that are more or less columnar .Their apical part projecting into the lumen of the gland, the nucleus is oval and lies basally in the cell. The lumen is narrow usually full of secretory vacuoles and secreted material. Large secretory granules are observed within cells. The semi-thin section show clearly the main outlines of the cells and the tracheae connected with the salivary gland tissues. (Figs. 3, 4).

C. Results of ultra structure

The glandular cells bear apically dense compact and long microvilli.(Figs.4,5,11). The lumen appeared full of secretory vacuoles with or without electron density, as well as secreted materials within the microvillar extensions.

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(Figs.4,5,10,11.) Basally, the basal cell membrane is deeply infolded to form a well developed basal labyrinth resting on a thick basement membrane and is associated with polymorphic mitochondria ,(Figs.5). The basal labyrinth bears hemidesmosomes between the cell and basement membranes (Fig. 7). Numerous tracheal insertions are found at the basement of the cells and in the intercellular space (Figs.6). The nuclei of salivary gland cells are large and well defined with irregular contours, and rich in heterochromatin (Figs.8). Rough endoplasmic reticulum is well developed has parallel cisternae (Figs.8) mostly near the nucleus and is dilated at the basal and apical region of the cell (Figs.9). Many secretory vacuoles are visible near the base of the microvilli and in the basal part of the cell (Figs.10, 11). A lot of these secretory vacuoles are extruded from the microvilli to the lumen; some of them have vacuolar membrane (Figs. 12).

IV. DISSCUSSION

The structures and functions of larval salivary glands in insects of medical and veterinary importance have been little studied [6]. Insect salivary glands are the largest exocrine organs and are essential for successful feeding [7].The larval stages of *C. tittlator* are voracious biontophagous parasites and consequently have well-developed digestive system and salivary glands. The tubular form of *C.tittilator* larval salivary glands are similar anatomically to those observed in other Diptera, particularly members of the Muscomorpha [8], including *Oestrus ovis* larva and *Gasterophilus intestinalis* [9].The salivary gland complex is a structure that can produce substances necessary for nutrient mobilization, usually digestive enzymes, which can be used to characterize feeding habits of insects [10].

Insect salivary glands generally have at least two regions, a secretory and a resorptive region. The major secretory component is saliva which is mainly composed of water which is transported from the hemolymph across cells of the salivary gland and into the lumen of the gland. Movement of water from the blood to the gland lumen is accomplished by active transport of K^+ and Na^+ ions from the hemolymph to the lumen down an osmotic gradient. Cells responsible for water transport generally have deep infoldings of the cell membrane and/ or dense microvilli on the side of the cell adjacent to the lumen of the gland. This serves to greatly increase the cell's luminal surface area, and also serves to enclose very narrow extracellular spaces into which ions are pumped. The enclosed nature of the spaces helps to contain the ions to keep their concentration high, thus facilitating the osmotic movement of water from the cell into the space [11].

In the present study the tubular part of the salivary gland was only investigated. Histologically the salivary glands of *C.titllator* is a monolayer of epithelial cells with a large oval central nucleus. Usually contains secreted material in the lumen. The cells of the glandular region of the salivary gland are more or less columnar with a well developed striated border. The semi-thin sections show clearly the main outlines of the cells and the tracheae connected with the salivary gland tissues. The cells of the tubular region of the salivary gland are not lined with cuticle. The epithelial cells of the salivary glands of adult *C.megacephgala* are lined with a thick cuticle layer along the apical boarder of the gland, but that no cuticular lining was apparent in the third larval instar of the same species[12]. The tubular form of the salivary glands in second and third instar larvae of *G.intestinalis* are similar to most dipterous insects[9]. They also reported that the epithelial cells apices of the ducts are lined with cuticule. Also the cuticle lines the efferent and deferent ductal cells in second and third larval stages of *Dermatobia.hominis [13]*.

The ultra structural investigation of *C.titillator* showed that the glandular cells bear apically dense compact microvilli. The microvilli at the apical portion of the glandular cells may serve to reinforce the structure of the gland or to resist osmotic pressure [9]. The basal cell membrane of the salivary gland cells in *C.titillator* forms a basal labyrinth, exposing a larger membrane surface to hemolymph active transport function. The basal cell membrane infoldings is similar to reports on salivary glands of male adult *C.megacephala* [12]. The infolds of the basal membrane in salivary gland of *Gastrophilus intestinals*, expose a large membrane surface to haemolymph and therefore may have an active transportation. The infoldings of the basal cell membrane and microvilli usually are associated with abundant mitochondria to provide the energy for the ion pumps. [9] The fine architecture of the salivary gland of *C.tittilator* shows an abundance of mitochondria and RER. The stacks of RER cistern is relatively dense in the cytoplasm this is similar to those described in salivary gland of adult *C.megacephala* [12].

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Intense tracheal ramifications indicate a high level of glandular activity and oxygen supply from the respiratory system and all these together indicate high metabolic activity. This has also been described in Triatominae [14] and *D.hominus* [13] and adult *C.megacephala* [12] The cytoplasm has secretory vesicles with or without electron density , as well as secreted material within the microvilli extensions. These secretions are probably enzymes which indicates glandular activity, this is similar to *D. hominis* L_1 [6],[13], and as adult *C.megacephala* [12]. The secretory products reach the lumen surrounded by membranes in Triatominae [15].

The most prominent feature of the salivary gland cell is the well developed polymorphic mitochondria, small mitochondria are more spread in apical region of cell and swollen mitochondria are widespread in basal region. Nucleus is large and well defined contains diffuse chromatin. The large nucleus is characteristic of the active cells, in which large quantities of nucleic acid move in and out of the nucleus to generate synthesis and secretion of proteins [13] The secretory region of the gland synthesizes proteins, such as salivary enzymes and other organic components of the saliva. Cells responsible for secretion of these components generally possess extensive endoplasmic reticulum, Golgi bodies, and secretory granules that synthesize and transport (intracellularly) the secretions. There may be one or several different types of cell in the secretory region [11].
Evangelista and Leite, [6],[13] could not confirm that enzymes secreted by the larval salivary glands and midgut of the first larval instar *D.hominis*, could initiate partial histolysis of the host's cutaneous tissues. In fly larvae known to produce myiasis, such as *Hypoderma lineatum* (Family Oestridae), secretory products containing protease are responsible for host penetration [16]. Innocenti et al [17] reported that they doubted the presence of enzymatic action of the salivary gland contents of Oestrus ovis larvae. Enzymes excreted by the salivary glands of Calliphoridae also permit external digestion, because the larvae live in semi liquid substrates, which they ingest. Some larvae of this family make these enzymes inside the gut lumen. [18].Roelfstra et al [9], suggested that these cells demonstrate an intense synthetic activity; the RER, free ribosomes, Golgi complexes and numerous tracheae insertions which supply the cells with oxygen and the highly abundant mitochondria support the hypothesis of important cellular synthesis and storage. Boonsriwong et al [12] reported that the intense salivary secretions and variable secretory products are synthesized by the salivary gland of male *C.megacephala*.The cell contacts clearly observed in salivary gland cells of *C.titillator* are the hemidesmosomes which are between the basal cell and basement membrane. Septate junctions in lateral cell membrane were not easily observed in *C.titillator*. Evangelista and Leite, [13] stated that the hemidesmosomes form lateral channels that link the cells and basement membranes. Roelfstra et a l[9] stated that the septate junctions link the adjacent cells tightly to one another allowing the cells to increase in volume when necessary, possibly to store synthesized products. Molecules diffuse between the cells across these junctions according to Evangelista and Leite, [13]. Further investigations are needed to elucidate the contribution of the salivary glands to digestion processes and the impact of salivary enzymes on host tissue during migration and maturation phases.

ACKNOWLEDGEMENT

I am grateful for my supervisor, Dr. Afaf Abdel-Meguid for her support cooperation and revising this manuscript.

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APPENDIX

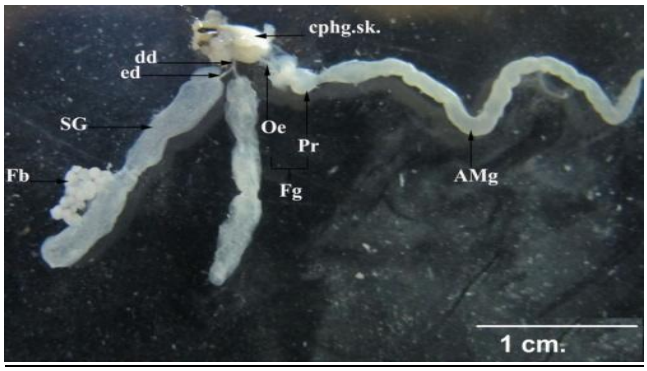

Fig.1: Photo micrograph of the salivary gland and part of the alimentary canal of the second instar larva of *Cephalopina titillator* showing translucent single median deferent duct (dd), bifurcated into short narrow efferent ducts (ed) connected to the tubular salivary glands(SG) .Fat bodies (Fb) are attached to the surface of salivary gland.The cephalopharyngeal skeleton (cphg.sk.) is followed by short simple tube (oesophagus (Oe)) that enters the proventriculus (Pr) , both form the foregut (Fg). The anterior midgut (AMg) begins at the posterior end of the proventriculus . scale bar = 1cm .

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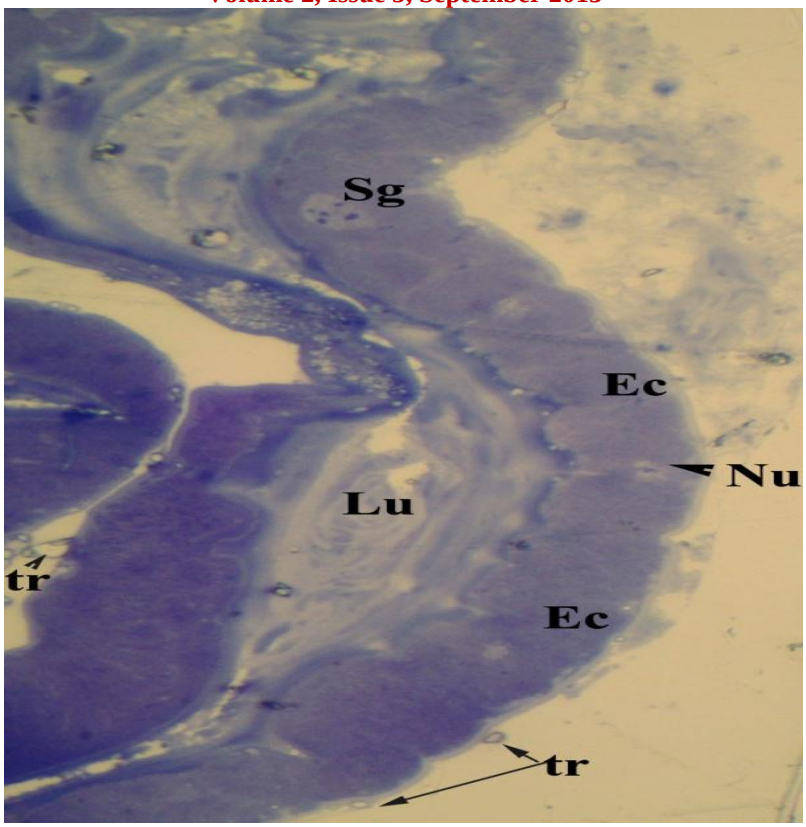

Fig.2: Photo micrograph of salivary gland of *Cephalopina titillator* (semi – thin section) showing : narrow lumen (Lu) , epithelial cells (Ec) nuclei (Nu) secretory granule(Sg) and trachea(tr) .(X400)

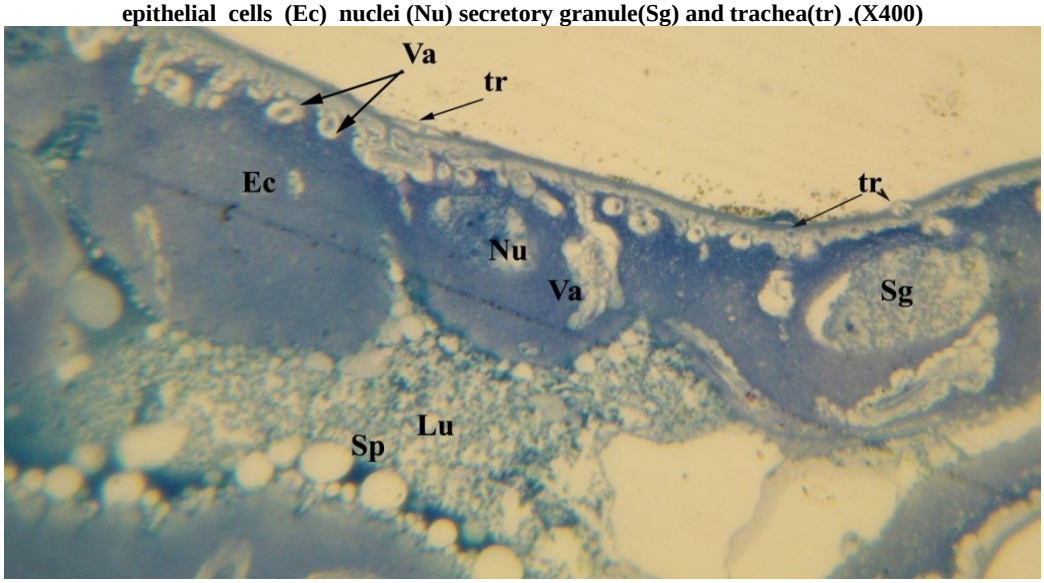

Fig.3: Photo micrograph of salivary gland of *Cephalopina titillator* (semi – thin section) showing : narrow lumen (Lu) full of secretory products (Sp) that are pinching off from the apices of the epithelial cells (Ec), large amount of vacuoles(Va) are present especially at the base of the cell, secretory granule (Sg), nucleus (Nu) which is basal and the tracheae (tr) at the base of the basement membrane and other are inserted in the cells.(X800) .

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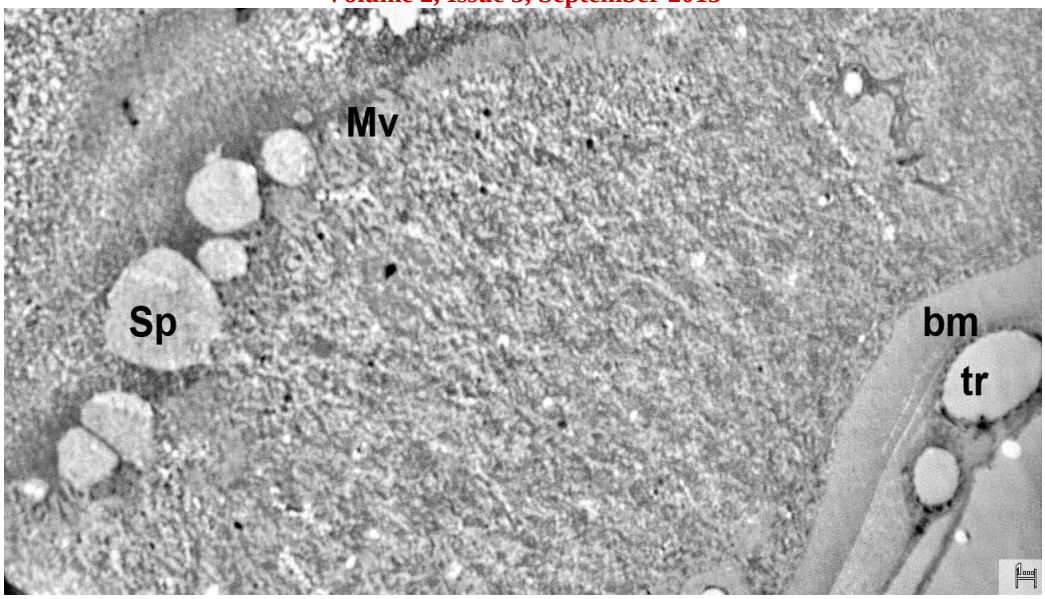

Fig.4: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: trachea(tr) , thick basement membrane (bm), microvilli (Mv) and secretory products (Sp) pinching off from the cell at the border of the microvilli. scale bar = 1 µm.

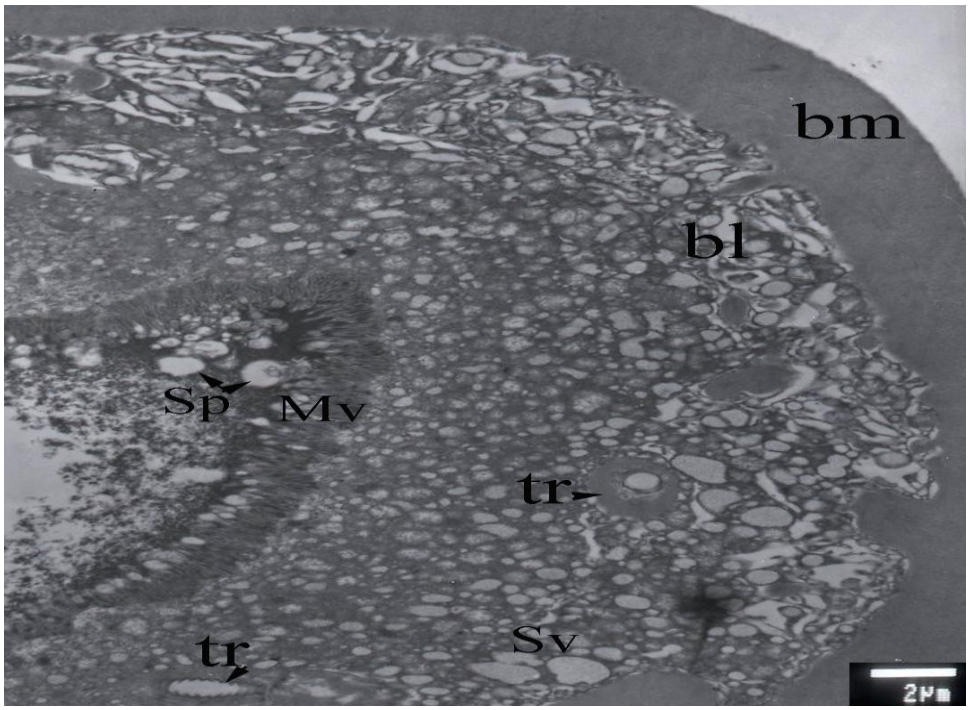

Fig.5: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: thick basement membrane (bm) , basal labyrinth (bl), trachea(tr) within the cell, secretory vacuoles(Sv) , microvilli (Mv) and secretory products (Sp) pinching off from the cell at the border of the microvilli to the lumen. scale bar = 2 µm.

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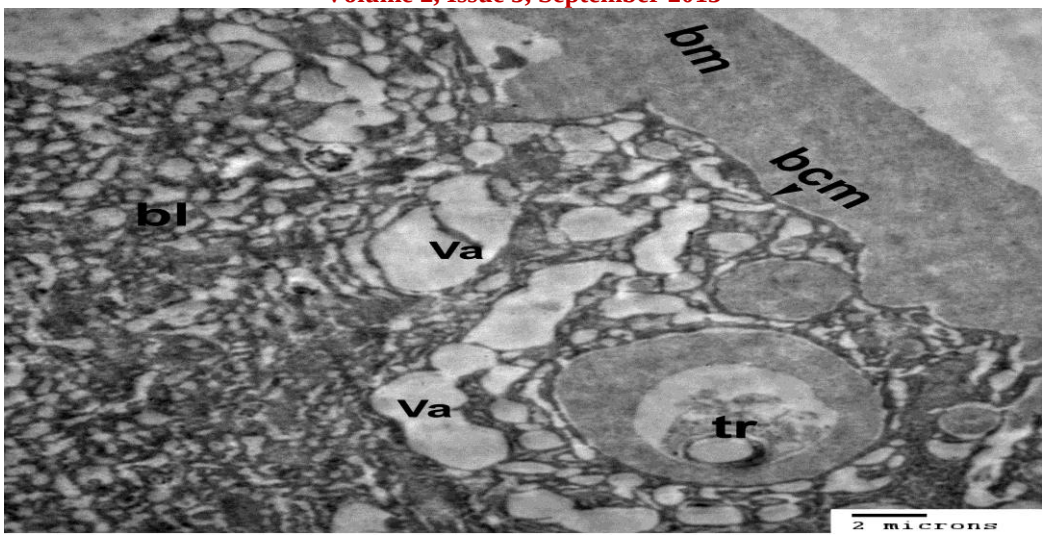

Fig.6: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: trachea (tr) inserted in the tissue , thick basement membrane (bm), basal cell membrane(bcm) ,basal labyrinth (bl) and vacuoles (Va). scale bar = 2 µm.

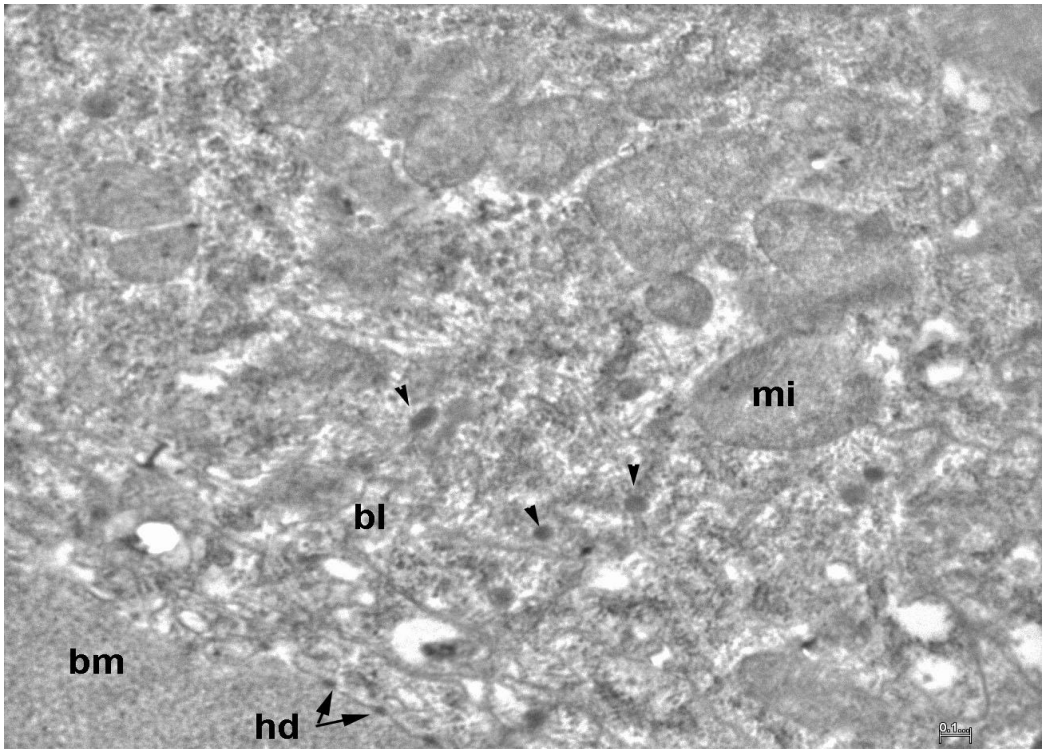

Fig.7: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: basement membrane (bm), basal labyrinth (bl), polymorphic and swollen mitochondria (mi) residual body( ) and hemidesmosomes (hd) connecting basal cell membrane and basement membrane. scale bar = 0.1 µm.

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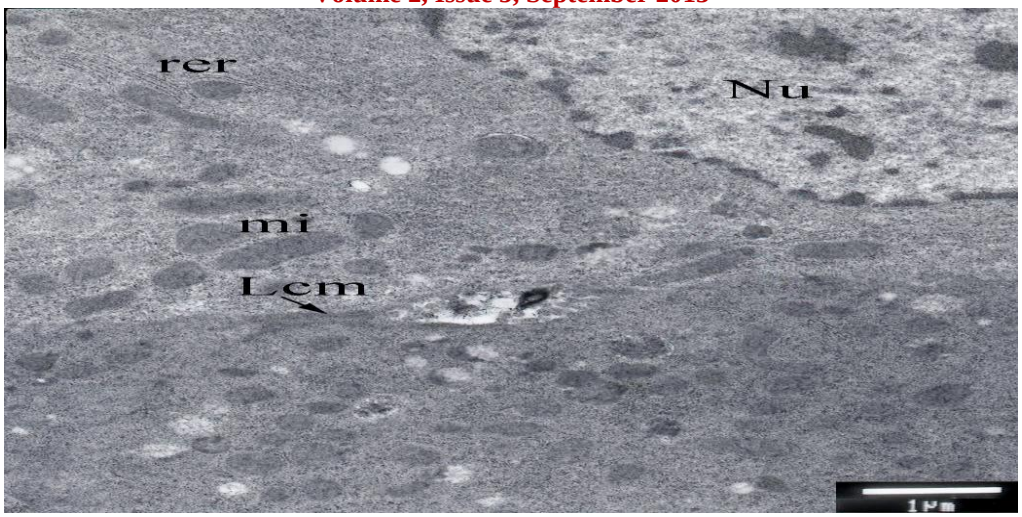

Fig.8:Electron micrograph of the salivary gland of *Cephalopina titillator* showing: polymorphic mitochondria (mi) , nucleus(Nu) , rough endoplasmic reticulum (rer) and lateral cell membrane (Lcm) . scale bar = 1 µm.

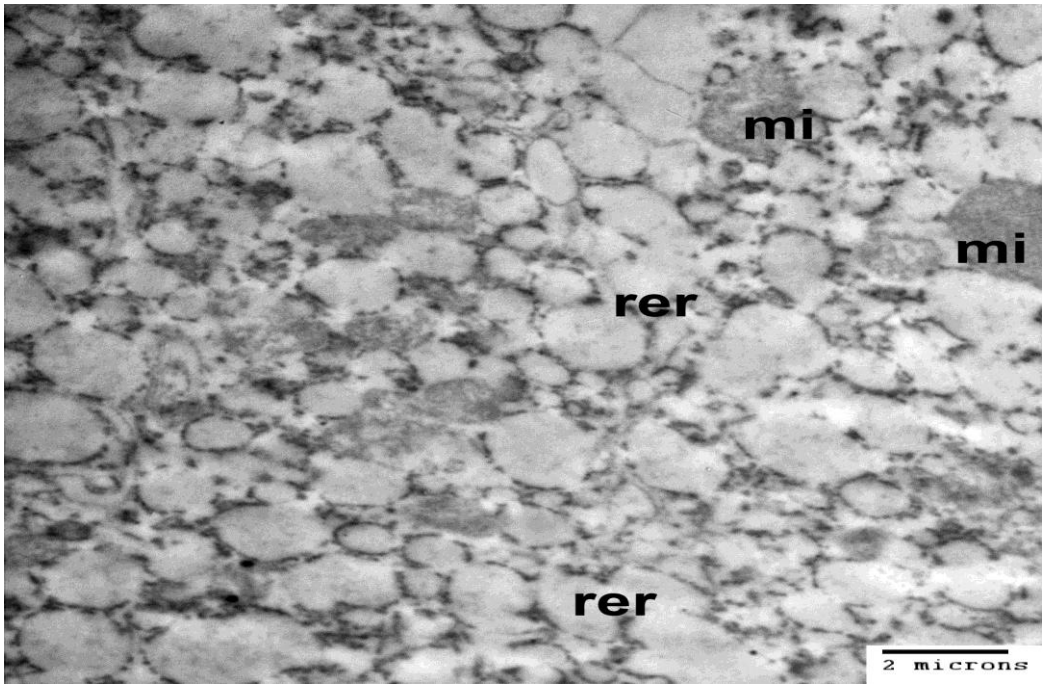

Fig. 9.: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: dilated rough endoplasmic reticulum (rer) and polymorphic mitochondria (mi). scale bar = 2 µm.

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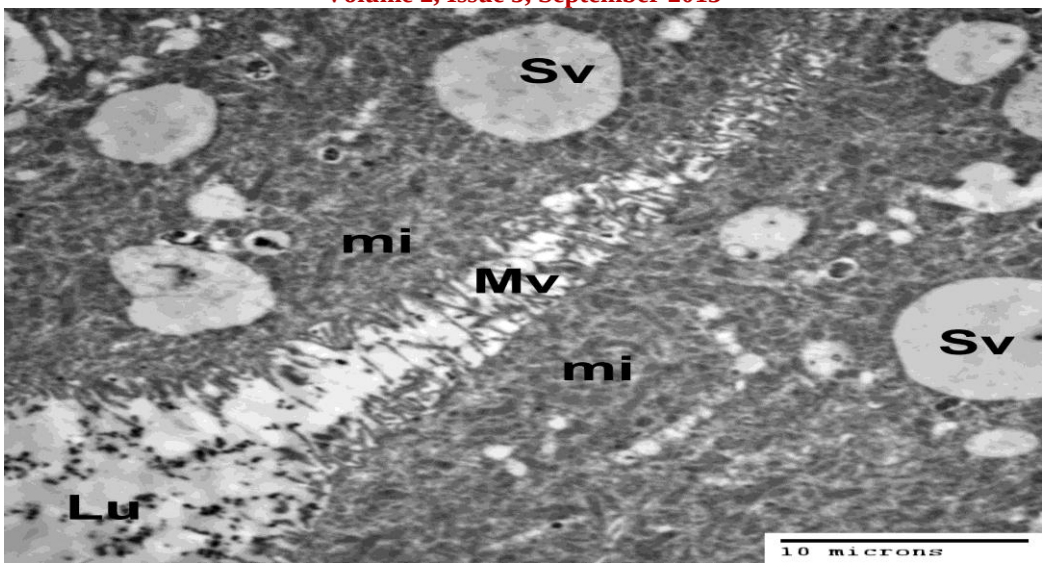

Fig.10: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: long and slender microvilli (Mv) , lumen(Lu) containg secretory products (Sp) , polymorphic mitochondria (mi) and secretory vacuoles (Sv) . scale bar = 10 µm.

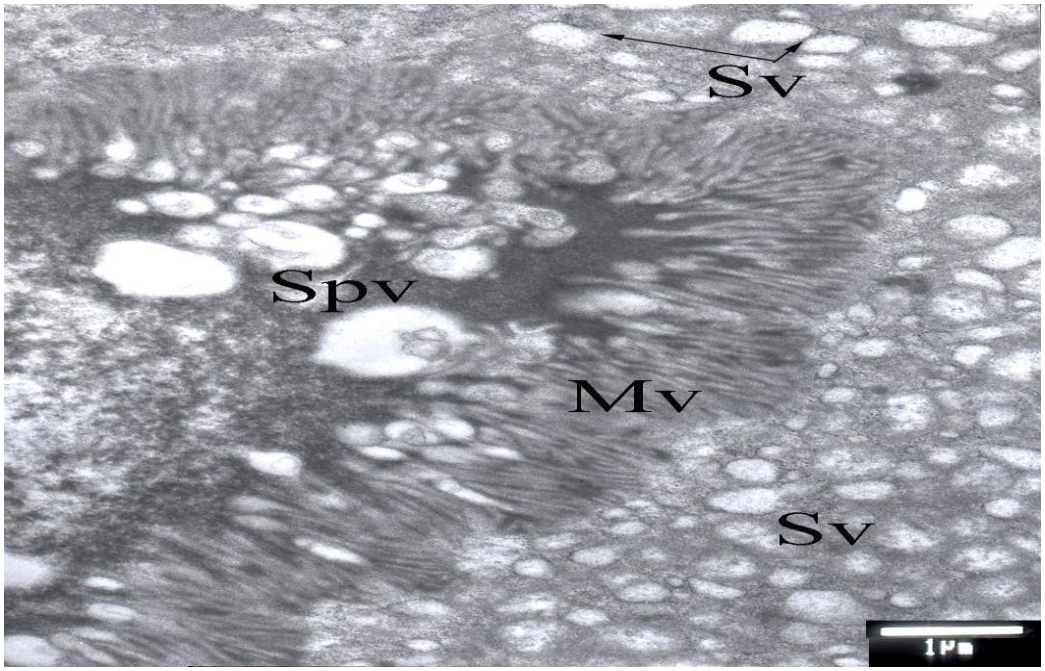

Fig.11: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: long, condensed and slender microvilli (Mv) , secretory products (Spv) pinching off from the cell at the border of the microvilli into the lumen and secretory vacuoles (Sv) . Scale bar = 1 µm.

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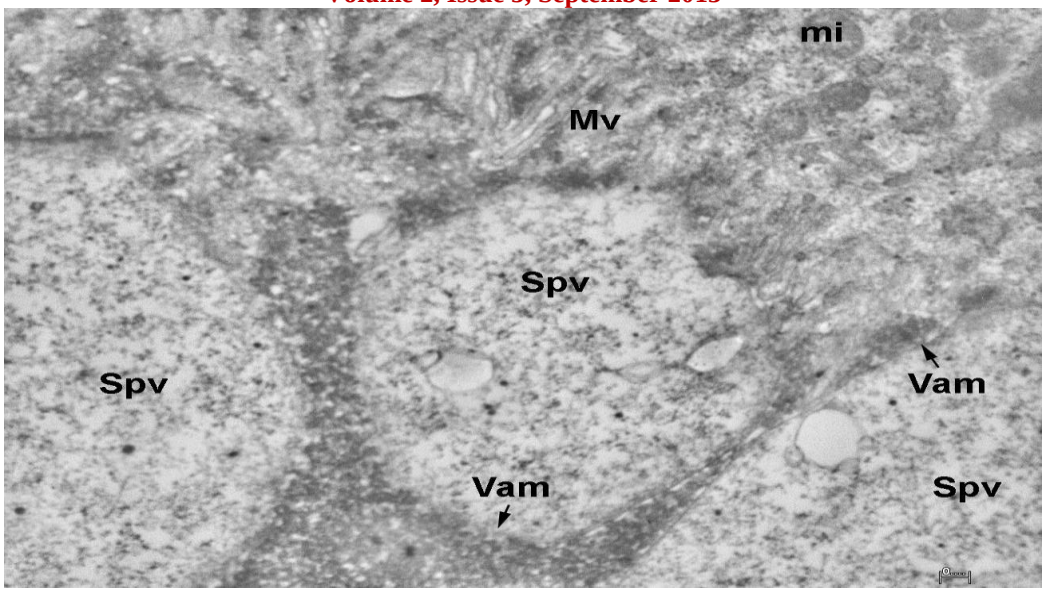

Fig.12: Electron micrograph of the salivary gland of *Cephalopina titillator* showing: mitochondria (mi), microvilli (Mv) and secretory product vacuoles (Spv) with vacuolar membrane (Vam) pinching off from the cell at the border of the microvilli to the lumen . scale bar = 0.1 µm.