ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.31398-31401, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SCANNING ELECTRON MICROSCOPIC STUDIES ON THE DEFENSE GLAND SYSTEM OF *PHEROPSOPHUS HILARIS* (COLEOPTERA: CARABIDAE)

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ARTICLE INFO

Article History:
Received 26th February, 2016
Received in revised form
17th March, 2016
Accepted 27th April, 2016
Published online 31st May, 2016

Key words:

Pheropsophus hilaris,
Demonstrate,
Sphincter muscles,
Carabid.

ABSTRACT

The pygidial defense gland of the *Pheropsophus hilaris* consists of two big gland (g^1 and g^2), two small reservoirs (r^1 and r^2) sac like structures filled with secretion with associated secretary tissues and basal eversible membrane structures. The complex structure consisting of two sets of secretary lower collection canals, collector reservoir, one – way valve, sphincter muscles, exit taken and exit nozzle. The bombardier beetle has been the subject of much discussion by creationists and evolutionists alike. Resent reports demonstrate and sophistication and accuracy with which these carabid beetles deliver a spray of hot quinines and steam of ward off predators. In this study the pygidial gland is studied under scanning electron microscopy and are shown to be quite complex this complexity could suggest an origin by design.

Citation: Iyyappan, V., Nagarajan, D. and Ramesh Kumar, T. 2016. "Scanning electron microscopic studies on the defense gland system of *Pheropsophus hilaris* (Coleoptera: Carabidae)", *International Journal of Current Research*, 8, (05), 31398-31401.

INTRODUCTION

The bombardier beetle has been a subject of interest for many years. This beetle is called a 'bombardier' because it ejects a hot, highly noxious spray of aqueous Benzoquinones, oxygen and steam as a defense mechanism against would – be predators Aneshansley *et al* (1969). This secretion is very accurately delivered via twin sets of spray nozzles located at the tip of the beetle abdomen and is most effective at stunning predators (Eisner. 1958). Bombardier beetle range in size from 2.0 mm overall length to 30 mm in length and can be found all over the world. They live under rocks or pebbles in cool and sandy soil. Aggregating in groups during the day time, they are usually active at night (Erwin, 1970). The structure of the defense system of the bombardier beetle, is quite complex, consisting of two sets of secretary lobes, collecting canals, collecting reservoirs, one – way valves, sphincter muscles, reaction chambers, exit tubes, and exit nozzles, Eisner *et al.,*(2000). The paired secretary lobes connect via long tubes to collecting reservoirs, each of which are surrounded by a thin layer of muscle and jointed to a reaction chamber by means of a one ways valve connected by a sphincter muscle (Crowson. 1981). The collection reservoir, the valve, and the reaction chamber function together to work as a pulse jet, with the spray emitted in pulses. (Dean *et al.,*1990) these reservoirs postubute that the beetle does not squeeze the collection reservoir and the sphincter muscle rapidly, but that the beetle sphincter even, steady pressure on the collection reservoir. Once these muscles around the reservoir squeeze the first amount of reactant through the valve into the reaction chamber, the resulting explosion causes the pressure to rise rapidly in the reaction chamber, forcing shut the one – way valve, (Eisner *et al.,* 1977). The products of the reaction the exit the chamber with a pop and a puff, and the pressure inside the reaction chamber lowers again, failing below the pressure of the collection reservoir, which is still being squeezed by the reservoir muscles. The cycle then repeat itself – the valve thus oscillates passively (Schlepf *et al.,* 1989). The secretary lobes secrete aqueous hydrogen peroxide and hydroquinones, which are stored in large quantity in the collecting reservoirs. It is reported that the same cells actually synthesize and separate both the hydrogen peroxide and the hydroquinones (Schlepf *et al.,* 1989). The stored liquids remain in the full reservoirs until needed. When the bombardier beetle is threatened (such as with a bite on the limb) it contracts its collection reservoirs, moving the hydrogen peroxide and hydroquinones into the reaction chamber through the valves. The reaction chamber is said to be lined with enzyme – secreting structures which produce peroxidases and catylases (Schildknecht *et al.,* 1970

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and Schlepf *et al.,* 1989). Although some studies state oxidases and enzyme are secreted and stored in the reaction chamber. (Eisner, 1970 and Dean *et al.,* 1990).When the hydrogen peroxide and hydroquinones come into contact with the enzyme, an explosive reaction takes place, yielding water, quinines, heat and gaseous oxygen, (Kofahl, 1981). The pressure of the free oxygen propels the mixture out of the reaction chamber through the spray nozzle, directed to the target (either at the predator or on the beetle's own integument as a protective measure) by way of flanges, line grooves, or spray deflectors.

The overall structure of the secretary lobes and collecting canals is said to resemble a cluster of grapes (Forsyth, 1972). The stalk being the final collecting canal leading to the collection reservoir. These authors state that each lobe is essentially a ball of cells which all face inward, aligned radially around a central collecting lumen. The secretary lobes are fingerlike, the collecting lumen is long and extends the length of the lobe, (Eisner *et al.,* 2000). Each secretary cell has an elongate secretary vesicle which is almost as long as the cell itself and is centrally located with a 'coated membrane' crowded with microvilli (Schlepf *et al.,* 1989). An efferent cuticular tubule or duct, leads out of the end of the vesicle towards the center of the secretary lobe. The duct extends past the end of the cell, and through a duct - carrying cell. A duct – carrying cell surrounds the duct, having its plasma membrane between it and duct, which in turn is surrounded by its own vesicular membrane. Finally, the duct terminates into the central collecting lumen in the middle of the secretary lobe. subsequent to the collecting lumen. The secretion travels through the collecting canal to the 'stalk', or the main collecting canal, which will then take the secretion to the collecting reservoir (Forsyth, 1972).The bombardier beetle has the ability to direct its defensive spray toward its aggressor with pinpoint accuracy. They show the ability to direct their deferent spray in almost all direction accurately enough to target the predators, (Nakatani *et al.,* 1996).

MATERIAL AND METHODS

Adult *Pheropsophus hilaris,* of both sexes, collected from the fields in the vicinity of Annamalai nagar, were used throughout the present investigation. The adult male and female insects are collected from the field were reared in wooden cages measuring 30x33x45 cm in the laboratory at the room temperature 28 ± 2^0c as suggested by the bottom of the cages was filled up with sand of about 10-15 inches. Since these beetles are living in crevices, brick stones are kept in the cages. To keep the moisture of the soil, water was sprayed regularly at equal intervals of every 12 hours. In order to provide sufficient aeration, the sides of the cages are fabricated with meshes. To allow sufficient light, one side of the cage is covered with glass. *Pheropsophus hilaris* was fed with wet Prawn, Fish meat, Larvae, eggs of pest insects, organic waste matters, dead and decay materials.

Pygidial glands were dissected and dried in vacuum for getting good moisture free specimen was needed. Then the samples were coated-gold with full deposition for 3 minutes using polaron SC 500 sputter coater. Few tungsten line coating was given this coating has given primary to prevent charging samples and clarity of pictures. Then the samples were mounted in stereo scan 440-model electron microscope UK. The ascertaining voltage given was 20kw and the beam current used was in between 18-25 p.a (pica amperes) notching distance was between 39mm to 1mm. The secondary electron images were taken for all the samples with varied magnifications from 50 x 10,000 (Kotze and Soley, 1990).

RESULTS AND DISCUSSION

The pygidial defense gland apparatus of *Pheropsophus hilaris* consists of a pair of defense gland, with associated secretary tissues and two reservoir r^1 and r^2 (Fig, 1 & 2). The defense gland system has already been well described by Jenkins (1957) in the gland cells, the filament layer surrounding the receiving canal probably does not represent accumulating secretion, structures similar to these filaments could neither be observed in the lumen of the receiving nor in the conducting canal, as it would be expected for secretion a secretion precursors. (Locke and Huie, 1979). According to Biemont *et al.,* (1992) such cuticular filament structures could contain enzymes or compounds modificating the secretion by passing into the lumen of the receiving canal. In this manner nontoxic precursors could be synthesized in the gland cell (Eisner *et al.,* 1964).

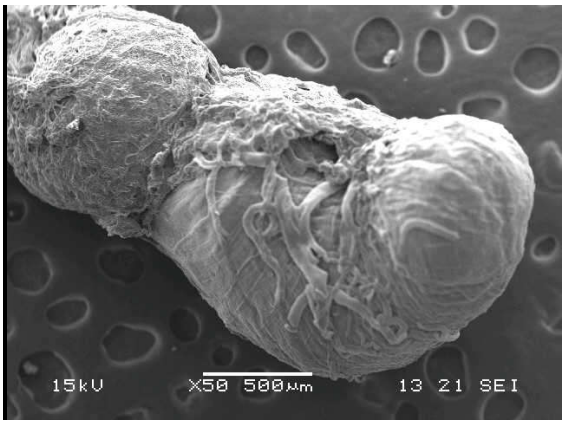

Fig. 1.

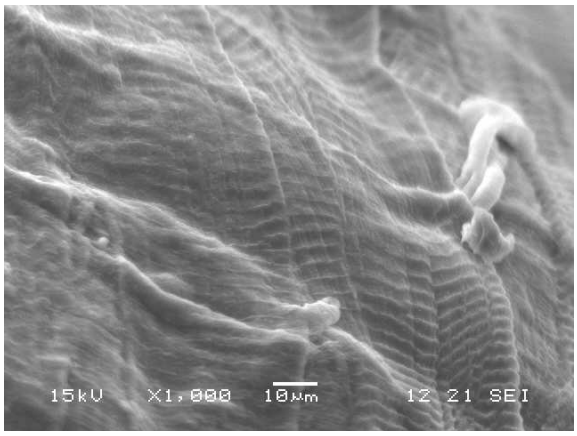

Fig. 2.

Jenkins (1957) describes the whole pygidial defense gland apparatus of the *Pheropsophus hilaris* as an invagination of the

pleural membrane. The duct structures of both secretary tissues g^1 and g^2, as well as the corresponding reservoirs are lined with Epicuticular material, which supports Jenkins' proposal of the glands' epidermal origin (Noirot and Quennedey, 1974: Quennedey, 1998). The pygidial gland of the *Pheropsophus hilaris* consists of numerous web like ruffled membrane had several pinocytotic pits. The cytoplasm the gland cell seems to be flattened sheet around the nuclei, which could be seen as prominent bulges near the centre of the cells. In some locations, these protuberances to be occurred in sub – peripheral regions while the outer margin of the membrane was appeared as rope like structure (Fig, 3 & 4). The invagination resembled like pirocytotic pits was seen inside.

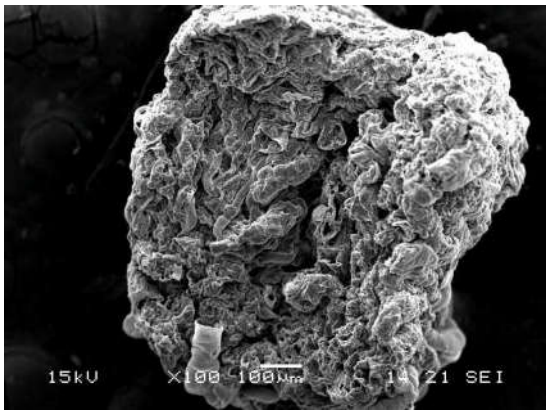

Fig. 3.

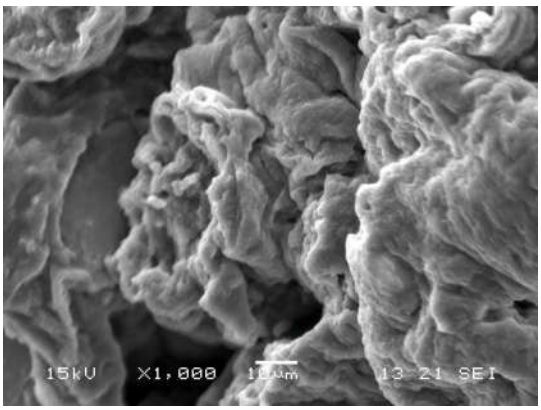

Fig. 4.

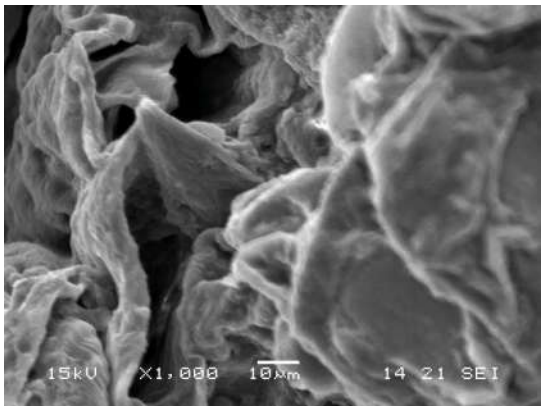

Fig. 5.

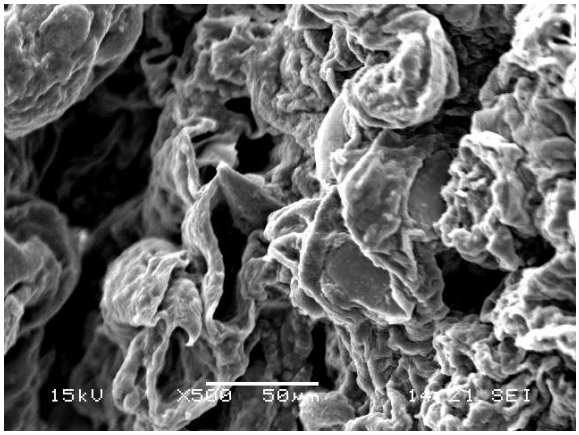

Fig. 6.

Minute blebs associated with cytoplasm invagination were also seen along the margin of the membrane. Penetration of macrophages and phagocytosis on such trophocytes were visible. The apices of secretary cell seems to be revealed the smooth secretary granules beneath the apical plasma membrane (Fig, 5 & 6).

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