UTTAR PRADESH JOURNAL OF ZOOLOGY

42(24): 629-636, 2021
ISSN: 0256-971X (P)

THE MORPHOLOGY OF ABDOMINAL SCENT GLANDS OF THE RED PUMPKIN BUG, *Coridius janus* (HETEROPTERA: PENTATOMIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Angelo Mark P Walag, University of Science and Technology of Southern Philippines, Philippines.
Reviewers:
(1) Ashok Kumar Meena, India.
(2) K. Nisha Pradeepa, India.

Received: 12 October 2021
Accepted: 20 December 2021
Published: 23 December 2021

Original Research Article

ABSTRACT

The Nymph of *C. janus* have three pairs of Orange coloured abdominal scent glands situated in the mid-dorsal region, underneath the tergum between the first and second, second and third, third and fourth, fourth and fifth segments. The first pair of glands are oval in shape (L 1.12±0.15mm, W 0.87±0.14mm,W 185.67± 18.60µg) as compared to other pairs. These glands bears muscles that regulate ejection of their secretion. The second (L 2.07±0.21mmW 2.72±0.33mm,W 298.65±16.20 µg) and third (L2.66±0.54mm,W 3.22±0.41mm,W 345.70±24.50 µg) abdominal scent glands are larger and roughly rectangular in shape and open dorsally separately on the tergum by two Ostioles. When the Nymph of bugs on being disturbed volatile scent is ejected out through a pair of ostioles.The scent secretion is defensive against predators.

Keywords: *C. janus*; nymph; abdominal scent glands; ostiole; scent secretion.

1. INTRODUCTION

Certain insects have produced various chemicals and behavioural defensive mechanisms to counter the attack of an incredible variety of animals as well as pathogenic micro-organisms. The scent secretions act as defensive or communication between the different species. The scent secretion of insect and other terrestrial arthropods have been subject of intermittent study ever since the formic acid was first time isolated and identified from ants (Wray, [1]). Now-a-days research is being intensified in this field to know the origin, structure and functions of the scent glands of insects.

Defence glands in general differ from pheromone glands in the possession of a reservoir in which the defensive material is stored for use because the

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quantities of chemicals involved are much greater than those employed as pheromones. Most defense glands develop as invaginations of the epidermis and are lined by cuticle, the obvious exception being salivary glands which produce defensive substances. The glands can be grouped into those which are not everted, but from which the defensive chemicals are expelled more or less forcibly, and those which can be everted and from which the defensive material diffuse away or its effective only on contact.

The defensive secretions are produced by ectodermal glands secrete out side of the body, exocrine glands. The basic structure of these glands is similar to whether they produce pheromones or defensive chemicals. Defensive glands are usually associated with a reservoir while pheromone glands are not scent glands (exocrine glands) secrete material outside of the insect and these glands are produced from the epidermis (Chapman, [2]).

Defensive glands are found in many orders of insects and occur on virtually all part of the body i.e., in the head, thorax, abdomen and often in more than one of these parts at the same time. The scent glands are known to occur in various orders of insects like Heteroptera, Hemiptera, Lepidoptera, Coleoptera, Hymenoptera, Diptera, Orthroptera, Dermoptera and Isoptera. Those glands which are confined in thorasic region are known metathoracic scent glands or repugnatorial glands or metasternal glands or stink glands or ventral glands while in the abdominal region of larvae and adult insects are called abdominal scent glands or stink glands or odoriferous glands. The histomorphology, structure of scent glands and chemical composition of scent secretions of Heteropteran bugs have been briefly reviewed.

Histomorphology: The detailed account of anatomy of scent glands of some heteropteran bugs was reported earlier (Dufour, [3]). Johanson and Braten [4] reported cuticular morphology of the scent gland areas of some Heteropterans. The scent glands of several members of hemiptera have been worked out (Remold, [5]; Gilby and Waterhouse, [6]; Calam and Scott, [7]; Carayon, [8]; Dhiman, [9] and Aldrich, [10]).

The Abdominal scent glands are in the abdominal region are called abdominal scent glands or odoriferous glands. The abdominal glands are exclusively present in larvae. But however some times a pair of abdominal scent glands are present in adults too. The study of abdominal scent glands of the larvae of certain hemiptera have been reported (Kunckel, [11]; Bordas, [12]; Usinger, [13], Karlson and Butenandt, [14]; Gupta, [15]; Stein, [16] and Aldrich et al., [17]. Janaiah, [18]; Leela Kumari, [19], Aldrich, [2], Surender [20]; Vidyasagar, [21]; Srinivasulu et al., [22]; Huai-jun xue & Wen-jun Bu [23]). These abdominal scent glands were degenerated and replaced by metathoracic glands in the adults.

All five instar larvae of the pentatomid bugs have three pairs of red or yellow abdominal scent glands, situated in the middle region, underneath the tergum., one benind the other. The first pair of non-functional abdominal scent glands becomes functional in adult bugs. Endopolyploid nuclei occur in the first pair of abdominal scent glands of the adults and larvae of both sexes of *Chrysocoris purpureus* (Venkat Reddy et al., [24]). Brindly, [25] gave an extensive account of scent apparatus in number of families (Naucoridae, Notonectidae, Corixidae, Acanthidae, Corridae, Myodocidsae, Pentatomidae, Coreoidae, Lygaeidae and Capridae). A comparative morphology of scent apparatus and reference to phylogeny of some stink bugs have been studied (Ahmad and Ali Kazmi, [26]).

2. MATERIALS AND METHODS

The insect namely *Coridius janus* (Fabr.) (Larvae) has been selected for the present investigation. The nimph of *C. janus* are field collected on the host plant of *Coccinia grandis* (Cucurbitaceae) in surrounding of the Kakatiya University, Warangal and varies areas of Khammam Dist. (T.S). The host plant *C.grandis* grows in slum areas. These bugs are also collected on the other intermediate host plant *Prosopis juliflora* (mimosaceae). *C. grandis* twists around the trees of *P.juliflora* and live together in the slum areas (Table 1).The adult bugs are not available throughout the year but these are available only from September to February and mainly appear in the rainy and winter seasons. These bugs are sluggish, gregarious, dull, flightless and red in colour with black spots on dorsal surface. These are stationary, phytophagous, and thrive only on the sap of the young shoots of the host plants (Table 2).

The eggs are deposited mostly in October. The eggs are small, round and white and beaded like. They are laid in a single pile of row looking like beaded chain. There are as many as piles of rows of eggs around the young shoots of the host plants and upper surface of the leaf (Fig. 2). Each row may contain 10-15 eggs. During early hours and evening time when the temperature is cool, the bugs are found mostly on along the stem and below the leaves of the host plants (Fig. 3). But as the temperature raises during sunny hours they take shelter under shadow spots of the shoots and starting part of the stem of the host plants and also it is observed that they dwell at the bottom of the stem of the host plant.

The larvae start emerging out from eggs in between August and September. All the five instar larvae will be hatched out by end of December. At the end of January all the instar larvae (Fig. 4) will undergo metamorphosis into adults (Fig. 1). The 5th instar larvae (Fig. 5) have 1.50 ± 0.075 cms. In body length, 0.80 ± 0.102 cms in body width and 230.00 ± 15.80 mg. in body weight (Table 3).

Rearing: The collected nymphs of *C. janus* from the host plants *C. grandis* with *prosopis juliflora* were brought alive into the laboratory and kept in a rearing cage. The nymphs are also reared on the host plant *C. grandis* grown naturally near by the laboratory.

The Rearing Cage : The rearing cage usually consists of an ordinary rectangular wooden box (length 34 cm, width 28 cm, breadth 22 cm) having many small holes on all the sides except bottom. A complete aeration takes place through these holes and the door has a fine iron wire mesh wet and dry bulb thermometer is fixed inside the cage for knowing humidity and temperature. Complete protections should be given to these bugs from the attack of red and black ants, rats and other organism. The adults and larvae were maintained at room temperature for two to three weeks on the leaves, shoots of *C. grandis.* During the high temperature moist sponges pieces are kept inside the cage, so that the percentage of humidity would be increased. Hence, the mortality rate will be less success of maintenance of these bugs in high temperature period is also possible.

3. RESULTS

Scent glands of *C. janus* consists of abdominal scent glands in larvae and metathoracic scent glands in male and female adult bugs. Abdominal scent glands are present in larval stages. These are three pairs of yellowish abdominal scent glands are present in all the five instar larvae of *C. janus.* These glands are arranged one behind the other in a row, in the mid dorsal region above the viscera in the abdominal segments of 2nd and 5th underneath the tergum and opens outside through dorsal surface, each gland with a pair of ostioles (Fig. 7).

The first pair of abdominal scent glands (I ASGL) are located in between first and second segments of the tergum. These are smaller in size, oval in shape brick red and they are very diminutive. These glands are present in all five instar larvae, but not functional in all the five instar larvae. These glands are open mid dorsally by through a pair of ostioles (Figs. 8, 9 10). A pair of regulatory muscles (GLM) are associated one on each side of the first pair of abdominal scent glands which have their origin on the tergum and insertion on the resilient neck of abdominal scent gland. The length of first abdominal scent gland 1.12 ± 0.15 mm, width is 0.87 ± 0.14 mm and weight is 185.67 ± 18.60 µg (Table 4).

The second abdominal scent gland (II AGSL) is large, roughly tetragonal, yellowish and some what boat shaped when distended with component. This gland is located in between third and fourth abdominal segments of the tergum and opens out dorsally each, with a pair of openings "ostioles". The length of second abdominal scent gland is 2.07 + 0.21 mm, width is 2.72 + 0.33 mm and weight is 298.65 + 16.20 µ*g* (Table 4).

The third abdominal scent gland (III AGSL) is slightly larger than the second abdominal gland. This gland is also tetragonal, yellowish and somewhat boat shaped structure when distended with distended with scent secretion. Third abdominal scent gland is present in between fourth and fifth abdominal segments of the tergum and opens out dorsally through a pair of opening called "Ostioles". The length of third abdominal scent gland is 2.66± 0.54 mm. Width is 3.22± 0.41 mm and weigh is 345.70± 24.50µg (Table 4).

The fist pair of abdominal scent glands are developed gradually from first instar to fifth instar larvae but this gland is non functional (?); whereas the second and third pairs of glands are fully developed and functional in all five instar larvae. During metamorphosis from fifth instar larva to adult, the second and third abdominal glands are gradually disappear but the first pair of non functional abdominal scent glands of fifth instar larvae become functional in adult bugs. However the traces of second and third abdominal glands remain left over in 10-15 days old bugs (Table 5).The micro capillaries were placed against the opening of second and third abdominal glands (Fig. 6). The scent secretions were collected from scent glands for identification of chemicals in the secretion.

Second Reservoir Muscles (II RM): On each side of the second tetragonal reservoir, two separate muscle threads are present, which are originate on the lateral margin of the third tergal plates and in between second and third tergal plates, insert on the mouth of second gland. These reservoir muscles help in regulating the opening of second gland reservoir (Fig.7).

Muscles attached to the neck of the II Reservoir (II MNR): These are five pairs of muscle fibres which originate on the beginning of the third segments of the tergum and insert on the neck of the third abdominal

scent gland and these muscles regulate the compressions of the seconds and third glands (Fig. 7).

Third Reservoir Muscles (III RM): Third reservoir locate in between fourth and fifth segments of the tergum. These two separate muscle fibres which originate from the margin of the fourth tergal plate and insert on the mouth of the third abdominal scent gland and these muscles help in operating the functioning of the third glands (Fig. 7).

Table 1. Insects species investigated and its host plants

Insect species				Host plants	
Scientific name	**Vernacular name**	**Botanical name**	**Family**	**Vernacular name**	**Locality**
Coridius janus (Fabr.) Larvae	Red bug	*Coccinia grandis* L. *Prosopis juliflora*	Cucurbitaceae Mimosaceae	Donda (Tel.) Circartumma (Tel.)	Warangal &khammam Warangal& khammam

Table 2. Details of Species investigated and its food

Common species	Common name	Order	Family	Food
Coridius janus (Fabr.)	Red bug	Heterptera	Pentatomidae	Sap from the shoots of *Coccinia grandis* L (Cucurbitaceae) and *prosopis juliflora* (Mimosaceae)

Table 3. Measurements in cm and weight in mg of body of larvae of *Coridius janus*

S.No.	Red bug	Body length(cms)	Body width(cms)	Body weight(mg)
1.	Larvae (v- instar)	1.50±0.075	0.80±0.102	230.00±15.80

Table 4. Measurement in mm and in µg of abdominal scent glands of *Coridius janus*

S.No.	Red bug	Length(mm)	Width(mm)	Weight(µg)
1.	First abdominal scent gland	1.12±0.15	0.87±0.14	185.67±18.60
2.	Second abdominal scent gland	2.07±0.21	2.72±0.33	298.65±16.20
3.	Third abdominal scent gland	2.66±0.54	3.22±0.41	345.70±24.50

Table 5. Development of abdominal scent glands of *Coridius janus*

S. No.	Larvae	I gland	II gland	III gland
1.	I instar Larva	Present, Non functional	Present, Non functional	Functional
2.	II instar Larva	Present, Non functional	functional	Functional
3.	III instar Larva	Endopolyploidy occurs	functional	functional
4.	IV instar Larva	Endopolyploidy continues	functional	functional
5.	V instar Larva	Endopolyploidy stop	functional	functional
6.	Imago	present	Slowly Atrophies	Slowly Atrophies
7.	One week adults female/male	Non-Functional (?) (No synthetic activity)	Completely degenerate	Completely degenerate

Muscles attached to the neck of third Reservoir (III MNR): These are four pairs of muscle fibres, originate from the beginning of sixth segments of the tergum and attached to the neck of the third abdominal scent gland and these muscles (MNR) regulate the compression of the third scent gland (Fig. 7). The striated muscle of the first pair of abdominal scent glands has its origin on the second segments of the tergum and its insertion on the mouth of the reservoir. The striated muscles help in operating the scent gland (Fig .8).

Histology: These glands are present in larvae of *C. janus.* In which first pair of abdominal scent gland is covered with yellowish layer and it is sac-like structure. The upper most layer is the epithelium which is made up of numerous cells. Each epithelium cell contains a growing nucleus and end-apparatus. The first pair of abdominal scent glands are developed from the first to fifth instar larvae but not appear to functional in all five stages. During post embryonic development of the cells of these glands gradually increase. The first pair of regulatory muscles which are attached to these glands show alternate light and dark bands. Their appearance are very clear in fifth instar larvae comparatively than other earlier instar larvae.

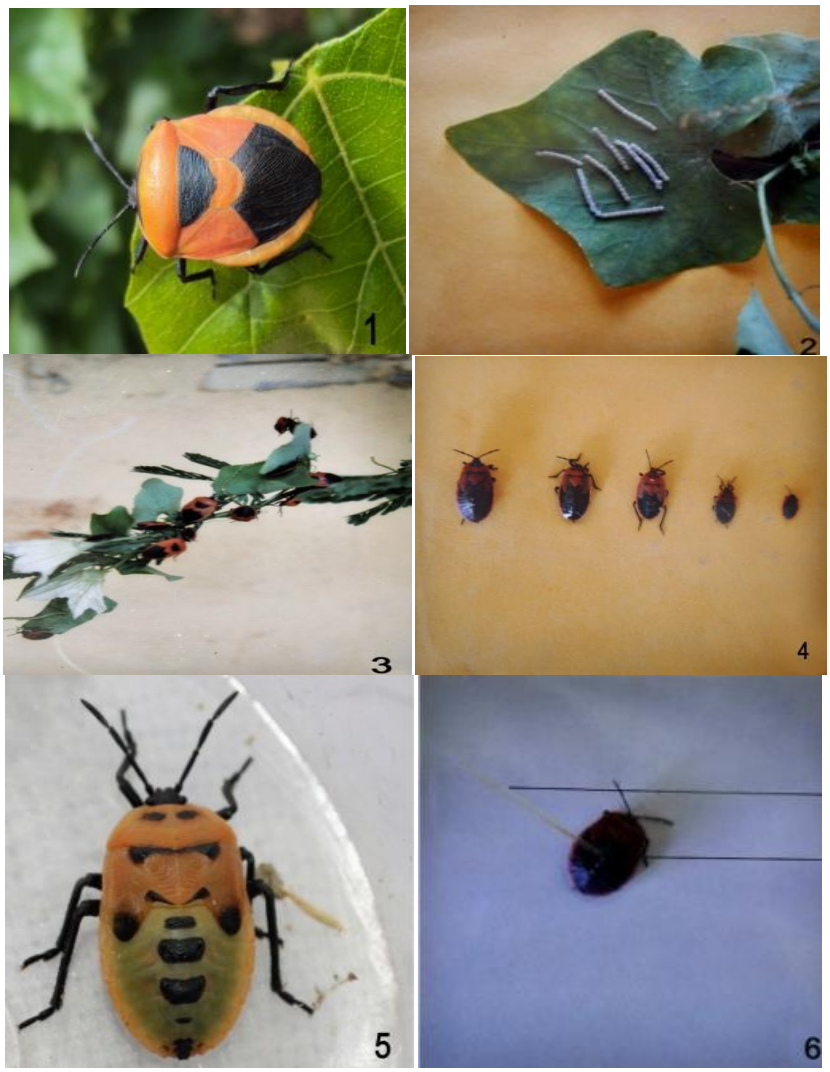

Fig. 1. Adult bug of *C. janus*
Fig. 2. Eggs on a leaf of host plant (*C. grandis* L.)
Fig. 3. The colony of various stages of larvae and adults of *C. janus*.
Fig. 4. Various instar larval stages of *C. janus* (from I instar to V instar larval stages)
Fig. 5. V instar larva of *C. janus*.
Fig. 6. Extraction of scent from the abdominal scent glands of *C. janus*
O = opening of abdominal scent gland; GC = Glass microcapillary

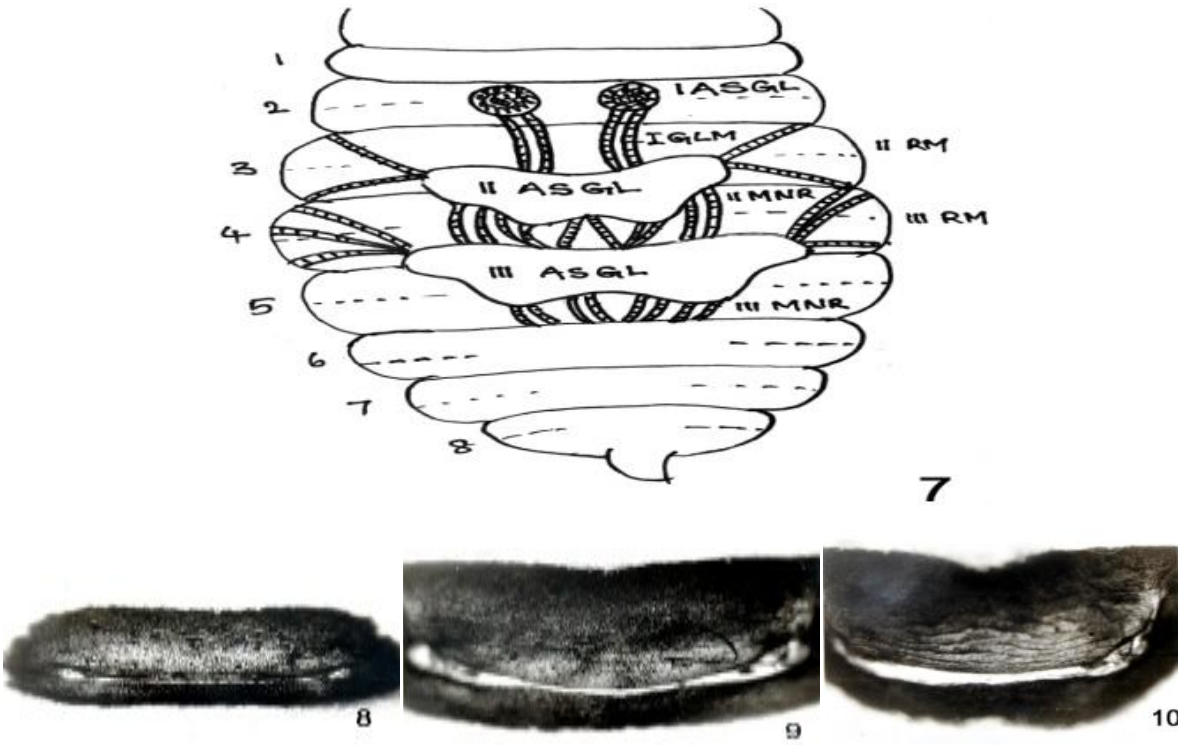

Fig. 7. Abdominal scent glands of *C. janus*

I ASGL = First abdominal scent gland
I GLM = First glandular muscle
II ASGL = Second abdominal scent glands
II RM = Second reservoir muscle
II MNR = Muscle attached to the neck of the second reservoir
III ASGL = Third abdominal scent gland
III RM = Third reservoir muscle
III MNR = Muscle attached to the neck of the third reservoir

Fig. 8. Opening of the first abdominal scent glands of *C. janus* Larvae
Fig. 9. Opening of the Second abdominal scent glands of *C. janus* Larvae
Fig. 10. Opening of the Second abdominal scent glands of *C. janus* Larvae

The remaining second and third abdominal scent glands of larvae also got covered by a yellowish outer epithelial layer and possesses on inner reservoir. The outer epithelial layer is similar to interstitial cells of the first pair of abdominal scent glands. The epithelial cells are secretory in function. These cells synthesize and secret the scent, while the end-apparatus of epithelial cells collects and transports scent secretions into reservoir of gland. The second reservoir muscle (II RM) and muscles attached to the neck of the second reservoir (II NMR) help in emitting the fluid from the reservoir.

The scent from the reservoir of second abdominal gland through a paired openings present on dorsal side of the abdominal tergum. The third reservoir muscle (III RM) and muscles attached to the neck of the third reservoir (III NMR) help in emitting the scent from the reservoir of third abdominal gland through a paired openings present on the dorsal side of the abdominal tergum. The second and third abdominal scent glands are functional in all instar larvae from first to fifth.

4. DISCUSSION

In the present investigation, the pentatomid bug, *C. janus* possesses three pairs of abdominal scent glands in the larvae. The presence of scent glands in hemipteran bugs is the striking feature and on being disturbed or irritated they eject out pungent, volatile and yellowish fluid with a jet speed. The chemical substances of the scent secretions are concerned with communication with other individuals of the same species, are called "Pheromones", or with other species known as "defensive secretions:. Defensive secretions against enemies often involves use of chemicals which are toxic or deterrent. The chemicals produced are often effective against a range of potential parasites and predators, both vertebrates and

invertebrates. Certain scent secretions of pentatomid and Heteropteran bugs . In the present study, *C. janus* shows that the scent gland complex is more or less similar to scent glands of several numbers of other pentatomid bugs which were described earlier (Gupta, [27]; Remold, [5]; Waterhouse and Gilby, [1]; Ahmad and Khan, [28]; Janaiah, [29,18]; Dhiman, [30]; Surender, [20]; Aldrich, [10]; Neelima Kumar and Kumar, [31]; Vidyasagar, [21]; C.H. Bottega et al. [32]).

In the present investigation, it is observed that all the five instar larvae of *C. janus* contained three pairs of abdominal scent glands, each gland arranged one behind the other in a row, in the mid dorsal region above the viscera under neath the tergum. The first pair of glands are separate in their position, small in size and ovel in shape hence, their secretions is very less and also not functional in all the five instar larvae. The second abdominal scent gland large, roughly tetragonal, yellowish and somewhat boat-shaped structure. The third abdominal gland slightly larger than the second abdominal scent gland, tetragonal in shape and yellowish in colour. The second and third pairs of glands are fully developed and functional in all five instar larvae. During metamorphosis from fifth instar larvae to adult, the second and third abdominal glands are gradually disappear but the first pair of non-functional abdominal glands of fifth instar larvae become functional in adult bugs (in few insects).

In *Coridius janus* it is found that the metathoracic glands start developing in larvae in the abdominal region. It is quite that the metathoracic glands did not replace the abdominal glands and further it is being confirmed that abdominal glands are present below the tergum in the larvae, on the other hand the metathoracic glands are found below the ventral nerve cord an the thorax of adult bug.

Insects of many varieties have developed various mode of chemical and behavioural defensive mechanisms. When disturbed, many hemipterans release a pungent and volatile fluide with an offensive odour. These secretions may be used by the bugs as defensive substances. In the present investigation, identification of scent secretions of *C. janus*(Fabr.) and the histomorphology, chemical composition of the scent secretion of larval abdominal scent glands.

5. CONCLUSION

C. janus possesses abdominal scent glands in larvae. The larvae contain three pairs of yellowish scent glands, arranged one behind the other in a row, in the mid dorsal region above the viscera in the abdominal segments of second and fifth underneath the tergum; each gland opens outside with a pair of ostioles. The first pair of glands smaller in size, oval in shape, where as second and third glands slightly larger, tetragonal in shape. Each abdominal scent gland is provided with regulatory muscles, on each side, to operate and control the glands. Each abdominal scent glands is covered with a yellowish layer, made up of epithelial cells, which are secretory in function. Each gland contains a growing gaint nucleus and a chitinous end-operatus with an elongated duct to drain the secretion into the reservoir where the scent is stored. The second and third gland reservoir muscles help in emitting the fluid from the reservoir.

ACKNOWLEDGEMENT

I am thankful to principal, SR & BGNR Govt. Arts & Science College, for his kind support, encouragement and institutional facilities. I am grateful to my Research supervisor Prof.C.Janaiah (Rtd) for encouraging, cooperation at various levels during the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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