www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 539- 550 (2016) DOI: 10.4194/1303-2712-v16_3_06

RESEARCH PAPER

Histological Changes of Y Organ in *Travancoriana schirnerae* during Moult Cycle and in de-Eyestalked Crabs

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Received 15 February 2016
Accepted 22 April 2016

Abstract

The Y organs of *Travancoriana schirnerae* were pale yellow, translucent, conical epidermal structures were located in the cephalothoracic region. The histology of the gland consisted of anastomosing lobules of epithelial cells separated by interconnected blood sinuses and capillaries. Two distinct cell types - larger and smaller - contributed to the cytology of the gland. The organ included medium sized lobules separated by indistinct blood sinuses and capillaries in the intermoult stage. The early premoult organ was characterized by large lobules with complete obliteration of the interlobular spaces and prominent haemal sinuses and capillaries. The larger cells with increased cytoplasmic volume, perinuclear granulations and nuclei containing extensive large chromatin granules were the prodigious features during early premoult. These cells demonstrated secretory vesicles during D_1 and D_2 stages and their mode of release appeared to be holocrine in nature. The gland showed large number of vacuoles with signs of degeneration in late premoult (D_4). The postmoult organ demonstrated smaller lobules with prominent interlobular spaces and indistinct blood sinuses and capillaries. Cellular degeneration and vacuolization were key features of the postmoult organ. Unilateral and bilateral eyestalk removal induced early premoult stages and produced marked changes in the morphology and histology of the organ during intermoult. In conclusion, the Y organ showed a cyclic change in activity in accordance with the moulting cycle.

Keywords: Blood sinuses, lobules, moulting stages, *Travancoriana schirnerae*, Y organ.

Introduction

The Y organs or ecdysial glands are ectodermally derived endocrine structures located in the cephalothorax, which are cytologically analogous to the prothoracic glands of insects and mediate several aspects of growth and reproduction in crustaceans (Chang *et al*., 1993). They are originated from the epidermis and either attached to the epidermis or become fully independent organs in various groups of Malacostraca (Bückmann, 1984). The regulation of Y organ activity is rather inquisitive; it seems to be mainly exerted by an inhibitory neuropeptide, moult inhibiting hormone or MIH, secreted from the X-organ-sinus gland complex (Skinner, 1985; Lachaise *et al*., 1993). The Y organs are the source of the moulting hormone, ecdysone which is secreted to the hemolymph as a precursor and converted into the active form, 20-hydroxyecdysone (Lachaise, 1990; Lachaise *et al*., 1993). Generally, moulting is found to be under the control of MIH and ecdysteroids (Skinner, 1985) and the Y organs play a decisive role in moulting and growth of crustaceans (Huberman, 2000). Gabe in 1953, first described the Y organs of Malacostraca and attributed a moult regulating functions to these glands. However, Gabe (1953) illustrated only minimal anatomical and cytological details of the organ, leaving much bewilderment regarding its precise location and identification among researchers. Later, Echalier (1955) established the role of these organs in moulting through bilateral extirpation experiments. Based on his work (1959), it has been speculated that the Y organs are the sites of ecdysteroid synthesis in crustaceans. Since then, numerous studies have been undertaken to depict the physiological role of this fascinating organ (Bollenbacher and O'Connor, 1973; Sonobe *et al*., 1991; Blais *et al*., 1994).

The Y organs have been extensively studied in marine brachyurans and natantian decapods. The moult related changes in the cytology of Y organs were reported in the portunid crab, *Portunus sanguinolentus* (Babu *et al*., 1989a). Zhi-junl *et al*. (2010) reported the histological changes of the Y organ in relation to ovarian development in the

swimming crab *P. trituberculatus*. The histology and the role of Y organs in moulting and growth have been characterized in *Penaeus japonicus* (Bourguet *et al*., 1977) and in *P. indicus* (Vijayan *et al*., 1993). The cytological aspects of Y organ were studied in the eyestalk ablated portunid crab *P. sanguinolentus* (Babu *et al*., 1989a). Furthermore, the endocrine function of Y organs has been demonstrated in several other species of decapods, isopods and amphipods (Passano and Jyssum, 1963; Maissiat, 1970; Blanchet, 1974).

The edible freshwater crab, *Travancoriana schirnerae* is a commonly distributed species in the wetlands of Wayanad, Kerala and is also known from the Southern Indian states of Karnataka and Tamil Nadu (Bahir and Yeo, 2007). Its meat forms a cheap source of animal protein for the poor, malnourished local tribes. A number of studies have emphasized the structural and functional aspects of Y organs in marine crabs (Babu *et al*., 1989a, b; Zhi-junl *et al*., 2010). Unfortunately, no studies have detailed the anatomical and cytological aspects of Y organs in freshwater crabs. The present investigation on histological changes in Y organs during different moult stages in *T. schirnerae* is reported to fill this gap which may possibly form the basis for further research on ultrastructural, cytochemical and physiological aspects of these organs. Moreover, the studies that elucidate the structure and activity of Y organs would contribute a better understanding of control of growth and moulting in crustaceans.

Materials and Methods

Adult male and female crabs (carapace width - 4.0 to 5.0 cm; 4.7±0.30) (n= 125) were collected from the paddy fields near Mary Matha Arts & Science College campus, Mananthavady, Wayanad (Kerala, India) during January 2013 to January 2014. Sampling was done twice in a month. The crabs were maintained in large cement tanks, fed ad libitum with chopped beef liver and boiled egg. The carapace width (CW) and wet weight were recorded for all the specimens collected. The moult stages were determined by observing the setal development of epipodite of the third maxilliped in males, pleopods in females and also by noticing the changes in the carapace texture (Anilkumar, 1980). For histological studies, the organs in different moult stages were fixed in Bouin's fluid, dehydrated in graded series of ethanol and embedded in melted paraffin wax. Sections of 5µm thickness were stained with Heidenhain's hematoxylin-eosin. Stained sections were observed under a Leica DM 500 Research microscope and photomicrographs were taken with a DG 330/120 camera, using Biowizard software. All the measurements were taken using an image analysis system of Biowizard software.

Eyestalk Ablation

Adult male and female crabs (CW 4.5-5 cm) of intermoult stage were used for this study. The individuals were left to acclimatize to the conditions of the laboratory for 72 hours before experimentation. The crabs (n= 30 each) were divided into three groups – control with intact eyestalks, -E1 with one eyestalk extirpated and -E2 with both the eyestalks extirpated. The surgical excision of the eyestalks was done using fine sterilized scissors. The trauma was lessened by applying ice to the wound. The wound was cauterized with a red-hot needle and swiped with sterilized cotton after the surgery. For bilateral eyestalk ablation, the second eyestalk was severed after 24 hours from the first. Both the control and experimental groups were sacrificed after 15 days and their Y organs were processed for histological studies.

Results

Morphology

The Y organs of *T. schirnerae* are paired, compact, conical and epidermal structures situated within the cephalothorax, anterior to the branchial chamber and postero-lateral to the eyestalks (Figure 1A, Figure 1B). The gland can be easily distinguished from the surrounding tissues by its pale yellow and translucent appearance. In adult animals, the size of the organ varied from 2.0 to 5.0 mm long and 1.0 to 3.5 mm wide.

Histology

Histologically, it was observed that the whole gland was composed of anastomosing lobules containing epithelial cell masses. The lobules were either in close apposition or separated by interconnected blood sinuses and capillaries. Frequently, hemocytes were noticed in the hemal spaces. The gland showed two cell types in the lobules which include smaller and larger ones (Figure 2A, Figure 2B). A clear cell boundary was apparently retained by both the cell types. The smaller cells measured 6.0 to10.0 µm (7.75±1.30) in width, polygonal in shape and much rarer in occurrence within the organ. They have round or oval prominent nuclei (3.0 to 5.0 µm in diameter; 3.06±0.93), showing strong basophilia. The centrally located nuclei revealed more evenly dispersed chromatin. The cytoplasm of smaller cells exhibited moderate basophilia and these cells were typically noted with high nucleocytoplasmic ratio (NPR) (0.50 to 0.66) (Figure 2B). The larger cells (11.0 to 27.5.0 µm in width; 18.06±5.13) demonstrated a round or oval shape and appeared to be more common. The nuclei were 3.0 to 10.0 µm in diameter (5.66±1.88), round or ovoid in shape and contained several distinctly aggregated basophilic chromatin granules. The nuclei were often eccentric and had one or two nucleoli. The cytoplasm was basophilic and granular in nature

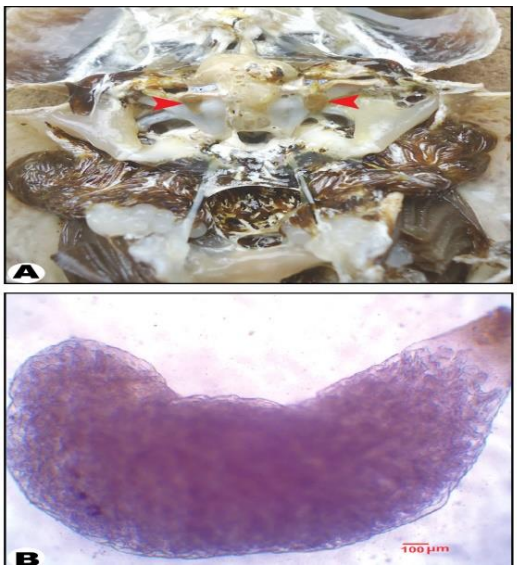

Figure 1. Y organ of *Travancoriana schirnerae*. A, Anatomical position of Y organ; B, Morphology of Y organ in intermoult stage. Y organ (arrow).

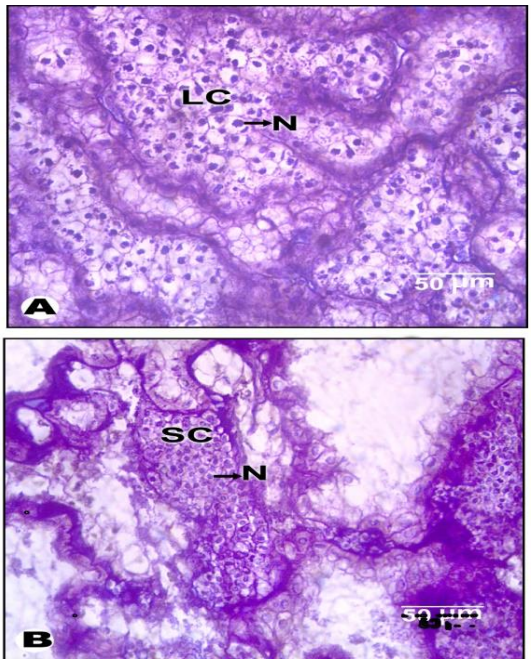

Figure 2. Histology of Y organ showing different cell types in *Travancoriana schirnerae*. A, Larger cell types of Y organ during early premoult D_1 stage; B, Smaller cell types of Y organ during intermoult stage. Larger cell (LC), nucleus (N), smaller cell (SC).

(Figure 2A). Generally, the NPR was reduced in larger cell types (0.20 to 0.47). At early premoult stages (D_1 and D_2), secretory vesicles became recognizable as large droplets in the cytoplasm of larger cells.

Moult Related Changes in the Histology of Y Organ

The Y organ showed substantial differences in the morphology and histology according to the moult stage of the animal.

Intermoult

During intermoult stage (C_4), the gland measured 2.0 to 3.0 mm (2.45±0.50) in length and 2.0 to 2.5 mm (2.17±0.24) in width. They appeared pale yellow in colour, translucent and flattened in nature. The lobules were medium sized (200.0 to 320.0 µm in length; 261.33±42) and inter and intra-lobular spaces found prominent (Figure 3A). The lobular epithelium measured 10.0 to 13.0 µm in width. There were no distinct blood sinuses and capillaries in the intermoult Y organ (Figure 3B). A few hemocytes were often

encountered in the inter-lobular hemal spaces. Both larger (70%) and smaller cells (30%) were apparent within the organ. Each lobule contained 25 to 30 larger cells. The larger cells measured 11.0 to 20.0 µm (14.79±3.15) wide and their nuclei (3.2±1.03; 2.5 to 5.0 µm;) appeared highly basophilic with granular chromatin. The nuclei were smaller when compared to other moult stages and the chromatin stained intensely with hematoxylin. Their cytoplasm was found to be homogeneous in nature (Figure 3C). A mild basophilia was occasionally found in the cytoplasm. However, a few larger cells with chromatin condensation were observed. Vacuoles were noticed in some areas of the gland. Furthermore, proliferation of the smaller cells (8.96±1.57; 6.0 to 10.0 µm) was another important observation of the intermoult Y organ, which ultimately appeared as patches within the gland (Figure 3D). These patches were either small with 20 to 35 cells or rather large with 70 to 100 cells. Their nuclei (3.15±1.01; 2.5 to 5.0 µm) showed a high basophilia with granular chromatin and cytoplasm was moderately basophilic (Figure 3D).

Premoult

D_1 Stage

The size and appearance of the Y organ (4.25±0.26; 4.0 to 4.5 mm in length and 2.5 to 3.0 mm in width; 2.83±0.24) varied markedly during the D_1 stage. The gland appeared large and pale brown in colour. The lobules were typically large (322.08±75.12; 220.0 to 500.0 µm) and closely packed without inter-lobular spaces (Figure 4A). The epithelium of lobule was found intact and increased in size (10.0 to 15.0 µm). The occurrence of large blood sinuses and numerous fine capillaries connected to these sinuses form another significant change during this stage (Figure 4B). The cells were densely packed and each lobule encompassed 30 to 46 larger cells. The larger cells (74%) were 13.0 to 25.0 µm in width (17.90±3.70). The most consistent and striking feature was nuclear granularity of larger cells. The nuclei showed characteristic fine granularity and moderate basophilia during early D_1 stage. At late D_1 stage, the nuclei presented numerous large chromatin granules and intense basophilia (Figure 4C). The nuclei (5.72±1.16; 5.0 to 7.5 µm) possessed one or two nucleoli. The cytoplasm was mildly basophilic and granular in nature (Figure 4C). In addition, small basophilic granules which tend to concentrate in peripheral cytoplasmic region of the cells. The larger cells showed enlarged cytoplasmic volume, which in turn resulted in obliteration of the intra-lobular spaces noted at intermoult. Appearance of intensely basophilic secretory vesicles (11%) (8.37±4.07; 2.5 to 15 µm in diameter) was another momentous observation of D1 stage (Figure 4D). The larger cells were found to accommodate single, round or oval secretory vesicles. Many of the secretory vesicles were observed towards the periphery of the larger cells. The cell boundaries of a few cells containing secretory vesicles were found broken. Very few vacuoles (5%) were noticeable inside the lobules. Smaller cells (6.0 to 10.0 µm) were scarce (10%) and their nuclei (3.0 to 5.0 µm) exhibited highly basophilic chromatin whereas cytoplasm was mildly basophilic in nature.

D_2 Stage

In the D_2 stage, the glands became greatly hypertrophied and measured 4.5 to 5.0 mm (4.85±0.24) in length and 3.0 to 3.5 mm (3.25±0.26) in width. The gland remained bulged and dark brown

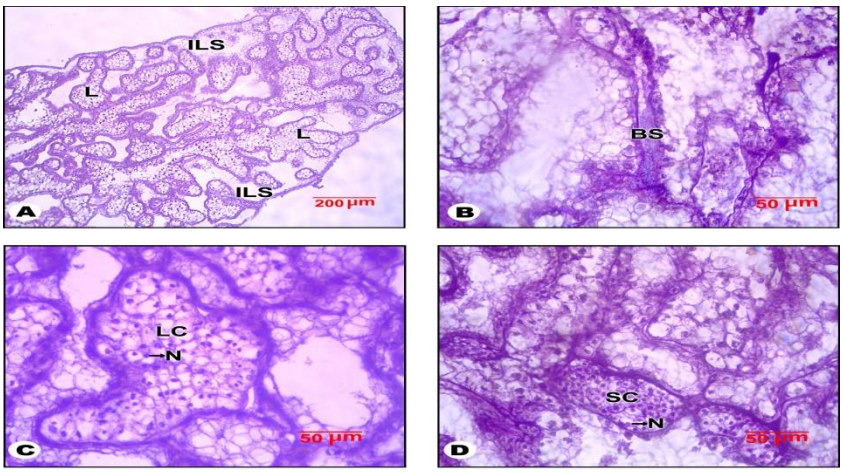

Figure 3. Y organ of *Travancoriana schirnerae* at intermoult stage. A, Y organ showing anastomosing lobules with interlobular spaces; B, Indistinct blood sinus of intermoult Y organ; C, Larger cells exhibiting high basophilic nuclei; D, Smaller cell proliferation during intermoult stage. Lobule (L), interlobular space (ILS), blood sinus (BS), larger cell (LC), nucleus (N), smaller cell (SC).

in colour. The size of the lobules significantly increased (425.7±108.31; 256.0 to 640.0 µm;) when compared to D_1 stage (Figure 5A). The lobular epithelium was intact, basophilic and further increased in size (12.0 to 17.0 µm). Large blood sinuses and innumerable fine capillaries were perceptible within the gland (Figure 5B). A few hemocytes were noticeable in capillaries and sinuses. The lobules were regularly packed with a huge number of larger cells (35 to 55). The size of the larger cells (69%) dramatically increased (24.47±4.42; 15.0 to 35.0 µm) than the D_1 stage (Figure 5C). Their nuclei (7.81±1.99; 5.0 to 10.0 µm) appeared highly basophilic and contained several distinctly aggregated large chromatin granules. The volume of cytoplasm was increased and they still retained the granular nature (Figure 5C). The perinuclear cytoplasm was rich in highly basophilic granules. The observed hypertrophy was more prominent in the larger gland cells near the hemocoelic sinuses. The secretory vesicles (10.43±3.96; 5.0 to 15µm) were seen more abundant (19%) during this stage (Figure 5D). Lobules with cells adjacent to the blood sinuses and capillaries were seen accumulated with a large number of secretory vesicles. These vesicles seemed to release their secretory product in to the sinuses and capillaries. The increase in percentage of vacuoles (10%) and a few secretory vesicles laden cells with indistinct cell boundaries indicate the holocrine mode of release of secretion. A few smaller cells (2%) were also evident; their nuclei showed strong basophilia with fine chromatin granules and cytoplasm mildly basophilic.

D_3 Stage

The Y organ measured 4.0 to 4.5 mm (4.22±0.26) long and 2.0 to 2.5 mm wide, appeared dull brown and flaccid in nature. The lobules were

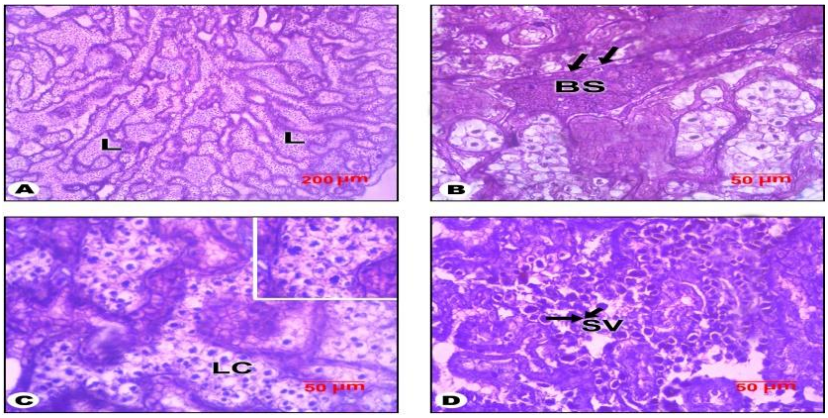

Figure 4. Y organ during early premoult D_1 stage in *Travancoriana schirnerae*. A, Y organ containing closely packed lobules without interlobular spaces; B, Presence of large blood sinus and numerous fine capillaries associated with sinuses; C, Larger cell nuclei exhibiting highly basophilic large chromatin (inset showing larger cell cytoplasmic granulation); D, Appearance of intensely basophilic secretory vesicles. Lobule (L), blood sinus (BS), larger cell (LC), secretory vesicle (SV), fine capillaries (arrows).

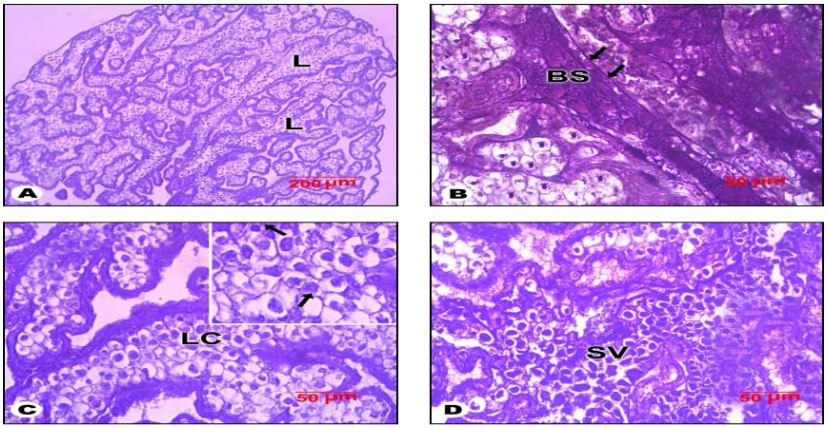

Figure 5. Y organ of early premoult D_2 stage in *Travancoriana schirnerae*. A, Y organ showing large lobules without interlobular spaces; B, Y organ with large blood sinus and innumerable fine capillaries; C, Larger cell much increased in size and nuclei presenting distinctly aggregated large chromatin granules (inset showing perinuclear cytoplasmic granulation); D, Large number of highly basophilic secretory vesicles associated with D_2 stage. Lobule (L); blood sinus (BS), larger cell (LC), secretory vesicle (SV), fine capillaries (arrows).

decreased in size (297.47±87.84; 200.0 to 500.0 µm). The lobular epithelium (12.0 to 15.0 µm) was intact and basophilic. The blood sinuses were large and a few capillaries were also evident within the organ. A few hemocytes were detected here and there in the hemal sinuses. Each lobule consisted of 24 to 37 larger cells. Intra-lobular space was perceptible in a small number of lobules. The larger cells (60%) measured 13.0 to 30.0 µm (18.76±4.67) in width and their nuclei (7.03±2.45; 5.0 to 12.5 µm) found basophilic with numerous large chromatin granules. The cytoplasm was basophilic and granular as observed in D_2 stage. An increase in the percentage of vacuoles (30%) could also be perceived. A few larger cells (10%) with indistinct cell boundaries were noticed within the lobules. No secretory vesicles were perceptible during this period.

Early D_4 Stage

In this stage, the organ appeared dull brown in colour, flaccid in nature and measured 4.0 to 4.1 mm (4.03±0.04) in length and 2.0 to 2.5 mm (2.27±0.26) in width. The lobules were decreased in size (223.63±63.76; 120.0 to 300.0 µm) and loosely arranged with inter-lobular spaces. The lobular epithelium degenerated and their nuclei showed pycnosis. A large blood sinus was still evident while hemal sinuses and capillaries were generally decreased during this stage. Each lobule contained a small number of larger cells (10 to 15) with intra-lobular spaces. Very few intact larger cells (19.42±5.44; 11.0 to 27.0 µm) were detectable within the gland. Their nuclei exhibited chromatin condensation and cytoplasm demonstrated mild basophilia. The vacuolization was more conspicuous than the D_3 stage and the gland showed signs of degeneration (Fig 6A).

Late D4 Stage

The lobules were small, shrunken and atrophied in nature with prominent inter and intra- lobular spaces (Figure 6B). The lobular epithelium was degenerated and disrupted. No blood sinuses and capillaries were distinct in this stage. Pycnotic nuclei were found scattered inside the lobules. The cellular degeneration was found to be the prodigious feature of Y organ (Figure 6B).

Postmoult

C_1 Stage

The Y organ of C_1 stage measured 3.5 to 4.0 mm (3.75±0.26) long and 2.0 to 2.5 mm (2.15±0.21) wide. They were small and pale brown in colour. The lobules exhibited a decrease in size (271.7±58.41; 222.0 to 388.0 µm;) and arranged loosely with pronounced inter-lobular spaces (Figure 6C). The lobular epithelium has a degenerated appearance. The lobules occupied lesser number of cells and intra-lobular spaces were more pronounced in this stage (Figure 6C). The larger cells were decreased in size (15.90±3.95; 11.0 to 15.0 µm). Their nuclei (5.88±2.44; 3.0 to 8.0 µm) exhibited chromatin condensation and cytoplasm was depleted and transparent in nature. Cellular degeneration was still apparent within the gland; a few vacuoles were perceptible within the organ (Figure 6D). The hemal sinuses and capillaries were not distinct.

C_2 Stage

In the C_2 stage, the organ measured 3.0 to 4.0 mm (3.4±0.5) long and 2.0 to 2.5 mm (2.12±0.23) wide. The lobules were small (185.23±64.27; 80.0 to 350.0 µm) with more pronounced intra and inter-lobular spaces than the C_1 stage (Figure 7A, Figure 7B). The lobular epithelium was degenerated in nature. The blood sinuses and capillaries were not discernible within the organ. The lobules with intact cells were typically not palpable and most of them exhibited degenerated appearance (Figure 7B).

C_3 Stage

In this stage, the Y organ measured 2.5 to 3.0 mm (2.66±0.25) long and 1.0 to 2.0 mm (1.62±0.51) wide. They were small and pale yellow in colour. The gland as a whole appeared shrunken in nature. The lobules showed further decrease in size (136.56±71.09; 50.0 to 320.0 µm;) and arranged loosely with large inter-lobular spaces (Figure 7C). The lobular epithelium fully degenerated in nature; degenerated cells and vacuoles were still evident in the lobules (Figure 7D). Neither blood sinuses nor capillaries were noticeable in the organ.

Effect of Eyestalk Ablation on the Histology of Y Organ

The morphology and histology of the Y organ of both the control and eyestalk ablated groups found inextricably varied. The control crabs remained in the intermoult stage whereas eyestalk ablation induced early premoult stages (D_1 and D_2) in the experimental crabs.

Histology of the Y Organ of Control Crabs

The whole gland measured 2.0 to 3.0 mm (2.36±0.49) in length and 1.0 to 2.0 mm (1.5±0.51) in width. They appeared small, pale yellow in colour, translucent and flattened in nature. The lobules were small (205.75±89.90; 81 to 437 µm in length) with prominent inter and intra-lobular cell free spaces (Figure 8A). The lobular epithelium (7.0 to 9.0 µm in width) was narrow and shrunken in nature and their cells (3.0 to 5.5 µm in width) demonstrated small,

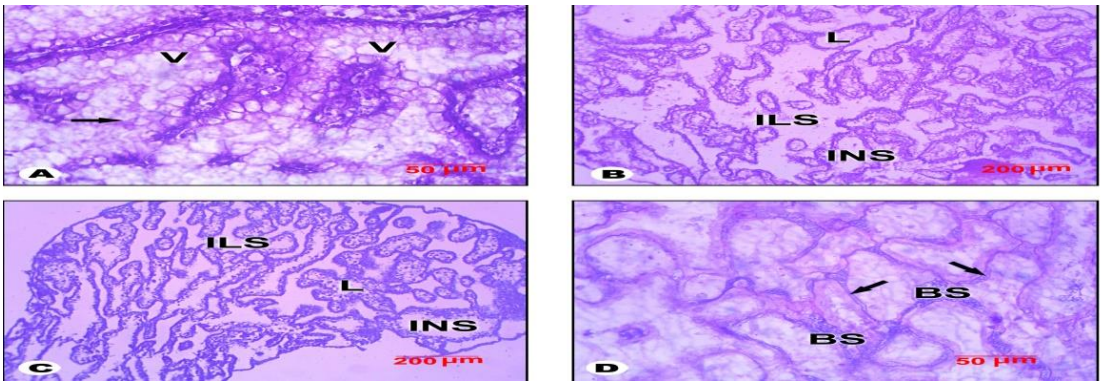

Figure 6. Y organ of *Travancoriana schirnerae* at late premoult D_4 and postmoult stages. A, Vacuoles and cellular degeneration at early premoult D_4 stage; B, Atrophied nature of Y organ with prominent inter and intralobular spaces at late D_4 stage; C, Y organ showing pronounced inter and intralobular spaces at postmoult C_1 stage; D, Y organ with indistinct blood sinuses and capillaries during postmoult C_1 stage. Lobule(L), blood sinus (BS), interlobular space (ILS), intralobular space (INS), vacuole (V), cellular degeneration (arrow in figure A), capillaries (arrow in figure D).

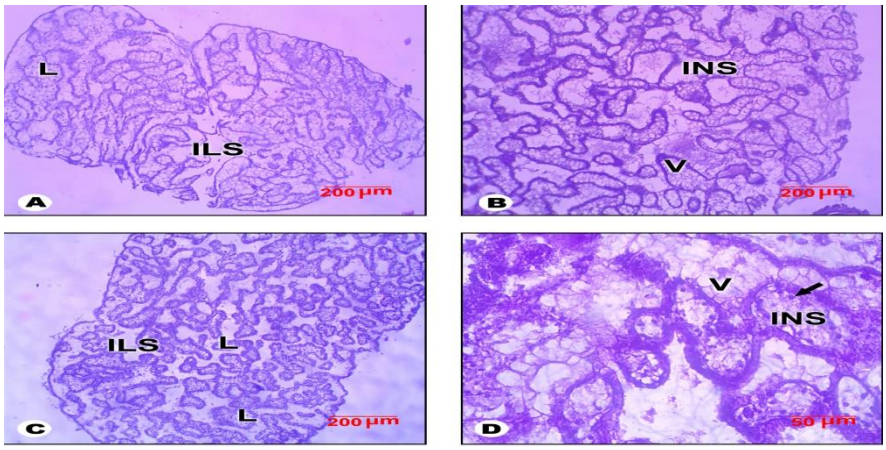

Figure 7. Y organ tissue of *Travancoriana schirnerae* at postmoult C_2 and C_3 stages. A, Organ showing smaller lobules accompanied by pronounced interlobular spaces during postmoult C_2 stage; B, Vacant lobules with degenerated cells and vacuoles in postmoult C_2 stage; C, Small shrunken lobules with prominent interlobular spaces in postmoult C_3 stage; D, Y organ containing fully degenerated cells and vacuoles during postmoult C_3 stage. Lobule (L), interlobular space (ILS), intralobular space (INS), vacuole (V), cellular degeneration (arrow).

basophilic nuclei (Figure 8B). The organ was characterized by indistinct blood sinuses and capillaries (Figure 8C). The lobules accommodated only few cells (20 to 30). These cells (15.30±3.67; 10.0 to 23.3 µm wide) portrayed small and condensed nuclei (3.72±1.29; 2.0 to 6.6 µm in diameter) and their cytoplasm appeared transparent. A few cells noted with intact boundaries while in large number of cells, boundaries were hardly discernible (Figure 8D). The whole gland exhibited signs of cellular degeneration.

Histology of the Y Organ of Unilateral Eyestalk Ablated Crabs

Of the 30 animals in which unilateral eyestalk ablation was performed, 20 showed changes in both the morphology and histology; 8 did not show obvious changes when compared to control crabs and 2 died during the experiment.

The gland appeared large, 3.5 to 4.5 mm in length (3.97±0.35) and 2.5 to 3.0 mm in width (2.78±0.25) and pale brown in colour. The lobules (250 to 903 µm; 507.64±143.31) were significantly large and closely packed with diminished inter and intra-lobular spaces when compared to control crabs (Figure 9A). The occurrence of large blood sinuses and numerous fine capillaries connected to these sinuses form another noteworthy character of -E1 crabs (Figure 9B). The gland also projected several small blood sinuses and numerous capillaries were seen encircling the lobules. Each lobule accommodated densely packed cells (40 to 100) with distinct cell boundaries, measured 16.0 to 31.0 µm in width (21.87±3.73). Their cytoplasm appeared mildly basophilic and granular (Figure 9C). The nuclei were large (9.28±1.78; 5.0 to 12.5 µm), round or oval, centrally or peripherally placed with intensely basophilic chromatin granules. A few cells contained oval, intensely basophilic secretory vesicles

(10.74±3.71; 3.5 to 21.4 µm diameter) (Figure 9D). Occasionally, the boundaries of a few cells carrying the secretory vesicles were found broken, releasing their contents. The gland showed remarkably few vacuoles (Figure 9E).

Histology of the Y Organ of Bilateral Eyestalk Ablated Crabs

Twenty four out of 30 crabs bilaterally destalked showed extensive changes in morphology and histology while 3 did not show any change and 3 died during the experimental period.

In bilaterally destalked crabs, the gland appeared greatly hypertrophied and measured 4.5 to 5.0 mm (4.79±0.25) in length and 3.0 to 4.0 mm (3.62±0.5) in width. The gland has a bulged appearance and dark brown in colour. The lobular size (600.41±190.45; 222.0 to 950.0 µm) was significantly increased with total annihilation of inter and intra-lobular spaces (Figure 10A, B). Large blood sinuses and innumerable fine capillaries were perceptible within the gland (Figure 10C). The lobules contained more number of cells (50 to 200) compared to -E1 crabs. The cells (28.68±4.47; 16.0 to 36.0 µm) displayed evidences of hypertrophy (Figure 10D). The observed hypertrophy was more prominent in cells near the haemocoelic sinus. Their oval nuclei (11.07±2.05; 6.0 to 14.0 µm) centrally or peripherally located with several large, distinctly aggregated basophilic chromatin granules. The nuclear hypertrophy was much more pronounced than - E1crabs signifying the

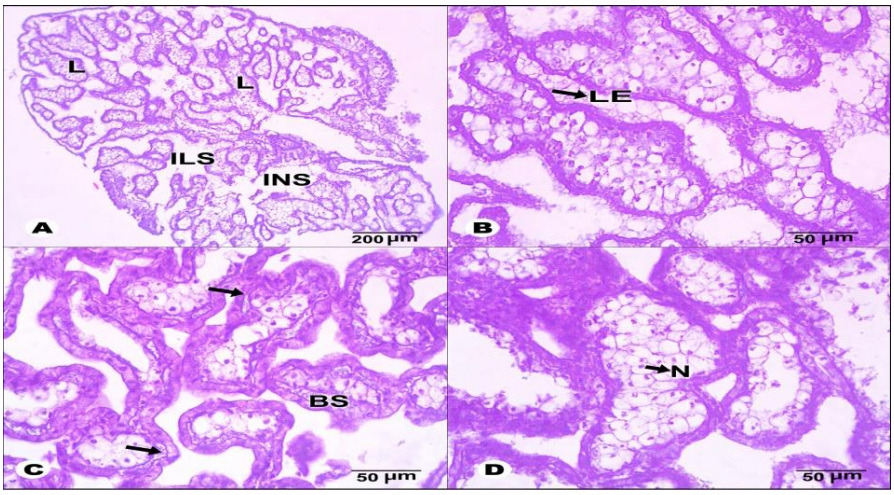

Figure 8. Histological sections of Y organ of a control specimen of *Travancoriana schirnerae*. A, Y organ with large inter and intra-lobular spaces; B, Narrow and shrunken lobular epithelium; C, Indistinct blood sinuses and capillaries; D, Cells in lobules showing small and condensed nuclei. Blood sinus (BS), inter-lobular space (ILS), intra-lobular space (INS), lobule (L), lobular epithelium (LE), nucleus (N), capillaries (arrow).

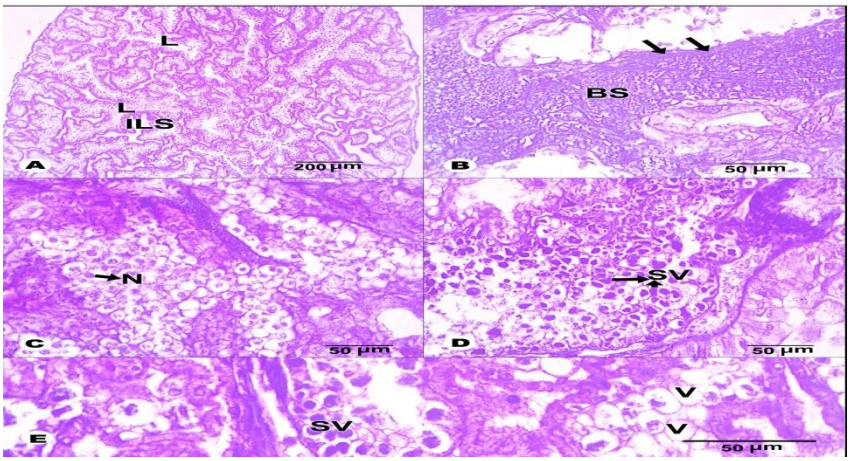

Figure 9. Histology of Y organ in unilaterally eyestalk ablated *Travancoriana schirnerae.* A, Y organ composed of closely packed lobules with reduced inter and intra-lobular spaces; B, Large blood sinus with numerous fine capillaries; C, Lobules containing densely packed cells showing chromatin granulation; D, Y organ with intensely basophilic secretory vesicles; E, Occurrence of vacuoles. Blood sinus (BS), inter-lobular space (ILS), lobule (L), nucleus (N), secretory vesicle (SV), vacuole (V), capillaries (arrow).

increased activity of cells. The abundance of secretory vesicles (17.78±3.47; 6.0 to 22 µm) observed in the lobules indicated the high synthetic activity of the organ. The secretory vesicles adjacent to the blood sinuses seemed to release their contents in to these sinuses suggesting the holocrine mode of release of secretion (Figure 11). The number of vacuoles found increased than -E1 crabs (Figure 12).

Discussion

The histological structure of the Y organ observed in *T. schirnerae* was identical to that described for other brachyurans which contained

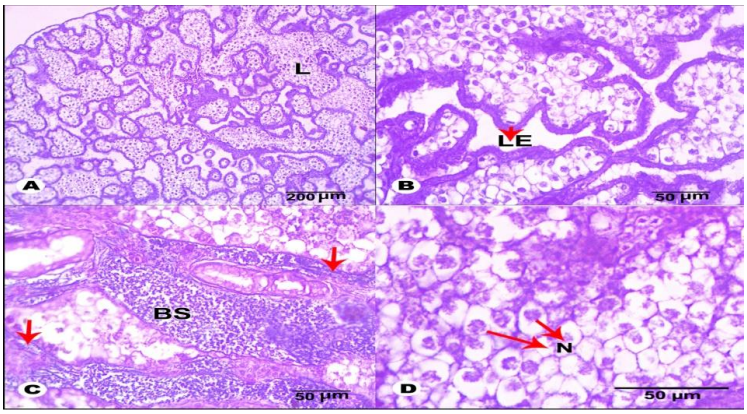

Figure 10. Histology of the Y organ of bilaterally destalked *Travancoriana schirnerae*. A, Y organ showing large lobules; B, Lobules with intact epithelium; C, Y organ with large blood sinuses and capillaries; D, Lobules with cellular hypertrophy. Blood sinus (BS), lobule (L), Lobular epithelium (LE), nucleus (N), capillaries (Arrow).

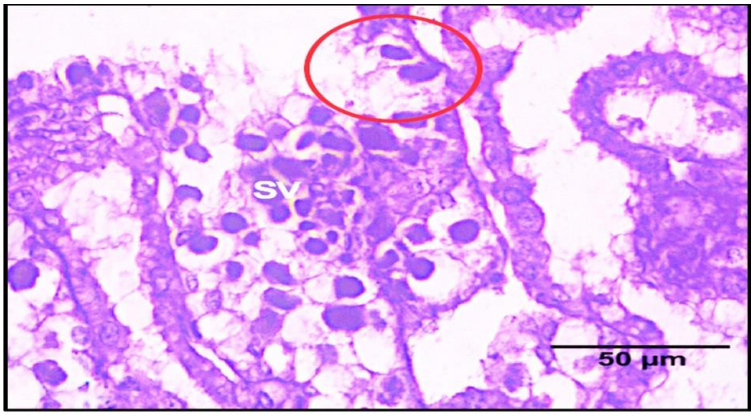

Figure 11. Y organ of bilaterally eyestalk ablated *Travancoriana schirnerae* showing holocrine mode of secretion. Secretory vesicle (SV), holocrine mode of release of secretion (Circle).

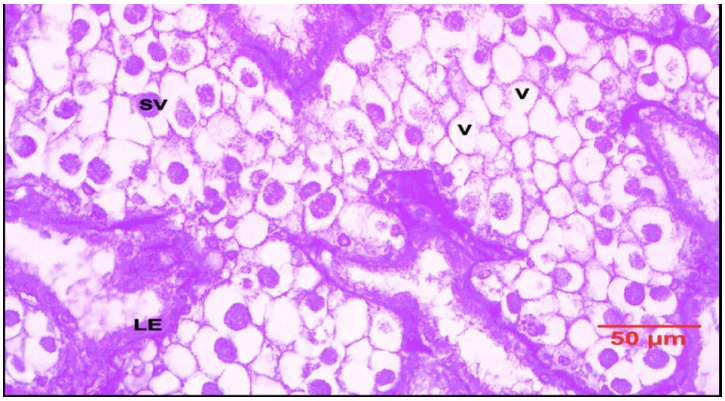

Figure 12. Y organ of bilaterally eyestalk ablated crab showing secretory vesicles and vacuoles. Lobular epithelium (LE), secretory vesicle (SV), vacuole (V).

anastomosing lobules interconnected by blood sinuses and capillaries (Babu *et al*., 1989a, b; Buchhlolz and Adelung, 1980). Likewise, in *Palaemon paucidens*, the organ was composed of many lobulated cell masses (Aoto *et al*., 1974). In *Metopograpsus messor*, the organ encompassed closely packed cells, which in some instances displayed lobulated appearance (Shyamal *et al*., 2014). On the other hand, the histological appearance of the gland was appreciably different in *Pandalus kessleri* (Aoto *et al*., 1974) and the organ represented as folded invaginations of the epidermis with many lumens.

It has been confirmed histologically that the Y organ of *T. schirnerae* was composed of two cell types. Madhyastha and Rangnekar (1972) illustrated two types of cells in the Y organ of *Varuna litterata* while Hoffman (1967) and Babu *et al*. (1989a) reported only one cell type. On the other hand, the organ was composed of smaller epithelial cells in *P. sanguinolentus* and *Charybdis annulata* (Babu *et al*., 1989a, b). It is clearly showed that in *T. schirnerae*, the smaller cells were distinct cell types as evidenced by their proliferation during intermoult stage. A possible explanation for this situation was noted in the review of Lachaise *et al*. (1993) wherein he mentioned that smaller cells with large nuclei and sparse cytoplasm were a constant feature of resting intermoult gland.

The histological structure of the Y organ of *T. schirnerae* found to be varied according to the moult cycle of the animal. In the intermoult stage, the organ contained medium sized lobules separated by indistinct blood sinuses and capillaries and prominent inter-lobular spaces. Such a situation has been demonstrated for other crustaceans also. In *P. paucidens*, the intermoult organ possessed many inter-lobular spaces and the organ cells exhibited small amounts of cytoplasm (Aoto *et al*., 1974). Babu *et al*. (1989a) stated that the intermoult Y organ contained profusely branched lobules separated by indistinct blood sinuses and capillaries with pronounced inter-lobular spaces. Hoffman (1966) mentioned that in the intermoult, new cells are being formed to compensate for those cells destroyed during moult cycle as well as for normal growth of the organ.

The present observations on the premoult Y organ of *T. schirnerae* showing the presence of large lobules, prominent hemal sinuses and capillaries, complete obliteration of the inter-lobular spaces, larger cells with high amount of cytoplasm and extensive nuclear granularity, all pointed towards the high synthetic nature of the gland. A Similar observation was made from the premoult Y organ of *P. sanguinolentus* (Babu *et al*., 1989a). An analogous situation was also noted in *P. paucidens* where the cytoplasmic volume reached its highest value and the chromatin became highly granulated during late premoult stage (Aoto *et al*., 1974). The only noteworthy change reported by Matsumoto (1962) in *Hemigrapsus* sp. was the progressive increase in cytoplasmic volume relative to the nuclei in the Y organ cells of premoult individuals.

In the present study, the presence of secretory vesicles in the D_1 and D_2 stages may indicate the secretory activity of the organ and this process might be associated with the accumulation of moulting hormone within the organ cells for the successive physiological changes during moult cycle. Ultrastructural studies in *M. messor* revealed that the secretion of the Y organ was at its peak in premoult crabs (Shyamal *et al*., 2014). The release of hormonal substances from organ cells was facilitated by the simultaneous vascularisation (Babu *et al*., 1989a). A possible explanation for the cytoplasmic vacuolization and cellular degeneration in the Y organ may be evidences for its holocrine mode of release of secretion (Babu *et al*., 1989a). Simione and Hoffman (1975) have also reported a holocrine mode of release of secretion in the Y organ of *Cancer irroratus*.

In the present study, the postmoult Y organ displayed small lobules with cellular hypotrophy and degeneration, prominent intra and inter-lobular spaces and indistinct hemal sinuses and capillaries. All taken together, convey an impression of inactivity of the organ and a similar trend was noticed in *P. sanguinolentus* (Babu *et al*., 1989a) and *P. paucidens* (Aoto *et al*., 1974), where the organ showed disintegration and subsequent decrease in the amount of cell cytoplasm. This phenomenon might be correlated with the transport of the hormonal substances to their respective target tissues and the reappearance of inter-lobular spaces may indicate the reduced vascular supply within the organ (Babu *et al*., 1989a).

Eyestalk ablation experiments in various crustaceans have adumbrated that the eyestalks are the source of MIH which generally inhibits moulting (Hinsch and Al Hajj, 1975). Interestingly, the changes noted in the histology of Y organ of unilateral and bilateral eyestalk ablated *T. schirnerae* revealed close resemblances with those of early premoult crabs. It is apparent that the removal of eyestalks also removed the MIH from the system, which induced the physiological functioning of the Y organ, resulting cellular hypertrophy. The organ cells with increased cytoplasmic volume, extensive chromatin and cytoplasmic granulations observed in the present investigation pointed towards the high synthetic nature of the gland. In the same way, in bilaterally destalked *P. sanguinolentus*, the Y organ cells showed evidence of hypertrophy wherein the cytoplasm increased in volume and nuclei appeared larger (Babu *et al*., 1989a). The hyper activity of Y organ cells following eyestalk ablation was noticeable in *C. irroratus* (Simione and Hoffman, 1975), *Pachygrapsus marmoratus* (Bressac, 1976) and *C. antennarius* (Hinsch *et al*., 1980). The organ demonstrated marked cytological changes following eyestalk ablation in the freshwater prawn *P. paucidens* (Aoto *et al*., 1974) and in the crayfish

Orconectes limosus (Gersch *et al*., 1977).

In general our observations led us to conclude that the Y organs showed cyclic changes in its morphology and histology in relation to moult cycle in the freshwater crab *T. schirnerae*. The two cell types recognized in the Y organ may possibly represent the different stages of the moulting cycle and not functional cell types. The presence of secretory vesicles in the early premoult stages might possibly indicate the accumulation of hormonal substance for the forthcoming physiological event, ecdysis. The results of the eyestalk ablation fortify the classical bihormonal hypothesis of moult control of decapod crustaceans, which establishes the fact that the moult cycle is controlled by the reciprocal action of two hormones, MIH from the eyestalk X organ-sinus-gland complex and ecdysteroids from the Y organ. Further research is needed at the ultrastructural level to clarify the structure and the physiological role of Y organ in this species.

Acknowledgements

This work was supported by a grant from Kerala State Council for Science Technology & Environment (Kerala, India).

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