MORPHOLOGY AND HISTOLOGY OF Y ORGAN IN RELATION TO GROWTH AND REPRODUCTION IN THE FRESHWATER CRAB *BARYTELPHUSA CUNICULARIS*

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ABSTRACT

This study aims to determine the morphological and histological changes of Y organ in relation to growth and reproduction in the female freshwater crab Barytelphusa cunicularis. The Y organs are ectodermally derived, lobulated endocrine glands entrenched in a brown fatty tissue in the cephalothoracic region and consisted of a network of lobules interconnected by many blood sinuses and capillaries. The gland contained two cell types: type I and type II and exhibited remarkable changes in gross morphology and histology in relation to growth and reproduction. The organ was very small and composed of only type I cells at stage I. Both the cell types and hemal sinuses were evident in the organ of stage II crabs. Type II cells with increased cytoplasmic volume and prominent blood sinuses and capillaries were characteristic of Y organ of stage III crabs. Large lobules with compactly packed type II cells were observed in stage IV. The organ displayed substantial differences in morphology and histology in relation to various phases of oogenesis, with least level of activity during avitellogenic and late vitellogenic phases and maximum activity during early and middle phases of vitellogenesis. During early vitellogenic phase, the organ exhibited type I cell proliferation. Large lobules with total elimination of intra and interlobular spaces and rich vascularization were the important features of the gland during middle phase of vitellogenesis. The gland has a vacuolated appearance showing signs of degeneration in late vitellogenic phase. To conclude, the Y organ of female B. cunicularis demonstrated discernible changes in morphology and histology in relation to various developmental stages and vitellogenic phases, indicating its role in growth and reproduction. Further research is yet to be carried out on ultrastructural profiles of the gland during various developmental and vitellogenic phases.

Keywords: Barytelphusa cunicularis, developmental stages, histology, oogenesis, Y organ

INTRODUCTION

The Y organs are epidermal structures located in the cephalothorax and are similar to the prothoracic glands of insects in position, appearance and function (Chang et al., 1993). In isopods and amphipods, these glands measured 60-300 µm and in decapods they were 1-3 mm in size (Diwan, 2005). The Y organ differs in gross morphology (conical in Brachyura, tenticular in Natantia and foliaceous in Isopoda) and location in different species of crustaceans (Spindler et al., 1980). The organ controls moulting, reproduction, calcium distribution and other physiological activities (Huberman, 2000). The gland synthesizes and releases the moulting hormone, ecdysone, into the hemolymph where it is converted to 20-hydroxy ecdysone (20-OH) (Chang and Kaufman, 2005). The gland seemed to produce another type of ecdysteroid, identified as 3-hydroxyecdysone (Lachaise et al., 1986; Spaziani et al., 1989).

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The synthesis and release of ecdysteroids are negatively regulated by the moult inhibiting hormone (MIH), an inhibitory neuropeptide secreted by the X-organ-sinus gland complex of the eyestalk. The inhibitory mechanism of MIH on Y organ has been extensively investigated in several crustaceans (Bliss, 1956; Passano, 1960; Passano and Jyssum, 1963). It has also been found that the synthesis of ecdysteroid is also inhibited by Xanthurenic acid (XA), a tryptophan derivative isolated from the eyestalk (Soyez and Kleinholz, 1977; Naya et al., 1988)

The Y organ was first described by Gabe in 1953 and later explained in several malacostracan crustaceans including shrimps (Vijayan and Diwan, 1993; Charmantier, 1994). Echalier (1955, 1959) provided an ambiguous illustration of their location in the shore crab *Carcinus maenas*. In vitro incubation of *C. maenas* Y organ revealed the production and release of 25-deoxy form of ecdysone (Lachaise et al., 1986, 1989). In the freshwater crayfish *Procambarus* c*larkii*, Sonobe et al. (1991) showed in vitro secretion of ecdysteroids by the Y organ.

Several light and electron microscopic investigations have been carried out on Y organ of marine, brachyuran and natantian decapods. Birkenbeil and Gersch (1979) detailed the ultrastructural changes in Y organ of the noble crayfish *Astacus astacus* in relation to moult cycle. In the Pacific rock crab *Cancer antennarius*, Hinsch et al. (1980) studied the fine structural changes in Y organ of normal and destalked crabs. Babu et al. (1989a) described histological changes of Y organ in the three-spot swimming crab *Portunus sanguinolentus* during moult cycle. Histological changes of Y organ in relation to moult cycle and under destalkation has been portrayed in the freshwater crab *Travancoriana schirnerae* by Smija and Sudha Devi (2016).

Very few studies explored growth, development or vitellogenesis related changes in the histological profile of Y organ. The failure of folliculogenesis by Y organectomy during mid-vitellogenic stages was observed in the shrimp *Lysmata seticaudata* (Charniaux-Cotton and Touir, 1973). In the amphipod *Orchestia gammarella*, amputation of Y organ resulted in decreased vitellogenin synthesis and ovarian growth (Meusy et al., 1977). The light microscopic observations of Y organ in the larval stages of *C. anthonyi* were made by Mc Conaugha (1980). Okumura et al. (1992) observed the correlation between ecdysteroid levels in the hemolymph and vitellogenic stages of *Macrobrachium nipponense*. Liu et al. (2010) reported the vitellogenic related anatomical changes in relation to vitellogenesis in the crab *P. trituberculatus*. Shyamal et al. (2014) examined variations in the secretory activity of Y organ in relation to moult and reproductive cycle in *Metopograpsus messor*.

The edible freshwater crab *Barytelphusa cunicularis* abundantly distributed in the streams of Wayanad forms an inexpensive means of nutrients to the malnourished local tribes. Unfortunately, no scientific study reported growth and vitellogenesis related changes in the anatomy of Y organ in freshwater crabs. The present investigation on morphological and histological changes of Y organ in relation to growth and vitellogenesis in the freshwater crab *Barytelphusa cunicularis* is reported to fill this gap.

MATERIALS AND METHODS

Adult female crabs of different developmental stages (carapace width 1.0 to 9.0 cm) and vitellogenic phases were collected from holes along the margin of streams near Chettapalam, Mananthavady, Wayanad (Kerala, India) during June 2016 to May 2017. The crabs were preserved in large cement tanks and fed with pulses and beef liver. The wet weight and carapace width (CW) were documented for all the specimens collected. The moult stages were determined by noticing the setae of epipodite of the third maxilliped in males and

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pleopods in females and also by checking the hardness of the exoskeleton (Anilkumar, 1980). The Y organs were dissected out; their size and wet weights were recorded. The excised organs were fixed in Bouin's fluid overnight. The tissue was dehydrated in graded alcohol, cleared in xylene and embedded in melted paraffin wax. Paraffin blocks were cut with a thickness of 5 µm and stained with Heidenhain's hematoxylin-eosin. The slides were examined under a Leica DM Research Microscope and photomicrographed with a DG 330/120 camera attachment using Biowizard software.

For characterization of the vitellogenic phases, the ovaries were carefully removed, weighed and the gonadosomatic index (GSI) was calculated as the percentage ratio of ovarian weight to the body weight. Micrometric measurements of a minimum of randomly chosen one hundred oocytes were taken from one half of the ovary with a calibrated ocular micrometer. The other half was preserved in Bouin's solution and processed for histology.

The gross morphology and histology of Y organ were studied during different developmental stages and oogenic phases. The distinct cell types found in the Y organ were classified based on the differences in their size and size and shape of the nuclei. Diameter of cells was measured and mean±SD of the diameter obtained for each cell type was calculated.

RESULTS

Morphology

The Y organs are lobulated endocrine glands of ectodermal origin, embedded in a brown fatty tissue of the cephalothorax in crustaceans. The size of the organ varied from 1-8 mm long and 1-4 mm wide in crabs of carapace widths ranging from 1-9 cm. In live specimens, the glands can be readily discernible from the surrounding tissue by their pale translucent appearance. The glands lie in flat depressions filled with hemolymph.

Histology

The organ consisted of anastomising lobules of epithelial cells detached by numerous interconnected haemal sinuses and capillaries. Each lobule was composed of 10-50 cells, enveloped by a branch of connective tissue (10.12±4.35 µm in width). The gland is comprised of two cell types: type I and type II. Type I cells were small (9.10±3.28 µm in diameter) with round nuclei (4.0±1.3 µm in diameter) and were characterized by high nucleocytoplasmic ratio (NPR) (0.38±0.10). The cytoplasm of these cells displayed moderate basophilia. Type II cells were considerably larger (16.33±4.54 µm in diameter) with elliptical nuclei. The large spherical or oval shaped nuclei (4.50±1.64 µm) of these cells were centrally placed and possessed central nucleoli and chromatin material scattered in the nucleoplasm. The cytoplasm was basophilic and granular in nature. Generally, the NPR was low in type II cells (0.25±0.08). Nerve supply was not visualized in the Y organ of *B cunicularis*.

Developmental Morphology and Histology of the Y Organ

The Y organ of *B. cunicularis* exhibited development-related changes in gross morphology and histology. The development of Y organ can be classified into 4 stages: stage I comprising crabs of CW 1-3 cm; stage II comprising crabs of CW 4-5 cm; stage III comprising crabs of CW 6-7 cm and stage IV comprising crabs of CW 8-9 cm.

Morphology and Histology of Y Organ of Stage 1 Crabs (1-3 cm CW)

In young crabs of CW 1-3 cm, the gland appeared small (2.5±0.5 mm in length and 1.5±0.5 mm in width), creamy yellow, translucent and weighed 1.3±0.5 mg (Table 1), (Fig. 1). The lobules of the gland were small (120.14±51.01 µm in length) and were enveloped by a thin

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layer of connective tissue (6.38±1.26 µm). The number of cells in a lobule ranged from 6-12. Type I cells formed the major cell types in Y organ of this stage and they were compactly packed (Fig. 5A). These cells measured 5.35±1.16 µm in width and have a round or ovoid shape. They have large, round, highly basophilic nuclei (2.88±0.75 µm) with peripherally arranged prominent nucleoli. The cytoplasm exhibited moderate basophilia and these cells were typically noted with high NPR (0.50±0.05). Type II cells were seldom detected. A few blood capillaries and sinuses were noted within the gland.

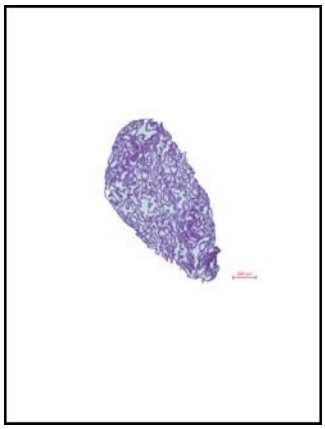

Figure 1. Y organ of *Barytelphusa cunicularis* during developmental stage I

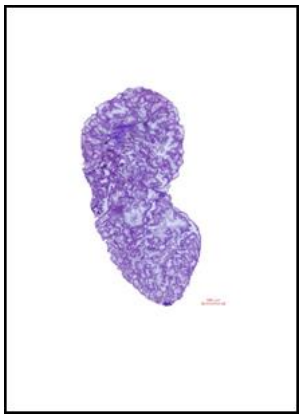

Figure 2. Y organ of stage II crabs

Morphology and Histology of Y Organ of Stage II Crabs (4-5 cm CW)

The gland appeared pale yellow and transparent. A significant increase in size (4.5±0.7mm long; 2.5±0.7 mm wide) and wet weight (3.5±0.7 mg) of the gland was noticed (Table 1), (Fig. 2). The lobular size (247.14±18.87 µm in length) and the thickness of lobular epithelium (6.94±1.45 µm) markedly elevated from the previous stage. Lobules displayed an augment in number of cells (8-19). Both the cell types were observed and the proportion of type I cells was found very high (90%). Type I cells were ovoid or round in outline and appeared noticeably larger (9.91±1.44 µm). Each cell has a highly basophilic round or oval nucleus and measured 3.88±0.79 µm in diameter. Their nuclei possessed distinct nucleoli with dense and granular chromatin. The cytoplasm contained moderate amounts of basophilic granules. Type I cells were characterized by a NPR of 0.38±0.02. Type II cells were larger in size (12.94±1.44 µm) and polygonal in shape. They have large acentric, round or oval, highly basophilic nuclei (4.62±1.25 µm). In majority of type II cells, intense basophilia of the nuclei was revealed and the nucleoli could not be seen. However, a few cells displayed moderate basophilia with prominent nucleoli. The cytoplasm was moderately basophilic and a few granules could be detected in the periplasm. The NPR was found low (0.34±0.06). Small blood sinuses and capillaries could be noticed throughout the gland (Fig. 5B).

Morphology and Histology of Y Organ of Stage III Crabs (6-7 cm CW)

The gland found enlarged in size (5.5±0.7 mm long; 3.2±0.3 mm wide) with a pale brown and flattened appearance and weighed 5.5±0.7mg (Table 1), (Fig. 3). The lobules showed a further increase in length from 247.14±18.87 µm to 278±18.32 µm. Progressive increase in the thickness (12.50±1.88 µm) of the lobular epithelium was perceptible. There was a significant rise in the number of cells in each lobule (27-31). A few type I cells (10.17±1.64 µm) were seen scattered amidst type II cells. They contained prominent, round or oval nuclei measuring 4.08±0.73 µm in diameter. The nuclei were positioned mostly within the centre of the cells. The nuclei evinced strong basophilia whereas the cytoplasm demonstrated moderate

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basophilia with small basophilic granules which tend to concentrate in the peripheral cytoplasm. The NPR of type I cells was found 0.37±0.06. A perceptible increase in the number and size of type II cells (15.52±2.02 µm) was evident (Fig. 5C). Their round or ovoid nuclei (4.51±1.13 µm) exhibited mild basophilia judged by the occurrence of chromatin granules. Type II cells were portrayed by a low NPR (0.28±0.03). Large blood sinuses and capillaries were seen interspersed among the lobules (Fig. 1C). Hemocytes were clearly discernable in the hemal sinuses of the gland. The most prominent feature of Y organ of this stage was the proliferation of epithelial cells (Fig. 5D).

Table 1. Comparison of Y organ in different developmental stages of *Barytelphusa cunicularis*

Developmental stages	Carapace width (cm)	Weight of crab (g)	Y organ			
			Colour	Length (mm)	Width (mm)	Weight (mg)
Stage I	1-3	4.49±4.20	Creamy yellow	2.5±0.5	1.5±0.5	1.3±0.5
Stage II	4-5	27.32±10.18	Pale yellow	4.5±0.7	2.5±0.7	3.5±0.7
Stage III	6-7	83.65±30.21	Pale brown	5.5±0.7	3.2±0.3	5.5±0.7
Stage IV	8-9	211.71±53.40	Deep brown	7.0±1.4	4.0±0.7	15.5±4.0

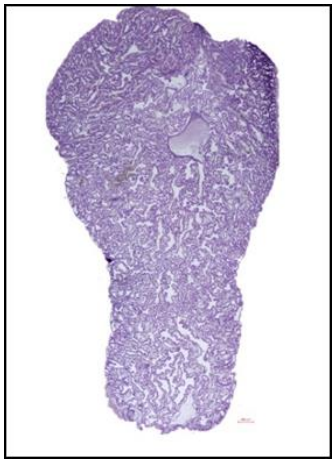

Figure 3. Y organ during developmental stage III

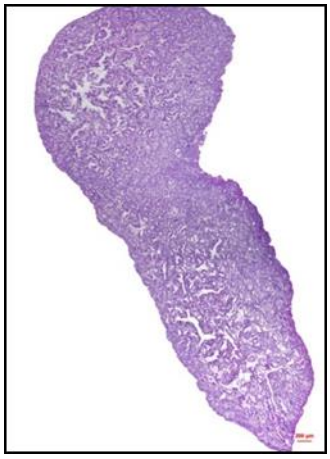

Figure 4. Y organ of *Barytelphusa cunicularis* during developmental stage IV

Morphology and Histology of Y Organ of Stage IV Crabs (8-9 cm CW)

The gland attained a size of 7.0±1.4 mm long and 4.0±0.7 mm wide and weighed 15.5±4.0 mg. The Y organ of this stage has a deep brown and flattened nature (Table 1), (Fig. 4). A corresponding increase in lobular size (521.28±103.19 µm in length) and thickness of the lobular epithelium (14.97±1.76 µm wide) was discernible. There was a pronounced rise in the number of cells in lobules (40-50) than the earlier stage. Only a very few type I cells could be perceived in this stage. A gradual increase in the size of type I cells (8-14 µm in diameter; 11.38±2.33) was apparent than the previous stage. The moderately basophilic round or oval nuclei (5.10±0.78 µm) were pushed to central or peripheral positions. The cytoplasm appeared moderately basophilic and the number of evenly scattered cytoplasmic granules increased. Type I cells of this stage were characterized by a low NPR (0.26±0.01). Type II cells (20.82±2.54 µm) dominated the Y organ during this phase (Fig. 5E). Their oval to spherical nuclei (4.64±1.14 µm) appeared more basophilic with 1-2 nucleoli. Their homogeneous cytoplasm showed an increase in volume. There was a reduction in type II NPR (0.27±0.03) when compared to that of stage III. The hemolymph sinuses and channels

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were clearly evident in the histological sections. Hemocytes were sharply detectable in the blood sinuses (Fig. 5F).

A, Section of Y organ of stage I crabs; B, Y organ portraying different cell types at developmental stage II; C, Organ with large blood sinuses and hemocytes in stage III crabs; D, Proliferation of epithelial cells in the Y organ of stage III crabs; E, Gland depicting larger lobules and type II cells during stage IV; F, Appearance of blood sinuses and hemocytes in the Y organ of stage IV crabs. BS: Blood sinus; H: Hemocyte; ILS: Interlobular space; L: Lobule; LE: Lobular epithelium; N: Nucleus; TI: Type I cell; TII: Type II cell; Arrow indicates proliferation of epithelial cells.

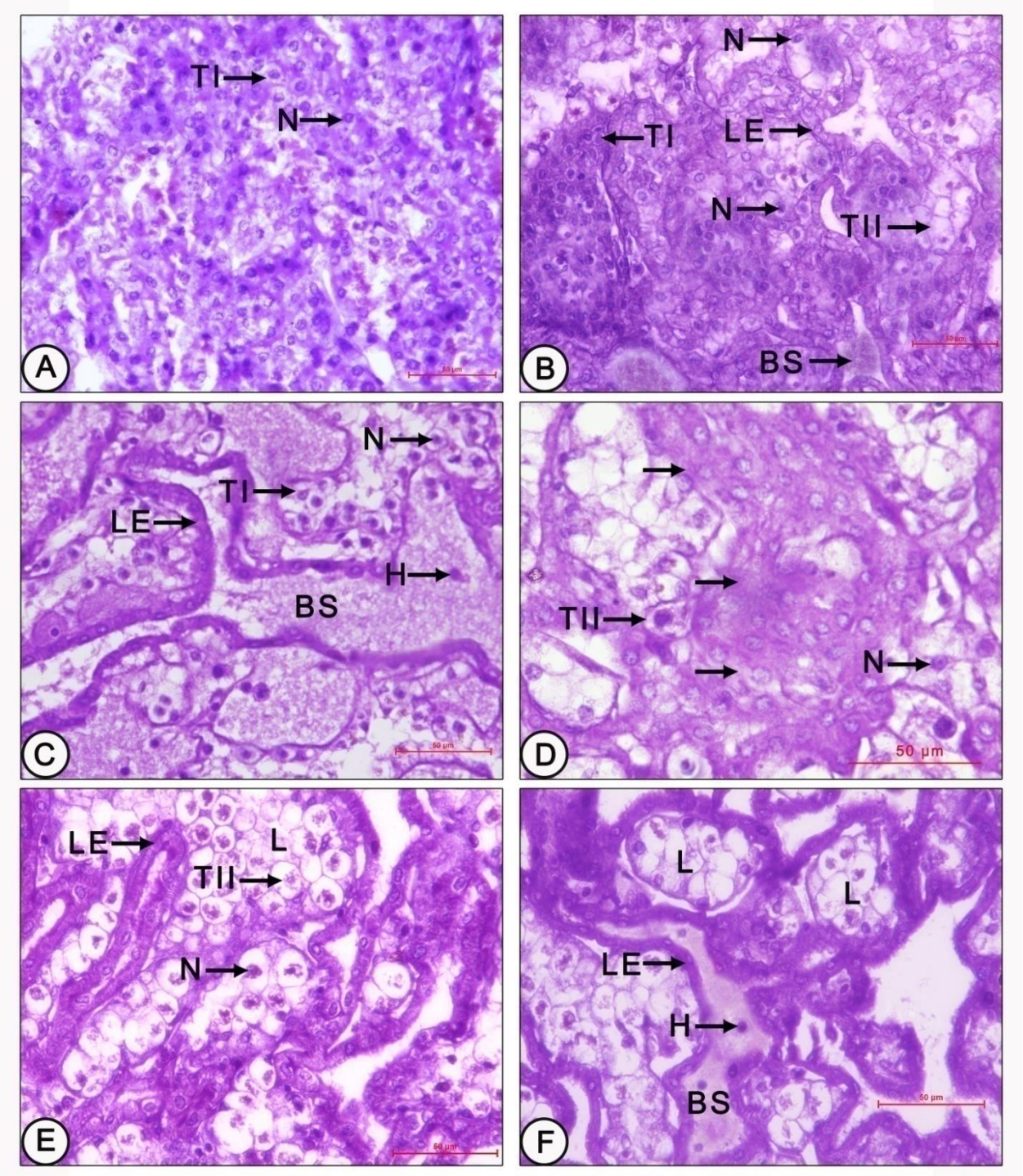

Figure 5. Photomicrograph of Y organ of *Barytelphusa cunicularis* during different stages of development

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Morphology and Histology of Y Organ In Relation To Oogenesis

In *B. cunicularis*, oogenesis was classified into four phases: avitellogenic, early vitellogenic, middle vitellogenic and late vitellogenic phases. The ovary exhibited a sequence of morphological and cytological changes during different reproductive phases. The Y organ showed substantial differences in the morphology and histology in relation to various phases of oogenesis.

Morphology and Histology of Y Organ during Avitellogenic Phase

The Y organ displayed the least level of activity during this stage of vitellogenesis. The gland was small, pale yellow and translucent, measuring 4.5±0.57 mm in length and 3.25±0.28 mm in width and weighed 9.5±0.2 mg (Table 2). The lobules appeared small (152.65±68.69 µm in length) and were loosely packed with prominent interlobular spaces (Fig. 6A). Lobules were regularly arranged and enveloped by a thin layer of lobular epithelium (4.85±2.65 µm wide). Each lobule contained 20-25 loosely packed cells with prominent intralobular spaces. The whole gland was composed of type I and type II cells. Type I cells (7.60±1.43 µm in diameter) were almost round in shape with round or oval basophilic nuclei (4.04±0.89 µm wide). The nucleus occupied either a peripheral or central position in the cell. The cytoplasm was midly basophilic in nature and cytoplasmic granules were seldom found. The NPR was 0.52±0.01. Type II cells formed the predominant cell types of the gland. They were visibly large, polygonal in shape and measured 12.66±1.72 µm in diameter. The nuclei were large (3.25±0.76 µm wide), round or oval and found highly basophilic. The cytoplasm was transparent and homogeneous in nature. Type II cells were typically noted with low NPR (0.25±0.03). The hemolymph sinuses and channels were lucid in the histological sections.

Morphology and Histology of Y Organ during Early Vitellogenic Phase

The size of the gland increased (5.5±0.50 mm in length and 4.0±0.36 mm in width) when compared with that of the previous phase. The gland appeared flattened and dark yellow and weighed 15.8±3.0 mg (Table 2). The lobules (254.42±46.02 µm in length) were closely packed and the interlobular spaces seemed to be reduced (Fig. 6B). It is evident that the thickness of the lobular epithelium (6.61±2.47 µm wide) increased. Each lobule was composed of closely packed type I and type II cells (25-30). Type I cells (8.10±1.23 µm wide) contained moderately basophilic centrally located nuclei (4.54±0.64 µm in diameter). They were round or oval in shape and the chromatin material was uniformly scattered in the nucleoplasm. The nucleus possessed mildly stained nucleoli (1-2). The cytoplasm was moderately basophilic and contained rod-shaped inclusions. The type I cells were observed with high NPR (0.56±0.01). Type II cells (13.46±1.92 µm wide) were quite abundant and noted as the major cell types of the gland. These cells have roughly spherical accentric nuclei, approximately 3.46±0.97 µm in diameter containing one or more peripherally or centrally located nucleoli and peripherally condensed chromatin. Interestingly, in some regions of the gland, the type I cells exhibited cell proliferation (Fig. 6C). Hemolymph sinuses and capillaries were evinced in the Y organ of this vitellogenic stage.

Morphology and Histology of Y Organ during Middle Vitellogenic Phase

The gland increased in size (7.75±0.18 mm length and 4.3±0.34 mm width) with advancement in vitellogenic phase. The gland appeared bulged (21.0±0.3 mg) and dark brown in colour (Table 2). The appearance of the gland varied markedly during the middle vitellogenic phase. The size of the lobules significantly increased (279.18±80.58 µm in length) when compared to early A, Y organ showing small loosely packed lobules with prominent interlobular spaces during avitellogenic phase; B, Lobules with compactly packed

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type I and type II cells during early vitellogenic phase; C, Y organ portraying type I cell proliferation during early vitellogenic phase; D, Y organ during middle vitellogenic phase illustrating lobules with large number of type II cells; E, Occurrence of blood sinuses and hemocytes in the gland during middle vitellogenic phase; F, Y organ during middle vitellogenic phase showing compactly packed cells with reduced intra and interlobular spaces . BS: Blood sinus; H: Hemocyte; ILS: Interlobular space; INS: Intralobular space; L: Lobule; LE: Lobular epithelium; N: Nucleus; TI: Type I cell; TII: Type II cell; Arrow indicates type I cell proliferation.

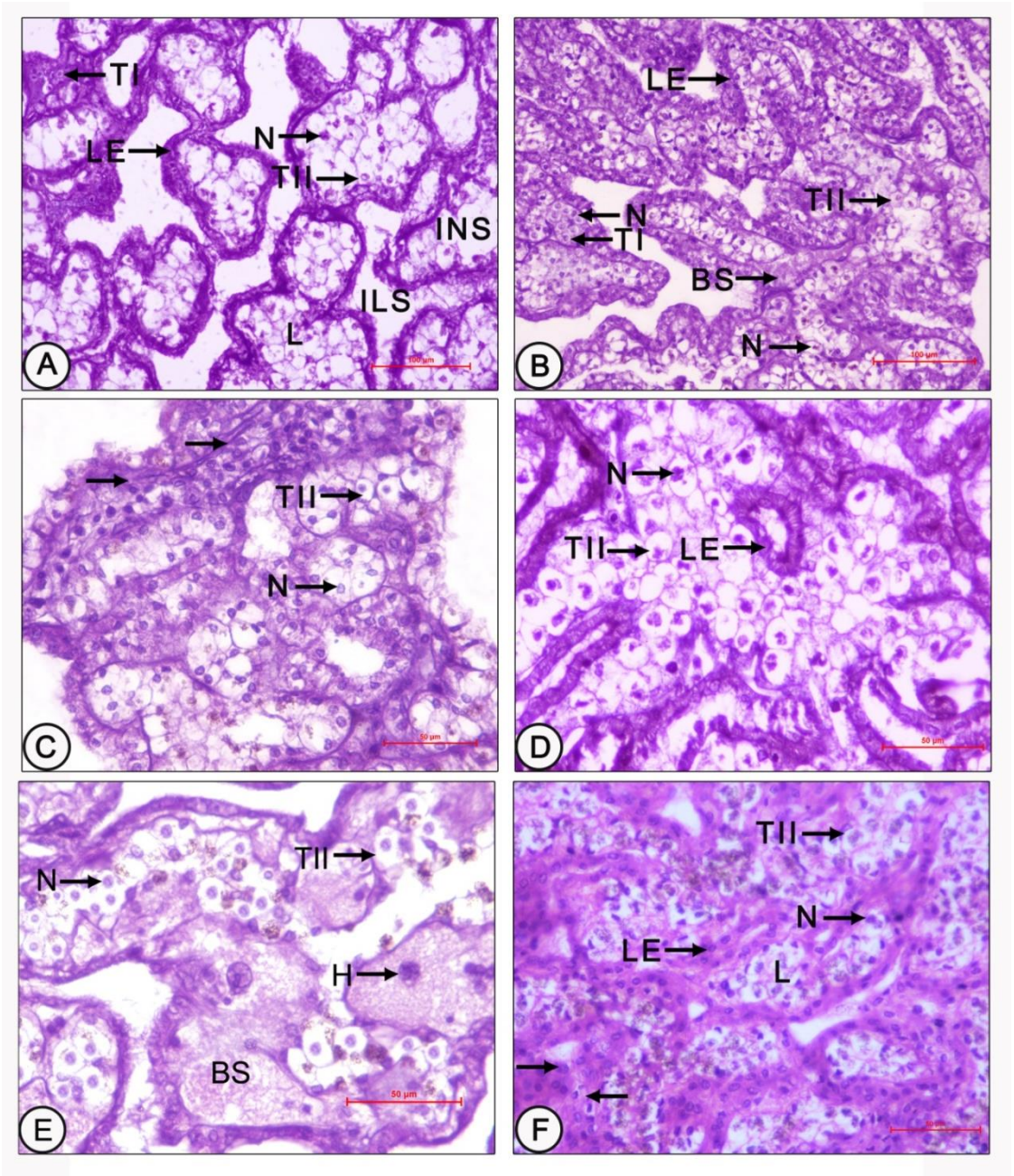

Figure 6. Histology of Y organ during different phases of oogenesis in *Barytelphusa cunicularis*.

vitellogenic phase. The lobules were closely packed without interlobular spaces. The lobular epithelium was intact, basophilic and attained a width of about 13.37±2.92 µm in width. Each lobule contained 30-40 cells. Only very few type I cells (8.24±1.37 µm wide) could be perceived in this stage. Their nuclei (4.68±0.78 µm in diameter) showed mild basophilia

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with fine chromatin granules and cytoplasm moderately basophilic. The lobules were regularly packed with large number of type II cells (Fig. 6D). The size of type II cells (20.50±2.44 µm wide) dramatically increased. Their nuclei (7.5±1.87 µm wide) were found moderately basophilic and encompassed solidly filled chromatin granules. Majority of the type II cells contained 1-2 nucleoli and some were observed without any signs of nuclei. The volume of cytoplasm was increased and retained the granular nature. The perinuclear cytoplasm was rich in highly basophilic granules. Type II cells were noted with reduced NPR (0.36±0.76). Large blood sinuses and capillaries were perceptible within the gland. Hemocytes were clearly discerned in the hemal spaces (Fig. 6E).

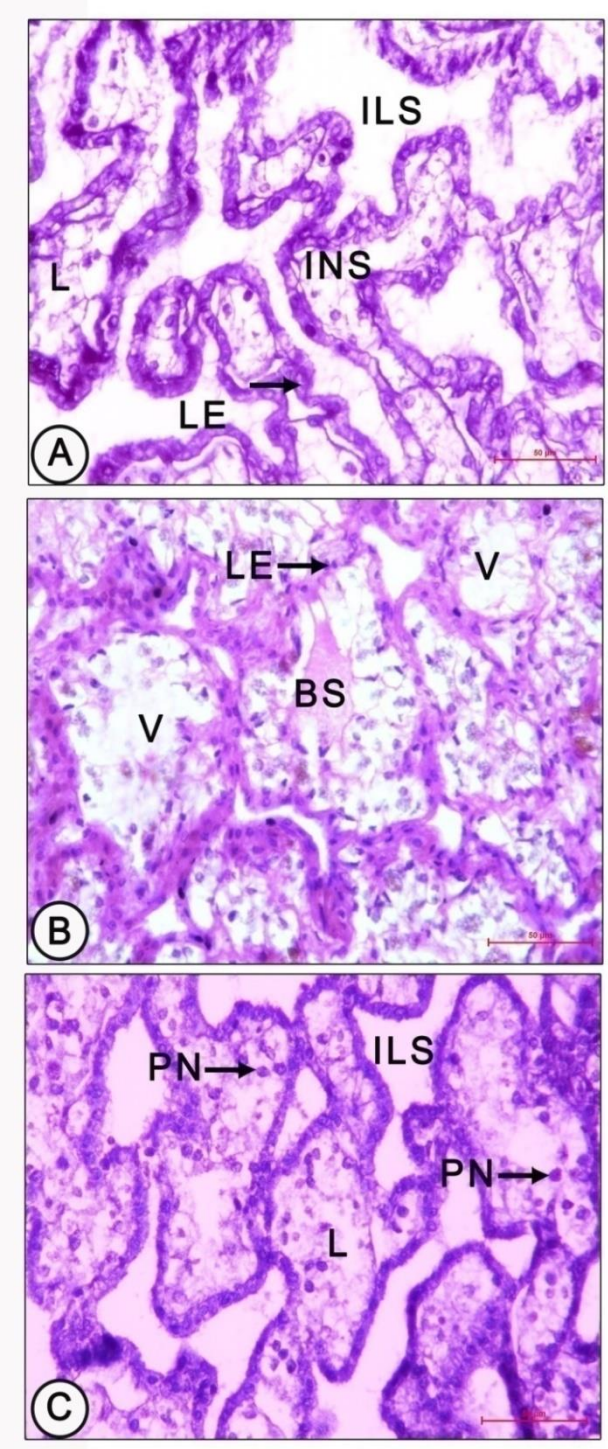

Figure 7. Y organ of *Barytelphusa cunicularis* during late vitellogenic phase.

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Morphology and Histology of Y Organ during Late Vitellogenic Phase

The gland appeared translucent, dark brown and flattened, measuring 5.71±0.19 mm in length and 4.2±0.21 mm in width and weighed 20.0±0.7 mg (Table 2). The size of the lobules (213.43±52.65 µm in length) and the thickness of lobular epithelium (6.33±2.37 µm wide) decreased notably. The inter and intralobular spaces were found prominent (Fig. 7A). The lobular epithelium was shrunken and has a degenerated appearance and their nuclei showed pycnosis (Fig. 7C). The lobules occupied lesser number of cells (10-20) and the intralobular spaces were more pronounced. Type I cells (8.19±1.28 µm wide) scarcely detected and were not easily recognizable. The type II cells were found decreased in size (13.14±1.23 µm wide) with indistinct nuclei (2.48±0.37 µm wide). The cytoplasm was depleted and transparent in nature. Cellular degeneration was a notable feature of Y organ of this phase. The occurrence of vacant lobules formed another significant change in this phase (Fig. 7B). The hemal sinuses and capillaries were inconspicuous.

Table 2. Comparison of Y organ during different phases of oogenesis in *Barytelphusa cunicularis*

Phases of oogenesis	Y organ			
	Colour	Length (mm)	Width (mm)	Weight (mg)
Avitellogenic	Pale yellow	4.5±0.57	3.2±0.28	9.5±0.2
Early vitellogenic	Dark yellow	5.5±0.50	4.0±0.36	15.8±0.3
Middle vitellogenic	Dark brown	7.7±0.18	4.3±0.34	21.0±0.3
Late vitellogenic	Dark brown	5.7±0.19	4.2±0.21	20.0±0.7

A, Organ depicting lobules with pronounced intra and interlobular spaces and shrunken lobular epithelium; B, Gland exhibiting vacant lobules; C, Lobules containing pycnotic nuclei and degenerated cells. BS: Blood sinus; ILS: Interlobular space; INS: Intralobular space; L: Lobule; LE: Lobular epithelium; N: Nucleus; PN: Pycnotic nuclei; TII: Type II cell; V: Vacant lobules.

DISCUSSION

The current study observed the morphological and histological changes in relation to growth and vitellogenesis in the Y organ of the freshwater crab *Barytelphusa cunicularis*. The Y organs are lobulated, epithelial structures anchored in a brown fatty tissue in the cephalothorax of *B. cunicularis*. Generally, the location and gross morphology of Y organs found varied in crustaceans. In *Uca pugnax*, Passano (1960) located Y organ ventral to the mandibular external adductor muscle above the junction of the branchiostegite. In *C. annulata*, the gland was situated in the facial region between the mandibular external adductor muscle and the branchiostegite at the anterolateral edge of the branchial chamber (Babu et al., 1989a). In *C. maenas* (Echalier, 1959), *C. irroratus* (Simione and Hoffman, 1975), *P. trituberculatus* (Taketomi and Hyodo, 1986) and *Pachygrapsus marmoratus* (Bressac, 1976), the gland was positioned on the ventral side of the body around the shell epimeral line. The Y organ was located at the anterolateral margin of the branchial chamber in the estuarine crab *Metopograpsus messor* (Shyamal et al., 2014). In *Orconectes limosus*, Chaudonneret (1956) identified the organ between the first mandible and the first maxilla. In the spiny lobster *Jasus lalandii* (Paterson, 1968) and *Palaemon paucidens* (Aoto et al., 1974), the organ was located between the pre-branchial and branchial chambers respectively. The gland was coupled to the prebranchial chamber and the inner wall of the branchiostegite in *Metapenaeus* sp. (Dall, 1965), *Penaeus japonicus* (Bourget et al., 1977) and penaeid shrimps (Bell and Lightner, 1988). The organ was positioned at the top of the pleural invagination of the maxillary segment in the mysid *Siriella armata* (Roudy and Saleuddin, 1989).

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Our present study accentuated the existence of perceptible changes in gross morphology and histology of the Y organ in relation to various developmental stages. In *B. cunicularis*, the gland becomes larger in size as development progresses and attains maximum size during stage IV. The observations of the present study are in agreement with the findings of Mc Conaugha (1980) in *C. anthonyi* where the gland length and width gradually increased from first to the fourth larval stage. In *M. messor*, the organ attained maximum size during moult reproductive season (Shyamal et al., 2014). The weight of the mandibular organ displayed a direct correlation with the body weight in *Oziotelphusa senex senex* (Nagaraju et al., 2004).

The general histological description of Y organ in *B. cunicularis* was identical to that described for other brachyurans (Babu et al., 1989a, b; Buchholz and Adelung, 1980). The Y organ of *B. cunicularis* contained lobules interconnected by blood sinuses and capillaries. Similarly, in *P. paucidens* (Aoto et al., 1974) and in the freshwater crab *T. schirnerae* (Smija and Sudha Devi, 2016), the organ was composed of many lobulated cell masses separated by interconnected blood sinuses and capillaries. In *C. irroratus*, the Y organ consisted of anastomising cords of epithelial cells separated by numerous interconnected haemocoelic sinuses and fine capillaries (Simione and Hoffman, 1975). On the other hand, the organ was represented as folded invaginations of the epidermis with many lumens in the shrimp *Pandalus danae* (Hoffman, 1967) and *P. kessleri* (Aoto et al., 1974). In mysids, the gland encompassed large number of nuclei clustered in a clear cytoplasmic zone (Vogt, 1935). In *S. armata*, the Y organ was composed of three lobes coupled to the integument by a cuticular peduncle (Roudy and Saleuddin, 1989). In *M. messor*, the organ has a lobulated appearance enclosing closely packed cells (Shyamal et al., 2014).

In the present study, the lobular size and number of cells in lobules were found to have increased as development progressed from stage I to stage IV. Observations of Mc Conaugha (1980) in *C. anthonyi* revealed that the gland becomes more composite through extensive folding and intertwining of the cellular cords as development advances.

Our histological studies confirmed the occurrence of two distinct cell types in *B. cunicularis* as reported for *Varuna litterata* (Madhyastha and Rangnekar, 1972) and *T. schirnerae* (Smija and Sudha Devi, 2016). In *P. sanguinolentus*, *C. annulata* (Babu et al., 1989a, b) and in *C. irroratus* (Simione and Hoffman, 1975), the organ was composed of small and large cells. On the other hand, the Y organ was composed of a single cell type in *C. anthonyi* (Mc Conaugha, 1980), *P. danae* (Hoffman, 1966) and *P. paucidens* (Aoto et al., 1974). In *B. cunicularis*, type I cells evinced basophilic nuclei, sparse cytoplasm and a high NPR. Correspondingly, Lachaise et al. (1993) portrayed a remarkable feature repeatedly observed in the cells of Y organ was their high NPR (Silen, 1954; Aoto et al., 1974; Le Roux, 1974; Simione and Hoffman, 1975).

The current investigation manifested that during the first stage of development, the Y organ remained compact with sparse cytoplasm and by the time it reached the fourth stage of development, the gland cells became hypertrophied due to the cytoplasmic enhancement. Similar reports were made by Mc Conaugha (1980) in *C. anthonyi*.

In the present study, the Y organ become more active with closely packed cells during early and middle vitellogenic periods and was inactive with a vacuolated appearance during avitellogenic and late vitellogenic periods. The vacuolated appearance seems quite comparable with what was reported in *C. maenas* (Noel et al., 1989) during stages of inactivity. Many studies reported changes in the histological profile of Y organ during various phases of gonadal maturation (Nagabhushanam and Farooqui, 1984; Hussain and Vasantha, 1985). In *M. messor*, Shyamal et al. (2014) observed that the Y organ of females engaged in breeding activity demonstrated high levels of secretory and cellular activity than

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that of reproductively inactive females, implicating the organ's involvement in reproduction. Ultrastructural studies of MO at different phases of vitellogenesis in *Sesarma quadratum* revealed a more advanced stage of development of the cells in the middle and late stages of vitellogenesis than the early stage of vitellogenesis (Syama, 2009). The secretory and cellular activities of MO of *Paratelphusa* sp. are enhanced throughout the breeding season and the MO become inactive during the non-reproductive season (Sarika et al., 2014). However, Liu et al. (2010) observed that the Y organ of *P. trituberculatus* was remarkably degenerated during ovarian development. .

The current investigation revealed that the Y organ plays an important role in the regulation of vitellogenesis in *B. cunicularis*. Though the primary function of Y organ is the regulation of moulting, it also plays a functional role in the control of vitellogenesis in many crustaceans (Souty et al., 1982; Gohar and Souty, 1984; Subramoniam and Kirubagaran, 2011; Sudha et al., 2012). Charniaux-Cotton and Touir (1973) observed the breakdown of folliculogenesis by Y organectomy during mid-vitellogenic stages in *L. seticaudata*. In *O. gammarella*, Y organectomy resulted in declined vitellogenin production and poor ovarian growth (Meusy et al., 1977). Okumura et al. (1992) observed a correlation between the ecdysteroid levels and oocyte developmental stages in *M. nipponense*. Steel and Vafopoulou (1998) observed elevated ecdysteroid titres in hemolymph of reproducing females in the terrestrial isopod *Oniscus asellus*. The presence of large amounts of ecdysteroids within the ovary is another indication to confirm the role of Y organ in the regulation of vitellogenesis in many crustacean species (Chang, 1997; Subramoniam, 2000). Despite the fact that many works have been carried out to ascertain the relationship between Y organ and vitellogenesis in many crustaceans, its functional role in the control of vitellogenesis is still controversial and more studies on the histological and ultrastructural profiles of the gland during vitellogenesis is yet to be determined.

CONCLUSION

Our observations revealed considerable differences in morphology and histology of the Y organ in relation to growth and vitellogenesis. Clear and specific changes in morphology and histology could be established in the Y organ of *B. cunicularis* during different developmental stages. The organ displayed substantial differences in morphology and histology in relation to various phases of oogenesis, with least level of activity during avitellogenic and late vitellogenic phases and maximum activity during early and middle phases of vitellogenesis. Further ultrastructural studies are required to on ultrastructural profiles of the gland during various developmental and vitellogenic phases.

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