Morphology and Histology of Mandibular Organ in Relation to Growth and Reproduction in the Freshwater Crab *Barytelphusa cunicularis*

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Abstract

This study depicts the morphology and histology of the mandibular organ in relation to growth and reproduction in the freshwater crab *Barytelphusa cunicularis*. The mandibular organs are a pair of ectodermally derived, vascularized glands positioned at the base of the mandibular tendon attached to the posterior abductor muscles of the mandibles. Two cell types could be visualized in the organ: type I and type II which are seen surrounded by hemolymph channels and sinuses. These cells exhibited changes in substructure during growth and reproduction. The gland was small in size with a few type I cells during stage I of development. The size of the gland increased and both the cell types were perceived in stage II crabs. Cord like arrangement of type II cells was characteristic of mandibular organ of stage III crabs. The gland attained maximum size with more number of hypertrophied cells by the time it reached stage IV. Our observations revealed that the organ was highly active during early and middle vitellogenic phases, evinced by the hypertrophy of cells and nuclei, compact arrangement of type II cells, abundance of hemocytes in the blood sinuses. However, during previtellogenic phase, the organ cells appeared loosely packed, hypotrophied with poor vascularization. The gland remained least active during late vitellogenic and oosorption phases judged by the presence of pycnotic nuclei and vacuolated appearance of cytoplasm with indistinct cell boundaries. These results strongly suggest a possible role for mandibular organ on regulating vitellogenesis in female *Barytelphusa cunicularis*.

Keywords: Mandibular organ, development, reproduction, *Barytelphusa cunicularis*, histology.
INTRODUCTION

Mandibular organs (MOs) in crustaceans are a pair of ectodermally derived, vascularized glands positioned at the base of the mandibular tendon attached with the posterior abductor muscle of the mandibles. The MO was first demonstrated in the crab Carcinus maenas by Le Roux (1968) and later identified in many other crustaceans. It plays an important role in the regulation of crustacean growth and reproduction (Aoto et al., 1974; Hinsch, 1981; Tamone and Chang, 1993; Laufer et al., 1998; Reddy and Ramamurthi, 1998; Jo et al., 1999).

Studies on several decapods have revealed that the MO synthesizes and secretes methyl farnesoate, a sesquiterpene, which is structurally similar to the juvenile hormone (JH) III of insects (Borst et al., 1987; Tobe et al., 1989; Borst and Laufer, 1990). This structural similarity implies that the MF of crustaceans might have roles similar to the JH III of insects (Riddiford, 1994; Wyatt and Davey, 1996; Homola and Chang, 1997). The synthesis and secretion of MF by MO is negatively controlled by an inhibitory neuropeptide, the mandibular organ inhibiting hormone (MO-IH) from the eyestalk. Amputation of eyestalks removes MO-IH inhibition resulting in MO hypertrophy and increased MF secretion in Orconectes virilis (Tsukimura et al., 1989). Besides roles in growth and reproduction, the MO is thought to be involved in the regulation of morphogenesis (Laufer et al., 1997; Abdu et al., 1998; Rotllant et al., 2000), behaviour (Borst et al., 1995; Sagi et al., 1994) and protein synthesis (Paulson and Skinner, 1988; Soroka et al., 1993).

Light and electron microscopic examinations carried out on MO in many species of crustaceans suggested that the secretory activity of the gland changed in relation to growth and reproduction (Le Roux, 1968; Laufer et al., 1986; 1987; Reddy and Ramamurthi, 1998). Hinsch (1980) observed that the mandibular organs were found active during vitellogenic phases in C. maenas and Libinia emarginata. Huiyang et al. (2003) analysed the developmental histology of MO in the mud crab Scylla serrata. In the freshwater crab Oziotelphusa senex senex, the changes in MO in relation to body weight, sex, moult and reproduction were described by Nagaraju et al. (2004). Syama (2009) detailed the histological and fine structural observations on MO of the mangrove crab Sesarma quadratum during different stages of ovarian maturation. Sarika et al. (2014) found that the secretory activity of the MO fluctuates during reproductive and non-reproductive seasons in the field crab Paratelphusa sp. Effect of eyestalk ablation on histology and fine structure of the mandibular organ in the freshwater crab Travancoriana schirnerae was investigated by Sudha Devi et al. (2017; 2018).

So far, very few studies concentrated the development or vitellogenesis related changes in morphology or anatomy of the MO in freshwater brachyurans. At this juncture, the present attempt to study the morphological and anatomical changes of MO with regard to growth and ovarian maturation was made in the edible freshwater crab Barytelphusa cunicularis.
MATERIALS AND METHODS

Juveniles and adult intermoult females in various developmental and vitellogenic stages (CW 1.0 to 12.0 cm) were collected from holes on the side walls of streams near Chettapalam, Mananthavady, Wayanad over a period of one year (June 2016-May 2017). The crabs were kept in plastic tubs, fed with cooked beef liver and pulses and maintained in the laboratory. The carapace widths (CW), moult stages and wet weights were documented for all the specimens collected. Mandibular organs from individuals in various stages of development (stage I, stage II, stage III and stage IV) and ovarian maturation (previtellogenic, primary, secondary and tertiary vitellogenic phases and oosorption phase) were dissected out; their size, shape and colour were noted. Tissues were then preserved in aqueous Bouin’s fluid for 24 hours, dehydrated in graded ethanol series, cleared in xylene and embedded in melted paraffin wax. Microtome sections of 5 µm thickness, stained with Heidenhain’s hematoxylin-eosin, were observed under a Leica DM 500 Research Microscope. Interested areas were imaged with a DG 330/120 camera using Biowizard software. Observations on morphology and anatomy of the MO were made with respect to various developmental and oogenic phases.

The colour and wet weight of ovaries were recorded for calculating the gonadosomatic index (GSI). One half of the ovary was carefully torn open and diameters of 50 randomly chosen oocytes were recorded using a calibrated ocular micrometer. Colour of ovary, GSI and oocyte diameter were the criteria used for characterizing the stages of maturation of the ovary.

RESULTS

Morphology

The MO of adult Barytelphusa cunicularis was a pale-yellow, C-shaped glandular tissue (about 2.0 mm long and 0.6 mm wide) located at the base of the mandibular tendon apodeme, associated with the muscles of the mandible. The gland was seen surrounded by layers of connective tissue which firmly attaches it to the tendon and was indistinguishable from the surrounding tissues.

The development of B. cunicularis from juvenile to adult can be divided 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 adult crabs of CW 8-12 cm.
Morphology and histology of mandibular organ of stage I crabs (1-3 cm CW)

In young crabs of CW 1-3 cm, the gland appeared very small (0.6±0.08 mm long; 0.25±0.07 mm wide), pale yellow and roughly oval in shape (Table 1) (Figure 1A). The organ was composed of a single type of small, oval cells (6.89±0.53 µm in diameter) with large, mild to moderately basophilic, ovoid or round, centrally located nuclei (3.44±0.33 µm wide) and small amounts of mildly basophilic cytoplasm. Each nucleus has a centrally placed single nucleolus and peripherally condensed chromatin. The cellular limits of these cells were found indistinct and the average NPR recorded was 0.49±0.01 (Table 2). No cord like arrangement of cells was noticed. The loosely packed cells were seen surrounded by hemolymph channels and sinuses. Both granular and agranular hemocytes were discernible in these channels and sinuses (Figure 2A).
Morphology and histology of mandibular organ of stage II crabs (4-5 cm CW)
The MO of this stage was pale yellow and translucent and showed an increase in size
(0.80±0.10 mm in length and 0.35±0.05 mm in width) from the previous stage (Table
1) (Figure 1B). The gland was composed of two cell types: type I and type II. Type I
cells (10.34±0.31µm in diameter) were round or ovoid, decidedly larger than those of
stage I and exhibited large intensely basophilic nuclei (6.89±0.23 µm in diameter)
with small amounts of moderately basophilic cytoplasm (NPR 0.61±0.06). Their
nuclei were positioned mostly in the centre with single nucleoli occupying central or
peripheral positions. Type II cells were seen scattered among type I. They were
noticeably large, polygonal in shape and measured 11.20±2.31 µm in diameter. They
have large round, spherical or oval, centric nuclei (3.44±0.77 µm in diameter)
containing 1-3 large, round, peripherally located nucleoli and ample moderately
basophilic homogenous cytoplasm. The chromatin was either condensed towards the
periphery or fibrillar in nature, seen scattered in the nuceloplasm. The NPR was found
in the range 0.24-0.36 (Table 2). The cell boundaries of both type I and II cells were
not very sharp. The mandibular organ at this stage displayed blood sinuses and
capillaries. Frequently, hemocytes were noticed in these sinuses (Figure 2B).

![Figure 2. Histology of mandibular organ during stages I and II of development.
A, Mandibular organ of stage I depicting cells with indistinct cellular limits; B,
Mandibular organ of stage II with decidedly larger type I cells. BS: Blood sinuses; H:
Hemocyte; N: Nucleus; NU: Nucleolus; TI: Type I cell.](image)

Morphology and histology of mandibular organ of stage III crabs (6-7 cm CW)
The MO appeared creamy white and showed a further increase in size (1.21±0.23 mm
long; 0.45±0.05 mm wide) by the time the crabs reached stage III (Table 1) (Figure
1C). An obvious increase in the number and size of glandular cells was evident at this
stage. Both the cell types with distinct boundaries were perceptible in MO of this
stage. Type I cells (12.06±0.42 µm in diameter) possessed moderately basophilic
large spherical nuclei (5.17±0.25 µm wide) with large, centrally placed nucleoli and peripheral chromatin. Their moderately basophilic cytoplasm appeared homogenous. The gland was rich in oval to elongate, large polygonal type II cells which measured 17.24±2.36 µm in width. Their large spherical nuclei (6.03±0.15 µm in diameter) occupied a centric or acentric position (Table 2). Each nucleus contained 1-2 clearly discernible nucleoli and peripheral chromatin. The cytoplasm was moderately basophilic and homogenous. An interesting feature noticed in MO of this stage was the formation of cellular cords. Blood sinuses and capillaries were fairly distinct with hemocytes in them (Figure 3A).

**Morphology and histology of mandibular organ of stage IV crabs (8-12 cm CW)**

The gland appeared milky white and got increased in size by the time it reached stage IV with a measurement of 1.75±0.18 mm length and 0.55±0.05 mm width (Table 1) (Figure 1D). A perceptible increase in the number and size of glandular cells than the preceding stage was noticed. Very few type I cells were evinced in MO of this stage (9.05±0.69 µm in diameter). The oval or polygonal type II cells (10.82±4.18 µm in diameter) possessed mildly basophilic nuclei (5.42±1.02 µm in diameter) with 2-3 peripherally occupied nucleoli and fibrillar chromatin (Table 2). The volume of cytoplasm was found increased which demonstrated a moderate basophilia. A notable feature in MO of this stage was the abundance of blood sinuses and capillaries containing large number of granular and agranular hemocytes (Figure 3B).

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**Figure 3. Photomicrograph of mandibular organ during stages III and IV of development.**

A, Cord like arrangement of gland cells with distinct cell boundaries and large blood sinuses in stage III crabs; B, Mandibular organ exhibiting densely packed hypertrophied type II cells, blood sinuses and hemocytes during stage IV. BS: Blood sinuses; H: Hemocyte; N: Nucleus; NU: Nucleolus; TI: Type I cell; TII: Type II cell.
Table 1: Mandibular organ morphology during different developmental stages of *Barytelphusa cunicularis*

<table>
<thead>
<tr>
<th>Stages of development</th>
<th>Carapace width (cm)</th>
<th>Mandibular organ</th>
<th>Shape</th>
<th>Colour</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stage I</td>
<td>1-3</td>
<td></td>
<td>oval</td>
<td>light yellow</td>
<td>0.6±0.08</td>
<td>0.2±0.07</td>
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<tr>
<td>stage II</td>
<td>4-5</td>
<td></td>
<td>kidney shaped</td>
<td>pale yellow</td>
<td>0.8±0.10</td>
<td>0.3±0.05</td>
</tr>
<tr>
<td>stage III</td>
<td>6-7</td>
<td></td>
<td>kidney shaped</td>
<td>Creamy white</td>
<td>1.2±0.23</td>
<td>0.4±0.05</td>
</tr>
<tr>
<td>stage IV</td>
<td>8-9</td>
<td></td>
<td>C-shape</td>
<td>milky white</td>
<td>1.7±0.18</td>
<td>0.5±0.05</td>
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Table 2: Cell types in mandibular organ during different stages of development in *Barytelphusa cunicularis*

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Type I cells</th>
<th>Type II cells</th>
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<tr>
<td></td>
<td>Cell diameter (µm)</td>
<td>Nuclear diameter (µm)</td>
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<tr>
<td>Stage I</td>
<td>6.89±0.53</td>
<td>3.44±0.33</td>
</tr>
<tr>
<td>Stage II</td>
<td>10.34±0.31</td>
<td>6.89±0.17</td>
</tr>
<tr>
<td>Stage III</td>
<td>12.06±0.42</td>
<td>5.17±0.25</td>
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<tr>
<td>Stage IV</td>
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</tbody>
</table>

Morphology and histology of MO in relation to ovarian maturation

Based on changes evident in the morphology and histology of the ovary, oogenesis in *B. cunicularis* was divided into five distinct phases: previtellogenic, primary, secondary and tertiary vitellogenic phases and oosorption phase. The current investigation recorded the changes in morphology and histology of MO in accordance with the different phases of oogenesis in *B. cunicularis*.

Morphology and histology of MO during previtellogenic phase

The gland was small (0.65±0.12 mm long; 0.25±0.05 mm wide) and light yellow in colour (Table 3). Cells were loosely arranged in cords. Though both the cell types were perceived in the MO, type II cells were the dominant cell types. Type II cells (14.74±3.17 µm wide) were large, polygonal or ovoid in shape. They possessed mildly or moderately basophilic, centrally or peripherally positioned round nuclei (4.34±1.02 µm wide). The nuclei enclosed clearly discernible one or rarely two peripherally located nucleoli (0.78±0.21 µm wide). They have ample cytoplasm which appeared moderately basophilic and homogenous in nature. Chromatin
arranged as a thin rim attached to the inner nuclear membrane. The NPR was 0.29±0.08. Type I cells (7.83±0.02 µm in diameter) were sparsely distributed. Blood sinuses and capillaries were evident with a few hemocytes (Figure 4A).

**Morphology and histology of MO during primary vitellogenic phase**

The gland appeared pale with an appreciable increase in size (1.60±0.36 mm in length and 0.35±0.05 mm in width) (Table 3). Gland was composed of closely packed oval to elongate type II cells measuring 15.13±16.16 µm in diameter. Their mildly basophilic, oval to elongate nuclei (4.73±1.03 µm wide) occupied mostly an acenral position. The nucleus contained 1-3 large, clearly discernible, centrally or peripherally positioned nucleoli (1.48±0.41 µm wide). Chromatin threads were seen scattered in the nucleoplasm. The cytoplasm was homogenous and showed an appreciable degree of basophilia. Type II cells were noted with low NPR (0.31±0.03). Blood sinuses and capillaries were frequently encountered in the gland. A characteristic feature of MO of this stage was the presence of copious amounts of hemocytes (7.36-9.52 µm wide) in the hemal sinuses and capillaries (Figure 4B).

![Figure 4. Light micrograph portraying mandibular organ of previtellogenic and primary vitellogenic phases of Barytelphusa cunicularis.](image)

A, Gland with loosely packed hypotrophied type II cells during previtellogenic phase; B, Compactly arranged type II cells with copious amounts of hemocytes during primary vitellogenic phase. BS: Blood sinuses; H: Hemocyte; N: Nucleus; NU: Nucleolus; TI: Type I cell; TII: Type II cell.

**Morphology and histology of MO during secondary vitellogenic phase**

The MO of this phase appeared snowy white with a progressive increment in size (1.85±0.07 mm in length; 0.56±0.05 mm in width) (Table 3). The gland was composed of compactly arranged cells in cords indicating signs of activity. The proportion of cell types was found varied; only very few type I cells could be noticed among the type II cells. Type I cells were round or oval in shape with a diameter of
about 8.44±0.02 µm. These cells often contained relatively large mildly basophilic nuclei (3.57±0.02 µm in diameter) with 1-2 round nucleoli (0.59±0.11 µm wide) and small amounts of cytoplasm (NPR 0.42±0.01). A prominent feature of MO of this phase was the hypertrophy of type II cells with increased cytoplasmic volume (37.19±6.39 µm in diameter). Their moderately basophilic spherical nuclei (10.90±1.02 µm wide) were mostly pushed towards the periphery. Large clearly discernible 1-2 circular nucleoli (2.38±0.41 µm wide) were seen to lie centrally or peripherally in the nucleoplasm. The fibrillar chromatin was seen scattered in the nucleoplasm. The cytoplasm exhibited homogeneity and showed mild to moderate basophilia (NPR 0.27±0.02). Blood sinuses and capillaries with granular and agranular hemocytes were apparent (Figure 5A). A few type II cells (10%) with vague cell boundaries were observed in some areas within the gland.

Morphology and histology of MO during tertiary vitellogenic phase

The gland appeared bulged, translucent and milky white with a measurement of 1.85±0.07 mm length and 0.56±0.01 mm width (Table 3). Both the cell types were perceptible in the gland which had a hypotrophied appearance. Type I cells were small (7.33±0.04 µm wide) and oval with relatively larger nuclei (4.23±0.03). Their nuclei appeared moderately basophilic, positioned centrally or peripherally. The cytoplasm was characterized by a mild basophilia. Polygonal type II cells (15.82±4.21 µm in diameter) were arranged in cords with round or oval, peripherally or centrally located, mildly basophilic nuclei (5.42±1.04 µm wide) and indistinct nucleoli (Figure 5B). The cytoplasm was homogenous and showed mild to moderate basophilia (NPR 0.34±0.01). Intercellular spaces were prominent with few blood sinuses and capillaries. Hemocytes were poorly detected in the hemal sinuses. The gland showed signs of inactivity indicated by cells with indistinct boundaries, pycnotic nuclei, conspicuous intracellular spaces and inconspicuous blood sinuses and capillaries (Figure 6A).

Figure 5. Photomicrograph illustrating histology of mandibular organ during secondary and tertiary vitellogenic phases.
A, Mandibular organ portraying hypertrophy of type II cells and nuclei during secondary vitellogenic phase; B, Mandibular organ at tertiary vitellogenic phase.
depicting type I and II cells with prominent intercellular spaces. BS: Blood sinuses; H: Hemocyte; N: Nucleus; NU: Nucleolus; TI: Type I cell; TII: Type II cell.

Morphology and histology of MO during oosorption phase

By this stage, the organ appeared creamy white, flaccid in nature and measured 0.95±0.01 mm in length and 0.03±0.02 mm in width (Table 3). The MO of this phase was discerned with a few type I (7.90±0.02 µm wide) and type II cells (14.93±3.47 µm in diameter), spotted among vacuoles and degenerating cells. Gland cells with distinct boundaries were rarely detected. Condensation of chromatin and depletion of cell cytoplasm displayed a degenerated appearance for the gland. Vacuolization was a prominent feature of the MO of this phase. Pycnotic nuclei were seen scattered inside the gland (Figure 6B). Blood sinuses and capillaries were indistinct.

![Figure 6. Section depicting mandibular organ of tertiary vitellogenic and oosorption phases.](image)

A, Another mandibular organ showing pycnotic nuclei, depleted cytoplasm and conspicuous intracellular spaces during tertiary vitellogenic phase; B, Mandibular organ containing pycnotic nuclei and vacuoles during oosorption phase. PN: Pycnotic nuclei; TII: Type II cell; V: Vacuole; Arrow indicates cells with depleted cytoplasm.

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<th>Table 3: Morphology of mandibular organ during various phases of oogenesis in <em>Barytelphusa cunicularis</em></th>
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<td>Phases of oogenesis</td>
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<tr>
<td>Previtellogenic</td>
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<td>Primary vitellogenic</td>
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<td>Secondary vitellogenic</td>
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<td>Tertiary vitellogenic</td>
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<td>Oosorption</td>
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DISCUSSION

The present study demonstrated the morphological and histological changes in the mandibular organ in relation to growth and vitellogenesis of the freshwater crab *Barytelphusa cunicularis*. The MO of *B. cunicularis* is a glandular tissue located at the base of the posterior abductor muscle of the mandible. A more or less similar location was noticed for the MO in *S. serrata* (Huiyang et al., 2003), *O. senex senex* (Nagaraju et al., 2004) and *T. schirnerae* (Sudha Devi et al., 2017; 2018). Le Roux (1974) observed the MO between the mandible and the maxillula in eucarid crustaceans. The mandibular organs were positioned posterior to the mandibles and closely associated with the paired chitinous tendons in *Callinectes sapidus* (Yudin et al., 1980). In *Procambarus clarkii*, the MO was placed at the dorsal side of the mandible, outside the base of the mandibular tendon (Xin and Sheng, 1998). Sarika et al. (2014) identified the MO posterior to the mandible and closely attached to the anterior portion of the paired tendons in *Paratelphusa* sp. Mandibular organ of *L. emarginata* was found in union with the chitinous ligaments of the mandibles (Hinsch, 1981).

The current investigation noted a remarkable increase in the gland size as development progressed. A comparable phenomenon was observed in *O. senex senex* where the organ size increased significantly with increase in body weight (Nagaraju et al., 2004). In *Eriocheir sinensis*, Wei-xin and Jian-feng (2004) found enhancement in MO size as growth advanced. Huiyang et al. (2003) observed an increase in MO size from first to the fourth stage of development in *S. serrata*. In *P. clarkii*, Taketomi and Nakano (2007) observed a positive correlation between the size of the organ and the size of the animal, attaining maximum size in fully grown animals. A comparable phenomenon was noticed in *Cancer anthonyi*, in which the length and width of the Y organ gradually increased from first to the fourth larval stage (Mc Conaugha, 1980).

Our histological studies clearly indicated the existence of two cell types in the MO of *B. cunicularis*. Many authors confirmed the presence of two cell types in MO. For instance, Hinsch (1981) distinguished light and dark cells in the MO of *L. emarginata*. Dorn (1973) identified dark cells in larvae and light cells in adults of *Oncopeltus fasciatus*. The MO cells from *Fenneropenaeus indicus* contained two cell types namely, dense and less dense cells (Vijayan and Diwan, 1994). The MO of *S. quadratum* also contained two cell types: secretory and non secretory cells (Syama, 2009). In *T. schirnerae*, two distinct cell types were identified based on the size of cell and nuclei (Sudha Devi et al., 2017; 2018). On the other hand, the organ was composed of a single cell type in *C. sapidus* (Yudin et al., 1980), *P. clarkii* (Xin and Sheng, 1998), *S. serrata* (Huiyang et al., 2003), *Portunus trituberculatus* (Kun et al., 2007) and *Paratelphusa* sp., (Sarika et al., 2014) and three cell types in *Homarus americanus* (Borst et al., 1994).

Our present study emphasized perceptible changes in the histology of MO in relation to various developmental stages. In *B. cunicularis*, the gland cells were small in size and few in number with indistinct cellular limits during stage I of development and by the time it reached stage IV, cells were more in number and hypertrophied. Similar
observations were made in *S. serrata*, wherein the gland cells were small in size during stage I and reached maximum diameter in stage IV (Huiyang et al., 2003).

A cord like arrangement of MO cells was observed in *B. cunicularis* as development advanced. These characters were similar to those observed in the MO of *C. sapidus* (Yudin et al., 1980), *P. clarkii* (Xin and Sheng, 1998), *E. sinensis* (Wei-xin and Jian-feng, 2004) and *T. schirnerae* (Sudha Devi et al., 2018). The cord configuration of MO cells was clearly demonstrated in the fine structural studies of MO of *Paratelphusa* sp. (Sarika et al., 2014) and *T. schirnerae* (Sudha Devi et al., 2017). On the other hand, the MO cells were arranged in groups to form cell clusters in *S. serrata* (Huiyang et al., 2003). Observations of Mc Conaugha (1980) in *C. anthonyi* revealed that the Y organ became more composite through extensive folding and intertwining of the cellular cords as development advanced.

The current results revealed that in young crabs (stage I of development), the gland cells remained small with sparse cytoplasm and in mature crabs (fourth stage of development), the gland cells got hypertrophied with a remarkable increase in the cytoplasmic volume. This is in agreement with the findings of Huiyang et al. (2003) in *S. serrata* where the cytoplasmic volume gradually increased from first to the fourth developmental stage.

Our observations showed appreciable changes in the morphology of MO in relation to the various phases of oogenesis. In *B. cunicularis*, the gland was small during non-reproductive (previtellogenic) phase and appeared remarkably large during the reproductive (early and middle vitellogenic) phase. In support of this research, in *Paratelphusa* sp., the MO attained maximum size during the reproductively active season and minimum during the reproductively inactive season (Sarika et al., 2014). The weight of the MO increased with the advancement of reproductive stage in female *O. senex senex* (Nagaraju et al., 2004). In *H. americanus*, Byard et al. (1975), Couch et al. (1979) and Waddy et al. (1995) observed an increase in MO size during ovarian maturation. In *P. clarkia* and *S. quadratum*, a progressive increment in size of the MO could be noticed with progress in development of the ovary (Wei-xin and Jian-feng, 2004; Syama, 2009).

The current research highlighted the fact that the MO appeared active during early and middle phases of vitellogenesis as evidenced by the increased gland size, hypertrophy of gland cells and distinct blood sinuses and capillaries with granular and agranular hemocytes and inactive during previtellogenic, late vitelligenic and oosorption phases as indicated by the presence of vacuolated areas, nuclear pycnosis, indistinct cell boundaries and poor vascularization. Likewise, in *Paratelphusa* sp., the MO was highly secretory during the reproductive period, characterized by the presence of Golgi bodies and extensive networks of SER and RER while during the non-reproductive period, the organ was found inactive with poorly developed RER and Golgi bodies (Sarika et al., 2014). In *S. quadratum*, the glandular cells were inactive during early vitellogenic period, but by the mid and late stages of ovarian maturation, they become more active as evinced by the occurrence of many Golgi bodies and secretory granules (Syama, 2009). In *C. maenas* and *L. emarginata*, Le
Roux (1968) and Hinsch (1980) reported that the MOs were more active during ovarian maturation. Laufer et al. (1986; 1987) reported that the MF synthesis by MO was minimum in juvenile and previtellogenic females whereas high in vitellogenic females.

Our observations revealed indistinct hemal sinuses and capillaries with poorly detected hemocytes during stage I of development and the non-reproductive periods whereas large blood sinuses and copious amounts of hemocytes were present in the MO during stage II, III and IV of development and the reproductive periods (primary and secondary vitellogenic phases). Huiyang et al. (2003) reported that the blood sinuses and blood cells were very few in early stages of development and were abundant in late stages of development in *S. serrata*. Likewise, in *Paratelphusa* sp., the MO cells were surrounded by a few hemocytes during the non reproductive periods and more number of granular and agranular hemocytes during reproductive periods.

The current investigation emphasized the fact that MO plays a key role in the regulation of vitellogenesis in *B. cunicularis*. Methyl farnesoate (MF), produced by the MO is known to be involved in the regulation of vitellogenesis in several crustaceans (Borst et al., 1987; Laufer et al., 1987; Nagaraju, 2007; Nagaraju, 2011; Sarika and Anilkumar, 2014; Hemalatha et al., 2016). Tsukimura and Kamemoto (1991) reported the effect of MF on ovarian development in *Penaeus vannamei*. In *P. clarkii*, MF treatment enhanced the growth of ovary (Laufer et al., 1998). Reddy and Ramamurthi (1998) observed that the early vitellogenic females of the crab *O. senex senex* entered late vitellogenic phase in response to MF administration. The ovarian index and oocyte diameter were significantly increased in females injected with MF in *P. indicus* (Nagaraju et al., 2002). In vitro and in vivo studies in *O. senex senex* have shown a direct correlation between MF production and ovarian development (Nagaraju et al., 2006).

**CONCLUSION**
This study revealed that the activity of MO in *Barytelphusa cunicularis* is entrained with growth and reproduction. The MO exhibited substantial changes in morphology and histology in accordance with the stages of development of the animal as well as the stage of oogenic cycle. The gland was small with least level of activity during early stages of development and attained maximum size and activity in adult. Likewise, the activity of MO was minimum during the non-reproductive phase and reached its peak during the reproductive phase (early and middle phases of vitellogenesis). Further fine structural studies are required to support these observations.

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