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).

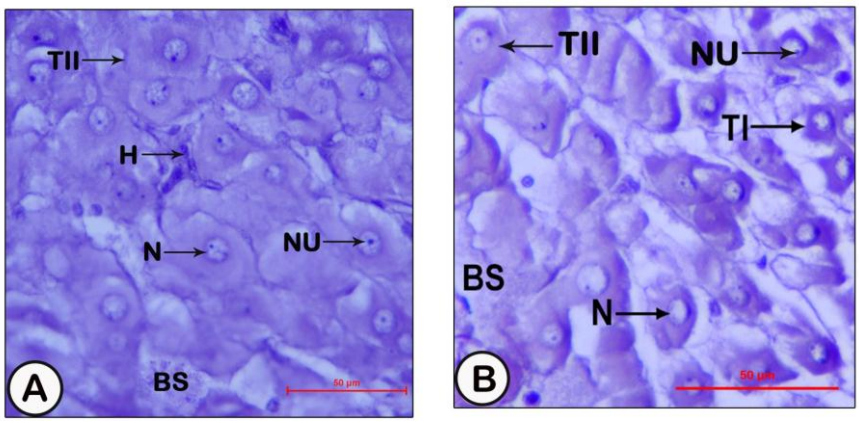

Figure 5. Photomicrograph illustrating histology of mandibular organ during secondary and tertiary vitellogenic phases.
A, Mandibular organ portraying hypertrophy of type II cells and nuclei during secondary vitellogenic phase; B, Mandibular organ at tertiary vitellogenic phase

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.

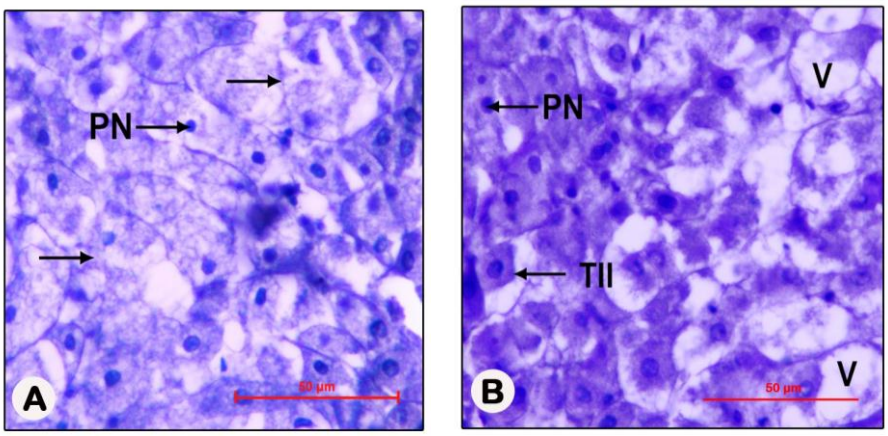

Figure 6. Section depicting mandibular organ of tertiary vitellogenic and oosorption phases.
A, Another mandibular organ showing pycnotic nuclei, depleted cytoplasm and conspicuous intracellular spaces during tertiary vitellogenic phase; B, Mandibular organ containing pycnotic nuclei and vacuoles during oosorption phase. PN: Pycnotic nuclei; TII: Type II cell; V: Vacuole; Arrow indicates cells with depleted cytoplasm.

Table 3: Morphology of mandibular organ during various phases of oogenesis in *Barytelphusa cunicularis*

Phases of oogenesis	Mandibular organ		
	Colour	Length (mm)	Width (mm)
Previtellogenic	Light yellow	0.6±0.12	0.2±0.05
Primary vitellogenic	Pale white	1.6±0.36	0.3±0.05
Secondaryvitellogenic	Snowy white	1.8±0.07	0.5±0.05
Tertiary vitellogenic	Milky white	1.8±0.07	0.5±0.01
Oosorption	Creamy white	0.9±0.01	0.1±0.02

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 vitellgenic 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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