Ovarian cycle and scanning electron micrographs of the spawned egg of female mantis shrimp *Oratosquilla massavensis* (Alexandria, Egypt)

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**Abstract**  Mantis shrimp *Oratosquilla massavensis* is an abundant marine crustacean in Egypt. It is common among the most important predators in many shallow, tropical and subtropical marine habitats. It is poorly understood as many species spend most of their life tucked away in burrows and holes. The objective of this study is to provide information on the histological characteristics of the ovary of female mantis shrimp *O. massavensis* and the morphology of the spawned egg, using scanning electron microscope. The ovaries showed a pronounced macroscopic differentiation in size and color with the maturation of the ovary, in six developmental stages namely: immature stage, previtellogenesis, primary vitellogenesis, secondary vitellogenesis, maturation and spent stage. Staining affinities of different structural components, size of different oocytes and nuclear sizes, as well as the follicular cells and their association with oocytes were used to differentiate between different oocyte developmental stages. Scanning electron micrographs of the spawned egg of *O. massavensis* revealed spherical forms of the egg with well noticed stalk or funiculus. The chorion is ornamented as a wrinkled layer with different textures. Two different yolky materials or matrices were observed, the first one constitutes a conical shaped hard matrix with glassy appearance, while the second one appears spongy with somewhat soft appearance.

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**Introduction**  
Mantis shrimps are benthic marine stomatopod crustaceans that live in defendable burrows. They are common predatory crustaceans in many sea coasts including the Gulf of Mexico (Caldwell and Dingle, 1976; Caldwell, 1991) along the coast of Brazil and in the Mediterranean Sea (Bauer, 1999), where, they entered from the Red Sea via the Suez Canal, when it was first recorded from Israel by Steuer (1936).

The study of the morphology and physiology of the reproductive systems is essential to define the reproductive cycles of...
animal species. Oogenesis is a complex process of cellular and molecular changes that occur during the formation, growth and maturation of the female germinal cells. The development of the oocyte is remarkable and the study of this process is essential to the understanding of reproduction though very little work was dealing with the histological structure of some Squilla sp. The first work was done by Yamazaki and Fuji (1980) on the reproductive system of Oratosquilla oratoria involving oogenesis which divided it into 4 stages, while Wortham-Neal (2002) showed that the gonad maturity of Squilla empusa divided into 3 stages. Recently, Kodama et al. (2009) investigated the reproductive biology of Oratosquilla raphidea into three stages based on histological observation. The developmental stages of oocytes in an individual ovary were synchronous, suggesting that almost all oocytes in an ovary spawned at the same time. Also, Yusli and Ali (2010) divided the gonad maturity of Harpiosquilla massavensis described in the present work was to investigate the morphology and the histology of different maturity stages of the ovary of females Oratosquilla massavensis collected from the eastern harbor in Alexandria sea water, Egypt and fine structures of the spawned eggs using scanning electron microscope hoping to yield detailed ontaxonomic features for this studied species.

Materials and methods

Females of O. massavensis were collected at night using commercial trawlers during 2009 from the Eastern harbor in the Mediterranean Sea, Alexandria, Egypt. The collected samples were transported to the laboratory in collecting bags with fresh sea water. The ovary was dissected out from 5 randomly selected individuals from each stage. Samples were fixed in aqueous 10% formalin and processed for light microscopy. Serial sections were cut at 5 μm and stained with Mayer’s hematoxylin and eosin. Slides were examined and photographed under Olympus microscope (CX31) equipped with an image analyzing system.

For scanning through electron microscope, the ripped eggs of female O. massavensis were fixed immediately in 2.5% buffered glutaraldehyde at 4 °C for 1 h. They were post-fixed at 1% osmium tetroxide at 4 °C for 1 h. After post fixation, eggs were dehydrated in ascending series of ethanol. Samples were dried in a critical point dryer. Dried specimens were mounted on metal stubs, sputter coated with gold and examined using JEOL JSU-5300 scanning electron microscope, at the Electron Microscope Unit at the Faculty of Science, Alexandria University, Egypt.

Results

According to the microscopical examination of the female gonads of O. massavensis, the ovaries could be differentiated into six different ovarian developmental stages according to their sizes, colors and histological observations (staining affinities of different structural components, location and organization of different oocyte inclusions and nuclear sizes). These stages are: immature stage, previtellogenesis, primary vitellogenesis, secondary vitellogenesis, maturation and spent stage.

Stage I (immature stage)

The ovary is thin, small and transparent with marked black pigments over its outer membrane (Fig. 1a). The length of the ovaries is about 6–7 cm and their width is about 0.3–0.4 cm. However, at this stage, all collected females had a white color of median triangular structure of the telson, which is enclosed inside the terminal part of the fused ovaries. Also, the color of abdominal tergites is transparent.

The ovary at this stage consists of two associated lateral lobes. These lobes are attached to each other by a complete middle sheath of connective tissue (Fig. 2). Each lobe is sheathed by two walls. The inner is the lobular wall and the other one is the outer ovarian wall. Each ovarian lobe contains a large number of undifferentiated basophilic cells which are found at the proliferation stage. These cells can be differentiated according to their sizes, affinities to stains and the nuclear shapes into gonial cells and follicle cells (Fig. 3). Each gonial cell appears rounded with an indistinct nucleus and deeply stained with hematoxylin and eosin, while the follicle cell is faintly stained with the above stains but has a distinct nucleus.

Stage II (previtellogenesis stage)

The ovaries of female O. massavensis at this stage are faint yellow in color, with 6–8 cm length and 0.5–0.6 cm width (Fig. 1b). Ovarian wall is reduced in size and appears with the same black pigments over its surface. The follicle cells have an oval shape. Its number becomes more than that of the previous stage and arranges around the oocytes, forming a sheath known as follicular sheath. The oocytes have a polygonal shape during this stage with a diameter of about 100 μm and are distinguished into four types. In the first type (type 1), the oocyte appears small in size, deeply basophilic and with a nearly central nucleus (Fig. 4). In the second type (type 2), the cell is larger than the first one, less basophilic and its nucleus is somewhat eccentric (Fig. 5). The third type of cells (type 3) became larger, their affinity to stain became less basophilic and their nuclei still remains somewhat eccentric. Examination of the third type of previtellogenic oocyte showed a clear peripheral unstained lipid globule at the periphery of the cell (Fig. 6). The last type of late previtellogenic cells (type 4) appears larger in size, faintly stained with hematoxylin and their nuclei are somewhat eccentric. In some cells of type 4, yolk droplets were gradually increased in size and spread from the periphery to the central region of the oocytes (Fig. 7). The peripheral unstained lipid globules were increased in number and occupy all the peripheral areas of the cell.

Stage III (primary vitellogenesis stage)

The ovaries of female O. massavensis at this stage became deep yellow in color with about 8–9 cm length and about 0.7–0.8 cm width and contains surface pigments (Fig. 1c). The ovarian wall became thinner. The oocytes appear larger with about 150 μm
Figure 1 A photograph of dissected adult female *O. massavensis* (a) at stage I of maturity (immature) showing a slightly thin transparent ovary (b) at stage II of maturity (previtellogenesis) showing a pale yellow ovary (c) at stage III of maturity (primary vitellogenesis) showing a deep yellow ovary (d) at stage IV of maturity (secondary vitellogenesis) showing orange color of the ovary (e) at stage V of maturity (maturation) showing bloody color of the ovary and (f) at stage VI of maturity (spent ovary) showing the cloudy color of the ovary.

diameter while the nucleus is centric and appears smaller with about 25 µm diameter. At this stage, the vitellogenic oocytes have a spherical shape due to the accumulation of yolk.

Figure 2 TS of ovary, showing stage I of maturity (immature ovary). OW: ovarian wall; FW: follicular wall; CT: connective tissue; OL: ovarian lobes of the ovary.

Figure 3 TS of ovary, at the stage I of maturity (immature ovary). Go: gonial cells; CT: connective tissue; FC: follicle cells distributed in-between gonial cells.

Figure 4 TS of ovary, demonstrating stage II of maturity. Showing type one of previtellogenic oocyte. Note: follicle cells (FC), N: nucleus; Nu: nucleolus.
materials. It has a homogenous highly, acidophilic cytoplasm with high contents of yolk and distinct lipid droplets. On the other hand, the nucleus has lesser basophilic affinity than the nucleolus and the chromatin granules became condensed inside the nucleus. The follicle cells surrounding the vitellogenic oocytes still form a sheath of flat cells (follicular wall) (Fig. 8).

Stage IV (secondary vitellogenesis stage)

Female ovaries at this stage have an orange color with about 8–10 cm length and about 1–1.2 cm width (Fig. 1d). This gonadal stage is characterized by the abundance of mature secondary oocytes with a high number of yolk globules (Fig. 9). Vitellogenic oocytes are surrounded by a sheath of flat follicle cells which expand around oocytes and are highly associated to the vitelline membrane.

Secondary oocytes of *O. massavensis* became rectangular in shape, due to their dense packing of acidophilic yolk inside the ovary and measure about 200 μm. The nucleus lost its spherical shape and migrated to the peripheral acidophilic zone of the oocytes and their affinity to hematoxylin increased intensively.

Stage V (maturation stage)

Ovaries of female *O. massavensis* at this stage became deep orange to bloody in color. Their length is about 9–10 cm, while their width is about 1.2–2 cm and occupied slightly over 50% of the abdominal cavity (Fig. 1e). The histological structure of the ovary at this stage showed a large ovarian lobular and thinner wall than that of the previous stage. It is entirely packed with large number of mature oocytes (Fig. 10). In addition, few oogonia and previtellogenic oocytes were observed in the...
center of the ovarian lobules. Mature oocytes at this stage, are characterized by their large size, reaching about 425 μm. The nuclear membrane of the mature oocyte became indistinct, since the cytoplasm was highly packed with yolk spheroids and yolk vesicles surrounding lipid globules, which tended to be homogeneous acidophilic in nature. At the end of this stage, a noticeable large number of fluid substances surround the oocytes as an extracellular yolk. This substance penetrates oocytes in between the yolk granules and surrounding the nucleus. The follicle cells were rarely found around the fully formed eggs.

**Stage VI (spent stage)**

Ovaries of females at this stage became cloudy in color and are easily distinguished from the immature stage in that their length is about 9–10 cm and about 1.5–2 cm width (Fig. 1f). Histological examination of the ovary at this stage revealed that the ovarian wall was increased again. In the early spent ovary, some mature ova still persisted in the ovary. Following ovulation, the border of some unspawned mature eggs became indistinct and appeared to undergo atresia. Oogonias and few oocytes are present (Fig. 11).

At the end of atresia, large number of phagocytic cells became apparent to occupy the ovarian follicles. In some cases, they invaded the wall of atretic oocytes and liquefied their cytoplasm. The phagocytic follicle cells digested and absorbed the yolky content by active phagocytosis and their cytoplasm became acidophilic.

*Scanning electron micrographs of spawned eggs*

Scanning electron micrographs of the brooded fertilized spawned egg of *O. massavensis* taken from females with bloody telson revealed spherical forms of the egg. Each egg is hung by a well noticed stalk or funiculus (Fig. 12). The egg is covered by the chorion (outer membrane) which was clearly observed in the processed egg (wrapped in some parts). The chorion can be discriminated into three regions: The marginal, central and the frontal regions. The first region (the marginal region) showed regular elevated smooth parts with elevated ridges, giving the appearance of sea waves (Fig. 13). The second region (the central region) showed an irregular slightly rough surface with irregular shaped projections (Fig. 14). It contained numerous dark spots and some of them appeared on the top of cone shaped elevations. The spots appeared to be a secretion released from conspicuous openings between the projections. The third region (the frontal region) which is in front of the egg or at the apex of the egg appears as a wrinkled cover with shallow elevation. The basal region of the processed egg pores with irregular outlines appears near the funiculus (Fig. 15).

The processed egg showed some uncovered parts with the outer chorion. The magnified SEM photographs for these parts showed the inner contents of the yolky egg and two different yolky materials or matrices. The first one constitutes a conical shaped hard matrix with glassy appearance (Fig. 16) while the second one appears spongy with somewhat soft appearance (Fig. 17). The first type of yolky matrix was found underneath the chorion in the apical part of the processed egg.
Figure 12 Scanning electron micrograph of the fertilized spawned egg of *O. massavensis*, showing an egg that is hung by a well noticed stalk or funiculus (F). The chorion can be discriminated into three regions (marginal chorion (MC), central chorion (CC) and frontal chorion (FC)).

Figure 13 Magnified part of the first region (marginal region) of the chorion (MC) of spawned egg, showing the numerous elevated ridges (ER).

Figure 14 Enlarged part of the outer surface of the second region of the chorion (central chorion (CC)), showing an irregular slightly rough surface with irregularly shaped projections.

Figure 15 Enlarged part of the basal region of the spawned egg, showing numerous pores (P).

Figure 16 Enlarged part of the outer surface of the third region of the chorion (frontal chorion (Fc)), showing the first material of yolky matrix with glassy appearance (glassy matrix (GM)).

Figure 17 Enlarged part of the outer surface of the third region (frontal chorion (Fc)), showing the second material of yolky matrix with spongy appearance (spongy matrix (SM)).
while the second type was located under the chorion in the basal part of the egg. A scanning electron microscopical examination of a spawned egg cut transversally showed that the egg was filled with a large amount of yolk globules and granules while it was embedded and coated by the above described yolky matrix (Fig. 18).

Discussion

The present investigation showed that the ovary of *O. massavensis* consists of two lobes which run along the length of the thorax, abdomen and telson. The two lobes are covered with an outer membrane and it is not easy to separate them from each other. Kodama et al. (2009) and Yusli and Ali (2010) recorded the same structure of the ovary of *O. oratoria* and *Harpiosquilla raphidea* in which the ovaries consist of two lobes that run through the body length anterior to the thoracic region and end posterior in the telson joined with the hepatopancreas.

The results also show that there are six ovarian colors during the ovarian cycle. These colors are transparent, pale yellow, yellow, orange, bloody and cloudy color. Some authors (George, 1963; Heegaard, 1963; Farmer, 1974; Fyhn and Costlow, 1977) recorded the color changes in the ovary during the maturation of crustaceans and suggested that this phenomenon was due to the synthesis of carotenoid pigments. Moreover, Harrison (1990) and Chang (1993) mentioned that in crustaceans, carotenoids had the ability to bind vitelline into lipo-glycol-carotene protein complex, by which the macromolecules accumulate in the oocyte cytoplasm.

In the present study, maturity of the ovary of *O. massavensis* can be easily determined externally by direct observation of the visible color of the ventral surface of telson and the color of abdominal tergites, which reflect the color of ovaries underneath them. This observation is in agreement with Deecaraman and Subramoniam (1980, 1983) and Wortham-Neal (2002).

The oocyte development in the adult *O. massavensis* as shown in the present anatomical and histological results was divided into six different stages: Immature stage, previtellogenesis, primary vitellogenesis, secondary vitellogenesis, maturation and spent stage. This seems to be similar to those observed in other mantis shrimps such as *Squilla holoschista* Deecaraman and Subramoniam (1983), *O. oratoria* (Yamazaki and Fuji, 1980; Kodama et al., 2009).

Histologically, the present study revealed that the ovarian wall decreased in thickness during the maturity stages of *O. massavensis*. This was noticed previously by Silva and Cruz-Landim (2006) who stated that the thickening of the ovarian wall of *Pandirius echinus* and *P. laevicauda* was reduced, indicating that they are being stretched to accommodate the growth of germ cells.

On the other hand, the present results showed that the follicle cells were scattered in between the immature stage of oocytes, while in the early developing stages a sheath of rounded or oval follicle cells surrounded the oocytes. A similar observation was recorded by Yano (1988) in the ovary of *Penaeus japonicus*. Talbot (1981) discussed the function of the follicle cells and mentioned that they are responsible for the formation of the chorionic egg membrane of the mature ovum of *Homarus americanus*.

At stage I (immature stage), ovaries of *O. massavensis* are characterized by the occurrence of a central proliferation zone, followed by gonial cells toward the periphery of the gonad. This has also been observed for *Macrobrachium aconthurus* (Carvalho and Pereira, 1981) and for *Armases rubripes* (Santos et al., 2009). The cytoplasm of the oocytes at stage I was basophilic until the primary vitellogenic stage. According to Raven (1961), this basophilic cytoplasm is especially important for the increase of the protoplasm, but not for the formation of the vitellus. Brown (2009) indicates that the basophilic ooplasm is due to activation of cells with the production of organelles. The formation of organelles such as mitochondria, Golgi complexes and abundant quantities of endoplasmic reticulum, ribosomes and fragmented glycogen may contribute to the blue staining of the ooplasm.

The presence of fatty yolk in significant quantities coincides, therefore, with the appearance of the yellow color in the primary vitellogenic stage (stage III). The yellow color in *Penaeus setiferus* ovaries was also associated to the increase of fatty yolk as mentioned before by King (1948).

A sheath of follicle cells was observed around pre-vitellogenic oocytes of *O. massavensis*. Accordingly, these cells could be involved in the process of vitellogenesis. On the other hand, Varadarajan and Subramoniam (1980) stated that in *Celbanarius clibanarius*, the lipoprotein from extraovarian sources when linked to carotenoid pigments may serve in facilitating the entry of lipoproteins into oocytes. This observation leads to the idea of extraoocytic production of this type of yolk in *O. massavensis* and these cells which are arranged at the periphery of the oocytes are possibly used for exchanging different substances that form part of the nutrient stock of the egg (protein, vitellogenin and lipids).

The spent stage is characterized by total post-elimination of the oocytes. In this phase, there is ovarian regeneration (with reabsorption), oocyte lysis and proliferation of the cellular elements of the connective tissue. This was also reported in *Palaemon paucidentus* (Kamiguchi, 1971), *Artemesia longinaris* (Christiansen and SceIzo, 1971) and *Armases rubripes* (Santos et al., 2009).

Ootaxonomy is based chiefly on the species-specificity of chorionic architecture, which is constant within the representatives of a species (Gaino and Abongiovanni, 1992). Egg envelopes are elaborated by follicle cells and laid down in a well
defined sequence (Gaino and Mazzinmi, 1990). The ultrastructural analysis of newly spawned eggs of O. massaverensis revealed that they were covered by the characteristic chorion with three different regions; the marginal, central and frontal regions. This may be due to different distributions of underlined yolk materials (El-Sherief, 1990).

The results also showed that there are numerous pores with irregular outlines at the basal region of the egg. By analogy, the presence of numerous marked pores throughout the chorionic membranes of Portunus is responsible for the spongy appearance of the ova (El-Sherief, 1987). In the present study, the spawned egg of O. massaverensis showed two different yolk materials or matrices which have an important role in the growth of the egg. In agreement with El-Sherief (1987) who indicated that the eggs of Portunus pelagicus enlarge due to the increased internal yolk content that also has a role in increasing the surface area of the attached membrane of the incubated egg during embryogenesis.

In summary, the ovarian maturity stages of mantis shrimp O. massaverensis can be determined on the bases of morphological appearance of the ovaries, the abdominal tergites and telson sternite and the histological structure of the female ovary. When external morphological characteristics of the ovaries were compared to histological descriptions, it was possible to observe modifications that characterize the process in different developmental stages throughout the ovarian cycle and, consequently, the macroscopic classification of the ovarian stages agrees with the modifications of the reproductive cells.

References


