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Oocyte Development and Female Reproductive Cycle in the Freshwater Crab *Travancoriana schirnerae*

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Abstract

The seasonal changes in the ovary of *T. schirnerae* were described based on histological and histochemical analyses. The development of oocytes was divided into ten stages: oogonia, chromatin nucleolus (1 to 3), perinuclear, primary vitellogenic, secondary vitellogenic (1 to 3) and tertiary vitellogenic. The seasonal ovarian development was classified into six phases: proliferation, previtellogenic, primary vitellogenic, secondary vitellogenic, tertiary vitellogenic and oosorption. Yolk accumulation started during primary vitellogenesis, when basophilic small yolk globules appeared at the cortical cytoplasmic region. The highly basophilic large yolk globules of secondary vitellogenic stage 1 underwent a series of morphological changes to become mildly basophilic yolk platelets in secondary vitellogenic stage 2. The ooplasm noted with strongly acidophilic large yolk platelets in secondary vitellogenic stage 3. The entire ooplasm formed an acidophilic, homogeneous matrix during tertiary vitellogenic stage. The primary vitellogenic stage oocytes showed a strong positive reaction to PAS and MBB, indicating the glycoproteinaceous nature of the yolk. In the secondary and tertiary vitellogenic oocytes, yolk platelets showed positive reaction for MBB, PAS and Sudan black B, demonstrating the lipoglycoproteinaceous nature of the yolk. In *T. schirnerae*, vitellogenesis extended from October to March and spawning occurred in April. It is concluded that *T. schirnerae* is an annual breeder accommodating a single ovarian cycle during the intermoult period.

Keywords: Histology, histochemistry, oogenesis, *Travancoriana schirnerae*, vitellogenesis.

Introduction

Being an essential element of breeding and fecundity, oogenesis has invited the attention of investigators for the past several years. A number of general studies have covered oocyte development and pattern of reproductive cycle in decapod crustaceans (Charniaux-Cotton and Payen, 1988). While considering brachyurans, most of the studies on oogenesis are concentrated in estuarine and marine species (Chiba and Honma, 1972; Sudha and Anilkumar, 1996). Investigations have also been pursued on various cytological and biochemical changes related to vitellogenesis in several marine brachyurans (Eurenius, 1973; Shyamasundari and Babu, 1984). The morphology of the reproductive system and the development of oocytes have been examined in the mangrove crabs *Goniopsis cruentata* (Desouza and Silva, 2009) and *Ucides cordatus* (Sampaio *et al*., 2011). Minagawa *et al*. (1993) reported the reproductive biology and the oocyte development in the red frog crab *Ranina ranina*. The light and electron microscopic details of oogenesis have been carried out in the blue swimmer crab *Portunus pelagicus* (Ravi *et al*., 2012). However, there is little information available on oocyte development and vitellogenesis in freshwater crabs (Joshi and Khanna, 1982). More detailed studies are necessary to ameliorate this situation and to achieve a proper understanding of how ovarian development occurs in this group.

The freshwater crab, *Travancoriana schirnerae* (Bott, 1969) abundant in the paddy fields and areca plantations of Mananthavady, Wayanad (Kerala, India), is also known from the Southern Indian states of Karnataka and Tamil Nadu (Bahir and Yeo, 2007). It is edible and forms a cheap source of animal protein to the poor, malnourished local tribes. Though some aspects of its reproductive biology such as mating pattern, gonadosomatic index, vas deferens factor and fecundity are known (Sudha Devi and Smija, 2013), no information is available on the female reproductive cycle of this species. The present study makes a holistic description of the oocyte development and

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female reproductive cycle in *T. schirnerae*. It is expected that the knowledge generated from this study may help in understanding the female reproductive biology and thereby large scale production of the species.

Materials and Methods

Sexually mature female specimens (n=165) (carapace width 4.0 to 5.0 cm; 4.6±0.29) were caught every month between June 2009 to March 2011 from the paddy fields near Mary Matha Arts & Science college campus, Mananthavady. The size and colour of ovary, oocyte diameter, CW of ovigerous, juvenile carrying females, CW of females in mating pairs and nature of spermathecae were the criteria in establishing the size at sexual maturity in females. The size at which males and females attained sexual maturity was 4.0 and 3.9 cm respectively (Sudha Devi and Smija, 2013)

Their carapace width (CW) and body weight were measured. The ovaries were dissected out, weighed and the gonadosomatic index (GSI) was calculated as the percentage of wet weight of ovary to body weight. One half of the ovary was fixed in Bouin's fluid, dehydrated in graded series of ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 5 to 6 µm thickness. The sections were stained with Heidenhain's hematoxylin-eosin for histological studies and with periodic acid Schiff (PAS), mercuric bromophenol blue (MBB), Azan, Mallory triple and Sudan black B for histochemical analyses. Photomicrographs were taken with a Leica DM 500 Research microscope equipped with a DG 330/210 camera using Biowizard software. The other half of the ovary was used for the characterization of vitellogenic stages. For this, the ovary was teared open; one hundred oocytes were randomly chosen and their diameter measured using a calibrated oculometer.

Results

Morphology of the Female Reproductive System

The reproductive system of *T. schirnerae* consisted of a pair of ovaries, oviducts, spermathecae and gonopores. The ovaries were elongated organs occupied dorsally in the cephalothorax. The two limbs of the ovary extended on either side of the alimentary canal and were linked together by a connecting bar which gives an H shape to the system. Spermathecae were saccular, roughly pear-shaped organs opening at the junction of the posterior ovarian lobe and the oviduct, in which sperms were stored after mating (Figure 1). The ovary continued as oviduct that led to the gonopore located on the sternite of the sixth thoracic segment.

Histology

The oocytes in the ovary of *T. schirnerae* underwent a series of morphological, cytological and histochemical changes during development and were categorized into ten sequential stages - oogonia, chromatin nucleolus stage (1 to 3), perinuclear stage, primary vitellogenic stage, secondary vitellogenic stage (1 to 3) and tertiary vitellogenic stage - based on the oocyte and nuclear diameter, appearance of nucleus and degree of yolk accumulation (Figures 2-6) (Table 1).

Oogonia

Small, oval germ cells (7.0 to 14.0 µm in diameter; 11.10±2.35), observable as cell nests in the

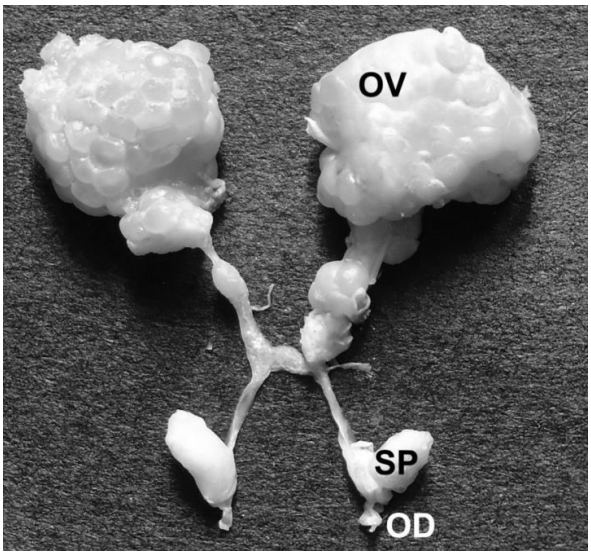

Figure 1. Female reproductive system of adult *Travancoriana schirnerae*. OV: Ovary; OD: Oviduct; SP: Spermatheca.

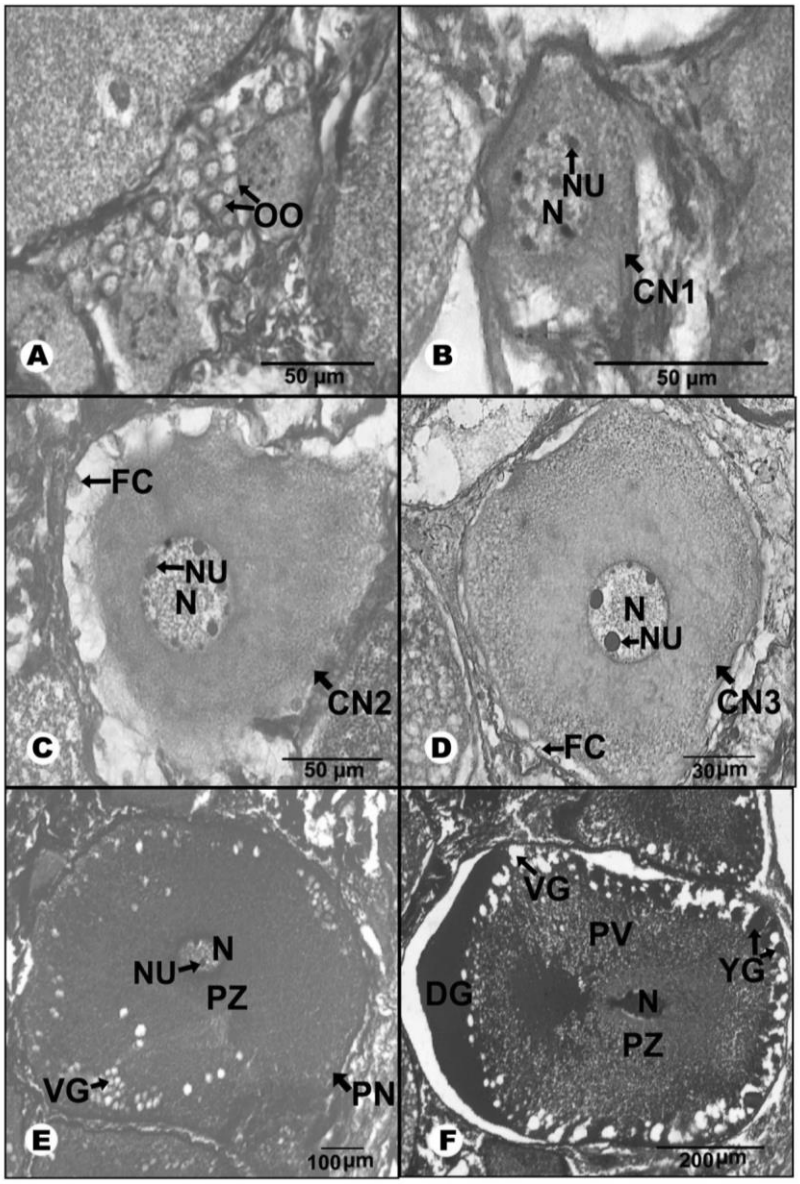

Figure 2. Germ cells at different developmental stages in the ovary of *T. schirnerae*.
(A) Oogonia, (B, C and D) Chromatin nucleolus stages 1, 2 and 3, (E) Perinuclear stage, (F) Primary vitellogenic stage. N: Nucleus; NU: Nucleolus; FC: Follicle cell; PZ: Perinuclear zone; VG: Vacuolated globule; YG: Yolk globule; DG: Dispersed yolk globule; OO: Oogonia; CN1: Chromatin nucleolus stage 1; CN2: Chromatin nucleolus stage 2; CN3: Chromatin nucleolus stage 3; PN: Perinuclear stage; PV: Primary vitellogenic stage.

central germinal zone of the ovary. Their nuclei appeared large (5.0 to 12.0 µm in diameter; 9.5±1.88) with condensed granular chromatin and extremely narrow cytoplasm (Figure 2A). These cells have a very high nucleus/cytoplasmic ratio (NPR) (0.70 to 0.90; 0.82±0.05).

Chromatin Nucleolus Stage 1

Oocytes of this stage lie close to the oogonia, appeared oval in shape and measured 25 to 60 µm (43.60±14.43) in diameter. A round or oval nucleus (15 to 30 µm; 23.70±5.25) was seen to lie centrally within the oocyte. Their chromatin appeared granular and 3 to 10 basophilic nucleoli (1.0 to 4.0 µm in diameter; 2.55±0.81) situated centrally or peripherally inside the nucleus (Figure 2B). The cytoplasm was moderately basophilic and showed mild reaction to PAS, MBB, Azan and Mallory triple (Figures 4A-4D). The oolemma was not well defined and follicle cells were not seen surrounding these oocytes. There was a slight reduction in the NPR than the previous stage (0.5 to 0.62; 0.55±0.04).

Chromatin Nucleolus Stage 2

These oocytes measured 65 to 195 µm (141.8±36.07) in diameter; bound by a well defined oolemma. Their large nuclei (25 to 50 µm; 40.8±9.78), often multinucleolated with 2 to 9

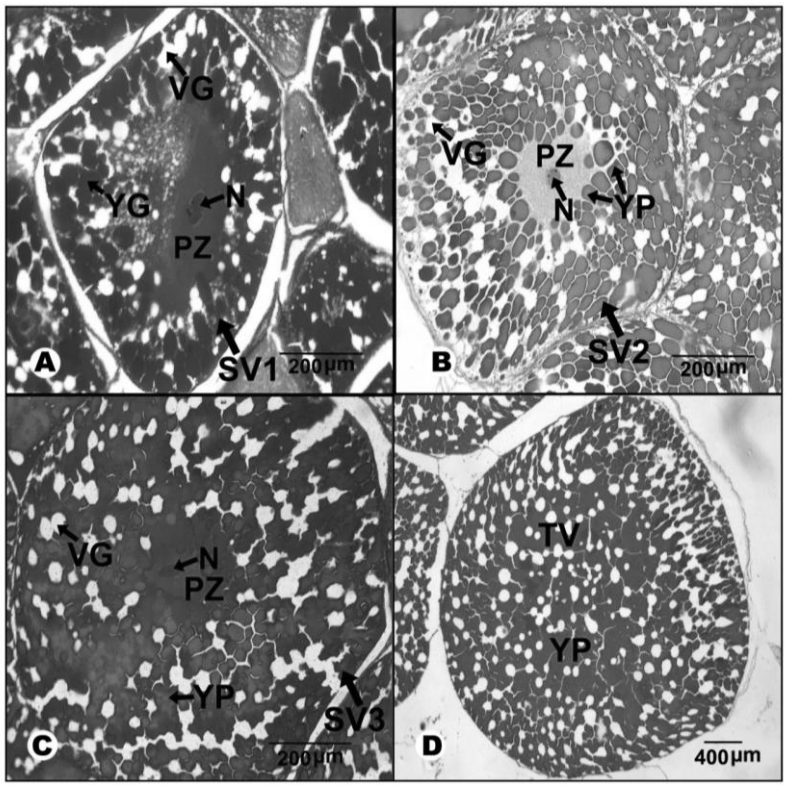

Figure 3. Germ cells at different developmental stages in the ovary of *T. schirnerae*.
(A, B and C) Secondary vitellogenic stages 1, 2 and 3, (D) Tertiary vitellogenic stage. N: Nucleus; PZ: Perinuclear zone; VG: Vacuolated globule; YG: Yolk globule; YP: Yolk platelet; SV1: Secondary vitellogenic stage 1; SV2: Secondary vitellogenic stage 2; SV3: Secondary vitellogenic stage 3; TV: Tertiary vitellogenic stage.

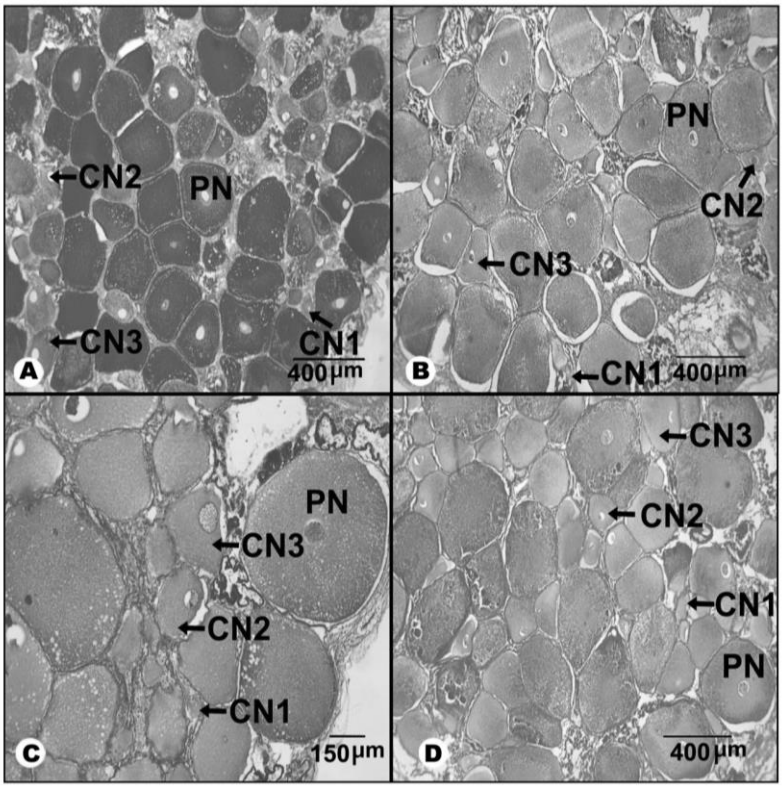

Figure 4. Histochemical analysis of previtellogenic phase ovary.
(A and B) Previtellogenic oocytes positive for PAS and MBB staining, (C and D) Previtellogenic stage oocytes stained with Azan and Mallory triple, respectively. CN1: Chromatin nucleolus stage 1; CN2: Chromatin nucleolus stage 2; CN3: Chromatin nucleolus stage 3; PN: Perinuclear stage.

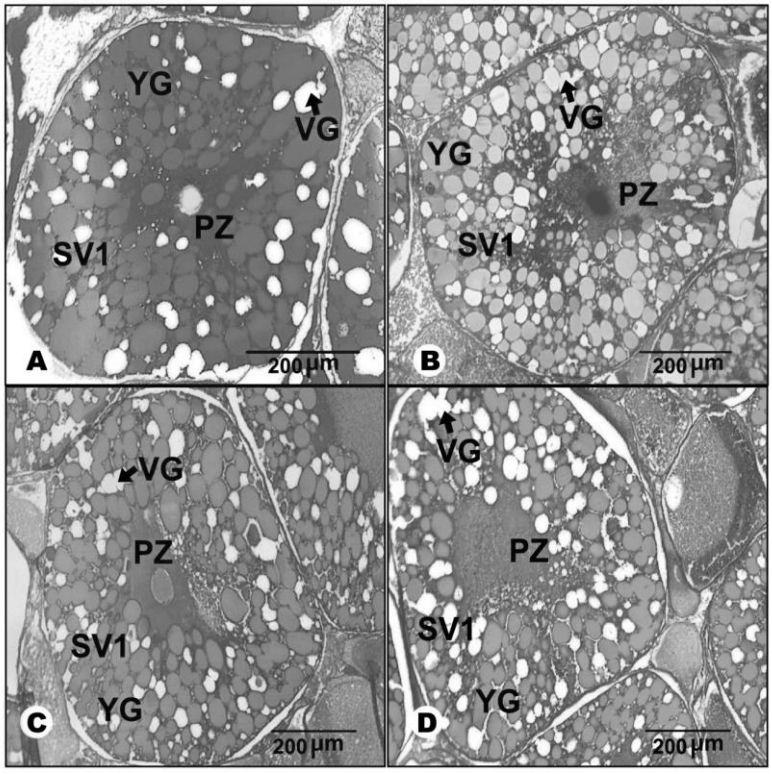

Figure 5. Histochemical staining of secondary vitellogenic stage 1 oocyte.
(A) Yolk globules staining intensely with PAS, (B) Perinuclear zone and yolk globules showing intense staining reaction to MBB, (C) Perinuclear zone moderately stained with azocarmine and yolk globules bright yellow with Azan, (D) Perinuclear zone exhibiting moderate reaction to aniline blue; yolk globules bright yellow with Mallory triple. PZ: Perinuclear zone; VG: Vacuolated globule; YG: Yolk globule; SV1: Secondary vitellogenic stage 1.

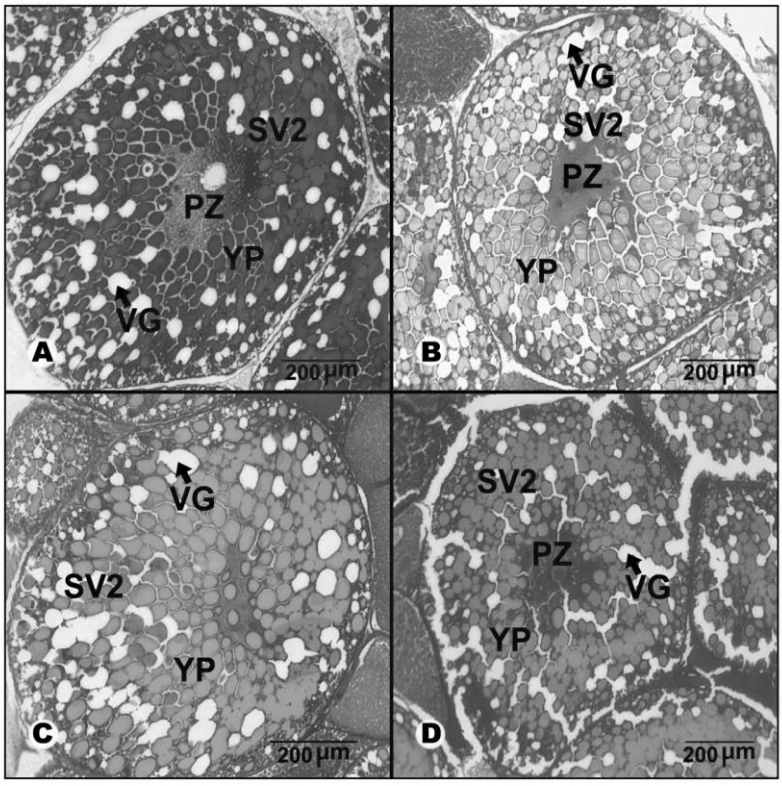

Figure 6. Histochemistry of ovary in secondary vitellogenic stage 2.
(A) Yolk platelets strongly positive for PAS, (B) Yolk platelets stained moderately with MBB, (C and D) Yolk platelets appeared orange yellow with Azan and Mallory triple, respectively. PZ: Perinuclear zone; VG: Vacuolated globule; YP: Yolk platelet; SV2: Secondary vitellogenic stage 2.

Table 1. Distribution of oocytes in different phases of the ovarian development in *Travancoriana schirnerae*

Phases of ovarian development	OO	CN	PN	PV	SV	TV	AO	SF
Proliferation phase	58	16	8				3	15
Previtellogenic phase	9	53	30				2	6
Primary vitellogenic phase	6	10	20	60			1	3
Secondary vitellogenic phase		10	10		80			
Tertiary vitellogenic phase	2	3				95		
Oosorption phase							10	90

OO: Oogonia; CN1: Chromatin nucleolus stage; PN: Perinuclear stage; PV: Primary vitellogenic stage; SV: Secondary vitellogenic stage; TV: Tertiary vitellogenic stage; AO: Atretic oocyte; SF: Shrunken follicle

nucleoli (1.8 to 10.0 µm; 6.34±2.61) which were peripherally placed (Figure 2C). The ooplasm stained moderately basophilic; displayed moderate reaction to PAS, MBB, azocarmine and acid fuchsin (Figures. 4A-4D). Very few basophilic follicle cells (2.4 to 6.0 µm in diameter; 3.6±1.07) were visible on the outer surface of these oocytes. The NPR found largely decreased (0.26 to 0.35; 0.31±0.02).

Chromatin Nucleolus Stage 3

These oocytes ranged in size from 215 to 270 µm (235.2±14.7) in diameter. Their nuclei further increased in size and measured 38 to 60 µm (47.8±7.53) in diameter with 3 to 4 circular peripherally positioned nucleoli (Figure 2D). The ooplasm was characterized by moderate reaction to PAS, MBB, azocarmine and acid fuchsin (Figures 4A-4D). The NPR further declined to 0.17 to 0.22 (0.20±0.01) compared to the previous stage.

Perinuclear Stage

This stage (280 to 465 µm; 366.3±51.57) was characterized by a perinuclear zone (25 to 73 µm wide; 46.73±16.67) and vacuolated globules (4.8 to 19.2 in diameter; 11.27±4.32) in the cortical region. There occurred a considerable increase in the oocyte and nuclear volume during this stage. The ooplasm became strongly basophilic and the highly basophilic nucleus (47 to 67 µm; 56.9±5.63) encompassed 2 to 3 large nucleoli (3.6 to 12.9 µm; 7.6±2.74) (Figure 2E). Follicle cells increased in number and surrounded the oocyte completely. The NPR showed a further reduction (0.12 to 0.16; 0.14±0.01). The peripheral ooplasmic area intensely stained for PAS and moderately stained with aniline blue. The perinuclear zone showed moderate staining reaction to PAS and acid fuchsin. The entire ooplasm appeared moderately stained with MBB and azocarmine (Figures 4A-4D).

Primary Vitellogenic Stage

The actual growth of the oocyte (480 to 530 µm; 501.3±14.7) begins from this stage. The commencement of vitellogenesis was characterized by the formation of small basophilic yolk globules (6.5 to 32 µm in diameter; 19.17±8.15) immediately beneath the oocyte membrane. The nuclei reached maximum size (56 to 72 µm; 63.0±7.11); nucleoli exhibited a further increase in diameter (8.0 to14 µm; 10.01±2.25) (Figure 2F). The NPR of these cells was found extremely low (0.11 to 0.13; 0.12±0.01). The entire ooplasm was more basophilic than the previous stage and strongly positive to PAS and MBB staining. Numerous, large (13 to 27.0 µm; 17.4±3.23) vacuolated globules were detected in the cortical region. The perinuclear zone becomes thicker (95 to 137 µm; 111.0±16.4) and stains strongly with PAS. In certain oocytes, the yolk globules dispersed to form a dense layer (14 to 26µm thick; 19.17±8.15) which is strongly positive to PAS and MBB, possibly suggesting the presence of polysaccharide and protein components of yolk. Follicle cells exhibited ovoid nuclei (4.7 to 7.6 µm in diameter; 6.5±1.37) and formed a complete epithelium (6.5 to 8.7 µm thick; 7.3±1.03) around the oocytes. The periplasm appeared strongly reactive to aniline blue while the central ooplasm gave an intense response to azocarmine and a mild response to acid fuchsin. The yolk globules stained light yellow with Azan and Mallory triple.

Secondary Vitellogenic Stage 1

The early secondary vitellogenic stage oocytes (550 to 680 µm; 603.3±33.2) portrayed dense, highly basophilic yolk globules in the peripheral ooplasm. Their nuclei (38 to 49 µm; 41.5±3.10) demonstrated a reduction in the number (1 to 2) and size of nucleoli (6 to11µm; 7.5±1.68) (Figure 3A). There was a significant decrease in the NPR (0.06 to 0.08; 0.07±0.01). The yolk globules (30 to 53 µm; 41.12±8.70) and vacuolated globules (26 to 49 µm; 35.6±9.64) increased in number and diameter, deposited in the cortical cytoplasm and seen extending towards the perinuclear region. Some of the yolk globules tend to fuse and organize as larger inclusions in the peripheral ooplasm. The perinuclear zone (85 to127 µm; 103.3±13.65) reflected a strong staining reaction to hematoxylin, PAS and MBB and presented moderate reaction to acid fuchsin, azocarmine and aniline blue. Follicle epithelium associated with the oocytes revealed round or elongate nuclei (4.2 to 6.8 µm; 5.05±0.83) with multiple nucleoli. The yolk globules stained intensely with PAS and MBB, bright yellow with Azan and

Mallory triple and moderately with Sudan black B, indicating the lipoglycoproteinaceous nature of the yolk (Figure 5A-5D).

Secondary Vitellogenic Stage 2

Oocytes of this stage (700 to 950 µm; 793.3±72.97) were characterized by the formation of mildly basophilic polygonal yolk platelets in the ooplasm. These yolk platelets (35 to 57 µm in width; 51.2±7.69) and vacuolated globules (32 to 54 µm; 41.2±6.37) were abundant and organized in the entire ooplasm except the perinuclear zone (Figure 3B). The yolk platelets were found strongly positive to PAS, signifying the presence of rich quantities of polysaccharides in them. These platelets were moderately reactive to MBB, stained orange yellow with Azan and Mallory triple, demonstrating the presence of basophilic and acidophilic components of yolk. The yolk platelets stained deep blue with Sudan black B. The nucleus reduced in size (35 to 45 µm; 37.7±4.85), appeared poorly basophilic and the NPR reached its minimal level (0.04). The perinuclear zone became thin (57 to 86 µm; 69.6±10.59), weakly basophilic and remained moderately stained with azocarmine and aniline blue (Figures 6A-6D). Follicle cells revealed elongate nuclei (3 to 5 µm; 3.75±0.84) and follicle epithelium found reduced (4 to 7 µm) in thickness.

Secondary Vitellogenic Stage 3

By this stage, notable increase in size of the oocytes (1000 to 1250 µm; 1160.3±66.19) takes place as a result of elaboration of well-developed eosinophilic yolk platelets (Figure 3C). The nucleus becomes condensed, shrinks rapidly to a diameter of 30 to 38 µm (32.6±4.61); its contours appeared indistinct with degeneration of nucleoli. As a result, the nucleus is less visible at this stage. The ooplasm loses its basophilia and appeared eosinophilic. The oolemma developed numerous evaginations and large numbers of micropinocytotic vesicles were observed both inside and outside the margin of the oolemma. These vesicles seemed to have an extraovarian origin which probably indicates the uptake and deposition of yolk during secondary vitellogenesis. The yolk platelets stained mildly with MBB, moderately with PAS and showed strong positive reaction to Sudan black B. The yolk platelets turned deep orange and bright yellow with Azan and Mallory triple, respectively. A narrow acidophilic perinuclear zone (46 to 50µm; 47.8±2.75) was still appreciable around the nucleus. Follicle epithelium is still observed around the oocytes; their nuclei (1.9 to 2.9 µm; 2.37±0.39) appeared fully condensed and streak-like.

Tertiary Vitellogenic Stage

Oocytes (1280 to 1500 µm; 1408±81.22) of this stage had completed vitellogenesis. Their nuclei were no longer visible and the eosinophilic yolk platelets fused to form a homogeneous matrix dispersed throughout the ooplasm (Figure 3D). Follicle epithelium is no more visible around the oocytes. The ooplasm appeared deep black with Sudan black B indicating the high lipid content of yolk.

Phases of Oogenesis

Based on the morphology and histology of the ovary, oogenesis in *T. schirnerae* can be divided into six phases: proliferation, previtellogenic, primary vitellogenic, secondary vitellogenic, tertiary vitellogenic and oosorption (Figures 7A-7F).

Proliferation Phase

April to May was considered as the proliferation phase of the ovary. Macroscopically, the ovary was small, white and transparent and the GSI was found generally low (0.207±0.01) (Figure 8). The ovarian epithelium was well developed (162 µm) and the ovary was dominated by oogonia (58%), organized as clusters in the germinal zone. The ovary exhibited chromatin nucleolus stage oocytes (16%) very close to the oogonia and perinuclear stage oocytes (8%) were seen distributed in the peripheral zone. Shrunken follicles (15%), pycnotic follicle nuclei and atretic oocytes (3%) were found scattered inside the ovary (Table 1) (Figure 7A).

Previtellogenic Phase

This phase extended from June to September. The ovary displayed a cream colour and the GSI showed a slight increase (0.319±0.04) compared to the previous phase. In previtellogenic phase, the ovary was configured with two main developmental stages - perinuclear stage oocytes (30%) and chromatin nucleolus stage (2 and 3) oocytes (51%). The germinal zone was occupied by oogonia (9%) and chromatin nucleolus stage 1 oocytes (0.2%). The perinuclear stage oocytes were positioned towards the periphery, adjacent to the ovarian wall. Shrunken follicles (6.%), pycnotic follicle nuclei and atretic oocytes (2%) found reduced in number (Table 1) (Figure 7B).

Primary Vitellogenic Phase

The ovary reached this phase in October. The ovary was light yellowish in hue and the GSI increased to 0.489±0.04. This phase was characterized by the predominance of primary vitellogenic stage oocytes (60%) and a few perinuclear stage oocytes (20%). The germinal zone was observed with a small percentage of oogonia (6%) and chromatin nucleolus stage oocytes (10%). Atretic oocytes (1%) and shrunken follicles (3%) were still apparent in the

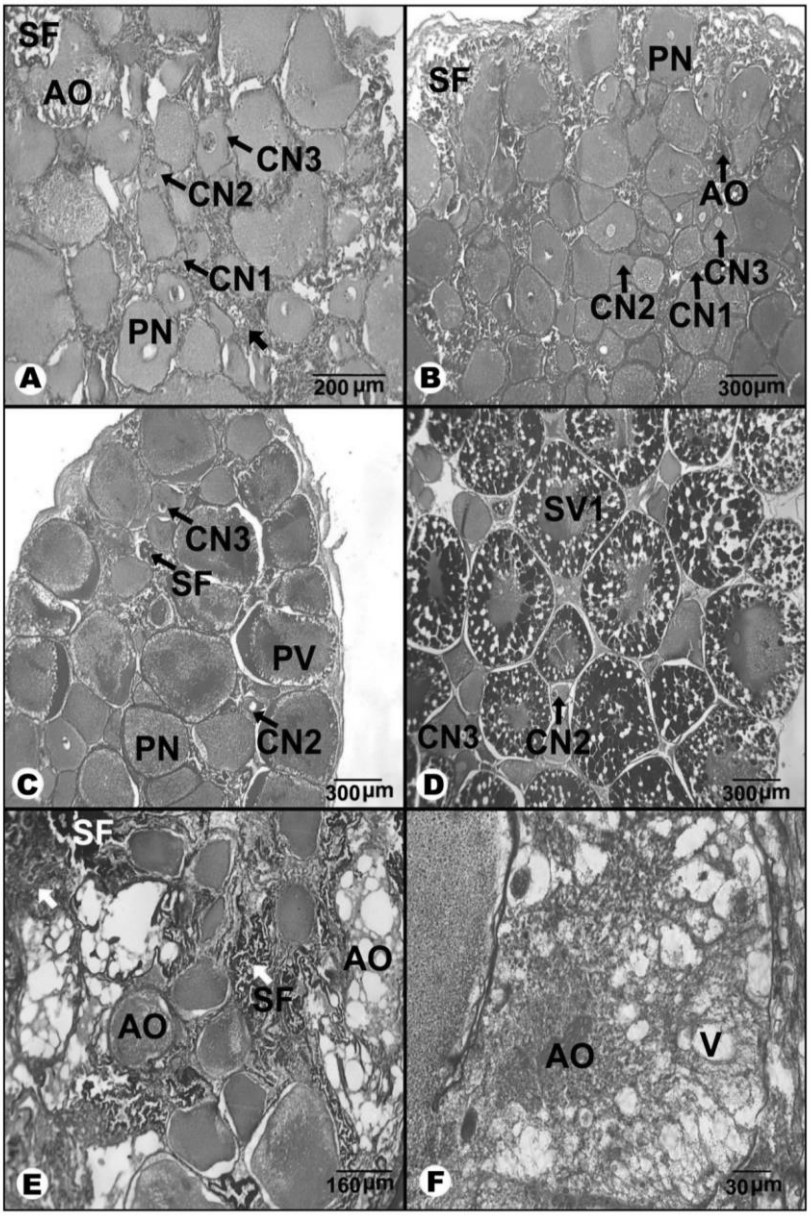

Figure 7. *Travancoriana schirnerae*: Phases of ovarian development.
(A) Proliferation phase (April to May), (B) Previtellogenic phase ovary in June to September, (C) Primary vitellogenic phase in October, (D) Ovary in secondary vitellogenic phase during November to January, (E and F) Ovary in oosorption phase showing atretic oocyte in April (spawning season). CN1: Chromatin nucleolus stage 1; CN2: Chromatin nucleolus stage 2; CN3: Chromatin nucleolus stage 3; PN: Perinuclear stage; PV: Primary vitellogenic stage; SV1: Secondary vitellogenic stage 1; AO: Atretic oocyte; SF: Shrunken follicle; V: Vacuole; Arrow indicates germarium in figure A and follicle nuclei in figure E.

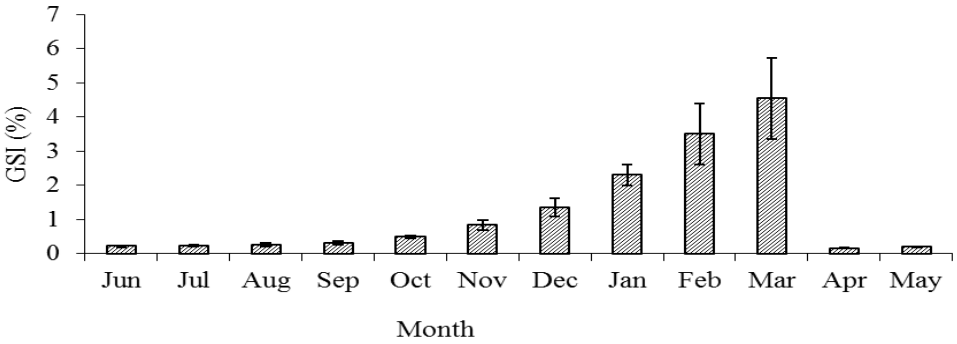

Figure 8. Monthly distribution of gonadosomatic index from June 2009-May 2011 in *Travancoriana schirnerae.*

ovary (Table 1) (Figure 7C).

Secondary Vitellogenic Phase

The ovary was in the secondary vitellogenic phase from November to January. During this phase, the ovary was considerably large, bright yellow with an appreciable increase in the GSI (2.296±0.30). Histological sections showed a prominence of secondary vitellogenic stage oocytes (80%) which occupied a considerable portion of the ovary; a few previtellogenic oocytes (20%) were found interspersed among the vitellogenic oocytes (Table 1). The size of the oocytes (secondary vitellogenic stage 1 and 2) ranged from 550 to 950 µm during November to December and 1000 to 1250 µm (secondary vitellogenic stage 3) in January (Figure 7D).

Tertiary Vitellogenic Phase

This phase extended from February to March. The ovary at this stage featured an orange yellow coloration. The GSI increased further, reached its peak in March (4.542±1.18). Histologically, the ovary was filled with tertiary vitellogenic oocytes (95%) and a few previtellogenic oocytes (5%) (Table 1).

Oosorption Phase

This phase occurred in the month of April which is considered as the spawning season. After spawning, the ovary showed drastic reduction in size, became flaccid, translucent and dirty white, making them difficult to distinguish from the proliferating ovary. The GSI found suddenly decreased (0.160±0.01). Empty spaces were observed in the ovarian stroma, along with considerable amount of atretic vitellogenic and previtellogenic oocytes. Atretic oocytes (10%) showed folding or collapsing of oocyte membrane with shrunken, vacuolated and degenerating ooplasm. The shrunken follicles (90%) appeared highly compressed or as loose sac like structures (Table 1). Large number of hemocytes was noticed in the ovarian stroma. Numerous pycnotic follicle nuclei were seen scattered in the ovary. The ovarian epithelium assumed a distinctly wavy and shrunken appearance (Figures 7E-7F).

Discussion

The present study elucidated the sequential oocyte developmental stages and female reproductive cycle of the freshwater crab *T. schirnerae*. In *T. schirnerae*, the germ cells were categorized in to ten developmental stages based on oocyte and nuclear diameter, appearance of nucleus and degree of yolk accumulation. A similar characterization was adopted by Minagawa *et al*. (1993) for *R. ranina*. Otsu (1963) proposed four stages for oocyte development in the freshwater crab *Potamon dehaani* based on changes in the cytoplasmic granulation of oocytes. In *Uca rapax* and in *G. cruentata*, the classification of germ cells was based on degree of vitellogenesis (Castiglioni *et al*., 2007; Desouza and Silva, 2009). In decapods, the germ cells are classified according to a range of criteria such as cell diameter, nuclear appearance (Mota and Tomé, 1965) and degree of vitellogenesis (Kulkarni *et al*., 1991).

In *T. schirnerae*, the germinal zone existed in the centre of the ovary, where oogonia and cords of small previtellogenic oocytes were situated. The central position of the germinative zone was also reported in other brachyurans such as *Libinia emarginata* (Hinsch and Cone, 1969) and *R. ranina* (Minagawa *et al*., 1993). Generally in brachyurans, the germinal zone existed longitudinally in the centre of the ovary, where oogonia proliferate and previtellogenic oocytes grow near the ovarian parenchyma (Kon and Honma, 1970; Chiba and Honma, 1972). In decapods, the location of the germinal zone varies considerably among species (Adiyodi and Subramoniam, 1983). In the land crab *Gecarcinus lateralis* (Weitzman, 1966), the ovarian capsule may constitute the germinal epithelium whereas in the prawn *Macrobrachium birmanicum choprai*, the germinative zone appeared in the ventrolateral region of the ovary (Singh and Roy, 1994).

Our observations on the strong basophilia associated with the perinuclear stage oocytes are supported by the ultrastructural observations in the crayfish *Cambarus virilis* (Beams and Kessel, 1962) and the brown crab *Cancer pagurus* (Eurenius, 1973) that the high level of basophilia observed in the previtellogenic oocytes are due to enrichment of the cytoplasm with ribosomes attached to the rough endoplasmic reticulum. Kessel (1968) observed a great number of ribosomes in the early-stage germ cells of lobsters of the genera *Homarus* and *Panulirus*. According to Krol *et al*. (1992), decapod previtellogenesis is exemplified by increased activity of various cytoplasmic organelles.

The vacuolated globules noticed in the peripheral ooplasm of perinuclear stage may act as frames and moulds for accumulation of yolk during primary vitellogenesis. In *T. schirnerae*, the vacuolated globules were observed during the period between previtellogenic and late secondary vitellogenic stages. This observation is concurrent with the description of large vacuolated globules occurring in the ooplasm of the previtellogenic oocytes in *Scylla paramamosain* (Islam *et al*., 2010).

The production and accumulation of yolk is a crucial event during oocyte development and both intra and extraovarian yolk synthesis was reported in crustaceans (Fainzilber *et al*., 1992; Riley and Tsukimura, 1998). The deposition of yolk in the vacuolated globules is considered as the initiation of primary vitellogenesis in *T. schirnerae*. In crustaceans, primary vitellogenesis is characterized by

endogenous yolk protein accumulation and the mode of yolk deposition varied in different groups. In *R. ranina*, oil globules appeared near the nucleus during initial stages of primary vitellogenesis (Minagawa *et al*., 1993). In *G. lateralis*, the lipid droplets were distributed at the periphery of the developing oocytes immediately before the accumulation of yolk (Weitzman, 1966). In *P. japonicus*, numerous acidophilic oil globules were noted in the primary vitellogenic oocytes (Yano, 1988). Abdu *et al*. (2000) noticed numerous oil globules in the entire cytoplasm of primary vitellogenic oocytes of the red claw crayfish *Cherax quadricarinatus*.

In *T. schirnerae*, secondary vitellogenesis is a prolonged phase and the size of the oocytes found dramatically increased as a consequence of yolk deposition. As the oocytes proceeded through secondary vitellogenic stages, the highly basophilic yolk globules of secondary vitellogenic stage1 underwent a series of morphological changes to become mildly basophilic yolk platelets in the secondary vitellogenic stage 2 and as development proceeded, the oocytes became fully acidophilic with large yolk platelets in the secondary vitellogenic stage 3. This process coincides with the observations made by Ando and Makioka (1999) and Brown (2009) in *P. dehaani* and *C. sapidus* respectively.

As stated in numerous studies, in *T. schirnerae*, the organization of follicle cells around the oocytes was observable during all developmental stages of the ovary. The follicle cells were grouped irregularly around the previtellogenic oocytes, developed an epithelium and reached maximum size during primary vitellogenesis, remained active till the end of mid secondary vitellogenesis, appeared inactive at the late secondary vitellogenic stage and hardly perceptible in tertiary vitellogenic stage. The follicle cells are, therefore, implicated as the possible cell type responsible for heterosynthetic yolk deposition in *T. schirnerae*. The investment of follicle cells around the oocytes termed as folliculogenesis is a prerequisite for heterosynthetic yolk deposition in brachyurans (Islam *et al*., 2010) and other crustaceans (Yano, 1988).

The importance of heterosynthetic yolk production is a topic which has gained considerable attention (Anderson, 1974). The incidence of plasma membrane evaginations and micropinocytotic vesicles in the secondary vitellogenic stage 3 oocytes, probably indicate signs of extraovarian yolk deposition in *T. schirnerae*. In crustaceans, the morphological evidence for the uptake of extracellular yolk materials during secondary vitellogenesis has been presented for a number of species (Santana, 2002). Duronslet *et al*. (1975) demonstrated that in the platelet phase oocytes (yolk granule stage oocytes) of *P. aztecus* and *P. setiferus*, numerous micropinocytotic vesicles which originate from the plasma membrane move into the cytoplasm and fuse each other giving rise to yolk spheres. Extraovarian yolk deposition through endocytosis has been reported in lobsters and crayfishes (Jugan and Van-Herp, 1989).

There is definite variation in the histochemical composition of the oocytes in concomitance with the growth of the ovary. The yolk globules in primary vitellogenic oocytes appeared strongly positive for PAS and MBB, indicating the glycoproteinaceous nature of yolk. In the secondary and tertiary vitellogenic oocytes, yolk platelets showed positive reaction for MBB, PAS and Sudan black B, demonstrating the lipoglycoproteinaceious nature of the yolk. Generally, in crustaceans, the primary yolk is considered to be glycoproteinaceous in nature (Charniaux-Cotton, 1985). In *G. cruentata*, the vitelline vesicles reacted intensely to bromophenol blue and PAS which showed the increasing concentration of proteins and glycoproteins within the oocytes (Desouza and Silva, 2009). In the intertidal crab *Menippe rumphii*, rich quantities of carbohydrates, proteins and lipids were observed in stage IV vitellogenic oocytes (Shyamasundari and Babu, 1984). On the contrary, in *R. ranina*, the oil globules and basophilic granules appeared in the primary vitellogenic oocytes found positive for Sudan black B and PAS respectively, signifying the glycolipid nature of yolk (Minagawa *et al*., 1993).

From the present investigation, it was corroborated that the animal followed an annual pattern for its reproductive activity and accommodated only one vitellogenic cycle in the intermoult period. In *T. schirnerae*, vitellogenesis extended from October to March and spawning occurred in April. A comparable situation has been recorded in the Calicut population of the freshwater crab *Paratelphusa hydrodromous* (Anilkumar, 1980). In *P. koolooense*, the development of ovary reached its maximum in the month of April (Joshi and Khanna, 1982). Conversely, the Trivandrum population of *P. hydrodromous* produced two broods annually, the first during March to April and the second during June to August (Anilkumar, 1980). In marine forms like *R. ranina* and *P. crassipes*, at least two broods have been noticed during the spawning season (Chiba and Honma, 1972; Minagawa *et al*., 1993).

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