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Histological Authentication of Reproductive Structures of Little Indian Squid *Loliolus (Loliolus) hardwickei*, Grey, 1849 (Cephalopoda: loliginidae)

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Abstract

The work deals with the oogenesis, spermatogenesis and associated reproductive structures of the little Indian squid *Loliolus (Loliolus) hardwickei*. Histological descriptions of the ovary, oviduct, testis and needham sac were made, the various changes observed during maturation were described and the functional significance was discussed. The female reproductive system consists of the ovary, glandular oviduct, lace-like oviducal funnel, paired nidamental gland, accessory nidamental glands and a seminal receptacle for sperm storage on the ventral side of the buccal cavity. The average size of the cells of the ovary varied from 7.635±3.466 µm (primary oogonia) to 1191.114±288.188µm (Ripe oocyte). Oocyte resorption or the presence of atretic oocytes was observed in some sections. The male reproductive system consists of the testis, vas deferens, spermatophoric organ, spermduct, the system of spermatophoric glands (SG) and spermatophoric sac (Needham sac) and penis. The spermatogenesis passed through the differentiation of primodial germ cells, primary and secondary spermatogonia, primary and secondary spermatocytes, rounded spermatids, and elongated spermatids to spermatozoa or sperms. The testis is connected to a thin-walled Needham sac by the spermatophoric duct. The testis is made up of numerous seminiferous tubules and is enveloped by tunica albuginea made up of connective tissue. Histological authentication of reproductive structures in *L(L) hardwickei* serves the specific purpose of providing a detailed understanding of the species' reproductive biology, which has implications for ecological, environmental, and conservation management efforts.

Introduction

The little Indian squid *Loliolus (Loliolus) hardwickei* Grey, 1849 is a tropical species present in the Indian Ocean and is distributed along the Northern Persian Gulf, along the coasts of India and Burma (Myanmar), and also throughout Indonesia (Jereb *et al.,* 2010). The histology of reproductive structures of *L. (L.) harwickei* is not studied so far, the works available are mostly related to the morphological aspects. Jereb *et al*. (2010) documented the occurrence of these species from the northern part of the Arabian Sea (Gujarat coast) and the east coast of India. Silas *et al.* (1986) identified this species as *Loliolus investigatoris* from Indian Coast. Lu *et al.* (1985), described and revised the morphological features of *L. (L.) harwickei* from Australian waters. Norman and Lu (2000) and Norman *et al.* (2016) mentioned this species in the checklist of the cephalopods of the South China Sea. Sajikumar *et al.* (2015) analyzed the intercohort growth rates of this species based on statolith analysis from the southwest coast of India. Neethu *et al.* (2018) studied the morphological and anatomical features of *L. (L.) hardwickei* in detail, using specimens caught from the Vizhinjam Coast. There is hardly any work available on the histology of reproductive structures of *L. (L.) harwickei.*

In most cephalopods, the male can be distinguished by the presence of a hectocotylus arm and by the white testis conspicuously visible through the mantle in the region between the fins, the females can be identified by the presence of ovary and nidamental glands present in the mantle cavity (Arnold and Arnold, 1977). However, the description of the microscopic maturity stages of the reproductive structures is laid chiefly on histological analytical studies. As cephalopods are strict gonochorists (dioecious), the females are found to be larger than males and vice versa. But the external sexual dimorphism becomes distinct only in sub-adult stages (Boletzky, 1989) and the gonads and accessory reproductive organs differentiate at different times in early development. Microscopic analysis of reproductive tissues enables the understanding of various cytological changes inside the testis, ovary and oviduct during the maturation process.

Peterson (1959) studied the anatomy and histology of the reproductive system of *Octopus bimaculoides.* Cowden (1968) studied the development of the oocyte of *Lolliguncula brevis* and classified it into ten developmental stages. Subsequently, numerous studies were made on oogenesis and spermatogenesis in other cephalopods, among them *Sepia* (Richard and Dhainaut, 1973), *Alloteuthis* (Bottke, 1974), and *Loligo pealii* (Arnold and Arnold, 1976; Takahashi and Yahata, 1973) merits much importance. Lum-kong (1992) described the histological and morphological aspects of the female accessory reproductive structure of *Loligo forbesi*. Other important studies on cephalopod species include the works of Wells (1960), Froesch and Marthy (1975) in *Octopus vulgaris* and Williams (1909) in *Loligo pealei*.

The female reproductive system of cephalopods consists of the ovary, accessory reproductive organs, and ducts. The accessory reproductive organs include the nidamental gland, the accessory nidamental gland, the oviducal gland and the seminal receptacle (Lum-kong, 1992: Arnold and Arnold, 1976). The male reproductive system consists of the testis, vas deferens, spermatophoric organ, spermduct, a system of spermatophoric glands (SG), Spermatophoric sac (Needham sac) and penis (Arnold and Arnold, 1976). The present study provides information on the histology and morphology of male and female reproductive structures, including gametogenesis in *L. (L.) hardwickei,* and also elucidates various cytological changes during the maturation process inside the ovary and testis. Attention was also given to elucidating different changes in different stages of oocyte and testis development.

Materials and Methods

Specimens of *L. (L.) hardwickei* (Figure 1) were collected from Vizhinjam Coast (8° 22 ’42.54” N Latitude and 76° 59’14.20” E longitude) along the South West Coast of India (Figure 2) from the fish vendors and also from the local fishermen operating boat seines during the night (light fishing). The catch was made at a distance of 3 to 10 km away from the shore within 20-30 m depth, using fibre glass coated plywood boats (18 feet long) fitted with an outboard engine. The mesh size of the gear ranged from 0.6 to 15 mm. The samples were incised longitudinally along the mid-ventral mantle to expose the internal structures. The reproductive tissues

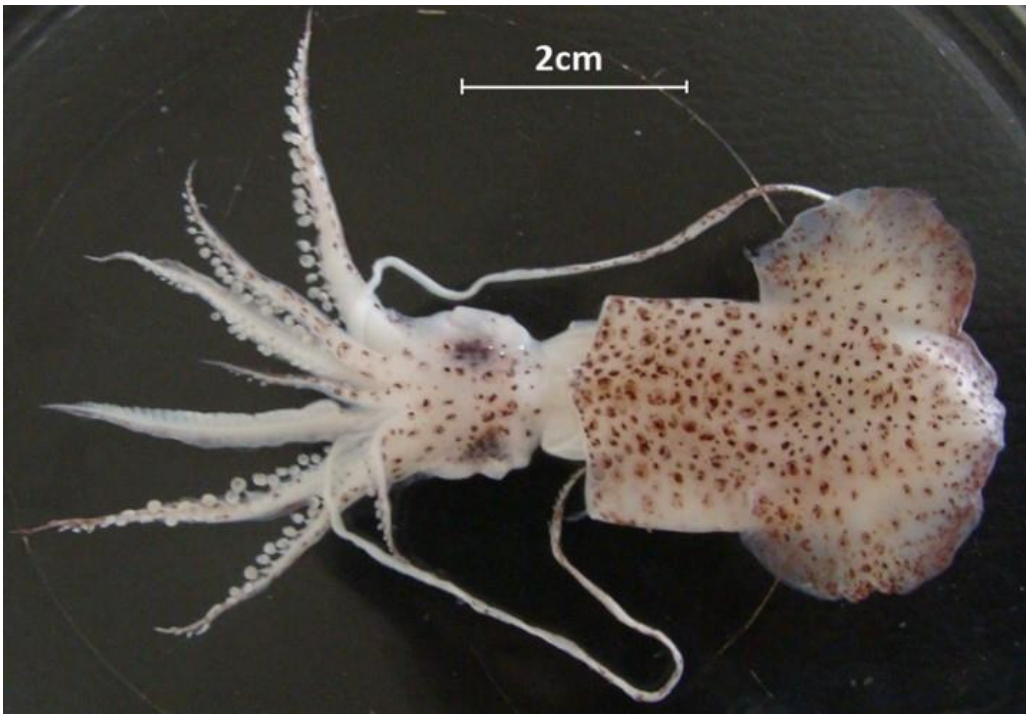


Figure 1. *Loliolus (Loliolus) hardwickei*

Figure 2. Sampling site

such as the testis, ovary, needham sac, spermatophoric duct and oviducts of specimens were dissected out and cut into convenient sizes. Tissue processing, paraffin embedding, sectioning, clearing, staining, and mounting were performed using standard histological techniques (Kiernan, 2008: Kerr, 2009). Tissues were fixed in 10% neutral buffered formalin for one week and then washed overnight in running tap water to remove the fixative. Later tissue samples were passed through grades of alcohol for dehydration and then through chloroform until they got completely immersed. The tissues were allowed to pass through 3 changes of paraffin for 3 hours and were then permanently embedded in paraffin blocks. The paraffin blocks carrying the tissues were sectioned precisely at 6µ thickness using a microtome (RADICAL Senior rotatory microtome, model: RMT-30 and Leica RM2235). After sectioning, tissue ribbons were carefully adhered over the glass slides smeared with egg albumen- glycerol - thymol mixture using a slide warming table. Slides were kept for 2-3 days to dry. The dried slides were stained in Harris-haematoxylin and counterstained with eosin. Before staining, the tissue slides were deparaffinized in 2 changes of xylene for 5 minutes and rehydrated in 2 changes of absolute alcohol for 5 minutes. Then the slides were kept in 2 changes of 95% alcohol and 70% alcohol for 4 minutes. After washing in distilled water, tissue slides were stained in Harris haematoxylin stain for 10-15 minutes. Then the slides were rinsed in tap water and distilled water and were immersed in 1% acid alcohol for 30 seconds until the tissues became pink. After washing, tissue slides were immersed in 0.2% ammonia water for 30 seconds to 1 minute for bluing. Slides were washed with tap water and distilled water for 10 minutes and counterstained with Eosin for 30 seconds to 1 minute. Then slides were subjected to 2 changes of 95% alcohol and absolute alcohol for 5 minutes each. After clearing in xylene for 10 minutes, tissue slides were mounted in DPX. Permanently mounted tissue slides were observed and photographed in the compound microscope (Leica DMLS) and stereo zoom microscope (Leica S8APO) equipped with the DFC295 camera. Sex and maturity stages (Stage I – Stage V) were assigned by direct observation, based on the type of cells present, their proportions, an abundance of connective tissue and the proportion of spermatozoa present in the sections. For assigning the maturity stages the universal maturity scales adopted for squids by Lipinsky (1979), Arkhipkin (1992) and Lipinsky and Underhill (1995) were used and also evaluated and compared with the works of Cowden (1968), Arnold and Arnold (1977) and Lopez-Peraza *et al.* (2013). Phases of

oocyte development were studied as suggested by Laptikhovsky & Arkhipkin (2001), Hoving *et al.* (2013) and Chen *et al.* (2018). The gonadosomatic index (GSI), spermatophoric complex index (SCI) and nidamental gland index (NGI) were also calculated and presented.

Results

Reproductive System

The reproductive system of both sexes of *L. (L.) hardwickei* lies along the dorsal inner wall of the mantle cavity and the reproductive structures are held in position by mesenteries.

Female Reproductive System

The female reproductive system consists of an ovary, oviducal gland, lace-like oviduct, paired nidamental gland and accessory nidamental glands. In addition to this, a seminal receptacle meant for sperm storage is present in females on the ventral side of the buccal cavity.

Differentiation of ovum (egg) - Oogenesis

1. Primary oogonia: The *primodial germ cells* differentiated to form the primary oogonia, found mostly in the ovarian sections of squids in reproductive stages I and II. The average diameter of the primary oogonia was 7.635±3.466 µm (Table 1). The nucleus (germinal vesicle) occupies almost all the area of the oocyte. Nucleoli were irregularly placed inside the nucleus. A prominent cytoplasm was lacking. At this stage most of the cells were rounded or ovoid, some were elongated and some were irregular in shape (Figure 3a). Follicle cells were lacking on the surface of oogonia. The oogonia were seen attached to the germinal epithelium.

***2. Secondary oogonia*:** Oogonia were small, ovoid, irregular or elongated cells (Figure 3a-c). The average diameter of the oogonia was 28.361±5.768 µm (Table 1). The nucleus was visible and its diameter was 19.463±2.907 µm. The nucleolus was irregular in shape. The cytoplasmic area was more prominent than in the previous stage. Follicle cells were absent. Secondary oogonia dominated in the ovary sections of squids in reproductive stages I and II. At the end of this stage, the oogonia began its transformation into the oocyte.

***3. Primary oocyte*:** The average diameter of the primary oocyte was 71.127±22.173µm (Table 1). Follicle cells were absent in most of the oocytes, if present they were in negligible numbers and were very thin and flattened (Figure 3a-c). The nucleus was prominent and its diameter was 37.869±11.237µm. Nucleolus could be noticed in most of the oocytes and its diameter was 24.601±9.205 µm.

4. Secondary oocyte: Oocytes with distinct cytoplasm and an enlarged nucleus (Figure 3a-d & f). Most of the oocytes were ovoid. The diameter of the oocyte increased to 129.486±30.886 µm. The diameter of the nucleus was 55.544±16.053 µm. The nucleolus was conspicuous and its size was 30.780±10.345 µm (Table 1). Follicle cells (primary follicular cells) were found multiplying (initial folliculogenesis) on the surface of the oocyte. Follicle cells were very thin and flattened (squamous).

5. Early previtellogenic oocyte (EPVO): The average diameter of the oocyte at the beginning of the yolkless stage was measured as 181.481±35.343 µm. The nucleus was observed in most sections, though in some sections nucleus was displaced. The average size of the nucleus was 62.994±17.022 µm. Nucleolus could be differentiated. The average size of nucleoli was 33.851±8.412 µm (Table 1). Follicular syncytium around the oocyte was fully accomplished and the follicular epithelium began to invaginate towards the germinal vesicle (Figure 4a-b). The shape of follicular cells progressed from squamous to round or cuboidal. The size (in longitudinal axis) of follicular cells in this stage was 5.729±1.43µm. The nucleus of follicular cells was larger as compared to the previous stage. Most of the oocytes were rounded or ovoid.

6. Late-pre- vitellogenic oocyte (LPVO): The average diameter of the oocyte at this stage was 293.896±105.387µm. Due to the invagination of follicle cell syncytium, the nucleus was displaced to one pole or disappeared in most oocytes. The average size of the

Table 1. Oocyte diameter (µM) in different maturity stages

L. (L.) hardwickei	*Oocyte diameter (in µm) (long axis was measured)*	*Diameter of the nucleus (in µm)*	*Diameter of the nucleolus (in µm)*
Cell type	AVG ± STDEV	AVG ±STDEV	AVG ± STDEV
Primary oogonia (POG)	7.635±3.466	-	Irregular nucleoli
Secondary oogonia (SOG)	28.361±5.768	19.463±2.907	Irregular nucleoli
Primary oocyte (PO)	71.127±22.173	37.869±11.237	24.601±9.205
Secondary oocyte (SO)	129.486±30.886	55.544±16.053	30.780±10.345
Early Pre-Vitellogenic Oocyte (EPVO)	181.481±35.343	62.994±17.022	33.851±8.412
Late Pre-Vitellogenic Oocyte (LPVO)	293.896±105.387	74.905±14.983	Nucleolus irregular
Early Vitellogenic oocyte (EVO)	819.836±240.189	Nucleus not visible	Nucleolus not visible
Late Vitellogenic Oocyte (LVO)	1231.085±338.138	Nucleus not visible	Nucleolus not visible
Ripe oocyte	1191.114±288.188	Nucleus not visible	Nucleolus not visible

nucleus was 74.905±14.983 µm (Table 1). The nucleolus seemed irregular in shape. This stage was characterized by the early signs of yolk formation. The follicular syncytium deeply invaginated into the ooplasm and occupied the maximum area of the oocyte. The follicular cells were columnar or cuboidal in shape with a diameter of 8.928±2.643 µm. Vitellogenesis or yolk formation has begun. Yolk granules were observed in some oocytes. Most of the oocytes were elongated or ovoid (Figure 5a-b).

7. Early vitellogenic oocyte (EVO): The size of the oocyte was increased to 819.836±240.189µm (Table 1). Folds of follicular syncytium occupy the major or entire parts of the oocyte, which obscured the nucleus. Few atretic or resorptive oocytes were also seen in sections. Yolk accumulation increased compared to the previous stage and could be seen between the follicular folds. Follicular cells were cuboidal in shape. (Figure 6a-b).

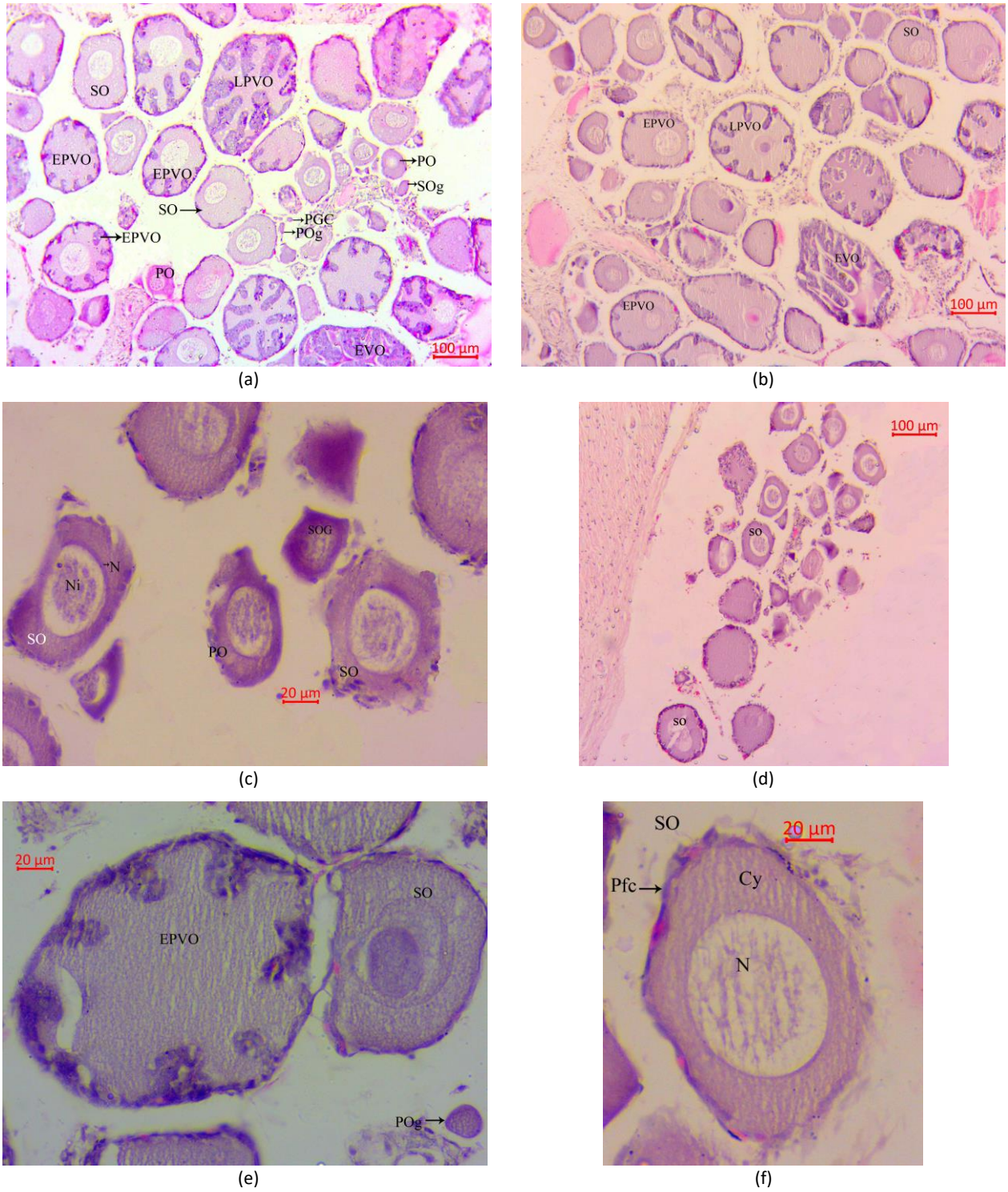
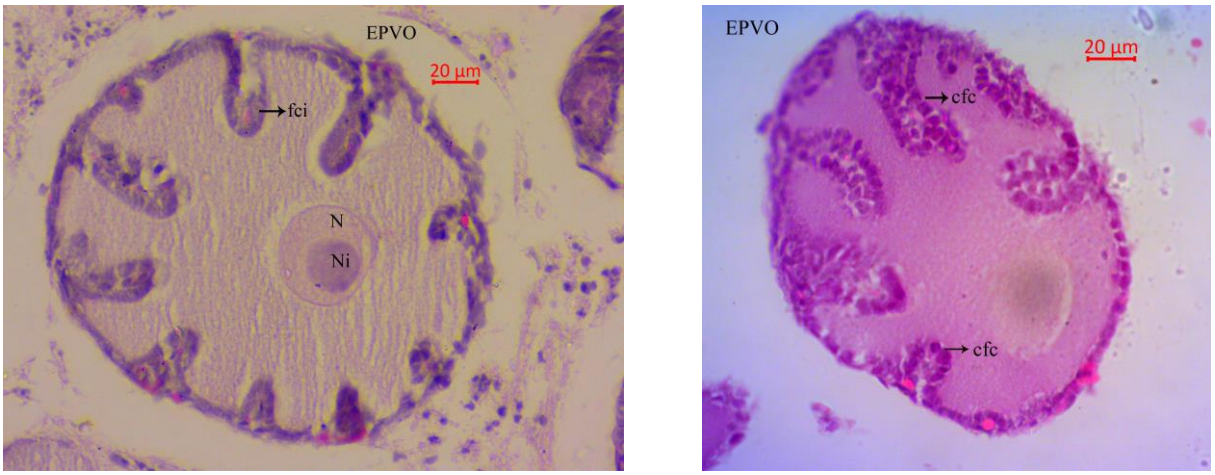
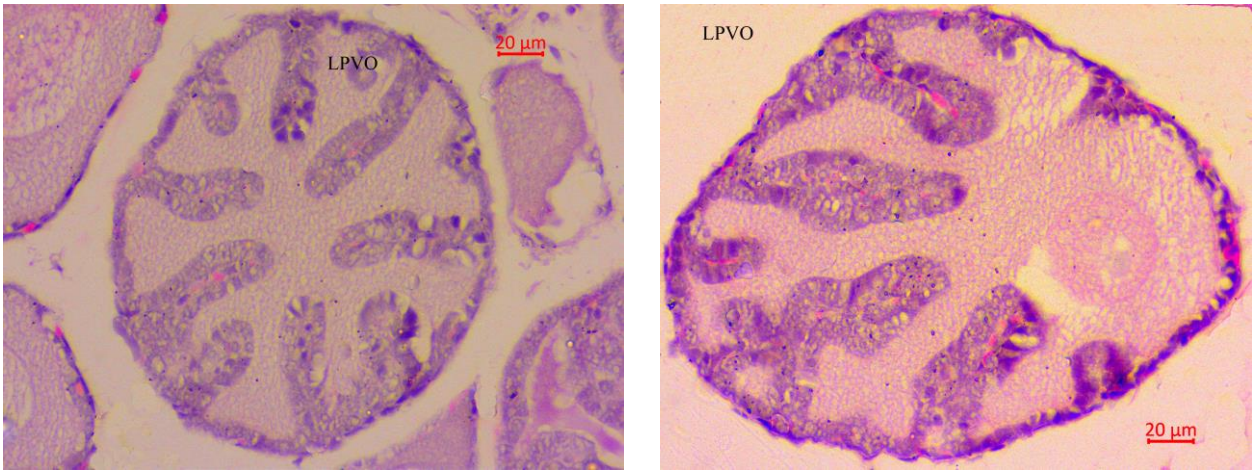


Figure 3. Oogenesis in *L.(L.) hardwickei* (a & b) Sections of ovary showing oocytes in various developmental stages; (c, d, e & f) Cells in early phases of development (PGC- primodial germ cells; POg- primary oogonia; SOg- Secondary oogonia; PO-primary oocyte; SO-secondary oocyte; N-Nucleus, Ni-Nucleolus, Pfc- primary follicle cells, Cy- cytoplasm).



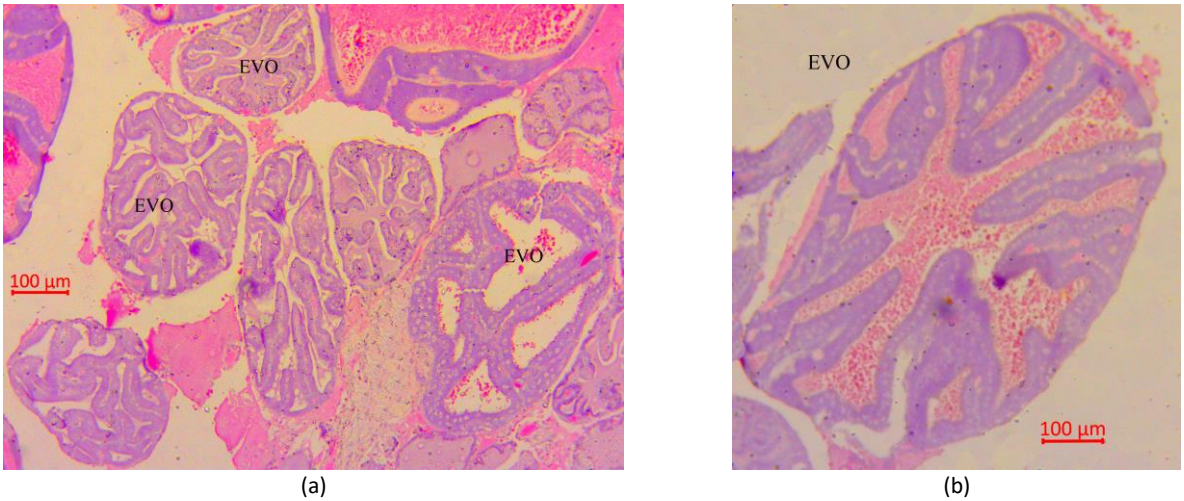
(a) (b)

Figure 4. (a&b) Early previtellogenic oocytes (EPVO- early previtellogenic oocyte; Pfc- primary follicle cells; fci- follicular cell invagination; n-nucleus; ni-nucleolus).



(a) (b)

Figure 5. (a&b) Late previtellogenic oocytes (LPVO- late previtellogenic oocyte).



(a) (b)

Figure 6. (a&b) Early vitellogenic oocytes (EVO- early vitellogenic oocyte).

8. Late- vitellogenic oocyte (LVO): (Figure 7a). The size and volume of the oocyte increased. The average diameter of the oocyte at this stage was 1231.085±338.138 µm (Table 1). Due to the greater accumulation of yolk, an increase in the size of oocytes was observed. The follicular folds began to recede towards the periphery of the oocyte and the degree of invagination was greatly reduced. Final disintegration of the syncytium of follicular cells (post-ovulatory follicles) could also be observed from this stage. The nucleus was obscured. This indicated the displacement of the nucleus to the animal pole and the accumulation of yolk in the vegetal pole. Several atretic or resorptive vitellogenic oocytes were found in sections.

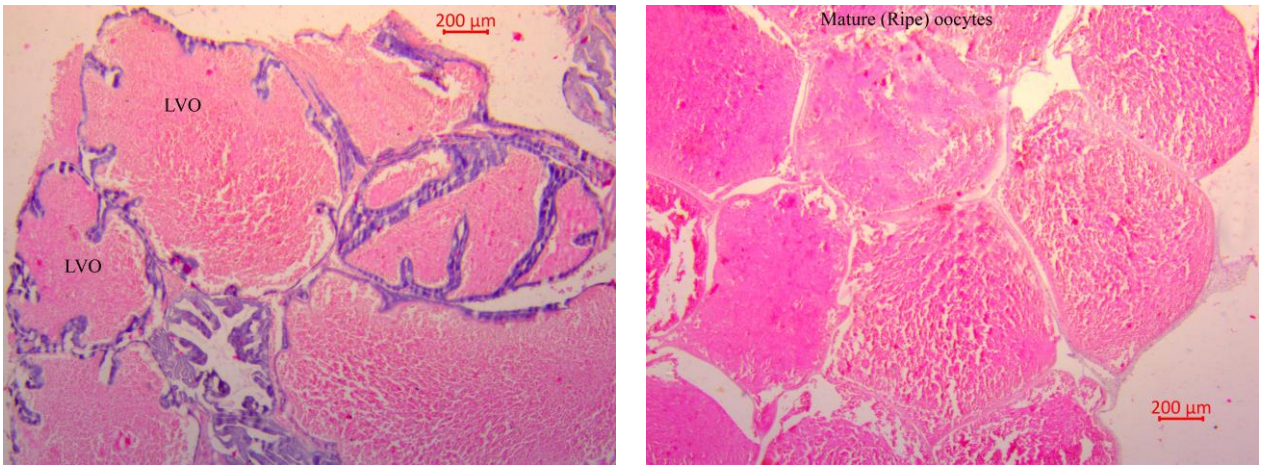
***9. Ripe or mature oocytes*:** The average size of ripe oocytes was 1191.114±288.188µm (Table 1). A distinct chorion can be seen around the oocyte. The oocyte becomes more ovoid or rounded (Figure 7b). A prominent animal pole with a nucleus and a vegetal pole with accumulated yolk were visible. The oocyte is ready to be released. Follicle cells completely disappeared from the oocytes.

10. Fully ripe/mature eggs: Ripe eggs were observed in the oviduct of spawning females (Stage V). Eggs were creamy white and the shape was either round or oval.

Atretic Oocytes

Atresia was observed in previtellogenic and vitellogenic oocytes. Atresic oocyte was found mostly in ovary sections of the specimens in the mature and spawning stages (Figure 8). Follicular cells of atretic oocytes were irregular and the follicular epithelium was found disintegrated.

Primary oogonia, secondary oogonia and primary oocytes were abundant in the histological sections of the ovary of stage I and stage II females. In the stage II ovary sections, along with primary oogonia, secondary oogonia and primary oocytes, the secondary oocyte and



(a) (b)

Figure 7. (a) Late vitellogenic oocytes; (b) Ripe or mature oocytes (LVO- Late vitellogenic oocyte).

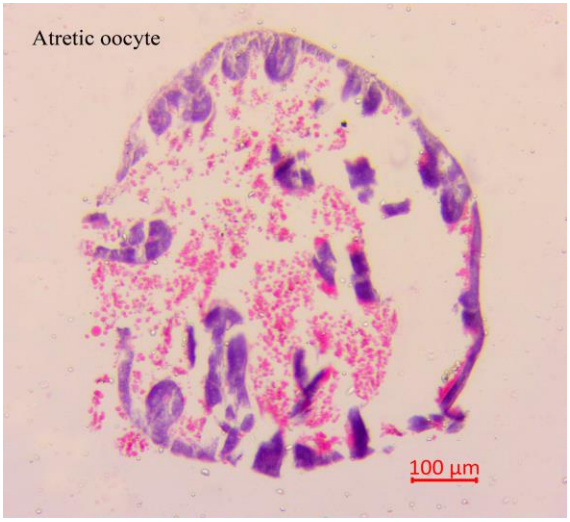


Figure 8. Atretic oocyte.

early previtellogenic oocyte were observed. At stage III very few oogonia and primary oocytes were observed, and secondary oocytes, early and late previtellogenic oocytes were abundant in the ovarian sections. In the sections of the stage IV ovary, oogonia were not observed. Very few primary and secondary oocytes were observed. Early and late previtellogenic oocytes and early vitellogenic oocytes were dominated in ovary sections. Very few late vitellogenic oocytes and ripe oocytes were also observed in some sections. In the sections of the stage V ovary, only post-vitellogenic (vitellogenic) oocytes and ripe oocytes were observed.

Oviducts

The oviduct lies ventrally on the visceral mass with proximal portions directed internally and distal portions facing outward. The distal oviduct opens to the exterior and is destined for the passage of ripe eggs. The Interior wall of the oviduct is lined by folded epithelium which consists of columnar cells and associated supporting cells. The jelly-like secretions produced by the oviducal gland and nidamental gland were found to envelop the eggs. Inside the oviduct, numerous lamellae could be seen. Images of sections of the oviduct are shown in Figure 9a-d.

Male Reproductive System

The male reproductive system consists of the testis, vas deferens, spermatophoric organ, spermduct, the system of spermatophoric glands (SG), spermatophoric sac (Needham sac) and penis. A spermatophoric duct connects the testis to the thin-walled Needham sac. The testis was whitish and bean-shaped and was made up of numerous seminiferous tubules and enveloped in a tunica albuginea made up of connective tissue.

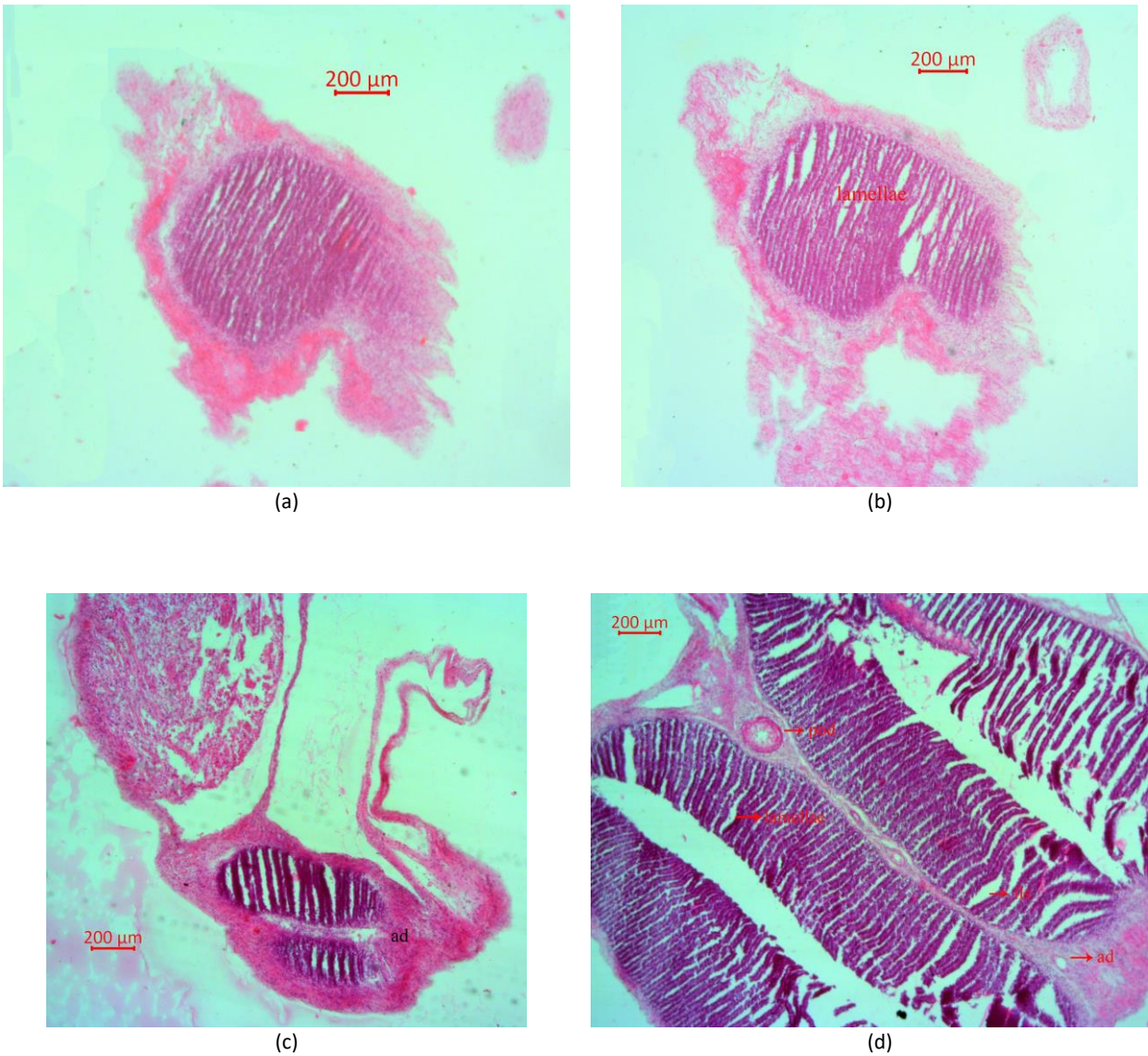


Figure 9. (a,b,c & d). Cross sections of the oviduct. (pod- proximal oviduct; ils –interlamellar space; ad-adjacent duct).

Reproductive Maturity Stages of L. (L.) hardwickei (Male)

Stage I: Immature (Physiological Maturation).

Seminiferous tubules and delimiting connective tissue could not be differentiated. A large number of isomorphic cells are present which comprise an abundant number of spermatogonia and a few large primary spermatocytes. The average GSI was 0.31±0.17 and the average spermatophoric complex index (SCI) was 0.31±0.16.

Stage II: Maturing

Seminiferous tubules were well-defined and delimited by connective tissue. Hectocotylisation in the left 4^{th} arm was more apparent than in the previous stage. Spermatogonia and primary spermatocytes were observed inside tubules of the histological sections of the testis at this stage. Secondary spermatocytes could also be seen from this stage. Intercellular space increased as compared to the previous stage.

Stage III: Mature.

Primary spermatocytes, numerous secondary spermatocytes and early spermatids (rounded spermatids) could be observed (Figure 9a). The centre lumen of the sections contains sperms (spermatozoa). Since the cells were closely packed, intercellular space was considerably reduced compared to the previous stages.

Stage IV: Fully Mature

Primary and secondary spermatocytes, numerous early and mature elongated spermatids and sperm (spermatozoa) could be seen towards the centre of the tubule section. The spermatids can be seen as bluish, elongated, spindle-shaped cells. A large number of rounded spermatids were also seen.

Stage V: Fully Mature (Spawning)

The seminiferous tubules appeared flaccid and deteriorated. A very large number of elongated spermatids and spermatozoa (sperms) could be observed in the sections.

The histological sections showing cellular differentiation during spermatogenesis are given in Figure 10 (a-c).

Needham Sac (Spermatophoric Sac)

The size of the needham sac varied with the number of spermatophores present inside and with the maturity stages. Needham sac is connected to the muscular penis via a tubule. Histological sections of the needham sac are shown in Figure 10 d-e. Numerous elongated spermatids were observed in the sections.

Discussion

The present study evaluated the morphological and cytological changes observed in the histological sections of gonads and other reproductive structures during the development of *L. (L.) hardwickei*. No other studies are available on the histological evaluation of gametogenesis (oogenesis and spermatogenesis) for this species. Histological analysis of the reproductive systems enables us to clarify the processes of maturation of gametes and to accurately define the phases of gonad development.

In the present study, we observed the occurrence of eggs (oocytes) in different maturity stages in the same ovary, the same was observed by other researchers in their studies, whereas the size and proportion varied in different species. Wells and Wells (1977) observed ova differing greatly in size during various stages of development in the same ovary of *Octopus* sps. While analysing the size-frequency of oocytes, the smallest oocytes were observed in stage I and the biggest oocytes were in stage V. In the final stages of oocyte maturation, the folliculogenesis and vitellogenesis peaked which increased the size of the oocyte. Sasikumar *et al.* (2015) also mentioned in their studies, that the eggs in the ovary wouldn't reach maturity at the same time due to the restraint in the physical capacity of the ovary in most cephalopods.

In the present study, 5 stage maturity scale was assigned for both male and female specimens of *L. (L.) hardwickei* based on morphometric characters of reproductive structures. In cephalopods, the duration and number of maturation stages of gonads showed variation in different species reported by different researchers. Arkhipkin (1992) proposed a 7-stage maturity scale for cephalopods. Lipinsky (1979) proposed a 6-stage universal maturity scale for the squids with emphasis on the squid, *Illex illecebrosus*. Many authors assigned 5-8 maturity stages in cephalopods, Hixon (1983) in squid *L. opalescens*, Rao (1988) in squid *L. duvaucelii*, Sauer and Lipinsky (1990) and Lipinsky and Underhill (1995) in choker squid *L.(V)reynaudii*, Butler *et al.* (1999) in squid *Loligo opalescens*, Laptikhovsky and Arkhipkin (2001) in the female cold water loliginid squid *Loligo gahi* assigned six-stage reproductive maturity scale. Boyle and Ngoile (1993) explained the development of squid *Loligo forbesi* male and female in 5 maturity stages. Diaz-Uribe *et al.* (2006) described the oocyte structure of jumbo squid (*Dosidicus gigas*) females based on a six-phase scale. Asokan (2000) assigned a 5-stage maturity scale in squid *L. duvaucelii.* For cuttlefishes *S. pharonis* and *S.dollfusi*, Gabr *et al.* (1998) designated a four-stage maturity scale based on histological and morphological observations. Olgac and Mehmet (2007) identified 6 stages in *S. officinalis*. In octopuses, Ines *et al.* (2002) identified 6 stages of maturity in *Octopus vulgaris* and Lopez-Peraza *et al.* (2013) identified 8 stages in females and 5 stages in male *Octopus rubescens* by histological

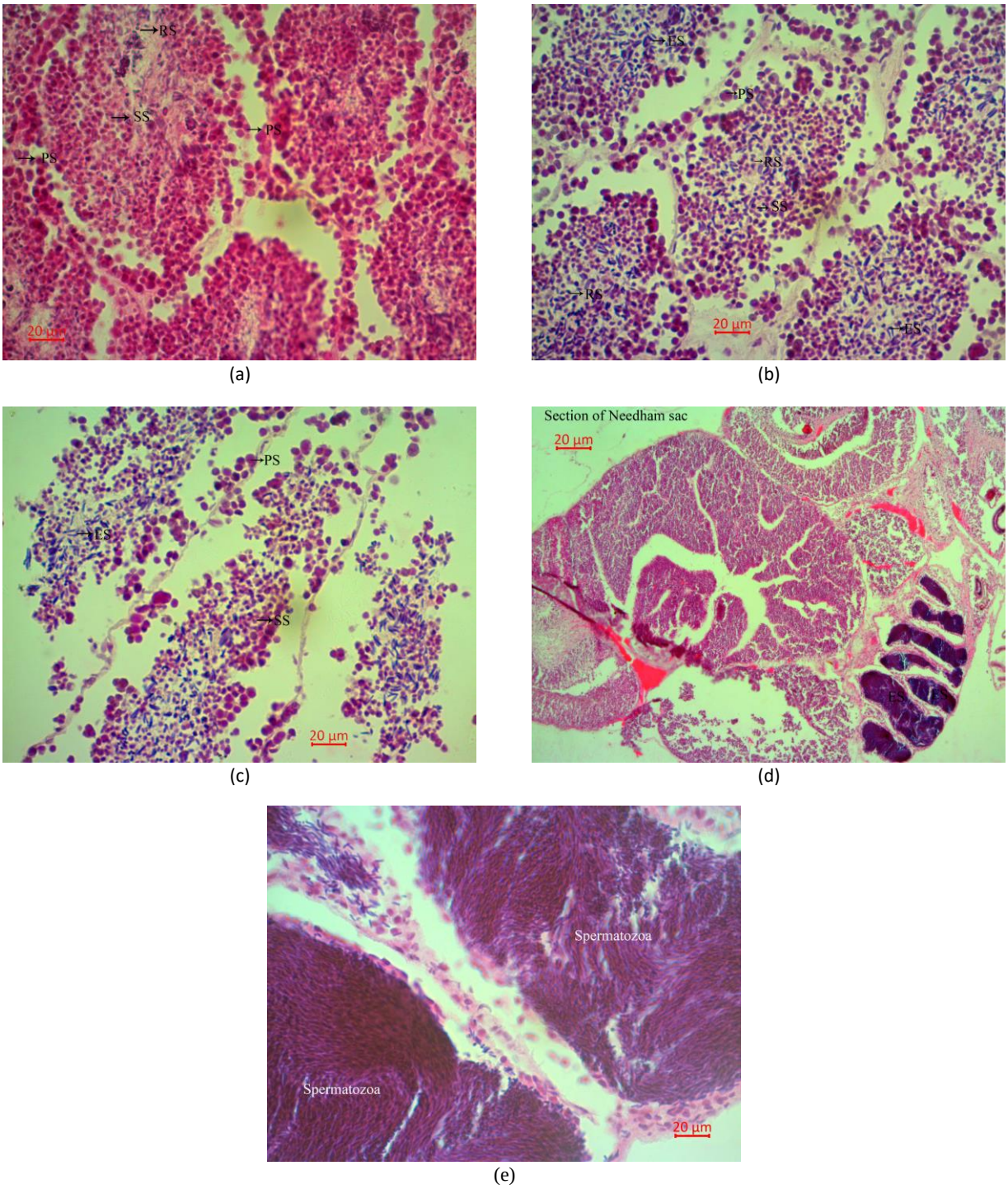


Figure 10. (a,b,c,d&e). Sections of *L. (L.) hardwickei* testis and Needham sac. (s-spermatogonia; ps-primary spermatocyte; ss-secondary spermatocyte; es-elongated spermatids; rs-rounded spermatids;).

and morphological analysis. Hussein and Saad (2015) validated the reproductive cycle of *Doryteuthis forbesii* via histological and statistical analysis noticed the changes in gonad during sexual maturation and determined reproductive maturity stages for oogenesis in 12 phases and spermatogenesis in 7 phases.

As mentioned by the previous researchers (Melo and Sauer, 1998; Lopez-Peraza *et al.,* 2013 and Sieiro *et al.,* 2016) the ovary is delimited by collagen fibrous tissue and the maturity stages could be recognised by analysing the thickness and invaginations of the layer of follicle cells, the absence or presence of chorionic membrane, the presence of pre or post-ovulatory follicles and oocytes in the state of reabsorption and presence of ovarian atresia in previtellogenic and vitellogenic oocytes. Ovarian atresia is considered to be the course of oocyte and follicle resorption which could alter the oocyte structure. Moreover, in the present study too, these characteristics were used to recognize various microscopic stages of gonadal development.

In the present study, while observing the histology sections of the ovary, we identified primodial germ cells,

primary oogonia, secondary oogonia, primary oocyte, secondary oocyte, early pre-vitellogenic oocyte, late pre-vitellogenic oocyte, early-vitellogenic oocyte, late-vitellogenic oocyte, ripe (mature) oocyte and fully ripe (mature) eggs during the oocyte development. Laptikhovsky and Arkhipkin (2001) also recorded the above stages of oocytes and histologically described sexual maturity stages of the cold water Loliginid squid *Loligo gahi* (Cephalopoda: Myopsidae) collected from the Falkland Shelf. Hoving *et al.* (2013) also delineated similar stages of egg cells during reproductive development in the mesopelagic squid *Lycoteuthis lorigerais.* While comparing the size of cells during the process of egg development with the results of previous researchers, showed variation with the current species. Laptikhovsky and Arkhipkin (2001) recorded a size range of 20-30 µm for secondary oogonia (pre-meiotic oocytes), 50-120 µm for primary oocytes, 180-300 µm for initial follicular oocytes (secondary oocytes), 250-400 µm for early yolkless oocyte, 500-800 µm for late yolkless oocytes, 1000-1300 µm for early vitellogenic oocytes and a minimum of 1600 µm for late vitellogenic oocytes in *L. gahi*. Hoving *et al.* (2013) in *L. lorigerais* recorded less than 40 µm for premeiotic oocytes, 40-80 µm for primary oocytes, 120-240 µm for simple follicular oocytes (secondary oocytes), 250-300µm for early yolkless oocytes, 400-450 µm for late yolkless oocytes, 500-700 µm for early vitellogenic oocytes, more than 900 µm for late vitellogenic oocytes and 1.2 mm to 1.5 mm for ripe ovulated eggs. Chen *et al.* (2018) described oocyte development in seven stages in the bigfin reef squid *Sepioteuthis lessoniana.* The cells types were classified as 1. oogonia, 2. primary oocytes, 3. multiple follicular oocytes 4. previtellogenic oocytes, 5. early vitellogenic oocytes, 6. late vitellogenic oocytes 7. ripe oocytes. According to them the average size of oogonia in *S. lessoniana* was <35µm, the primary oocytes were 35–300µm, multiple follicular oocytes were 0.3–1.2 mm, previtellogenic oocytes were 1.2–1.8 mm, early vitellogenic oocytes were 1.8–3 mm, late vitellogenic oocytes were 3–5 mm, ripe oocytes were 5–5.5 mm. However, in the present study the size of the late vitellogenic oocytes (1231.085±338.138 µm) was found to be larger than the ripe or mature oocytes (1191.114±288.188µm) this may be due to the change in shape from ovoid to spherical in the later stage of ova development. From the previous studies, it was clear that the size of oocytes differed in different species of cephalopods. According to Hunter & Macewicz (1985), Melo & Sauer (1998) and Bush *et al.* (2012) the postovulatory follicles persist in the ovary after ovulation and are used as indicators of previous spawning activity. According to Hoving *et al.* (2013), the gonadosomatic index increased with the mantle length. In the present study the GSI increased gradually from stage I to IV, except in stage V. The reduction in GSI in the Vth stage could be due to the beginning of the spawning activity.

Some important studies conducted in the oogenesis of cephalopods include those of Takahashi and Yahata (1973) who described the process of oogenesis in the squid *Todarodes pacificus* into eight stages, 1. oogonium stage; 2. synaptic stage; 3. early yolkless stage; 4. late yolkless stage; 5. early yolk formation stage; 6. middle yolk formation stage; 7. late yolk formation stage; 8. maturation stage. Cowden (1968) described the oocyte maturation process of *Loligo brevis* into 10 stages (cited in Arnold and Arnold, 1977) and the histological descriptions have shown similarity with the structure of oocyte of *L. (L.) hardwickei*. According to Cowden (1968) and Arnold and Arnold (1977) in the first two stages, there is little change in the size of the oocyte but from stage III (early previtellogenic oocyte stage in the present study) onwards size started to increase and the follicular epithelium invaded the oocytes. At the start of stage IV(late previtellogenic oocyte stage and early vitellogenic stage in the present study), the follicular epithelium invaded the oocytes (Cowden, 1968). Bottke (1974) and Arnold and Arnold (1976) observed the occurrence of a vast array of interdigitations on the plasma membranes of oocytes and the follicle cells, they also noticed the regression of some oocytes while vitellogenesis advances. Arnold and Arnold (1977) in their study observed the follicular cells at the beginning of folliculogenesis as small spindle-shaped cells, later by stage IV they become cuboidal and showed a high mitotic rate. The appearance of follicular cells as squamous (thin and flattened) at the initial stages of oogenesis and its transformation to round or cuboidal shape in the later stages in the present study

In the present study, in the sections of the oviduct of *L. (L.) hardwickei*, numerous lamellae were noticed and the interior wall of the oviduct was found with a lining of folded epithelium which consists of columnar cells and supporting cells. Lum-Kong (1992) also observed the same in the histological sections of the oviduct of *Loligo forbesi* (Cephalopoda: Loliginidae).

The process of spermatogenesis happens similarly in almost all cephalopod species reported and the stages of spermatogenesis observed in the present study were practically similar to those described by Sauer and Lipinsky (1990) in *Loligo vulgaris reynaudii*, Lopez-Peraza *et al.* (2013) in *O. rubescens* and Ines *et al.* (2002) in *Octopus vulgaris*. In the present study, histological sections of the needham sac were also studied and briefly explained the structure of the spermatophore. Histological sections of the needham sac revealed stored spermatozoa in the spermatophores, seen embedded in the needham sac. The morphology of spermatophores is significant in the identification of different cephalopods, because of its morphological variations among different species. The studies on the ontogenic aspects of morphology, size, structure and production of spermatophores in Ommastrephid squids (Suborder: Oegopsida) by Nigmatullin *et al.* (2003) was referred to analyse the structure of spermatophores of

L. (L.) hardwickei in the present study. Hoving *et al.* (2008) noticed an increase in the spermatophore length with an increase in mantle length in the mesopelagic squid *Octopoteuthis sicula* (Ruppell 1844) (Cephalopoda: Octopoteuthidae) collected from Southern African waters. In the present similar observations were made. Fields (1965) opined that the size of spermatophores varied with the size of squids and larger squids tend to have relatively larger sperm masses. Fields (1965) also opined that the spermatophores produced by one animal are usually uniform in size but may vary as much as 10% and lengths are roughly proportional to the mantle length of the animal. *L. (L.) hardwickei* tend to have smaller spermatophores as compared with other loliginid squids such as *U.* (*P.) duvaucelii*, *S. lessoniana, U. (P.) singhalensis, U. (P.) edulis* and *U. (P.) sibogae*. This presents a significant relationship between body size and spermatophore size among cephalopods. According to Hoving *et al.* (2008), the spermatophore length increases with an increase in mantle length in the mesopelagic squid *Octopoteuthis sicula* (Cephalopoda: Octopoteuthidae) collected from Southern African waters. In the present study also, the size (DML) of the specimens influenced the size of the spermatophore, an increase in spermatophore length was recorded in squids with higher dorsal mantle length. According to Duysak *et al.* (2014), spermatophore lengths increase with reproduction periods in *S. officinalis.* Hoving *et al.* (2010) also described some aspects of morphometry of the spermatophore of *Histioteuthis miranda.* Histological sections of the needham sac of *L. (L.) hardwickei* revealed stored spermatozoa. In addition, to identifying the maturity stages, histological studies of reproductive structures are useful for understanding the life cycle, spawning season and area of spawning of cephalopods.

Conclusion

The effective and sustainable management of fishery requires an understanding of the biology of species which contributes to the fishery. Cephalopods form an important fishery and are of great economic significance not only to the fishermen in the mechanized sector but also to the fishermen in the artisanal sector. The present study evaluated the morphological and cytological changes observed in the histological sections of gonads and other reproductive structures during the development of *L. (L.) hardwickei*. Histological analysis of the reproductive systems enables us to clarify the processes of maturation of gametes and to accurately define the phases of gonad development.

Ethical Statement

We have purchased dead specimens of *Loliolus (Loliolus) hardwickeii* from the market and also from the fishermen operating boat seine along the Vizhinjam coast. Surgical procedures were performed on the dead purchased samples; hence we are unable to address the ethical requirements.

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Author Contribution

Dr Neethu Raj Panickar conceptualized the methodology for the study, collected the data and wrote the original draft under Dr M K Anil's scientific and administrative supervision. Dr Rohini Krishna M V has done a review, editing and analysis.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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