Histological studies of Sepiella inermis and Sepioteuthis lessoniana
Histological studies of *Sepiella inermis* and *Sepioteuthis lessoniana*

**Introduction**

Cephalopods are soft bodied animals which are distinctly different from their counterparts in Phylum Mollusca. They are morphologically and behaviourally complex. The histological studies of the anatomy of these two cephalopods viz, *S. inermis* and *S. lessoniana* becomes imperative owing to their deviation from the general anatomy of a typical cephalopod. Histological studies are carried out for the better understanding of the tissues and their organization.

**Materials and methods**

Fresh specimens of the spineless cuttlefish *Sepiella inermis* and the big fin squid *Sepioteuthis lessoniana* were collected from the landing centers and brought to the laboratory. Body parts such as the buccal mass, digestive gland, stomach, gills, spiral caecum, testis and ovary were carefully dissected out. These were preserved in 10% phosphate buffered neutral formalin (Bullock *et al.*, 1976). The parts were embedded in paraffin wax after dehydration in increasing alcohol concentration. Histological sectioning and staining were carried out at Vaishnavi Labs, Chennai. The parts were sectioned at 5 μm and Hematoxylin and Eosin were used for staining. Microphotographs were taken using the trinocular microscope.
The Director,  
SDMRI, 44, Beach Road,  
Thiruvananthapuram.

Sir,

Sub: Conduct of Public viva-voce Examination  
- information - reg

I am, by direction, to inform you that the Public viva-voce examination on the Ph.D thesis entitled "Comparative study on the cuttlefish Sepiella inermis Orbigny, 1848 and the squid Sepioteuthis lessoniana Lessom, 1830 of southeast Coast of India" submitted by Mr. Ditty Chacko, Lovedale, Akloor PO, Mattom Via, Thrissur, Kerala - 680 603 has been scheduled on 19.12.2008 at 11.00 AM at SDMRI, Thiruvananthapuram.

I am also to inform you that this may kindly be informed to the staff and research scholars of your department. Copies of the above thesis are available in the Dept of Library, M.S.University, Thirunelveli and SDMRI, Thiruvananthapuram for reference and for clarifications at the time of Viva-voce examination.

No TA/DA will be paid to the participants in this regard.

Yours faithfully,

[Signature]

COORDINATOR

Copy to: The Librarian, M.S.University.
Observations

Buccal mass

Buccal mass consists of dense musculature. It encapsulates the radula, beak and the lips. Followed by the capture of the prey, it is bitten using the beak and scrapped using the radula of the buccal mass before being sent into the oesophagus. Section of the buccal mass (2.5 X magnification) of *S. inermis* and *S. lessoniana* is given in figures 1 & 2 respectively.

The figures suggest that the buccal masses are structures with heavy musculature. The beak (B), is visible in both the buccal masses, which is hard chitinous in nature and is used to bite and cut the prey. In Fig 1, salivary papillae (SP) is visible along the inner margin of the buccal opening. In *S. lessoniana*, buccal mass cells with secretory activity is visible along the inner margin of the buccal opening. From the figures, lobes of buccal mass (L) and musculature (M)
are clearly visible. Muscle fibers are closely arranged which constitutes the major portion of the whole buccal mass. The cells with secretory activity (S) and salivary papillae (SP) are having columnar cells.

**Digestive gland (Liver)**

The section of liver of *S. inermis* and *S. lessoniana* are shown in figures 3 (a & b) and 4 (a & b) respectively. Polygonal hepatocytes (HC) were clearly visible in the liver of both the species. These paranchymatous hepatocytes appeared to form a homogenous mass. A distinct centrally placed nucleus (N) was observed in the hepatocytes. Blood lacunae (L) and bile canals (BC) could also be observed from the sections.

![Fig 3a: S. inermis liver (2.5 x). 'L'- Blood lacunae; 'HC'- Hepatocytes; 'BC'- Bile canals](image1)

![Fig 3b: S. inermis liver (40 x). 'N'- Nucleus; 'HC'- Hepatocytes; 'BC'- Bile canals](image2)
Stomach

In the section of *S. inermis* stomach (Fig 5 a & b), thick musculature was visible in the outer layers. The lacunae (L) and gastric glands (GG) were prominently seen. Above the musculature (M) submucosal coverings were noted. Between the submucosal coverings and the musculature existed the connective tissue (CT). Secretory cells were noted. Rugose-like projections (R) were noted inside the stomach. Higher magnification (40 x) of the stomach showed the thick musculature because of its carnivorous feeding habits.

Section of *S. lessoniana* stomach (Fig 6 a & b) also showed thick musculature (M). The lacuna (L) was large with Rugose-like projections (R).
Gastric glands (GG) with secretory function were noticed. Mucus cells (Mu) were found to line the walls of the lacunae.

Fig 5a: *S. inermis* stomach (2.5 x). 'M'- Musculature; 'CT'-Connective tissue; 'GG'-Gastric gland; 'L'-Lacunae; 'R'-Rugose-like projecton

Fig 5b: *S. inermis* stomach (40 x). 'M'- Musculature

Fig 6a: *S. lessoniana* stomach (2.5 x). 'M'- Musculature; 'GG'-Gastric gland; 'L'-Lacunae; 'R'-Rugose-like projecton

Fig 6b: *S. lessoniana* stomach (40 x). 'M'- Musculature; 'GG'-Gastric gland; 'Mu'-Mucus cells
Gills

The gills of *S. inermis* and *S. lessoniana* have a series of alternating primary lamellae that extend laterally from the gill axis. Section through the ctenidia at 2.5 x magnification showed continuous design of the gill filaments. The gill sections of *S. inermis* and *S. lessoniana* are given in figures 7 (a & b) and 8 (a & b) respectively. The Rachis (RA) was noted to be made up of soft tissues and blood lacuna (BL). On further magnification of the gill filament, filamentous cells (FC) filled with blood cells were observed. Branching of the blood vessels was distinguished. There was a rich capillary bed observed in the sections of the ctenidia.

![Fig 7a: S. inermis gill (2.5 x). ‘BL’- Blood lacuna; ‘RA’- Rachis; ‘CV’- Capillary vessel](image)

![Fig 7b: S. inermis gill (40 x). ‘FC’- Filamentous cells](image)
**Spiral caecum**

Sections of the spiral caecum of *S. inermis* and *S. lessoniana* at different magnifications are given in figures 9 (a & b) and 10 (a & b) respectively. Secretory cells could be seen abundantly which indicates heavy secretory activity. The spiral caecum appeared to be composed of columnar cells with secretory activity. The secretions enter into the central canals in between the columnar cell layers.
Fig 9a: *S. inermis* hepatic ceacum (2.5 x). ‘C’- Columnar cells

Fig 9b: *S. inermis* hepatic ceacum (40 x). ‘C’- Columnar cells; ‘S’- Secretory cells

Fig 10a: *S. lessoniana* hepatic ceacum (2.5 x). ‘C’- Columnar cells; ‘S’- Secretory cells

Fig 10b: *S. lessoniana* hepatic ceacum (40 x). ‘C’- Columnar cells; ‘S’- Secretory cells
**Testis**

In both *S. inermis* (Figs 11 a & b) and *S. lessoniana* (Figs 12 a & b) the cross sections of the testis appeared to have a laminated appearance due to the presence of successively arranged seminiferous tubules (ST). A central lumen (L) could be seen in the central part of the seminiferous tubules. This was clearly noticed in the testis of *S. lessoniana*. Surrounding to the lumen various germinal cells (GC) were observed. Between the seminiferous tubules, interspaces (IS) were found to be developed. Spermatogonia (SG) were observed surrounding the somatic cells (SM) in the testis sections of *S. inermis*.

![Fig 11a: *S. inermis* testis (2.5 x). 'ST'- Seminiferous tubules; 'IS'- Interspaces](image1)

![Fig 11b: *S. inermis* testis (40 x). 'GC'- Germinal cells; 'SM'- Somatic cells; 'SG'- Spermatogonia](image2)
**Ovary**

The ovary of *S. inermis* and *S. lessoniana* are shown in figures 13 (a & b) and 14 (a & b) respectively. Ovarian follicles (FC) were noted along with the oviduct (OV). Inside the ovarian follicles, oocytes (OO) were visible. The follicular walls were found to ingress into the middle of the follicle as a fold (FI). The ovarian follicles of *S. inermis* were in abnormal shapes (FA). On magnification the ovarian follicles and the oocytes inside were clearly visible in both *S. inermis* and *S. lessoniana*. The ingressing walls of the ovarian follicles were noted to be composed of columnar epithelial cells.
Fig 13a. *S. inermis* ovary (2.5 x). ‘FC’- Ovarian follicles; ‘OV’- Oviduct; ‘FA’- Abnormal follicles

Fig 13b. *S. inermis* ovary (40 x). ‘FI’- Follicular fold; ‘OO’- Oocytes

Fig 14a. *S. lessoniana* ovary (2.5 x). ‘FI’- Follicular fold; ‘OO’- Oocytes

Fig 14b. *S. lessoniana* ovary (40 x). ‘FI’- Follicular fold; ‘OO’- Oocytes
Discussion

Feeding in cephalopods is accomplished by the buccal mass. In *S. inermis* and *S. lessoniana*, the buccal mass appeared in a spherical structure within a sinus formed by the base of the arms. The buccal mass is composed of structures such as upper and lower beaks attached to the mandibular muscles. The beak is responsible for cutting the prey into small pieces. The dense musculature is responsible for the opening and closing of the buccal mass (Uyeno and Kier, 2006). The salivary papillae present inside the buccal mass region posses secretory cells suggesting the release of certain secretions during the time of feeding.

The digestive gland was found to be the biggest gland in both *S. inermis* and *S. lessoniana*. The digestive gland and hepatopancreas are paired in *Sepia* consisting of a brownish liver and a whitish pancreatic region (Meglitsch and Schram. 1991). In the present study, the liver sections showed to be composed of homogenous mass of hepatocytes arranged in lobules. The hepatocytes were nucleated with lacunae in between. Similar studies have been reported earlier in *Sepia pharaonis* (Samuel, 2003). Digestive gland cells have been reported to be coated with connective tissue membrane in the gastropod *Hemifusus pugilinus* (Benny, 1996).
The stomach was found to be lined with columnar epithelium with secretory activity. Thick layers of musculature could be made out clearly. The stomach in cephalopods is lined with chitin and is presumably a derivative of the stomodeum (Meglitsch and Schram, 1991). The caecum is typically coiled in *S. inermis* while it is straight in *S. lessoniana*. Absorption of the nutrients takes place mainly in the caecum and the columnar cells aids in absorption.

The gills of cephalopods are modified into ctenidia with a central axis containing the main afferent and efferent blood vessels, and flattened gill filaments containing capillary beds. Both *S. inermis* and *S. lessoniana* had only a single pair of gills. The branchial canal can be distinctly made out in the ctenidia of *S. lessoniana*, while it is absent in *S. inermis*. This was one of the primary features used earlier to separately classify sepioids and teuthoids (Young and Vecchione, 2006). In the gills of the gastropod *Hemifusus pugilinus*, the epithelium is having a ciliated cuticle as well as a small number of mucocyte cells (Benny, 1996). In both *S. inermis* and *S. lessoniana*, three types of lamellae viz, 1°, 2° and 3° lamellae were observed as mentioned by Schipp *et al.* (1979). The lamellae were noted to be rich in blood vessels where the oxygenation of the deoxygenated blood takes place.
In the testis of *S. inermis* and *S. lessoniana* studied, the differentiation of the germ cells took place from the center of the seminiferous tubules to the periphery. Similar observation was made by Rahim and Chandran (1984) in the testis of the warm water squid *Loligo duvauceli*. As the animal matures, the central lumen in the seminiferous tubules gets obliterated (Rahim and Chandran, 1984). In the section of the testis of *S. inermis*, the central lumen was not visible stating that it was a mature testis, while in *S. lessoniana* central lumen was present stating that it was a maturing testis. Maturing testis is characterized by the presence of spermatocytes in the center of the seminiferous tubule (Gabr *et al.*, 1998).

In the ovary of *Sepia dol fusii*, during the pre-spawning state the follicular epithelium is found to invade the oocytes as follicles of tissues with higher mitotic rate, and make the end of their maximum penetration into the oocytes in the formation of the scyncitium (Gabr *et al.*, 1998). The folding of the follicular epithelium into the oocytes was also observed in the present study in both *S. inermis* and *S. lessoniana*, suggesting that the animals were in pre-spawning state. The folds of the follicular syncytium are active in the vitellogenesis and formation of the chorion. The number of oocytes was observed to be low in the follicles with heavy infolding. The ovarian follicles found in abnormal shape in *S. inermis* may be ruptured ones with the oocytes released into the oviduct.
The histochemical study carried out in *S. inermis* and *S. lessoniana* in the present study could be helpful in the investigations of the micro-taxonomical characters in the future. The changes in the cellular make-up at different maturity stages will help in the better understanding of the cephalopod biology. The present study will also form a basis for future biological studies in the cephalopods.