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Morpho-anatomical study of *Lingula anatina* Lamarck, 1801 from West Bengal-Odisha coast, India

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Original Article

Abstract
Lingula anatina, the living inarticulate brachiopoda has been reported in the present paper from the intertidal belt of Subarnarekha estuary at the confluence of Bay of Bengal. The genus *Lingula* being the oldest of all living genera is considered as living fossil which reveals an ancient lineage to Pre- Cambrian period. The morphological (shape and sizes of shell, pedicle, muscle, lophophore, mantle digestive system, coelom, nephridium and gonads etc) and microanatomical (Histological features of different parts of body) studies of this newly recorded species from West Bengal- Orissa coast, India have been undertaken and presented.

Keywords: *Lingula anatina, Subarnarekha estuary, morphology, microanatomy.*

Introduction

The intertidal belt of West Bengal coast including Midnapore (east) district in its extent of 60 km, encompasses a diversified habitat and niche which accommodate a good number of faunal components (Chakraborty, 2010 and 2013) including brachiopods- *Lingula anatina*. This two shelled marine benthic brachiopods represent one of the important minor animal phyla commonly known as lamp shells. Although about 30000 species and 120 genera under the phylum of Brachiopoda have been described in the fossil records of the lower Cambrian Period of which only about 300 species of brachiopods still exist. (Pennington *et al.*, 1999; Stricker, 1999). The brachiopod of the family Lingulidae is being represented by only two living genera- *Glottida* and *Lingula* (Emig and Bitner, 2005). This rare group of benthic animal belonging to family Lingulidae has been reported from some river mouths of Indian coasts (Rao, 2004). The present study has elucidated the morpho-micro anatomical details of the species which is a new record from the confluence of Subarnarekha estuary and Bay of Bengal, India.

Material and methods

In the present work, three contrasting study sites viz. site-I (S-I), site-II (S-II) and site- III (S-III) located along the intertidal

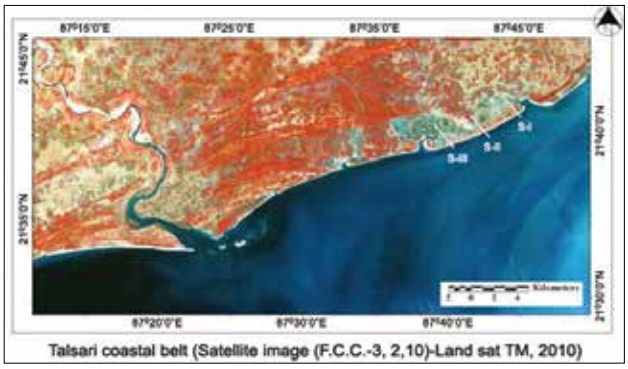

Fig 1. Geographical situation of three study sites.

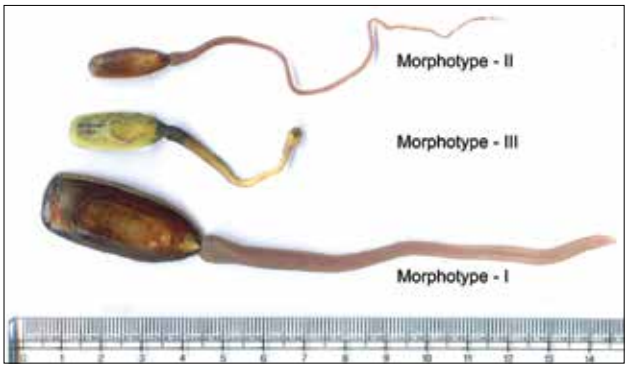

Fig 2. Adults of *Lingula anatina* showing three different morphotypes.

belts of an ecotone in the confluence of Subarnarekha estuary (Fig.1) with Bay of Bengal, West Bengal-Orissa coast, India have been selected. Random samplings of three different adult morphotypes (Morphotype-I at S-I, Morphotype-II at S-II and Morphotype-III at S-III) of *Lingula anatina* were made along three transects lying on different zones of intertidal belts at low tide level (LTL), mid tide level (MTL), and high tide level (HTL): at Talsari (Longitude 87°5′E to 88°5 E and Latitude 20°30′ N to 22°2′ N) for 24 months, 6 seasons and 2 years from June, 2009 to July, 2011. Five quadrates measuring 1m^2 areas were placed randomly on each transect and the bottom dwelling faunal components especially brachiopod, were unearthed, counted and their mean population density was expressed as no./m^2. Shell lengths, shell width and pedicle lengths were recorded with the help of slide calipers and with a centimeter scale.

The desired tissues of adult animals were fixed for a minimum of 24 hours in 4% formalin solution, processed through alcoholic grades (70%, 90%, and 100%) and embedded in wax following standard methods of Bancroft *et al*., 1996 and Shyamasundari and Rao, 2007. Sections of 4-5 μm in thickness were cut from paraffin blocks with the help of a rotary microtome (AMBALA DJ. H4. RH) followed by dewaxing. Slides were stained with Harris's Haematoxylin and Eosin following standard methods of Gurr, 1962 and Bancroft *et al*., 1996. Stained sections were examined and photographed using a light microscope (Model no- micro imaging GmbH, serial no- 3109003139, Carl Zeiss- Germany).

Results

Physiography

The physiography of the study sites along with morpho-anatomical and micro-anatomical descriptions of *Lingula anatina* are presented. Three morphotypes (Fig. 2) of this species were observed at 3 different intertidal mudflats experiencing total exposure during extreme low tide. The present study area is characterized in having mixed sand and mudflats endowed with degraded mangroves. The eco-geographical characteristics of three study sites are presented in Table 1 and Fig.1. Fourteen plant species under 10 families and 32 animal species under 20 families have been recorded during the study period, but they are not reported in this paper.

Systematic position

Kingdom: Animalia C. Linnaeus, 1758. , Phylum: Brachiopoda Duméril, 1806, Family: Lingulidae Menke, 1828, Genus:

Table. 1: Eco- geological features of study sites:

Characteristic features	S-I	S-II	S-III
Beach plan shape	Barrier beach	Barrier beach	Barrier beach
Beach width (m)	210	195	180
Beach slope	1.6	1.5	1.8
Wave length (m)	0.85	0.82	0.88
Tidal amplitude (m)	6.2	5.9	6.0
Textural composition	Sandy	Sandy loam	Sandy loam
Beach mark	Present	Present	Present
Back wash ripple	Well characterized	Moderately visualized	Least visualized
Sand dunes (height)	7-10 (m)	5-8 (m)	4-8 (m)
Mangrove vegetation	Devoid of any mangrove vegetation	Only one mangrove species- *Acanthus ilicifolius* present as dominant species along with scanty occurrence of *Saueda* sp. and *Salicornia* sp.	Considerable numbers of mangroves were found to exist with *Avicennia alba* and *A. marina* as dominant species.

Lingula Bruguière, 1797, Species: *anatina* Lamarck, 1801 Scientific name: - *Lingula anatina* Lamarck, 1801.

Morpho- anatomical study

The shell is characterized in having two dissimilar valves which appeared to be oblong, ovate, elongate, bilaterally symmetrical, endowed with parallel lateral margins, sharp beaks and straight edges (Table 2 and Figs.2 to 5). Outer surface of the valves were mostly found to be smooth but indicating growth lines. The inner surfaces of the valves were designed with different ornamental features. The ventral valve possesses two hinge teeth fitting into opposing sockets into the dorsal valve. Some bristles have been found at the anterior margin of both valves, marked with lines on upper side. Lower sides of the valves are endowed with signs of muscle scars and mantle canals.

A long extension, in the form of pedicle has been emerged from the posterior end of ventral valve as an elevation of ventral mantle lobe. It includes evagination of the main coelom. Pedicle is the anchoring device of *L .anatina* (Table 2 and Fig. 4).The studied species has been observed to possess three pairs of oblique muscles, one pair of posterior adductor muscle and one pair of anterior adductor muscle. Besides one pair of lateral muscle has also been found in the *Lingula anatina* (Fig.4).

During present investigation it was observed that anterior body wall of *Lingula* projects into the mantle cavity forming a cilliary pumping device on both sides of mouth called lophophore. These spirolophous type lophophore was supported by a single arm called brachium. Functionally the organs were active in the transporting and capturing food, accumulation and removal of faeces, the sorting of particles, and pumping of water required for respiration (Fig.4, 5A, and 5B).

The anterior body wall extensions of the animal formed mantle and the space occupied between the mantle lobes are called as the mantle cavity. The present study has shown that the innermost organs of *Lingula anatina* are being protected with this thin flat extension both ventrally and dorsally (Fig. 4).

Table. 2: External morphological characteristics of 3 morphotypes of *Lingula anatina*.

Characteristics features	Morphotypes-I	Morphotype- II	Morphotype- III
Shell colour	Deep greenish brown	Deep brown	Pale greenish
Shell length (cm)	4-6	1-2.5	3-4
Shell width (cm)	1.4-2.6	0.1-1.2	1.1-1.9
Shell ornament	Well marked internally	Moderately marked internally	Visible internally
Growth lines on shell	Prominent	Not well prominent	Prominent
Pedicle length(cm)	5-30	20-35	5-15
Pedicle colour	Blackish	Grey	Whitish
Population Density	8- 98 no/m^2	284-3254 no/m^2	84- 216 no/m^2

Fig 3. Shell of *Lingula anatina* (internal view).

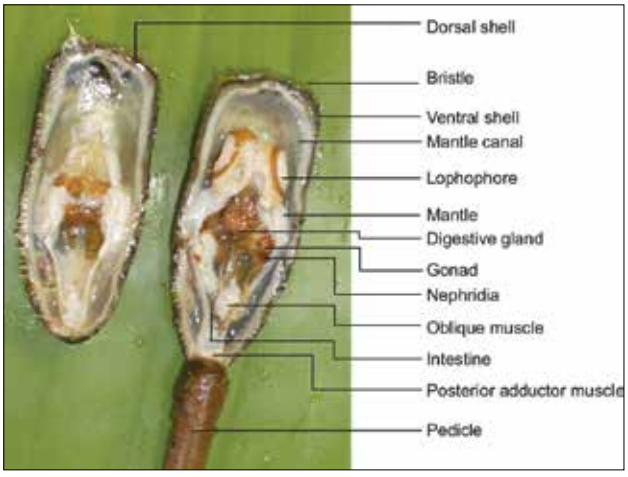

Fig 4. Morphoanatomical details of *Lingula anatine*.

Fig 5. Lophophore of *Lingula anatina* (5A: dorsal view and 5 B: ventral view).

The digestive system originated from mouth as a transversely elongated slit in the centre of the lophophoral base between the brachial fold and tentacular fringe and terminated after convolutions of intestine at the right side into the mantle cavity. The result of gut content analysis revealed the presence of fragmented mangrove leaves, planktonic materials and detritus inside the gut (Fig.4).Two conspicuous digestive glands with four ducts surrounding the stomach having pale yellowish in colour have been observed (Fig.4).

In *Lingula anatina*, coelom has been shown to accumulate a moderate amount of coagulable pinkish fluids with free cells called coelomocytes- spherical, amoeboid and phagocytic in nature. A large quantity of spicules has also been found in the coelomic fluid during present work (Fig.14).

Each nephridium of studied animal consisted of a large funnel which is shaped with nephrostome and a tubular part that open through nephridiopore into the mantle cavity (Fig.4). It was found that in L. *anatina* a pair of gonads are running along the digestive tract. Male possesses pale whitish testis while female is having deep yellowish-red ovary at the time of breeding season preferably during premonsoon (Fig.4).

Microanatomical study

Microanatomical study has revealed that lophophores of studied animal presently containing a central canal, a thick muscle layer, a connective tissue layer and cuticular layer. Several lateral canals, tentacular fringes and glandular cell layers were also observed. The transverse section through lophophoral tentacle has demonstrated several features like cilliary root region, mucous cells, and connective tissue layers surrounding the tentacular canals, centrally positioned blood vessels and coelomic epithelium layers (Fig.6, 6B and 7).

The gut or digestive tract seems to be covered externally with peritoneum. Beneath this, a muscle layer is found. Numerous glandular cells or goblet cells have been found in between epithelial cell layers and muscle layers. The innermost portion of digestive tract is covered with cilia (Fig. 10).

The present observation has revealed that a set of conspicuous digestive glands are present surrounding the stomach and fill much of the interior space. The acini of digestive glands found to contain three principal types of cells. At the exterior

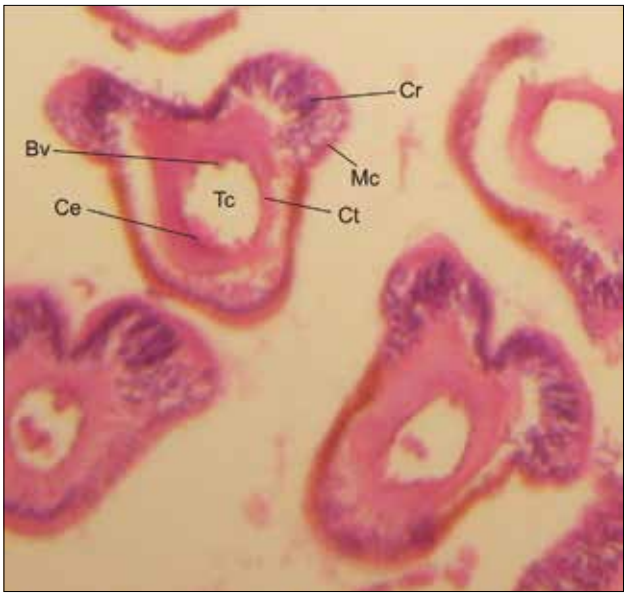

Fig 7. Transverse section of Lophophoral Tentacle (40x × 15 xs).

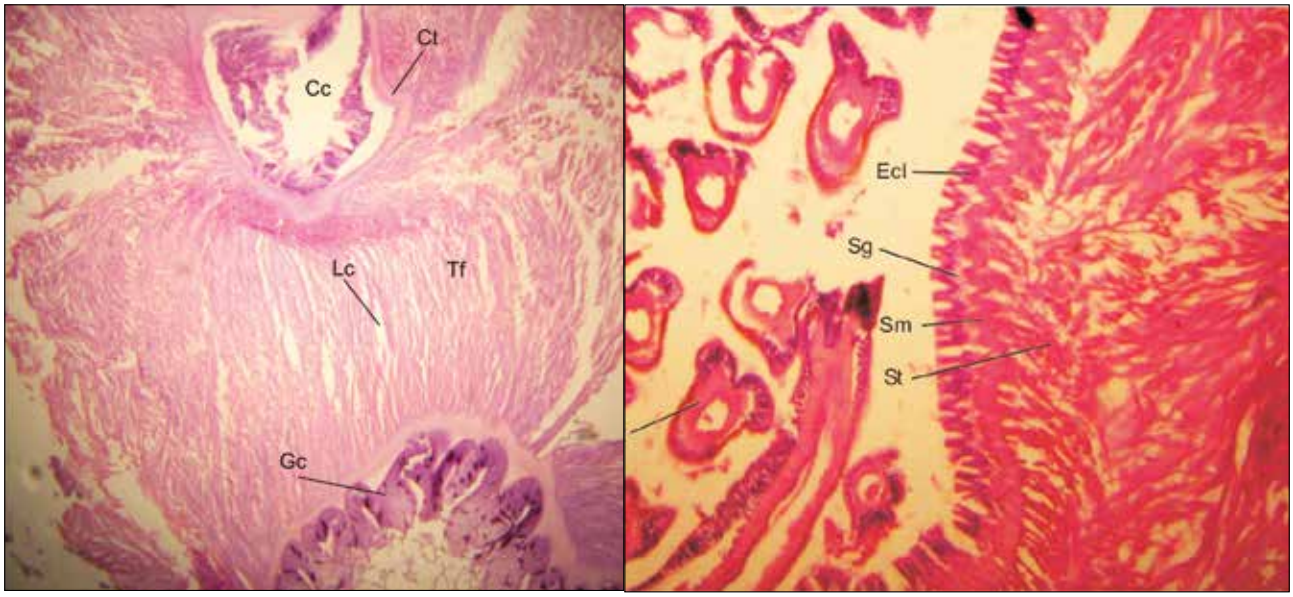

Fig 6. 6A and 6B: Transverse section of Lophophore (40x × 15 x).

Fig 8. Transverse section of Digestive Gland (40x × 15 xs).

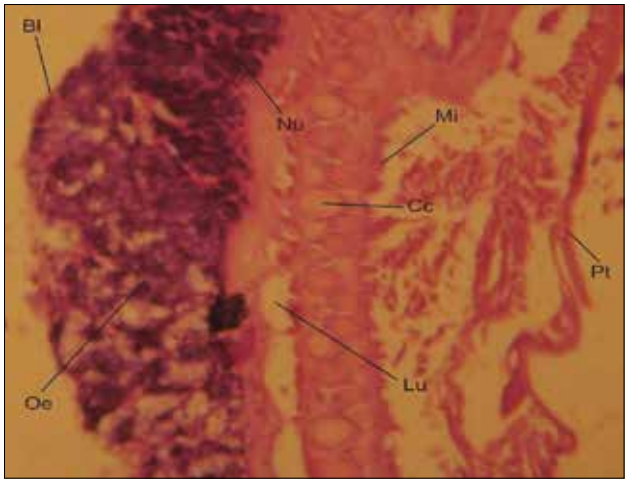

Fig 9. Transverse section of Mantle (40x × 15 xs).

of each acinus, a connective tissue layer is present. Numerous secretory granules and lipid droplets were also present within and acinus (Fig. 8).

The connecting layer of mantle with body wall has been characterized with the presence of periostracum. The outer epithelium layer and basal lamina have been found to be well characterized with the extension of lumen, beside which glandular core cells have been found to be situated. The outer epithelium layer displays the presence of distinctive nucleus (Fig. 9). Transverse section through adductor muscle exhibited the presence of two distinctive area i.e. translucent area containing complex muscle fibers and opaque area containing smooth muscle fibers. In mature individuals pedicle consists of a concentric cuticle layer, a one cell columnar epithelial cell layer, a connective tissue layer and a thick, crisscross, spiral

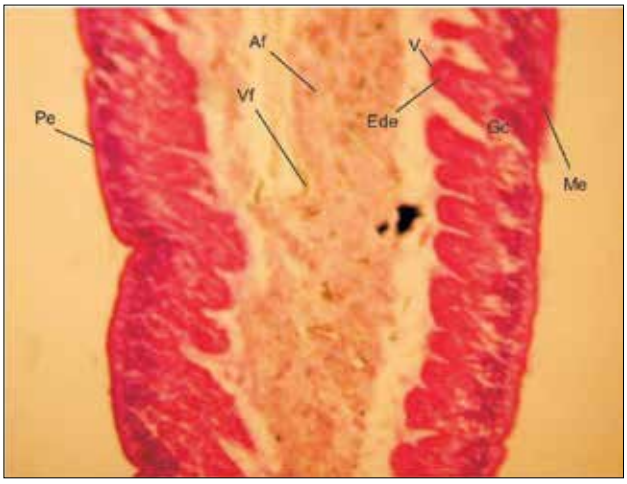

Fig 10. Transverse section of Gut (40x × 15 xs).

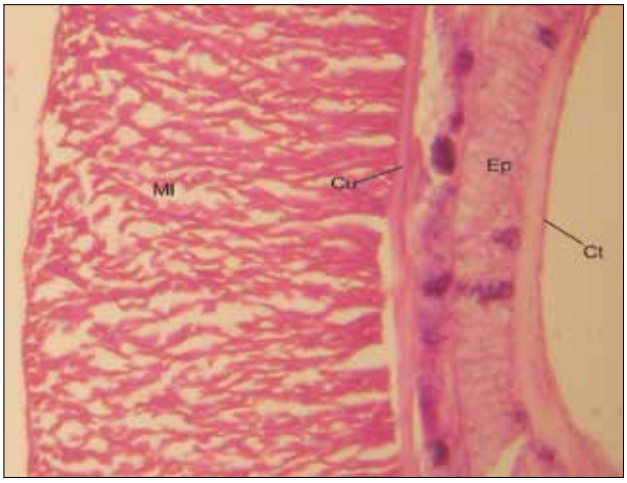

Fig 11. Transverse section of Pedicle (40x×10 xs).

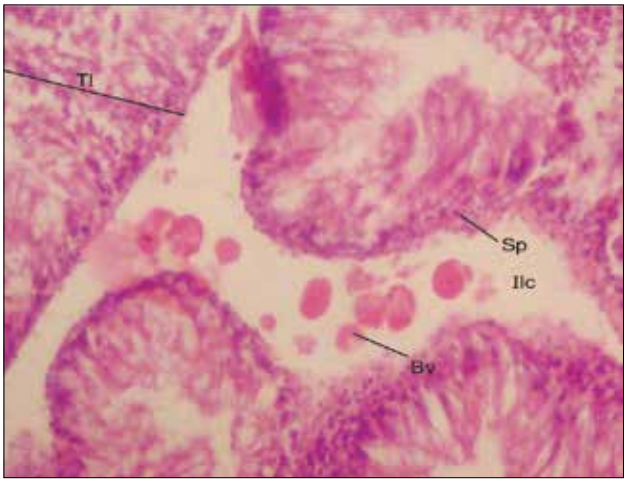

Fig 12. Transverse section of Male Gonad (40x × 15 xs).

fibrous muscle layer (Fig.11). Transverse section through nephridia revealed the presence of connective tissue layers at outer portion and epithelial cell layer, next to connective tissue

layer. Numerous deep brownish mesenteric cells are observed during present investigation after the epithelial cell layer.

The ripe male gonads are found with pale yellowish colour during breeding season. The transverse section through testis lobule demonstrated presence of numerous young sperms within testis lobule. Some interlobular cells and blood vessels have also found in inter lobular space (Fig.12). Each follicular cell of female gonad exhibits the presence of a distinctive ovum

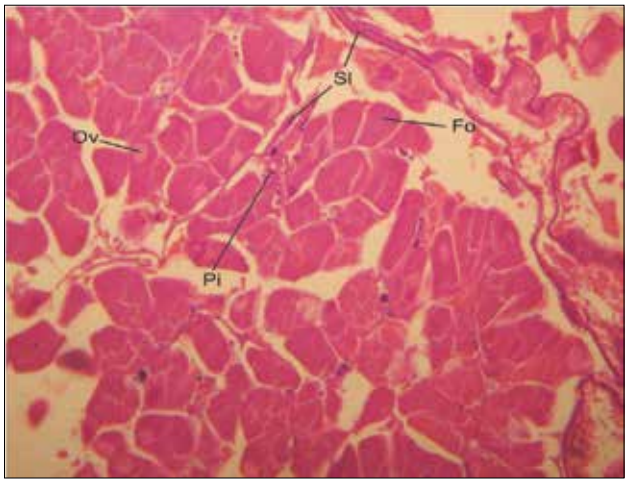

Fig 13. Transverse section of Female Gonad (40x×15x).

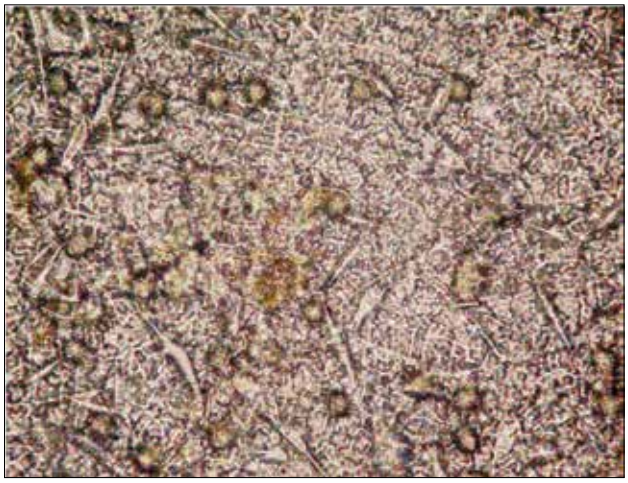

Fig 14. Spicules of Lingula anatina.

which is light pinkish in colour. Each lobule is characterized by supporting layers. Some deep violet pigmented granules have been observed situated within the lobule (Fig.13).

Discussion

Since the publication of Darwin's theory of evolution in 1859, lingulids have probably been the most widely quoted examples of arrested evolution. This, to some degree, may be because of few anatomical features which are impressed either on or inside valves so these may not adequately reflect the extent of change incurred during lingulid evolution (Zhang *et. al.*, 2005).

The individuals of 3 contrasting study sites have displayed common morphoanatomical and microanatomocal features. Scanty information is available regarding *Lingula anatina* in the Indian subcontinent. Only report on mass mortality of *Lingula anatina* in Parangipettai waters of Bay of Bengal was published by Ramamoorthi *et al.*, 1973. No report on the morphoanatomy and microanatomy has been made so far in order to establish the species status of this fauna which is in tune with the works of Emig, 1984.

The valves attained maximum length and width which has been manifested by the broadest area near the posterior part of the pedicle. The lateral edges appeared to be parallel at full matured state. The straight anterior edge has slightly angular projection in the middle with rounded angles. The ventral valve showed a more or less strongly rounded pedicular groove, continuing with the inner surface of the valve. The present study has attempted to record the species *Lingula anatina*, a living fossil (Goodnight *et al.*, 1970) based on the morpho-microanatomical study.

The external surface of the shell is smooth and the lines of growth are easily detectable. The inner surface of each shell valve is ornamented with various channels. *Lingula anatina* always displays brilliantly coloured shell. Pedicle, when contracted, is about one and a half to twice as long as the shell. Root like extension at the end of pedicle has been developed to ensure adherence to the substratum. Some matured individuals often showed very short pedicle indicating that after loss of some portion of pedicle it becomes regenerated. Pseudosiphon formation of setae found in *Lingula anatina* is an adaptation to an infaunal lifestyle. Lophophore and mantle of this animal are being considered as functionally integrated organs which are active in the transport and capture of food and removal of feaces.

The presence of spherical amoeboid phagocytic cells, spicules and heme -erythrine in the coelomic fluids of *Lingula anatina* has also been recorded by Yatsu, 1902. Hemerythrine being an analogous to hemoglobin was supposed to display low oxygen affinity and thereby increases Bohr Effect, facilitating oxygen transport in order to enable the lingulids to tolerate low oxygen condition (Manwell, 1960). This biological attribute likely provides lingulids with the necessary mechanism to proliferate during the early Triassic (Peng *et al.*, 2007.) According to Hyman, 1959 all brachiopods possess at least one pair of metanephridia. During pre monsoon period,

enlargement of gonads and deeping in colouration indicate their breeding period.

In L. *anatina*, anterior body wall projects into the mantle cavity forming ciliary pumping device that help in feeding and respiration called lophophore which is spirolophous in nature and corroborates with the findings of Hyman, 1959. Histologically, the lophophore includes three main elements- an epidermal cover of a selectively ciliated epithelium, a variably complex connective tissue tube and inner coelomic epithelium underlaind by muscle fibers.

According to Emig, 1997 the gut is essentially a variably elastic connective tissue tube encompassed by two layers of muscles. The gut is encased by a thin ciliated coelomic epithelium. A number of different types of cells lining the acini reflect their role as the main site of intracellular digestion (Storch and Welsch, 1975).

The digestive glands are enriched with the presence of a columnar ciliated epithelium containing secretory droplets and sparsely interspersed with darkly staining gland cells. The epithelium is to be covered externally with very thin connective tissue and the peritoneum. Muscle cells are confined to the ducts.

The outer mantle epidermis is cubical and besides secreting much of the shell, it produces mantle papillae which occupy the vertical tubules or canals. The cells toward mantle edge becoming more columnar and armed with setae or bristles. The setae have been born by mantle lobes and were generally greater anteriorly and smaller posteriorly. The glandular zone was well developed in the studied specimen which had been also established by Hyman (1959).

The adductor muscle of the *Lingula* has been well documented by Kuga and Matsuna, 1988. Each adductor muscle is endoweded with smooth and obliquely striated components segregated into an anterior opaque and a posterior translucent portion that are constructed to smooth and obliquely striated muscles respectively.

The pedicle, a fleshy stalk has emerged through a hole, called pedicle foramen situated at the posterior end of ventral valve. The pedicle appeared to be used for anchoring and rotation of L. *anatina*. It contains a central coelomic cavity and an outer transparent cuticle. Thus fluid skeleton formed by the perivisceral coelom acted as a classical hydrostatic skeleton (Trueman & Wong, 1987).

The transverse sectional view of nephridia of L. *anatina* has revealed that the inner portion of nephridium is covered by a highly microvillus ciliated epithelium interspersed by secretory probably mucous cells. The fold of the nephrostome has been thickened by connective tissue layer. The brownish granules present in the epithelium tube presumably serve the function of excretion that often conferred a colour on the nephridium.

In males, the spermatozoa are formed in the testis which is restricted to the ileo-parietal band and pallial sinuses. Exterior to the supporting substances of ileo-parietal band there are a thicker layer of clusters of heads of young spermatozoa (Yatsu, 1902).The outer surface of the later layer resembles ciliated because of the presence of tails of spermatozoa.

The ova have been found to be slightly rounded or prism shaped. According to Yatsu, 1902 the follicle of the ovum is formed at the expense of interstitial cells, which become extremely flattened so that their presence is perceived only by their compact and spindle shaped nuclei. The yellowish brown pigmented granules have been observed along the supporting substance of the ileo- parietal band and among the ova during the present study.

Based on the ecological and detailed morpho-microanatomical study of the present brachiopod specimen which corroborate very closely with the findings of Emig (1984), the present authors are convinced to claim this species as *Lingula anatina* to be recorded for the first time from the east coast of India.

Acknowledgements

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