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Macroanatomy and 3-dimensional modeling of the tentacular head of *Phascolosoma arcuatum* (Grey, 1828)

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Abstract: **The macroanatomy of the tentacular head of Phascolosoma arcuatum belongs to phylum Sipuncula has been studied under light microscopic methods and computer aided graphics analysis. The tentacular head structure has been reconstructed using computer aided graphics analysis using series histological images on computer aided software platform. The P. arcuatum has only five, semitransparent, pigmented, lobed tentacles in tentacular crown with large tentacular coelomic connections. We found that the tentacles are lined by ciliated columnar epithelium with large number of mucosecretory cells, indicating their deposit feeding habit. Fewer number of tentacles, body wall and there burrowing habit supports there integumentary breathing habit. A thick striated collar encircles the tentacles forming the ventral wall of the mouth channel and protects tentacles from mechanical damage when retracted. These structural peculiarities revealed from macroanatomical analysis are strongly suggestive about the important role of the collar region of the tentacular crown of the head of P. arcuatum in relation to its burrowing habit to its concern habitat.**

Keywords: **Phascolosoma arcuatum, Deposit feeding, Tentacular Introvert, Anatomical modeling.**

I. INTRODUCTION

Phascolosoma arcuatum (Gray, 1828) is benthic nonsegmented vermiform marine coelomate [19], commonly called "peanut worm", belongs to phylum Sipuncula, found in mud flat of Sundarban mangrove [8]. The genus *Phascolosoma* has narrow tube like 'eversible introvert' and 'peanut' like trunk [7]. Anterior tip of eversible introvert consists of tentacular crown, cerebral ganglia with nerve ring around the oesophagus, simple unbranched contractile vessel and single unsegmented ventral nerve running up to the terminal end of the trunk [19]. There are nearly more or less 150 sipunculan species can be found all over the world [7], where *P. arcuatum* is the only sipunculan representative found in Sundarban mangrove [8]. Still the classification of phylum Sipuncula is mainly based on the macro and micro anatomical structures due to unavailability of detail molecular based information. In this phylum the arrangements of the tentacular apparatus has macrotaxonomic importance [13] and gives important clues about its habits in relation to its habitat [20]. The morpho-anatomy of the tentacular apparatus differs considerably among the taxa of this phylum [14]. According to orientation of the tentacular apparatus, members of this phylum can be divided as 'integumentary breather' with nonselective detritus feeding habit, 'introvert and tentacle breathers' with selective deposit feeding habit and 'tentacle breathers' with cilliary suspension feeding habit [6, 1]. Due to their retracting behavioral response to any external stimuli and small size, it is very difficult to study macroantomical organization of the tentacular crown when it is retracted. Micro-Computed Tomography (MicroCT) is the prime method to study such structures. But this method is highly expensive and less available in eastern world. Here in this study we reconstructed the retracted tentacular head region of *P. arcuatum* in a less expensive way by simple image stacking method using histological images and WINDOWS based image analysis software applications. The present study is emphasized on macro anatomical peculiarities of the tentacular head of *P. arcuatum* by light microscopy and histological image based computer aided modeling technique to find its functional relationship with its concerned environment.

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II. MATERIALS AND METHOD

A. Specimen collection and macroanatomical study:

The specimen was collected from the mud flat of the different canal mouth of Gangasagar island ($21^0$38'02"N, $88^0$04'50"E) of West Bengal, India. Collected specimens are taken to the laboratory by plastic container containing same mud from where they collected. In laboratory, the specimens were settled in glass container in same mud for at least 7 days with maintained moisture and salinity (approx 20-25ppt). Only the healthy organisms with body length more than 5cm±0.5 (when retracted) were selected for the morpho-anatomical analysis. 7.5% isotonic $MgCl_2$ [1] was used to relax the specimen before dissection. The tentacular head was depicted quickly from the fully relaxed organisms and wash several times in 0.1M phosphate buffer saline prepared in FSW (Filtered Sea Water) before fixation. Depicted head was fixed in 10% Neutral Buffered Formalin (NBF) prepared in the same buffer at 4^0C for overnight. For morphological characterization, the whole head was further dissected carefully under dissecting microscope equipped with LED light source and observed and scaled digital micrograph was taken for further analysis.

B. Histological study, Image analysis and model construction:

For histological study NBF fixed head was cryoprotected by 15% and 30% sucrose in 0.1M phosphate buffer for 2 hour at room temperature and overnight at 4^0C, respectively. About 5-7µm transverse and longitudinal sections were cut in cryomicrotome (Leica CM 1850; Germany) at -20^0C. The sections were stained by routine Hematoxiline-Eosine stain [5], methylene blue [4], alcian blue method [5]. For model construction, series of 4-5µm sections were collected on gelatin coated slides, stained and micrograph were taken under light microscope (Leica DM 3000; Germany) equipped with camera attachments. All collected images were analyzed with the help of computer aided software's like ImageJ, Amira etc. The general computational analysis and image stacking was done in ImageJ. The stacked histological images are analyzed with Volren module at different alpha scale on texture-based volume rendering (VRT) mode to render segmented regions with colormap on Amira 5.3.3 software application [12, 15]. During stacking to get proper depth (Y-axis) similar number of multiple images was taken in consideration to adjust the stacking depth.

III. RESULT

The tentacular introvert of *P. arcuatum* possesses tentacular crown with tentacles, C-shaped coller, bilobed ganglia, single nuchal lobe and cavity above the ganglia, simple tubular contractile vessel and mouth opening. Behind the crown there are 25-28 complete and 4-5incomplete rings of hooks are arranged in the anterior introvert. The crown is composed of circularly arranged five distinct semitransparent, unbranched, lobed, tentacles (Fig 1. A-C, T1-T5) surrounded by an incomplete circular thick collar (Fig 1. A-C). The tentacles emerge from a common circular base, situated above the nerve ring.

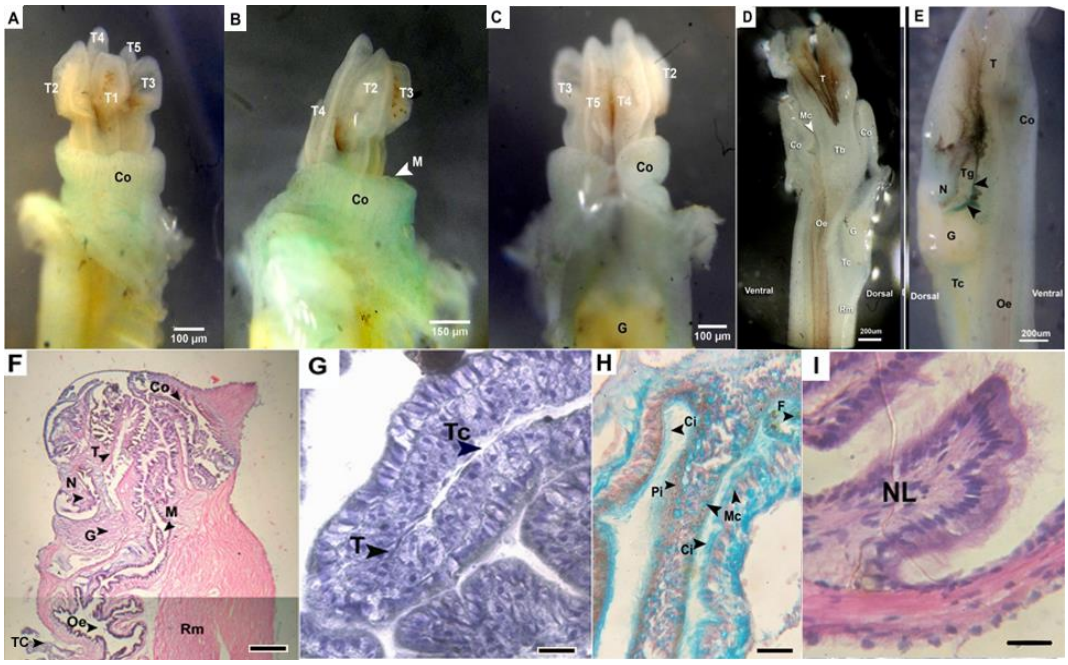

Figure 1: Different views of tentacular Head of *P. arcuatum*.

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A- Ventral view, B- Side View, C- Dorsal view, D- Longitudinally dissected head through mid region of the head when extended, E- Longitudinally dissected head through mid region of the head when retracted, F- Longitudinal histological section through mid region of the head ; Images are superimposed to show the complete arrangement of introvert (Scale: 120µm) (H&E), G to I- Histology of Tentacular epithelium. G. Cellular arrangement of tentacular epithelium. (Methylene blue method) (Scale: 25µm) H- Mucusecretory cells (Alcian blue method) (Scale: 30µm). I- Nuchal epithelium (Scale: 10µm) (H&E).

Abbreviations: Ci- cilia, Co- collar, Cv- contractile vessel, F- food particles, G- ganglia, T,T1to T5- tentacles, M- mouth, Mc- mouth channel, Mu- mucus cells, NL- nuchal lobe, Nu- nuchal organ, Oe- oesophagus, Pi- pigments, Rm- retractor muscle, T- tentacles, Tb- tebtacular base, Tc- tentacular coelom, TE- tentacular epithelium, Tg- tentacular groove.

When fully extended, the average height and width of the tentacular crown is 1-1.2mm and 0.6-0.8mm respectively above the ganglia. The base of the tentacles shows common stalk like appearance with 200-250µm in length and 90-110µm in transversally sectioned crown, which covers 25-30% of the total tentacular area (Fig 1: D). The dorsal tentacle T4 & T5 is little longer (500-550µm) than the T1, T2, T3 (450-480µm) above the collar. The inward surface of the tentacles is wavy and highly pigmented, the outer surface is smooth and devoid of pigments (Fig 1: D, E). Each tentacle possesses tentacular coelom connected to the single simple branched contractile vessel through the common tentacular coelomic connection runs below the ganglia (Fig 2.C). The 10-20 µm long layer of columnar epithelium (Fig 1. G-I) is continuously lined all over the tentacular surface with 5-8µm long cilia. The surface of the tentacles possesses high number of mucus cells (Fig 1. H) with average diameter of 40-70µm. The collar region of the crown is longitudinally striated, greenish in colour, C-shaped, about 90-100µm in thickness & 550-600µm which incompletely circles the tentacular base and supplied with tentacular coelomic connections (Fig 1. C, Fig 2. C & D). The collar is discontinued at the dorsal surface of the nuchal cavity by forming a hinge (Fig 1. C). The tentacular coelom at collar region covers 23-25% of the total collar area. About 1mm^2 area of collar region possesses 75-90 epithelial nucleus and 12-17 fibroblast nucleus (Fig 1. F-I). The mouth channel is shifted towards the dorsal surface and situated opposite to the ganglia. Ventrally it is surrounded by the collar and dorsally by the base of the tentacles (Fig 1. D, Fig 2. C). The diameter and length of the mouth channel about 60-80µm and 250-300µm respectively. There is no direct connection present between the mouth channel and the nuchal cavity (Fig 1. E). When retracted, the collar shifted upward with the introvert and mouth channel become extended (Fig 1. E). In Volren model (Fig 2. A-D) both without colour map (Fig 2. A) and with colour map (Fig 2. B-D) the actual density is demarcated by dense and light area in the model; where the whitish region represents the denseness of the area. Here, we found, in retracted condition the collar extends with the introvert and forms a closed cup like structure which circles the whole tentacular crown (Fig 2. C-D). At the terminal portion, the collar is continuous where at the middle portion the collars become discontinued (Fig 2. C). There is a common tentacular coelomic connection throughout tentacular crown are demarcated as light area at the dorsal side below the ganglia and the light area at opposite side of the ganglia shows the mouth channel and oesophagus (Fig 2. A, B). In colour mapped model, at the tentacular region the dense area can be found due to presence of compact epithelium of the tentacles and collar (Fig 2. C, D). At ganglia there are mostly dense area is situated at the dorsal side where the dense sub regions of the ganglia and retractor muscle shows highly dense muscular regions (Fig 2. B, D).

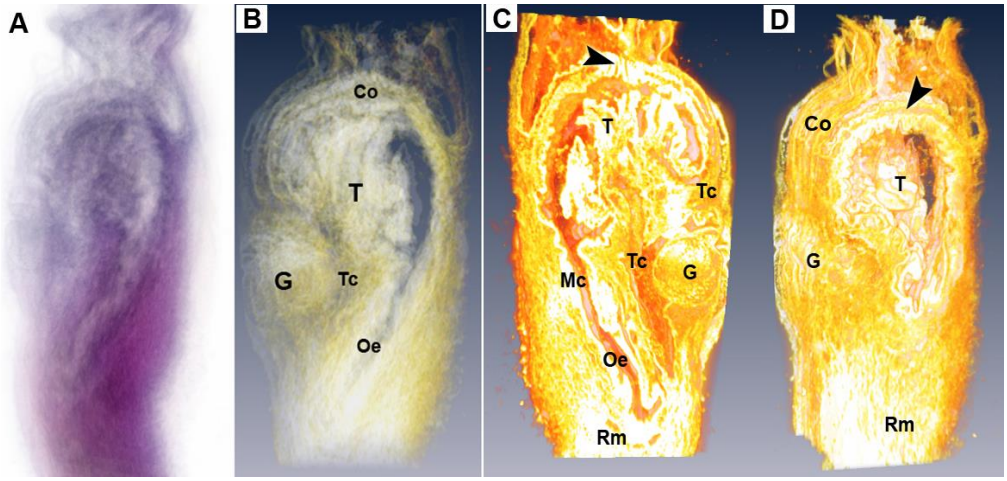

Figure 2: Volren Model of retracted head region.

A & B- Vorlen model normal and colour map images with alpha scale, dense or light regions are directly proportional to the tissue density. C-middle region facing color map 3D model (VRT) Arrow shows collar is discontinuous. D-Side region facing color map 3D model (VRT); Arrow shows continuous collar.

Abbreviations: Co-collar, G- ganglia, T- tentacles, Mc- mouth channel, Oe- oesophagus. Rm- retractor muscle, T-tentacles, Tc- tentacular coelom.

IV. DISCUSSION & CONCLUSION

The tentacular apparatus of *P. arcuatum* possesses fewer tentacles with tentacular coelom, coller, bilobed ganglia, nuchal apparatus, mouth and rings of hooks which follow the basic structural pattern of class Phascolosomatidae [1, 2, 7]. Till date, there is no direct evidence describing the feeding mechanism of class Phascolosomatidea, they may feeds on detritus in the mud they engulfed [3, 6]. Highly ciliated fewer numbers of tentacles with large number mucus cell suggests that they are mainly nonselective deposit feeder. From the anatomy and model analysis we assume that, at first it may extends its introvert through the mud, the secreted mucus at the tentacles helps to gather the mud particles collected by the tentacles. During tentacular retraction the mouth channel extends with the extension of the collar which forms the base of the mouth. Firstly tentacular cilia and then esophageal cilia may drive the organic deposits towards the intestine. Other than morphological differences no definite histological difference was observed among the tentacles under light microscope. Showing in figure 2. B&C, the tentacular coelom is a secondary coelom, connected to a simple contractile vessel which acts like a storage tank of tentacular coelomic fluid and helps in peristalsis movement of the tentacles and collar [3]. The tentacular groove and the anterior dorsal margin of ganglia forms nuchal lobes are thought to be the chemosensory region of many sipunculans [16]. In *P. arcuatum* the distinct bilobed sensory structures is nuchal lobe where optic ocilli or eye spot is unavailable [18]. The anatomical modeling directly represents the virtual model of the tentacular crown of *P. arcuatum* when it is retracted. Generally it is very difficult to observe the arrangement of the crown when it is retracted. The previous structural descriptions about the head of any sipunculan species were based on images acquired from Scanning Electron microscopic images when it is extended. Here, we reconstructs the retracted tentacular crown region from the histological images prepared by general H&E staining to get overall impression of the structures and there reflection in the virtual model. This modeling technique significantly represents the importance of the collar region when tentacular crown is retracted. When retracted the collar extends and forms a closed cup shaped covering around the crown which may helps to protect delicate tentacles from mechanical injury. Histological and modeling images (Fig: 1. F, Fig 2. A-D) clearly shows the continuity of tentacular coelom inside the collar region. Class Phascolosomatidea is considered as “integumentary breathers” due to their burrowing nature, presence of coelomic invaginations [17, 21], tentacular crown with fewer tentacles and simple contractile vesicle [1]. The presence of green and greenish yellow pigment granules in sub-epidermal connective tissue is one of the oldest characteristic features of *Sipunculus nudus* [23]. Apart from that the appearance and role of such greenish pigments is still under investigation [11]. The presence of such sub-epidermal pigments may arise from different metabolic processes [11]. The presence of such pigments in *Phascolosoma arcuatum* may show basic functional similarity or metabolic patterns with other sipunculan groups. In conclusion, anatomical and histological evidences of crown arrangement support that *P. arcuatum* has nonselective feeding habit and tentacles do not majorly helps in gaseous exchange. From modeling study we hypothesize that the collar region plays an important role in protection of the tentacles which helps them to cover the tentacles during retraction and support its deep burrowing nature in the mudflat.

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