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STUDIES ON THE CEPHALOCHORDATES OF MADRAS COAST

IV. Structure of the pharyngeal region and its associated organs

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Although much work has been done on the anatomy of the different systems of *Branchiostoma lanceolatum* of the temperate and other waters (see Barrington, 1965 ; Godeaux, 1967) very little has been published so far on the anatomy of the tropical forms of *B. lanceolatum*. The present paper describes the structure of the pharyngeal region and also brings to light other interesting features in the pharyngeal morphology not noted before. Specimens of *B. lanceolatum* dredged from the inshore area of Madras constituted the material for study. The methods employed for anatomical study have already been described (Azariah, 1966).

Prepharyngeal Organs

Cirri and its skeleton: The oral cirri when seen in transverse section show a central skeletal rod (CR) surrounded by a single layer of cells (E) and separated by a space occupied by blood vessel (Fig. 1). The cells forming the outer layer are rectangular with a centrally placed rounded nucleus(N). This layer is raised into sense papillae (SP) the cells of which have tapering outer extremities. The nuclei are at the base of these cells and are elongated. The central rod consists of a thin structureless outer sheath enclosing a homogenous substance. In the space between the outer layer and the rod, blood (BL) can be seen in a clotted condition. All the cirri are connected at the base by a skeletal ring whose detailed histology is similar to that of the central rod. Bundles of muscle cells are seen attached to the basal portion of the rod of the cirri and it is likely that they move the cirri.

Wheel organ: The inner epithelium of the vestibule consists of thin cylindrical cells. They have darkly staining nuclei. Some of the cells have vacuoles. Where the wheel organ lobes occur the cells of the epithelium are taller and present thickened ridges. In the centre of the ridges the cells are again shorter, thus giving rise to grooves. These short cells bear cilia. The cells of the wheel organ stain darker than the rest of the epithelium.

Ciliated pit organ: The ciliated pit organ, measuring 350 μ in diameter, occurs in the middle part of the roof of the vestibule (Fig. 2). Anteriorly the pit appears as a shallow depression of the general epithelial cells (E) which are cylindrical like the rest of the vestibular cells and are ciliated. Towards the centre of the pit, the depression deepens (Fig. 3) and then becomes shallow again. Thus, the ' ciliated pit ' is a definite ciliary organ. In transverse sections it appears like a funnel inserted into the lining layer of the vestibule. But as can be seen from figure 2, the layer of cells bearing the cilia appears to be the well developed thickened epithelium with a very thick peduncular development of connective tissue (CO). These two layers of the

ciliary organ appear continuous with the vestibular lining all round the ciliary organ. Since the ' pit ' does not lead into the interior, it is probable that the lining serves to increase the mucus secreting layer. This fact was tested by increasing the concentration of particulate matter and observing the activity in a live specimen. It was found that copious secretion of mucus was stimulated and it is probable that food particles were trapped in this thick mucus which was swept into the pharynx.

PHARYNGEAL REGION

Velar membrane and oral aperture: Behind the vestibule, i.e., below the apex of the seventh myotome, occurs the velar membrane. This is funnel-shaped and leads to the oval-shaped opening of the ' mouth '. The opening is situated more ventrally and not in the centre of the velum since the dorsal side of the funnel is 120 μ whereas the ventral side is 160 μ in length. The structure of the velum shows that the outermost layer is a continuation of the vestibular lining. The ventral area round the mouth consists of cells which are ciliated. The lateral edges of the mouth are produced into ten tentacular processes which extend behind the limit of the mouth. Cells occurring at the anterior surface of the velar membrane are similar to those of vestibular region and the cells lining the posterior region resemble the cells found in the pharyngeal epithelium. Between the two lie the connective tissue and muscle fibres which are more on the dorsal side than on the ventral side. The muscle fibres on the dorsal side are extended into the anterior part of the velar tentacles which, probably, are contractile.

Pharynx: Transverse sections of six lancelets of different size (age) groups were prepared. A preliminary examination of these sections through the anterior end of the pharynx shows : (1) that the endostyle (EN) starts between the 8th and 9th myotome and the epipharyngeal groove (EG) starts at about the 9th myotome (Fig. 5) ; (2) that there are no peripharyngeal grooves in these forms. Instead there are two short lateral grooves (LG) which start anteriorly about the apex of the 8th myotome and run backwards and upwards to join the epipharyngeal groove at the point of its formation (see Figs. 4 and 5). These two lateral grooves have a maximum length of 500 μ and do not go down the sides to meet the endostyle. The monograph of Willey (1894) mentions the endostyle as extending far anterior to the commencement of the epipharyngeal groove and earlier authors describe the peripharyngeal groove as really long grooves embracing the pharynx and uniting with the front tip of the endostyle, (see Orton, 1913, page 227). So a careful and detailed examination of all the sections of amphioxus through this region was made. This study shows that the posterior edges of the funnel-shaped velum (V) were pushed into the cavity of the pharynx. The velar tissue thus distinguished part of the pharynx dorsal to it and a part of the pharynx ventral to it in the course of fifteen sections (150 μ). Further, Figure 4, section taken more posteriorly, shows only the portion of the lateral edges of the velar tissue. It is in this region that the lateral grooves are first distinguished. Anterior to this, careful examination showed neither the lateral groove nor the endostyle. Figure 5 which corresponds to the sections through the region of the 9th myotome, the lateral grooves reach the ceiling of the pharynx and help to constrict it to form the epipharyngeal groove. It also shows the beginning of the endostyle as a shallow groove on the floor of the pharynx.

(i) *Lateral grooves :* Each lateral groove is 500 μ long and is formed by the epithelium lining the pharynx. Lining the groove are tall cylindrical cells with vacuolated cytoplasm and a peripherally situated nucleus (Fig. 6). The nuclei do

not take Mallory's triple stain avidly nor does the cytoplasm. The lateral grooves measure 6.02 μ across at its commencement but about the middle of their length, the groove broadens to 15.05 μ and has a depth of 18.06 μ. The two side walls which measured about 6 μ at the thick edges, increase to twice their thickness. However, where the lateral groove ascends to form the epipharyngeal groove it becomes broadened to 24 μ and is more shallow. The lateral grooves are ciliated.

(ii) *The epipharyngeal groove :* This groove commences about the level of the 9th myotome and extends upto 28th myotome where the pharynx opens into the oesophagus. Its commencement is facilitated by the dorsal ends of the lateral grooves constructing the roof of this groove (Fig. 5). The cells forming the epipharyngeal groove are, however, distinguishable from those of the lateral grooves by their greater affinity for stains. In the region of the 10th myotome (Fig. 7) the groove becomes narrow and well-defined ; the two lateral walls are thickened, by tall cylindrical cells. The cells forming the roof of the groove as well as those forming the sides take the stain more deeply and bear cilia at their inner ends. The nuclei are centrally situated in the dorsal cells but scattered in the lateral cells. The groove measures about 72 μ at its commencement and posteriorly it narrows gradually to measure between 30 and 45 μ. While joining the oesophagus it has a depth of about 45 μ.

(iii) *The endostyle :* The commencement of the endostyle (EN) varies in different specimens. In some, sections through the 8th myotome show the endostylar formation on the floor of the pharynx while in others the commencement is evident only in sections passing through the apex of the 9th myotome. The endostyle extends upto the oblique junction between pharynx and oesophagus. At the point of commencement the endostyle is shallow and broad but becomes deepened and concave more posteriorly. This concavity increases and the groove appears as an incomplete tube where the pharynx joins the oesophagus.

As in the case of epipharyngeal and lateral grooves, the endostyle is formed by a single layer of pharyngeal epithelium. When the groove first appears it is in continuation with the whole pharyngeal epithelium and very shallow (Fig. 5). It assumes the shape of a definite groove only about the level of the 10th myotome. Haematoxylin preparations show vacuolated cells with rounded nuclei. They are the mucus secreting cells (MC). The rest of the cells forming the endostyle are cylindrical in shape with oval shaped nuclei and bear cilia.

These two kinds of cells are arranged in two separate groups i.e. there are four groups (I, II, III, IV) of secreting cells arranged alternately with five groups (1, 2, 3, 4, 5) of ciliated cells. Recently Olsson (1963) has described the nature of secreted substances by the secreting cells of endostyle. The odd ciliated block (3) of cells occupying median position within the groove can be distinguished by the fact of their longer cilia. However, in the very anterior and posterior tips of the endostyle there are only one group of mucous secreting cells. The five groups of ciliated cells help in moving the mucus threads and other secreted products formed by the secreting cells.

The endostyle is supported by structureless skeletal pieces (SP). Lankester (1889) in describing this skeletal support says 'it consists of a number of pieces following one another, corresponding in number to the primary gill slits, each piece being composed of a loosely joined overlapping right and left half'. Though this description is true in the main, a few more details may be added. The skeleton consists of a series of

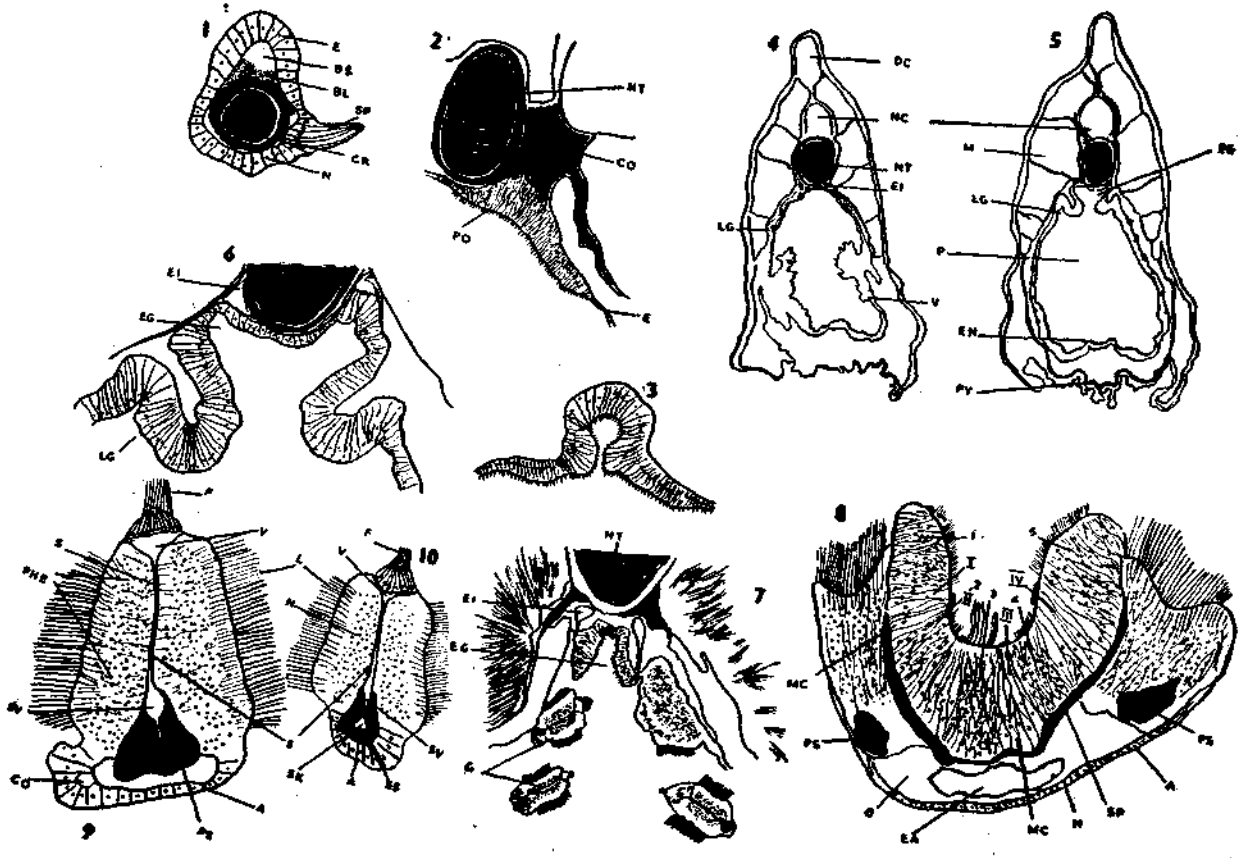

FIG. 1. Transverse section through the oral cirri. FIG. 2. Transverse section through the anterior region of the preoral ciliated pit. FIG. 3. Transverse section through the middle of the preoral ciliated pit. FIGS. 4 and 5. Transverse sections through the region of 8th and 9th myotomes respectively. FIG. 6. Epithelium lining the lateral groove. FIG. 7. Structure of the epipharyngeal groove. FIG. 8. Structure of endostyle. FIG. 9. Primary gill bar. FIG. 10. Secondary gill bar. EI: Epibranchial artery, DC: Dorsal fin chamber, MY: myotome, NC: Nerve cord, NT: Notochord, PO: Ciliated pit organ. PY: pterygeal muscle. (Rest of the letterings are referred to in Text).

skeletal plates one on either side. The plates of series on each side occupying the region of the primary gill slits, but as the gill slits on either side alternate in position, the plates on each side also alternate. Ventrally the two plates overlap to an extent of about 20 μ. Thus, the segments of endostyle are not supported on each side. The primary gill bar forks ventrally, each branch of the adjacent bars goes below the plates between and support it. Microscopically the endostylar skeleton is of the same consistency as that of the pharyngeal skeleton. Below the endostylar skeletal plate is the subendostylar coelom (C) bounded by the atrial-epithelium (A) with elongated nuclei. In this region runs the endostylar blood vessel (EA).

(iv) *Gill bars :* The lateral walls of the pharynx are cut into gill slits. The gill slits are added proportionately to the length of the lancelets. Thus, it was found that the lancelets measuring 14 mm. in length had 70 gill slits ; 18 mm.- 80 gill slits ;

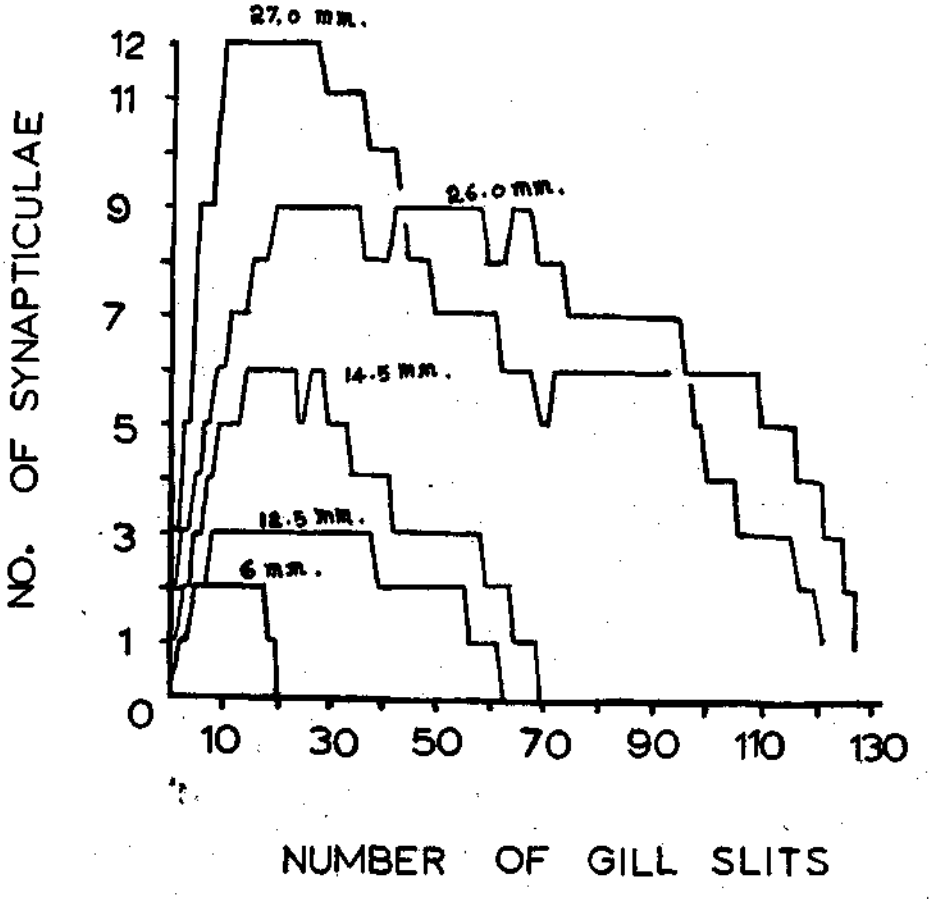

FIG. 11. The relationship between the number of synapticulae and gill slits.

and 23 mm.- 124 gill slits (Fig. 11). The number of gill bars (primary and tongue bars) is 124 in a specimen of 23 mm. Of these the primary gill bars are 63 in number.

The same number of gill slits (124 in 23 mm.) has been counted in the European form having a length of 50 mm. The structure of the pharynx can be described under two heads : (1) the pharyngeal epithelium, (2) pharyngeal skeleton.

(1) *Pharyngeal epithelium :* The lining epithelium of the pharynx covers the gill bar on three sides, the fourth side is covered by the atrial eipthelium (Figures 9 & 10 PHE, A). The epithelium covering each gill bar can be distinguished into two kinds of cells : (a) the epthelium covering the inner end of the gill bar consists of single layer of cells arranged in a conical manner to give a tapering end to the gill bars. The outer ends of the cells have short frontal cilia (F) (b) the pharyngeal epithelium covering the two sides of the gill bar also consists of a single layer of tall cells with scattered nuclei and bear long lateral cilia (L). Separating the two layers of cells medially is the septal membrane (S) which is hollowed out to permit two blood vessels one at the inner end (V) and one at the outer end (SV). The nuclei of the cells stain deeply and are circular in form.

The gill bars are bounded on the side facing the atrium by elongated columnar cells constituting the atrial epithelium with round nuclei. Brown pigments are found in the cells near the pharyngeal epithelium. The atrial epithelium is devoid of cilia.

(2) *The pharyngeal skeleton*

(*a*) *Skeleton of the primary bar :* Each bar is supported by two skeletal rods (PS) pressed together and separated by only a narrow interspace as seen in a cross section. Such a double rod runs through the atrial edge of the gill bar. The two halves separate at the ventral end in such a way that each supports the edge of an endostylar plate. The dorsal end of the rod also forks and each half arches over to unite with those of the adjacent primary gill bar skeleton.

(*b*) *Skeleton of the tongue bar :* From the middle of the arch, the skeletal rod (SS) of the tongue bar descends ventrally but does not fork. It has a simple termination. The skeletal rod of the secondary gill bar is thinner measuring 28 μ across whereas that of the primary gill bar is doubled. The rod of the tongue bar is hollow and contains the blood vessel (SK).

(*c*) *Cross bars :* The vertical skeletal rods, the primary and secondary gill bars are connected across the gill slit by cross bars or synapticula which are covered by thin epithelium. The cross bars are hollow, the lumen measures 3.8 μ. The lumen of the tongue bars are continuous with the cavity of the synapticula and serve as passages for blood vessel. These blood vessels form a network of vessels running vertically through the tongue bars, primary bars and horizontally through the synapticulum. The number of cross bars in the anterior, middle and posterior gill slits of the same specimen varies. Further, the younger lancelets have lesser number of cross bars than the older specimens. To show that this variablity has no relation to either the length or the age of the specimen, a detailed study was made. Moreover, the ventral part of the pharyngeal skeleton is relatively crowded with cross bars than the dorsal part. It is therefore, concluded that the synapticulae are added to the pharyngeal skeleton from the base. Figure 11 shows the relationship between the number of synapticulae and the vertical gill slits. It can be seen in the graph that in the young form of 6.00 mm. in length, the maximum number of synapticulae joining the skeletal rods of the gill bars is only 2 ; in 12.5 mm. forms it is 3 ; in 14.5 mm. it is 6 ; in lancelets of 26 mm. length the number increases to 9

and in the 27 mm. long lancelets 12 cross bars were found as maximum. From these data one can infer that the number of synapticulae increases with age but the increase is not proportional to the length.

(3) *Blood supply to the gill:* Both primary and tongue bars (Figs. 9 & 10) have three blood vessels, namely visceral (V), somatic (SV), and skeletal (SK) blood vessels (Benham, 1894). Willey (1894) calls them as internal, external and coelomic vessels respectively. The inner and outer edges of the septal membrane of the gill slit lodge the hollow of the skeletal rod and in the case of primary gill bar the vessel is enclosed in the atrial epithelium just near the pharyngeal coelom. The three vessels of the primary bar spring on the ventral side from the endostylar blood vessel through an enlarged vascular bulbil which is clearly seen in a sagittal section. All the three vessels at the dorsal end of the gill bar unite to form a common efferent vessel which joins the epibranchial artery. The blood supply for the tongue bar is recuited from the blood vessels of the primary bar through the vascular connections between the gill bars. The cross vessels vary in number according to the number of the skeletal cross pieces. The blood, thus, collected in the tongue bar runs upwards to empty into the epibranchial artery separately. The blood vessels of the tongue bar are connected only with the epibranchial artery on the dorsal side whereas on the vental side they are connected with each other.

Diffusion of oxygen from the surrounding water into the blood can take place in all the three vessels of the primary bar whereas in the secondary bar it is possible only in the two vessels namely the somatic and visceral since the skeletal vessels is inside the skeleton. The secondary gill bar, though useful in detaining minute particulate food matter, is of subsidiary importance so far as respiration is concerned. This lends support to the conclusions from the experiments reported by Azariah (1968) that the pharynx is concerned primarily with food procuring.

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