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Microanatomical study of thymus gland of *Aplocheilus panchax* (Hamilton, 1822)

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A B S T R A C T

Aplocheilus panchax is an indigenous larvivorous fish (Teleost: Cyprinidontiformes) of South- East Asia. Microanatomically, the thymus gland of *A. panchax* is triangular in shape and almost close to the base of third and fourth gill arches within the pharyngeal wall of the opercular cavity. The cortex and medulla is not well demarcated. Thymus is also closely packed with thymocytes. Mucous cells are noted at the periphery region of thymic stroma. In the transmission electron microscopical (TEM) observation shows a few thymic components (e.g., thymocytes, thymoblast cells, thymopharyngeal epithelial cells, reticular epithelial cells, goblet cell, granulated leucocytes *etc.*) within the thymopharyngeal region. Different cellular architecture of the thymopharyngeal part in thymic stroma has been examined to correlate its functional significance of the concerned region.

Introduction

Thymus gland is the lymphohaemopoietic organs in fish (Zapata, 1981) and it was first reported in *Lophius piscatorius*, the angler fish (Stannius, 1850). This gland is present in all fishes (Lele, 1934 – 35) except Agnathans (Good *et al*, 1966), although they possess small mononuclear cells supposed to be the lymphocytes (Kendall, 1992). Among the commercially important fishes, (e.g., Salmonids, Carps, Catfishes, Tilapias, *etc.*) the thymus gland are well studied (Zapata, 1981; Chilmonczyk, 1992). The role of thymus gland in higher vertebrates in relation to aging, neuroendocrine action, breeding, behavior, *etc.* are not well nurtured. In India, the work related to thymus biology was first reported by Sailendri and Muthukkaruppan (1975) on *Tilapia mossambica*. Owing to that the unique structure and its variety of cells of thymus gland is always an intensive part in thymic research. *Aplocheilus panchax* is a trash fish and they are distributed all over South-East Asia. Generally they are very

fast swimmers, surface dwelling and they can tolerate in among freshwater, brakishwater (Jayaram, 1999) and polluted water respectively. This paper focused on light and transmission electron microscopical studies on thymus gland of *A. panchax* with special reference to the cell structure of the surface part thymic environment.

Materials and Methods

'Least concern' fish species *Aplocheilus panchax* was collected from different water bodies of Village Rupasgori (Howrah, West Bengal, India; Lattitude 22º46′N and Longitude 87º96′E). Fresh, young, *A. panchax* [Weight 2-3 gm. & Length 3-6cm.] was acclimatized in laboratory for light microscopical (LM) work. Microanatomical studies were conducted following standard literatures by Mohammad et al., 2007 and De *et al*., 2015. The thymus gland was removed from the opercular cavity of the studied species and was instantly infiltrated with freshly prepared aqueous Bouin's fluid for 24 hour at room temperature (20ºC-25ºC). After fixation the tissue were then washed and subsequently dehydrated through graded ethanol, cleared with xylene and embedded in paraffin. Sections were cut at 5 µm thick by using rotary microtome. The section were then stained with haematoxylin-eosin and examined under light microscope (Model No micro imaging GmbH, serial no- 3109003139, Carl Zeiss-Germany). Microanatomical study were carried out through semithin section, thymus tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4**)** at 4^{o}C for a period of 2hours and washed in 0.1 M phosphate buffer. Post-fixation was done by 1% osmium tetraoxide (OsO_4) in 0.1 (M) phosphate buffer (pH 7.2-7.4) for 1 hrs. and dehydrated in a graded ethanol. Infiltration and embedding were done by the resin mixture and then sections were cut by a glass knife using the ultramicrotome. Semithin sections (0.5µm) were stained with 0.1% toluidene blue (1% sodium borate) and viewed under light microscope (LM). For transmission electron microscopic (TEM) study, the ultrathin sections (600-800) Å were cut by ultramicrotome and stained with uranyl acetate and lead citrate and examined under transmission electron microscope (TEM: MORGAGNI – 268D), operated at 40kV.

Results and Discussion

Anatomically, the thymus glands of *Aplocheilus panchax* (Figure-1)is a triangular shape and well connected in front of third and forth gill arches in the opercular cavity (Figure-2). In *A. panchax*, the thymic tissue is not differentiated into a cortex and medulla respectively (Figure-3). Mucous cells are noted at the periphery region of thymic stroma (Figure-3). Thymic environment consists of e.g., thymocytes, thymoblast cells, thymopharyngeal epithelial cells, reticular epithelial cells, goblet cell, granulated leucocytes *etc.* is also identified in this fish. Thymocytes are the chief components and intimately packed within the thymus tissue (Figure-3). These cells are frequently observed within the thymic tissue. Thymocytes are very small and round in shape (Figures-3, 6, 7). The nucleus is prominent and the condensation of chromatin is more towards the periphery of the nuclear envelope (Figures-6, 7). Nucleus is well chromatinized within the thymoblast cell (Figure-6). The thymopharyngeal epithelial cell (TPE) is observed with long prominent nucleus. Heterochromatin materials are distinctly distributed within the peripheral part of the nuclearplasm. The cellular junction *i.e*., desmosome is observed between two adjacent TPE cells (Figure-4).

The reticular epithelial cell shows extended cytoplasmic processes (Figures-6, 7). The occurrences of Goblet cells are evident within thymic stroma of *A. panchax*. The cytoplasm of these cells is filled with many secretory vesicles (Figure-5). The cytoplasm of developing granulated leucocytes contains distinctive granules and mostly similar in size. The cytoplasm of the granulocytes contains distinctive granules and mostly similar in size. Nucleus is round in shape. Villiform vacuoles are also found near the granules (Figure-6).

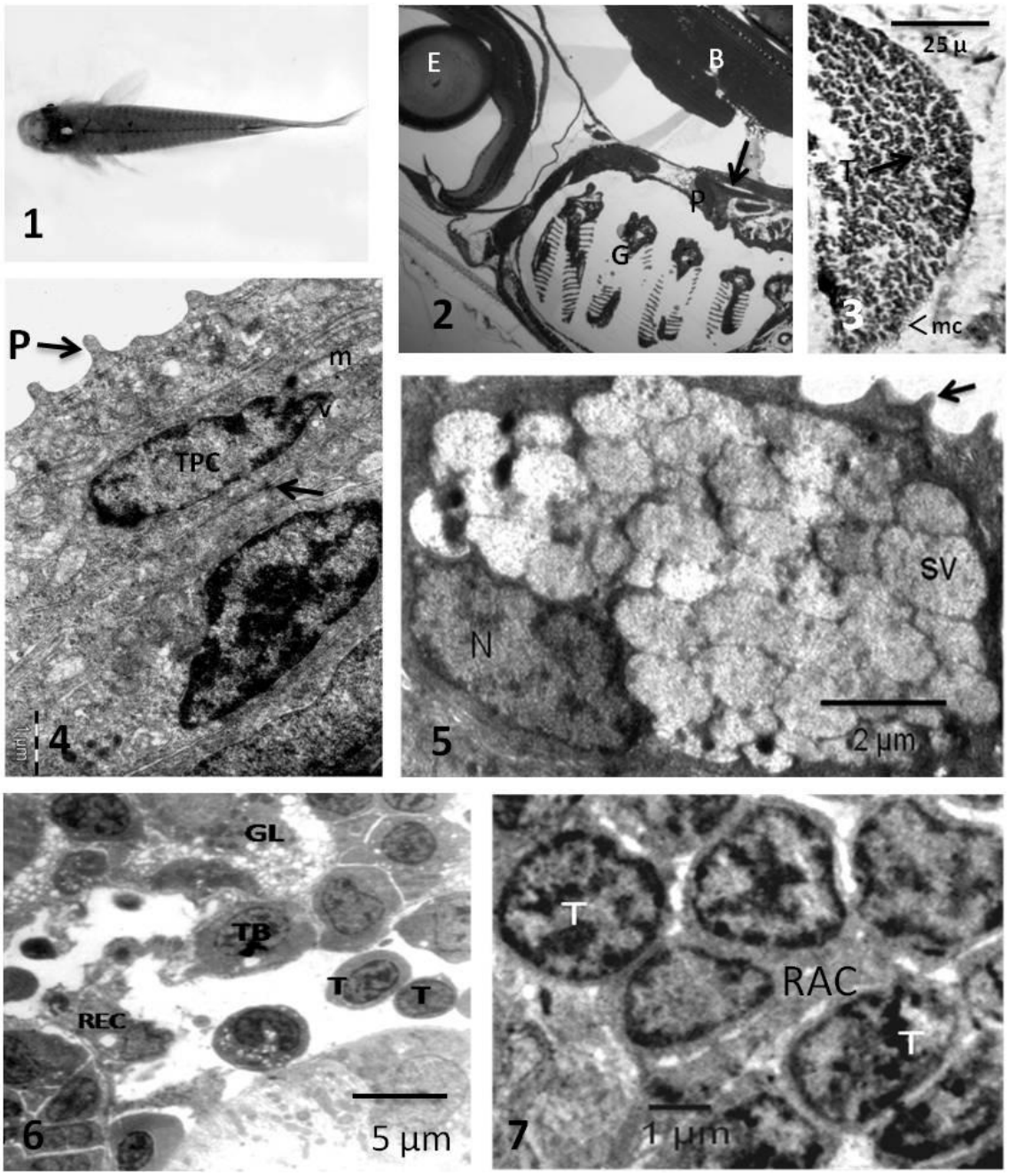
The present work emphasized on the anatomical based light and electron microscopical studies of the thymus of *A. panchax*. The character of a particular fish species may depends on the diverse position of thymus gland (Xie *et al*., 2006). Mostly, the thymus tissues retain its association with the pharyngeal epithelium. In bony fish, the thymus is intimately associated with the pharyngeal epithelium during development (Grace and Manning, 1980). In *Siniperca chuatsi*, a mandarin fish where the thymus gland is very close to pharyngeal epithelium and connected to the pharyngeal cavity (Xie *et al*., 2006).

The paired thymus glands of *A. panchax* are located near 3rd and 4th gill arches in opercular cavity and well connected with the pharyngeal epithelium. The junction of thymic cortex and medulla is not properly separated in many fish species (Liu *et al*., 2004; Mohammad *et al*., 2007). In *A. panchax* there is also no clear differentiation of thymic cortex and medulla which is a contradictory part in the thymic research (De and Pal, 1998). The thymus gland contains of thymocytes, thymoblast cells, thymopharyngeal epithelial cells, mucous cell (goblet cell), reticular epithelial cells, developing granulated leucocytes, *etc*. in *A. panchax* which shows similarities with other higher vertebrates. The colonization by the lymphocytes of the pharyngeal epithelium is very important steps in the developmental histogenesis in thymus of *Dicentearchus labrax* (Zapata *et al*, 1996). In *A. panchax* the organization of thymocytes are broadly similar to those observed in some teleosts (De *et al*., 1997; Bowden *et al*., 2005; Mohammad *et al*., 2007).

Thymoblast cell is frequent in thymic cell mass and well chromatinized nuclei with different level of condensation (De and Pal, 1998) which is very similar in *A. panchax*. Thymic pharyngeal epithelial cell (TPC) is very prominent and close to pharyngeal structure. These epithelial cells have been recommended to perform as a blood thymic barrier, as suggested for other vertebrate species (Raviola and Karnovsky, 1972; Zapata, 1981). Reticular epithelial cell (REC) with cytoplasmic processes is well observed in *A. panchax* and this type of similar observation is also reported in Australian lungfish, *Neoceratodus forsteri* (Mohammad *et al*., 2007).

In carp, reticular epithelial cells are seen with long nuclei, loose chromatin and cytoplasm that is granular with processes between lymphocytes (Schneider, 1983). Although the presence of a thymic epithelial network in a variety of fish species is well established (Castillo *et al*., 1991; Abelli *et al*., 1996). Very little information is available about the granulocytes of bony fishes. Granulocytes have been observed in thymus of several fish species (Romano *et al*., 1998). In *A. panchax*, granulated leucocytes are present and this type similar observation is reported in *Cyprinus carpio* (Rowley *et al*., 1988). Granulocyte accumulation on the apical zone possibly will imply the value of the thymus in protection against pathogens (Xie *et al*., 2006).

Figure.1 – The external morphology of *Aplocheilus panchax* (Hamilton, 1822), a larvivorous fish (Not to Scale); **2** – Semithin section also indicates the position of thymus gland within the head of *A. panchax*. [thymus (T), gills (G), eye (E) and brain (B).] [Mag. 100 X (approx.)]; **3** – The histological section of the thymus gland shows thymocytes (T), mucous cell (mc) etc. in *A. panchax*; **4** – Electron micrograph shows thymopharyngeal region in thymus gland of *A. panchax*. [pharyngeal wall (P), thymopharyngeal epithelial cell (TPC), Mitochondria (m), vacuoles (v), cellular junction (arrow)]; **5** -The mucous cell (Goblet cell) in which the cytoplasm is filled with many secretory vesicles . [nucleus (N), secretory vesicle (sv), pharyngeal wall (arrow)]; **6**- Thymic stroma of *A. panchax* shows the thymocytes (T), thymoblast cells (TB), reticular epithelial cell (REC) and granulated leucocytes (GL); **7** – The reticular epithelial cell (REC) shows cytoplasmic processes within thymic tissue of *A. panchax*. Thymocytes (T) are also observed.



So, the cellular association within the peripheral part of the thymic stroma may help in defending the thymus gland of this vary fish. It appears that the thymic microenvironments and the tissue organizations of the fishes are frequently related to those of higher vertebrates (De and Pal, 1998). In spite of the fact that the thymus glands in the fishes received quite uneven attention from the zoologist (Deanesly, 1927-28). The brief explanation is based on the structural details of the thymus of *A. panchax* which is susceptible to pollutant in relation to their growth and development.

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