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Short Communication

Anatomical, histoarchitectural and topological studies on the olfactory organ of freshwater garfish, *Xenentodon cancila* (Hamilton, 1822)

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Abstract: The olfactory structure of *Xenentodon cancila* (Hamilton, 1822) were explored by advancement in microtomy, staining and ultrastructural practices. The unique feature of the olfactory system was that the olfactory cavity, an open groove with an obtruding sole lamella, no rosette like organization. The lamella was constituted of the central core, lined on both sides by well-organized epithelium. The central core usually consisted of connective tissue fibres and blood capillaries. The epithelium exhibited compact cellular distribution and made up of receptor cells, supporting cells, lymphatic cells, inner most basal cells and almost never mucous cells. Morphologically specific two types of receptor neurons were recognizable: ciliated and microvillous, comprising sensory terminals. The cellular constitution of olfactory mucosa was explained with olfactory sensitivity of the fish necessitated.

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Introduction

Olfaction is the crucial chemosensory channel, playing essential role in the behaviour of teleosts. It is involved in food finding, enemies' recognition, mate selection, parental behaviour, shoaling, migration and in many more approaches (Song, 1987). Olfaction is accomplished by olfactory receptor cells on the mucosal surface which remains directly exposure to surrounding aquatic environment (Singh et al., 1995). Among vertebrates, fishes exhibit considerable variations in the anatomical and morphological features of the olfactory organ as depicted by Kasumyan (2004), Hansen and Zielinski (2005), Ghosh (2012), Kuciel et al. (2013), and Oliver and Oliver (2019). Olfactory mucosa consists of a mosaic of sensory cells, classified as ciliar, microvillar, rod and crypt receptor neurons which have high particularity and sensitivity to chemical stimulation. In teleostean fishes, the structural specialization and function of olfactory organ is related to their ecological niche (Hara, 1994). The structural peculiarity of the olfactory organ in needlefishes has been attracted by researchers (Singh, 1972, 1977; Theisen et al., 1980).

Xenentodon cancila (Hamilton, 1822) is a carnivorous silver needlefish, feeds preferably on insects, crustaceans and small fishes (Gupta and Banerjee, 2017). There is no report about the cellular details in the olfactory mucosa of Asian garfish. Thus an attempt has been taken to investigate the olfactory structure in *X. cancila* (Beloniformes: Belonidae) at the light and scanning electron microscopic level.

Materials and Methods

Collection of sample: A total fourteen mature specimens of *X. cancila* (18.5-24.5 cm) were collected from Damodar River, nearby Jamalpur (23.061089°N, 87.992584°E) of Purba Bardhaman, West Bengal. The specimens were identified based on Misra (2003). Fishes were anaesthetized with 0.01% ethyl 3-aminobenzoate methanesulfonate (MS-222; Merck) solution and sacrificed following the protocol of the institutional animal ethics committee. The olfactory organs were dissected out precisely to display the position under a stereoscopic binocular microscope (Magnus MS24) and rapidly processed for histology

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Figure 1. The olfactory organ of *Xenentodon cancila*. (A) Lateral aspect of *X. cancila* showing the olfactory pit (arrow). (B) Enlarged view of head showing the position of olfactory organ (arrow) in front of eye (E). (C) Dissected dorsal view of head region shows olfactory organ (O) along with elongated olfactory tracts (arrows), olfactory lobe (OL), cerebrum (CB), optic lobe (OPL), cerebellum (CL) and medulla oblongata (MO).

and scanning electron microscopy.

Histological preparation: Olfactory tissues were immersed in Bouin's fluid for 18 h. After fixation, the tissues were washed in 70% ethanol, dewatered in an ascending series of ethanol, cleared in dimethyl-benzene and embedded in paraffin (56-58˚C, Sigma-Aldrich). Serial sections of tissue block were cut at 4 µm thickness using a rotary microtome (Weswox MT-1090A). Tissue sections were stained with Delafield's Haematoxylin-Eosin (Romies, 1968) and Mallory's Triple (Mallory, 1936). The staining slides were viewed and photographed under light microscope (Carl Zeiss Primo Star) equipped with microscope camera (Tucsen 5.0 MP).

Scanning electron microscopic preparation: Ahead removal of the olfactory organs, they were rinsed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 10 min. Then the olfactory organs were dissected out and washed with 1% polyoxyethyl-enesorbitan monopalmitate solution (Tween 40; Merck) to remove the adhering mucus and debris from the surface. The samples were fixed in glutaraldehyde of same concentration for overnight at 4˚C and additionally fixed in 0.1 M phosphate buffered (pH 7.4) 1% solution of osmium tetroxide (OsO4*;* Sigma-Aldrich) for 1 h at room temperature. After dehydration through a graded acetone series followed by isoamyl acetate, samples were dried by CO_2 critical point apparatus. After sputtered with platinum (using BT-150 Sputter coater, Hind High Vacuum Co. Pvt. Ltd.), the olfactory organs were observed and photographed in the scanning electron microscope (Zeiss Evo 18).

Results and Discussion

Anatomy: Paired olfactory organs of *X. cancila* are located in the depression of ethmoidal region of the skull (Fig. 1A). They are placed on the dorso-lateral side of the head, near the eyes in the shape of simple open pits. The usual olfactory rosette is absent, replaced by a protruding solid lamella (Fig. 1B). This laminate rosette has stubby base which is lodged in the ground of the groove. The thin elongated olfactory tracts which are composed of a bundle of olfactory fibres, connect the olfactory organ and olfactory lobes of forebrain (Fig. 1C).

Histology: The olfactory lamella is made up of central core, enclosed by pseudostratified olfactory

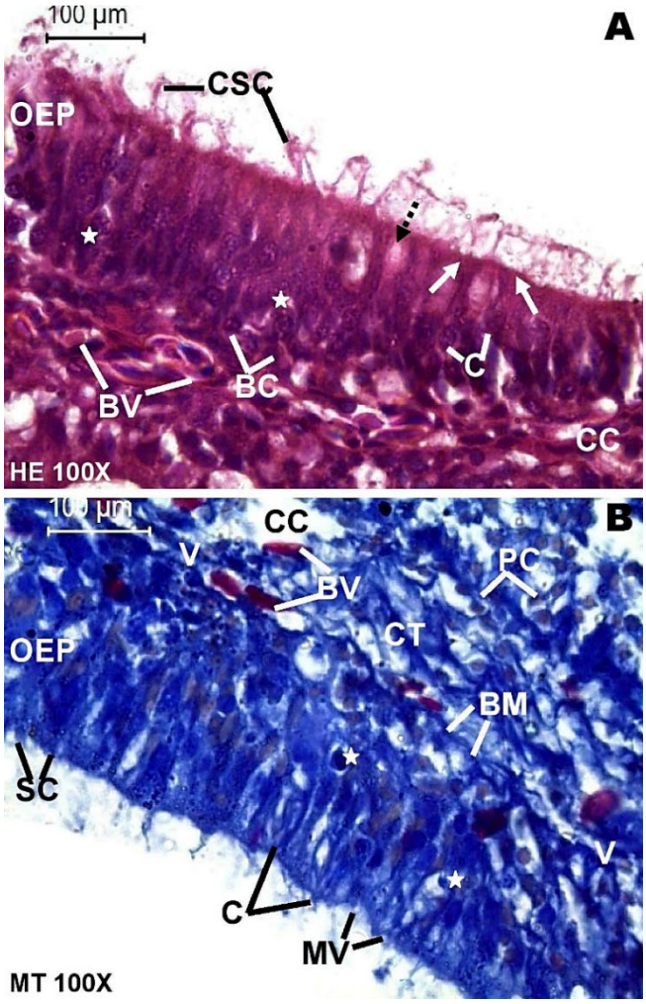

Figure 2. Histology of the olfactory lamella of *Xenentodon cancila* stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's Triple (MT). (A) Olfactory epithelium (OEP) exhibiting ciliated receptor cells (C) with apical protrusions (solid arrows), ciliated supporting cells (CSC), mucous cell (broken arrow), lymphatic cells (asterisks) and basal cells (BC). Central core (CC) contains blood vessels (BV). (B) Pseudostratified olfactory epithelium (OE) shows ciliated receptor cells (C), microvillous receptor cells (MV), non-ciliated supporting cells (SC), lymphatic cells (asterisks) and basal cells (arrow heads). CC consists of connective tissue (CT), blood vessels (BV) and pigment cells (PC). Note the presence of basement membrane (BM) in between OEP and CC.

epithelium on both sides (Fig. 2A-B). The basement membrane separates the olfactory epithelium from the central core (Fig. 2B). The central core is composed of connective tissue, nerve fibres, fibroblasts and pigment cells. Blood capillaries are encountered in this region. The olfactory epithelium is comprised of supporting cells, basal cells, rarely occurring mucous cells, lymphatic cells and notably two types receptor neurons: ciliated and microvillous cells. The cell types are evenly differentiated by the morphology, contour, staining property and their position in the mucosa. The ciliated receptor cells are compactly arranged in proximal zones, characterized with spindle shaped body having basally located dark nuclei and extension of dendrites bearing apical swelling over the epithelial surface (Fig. 2A-B). Relatively smaller microvillous cells are closer to epithelial lining; contain gentle stained rounded nuclei (Fig. 2B). The non-ciliated supporting cells are intermingled in the epithelium, provided with intensely stained nuclei and faintly discernible chromatin material. The ciliated supporting cells are columnar in shape, having acentally located nuclei and granular cytoplasm (Fig. 2A). The mucous cells are rarely observed in the surface zone. They are empty in nature due to release of muciferous contents. Oval shaped lymphatic cells are scattered throughout the mucosa, contain profoundly stained nuclei and discreet cytoplasm (Fig. 2A-B). The nuclei hold maximum of cell size. The rounded basal cells with large central nuclei are buried in the distal zone of mucosa, over the basement membrane.

Scanning electron microscopy: The olfactory organ is rapheless and contains one lamella. The surface of lamella contains structurally distinct ciliated receptor cells, microvillous receptor cells along with ciliated supporting cells and stratified epithelial cells. Sporadically occurring ciliated receptor cells have cylindrical projected sensory dendrites on the free surface (Fig. 3A, C). Microvillous cells bear murky carpet of tiny microvilli, display sculpt surfacing (Fig. 3A). The microvilli are stubbier in comparison to cilia. The sensory receptor cells are overlapped in the mucosa. Mass of the ciliated supporting cells show squashy appearance. They bear nonsensory ciliary endings emerge from cell bodies (Fig. 3B-C). Stratified epithelial cells are arranged like epidermal outside pattern, marking with microridges (Fig. 3C-D). A very few number of mucous cells along with secreted mucin are observed in between stratified epithelial cells.

Chemical stimuli are exposed by apical parts of

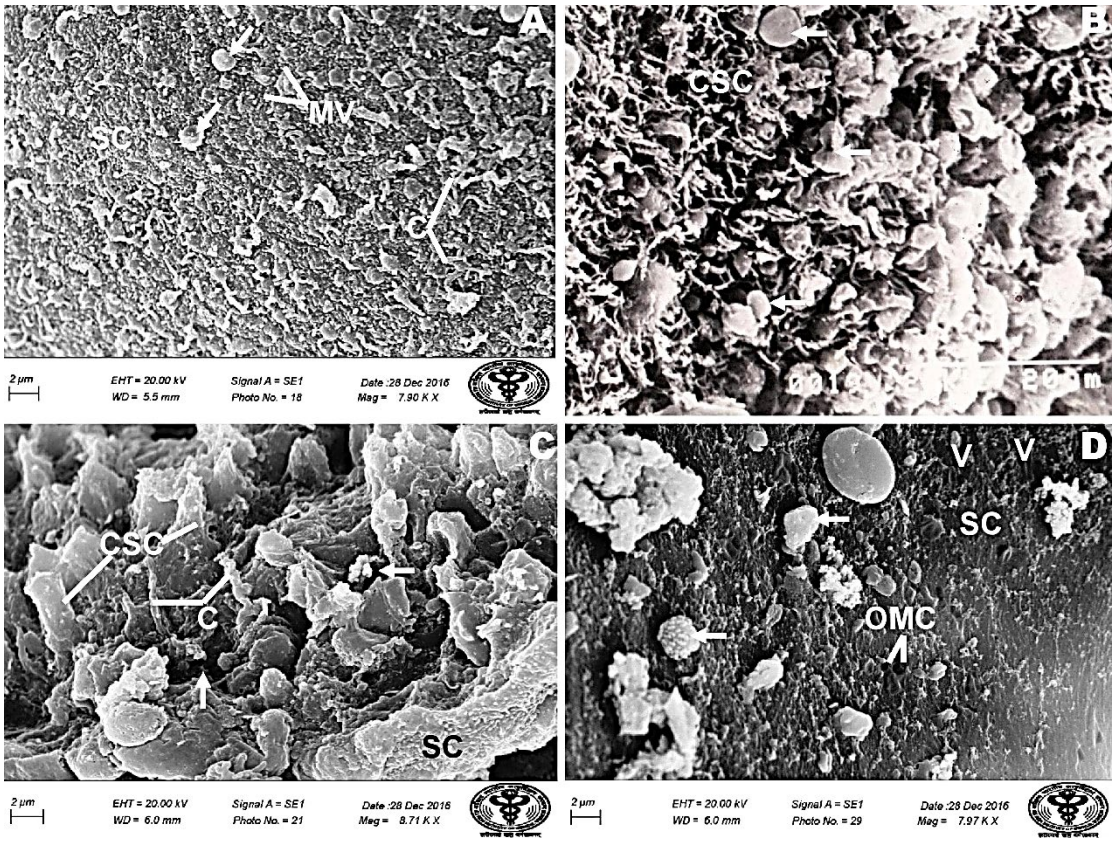

Figure 3. Scanning electron micrographs of olfactory lamella of *Xenentodon cancila* (A) Surface of lamella shows ciliated receptor cells (C), microvillous receptor cells (MV) and stratified epithelial cells (SC). Arrows mark mucin masses over SC. (B) Mucosal surface shows thick ciliated supporting cells (CSC) and secreted mucus clumps (arrows). (C) Part of epithelium displays ciliated receptor cells (C) with prolonged dendrites, ciliated supporting cells (CSC), stratified epithelial cells (SC) and opening of mucous cells (arrows). (D) Olfactory epithelium furnishes stratified epithelial cells (SC) with microridges (arrow heads) and aperture of mucous cells (OMC). Solid arrows mark lump of mucin on top of SC.

sensory cells bounded to the olfactory mucosa and admissible behaviours expressed by fish species. In *X. cancila*, the olfactory cues are detected by unilamellar olfactory organs and conveyed precisely to the central nervous system by olfactory tract. *Xenentodon cancila* have a pair of unilamellar olfactory organ, appearing as an open pit, without nostrils, which is related to their behavioural adaptations. The results showed that *X. cancila* belongs to Teichmann's (1954) second group of eye fishes i.e., sight feeder and Pol Gerard (1954) first category i.e., anosmic in which olfactory organ is regressed and seek their food by vision.

Morphologically distinct ciliated and microvillous neurons on the mucosa are responsible for appropriate reception of olfactory sensation. Stratified epithelial cells bearing microridges are thought to safeguard for supporting tissues while disclosed to water forces and help in mechanical dissociation (Uehara et al., 1991). Numerous ciliated supporting cells are able to generate a gentle water flow over the epithelial surface and perform as guides allowing chemicals to contact with the receptor sites of sensory neurons. Same finding was cited by Singh and Singh (1989) in the olfactory organ of *Heteropneustes fossilis*. Occurrence of lymphatic cells in the epithelial layer are function as part of cell immunity (Lieschke and Trede, 2009; Kim et al., 2019). Mucous cell discharges mucus which shields the epithelium from mechanical rubbing. Graziadei and Metcalf (1971) mentioned that the basal cells in the deeper layer of mucosa are capable to differentiate into receptor cells while Ojha and Kapoor (1973) reported their conversion into supporting cells.

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