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Original Article

Histological characterization of the olfactory organ in Schilbid Catfish, *Clupisoma garua* (Hamilton, 1822)

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Abstract: Fishes have a good sense of smell and are able to ascertain odour with the help of a pair of olfactory organs connected to the olfactory lobes of the forebrain by means of olfactory tracts. The functional anatomy and structural characterization of olfactory organ in the freshwater Indian Catfish *Clupisoma garua* (Hamilton, 1822) was investigated by light microscopy. The paired well-developed olfactory organs were located in nasal cavity having two exterior apertures: incurrent and excurrent nares. The olfactory rosettes were elongated structure, possessed 40±02 lamellae on each side of the narrow median raphe. Histologically, each lamella consisted of two principal layers: an epithelium consisted of sensory and non-sensory cells and a central core, which was composed of connective tissues, nerve fibers and blood vessels. The sensory epithelium was composed of three types of receptor cells: two described as classical bearing cilia or microvilli and third bearing rod like dendritic terminal. Synapses between primary and secondary neurons were formed. The indifferent epithelium comprised the greater surface area of the olfactory lamella, was typified with ciliated non-sensory cells, secretory mucous cells, mast cells and supporting cells. Undifferentiated basal cells were scattered in the deeper part of the epithelium above the basement membrane. Organization of various cells on the olfactory epithelium was correlated with essential life process of the fish concerned.

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Introduction

Olfactory organ in fishes is of primary importance because it is innately a chemoreceptor and performs an indispensable role in detection and location of food, recognition of sex, exposure of predators, parental behaviour and migration. Olfaction results from stimulation of the sensory receptor cells lining the olfactory mucosa, which is innervated by the olfactory nerve. A large number of researchers investigated diverse views of the olfactory organ of teleosts (Fishelson, 1995; Hansen and Zielinski, 2005; Sinha, 2008; Chakrabarti and Ghosh, 2009; 2010; Waryani et al., 2013, Kim and Park, 2016; Ghosh, 2018). Variation in the morphology of the olfactory organ correlates with the enormous diversity of life-styles among fish species, the long, divergent evolutionary history of primary aquatic vertebrates and their inferred actual ecological adaptations (Zeiske et al., 2009). The knowledge of the cellular organization of the olfactory organs in schilbid catfish is almost unknown. Considering the dearth of information, the present study was undertaken to describe the morphology and histoarchitecture of the olfactory epithelium of *Clupisoma garua* (Siluriformes; Schilbeidae); a bottom dweller river catfish which feeds on mollusks, insects, small fishes and decaying matter (Talwar and Jhingran, 1991).

Materials and Methods

This study used 16 samples of adult *C. garua* (17±1.57 cm in total length), caught from the river Ganga at Kalyani, Nadia, West Bengal. The fishes were deeply anaesthetized with benzocaine (4 mg/L) and decapitated following the guidelines of the institutional animal ethics committee. The olfactory apparatus with the brain was dissected out under a Zeiss Stemi 2000-C stereoscopic binocular microscope and further processed for respective

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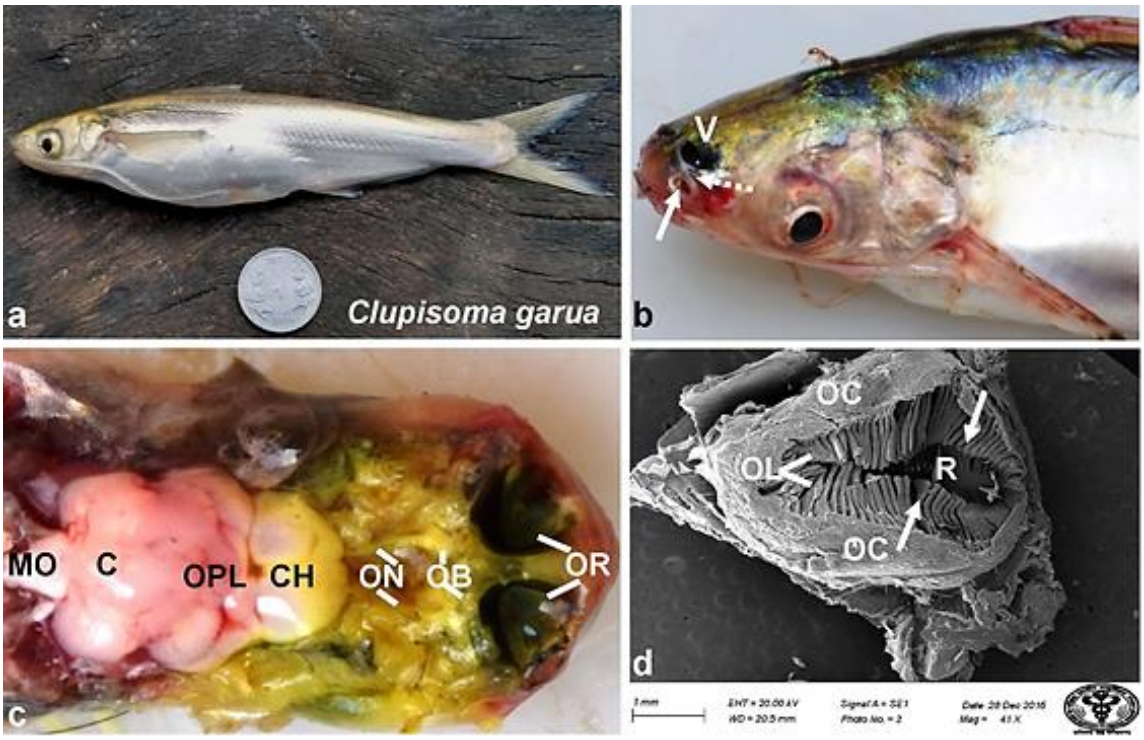

Figure 1. Photographs showing the position of nostrils, olfactory rosette and connection of olfactory organ with brain in *Clupisoma garua*. (a) Complete structure of *Clupisoma garua*, (b) Dorsolateral view of the head showing the anterior nostril (solid arrow), posterior nostril (arrow head) and nasal bridge (broken arrow), (c) Dorsal view of dissected olfactory rosette (OR) showing the relationship of the olfactory bulb (OB), olfactory nerve (ON), cerebral hemisphere (CH), optic lobe (OPL), cerebellum (C) and medulla oblongata (MO), and (d) Olfactory rosette within olfactory chamber (OC) holds a series of olfactory lamellae (OL) radiated from central raphe (R). Note the presence of linguiform processes (arrows) on OL.

studies.

For histological preparation, the olfactory tissues were immediate fixed in aqueous Bouin's fluid for about 24 h. Fixated tissues were thoroughly washed in 70% ethanol, dehydrated through ascending grades of ethanol, cleared in xylene and then embedded in paraffin wax of 56-58°C during 1 h 30 min. Serial transverse sections were cut in 4 µm thick using a rotary microtome (Weswox MT-1090A). Sections were firmed on glass slides, deparaffinized and stained with Romies Azan (RA) and Mallory's Triple (MT) stain (Mallory, 1936). The staining slides were observed and photographed using a LEICA EC3 light microscope at different magnifications.

For scanning electron microscopy, the rosettes were taken out and fixed in glutaraldehyde 2.5% in 0.1M sodium phosphate buffer (pH 7.4) for 4 h, postfixed in 1% osmium tetroxide in phosphate buffer. The rosettes were then repeatedly washed in same buffer, dehydrated in a growing acetone series and subsequently processed through critical point drying method. The dried samples were mounted on aluminium stubs, coated with gold palladium and documented photographically with a ZEISS EVO 18 scanning electron microscope.

Results

General anatomy: Paired olfactory organ of *C. garua* (Fig. 1a) are located on the nasal cavity on the dorsolateral side of the head and at a distance from the eyes, near to the rise of maxillary barbels (Fig. 1b). Each nasal cavity contains two separate openings, the anterior inlet and a posterior outlet. A nasal bridge distinguishes these openings. The anterior nostril is roughy rounded, whereas posterior nostril is crescentic and occupied a transverse position. The olfactory apparatus consists of nasal cavity, anterior and posterior nostrils, olfactory rosette, olfactory bulb and olfactory nerve terminated to the telencephalon (Fig. 1c). The deck of the nasal cavity is lined by mucosa, which is aroused from the bottom into a series of lamellae to construct olfactory rosette. A very small

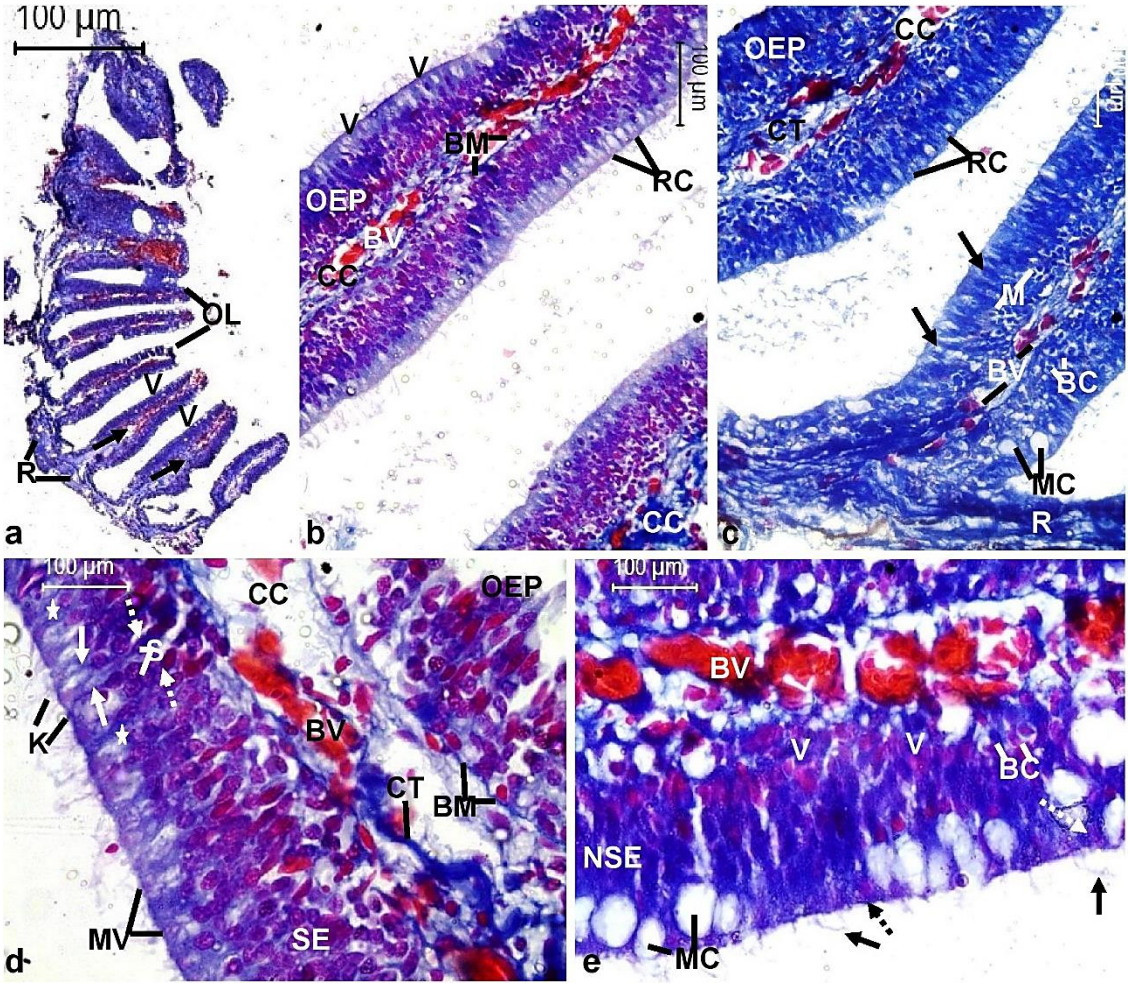

Figure 2. Photomicrographs of the transverse section of the olfactory organ in *Clupisoma garuaa* stained with Romies Azan (RA) and Mallory's Triple (MT) stain. (a) Section of olfactory lamellae (OL) showing olfactory epithelium (OEP) (arrow heads) and central core (CC) (solid arrows), radiated from raphe (R) (RA 40X), (b) Sensory OEP lined with ciliated receptor cells (RC) and microvillous cells (MV). CC contains blood vessels (BV) distinguished from OEP by basement membrane (BM) (RA 400X), (C) OEP typified with RC, rod cells (solid arrows), mucous cells (MC), mast cells (M) and basal cells (BC). Note the presence of connective tissue (CT) and BV in CC. R marks raphe (MT 400X), (d) Magnifying sensory epithelium (SE) shows rod cells (asterisks), MV, synaptic contact (S) in between primary (solid arrows) and secondary RC (broken arrows). Note knob like structure of ciliated RC on the epithelial surface and presence of BV and CT in CC, separated from OEP by BM. (RA 1000X), and (e) Non-sensory epithelium (NSE) shows MC, BC, mast cells (arrow heads), supporting cells (broken arrows) and ciliated non-sensory cells (solid arrows). Note CC with copious BV (MT 1000X).

amount of fibrous connective tissue is observed, which engrossed the olfactory organ within the nasal cavity.

The olfactory rosette is an elongated structure having concave dorsal and convex ventral appearance inhabited the major space of the corresponding chamber (Fig. 1d). It consists of 40±02 leaf lets, the olfactory lamellae, arranged on each side of the median raphe. The number of lamellae on the either side of the rosette exhibits bilateral symmetry. The lamellae are affixed to the wall of olfactory chamber by their ventral borders and to the raphe by their proximal ends. The dorsal portion of the lamellae is characterized with linguiform processes at their distal ends. Each lamella is broad anteriorly and narrow posteriorly.

Histology: The lamella of the olfactory organ consists of olfactory epithelium, which is pseudostratified in nature, enclosing a connective tissue layer, central core (Fig. 2a). The central core is distinguished from the epithelium by a basement membrane, made up of loose fibres, connective tissues and blood vessels (Fig. 1b-e). The epithelium is dissociated into two regions: sensory and indifferent, which are regularly interspaced. The sensory epithelium is embossed with morphologically distinct ciliated, microvillous and rod

receptor cells. Ciliated receptor cells are columnar in shape with a basally located cell body and a thin long dendrite, which formed a swelling, the olfactory knob at the free surface of the epithelium. In some areas, the axonal end of primary neurons is synapsed with the dendrite tips of the secondary neurons (Fig. 2d). Microvillous receptor cells are of small size and have broad apical surface but without cilia. The cell body and light stained nucleus are located in more superficial layer in the epithelium (Fig. 2b). The rod receptor cells are scattered along the surface zone of the mucosa, consisted of basally placed vesicular nuclei and narrow protruded dendrons (Fig. 2c).

The indifferent epithelium is typified with supporting cells, ciliated non-sensory cells, mast cells and mucous cells. The supporting cells give the basic structure of the olfactory epithelium. They are elliptical in shape with intensely basophilic nuclei and faintly stained less granular cytoplasm (Fig. 2e). Ciliated non-sensory cells are columnar and composed of numerous long cilia at their surface. Deeply stained round nucleus is placed in the middle of the cell. The mast cells are rounded having relatively smaller amount of cytoplasm and centrally placed nuclei. (Fig. 2c, e). Mucous cells are fairly larger and oval, distributed at the margin of the olfactory epithelium in between other non-sensory cells. Nucleus is present towards the basal ends. The basal cells appeared to be stem cells, are grouped in the deeper part of the epithelium just above the basement membrane. They are small, round in shape with an enormous central nucleus.

Discussion

Olfactory mucosa containing the sensory neurons is typically located on the floor of the nasal chamber, which is often folded, forming olfactory lamellae (Hara, 1975). The present study reveals that olfactory rosette of *C. garua* is elongated texture which can be placed under Bateson's (1889) rosette type 2, Burne's (1909) rosette column II and Teichmann's (1954) 1st group. According to Teichmann (1954) the elongated olfactory organ belongs to the category of "nose" fishes, which means that, *C. garua* having a predominantly developed sense of olfaction. The presence of 40±02 lamellae around a central raphe designated as macrosmatic, furnishes their bottom dwelling habit. The capacious surface area furnished by the olfactory lamella increases and sensitivity and efficacy of the olfactory system (Zeiske et al., 1976). In *C. garua*, the paired olfactory chambers are communicated to surrounding aquatic environment by means of two opening, through which water enters and leaves. Adequate ventilation is needful to bring the odorants in the nasal chamber for perceiving the chemical signals (Belanger et al., 2003). Hara (1993) reported that ventilation of the nasal chamber occurs by either forward movement of fish or synchronous beating of ciliated non-sensory cells.

In the present study, the sensory epithelium of *C. garua* is consisted of ciliated, microvillus and rod receptor cells. The ciliated receptor cells correspond to type I cell of Yamamoto and Ueda (1978), whereas microvillus to those of type II cells of Muller and Marc (1984) and rod cells of those type IV cells of Ichikawa and Ueda (1977). The ciliated receptor cells dominate over the microvillous and rod receptor cells. The ciliated receptor cells with knobs are of special interest because they are able to detect chemical changes in the surrounding environment. The long and well-developed dendrite process of the receptor cell enables the fish to smell its food even in muddy surrounding. Bhute and Baile (2007) advocated that the microvillous receptor neurons perceive and process signals of pheromone, which is an important step of breeding in *Labeo rohita*. On the other hand, Bakhtin (1977) and Bannister (1965) reported that microvillous cells in the olfactory surface of *Squalus acanthias* and teleostean fishes are predecessors of ciliated receptor cells. Zielinski and Hara (1992) and Moran et al. (1992) have mentioned that rod shaped processes represent the dendrite apical processes of olfactory receptor cells. Hernadi (1993) proposed that the occurrence of the rod-shaped olfactory neuron has been observed in the presence of a new physiological condition.

The epithelial surface is covered with non-sensory cilia, which propel streams of incoming water and/or

dissolved chemicals between the lamellae and act as guides enabling chemicals to reach olfactory terminals containing receptor sites (Singh and Singh, 1989). The supporting cells have been suggested to perform several functions: secretory, absorbing and glial (Yamamoto and Ueda, 1978; Hernádi, 1993; Theisen, 1972; Hansen and Zeiske, 1998). Mast cells in the olfactory mucosa are believed to play an important role in reproduction of Baltic trout (Bertmer, 1982) and Indian major carp, *L. rohita* (Bhute and Baile, 2007). Mast cells can change metabolic activity of receptors and thereby the sensitivity of olfactory epithelium. The mast cells are thought to cause fluctuations in the production of mucus over the olfactory epithelium. As the terminal mucus film is believed to be an important factor in the olfactory process this may also influence the variations in the olfactory sensitivity (Moulton and Beidler, 1967). The basal cells are located in the deeper layers of the epithelium. Graziadei and Metcalf (1971) advocated that the basal cells are able to differentiate into receptor cells while Ojha and Kapoor (1973) reported their transformation into supporting cells. Mucous cells secret mucin to shield the epithelium from mechanical abrasion. The secreted mucin from the mucous cells probably helps for coagulating microscopic debris and thereby keeps the receptor ready for new stimuli. Zeni and Stagni (2002) opined that the mucus covering the olfactory lamellae constitutes an important medium in which the odorants are diffused like that of other olfactory systems of vertebrates.

In conclusion, elongated olfactory rosette bounded by 40±02 lamellae associated with a predominantly developed sense of olfaction for exploring the surrounding in which *C. garua* live. It is a macrosmatic species, can be classified as “nose fishes” whose olfaction is better developed than vision. During forward rhythm, unidirectional water flows into anterior opening and passes out through the posterior one after bathing the olfactory epithelium. Ascendant receptor cells in the epithelial lining manifest the olfactory stimuli and assess the surrounding habitat.

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