I.J.S.N., VOL. 2(1) 2011: 1-6 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

FUNCTIONAL MORPHOANATOMY OF OLFACTORY SENSORY EPITHELIAL CELLS OF *PSEUDAPOCRYPTES LANCEOLATUS* (BLOCH AND SCHNEIDER)

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ABSTRACT
Olfaction in fish is an important type of chemoreception which is mediated through an olfactory epithelium of olfactory apparatus. The olfactory epithelium of *Pseudapocryptes lanceolatus* is a pseudostratified structure and includes different types of cell *viz.*, sensory receptor cells, supporting cells, basal cells, goblet cells, *etc.*. The sensory receptor cells are bipolar neuron and may categorized as crypt cell, microvillous sensory receptor cells and ciliated sensory receptor cells. These sensory receptor cells are morphologically different. The length of the dendron apparently differs among the sensory receptor cells and results different location of the perikaryon of respective sensory receptor cells within the olfactory epithelium. The tip of the dendron swells to form olfactory knob. The structures of olfactory knobs in these sensory receptor cells are also anatomically and functionally variable. It is assumed that the variable length of dendron along with their variable structures is both probably responsible for the recognition of different odorants during olfaction in *P. lanceolatus*

KEYWORDS*: P. lanceolatus*, Chemoreception, olfactory, crypt, perikaryon, *etc.*

INTRODUCTION
Olfaction is the most primitive type of special sense found in animals (Laska and Teubner, 1998; Buck, 2000; Dominy *et al.*, 2004). It is mediated through olfactory apparatus. The structures of olfactory apparatus in some invertebrates like arthropods, nematodes, molluscs, *etc.* were studied by several authors (Steinbrecht and Muller, 1976; Chapman, 1982; Chase, 1982; Yi and Emery, 1991; Catalá, 1996; Chou *et al.*, 1996; Murphy and Hadfield, 1997). The olfactory system of animals of different group of vertebrates *i.e.*, fish to mammals were also reported (Burne, 1909; Bertmar, 1981; Jouventin and Robin, 1983; Iwahori *et al.*, 1987; Calof and Chikaraishi, 1989; Das and Datta, 1992; Døving *et al.*, 1993; Dial and Schwenk, 1996; Dawley, 1998; Buck, 2000; Hansen *et al.*, 2005; De and Sarkar, 2009) in different aspects. Olfaction in fishes is the most highly developed sense of all vertebrates (Kleerekoper, 1969). The olfactory apparatus of fish is generally consist of olfactory chambers, olfactory lamellae or olfactory rosette, accessory nasal sacs, olfactory bulb, olfactory nerve tracts and olfactory lobe of the brain (Døving *et al.*, 1977; Datta and Das, 1980). The olfactory apparatus is externally covered by an epithelium which plays a crucial role in olfaction (Hara, 1971). The histological components of the olfactory epithelium were first studied by Bannister (1965) in two Cyprinid fishes. The epithelial structures along with their distribution within the olfactory epithelium of Indian gobiid fishes are still less understood. *Pseudapocryptes lanceolatus* (Bloch and Schneider), a teleostean: gobiid - a mud skipper of gangetic Bengal. This fish has a special ability of dual mode of respiration *i.e.*, aquatic and aerial respiration (Das, 1934). This study focused on the identification, distribution and characterization of the sensory epithelial components of *P. lanceolatus* under light microscope (LM) and transmission electron microscope (TEM) to correlate their role in olfaction.

MATERIALS AND METHODS
Adult, live, sex-independent *P. lanceolatus* species were collected from the local markets of Diamonhourbour of South 24 Parganas, West Bengal and brought to the laboratory. Specimens were acclimatized and anaesthetized with MS–222 (100-200mg./lit.). Olfactory tissues were procured by dissecting the dorsal side of the head and fixed in 2.5% gluteraldehyde in 0.1 (M) phosphate buffer (pH. 7.2-7.4) for 1hour at 4°C. After completion of primary fixation, olfactory tissues were rinsed in the same buffer and then further fixed in 1% osmium tetraoxide in 0.1 (M) phosphate buffer (pH. 7.2-7.4) for 1hour at 4^0 C. Dehydrated by graded ethanol and embedded in araldite. Semithin sections (1µm) were cut for light microscopical (LM) study using 0.1% toluidene blue (1% sodium borate) for staining and viewed under light microscope (LM). For transmission electron microscopical (TEM) study, the ultrathin sections (70 – 90 nm) 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
In *P. lanceolatus*, the olfactory apparatus is present at the ethmoid region of the head in between anterior and posterior nostrils. The olfactory apparatus of this species comprises of olfactory lamella along with accessory nasal sacs *viz.*, ethmoidal sac and lachrymal sac, semi-enclosed within the olfactory chambers, present at the either side of the head. The olfactory apparatus is a unilamellar, tube-shaped structure and externally lined by pseudostratified epithelium (Fig. 1). The olfactory epithelium completely

covers the nasal cavity and does not show any secondary folding. The thickness of the olfactory epithelium of *P. lanceolatus* approximately ranges form 40- 70µm. The olfactory epithelium of *P. lanceolatus* comprises of sensory receptor cells, supporting cells, basal cell, goblet cell, *etc.*. These cells are morphologically distinct and intermingled within the olfactory epithelium (Fig. 1). Among the other type of the epithelial cells, sensory receptor cells are bipolar neuron and possess dendron, perikaryon and axon. The dendron of the sensory receptor cells reached to the lumen of the nasal cavity where they terminated as olfactory knob. There are three different types of sensory receptor cells present within the olfactory epithelium of *P. lanceolatus viz.*, crypt cell, microvillous sensory receptor cells and ciliated sensory receptor cell (Fig. 2). Apparently the length of the dendron differs among these sensory receptor cells. The perikaryons of these sensory receptor cells are located at the different strata of the olfactory epithelium (Fig. 2). The crypt cell is pear-shaped in structure and located at the apical part of the olfactory epithelium. This cell possesses very short dendron. The perikaryon of the microvillous sensory receptor cell is present at the middle part of the olfactory epithelium. This cell shows moderate length of dendron. The perikaryon of the ciliated sensory receptor cell with very long dendron is marked at the lower part of the olfactory epithelium (Fig. 2 & 3). The tip of the crypt cell shows microvilli and sunken cilia. The microvilli are located around the cilia on the apical rim of the cell. The nucleus of the crypt cell is present at the middle portion of the cell. The cytoplasm of this cell is quite electron dense and shows filamentous mitochondria and free ribosomes (Fig. 4). In microvillous sensory receptor cell, the olfactory knob shows large number of microvilli. The olfactory knob of this type of receptor cell is less pronounced than ciliated sensory receptor cell (Fig. 5). Centrioles are located just beneath the olfactory knob. The cytoplasm of the dendron in microvillous sensory receptor cell contains granulated particle and electron lucent vesicles (Fig 5). The oval shaped nucleus is present apparently in centre of the perikaryon (Fig. 3). The olfactory knob of the ciliated sensory receptor cell possesses 4-6 numbers of cilia. The olfactory knob of the ciliated sensory receptor cell is very pronounced than other types of sensory receptor cells. The cilia show (9+2) arrangement of microtubules. The basal body of the cilia is present just beneath the plasma membrane of olfactory knob and associated with centrioles along with several neurofilaments. The basal body does not any striated rootlets (Fig. 6). The axon of the sensory receptor cells including crypt cell, microvillous sensory receptor cells and ciliated sensory receptor cell are runs towards the basal lamina (Fig. 3). Supporting cells and goblet cells are also marked in the olfactory epithelium of *P. lanceolatus*. Small polygonal basal cells are lying at the deeper part of the olfactory epithelium adjacent to the basal lamina (Fig. 1). Beneath the basal lamina a thin layer of aggregation of the axons of sensory receptor cells, connective tissues, blood capillaries *etc.* are present to form the fila olfactoria.

DISCUSSION

The olfactory apparatus is an important chemosensory organ of fish. This organ is externally lined by olfactory epithelium which is generally raised from the floor of the olfactory chambers and often folded to form olfactory lamellae (Hara, 1975). The number, shape and arrangement of the olfactory lamellae vary considerably among the different teleosts (Yamamoto, 1982; Zeiske et al., 1992). In *P. lanceolatus*, olfactory rosette is absent in olfactory apparatus and shows single olfactory lamella at the either side of the head (De and Sarkar, 2010). Like other teleosts, the olfactory apparatus of *P. lanceolatus* is externally lined by pseudostratified olfactory epithelium and includes three easily distinguishable types of sensory receptor cells. Apart from the common epithelial cells *viz.*, sensory receptor cells, supporting cells, basal cells, *etc.*, some other type of the epithelial cells are also studied in the olfactory epithelium of different teleosts such as goblet cell (Datta and Bandopadhyay, 1997), mast cell and labyrinth cell (Chakrabarti, 2005) *etc.*. The functions of theses type of cells in relation to olfaction are still hardly known to us. The sensory receptor cells are bipolar neuron in nature. The presence of sensory receptor cells within the olfactory epithelium of *P. lanceolatus*, may indicate that this epithelium probably plays a decisive role in olfaction. This sensory epithelium can perceive different chemical cues from the external environment and analyze the chemical nature of the surrounding aquatic environment (Døving, 2003). The olfaction in fish is generally interrelated with the water ventilation by sniffing process (Nevitt, 1991). There are two prominent accessory nasal sacs *viz.*, ethmoidal sac and lachrymal sac are present in the olfactory apparatus of *P. lanceolatus* which may regulate the water ventilation over the olfactory epithelium (De and Sarkar, 2009). Probably this is the initial step of fish olfaction where water soluble chemicals are transported to the sensory surface of the olfactory apparatus (Cox, 2008). The olfactory processing initiates at the apical tip of the olfactory receptor neurons (Buck and Axel, 1991). The morphological variation is evident among the apical part of three types of sensory receptor cells present within the olfactory epithelium of *P. lanceolatus.* The functional difference of ciliated and microvillous receptor cell indicate a relation with the length of the dendron (Hamdani *et al.,* 2001). Accrding to Hamdani *et al.,* (2001) microvillous sensory receptor cells with comparatively short dendrite, mediates the feeding behaviour in Crucian carp (*Carassius carassius*). Where as ciliated sensory receptor cells having long dendrites participated in alarm reaction elicited by pheromones (Hamdani and Doving, 2002). The third type of receptor cell *i.e.*, crypt cell may responsible for the reproductive behaviour of fish (Hansen *et al.,* 2003; Hamdani and Doving, 2006). The variation of the length of dendron probably results the differential location of the perikaryon of respective sensory receptor cells in *P. lanceolatus*. Thus it is assumed that the variable lengths of dendron along with their apical structures are both responsible for the recognition of different odorants during olfaction in *P. lanceolatus* and may elicits different behavioural responses like feeding, alarming, reproductive, recongnition of species, *etc.*. The axon of the different sensory receptor cells are accumulated within the olfactory epithelium and penetrate the basal lamina as bundles (Hansen and Zeiske, 1998). The similar bundle of axon of sensory receptor cells are also noticed in the fila olfactoria region of *P. lanceolatus* but it is quite difficult to demonstrate their projection into olfactory bulb as medial olfactory tract or lateral olfactory tract under light microscope. The olfactory epithelium is a unique structure with different sensory receptor cells which may be replaced by the progenitor basal cells through out the life (Zeiske *et al.*, 1992). The fish olfactory system may also sensitive to chemical pollutants present in the aquatic system as it directly exposed to the external environment (Scott et al., 2003). As the vertebrate olfactory system is equipped with different types of odorant receptors (Buck and Axel, 1991), so this system can be use as an odorant receptor based biosensor for screening the chemicals present in the environment (Gon Song *et al.*, 2008).

ACKNOWLEDGEMENTS
We are very much thankful to Prof. A. K. Chakravarty, Emeritus Professor, North Bengal University; Prof. N. C. Datta, Department of Zoology, University of Calcutta, Kolkata and Dr. T.C. Nag, Associate professor, Electron Microscope Unit, Department of Anatomy; (DST - SAIF): AIIMS, New Delhi, India for their kind help and necessary guidance.

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FIGURES

FIGURE 1 – The photomicrograpgh shows the pseudostratified olfactory epithelium of *P. lanceolatus* covers the nasal cavity (nc) and indicates the epithelial components *viz.*, bc – basal cell, bl – basal lamina, cc – crypt cell, csc – ciliated supporting cell, gc – goblet cell, msc – microvillous supporting cell, ok – olfactory knob, pk of csrc – perikaryon of ciliated sensory receptor cell, pk of msrc - perikaryon of microvillous sensory receptor cell. [Scale - 50μm].

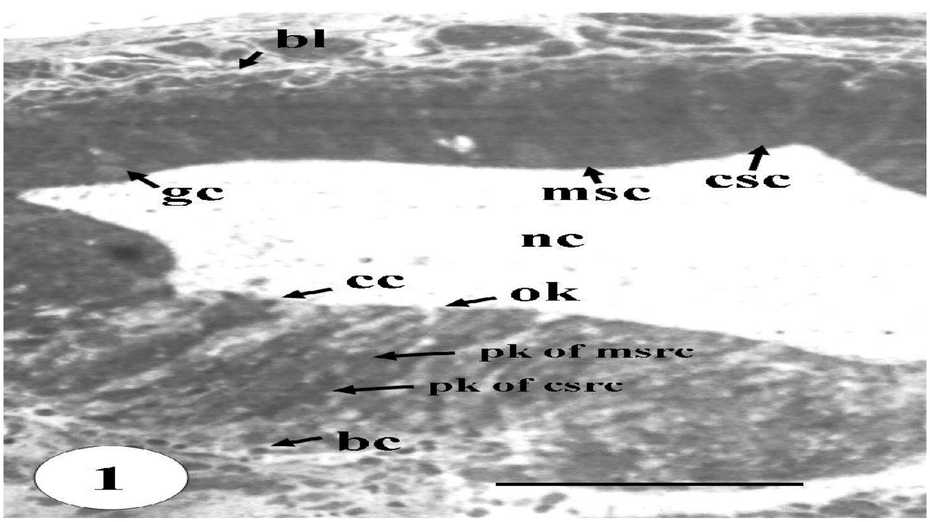

FIGURE 2 – The schematic representation of crypt cell, microvillous sensory receptor cell, ciliated sensory receptor cell, basal cell and basal lamina at the different part of the olfactory epithelium of *P. lanceolatus*. [Not to scale]

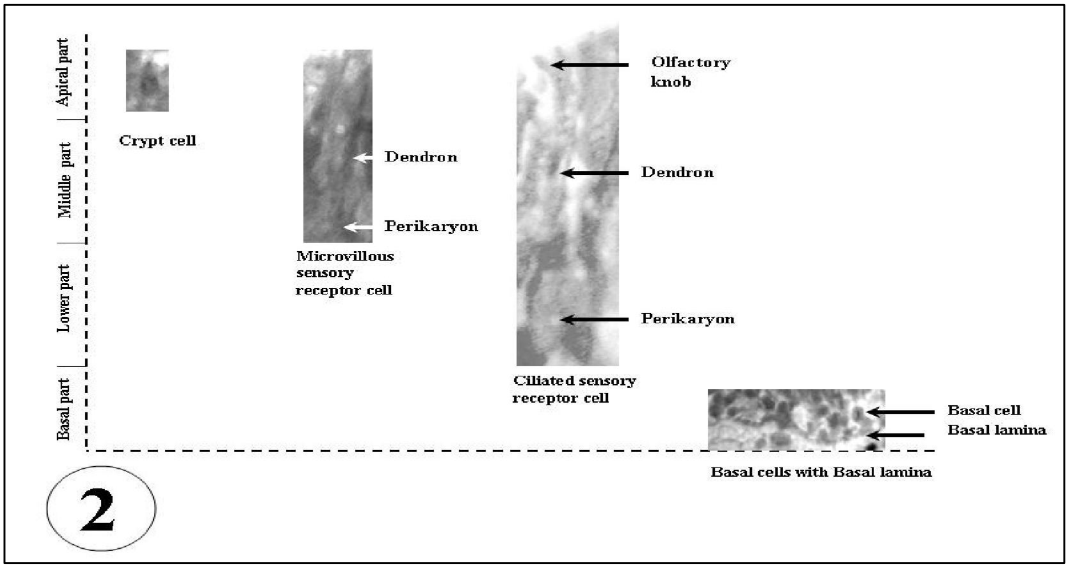

FIGURE 3 – The diagram represent crypt cell, microvillus sensory receptor cell, ciliated sensory receptor cell, ciliated supporting cell, microvillous supporting cell, basal cell of the olfactory epithelium of *P. lanceolatus*. [Not to scale]

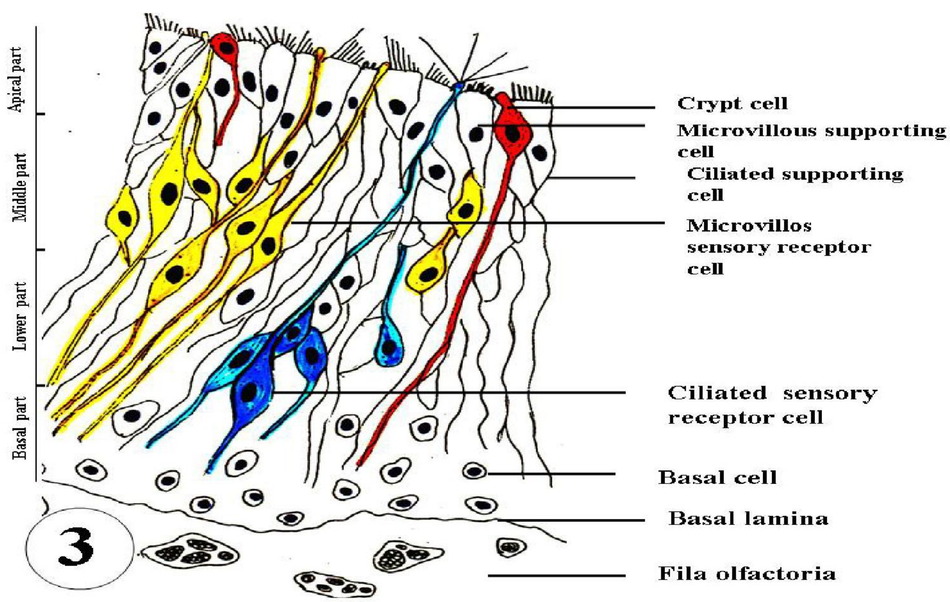

FIGURE 4 – The electron micrograph shows crypt cell with several microvilli (m) and sunken cilia (c) [Mag. 2800X (approx.)].

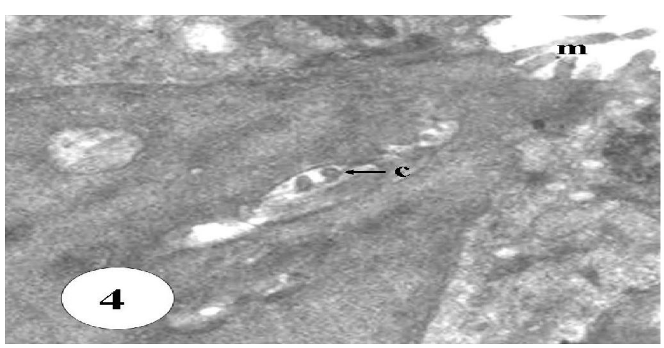

FIGURE 5 – The tip of the microvilllous sensory receptor cell indicates numerous microvilli (m). The cytoplasm of the dendron shows electron lucent vesicles (vs) and granulated particles [Mag. 5500X (approx.)].

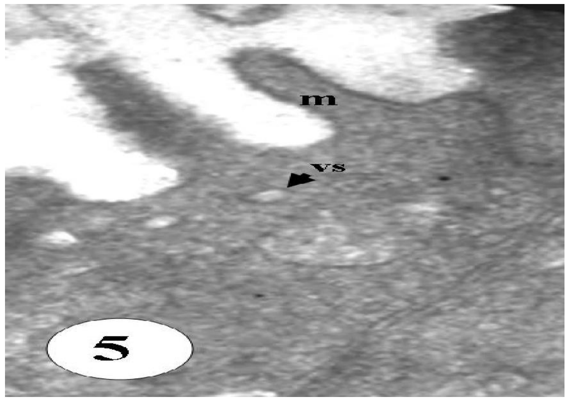

FIGURE 6 – The olfactory knob of ciliated sensory receptor bears cilia (c) with distinct basal body (bb) and neurofilaments (nf) [Mag. 4000X (approx.)].