ANATOMY AND ULTRASTRUCTURAL OBSERVATIONS OF NASAL NEUROEPITHELIUM OF PSEUDAPOCRYPTES LANCEOLATUS (BLOCH AND SCHNEIDER)

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ABSTRACT
The olfaction in fish is generally related with water ventilation process. This study emphasized on the surface structures of the olfactory neuroepithelium and cytological details of the ciliated epithelial elements (i.e., ciliated sensory receptor cells and ciliated supporting cells) in relation to olfaction during water ventilation in a mudskipper, Pseudapocryptes lanceolatus; a teleostean: gobiiid fish of South- East Asia. The olfactory apparatus is well connected with anterior and posterior nostril respectively through the integral nasal cavity of olfactory lamella. The nasal cavity is lined by pseudostratified olfactory neuroepithelium. Under scanning electron microscopical (SEM) study, the surface of the olfactory neuroepithelium of P. lanceolatus shows olfactory knobs with either cilia or microvilli, flat surfaces with numerous cilia, polygonal microridges (average width 0.2 μm), mucous secretory pores with different diameter (0.5 μm to 1.5 μm), mucous secretion, etc. The morphological and subcellular differences of the ciliated sensory receptor cell and ciliated supporting cell have been studied under transmission electron microscope (TEM). The fine structural details indicate that ciliated supporting cell are non sensory in nature and directly involved in water ventilation but the ciliated sensory receptor cell may play crucial role in detection and discrimination of chemical cues from the external aquatic environment.

KEY WORDS: Pseudapocryptes lanceolatus, olfactory, pseudostratified, neuroepithelium, microridges, etc.

INTRODUCTION
Olfaction appears first among the special senses in phylogeny and exists in all vertebrates including fish (Strausfeld and Hildebrand, 1999). This sense is mediated by olfactory apparatus (Burne, 1909). The olfactory apparatus of fish generally comprises of olfactory chambers, olfactory rosette, accessory nasal sacs, olfactory nerve tracts, olfactory bulbs and brain (Hamdani and Døving, 2007). Although fish lives in aquatic environment but their olfactory system shares many common characteristics with those of terrestrial vertebrates (Hino et al., 2009). Anatomically the olfactory apparatus of teleostean vertebrates shows wide range of diversity in different aspects viz., shape, number, arrangement of the olfactory lamellae, and distribution of the sensory and non-sensory epithelium, etc. (Kleerekoper, 1969; Kapoor and Ojha, 1972; Jakubowski and Kunysz, 1979; Døving, 1986; Bandopadhyay and Datta, 1996; Hansen and Zeiske, 1998; Hansen et al., 1999; Mana and Kawamura, 2002; Belanger et al., 2003; Mandal et al., 2005; Kumari, 2008; De and Sarkar, 2009). In higher vertebrates, an odor perception begins when odorants interact with the sensory surface of the olfactory epithelium (Gon Song et al., 2008; Mayer et al., 2008). Fish can detect and discriminate water soluble chemicals through the sensory surface during water ventilation over the olfactory epithelium (Cox, 2008; Hino et al., 2009). The discrimination of water soluble odorants is very important for several life functions in fish such as feeding, alarming, reproduction, migration, etc. (Hara, 1992). Notwithstanding that the surface topographical details of the olfactory neuroepithelium in relation to water ventilation in most of the teleostean fishes are hardly explored. Pseudapocryptes lanceolatus is a teleostean: gobiiid fish of South-East Asia. It is a common mudskipper of coastal area in West Bengal (Das, 1934). The olfactory apparatus of P. lanceolatus shows single olfactory lamella on the either side of the head (De and Sarkar, 2009; Sarkar and De, 2011b). The olfactory apparatus of P. lanceolatus is externally lined by olfactory neuroepithelium (Sarkar and De, 2011a). The present study emphasized to unfold the surface topography of the olfactory neuroepithelium as well as the fine structural details of the ciliated olfactory neuroepithelial components in P. lanceolatus in relation to their role in olfaction.

MATERIALS AND METHODS
Anatomical work supported by medical analog X-ray study
Live, adult (10±5) gm., sex-independent P. lanceolatus collected from the local markets of east Midnapore and South 24 Parganas West Bengal, India respectively and brought to the laboratory for anatomical work. Fishes were acclimatized with the laboratory conditions. Live P. lanceolatus were anaesthetized by MS-222 (100-200 mg./lit.). Anaesthetized specimens having (15 – 20) cm. body length were selected for medical analog X-ray radiograph study. The olfactory apparatus was dissected out from the dorsal side of the head and fixed in aqueous Bouin’s solution. The olfactory apparatus were mounted on a glass slide using glycerine as mounting medium and studied under light microscope (LM).
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**Light microscopical (LM) study**

The olfactory tissues of *P. lanceolatus* were procured and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for 1 hour at 4°C. After completion of the primary fixation, tissues were then processed for microanatomical study. Olfactory tissues were rinsed in the same buffer and fixed in 1% osmium tetroxide (OsO₄) in 0.1 M phosphate buffer (pH 7.2-7.4) for 1 hour at 4°C. Dehydrated through graded ethanol and embedded in araldite mixture. Semithin sections (1 μm) were cut by ultramicrotome (Leica ultracut), followed by staining with 0.1% toluidene blue in 1% sodium borate at 54°C and examined under light microscope (LM).

**Scanning electron microscopical (SEM) study**

For scanning electron microscopical (SEM) study, the anterior part of the head and dissected olfactory apparatus of *P. lanceolatus* were separately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) at 4°C for 1 hour. After rinsing in the same buffer and the specimens were dehydrated in graded chilled acetone. Then the specimens were critically point dried (CPD) in liquid Carbon di-oxide (CO₂), mounted on aluminium stabs and coated with gold respectively. The specimens were observed under scanning electron microscope [SEM: LEO - 435] operated at 15-20 kV in 0° tilt angle.

**Transmission electron microscopical (TEM) study**

Olfactory lamella of *P. lanceolatus* was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for 1 hour at 4°C for primary fixation. Rinsed in the same buffer and fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2-7.4) for 1 hour at 4°C. The olfactory lamella was then dehydrated with graded ethanol, embedded in araldite mixture and incubated at 56°C for 48 hours. Ultrathin sections (70 – 90 nm) were cut by ultramicrotome (Leica ultracut) and stained with uranyl acetate and lead citrate and viewed under transmission electron microscope (TEM: MORGAGNI – 268D), operated at 40kV.

**RESULTS**

*P. lanceolatus* shows two pairs of nostrils viz., anterior nostril and posterior nostril (Figs. 1b and 2b). The anterior nostrils are short, tube like structure. It is located just behind the upper lip of *P. lanceolatus* and projected downwardly (Fig. 2c). The posterior nostrils are oval shaped aperture and located at the anterior edge of the eye (Figs. 2b and 2c). Apparently the diameter of posterior nostril is grater than anterior nostril. The nasal flaps are absent in both anterior and posterior nostrils of *P. lanceolatus*. In between the anterior and posterior nostril, the olfactory apparatus is present just beneath the dorsal surface of skin (Fig. 1b). The olfactory apparatus of *P. lanceolatus* is a unilamellar structure i.e., presence of single olfactory lamella on either side of head. The ethmoidal sac and lachrymal sac are connected with olfactory lamella at ventrocaudal and dorsocaudal region respectively (Fig. 1b). The X-ray radiograph indicates that the olfactory apparatus is guarded by different type of bones viz., ethmoid, lachrymal, maxilla, premaxilla, parietal, post parietal, etc. (Fig. 1a). Tubular channel viz., nasal cavity is the integral part of olfactory lamella (Fig. 4). This cavity is extended from the anterior tip to the posterior part of the olfactory lamella (Fig. 4). The nasal cavity is completely enclosed by olfactory neuroepithelium (Figs. 3 and 4). It is a pseudostratified structure. The olfactory neuroepithelium comprises of different types of cells viz., sensory receptor cell, supporting cell, goblet cell and basal cell (Fig. 3). The sensory receptor cells are bipolar neuron and possess dendron, perikaryon and axon. The tip of the dendron is protruding to form olfactory knob (Fig. 3). On the basis of the morphological demarcations, two types of sensory receptor cells are marked i.e., microvillous sensory receptor cell and ciliated sensory receptor cell. The perikaryons of the microvillous sensory receptor cell and ciliated sensory receptor cell are located at the different position of the olfactory neuroepithelium (Fig. 3). The cytoplasm of the sensory receptor cell is denser than supporting cell. The supporting cells are columnar in nature with flat apical surface and may be divided into ciliated supporting cell and microvillous supporting cell. At the basal region of the olfactory epithelium shows polygonal basal cells (Fig 3). Beneath the basal lamina, a zone of connective tissue and several bundles of axon are marked (Fig. 3). Under scanning electron microscopical (SEM) study, the olfactory epithelium shows numerous olfactory knobs (Fig. 5). Generally the olfactory knobs are recognized as the protrusion of apical tip of dendron in sensory receptor cells and projected into the lumen of the nasal cavity. In ciliated sensory receptor cell, the olfactory knob possessseveral cilia (Figs. 5 and 6). The number of the cilia ranges from 4 to 6 (Fig. 6). The olfactory knob of the microvillous sensory receptor cell shows microvilli (Fig. 5). The other portion of the olfactory epithelium also shows tufts of cilia radiating from the apical flat surface of the ciliated supporting cells (Figs. 5 and 6). The number of the cilia varies from 10 to 12 (Fig. 6). The density of these cilia is apparently greater in the lateral part of the olfactory epithelium. A narrow zone of the polygonal microridges is present and separates the ciliated zone. The average width of this microridge is measured approximately 0.2 μm (Fig. 7). Some mucocous secretory pores along with mucous balls are clearly noticed in the olfactory epithelium of *P. lanceolatus* (Fig. 6). The diameter of these pores ranges from 0.5μm to 1.5μm (Fig. 6). Under transmission electron microscope (TEM), the subcellular details on apical part of the ciliated sensory receptor cell and ciliated supporting cell are also characterized. The olfactory knob of the ciliated sensory receptor cell is a pronounced structure and shows cilia (Fig. 8). Each cillum ends in a long slender distal lash and supported by several microtubules (Fig. 8). The transverse section of cilia indicates (9+2) arrangement of microtubules. Each cillum has a basal body associated with centrioles and several neurofilaments (Fig. 8). The basal body has no striated rootlets. The cytoplasm of ciliated sensory receptor cell is more electron dense than neighboring ciliated supporting cell (Figs. 8 and 9). On the other hand, the apical part of the ciliated supporting cell shows broad and flat surface (Fig. 9). Each cillum of the ciliated supporting cell is well supported by microtubules (Fig. 9). The cilia are associated with basal body and they are present just beneath the plasmamembrane. The basal body of the ciliated supporting cell indicates striated rootlets (Fig. 9). Neurofilaments are absent in ciliated...
A large number of mitochondria are arranged at the apical portion of the cell (Fig. 9). The mitochondrial matrix is relatively dense and cristae are well marked. Minute electron dense granule like structures (?) are also appear in a distinct fashion at the upper cytoplasmic portion of the ciliated supporting cell (Fig. 9).

**FIGURES**

Fig. 1a – The X-ray radiograph shows the ethmoid region of *P. lanceolatus* and this region indicates olfactory chambers (oc) and guarded by different type of bones viz., ethmoid (eth), lacrimal (l), maxilla (m), neurocranium (n), premaxilla (pm), parietal (p) and post parietal (pp). [Scale – 5 mm.]
Nasal neuroepithelium of *P. lanceolatus*

Fig. 1b - Diagrammatic representation of olfactory apparatus of *P. lanceolatus* shows anterior nostril (AN), olfactory lamella (OL), ethmoidal sac (ES), lachrymal sac (LS), olfactory chamber (OC) and posterior nostril (PN). [Scale - 1 mm]

Fig. 2a – The photograph shows the head of an adult *P. lanceolatus*. [Scale – 5 mm.]

Fig. 2b – The scanning electron micrograph shows the position of anterior nostril (AN) and posterior nostril (PN) of *P. lanceolatus* [Scale – 300 μm]

Fig. 2c – The micrograph shows tube shaped anterior nostril (AN) of *P. lanceolatus*. [Scale - 30 μm]

Fig. 2d – The posterior nostril (PN) of *P. lanceolatus* is an oval shaped aperture [Scale – 300 μm]

Fig. 3 – The transverse section of the olfactory lamella in *P. lanceolatus* shows pseudostatified olfactory epithelium (OE) which completely encloses the nasal cavity (NC). This epithelium comprises of sensory receptor cells (SRC), supporting cells (SC), goblet cells (GC) and basal cells (BC). The cellular components of the olfactory epithelium are well marked under light microscope, such as olfactory knob of ciliated sensory receptor cell (Ok of cSRC), olfactory knob of microvillous sensory receptor cell (Ok of mSRC), perikaryon of microvillous sensory receptor cell (P.), perikaryon of ciliated sensory receptor cell (P.), ciliated supporting cell (cSC), microvillous supporting cell (mSC), basal cells (BC), basal lamina (BL). Beneath the basal lamina (BL) of the olfactory epithelium, bundles of axons (AB), blood vessel (BV) are also observed. [Scale - 20 μm]

Fig. 4 – The longitudinal section of the olfactory lamella of *P. lanceolatus* shows nasal cavity (NC). The nasal cavity is enclosed by olfactory epithelium (*). [Scale - 30 μm]

Fig. 5 – The surface structures of the olfactory epithelium indicates olfactory knob of ciliated sensory receptor cell (cSRC), olfactory knob of microvillous sensory receptor cell (mSRC), ciliated supporting cell (cSC), etc. [Scale - 3 μm]

Fig. 6 – Scanning electron microscopical (SEM) photograph shows the olfactory epithelium of *P. lanceolatus*, includes mucous secretory pores (→), olfactory knob with cilia of ciliated sensory receptor cell (cSC) and several cilia radiated from the apical flat surface of ciliated supporting cell (cSC) [Scale - 2 μm]

Fig. 7 – A narrow zone of polygonal microridges (mr) are identified in the olfactory neuroepithelium of *P. lanceolatus* [Scale - 6 μm]

Fig. 8 – The electron micrograph shows the olfactory knob of ciliated sensory receptor cell with cilia (c) and basal body (bb). [Scale – 200 nm]

Fig. 9 – The ciliated supporting cells are also marked under transmission electron microscope (TEM). Cilia (c), basal body possesses striated rootlets (sr), the apical part of the cell indicates large number of mitochondria (m), granulated particles (?) (→), etc. are marked. [Scale - 1 μm]

**DISCUSSION**

Olfaction is an important sensory modality of fish (Hara, 1992). Generally olfaction in fishes is related with water ventilation through the olfactory apparatus (Nevitt, 1991). Through this type of chemical sense, different chemical odors are transported into the nasal cavity (Cox, 2008).

The prominent presence of anterior and posterior nostrils in *P. lanceolatus* may act as an avenue for incidental and excurrent of water respectively. The detail on the water ventilation process of teleostean species through the olfactory apparatus is still an obscure part. A pumping mechanism of accessory nasal sacs may regulate the water ventilation process through the olfactory apparatus (Burne, 1909; Døving et al., 1977; Sinha and Sinha, 1990).

According to Nevitt (1991), the water ventilation is also related with protrusion of jaw in fishes. Døving et al., (1977) classified fishes into two categories viz., isomates and cyclosmates respectively. In isomates, water movement is achieved by the ciliary action of the apical support cells of the olfactory epithelium. In other category viz., cyclosmates, water circulation is created by the pumping mechanism of accessory nasal chambers. In *P. lanceolatus*, the olfactory apparatus is associated with accessory nasal sacs viz., ethmoidal sac and lachrymal sac.

The surface structure of the olfactory epithelium of *P. lanceolatus* also shows densely arranged cilia of ciliated supporting cells. The fine ultrastructural details indicate that this cell is non sensory in nature. Thus it is assumed that synchronous beating of cilia of supporting cell and pumping mechanism of accessory nasal sacs may both responsible for the unidirectional flow of water through the nasal cavity of *P. lanceolatus*. This may be recognized as the first step of fish olfaction *i.e.*, transport of odorants *(e.g. amino acids, steroids, protaglandins, etc.)* from the external environment to the sensory surface of the olfactory organ (Cox, 2008). The distribution of sensory and nonsensory areas in the olfactory epithelium is variable in fish (Yamamoto, 1982). The arrangement of sensory and nonsensory region of olfactory epithelium in *P. lanceolatus* differs from the other teleostean species to maximize their probability of interaction with the water soluble chemicals during olfaction. The presence of goblet cells and mucous secretory pores on the olfactory epithelium of *P. lanceolatus* may be involved in the secretion of mucous. This mucous possibly protect the olfactory epithelium from the external hazards and also helps in smooth flow water through the nasal cavity. The microvillous supporting cells with polygonal microridges may hold the thin mucous film for protecting the sensory epithelial components (Mandal et al., 2005).

Odorant stimulate olfactory sensory receptor cells by first being absorbed into the mucous layer and bound to specific G protein carrier and may initiates a multistep reaction cascade of olfaction (Getchell, 1986; Bigneti et al., 1988; Reed, 1990). The discovery of olfactory adenyl cyclase (Pace et al., 1985), it has become clear that the majority of vertebrate olfactory receptor neurons achieve odor transduction through a G-protein–coupled cascade that uses cyclic adenosine 3′: 5′-monophosphate (cAMP) and Ca²⁺ as second messengers (Kleene, 2008). In teleost fish, the olfactory sensory receptor cells may employ adenylate
cyclic (AC)/cyclic-AMP (cAMP) or phospholipase C (PLC)/ inositol triphosphate (IP₃) as second messenger systems (Sorensen and Caprio, 1998; Hansen et al., 2003). The olfactory processing initiates at the apical tip of the olfactory receptor neurons (Buck and Axel, 1991). Probably the olfactory knob of *P. lanceolatus* and its associated components like cilia and microvilli of sensory receptor cells are the most specialized structure that can interact with the water present within the nasal cavity during water ventilation process to sense different odors. The variation of the apical structure may indicate the functional difference of sensory receptor cells in *P. lanceolatus*. According to Hamdani et al., (2001) microvillous sensory receptor cell may mediates the feeding behaviour in Crucian carp (*Carassius carassius*) where as ciliated sensory receptor cell participate in alarm reaction elicited by pheromone (Hamdani and Døving, 2002). Thus these sensory epithelial components may crucial to evoke different behavioural pattern in teleosts including *P. lanceolatus*. Moreover, the constant exposure of other harmful chemicals like pesticides, heavy metals, etc. may cause cytotoxic effect on different sensory components of the olfactory epithelium (Hansen et al., 1999; Bettini et al., 2006). Thus we believe that an effective xenobiotic biotransformation metabolism is needed to protect the sensory components of the olfactory epithelium as proposed by Marini et al., (1998) and Ling et al., (2004).

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