

Histoarchitecture and scanning electron microscopic studies of the olfactory epithelium in the exotic fish *Puntius javanicus* (Bleeker)

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Abstract. The olfactory epithelium of *Puntius javanicus* (Bleeker) was examined methodically with a light microscope (LM) and a scanning electron microscope (SEM). The ovoid olfactory apparatus consists of 25 to 26 primary lamellae arranged on both sides of the narrow median raphe. The sensory epithelium occupies the upper middle and apical half of the olfactory lamellae and consists of receptor cells (both ciliated and microvillus) and mucous cells. The region from basal part of the lamellae to the junction of the median raphe is covered with non-sensory epithelium. The non-sensory epithelium is composed of non-sensory supporting cells, stratified epithelial cells with fingerprint like microridges, and mucous cells.

Keywords: Histoarchitecture, topological organization, olfactory epithelium, *Puntius javanicus*

Introduction

Olfaction and gustation are the two major chemosensory systems that enable fish survival in aquatic environments. These are relatively simple structures comprising a mosaic of receptors arranged

between supporting cells (Graziadei 1971). The teleostean olfactory organ displays numerous variations especially in gross structure and size due to differences in habits. The surface architecture of the olfactory epithelium in fishes have been investigated by various authors (Zeiske et al. 1976, Cancalon 1983, Singh 1994, Mandal et al. 2005, Bhute and Baile 2007, Chakrabarti and Hazra Choudhury 2008, Chakrabarti and Ghosh 2009). Studies revealed that enormous diversity exists regarding the shape, number, and arrangement of the olfactory lamellae, the distribution of sensory and non-sensory epithelium, as well as variations in receptor cells in different teleosts. However, various cells lining the olfactory epithelium and their functional aspects in relation to the feeding habits of the fish are few. Thus, the present study is an attempt to examine the histology and SEM structure of the olfactory epithelium of the exotic freshwater teleost, *Puntius javanicus* (Bleeker).

Materials and Methods

Healthy adult *Puntius javanicus* were obtained from local freshwater bodies. The fish were anesthetized with MS 222. The olfactory rosette was perfused *in vivo* with a 2.5% glutaraldehyde solution in a 0.1M Cacodylate buffer (pH 7.4) for 10 minutes. The rosettes were then carefully dissected out from the

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dorsal side under a stereoscopic binocular microscope. The adhering mucus was rinsed away with a pleuronic F 68 solution. After rinsing in 0.1M Cacodylate buffer (pH 7.4), the tissues were infiltrated with 2.5% glutaraldehyde for 24 hours at 4°C. After fixation, the tissues were removed, rinsed in the same buffer, pH 7.4, for 10 minutes and subjected to post-fixation in 1% OsO₄ in 0.1M Cacodylate buffer (pH 7.4) for two hours. The tissues were washed thoroughly in buffer and dehydrated through graded series of acetone followed by iso-amyl acetate and subjected to the critical point drying method with liquid carbon dioxide. The olfactory rosettes were mounted on metal stubs, coated with gold with a thickness of approximately 20 nm, and scanned in a Hitachi S 530 SEM. Some tissues were also fixed in Bouin's fluid for 16 to 18 hours to facilitate obtaining a better understanding of the orientation of different cells. The tissues were then processed following routine histological procedures and stained with Mallory's triple stain.

Results

According to the SEM examinations, the olfactory rosette is almost oval in outline with a rostro-caudally oriented median depression from which 25-26 primary lamellae radiate to the left and right sides (Fig. 1a). The outer margins of the lamellae are free, while their inner margins are attached to the raphe. The raphe extends along the long axis of the rosette (Fig. 1a). The lamellae on the right side are longer at 1 to 1.5 mm in length while those on the left side are 0.5 to 0.8 mm in length. The apical part of the lamellae on the left and right sides have tongue-shaped structures (Fig. 1a). Histologically, each olfactory lamellae consist of an olfactory epithelium that surrounds the central lamellar space, the central core, which contains blood vessels, connective tissues, and nerve fibers. The olfactory epithelium is separated into sensory and non-sensory regions. The sensory epithelium occupies the apical third of the olfactory lamellae (Fig. 1b). The sensory epithelium is

composed of large number of receptor cells and supporting cells (Fig. 1c). Supporting cells and receptor cells are arranged in alternate rows. The receptor cells are bipolar with a cell body and a long dendrite. The cell body contains a round prominent nucleus (Fig. 1c). The SEM examinations revealed that the sensory epithelium comprises a tuft of ciliated receptor cells interspersed with a few stratified supporting cells and blood cells (Figs. 1d and 1e). The apical surface of the stratified epithelial cells is either elongated or oval, and is equipped with fingerprint-like microridges (Fig. 1e). The receptor cells are so dense that the free surface of the ciliary patches is not visible under SEM. Scattered microvillous cells are present in between ciliated receptor cells (Fig. 1e). Histologically, the surface zone of the non-sensory epithelium is comprises basically non-ciliated supporting cells, stratified epithelial cells, mucous cells, and a few scattered receptor cells in between supporting cells and stratified epithelial cells (Fig. 1f). According to SEM studies, the transitional zone of the sensory and non-sensory epithelium are supported by ciliated receptor cells and supporting cells. The apical surfaces of the non-ciliated supporting cells are provided with labyrinth-patterned microridges while the microridges on the apical surfaces of stratified epithelial cells are arranged linearly. In both the supporting and stratified epithelial cells there are deep concavities between the microridges (Figs. 1g and 1h). Mucous cells are scattered among the supporting and stratified epithelial cells. Secreted mucin masses intermingle with ciliated receptor cells forming patches of sensory cells in between supporting cells (Fig. 1f). There are a very few ciliary patches of sensory cells surrounded by stratified epithelial cells in the basal region of the surface epithelium of each lamella adjacent to the raphe (Fig. 1h).

Discussion

The olfactory epithelium is located on the floor of the nasal chamber and is often folded to form lamellae

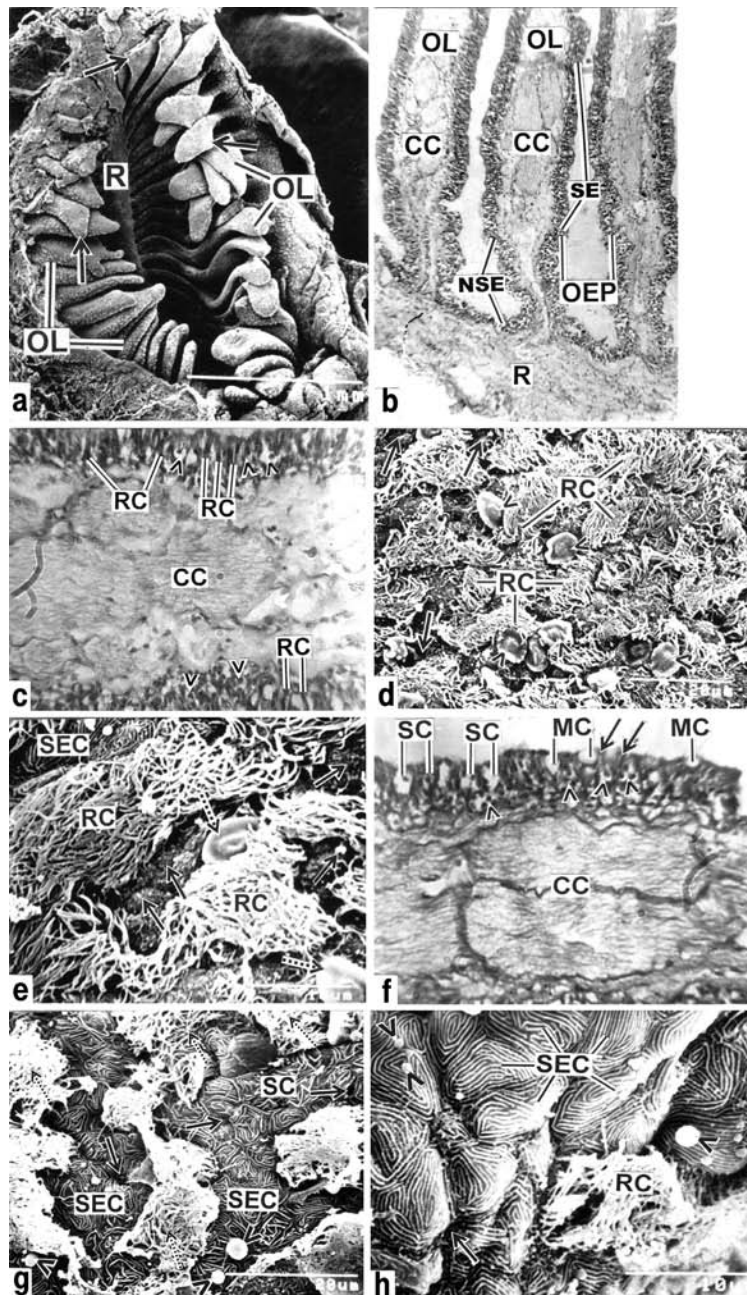


Figure 1. Photomicrographs of the olfactory epithelium of *Puntius javanicus* by scanning electron microscopy (SEM) and histological technique (Mallory's Triple: MT). a-h. Oval shaped olfactory rosette showing olfactory lamellae (OL) radiating from the median raphe (R). Note tongue shaped structure (arrows) on the apical part of the lamellae. (SEM) \times 50 (a). Olfactory lamellae (OL) consist of sensory (SE) and non-sensory (NSE) epithelium (OEP) that surrounds the central core (CC). Note the attachment of OL with the raphe (R). (MT) \times 100 (b). Section of sensory OEP provided with receptor cells (RC) and supporting cells (arrow heads). Note prominent nucleus of RC. (MT) \times 400 (c). Sensory epithelium showing tuft of ciliated receptor cells (RC) interspersed with microvillus cells (solid arrows) and blood cells (arrow heads). (SEM) \times 4000 (d). Showing dense RC and stratified epithelial cells (SEC) provided with finger print like microridges. Note presence of microvillus cells (solid arrows) in between RC and blood cells (broken arrows). (SEM) \times 5000 (e). Section of non-sensory epithelium, showing non-ciliated supporting cells (SC), stratified epithelial cells (arrow heads) and mucous cells (MC). Note the presence of few scattered receptor cells (solid arrows). (MT) \times 400 (f). Transitional zone between sensory and non-sensory olfactory epithelium showing dendrite patches of receptor cells (broken arrows) in between non-ciliated supporting cells (SC) and SEC. Note presence of MC (solid arrows) and mucin droplets (arrow heads) over the SEC. (SEM) \times 4000 (g). Basal region of non-sensory epithelium provided with a very few dendrite process of receptor cells (RC) surrounded by SEC. Note MC (solid arrow) in between SEC. Arrow heads indicate mucin droplets. (SEM) \times 6000 (h).

(Hara 1975). It shows considerable diversity which reflects degrees of development and ecological habitats (Zeiske et al. 1992). The number and shape of the olfactory lamellae are related to the space available in the olfactory cavity of the fish; thus they represent adaptations that maximize the sensory area under given restrictions (Zeiske 1973, 1974). The oval shaped olfactory rosette of *P. javanicus* consists of 25 to 26 lamellae arranged on either side of the median raphe and is similar to other type VI cyprinid olfactory epithelia as identified by Yamamoto and Ueda (1978).

The distribution of sensory and non-sensory epithelia on the surface of the lamellae varies greatly in different fish species (Yamamoto 1982). In the present study of *P. javanicus*, the receptor epithelium is restricted to the upper portion of the lamellae. The receptor epithelium consists of two types of sensory dendrites, the ciliated and the microvillous. The present study revealed that the ciliated receptor cells dominated over the microvillous receptor dendrites. In contrast to the ciliated receptor cells, the microvillous receptor cells had a slightly sunken apex. This conforms to the findings of Camacho et al. (2010) regarding the olfactory epithelium of sturgeon. In *P. javanicus* the ciliated receptor cells are of special interest because they form part of the olfactory transduction mechanism, are stimulated by odour-bearing substances, and also enable the fish to detect food. The microvillous receptor cells might form a different olfactory transduction mechanism for pheromones or amino acids. Hansen et al. (2003) reported that the olfactory epithelium of channel catfish contains three intermingled types of olfactory receptor neurons: ciliated, microvillous, and crypt, which are responsible for the detection of bile salt and amino acid odorants.

In *P. javanicus* the non-ciliated supporting cells and stratified epithelial cells in the non-sensory epithelium are believed to give mechanical support to other sensory cells. The transitional zone between sensory and non-sensory olfactory lamellae contains patches of dendrites. This suggests that the olfactory sensation might extend up to this zone. The labyrinth pattern of microridges and the microridges arranged

linearly on the apical surface of the non-ciliated supporting cells and stratified epithelial cells play major roles in anchoring the mucous film secreted by neighboring mucous cells to protect the olfactory epithelium from different hazardous substances. The mucous cells are distributed in between the non-sensory and epithelial cells in the middle region of the olfactory lamellae. The mucin secreted by mucous cells probably helps in binding microscopic debris and keeps the sensory cells ready for new stimuli. This conforms with the findings of Bandyopadhyay and Datta (1998). However, the ciliary patches of non-sensory supporting cells adjacent to the raphe might be responsible for slowing the water current across the olfactory chambers to enable better sensory cell water quality monitoring.

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Streszczenie

Budowa nabłonka węchowego u egzotycznego gatunku *Puntius javanicus* (Bleeker) w oparciu o badania przy użyciu elektronowego mikroskopu skaningowego

Nabłonek węchowy *Puntius javanicus* (Bleeker) był systematycznie badany przy pomocy mikroskopu świetlnego (LM) oraz elektronowego mikroskopu skaningowego (SEM). W zarysie obła rozeta aparatu węchowego składa się z 25 do 26 głównych lameli ułożonych po obu stronach wąskiego szwu środkowego. Nabłonek węchowy zawiera centralny szew zwany rdzeniem centralnym. Nabłonek receptorowy zajmuje środkową oraz górną połowę lameli węchowych. Patrząc na to z drugiej strony, region od podstawy lameli do połączenia z szwem środkowym jest ukryty pod nabłonkiem. Z badań przy pomocy SEM wynika, iż nabłonek sensoryczny posiada

zarówno urzęsione jak i mikrokosmkowe komórki czuciowe, które są wymieszane i rozmieszczone wzdłuż nabłonka. Z kolei w nabłonku wyściółkowym znajdują się komórki podporowe, uwarstwione komórki nabłonkowe przypominające mikropręgi linii papilarnych odcisku palca oraz komórki śluzowe. Histologicznie nabłonek węchowy składa się z komórek sensorycznych i komórek podporowych ułożonych naprzemiennie w rzędach, natomiast nabłonek wyściółkowy jest objęty nie urzęsionymi komórkami podporowymi, uwarstwowionymi komórkami nabłonkowymi, komórkami śluzowymi oraz niewieloma rozrzuconymi komórkami sensorycznymi.