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RESEARCH ARTICLE

# Surface architecture and histoarchitecture of the olfactory rosette of freshwater lesser spiny eel, *Macrognathus aculeatus* (Bloch)

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**Abstract**. The histology and micro-architecture of different cells lining the olfactory epithelium in lesser spiny eel, *Macrognathus aculeatus* (Bloch), were analyzed with light and scanning electron microscopes. The leaf-like elongated olfactory rosette of the fish consists of 16 to 18 flat lamellae arranged on both sides of a narrow median raphe. Each lamella comprises a very restricted area of sensory epithelium on the extreme left corner, with the rest non-sensory epithelium. The sensory epithelium has two types of receptor cells–ciliated and microvillous, while the non-sensory epithelium comprises stratified epithelial cells, labyrinth cells, and mucous cells. The orientation of different cells on the olfactory epithelium has been correlated with the functional significance of the fish concerned.

**Keywords**: cellular organization, function, olfactory epithelium, *Macrognathus aculeatum*

## Introduction

The olfactory system is one of the crucial sets of chemosensory organs, and its function ranges from searching for food, detecting sexual partners, to avoiding predators and other very dangerous situations (Hara 1992, Sorensen and Caprio 1998). In teleosts, there is considerable variation in the size, shape, lamellar arrangement, and the sensory and non-sensory areas of olfactory organs because of differences in their habits (Hara 1975). A number of researchers have studied the histoarchitectural peculiarities of the olfactory epithelium in fish (Zeiske et al. 1987, Hara and Zielinski 1989, Mandal et al. 2005, Ferrando et al. 2007, Ghosh and Chakrabarti, 2009). As a complement to these studies, the fine anatomical structure of the olfactory epithelium of different teleosts has been investigated by various authors (Jakubowski 1981, Singh 1994, Mana and Kawamura 2002, Bhute and Baile 2007, Ma and Wang 2010, Chakrabarti and Ghosh 2010). However, there is a dearth of knowledge regarding the arrangement of olfactory lamellae, the distribution of sensory and non-sensory epithelium, the orientation of various cells lining the olfactory epithelium, and their functional aspects in mud-dwelling teleosts. Therefore, the purpose of the present study is to examine in detail the histology and surface architecture of the olfactory epithelium of lesser spiny eel, *Macrognathus aculeatus* (Bloch).

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## Materials and Methods

Adult *M. aculeatus* weighing 60 to 62 g (20 to 22 cm in length) were caught in water bodies and sacrificed by decapitation within a few second of capture

following the guidelines given by the Institutional Ethical Committee. The olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in a 0.1M phosphate buffer (pH 7.4) for 10 minutes. The rosettes were then carefully dissected out from the lateral side of the head under a stereoscopic binocular microscope. The adhering mucus was rinsed away in a heparinised saline (heparin sodium salt 10,000 IU mixed in 0.67% NaCl) solution. After rinsing in a 0.1M phosphate buffer (pH 7.4), the tissues were transferred to 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 minute and post-fixed in 1% osmium tetroxide in a 0.1M phosphate buffer (pH 7.4) for two hours. The tissues were washed thoroughly in buffer and dehydrated through graded series of acetone followed by isoamyl acetate and subjected to the critical point drying method with liquid carbon dioxide. After having been dried, the olfactory rosettes were coated with gold palladium at a thickness of approximately 20 nm and viewed under a Hitachi S 530 SEM.

For the histological study, some tissues were fixed in aqueous Bouin's fluid for 16 hours and subsequently dehydrated properly through graded series of ethanol, cleaned with xylene, and embedded in paraffin. Sections were cut 3-4 μm thick, and deparaffinized sections were stained with Delafield's Haematoxylin-Eosin and Mallory's triple stain.

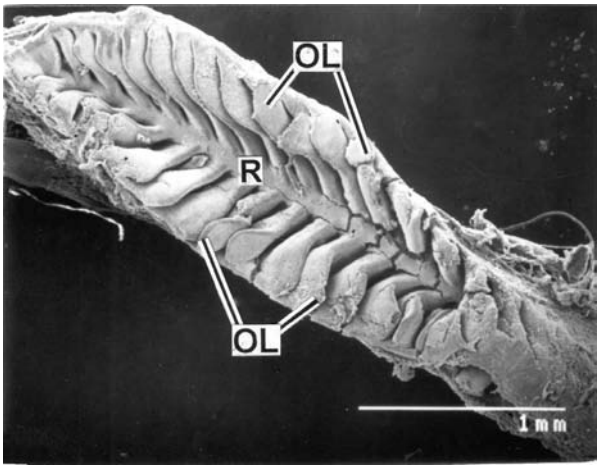


Figure 1. Photomicrographs of the olfactory epithelium of *Macrognathus aculeatus* by scanning electron microscopy (SEM) and histological sections stained with Mallory's triple (MT) and Delafield's Haematoxylin-Eosin (HE) stain. Leaf like elongated olfactory rosette showing olfactory lamella (OL) and median narrow raphe (R) (SEM) × 40.

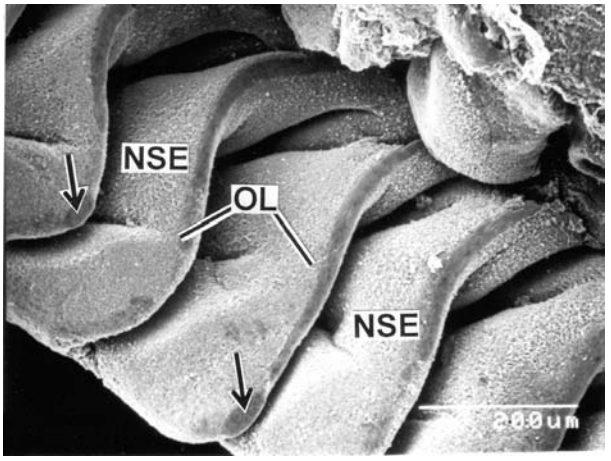


Figure 2. Higher magnification of flat OL showing restricted area of sensory epithelium (arrows) encircled by non-sensory (NSE) epithelium (SEM) × 1500.

## Results

According to SEM observations, the leaf-like elongated olfactory rosette of *M. aculeatus* consists of a series of 16 to 18 flat, wide lamellae arranged on both left and right sides of the narrow, median raphe. The outer margins of the lamellae are attached to the wall of the olfactory chamber, while their inner margins are attached to the raphe. The size and shape of the lamellae vary according to their position in the rosette. The anterior lamellae are smaller than the posterior lamellae (Fig. 1). Each flat olfactory lamella bears a restricted sensory area on its extreme left corner while the remaining large part of the lamella is covered with non-receptor epithelium (Fig. 2). Histologically, the olfactory lamella are comprised of two layers of epithelium, a small segment of which is sensory epithelium (Fig. 3). However, the two layers of epithelium are separated by the central core, which contains blood vessels and nerve fibers (Fig. 3). The surface zone of the sensory epithelium is mainly lined with receptor cells and microvillous cells on one side of the lamellae, and non-sensory epithelium on the other side that has ciliated

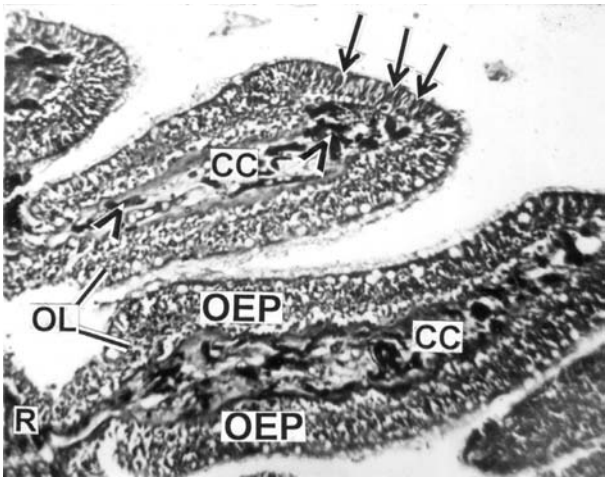


Figure 3. Section of OL based on raphe (R) showing the olfactory epithelium (OEP) separated by central core (CC) having blood vessels (arrow heads). Solid arrows indicate a small portion of sensory epithelium (MT) × 100.

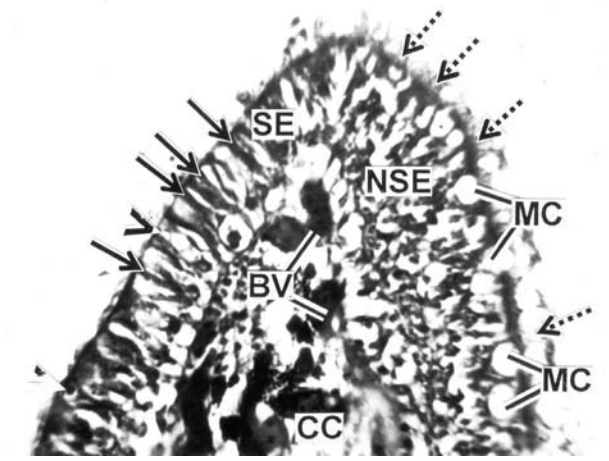


Figure 4. OEP provided with sensory epithelium (SE) lined with receptor cells (solid arrows) and microvillous cells (arrow heads). Non-sensory epithelium (NSE) on another side provided with ciliated supporting cells (broken arrows) and mucous cells (MC). Note CC with blood vessels (BV) (MT) × 400.

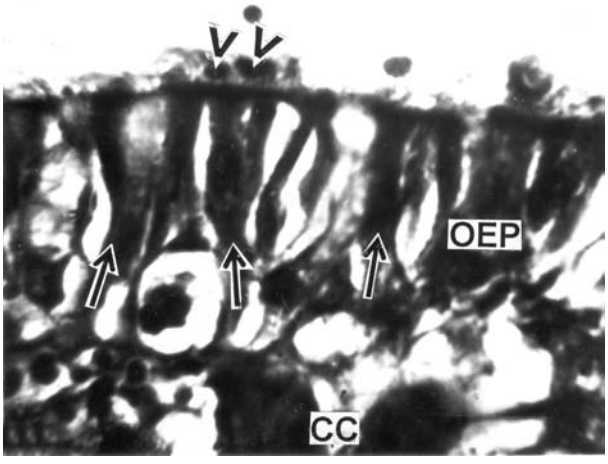


Figure 5. Receptor cells (solid arrows) provided with knob-like structure (arrow heads) on the free epithelial surface. CC indicates central core (HE) × 1000.

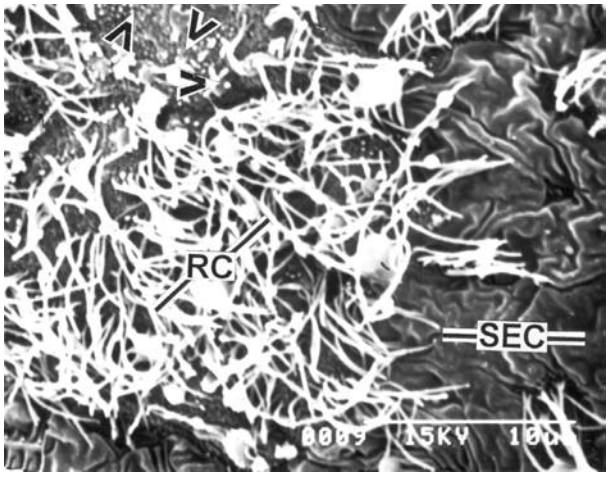


Figure 6. Dendrite process of ciliated receptor cells (RC) and minute dendrite process of microvillous receptor cells (arrow heads). Note the presence of stratified epithelial cells (SEC) adjacent to RC (SEM) × 4000.

supporting cells and mucous cells (Fig. 4). The dendrite process of each receptor cell extends as a narrow cylindrical process with a knob-like structure on the free epithelial surface (Fig. 5). According to SEM studies, the surface of the olfactory lamellae also have restricted sensory areas with ciliated receptor cells surrounded by stratified epithelial cells (Fig. 6). The microvillous receptor cells are few in number and are somewhat submerged into the thickness of ciliated receptor cells (Fig. 6). In SEM studies at higher magnification in another region of sensory epithelium indicated that microvillar cells are clearly discernible at the base of the ciliated receptor cells (Fig. 7).

Histologically, the surface zone of the non-sensory epithelium is basically composed of ciliated supporting cells, stratified epithelial cells, mucous cells, and labyrinth cells (Fig. 8). The labyrinth cells are comparatively large and located at the surface layer of non-sensory epithelium and have prominent nuclei (Fig. 8). According to SEM studies in some areas of non-sensory epithelium, the labyrinth

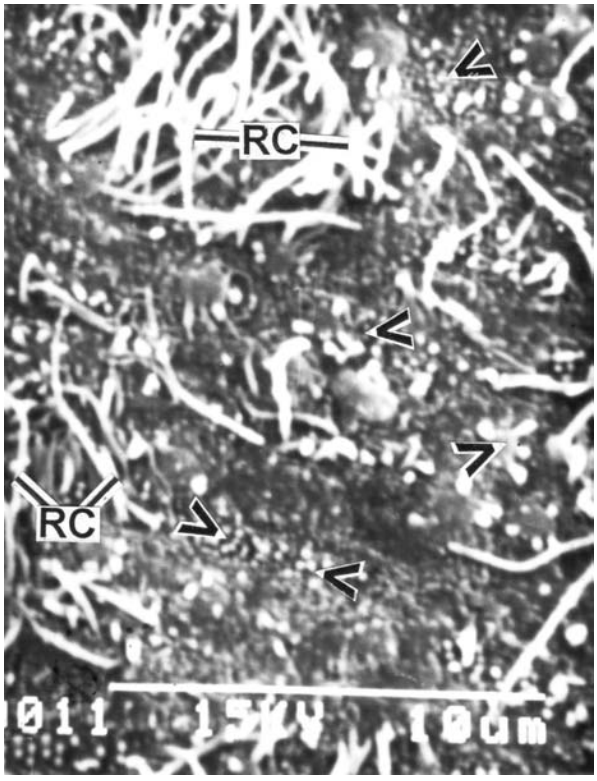


Figure 7. Sensory epithelium showing microvillous receptor cells (arrow heads) and patches of ciliated RC (SEM) × 5000.

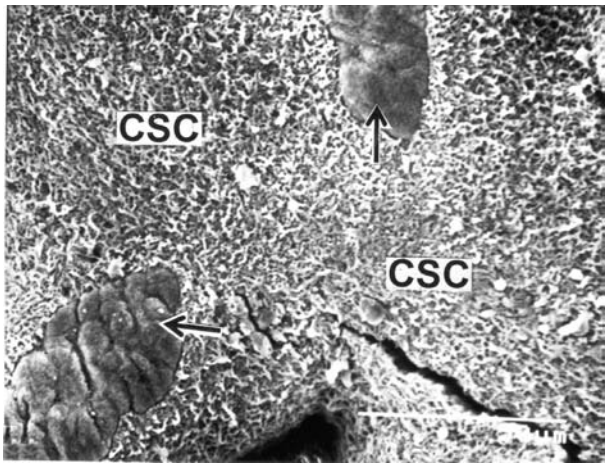


Figure 9. Non-sensory epithelium showing labyrinth cells (arrows), scattered in between the dense mat of ciliated supporting cells (CSC) (SEM) × 800.

cells present are of a peculiar structure and are scattered in and among the dense mat of ciliated supporting cells. They are ovoid in appearance with prominent infoldings (Fig. 9). Further, the surface area of non-sensory epithelium of each lamella has densely packed ciliated supporting cells and

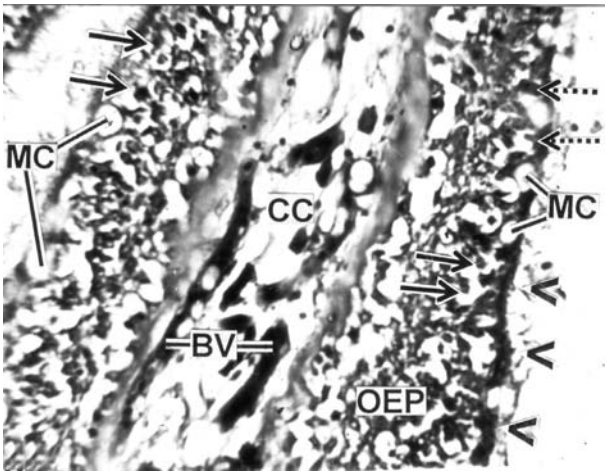


Figure 8. Non-sensory epithelium having ciliated supporting cells (arrow heads), stratified epithelial cells (solid arrows), mucous cells (MC) and labyrinth cells (broken arrows). Note the presence of blood vessels (BV) in the CC (MT) × 400.

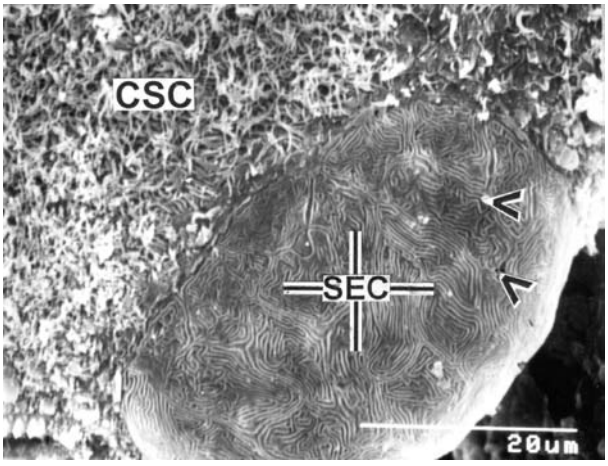


Figure 10. The surface of non-sensory epithelium is provided with densely packed ciliated supporting cells (CSC) and stratified epithelial cells (SEC) having unbranched microridges. Note the presence of opening of mucous cells (arrow heads) in between SEC (SEM) × 2000.

stratified epithelial cells. The opening of mucous cells are located in between the stratified epithelial cells. The apical surfaces of the stratified epithelial cells are provided with unbranched microridges arrange in a concentric whorl (Fig. 10).

## Discussion

The multi-lamellar peripheral olfactory organ in fish supplies them with an acute sense of smell at various

stages in their life history, such as feeding and reproduction, which are mediated through olfactory cues (Hara 1992). The olfactory epithelium shows considerable diversity, reflecting the degree of development and ecological habitats (Zeiske et al. 1992). The present study reveals that the elongated olfactory rosette of *M. aculeatus* consists of 16 to 18 lamellae arranged on either side of the narrow, median raphe. This means this species can be included in Teichmann's (1954) group of nose fishes comprising solitary and nocturnal predators. This species is usually a bottom dweller at night, but it can also come to the mid level occasionally during the day. The distribution of sensory and non-sensory epithelia on the surface of lamellae varies greatly among different fish species (Yamamoto 1982). In *M. aculeatus* the sensory receptor epithelium is restricted to the extreme left corner of the flat lamella. This is an unique feature of the olfactory epithelium in this fish which occupies a specific ecological habitat, and thus mobilizes different olfactory cues. Zielinski and Hara (1988) and Hara and Zielinski (1989) also identified definite aggregations of ciliated receptor cells and confirmed their olfacto-sensory functions. In the present study, the sensory epithelium of *M. aculeatus* exhibits two morphologically distinct types of receptor cells – ciliated and microvillous receptor cells. They occur together but in different proportions. The present study reveals that the ciliated receptor cells dominate the microvillous receptor cells. These receptor cells are of special interest because they form part of the olfactory transduction mechanism, are stimulated by odor bearing substances and also enable the fish to accept meaty foods like bloodworms and small shrimps. In the present study in contrast to the ciliated receptor cells, the microvillous receptor cells consist of minute dendrites with a slightly sunken apex. This conforms to the findings of Camacho et al. (2010) regarding the olfactory epithelium of sturgeon. Zeiske et al. (2003) observed that the ciliated and microvillous olfactory receptor cells occur together in the olfactory organ of the genus *Acipenser*, but in different proportions in different species. In *M. aculeatus* the microvillous receptor cells might form a different olfactory transduction mechanism for pheromones in the regulation of reproductive activities in the environment. In the transitional zone of sensory and non-sensory epithelium of *M. aculeatus* the epithelial cells are provided with complex labyrinth pattern microridges which actually protects the sensory epithelium from mechanical abrasion.

The non-receptor epithelium consists of ciliated supporting cells, stratified epithelial cells, labyrinth cells, and mucous cells. The ciliated supporting cells are responsible for creating a water current in the olfactory chamber as well as the lamellar surface for better monitoring of the water quality by the receptor cells. The stratified epithelial cells are provided with microridges arranged in a concentric whorl. Such microridges located on the epithelial cells play a major role in anchoring a thin mucus film over the epithelial membrane to protect the olfactory epithelium from different hazardous substances. On the other hand, the labyrinth cells on the surface of the non-sensory epithelium serve as excretory cells for osmoregulation. In this way, they can influence the olfactory organ to function optimally in water of different salinities. Shirai and Utida (1970) speculated that the labyrinth cells may be involved in electrolyte transport because they are structurally similar to chloride cells found in fish gills. Ruzhinskaya et al. (2001) also demonstrated the presence of typical chloride cells in the olfactory epithelium of some sturgeons, salmonids, cyprinids, and percids. They also reported that these cells are present in the areas both of indifferent and sensory epithelium and provide active transport of ions between the inner and outer media to maintain ion and osmotic homeostasis. In the non-sensory epithelium the mucous cells are distributed between the stratified epithelial cells. The mucin secreted by mucous cells probably constitutes an important medium in which odorants are diffused. In addition the mucin secreted from mucous cells probably helps the smooth flow of water in the olfactory chamber by binding microscopic debris, which is ejected out through the posterior nostril. This conforms with the findings of Bandyopadhyay and Datta (1996) in the olfactory epithelium of Stinging catfish, *Heteropneustes fossilis* (Bloch).

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## Streszczenie

### Budowa anatomiczna i histologiczna rozety węchowej u długonosa ciernistego *Macrognathus aculeatus* (Bloch)

Narządy węchu są jednymi z kluczowych organów wykorzystywanych przez ryby do lokalizacji pokarmu, czy unikania drapieżników. Receptorami odbierającymi bodźce chemiczne są komórki węchowe. W pracy przedstawiono budowę anatomiczną oraz strukturę rozety węchowej długonosa ciernistego *Macrognathus aculeatus* (Bloch). Badania przeprowadzono za pomocą mikroskopu świetlnego oraz elektronowego mikroskopu skaningowego. Rozeta aparatu węchowego jest w zarysie obła i składa się z 16 do 18 blaszek głównych ułożonych po obu stronach wąskiego szwu środkowego. Komórki receptorowe zajmują jedynie ograniczoną część powierzchni blaszki. W skład nabłonka receptorowego wchodzą urzęsione komórki receptorowe i nieurzęsione komórki z mikrokosmkami. Część pozbawioną receptorów pokrywa wielorzędowy nabłonek, labirynt błon i komórki gruczołów śluzowych.