

The structural organization and functional aspects of the olfactory epithelium of tigerperch, *Terapon jarbua* (Forsskål, 1775) (Perciformes: Terapontidae)

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Abstract: The structure and function of different cells lining the olfactory epithelium in *Terapon jarbua* (Forsskål, 1775) were investigated by light and scanning electron microscopy. The olfactory rosette is oval in structure and consists of 18-20 primary lamellae arranged on a median raphe. A large part of the lateral surface of the olfactory lamella is covered with nonreceptor epithelium, whereas the receptor epithelium occupies the middle region. The sensory receptor epithelium is made up of receptor cells, rod cells, microvillar cells, labyrinth cells, and mucous cells. The nonsensory epithelium consists of ciliated supporting cells, mucous cells, and stratified epithelial cells. Different cells on the olfactory epithelium were correlated with the functional significance of the fish concerned.

Key words: SEM, histoarchitecture, olfactory epithelium, *Terapon jarbua*

Introduction

The olfactory organs of fish are of immense importance because they are essentially chemoreceptors, capable of detecting water soluble compounds in order to provide the fish with information about the surrounding environment. Olfaction plays an important role in mediating behaviors that range from feeding and predator detection to social interaction and reproductive synchrony (Sorensen and Caprio, 1998). In teleosts, there is considerable variation in the size, shape, lamellar arrangement, and sensory and nonsensory areas of the olfactory organ (Hara, 1975). Accordingly, a number of researchers have studied the histological peculiarities of the olfactory epithelium in fish (Ojha and Kapoor, 1973; Zeiske et al., 1987; Hara and

Zielinski, 1989; Mandal et al., 2005; Ferrando et al., 2007; Chakrabarti and Hazra Chowdhury, 2008; Ghosh and Chakrabarti, 2009).

A number of researchers have also used scanning electron microscopy (SEM) to reveal extensive information that indicates the characteristic features of the olfactory epithelium in different teleosts (Caprio and Raderman-Little, 1978; Jakubowski, 1981; Singh and Singh, 1989; Zhang et al., 1994; Mana and Kawamura, 2002; Arvedlund et al., 2007; Bhute and Baile, 2007; Chakrabarti and Ghosh, 2010). Studies show that enormous diversities exist regarding the modification and distribution of the sensory and nonsensory epithelia as well as the abundance of various receptor cells in different teleosts. Wang and Huang (1999) described the effects of tributyltin on

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the olfactory structures of tigerperch, *Terapon jarbua*.

The tigerperch is native to the Digha coast and adjoining brackish water creeks. This bottom-dwelling teleost displays reproductive behavior that may be mediated through intraspecific communication by pheromones. In the present study, therefore, an attempt was made to examine more closely the histology and surface architecture of the olfactory epithelium of an economically euryhaline teleost, *Terapon jarbua* (Forsskål, 1775).

Materials and methods

Healthy adult fish of *Terapon jarbua* were collected from the Junput fish farm in Purba Medinipur, West Bengal, India. Fish were anesthetized with MS 222. The olfactory rosette was perfused in vivo with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 10 min. The rosettes were then carefully dissected from the dorsal side under a stereoscopic binocular microscope. The adhering mucus was removed by rinsing with Pluronic F-68 solution. After being rinsed in 0.1 M cacodylate buffer (pH 7.4), the tissues were infiltrated with 2.5% glutaraldehyde for 24 h at 4 °C. After proper fixation, the tissues were removed, rinsed in the same buffer (pH 7.4) for 10 min, and subjected to postfixation in 1% OsO₄ in 0.1 M cacodylate buffer (pH 7.4) for 2 h. The tissues were washed thoroughly in cacodylate buffer and dehydrated through an ascending series of acetone followed by isoamyl acetate, and then subjected to the critical point drying method with liquid carbon-dioxide.

The olfactory rosettes were mounted on metal stubs, coated with gold to a thickness of approximately 20 nm, and scanned in a Hitachi S-530 scanning electron microscope. Some tissues were also fixed in Bouin's fluid for 16-18 h for a better understanding of the orientation of different cells. The tissues were then processed following a routine histological procedure and stained with Mallory's triple stain.

Results

According to SEM studies, the oval olfactory rosette of *T. jarbua* consists of 18 to 20 primary lamellae in each left and right rosette. 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 (Figure 1). The posterior third of the lamellae is provided with a linguiform process (Figures 1 and 2). A large part of the lateral surface of the olfactory lamella is covered with nonreceptor epithelial cells, whereas the receptor epithelial cells mainly occupy the middle region of the olfactory lamella (Figure 3).

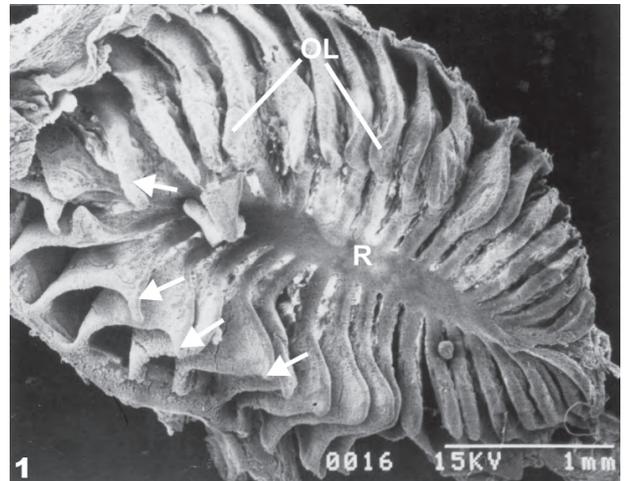


Figure 1. Photomicrograph from scanning electron microscopy (SEM) of the oval olfactory rosette, with different shapes of olfactory lamellae (OL) radiating from the median raphe (R). Arrows indicate the linguiform process of the OL (SEM, '50).

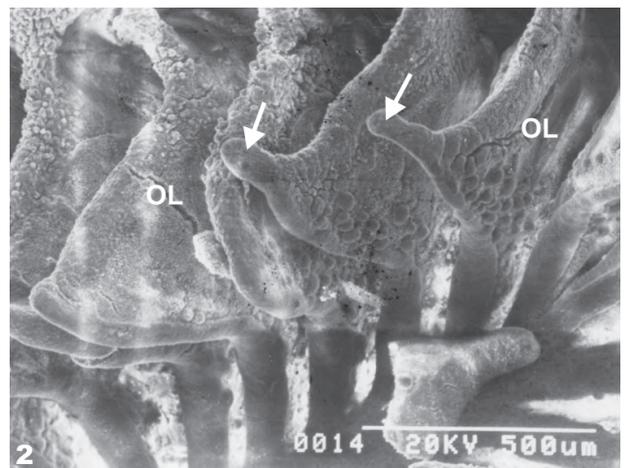


Figure 2. Linguiform process of the OL (arrows), in higher magnification (SEM, '200).

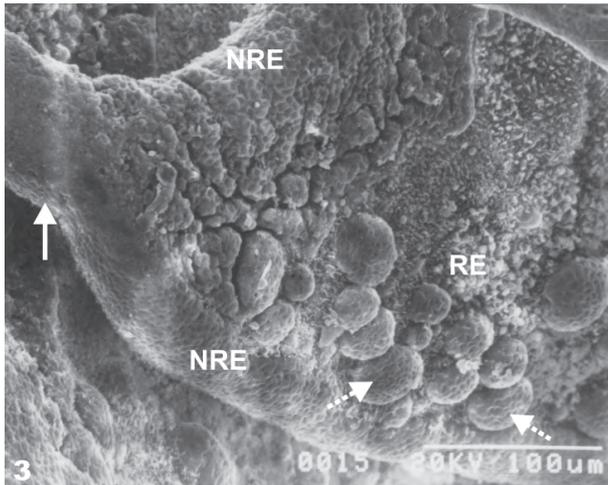


Figure 3. Lateral surface of the OL showing a restricted area of receptor epithelium (RE) encircled by major areas of nonreceptor epithelium (NRE). Note the labyrinth cell (LC; broken arrows) between the RE and NRE. Solid arrow indicates the stalk of the OL (SEM, '600).

Histologically, the olfactory epithelium is separated into sensory and nonsensory regions. The tip and base of the olfactory lamella is nonsensory, while the remaining middle portion is sensory. The surface zone of the middle olfactory epithelium is mainly lined with receptor cells, rod cells, labyrinth cells, and mucous cells. The dendrite of each primary receptor cell extends as a narrow cylindrical process up to the free olfactory epithelial surface. The nucleus of the receptor cells is more or less oval (Figures 4 and 5). The labyrinth cells are scattered in the superficial layer of the olfactory epithelium, in between sensory receptor cells. They are ovoid in appearance with basal nuclei (Figures 4 and 5). The apical end of the rod cells protrude as a simple rod-like structure from the epithelial surface (Figure 4). The globular mucous cells are scattered in between the receptor cells, rod cells, and labyrinth cells (Figure 4).

According to SEM studies, the surface of the sensory epithelium features some 'pock' marks in between the receptor and rod cells, representing the apical surface of labyrinth cells having shallow depressions (Figure 6). The receptor cells bear 5 to 6 cilia (about 4 to 6 μm in length) on the epithelial surface in between rod cells. The rod cells measure about 3 to 8 μm in length and 1 to 1.5 μm in diameter at the base, and they are oriented in the same direction (Figure 7). The microvillar receptor cells are few in

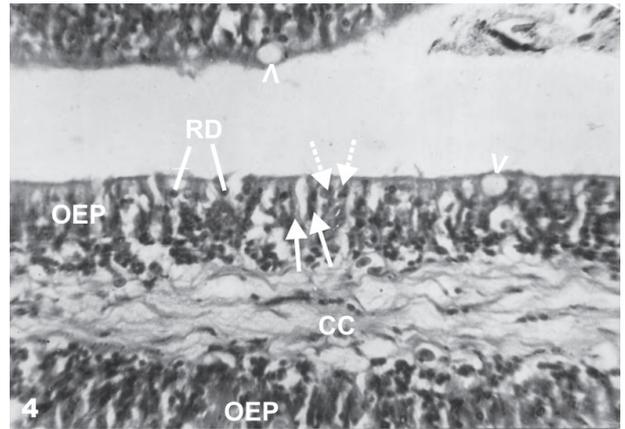


Figure 4. Section of the sensory olfactory epithelium (OEP) separated by the central core (CC), stained with Mallory's triple (MT) stain. Note the presence of receptor cells (RC; solid arrows), LC (broken arrows), rod cells (RD), and mucous cells (arrow heads) in the OEP (MT, '400).

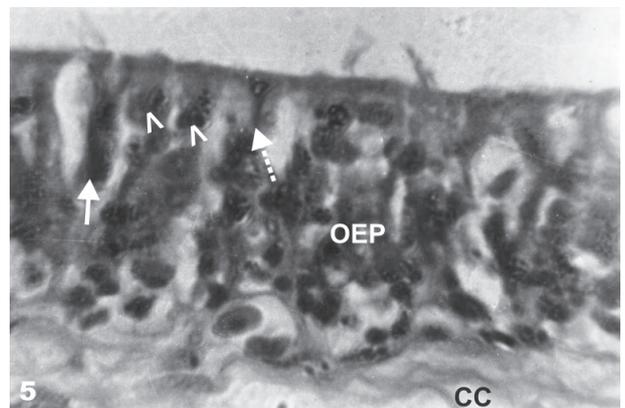


Figure 5. Section showing the dendrite process of the cylindrical RC (solid arrow), which extends up to the surface of the epithelium. Note the oval nucleus of the LC (arrow heads). The broken arrow indicates rod cells (MT, '1000).

number and include tufts of microvilli. These cells are somewhat submerged into the thickness of the ciliated receptor cells and rod cells (Figure 7).

SEM study revealed that the nonsensory epithelial surface is composed of prominent folds of stratified epithelia, with long furrows between them (Figure 8). Between these epithelial folds, patches of ciliated supporting cells (5 to 6 μm) are present, which contain labyrinth-patterned microridges with deep channels between them. Mucous cells with mucus plugs are located between the stratified epithelial

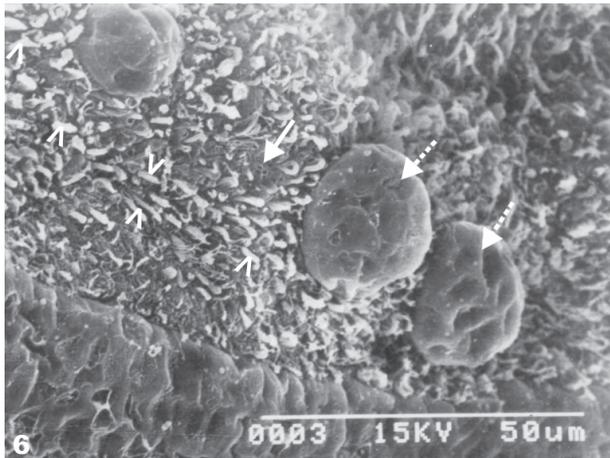


Figure 6. LC pictured with depressions (broken arrows) between large numbers of rod cells (arrow heads) and sensory cells (solid arrow) (SEM, '1000).

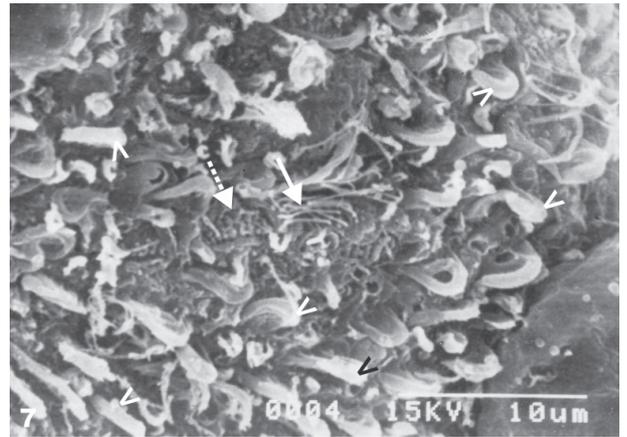


Figure 7. Sensory epithelium, showing RC with 5 to 6 cilia (solid arrow) in between rod cells (arrow heads). The broken arrow indicates microvillous cells (SEM, '2000).

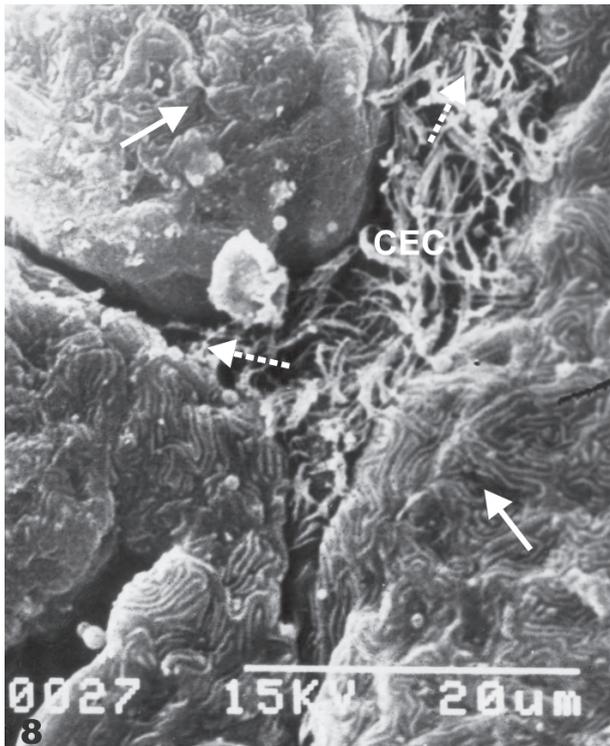


Figure 8. Prominent folds of stratified epithelial cells (SEC; solid arrows), with deep furrows in between (broken arrows). Patches of ciliated supporting cells (CEC) can also be noted in the channels between the folds of the SEC (SEM, '2000).

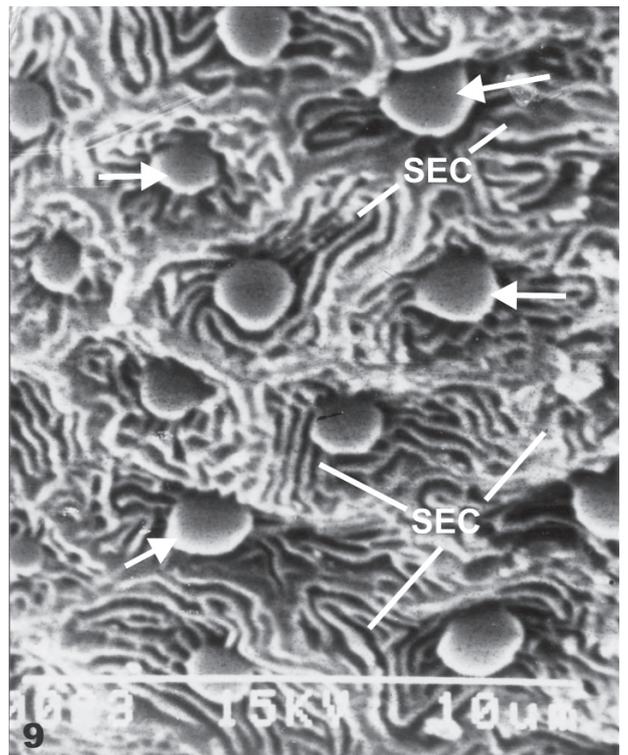


Figure 9. SEC under a higher magnification, showing the labyrinth pattern of microridges (MR). The presence of MC with mucus plug (arrows) can also be observed between the SEC (SEM, '3000).

cells (Figure 9). The SEM examinations revealed that the surface epithelium of the raphe is represented by stratified epithelial cells (5 to 7 μ m) interspersed with mucous cells. The unbranched microridges are

arranged in a concentric whorl on the apical surface of the epithelial cells. Secreted mucin droplets are deposited over the microridges of the epithelial cells (Figure 10).



Figure 10. Surface epithelium of raphe showing SEC with unbranched MR arranged in a concentric whorl. Mucin droplets (arrow heads) are also shown over the MR of the SEC (SEM, $\times 3000$).

Discussion

The multilamellar peripheral olfactory organs in fish have an acute sense of smell, and various aspects of the life history, such as feeding and reproduction, are mediated through olfactory cues (Hara, 1992). The number and shape of the olfactory lamellae are related to the space available in the olfactory cavity of the fish and therefore represent an adaptation that maximizes the sensory area under a given restriction (Zeiske, 1973, 1974). The present study reveals that the olfactory rosette of *T. jarbua* is oval in shape and consists of 18-20 lamellae arranged on either side of the median raphe. In addition, the posterior third of the lamellae is provided with a linguiform process. Thus, the total olfactory area of this fish is considerably greater than the total retinal area. This entitles it to belong to Teichmann's (1954) group of nose fishes, comprising solitary, nocturnal predators (Ojha and Kapoor, 1973).

The distribution of sensory and nonsensory epithelia on the surface of the lamellae shows a great variation in different fish species (Yamamoto, 1982). In the present study, the surface of the sensory epithelium in *T. jarbua* is restricted to the middle region of the olfactory lamellae while the tip and base of the lamellae are provided with nonsensory epithelial cells. This is a unique feature of the olfactory epithelium in this fish.

The sensory epithelium of *T. jarbua* exhibits 3 morphologically distinct types of receptor cells: ciliated, microvillous, and rod cells. They occur together, but in different proportions. The present study reveals that the rod receptor cells dominate over the ciliated and microvillous receptor cells. The receptor cells, with rod-shaped dendrite endings, are distributed randomly in the epithelium. The sensitivity of the rod receptor cells may change in the euryhaline *T. jarbua* when it migrates into sea water from brackish water or vice versa. Hernadi (1993) also theorized that the rod-shaped olfactory neuron may have occurred as a result of the presence of a new physiological condition. On the other hand, the ciliated and microvillar receptor cells are of special interest because they may form a different olfactory transduction mechanism stimulated by odor-bearing substances. Zeiske et al. (2003) observed that the ciliated and microvillar olfactory receptor cells occur together in the olfactory organ of the genus *Acipenser*, but in different proportions in different species.

In the present observation of the sensory epithelium of *T. jarbua*, crypt olfactory sensory neurons were difficult to find in histological and scanning electron microscope studies. The olfactory epithelia of almost all teleosts contain ciliated and microvillous olfactory sensory neurons, which, though described only recently, have been reported in many species and seem to represent a conserved trait in the olfactory systems of fish (Hansen and Finger, 2000). In the present study, the microvillous cells might play an important role in the transduction of environmental signals stimulating the pituitary and gonads. Biju et al. (2003) revealed that the microvillous cells of the olfactory epithelium of *Cirrhinus mrigala* play an important role in the regulation of reproduction. The labyrinth cells on the surface of the olfactory epithelium may serve as excretory cells for osmoregulation; in other words, they may excrete Na^+ and Cl^- . In this way, they may cause 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 the chloride cells found in fish gills. Ruzhinskaya et al. (2001) also demonstrated the presence of typical chloride cells in the olfactory epithelium of *Acipenser maerii*, *A. ruthenus*, *Salmo*

gairdneri, *Carassius auratus*, *C. carassius*, *Perca fluviatilis*, and *Oreochromis mossambicus*. They also reported that these cells are present in the areas of both indifferent and sensory epithelium and provide the active transport of ions between the inner and outer media in order to maintain ion and osmotic homeostasis.

The apical surface of the nonsensory epithelium is provided with tufted, ciliated supporting cells, which are responsible for creating a current of water in the olfactory chamber as well as the lamellar surface for better monitoring of the water quality by the receptor cells. Furthermore, the nonsensory epithelium and the epithelium of the raphe consist of stratified epithelial cells with labyrinth-patterned or unbranched microridges on the apical surface; these cells help to hold a mucus film over the epithelium

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