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Histological and ultrastructural studies of the olfactory epithelium of spotted butter fish *Scatophagus argus* (Linnaeus)

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The olfactory epithelium of Scatophagus argus *(Linnaeus) was investigated by light and scanning electron microscopy. The elongated olfactory organ is made up of 20 to 22 primary lamellae arranged on both sides of the narrow median raphe. Sensory and non-sensory regions are located separately on each lamella. The sensory epithelium occupies the upper apical broad half and extreme basal part of the olfactory lamellae whereas the middle slender part is covered with non-sensory epithelium. The sensory epithelium consists of ciliated, microvillus, and crypt cells. The non-sensory epithelium is made up of stratified epithelial cells having different patterns of finger-like micro-ridges and mucous cells. Different cells lining the olfactory epithelium have been correlated with the functional views of the fish concerned.* (Folia Morphol 2011; 70, 2: 74–79)

Key words: cellular organisation, olfactory epithelium, function, *Scatophagus argus*

INTRODUCTION

The olfactory organ of fish mediates responses to a multitude of different stimuli that enable fish survival in the surrounding aquatic environment. These are relatively simple structures comprising a mosaic of receptors arranged between supporting cells [12]. The teleostean olfactory organ displays numerous species variations especially in gross structure and size, due to differences in their habits. Herbivorous *Puntius javanicus* and *Puntius sophore* have oval shaped olfactory rosettes consist of 25 to 26 and 16 to 18 lamellae, respectively [8, 11]. On the other hand, in *Etroplus suratensis*, an algal feeder, the olfactory rosette consists of radially arranged lamellae [10], and in *Wallago attu*, a carnivorous fish, the olfactory rosette is elongated and made up of 62 to 64 olfactory lamellae [7]. The fine anatomical structure of the olfactory epithelium of different teleosts have been investigated through the electron microscope by various authors [8–10, 17–21]. Studies revealed that enormous diversities exist in different teleosts regarding the shape, number, and arrangement of olfactory lamellae and distribution of sensory and non-sensory epithelium on the olfactory lamellae depending upon various factors including food searching, migration, predator avoidance, and reproduction. However, there is a dearth of knowledge regarding the various cells lining the olfactory epithelium and their functional aspects in brackish water teleosts. Therefore, the purpose of the present study was to work out in detail the histology and the surface architecture of the olfactory epithelium of *Scatophagus argus*, which plays a meaningful role in detecting the odoriferous substances in the saline ecosystem.

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MATERIAL AND METHODS

Adult healthy fish of *S. argus* (9 to 11 cm in length) were collected from Junput brackish water fish farm, West Bengal. The collected fish were killed by decapitation. For scanning electron microscopy (SEM), the olfactory rosettes after dissection were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.3) for 30 min. The olfactory rosettes were dissected from the olfactory chamber under a stereoscopic binocular microscope to unravel the olfactory apparatus. The adhering mucus of the epithelial surface was removed by repeated rinsing with heparinised saline (heparin sodium salt 10,000 IU dissolved in 0.67% NaCl solution). After being rinsed in 0.1 M cacodylate buffer (pH 7.3), tissues were infiltrated with 2.5% glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.3) for 24 h at 4°C. After fixation the tissues were put away, rinsed in the same buffer for 10 min and subjected to post fixation in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.3) for 2 h. The tissues were cleansed with buffer and dehydrated through a graded series of acetone, followed by isoamyl acetate and subjected to critical point drying method. The olfactory rosettes were carefully mounted on metal stubs coated with gold palladium with a thickness of approximate 20 nm. The tissues were then scanned in a Hitachi S-530 SEM.

For histological studies the tissues were fixed in Bouin's fluid for 16–18 h and were dehydrated properly through an ascending series of ethyl alcohols, cleared with xylene, and embedded in paraffin wax at 56–58°C under a thermostat vacuum paraffin embedding bath for a period of 1 h. Sections were cut at 4 mm thick and stained with Mallory's triple stain.

RESULTS

According to SEM examination, the elongated olfactory apparatus of *S. argus* is provided with a convex ventral and concave dorsal surface having 20 to 22 primary lamellae (0.6 to 0.8 mm in length) that radiate to the left and right side of the rosette (Fig. 1). The outer margins of the lamellae are free, while the inner margins are attached to the raphe. The apical part of the lamellae are flat and tongue shaped while the middle and bases are slender (Figs. 1, 2). Each olfactory lamella bears a broad space of receptor area on the apical end and small aggregation of receptor area in between the base of the lamella adjacent to the raphe. The non-sensory epithelium occupies mainly the lateral surface of the middle region of the olfactory lamella (Fig. 2).

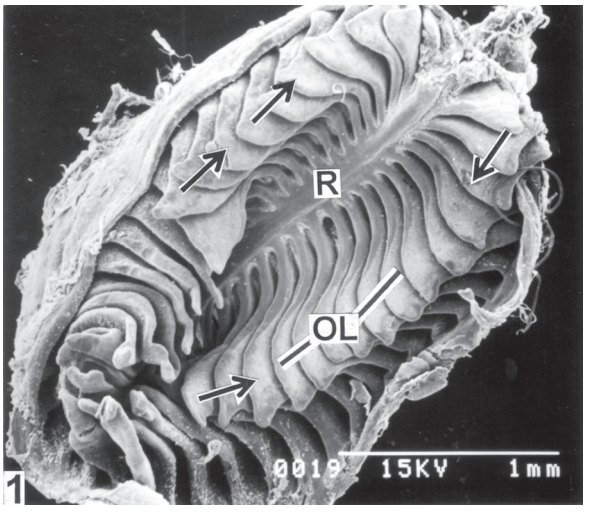

Figure 1. Elongated olfactory rosette exhibiting different shapes of olfactory lamellae (OL) radiating from median raphe (R). Arrows indicate tongue shaped apical part of the OL; SEM × 40. Information: Photomicrographs of the olfactory epithelium of *Scatophagus argus* by scanning electron microscopy (SEM), and histological sections stained with Mallory's triple (MT) stain.

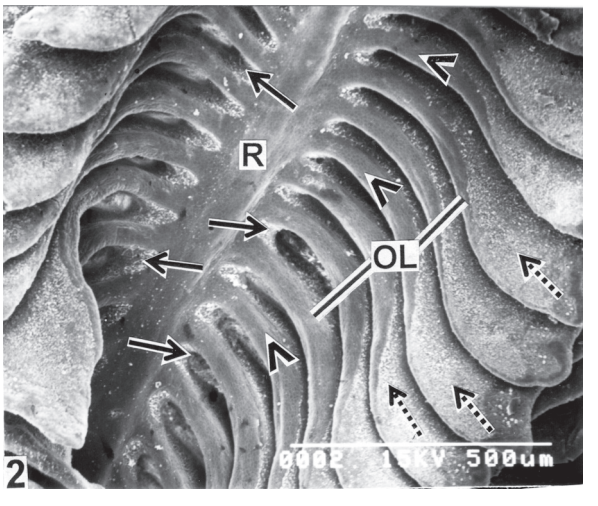

Figure 2. Olfactory lamellae (OL) provided with receptor area on the flat apical ends (broken arrows) and the base of the lamellae (solid arrows) adjacent to the raphe (R). Arrowheads indicate the non-sensory epithelium; SEM × 100. Information as in Figure 1.

Histologically, the surface of the sensory epithelium is composed of a large number of elongated receptor cells, supporting cells, and mucous cells. All cells are closely packed in the olfactory epithelium (Fig. 3). The sensory epithelium is also supported by prominent crypt cells and microvillus cells (Fig. 3).

According to the SEM study the surface of the olfactory epithelium is made up of receptor cells and stratified epithelial cells, leaving mucous cells in be-

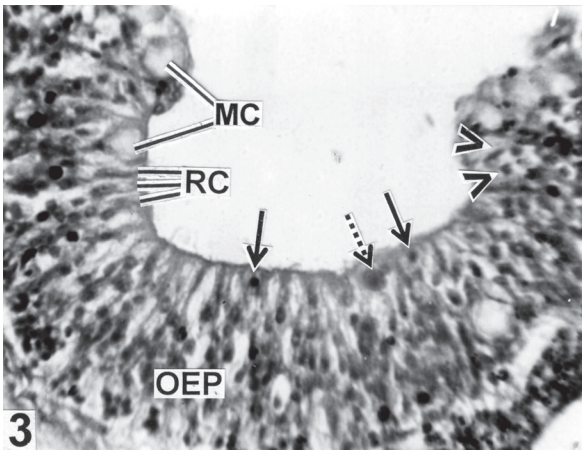

Figure 3. Sensory olfactory epithelium (OEP) composed of receptor cells (RC) supporting cells (arrow heads) and mucous cells (MC). Note the presence of crypt cells (broken arrows) and microvillus cells (solid arrows); MT × 400. Information as in Figure 1.

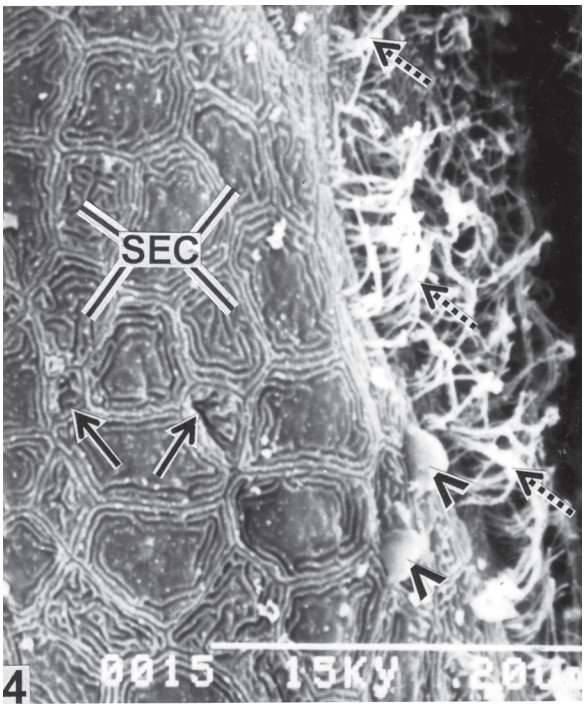

Figure 4. Sensory olfactory epithelium showing dense mat of receptor cells (broken arrows), stratified epithelial cells (SEC). Note the presence of mucous cells (solid arrows) and mucin droplets (arrowheads) in between SEC; SEM × 2000. Information as in Figure 1.

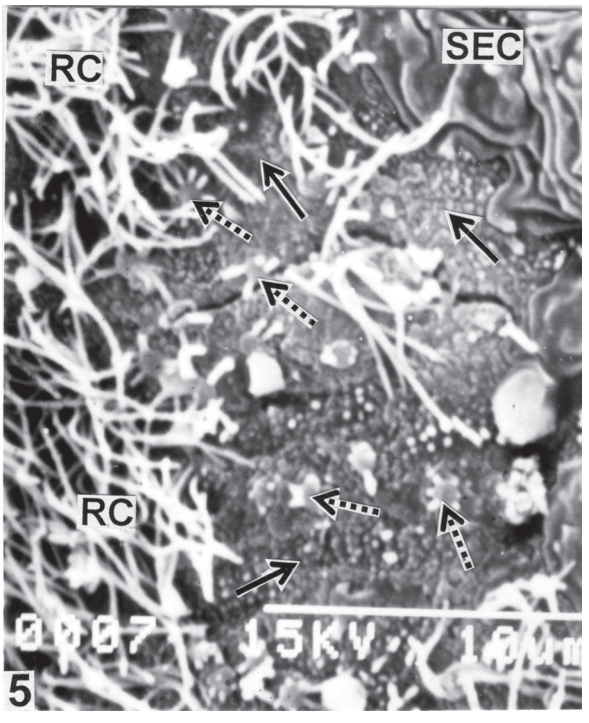

Figure 5. Dendrite process of cylindrical receptor cells (RC) and stratified epithelial cells (SEC). Note presence of microvillar cells (solid arrows) and crypt cells (broken arrows) in between RC; SEM × 3500. Information as in Figure 1.

tween (Fig. 4). The olfactory receptor cells are located in groups and have three types on the basis of the structure on their apical part, i.e. flagellar, microvillar, and crypt cells. The dendrite process of cylindrical receptor cells of sensory epithelium extend as a flagellated process. The microvillar receptor cells are few in number and are provided with microvilli and submerged into the thickness of the flagellar receptor layers (Fig. 5). In contrast to these flagellar or microvillar receptor cells a slightly sunken apex of crypt cells has also been recognised because they have a peculiar arrangement of inconspicuous microvilli (Fig. 5).

Histologically, the surface zone of the non-sensory epithelium is basically comprised of stratified epithelial cells with prominent nuclei and mucous cells. A few scattered flagellar receptor cells are present in between the stratified epithelial cells (Fig. 6). Under the SEM study the basal region of the surface epithelium of each lamella adjacent to the raphe is provided with patches of sensory receptor cells in between the stratified epithelial cells (Fig. 7). Mucous cells are located in between the stratified epithelial cells. The apical surfaces of the stratified epithelial cells are provided with unbranched micro-ridges arranged in a concentric whorl. The raphe is represented by compactly arranged stratified epithelial cells. The unbranched micro-ridges on the apical surface of the epithelial cells are also arranged in a concentric whorl. Scattered mucous cells with a mucous plug are located in between the stratified epithelial cells (Fig. 8).

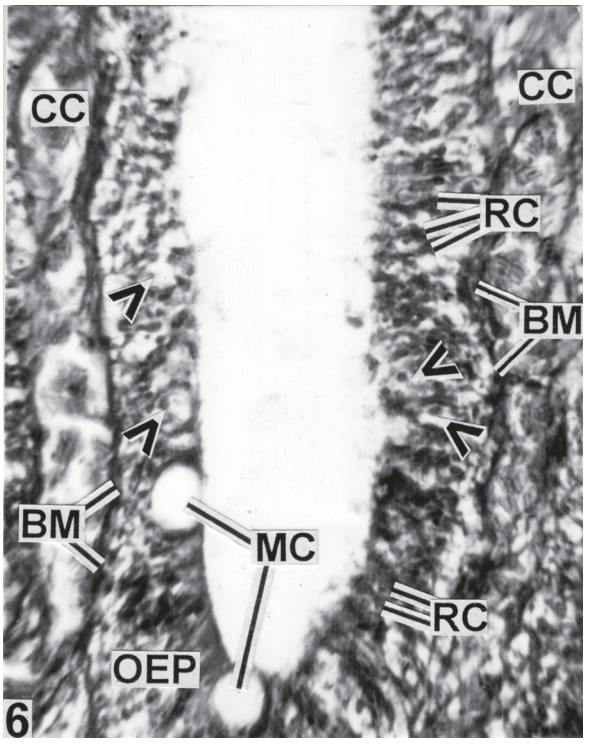

Figure 6. Section of non-sensory olfactory epithelium (OEP) showing stratified epithelial cells (arrowheads), mucous cells (MC), and a few scattered receptor cells (RC). Note the presence of basement membrane (BM) in between central core (CC) and OEP; MT × 400. Information as in Figure 1.

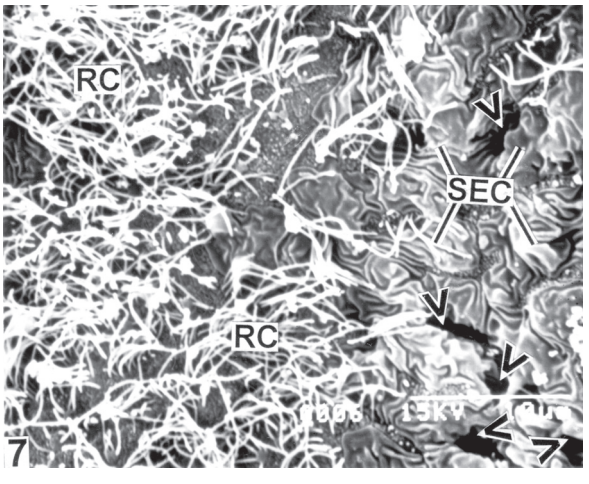

Figure 7. Dendrite patches of receptor cells (RC) in between the stratified epithelial cells (SEC). Note the presence of mucous cells (arrowheads) over the SEC; SEM × 3000. Information as in Figure 1.

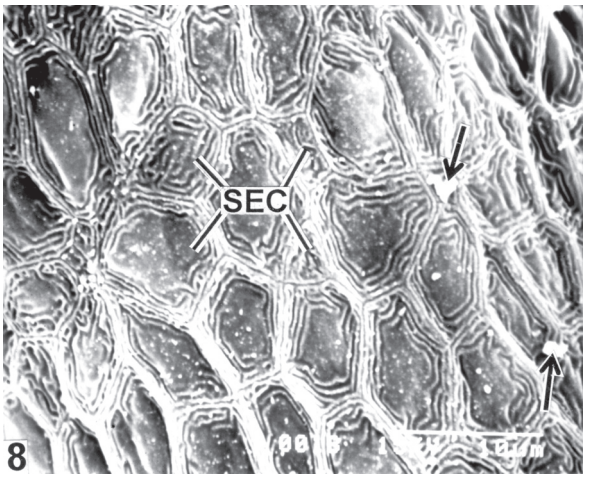

Figure 8. Compactly arranged stratified epithelial cells (SEC) on the raphe. Note peculiar arrangement of micro-ridges on the SEC. Note also the presence of a mucous plug (solid arrows) over the SEC; SEM × 4000. Information as in Figure 1.

DISCUSSION

The olfactory epithelium shows considerable diversity, reflecting the degree of development and ecological habitat [25]. The presence study reveals that the elongated olfactory rosette of *S. argus* consists of 20 to 22 lamellae arranged on either side of the median raphe. This means that it belongs to the Teichmanns [22] group of nose fishes comprising solitary and nocturnal predators [3]. The distribution of the sensory and non-sensory epithelia on the surface of the lamellae shows great variation in different fish species [23]. The surface of the olfactory lamellae of *S. argus* can be distinguished as sensory or non-sensory or by different region. In *S. argus* the sensory receptor epithelium is restricted to the apical tongue-like portion of the lamellae and the base from which the lamellae arise. This is a unique feature of the olfactory epithelium in this fish occupying a specific ecological habitat and thus mobilising different olfactory cues. Zielinski and Hara [27] and Hara and Zielinski [16] also identified definite aggregations of ciliated receptor cells and confirmed their olfacto-sensory functions.

In the present study of *S. argus*, the receptor epithelium consists of three types of extensions of sensory dendrites: the flagellated, microvillus, and crypt cells. Hansen et al. [15] opined that the olfactory epithelium of channel catfish contains three intermingled types of olfactory receptor neurons: ciliated, microvillus, and crypt, which are responsible for the detection of bile salt and amino acid odorants. The present study reveals that the flagellated receptor cells dominated over the microvillus and crypt cells. The flagellated receptor cells are of special interest because they form part of the olfactory transduction mechanism, are stimulated by odour-bearing substances, and because they also enable the fish to detect food. Zeiske et al. [26]

reported that the ciliated and microvillar receptor cells are common but in different proportions in different species. In *S. argus* the microvillus receptor cells consist of minute dendrites having a slightly sunken apex in contrast to the flagellated receptor cells. The microvillus receptor cells might form a different olfactory transduction mechanism for pheromones or amino acids. Bhute and Baile [5] also advocated that the receptor neurons perceive and process signals of pheromone, which is an important step in the breeding pattern of *Labeo rohita*. Camacho et al. [6] reported more or less similar positions of microvillus cells in the olfactory epithelium of sturgeon. However, Bakhtin [2] and Bannister [3] reported microvillus cells in the olfactory surface of *Squalus acanthias* and teleostean fishes and opined that these cells are predecessors of ciliated receptor cells. In addition to the two aforesaid classical receptor cells, another sensory cell is present in the receptor epithelium of the *S. argus*: the crypt cell. Although crypt cells occur regularly in all lamellae, the number is low. The most striking characteristic of this cell is the fact that it bears 5 to 6 short cilia oriented on the apical rim of the cell. Similar cells have been reported before by Andres [1] and Zeiske et al. [24] in the olfactory epithelium of fish. Crypt cells have also been found in catfish, sword tails, and needle fish [14]. Furthermore, Hansen and Zeiske [13] support that the crypt cell is a receptor neuron in the peripheral olfactory organ of the zebra fish, *Danio rerio*. The present study suggests that the peculiar structure of the crypt cells in the sunken position on the olfactory epithelium of *S. argus* may be involved in the transduction mechanism for pheromones in the environment. The present study supports the view that the features of crypt olfactory sensory neurons share the features of microvillus cells. In the transitional zone of receptor and non-receptor epithelium few scattered flagellated receptor cells are responsible for better monitoring of the water quality even up to this zone.

The non-receptor epithelium consists of stratified epithelial cells provided with micro-ridges arranged in a concentric whorl. Such micro-ridges located on the epithelial cells play a major role in the anchorage of a thin mucus film over the epithelial membrane to protect the epithelium from different hazardous substances. The mucous cells are distributed in between the sensory and non-sensory epithelial surface of the olfactory lamellae. The mucus covering the olfactory lamellae constitutes an important medium in which odorants are diffused. On the other hand, the mucin probably helps in the binding of microscopic debris and keeps the sensory cells ready for new stimuli. This corresponds with the findings of Bandyopadhyay and Datta [4].

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