VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 69, No. 3, pp. 154–159

ISSN 0015–5659
www.fm.viamedica.pl

Cellular organisation and functions of the olfactory epithelium of pearl spot *Etroplus suratensis* (Bloch): a light and scanning electron microscopic study

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[Received 12 June 2010; Accepted 29 June 2010]

The cellular organisation of the olfactory rosettes of Etroplus suratensis *was studied by light and scanning electron microscopy. The oval shaped olfactory rosette of the fish consists of 12 lamellae radiating from a central raphe. The olfactory lamellae are comprised of restricted areas of sensory epithelium and broad areas of non-sensory epithelium in the apical, middle, and basal regions. The sensory epithelium contains three types of receptor cells: microvillus, ciliated, and rod cells, as well as labyrinth cells and supporting cells. The non-sensory epithelium consists of stratified epithelial and mucous cells. The transitional region between the sensory and non-sensory epithelium consists of ciliated receptor cells, mucous cells, and stratified epithelial cells. The different cells on the olfactory epithelium were discussed regarding the functional significance of the fish concerned.* (Folia Morphol 2010; 69, 3: 154–159)

Key words: cellular architecture, olfactory epithelium, function, *Etroplus suratensis*, light and SEM study

INTRODUCTION

Olfactory cues play an important role in the behaviour of fish, such as the procurement of food, recognition of sex, defence against predators, parental behaviour, and orientation [13]. Olfaction is a mediator of chemical signals and is involved in diverse teleost behaviour [10]. The olfactory organ of fish is the only vertebrate organ in which nerve cells are directly exposed to the aquatic ecosystem. Many reports are available on scanning electron microscopic (SEM) studies of the olfactory organ of different teleosts [1, 2, 4–6, 18, 25]. The studies revealed that enormous diversity exists regarding the modification, distribution of the sensory and non-sensory epithelium, and the abundance of various receptor cells in different teleosts. An effort has therefore been made in the present observation to describe the structural organisation and the different functional views of the olfactory epithelium of brackish water teleost *Etroplus suratensis* (Bloch).

MATERIAL AND METHODS

Ten live, mature *E. suratensis* fish weighing 199.33 ± 2.08 g (13 to 15 cm in length) were procured from Junput brackish water fish farm, West Bengal. The specimens were anaesthetised under tricaine methanesulfonate. The heads of the *E. suratensis* were dissected from the dorsal side under a stereoscopic binocular microscope to remove the olfactory rosette.

For SEM study purposes, the olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 30 minutes. The rosettes were then dissected and the

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adhering mucus of the epithelial surface was removed by repeated rinsing with heparin solution (heparin sodium salt 10,000 IU dissolved in 0.67% NaCl solution). After rinsing in 0.1 M phosphate buffer (pH 7.4), the tissues were again fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4) for 24 hours at 4°C. After fixation, the tissues were rinsed in the same buffer for 10 minutes and post fixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) for two hours. The tissues were washed thoroughly in buffer and dehydrated through a graded series of acetone followed by isoamyl acetate and subjected to the critical point drying method. The dried tissues were coated with gold palladium and observed under a Hitachi S-530 SEM.

For histological study, the tissues were fixed in Bouin's fluid for 16–18 hours and were dehydrated properly through an ascending series of alcohols, cleared with xylene, and embedded in paraffin. Sections were cut at 4 μm thick. After routine histological procedure the sections were stained with Mallory's triple stain.

RESULTS

The oval shaped (2.5 mm × 2 mm size) olfactory rosette of *E. suratensis* occupies the entire cavity of the olfactory chamber and is composed of 12 club shaped radial lamellae radiating from a central raphe with a convex ventral and concave dorsal surface (Fig. 1). Each lamella is dorsally attached to the wall of the olfactory chamber but ventrally rests on a limited space of the raphe (Fig. 1). Each olfactory lamella bears a limited swollen space of receptor area (Fig. 1). Histologically, the olfactory lamellae are based on a raphe and composed of two layers of thin olfactory epithelium separated by a wide central lamellar space, the central core — which is made up of loose connective tissue, blood capillaries, and nerve fibres (Fig. 2). The sensory olfactory epithelium is composed of a large number of primary and secondary receptor cells and supporting cells. All cells are closely packed in the olfactory epithelium (Figs. 3, 4). The dendrite process of each primary receptor cell extends as a narrow cylindrical process up to the free epithelial surface. The secondary receptor cells are mainly present below the primary receptor cells and they do not extend up to the surface epithelium or surface mucosal layer. The nuclei of the receptor cells are more or less oval (Figs. 3, 4). In between the receptor cells, few labyrinth cells are distributed in the olfactory epithelium (Fig. 4).

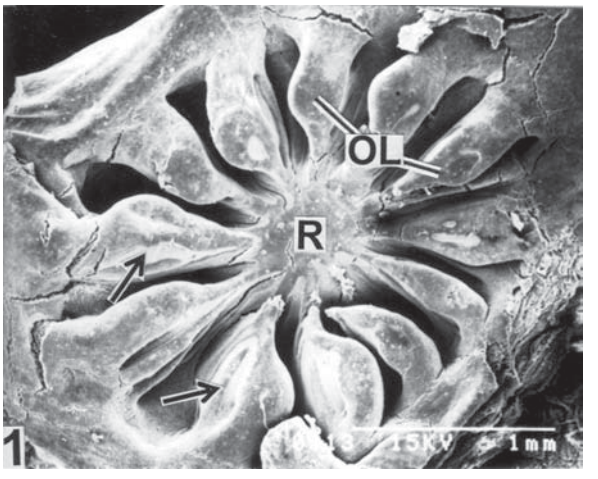

Figure 1. Showing oval olfactory rosette with different shapes of olfactory lamellae (OL) radiating from central raphe (R). Note limited swollen areas of sensory epithelium on the OL (solid arrows); SEM × 50.

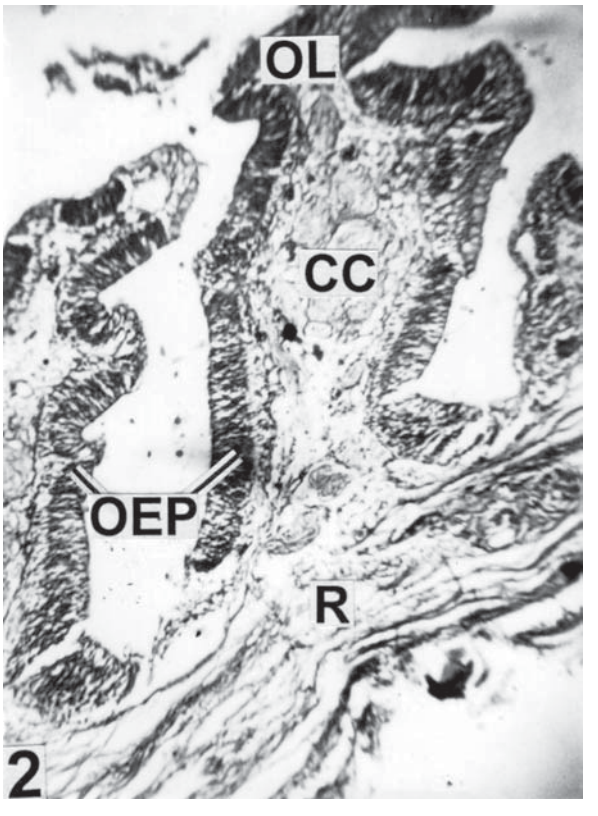

Figure 2. Section of olfactory lamellae (OL) showing thin olfactory epithelium (OEP) separated by broad central core (CC); R — raphe; MT × 150.

According to SEM studies, the surface of the olfactory lamella is provided with restricted sensory area surrounded by broad areas of non-sensory epithelium (Fig. 5). At higher magnification, the apical surface of the sensory epithelium can be seen

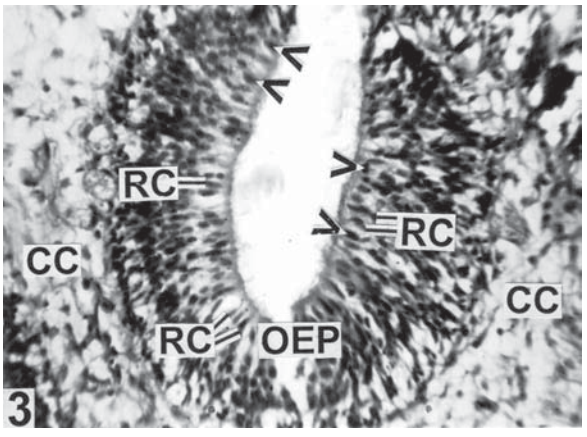

Figure 3. Sensory olfactory epithelium (OEP) showing various stages of receptor cells (RC) and supporting cells (arrow heads); CC — central core; MT × 400.

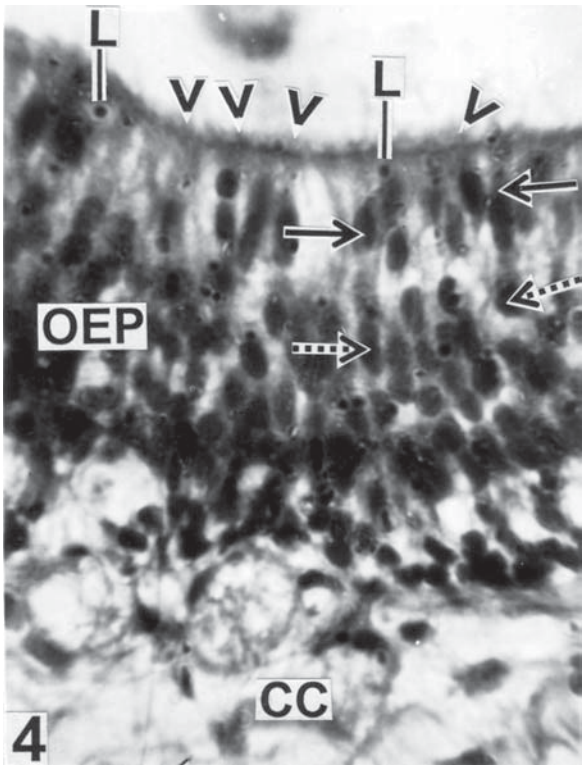

Figure 4. Sensory olfactory epithelium (OEP) showing primary (solid arrows) and secondary (broken arrows) receptor cells. Note extended dendrite process of receptor cells (arrow heads); CC — central core; L — labyrinth cells; MT × 1000.

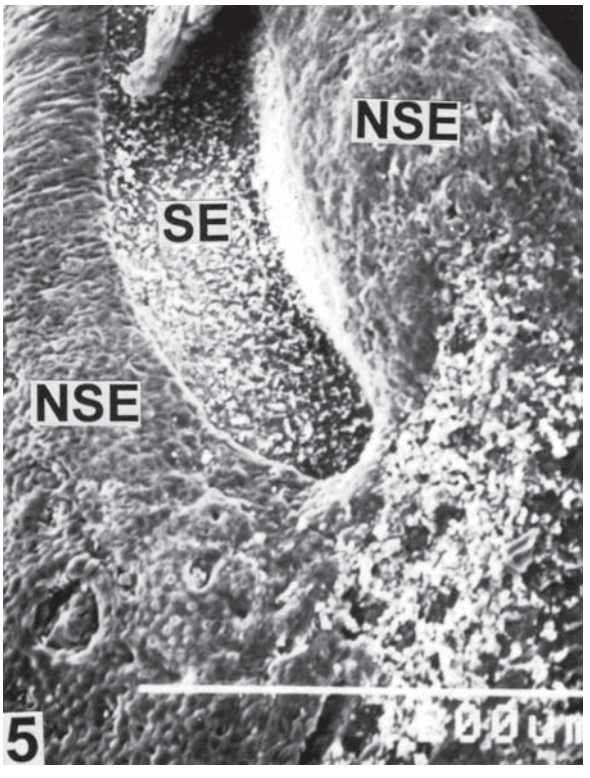

Figure 5. Higher magnification of SEM view of olfactory lamellae showing restricted area of sensory epithelium (SE) encircled by non-sensory epithelium (NSE); SEM × 200.

Figure 6. Apical part of sensory epithelium showing prominent ciliated receptor cells (arrows). Arrow heads indicate labyrinth cells; SEM × 5000.

to have prominent ciliated receptor cells (4 to 5 μm in length) and some 'pock' marks (2 to 2.5 μm in width) representing the apical surface of labyrinth cells (Fig. 6). The middle part of the sensory epithelium is mainly comprised of a dense population of receptor cells (Fig. 7). On the other hand, the apical part of some areas of sensory epithelium consist of three types of receptor cells: microvillus cells (2.5 μm), rod cells (5 to 6 μm), and ciliated receptor cells (4 to 5 μm), which may be distinguished on the basis of the structure of the dendrite end (Fig. 8).

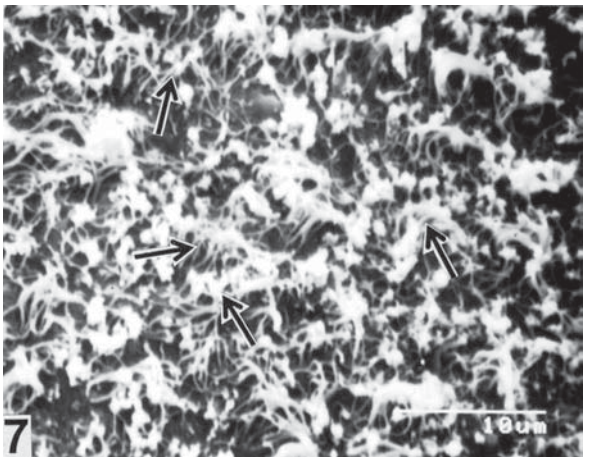

Figure 7. Sensory epithelium of the middle part of olfactory lamellae showing dense mat of ciliated receptor cells (solid arrows); SEM × 5000.

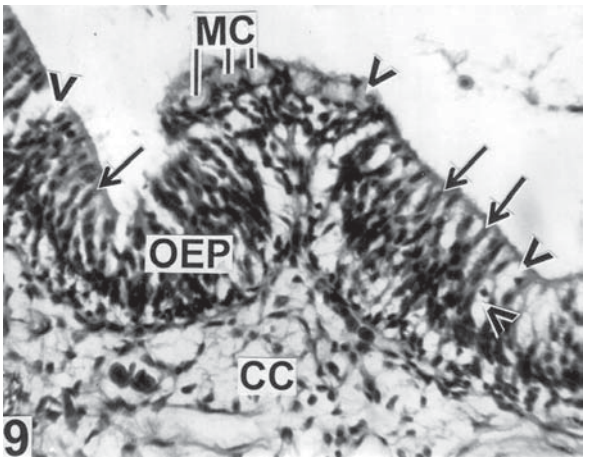

Figure 9. Section of transitional zone of sensory and non-sensory olfactory epithelium showing stratified epithelial cells (arrow heads), mucous cells (MC), and few ciliated receptor cells (solid arrows); CC — central core; OEP — olfactory epithelium; MT × 400.

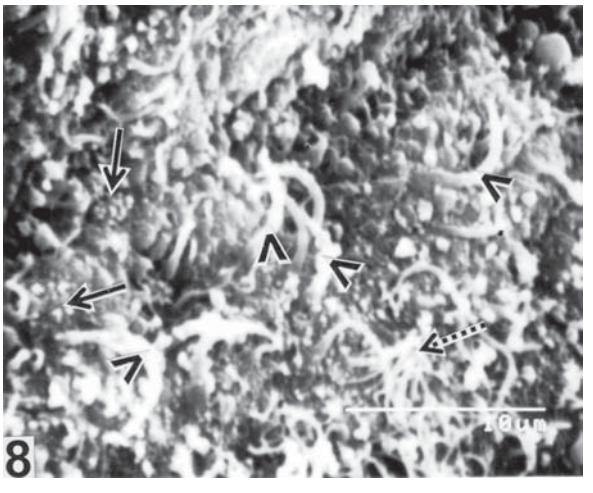

Figure 8. Basal part of sensory epithelium showing ciliated receptor cells (broken arrow), rod cells (arrow heads), and microvillus cells (solid arrows); SEM × 5000.

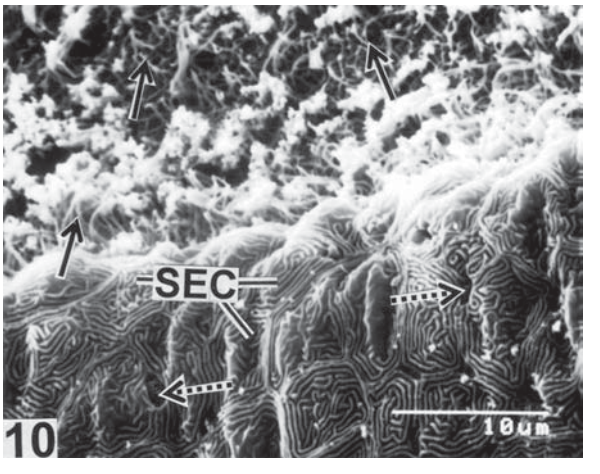

Figure 10. Transitional zone of sensory and non-sensory olfactory epithelium showing ciliated receptor cells (solid arrows), stratified epithelial cells (SEC) having labyrinth-pattern microridges, and mucous cells (broken arrows) in between SEC; SEM × 5000.

Histologically, the transitional zone of the sensory and non-sensory olfactory epithelium is comprised of stratified epithelial cells, mucous cells, and few ciliated receptor cells (Fig. 9). Under SEM study, the non-sensory epithelial surface is provided with compactly arranged, stratified epithelial cells (8 to 9 μm in height), leaving mucous cells in between. However, in between the stratified epithelium, patches of ciliated receptor cells are also present (Fig. 10). Under SEM study, the surface epithelium of the raphe is represented by stratified epithelial cells (8 to 9 μm) interspersed with the openings of mucous cells. However, the apical surfaces of the stratified epithelial cells are provided with labyrinth-pattern microridges leaving shallow channels in between (Fig. 11).

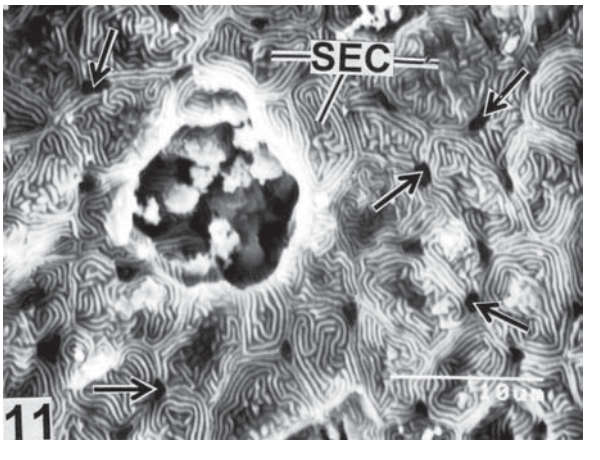

Figure 11. Surface epithelium of raphe showing definite arrangement of stratified epithelial cells (SEC) and mucous cells (solid arrows) in between SEC. Note labyrinth pattern of microridges on SEC; SEM × 5000.

DISCUSSION

The multi lamellar peripheral olfactory organ in fish gives them an acute sense of smell in various aspects of their life history, such as feeding and reproduction, which are mediated through olfactory cues [9]. The olfactory mucosa containing the olfactory sensory neurons is typically located on the floor of the olfactory chamber, which is often folded, forming olfactory lamellae [8]. The number and shape of the olfactory lamellae are related to the space available in the olfactory cavity of the fish and, therefore, represent the adaptation which maximises the sensory area under a given restriction [23, 24]. The present study reveals that the olfactory rosette of *E. suratensis* is oval in shape and consists of twelve lamellae arranged radially from the central raphe. According to Teichman [19], the oval type of olfactory organ falls under the category of 'eye-nose' fish, which means that this category of fish possess similarly developed optic and olfactory faculties. The distribution of sensory and non-sensory epithelia on the surface of olfactory lamellae shows great variety in different fish species [20]. In the present study, in *E. suratensis* the surface of the sensory epithelium was restricted to the middle region of the olfactory lamellae while the tip, base, and middle broader part of the lamellae are provided with non-sensory epithelium. This is a unique feature of the olfactory epithelium in this fish. Histologically, the olfactory lamellae of *E. suratensis* comprise two layers of olfactory epithelium sandwiching a central core. Both the epithelia are of unequal thickness; the middle region of the olfactory lamellae is thicker than the apical part. The reason may be the fact that epithelia of the middle region of lamellae possess a rich population of receptor cells as compared to the epithelium of the apical lamella. Yamamoto and Ueda [22], and Cancalon [3] and Yamamoto [20] have reported three distinct classes of receptor cells depending on dendrite pattern: ciliated receptor cells, microvillus receptor cells, and rod cells. In the present study, in *E. suratensis* the sensory epithelium mainly consists of three morphologically distinct types of receptor cells: ciliated, microvillus, and rod cells. They occur in different proportions in different areas of the sensory epithelium. The present study reveals that the ciliated receptor cells dominate over the microvillus and rod receptor cells. In the present observation, the ciliated receptor cells correspond to the type I cells of Yamamoto and Ueda [22], whereas the microvillus correspond to the type II cells of Muller and Marc [15], and the rod cells to the type IV cells of Ichikawa and Ueda [12]. The olfactory epithelial cells with rod shaped processes have been described as usual receptors [21] by Yamamoto [20] while Muller and Marc [15] reported their existence and ultrastructural integrity but advocated against their receptive nature in goldfish and catfish. However, on the basis of experimental work and developmental studies, Zielinski and Hara [28, 29] and Moran et al. [14] have established that the rod shaped processes of these cells represent the dendritic apical processes of olfactory receptor cells. The present study reveals that the receptor cells with rod shaped dendrite endings were distributed randomly in the epithelium. The sensitivity of the rod receptor cells may change in the euryhaline *E. suratensis* when they migrate into sea water from brackish water or vice versa. Hernadi [11] proposed that the occurrence of the rod shaped olfactory neuron has been observed in the presence of a new physiological condition. On the other hand, the ciliated and microvillus receptor cells are of special interest because they may form a different olfactory transduction mechanism, are stimulated by odour-bearing substances, and enable the fish to detect food. Zeiske et al. [26] and Chakrabarti and Ghosh [4] observed that the ciliated and microvillus olfactory receptor cells occur together in the olfactory organ of *Acipenser* and *Catla catla* but in different proportions. Hansen et al. [7] 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 labyrinth cells on the surface of the olfactory epithelium serve as excretory cells for osmoregulation and ion regulation. In this way, they may cause the olfactory organ to function optimally in waters of varying salinities. Shirai and Utida [17] speculated that the labyrinth cells may be involved in electrolyte transport because they are structurally similar to chloride cells found in fish gills. On the other hand, the non-ciliated supporting cells in the sensory epithelium are believed to give mechanical support to other sensory cells.

The transitional zone between sensory and non--sensory olfactory lamellae contains receptor cells with patches of dendrites. This suggests that the olfactory sensation may extend up to this zone. In *E. suratensis* the non-sensory epithelium and the epithelium of raphe consists of stratified epithelial cells provided with labyrinth-pattern microridges on their apical surface that help to hold the mucus film

over the epithelium to protect the olfactory epithelium from different hazardous substances. The mucous cells are distributed between stratified epithelial cells, and the secreted mucin from the mucous cells probably helps in binding minute particles and keeps the sensory cells ready for new stimuli. This is in agreement with the findings of Rahamani and Khan [16]. Zeni and Stagni [27] opined that the mucus covering the olfactory lamellae constitutes an important medium in which the odorants are diffused, like that of other olfactory systems of vertebrates.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Dr. S. Chakraborty, Scientist-in-charge of the USIC, Burdwan University, for his technical support and also are thankful to the Department of Science and Technology, New Delhi for providing necessary instrumental facilities for this research work.

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