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Cytoarchitectural and surface ultrastructural analysis of the olfactory epithelium of *Oreochromis nilotica* (Linnaeus)

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The olfactory organ of Oreochromis nilotica *was studied by means of light and scanning electron microscopes. The oval shaped olfactory apparatus consists of 19–20 lamellae radiating from a central raphe. The receptor epithelium occupies the restricted area of the middle swollen region of the lamellae and is framed with receptor cells (both ciliated and microvillous) and rod cells. The larger part of the lateral surface of the olfactory lamella is covered with non-receptor epithelium, which is made up of stratified epithelial cells and mucous cells. The functional significance of various cells lining the olfactory epithelium of this fish are discussed.* (Folia Morphol 2011; 70, 3: 143–148)

Key words: histology, surface architecture, olfactory epithelium, function, *Oreochromis nilotica*

INTRODUCTION

The olfactory organ of fish directly interacts with the environment; therefore, sensory cells of the olfactory epithelium detect water-soluble compounds to convey information about the surrounding environment. Olfaction plays an important role in mediating behaviour, from feeding and predator detection to social interaction and also reproductive synchrony [16]. Many reports are available on the morpho-histological peculiarities of the olfactory epithelium of fish [6, 7, 9, 13, 20]. As a complement to those studies of histology, SEM has been employed by different workers [1, 4, 11, 12, 23]. These studies have revealed that enormous diversities exist regarding the modification and distribution of the sensory and non-sensory epithelium as well as an abundance of various receptor cells in different teleosts. An attempt has therefore been made in the present study to examine the histology and the surface architecture of the olfactory epithelium of *Oreochromis nilotica* because in this way the function of the olfactory organ may be better analysed in the aquatic ecosystem. This fish lives in both freshwater and brackish water and displays reproductive behaviour that may be mediated through intraspecific communication by pheromones.

MATERIAL AND METHODS

Living healthy adults of *Oreochromis nilotica* were obtained from freshwater bodies. The fish were anaesthetised with MS 222. The olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 10 minutes. The rosettes were then carefully dissected out from the dorsal side under a stereoscopic binocular microscope. The adhering mucus was taken away by rinsing in 1% Tween 40 solution. After being rinsed in 0.1 M cacodylate buffer (pH 7.4), the tissues were transferred to 2.5% glutaraldehyde solution for 24 hours at 4°C. After fixation the tissues were removed, rinsed in the same buffer pH 7.4 for 10 minutes, and post fixed in 1% osmium tetroxide in 0.1 M ca-

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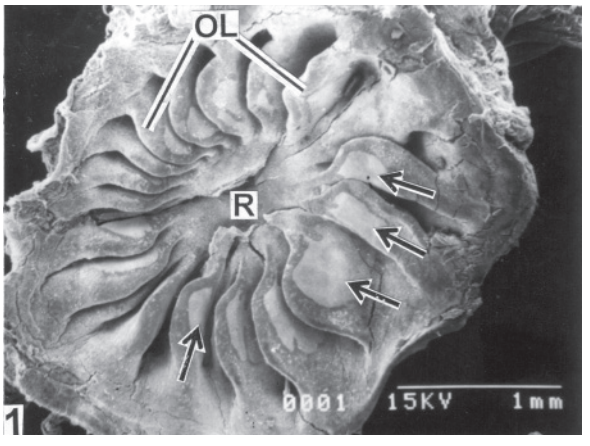

Figure 1. Oval shaped olfactory apparatus showing olfactory lamellae (OL) radiating from raphe (R). Note restricted swollen portion of receptor epithelium of lamellae (arrows); SEM × 50 [Figures 1–10: Scanning electron micrographs (SEM) and histological photomicrographs of the olfactory epithelium of *Oreochromis nilotica*].

codylate buffer (pH 7.4) for 2 hours. The tissues were subsequently dehydrated through a graded series of acetone followed by isoamyl acetate and subjected to critical point drying method with liquid carbon dioxide. After being dried the olfactory rosettes were coated with gold palladium with a thickness of approximately 20 nm and scanned in a Hitachi S-530 SEM.

For histological study, some tissues were fixed in Bouin's fluid for 16 h and were subsequently dehydrated properly through a graded series of ethyl alcohols, cleaned with xylene, and embedded in paraffin. Sections were cut at 3–4 μm thick, and deparaffinised sections were stained with Delafield's Haematoxylin-Eosin stain.

RESULTS

According to SEM studies the oval shaped olfactory rosette of *Oreochromis nilotica* occupies the entire cavity of the olfactory chamber and is composed of 19–20 club shaped radial lamellae (0.6 to 1 mm) radiating from a central raphe (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). The size and shape of the lamellae vary according to their position in the rosette. A large part of the lateral surface of the olfactory lamella is covered with non-receptor epithelium, whereas the receptor epithelium occupies mainly in the middle swollen region of the olfactory lamella (Fig. 1). Histologically, the olfactory lamellae are based on a raphe and are composed of two layers of olfactory epithelium separated by a central core which is made up of

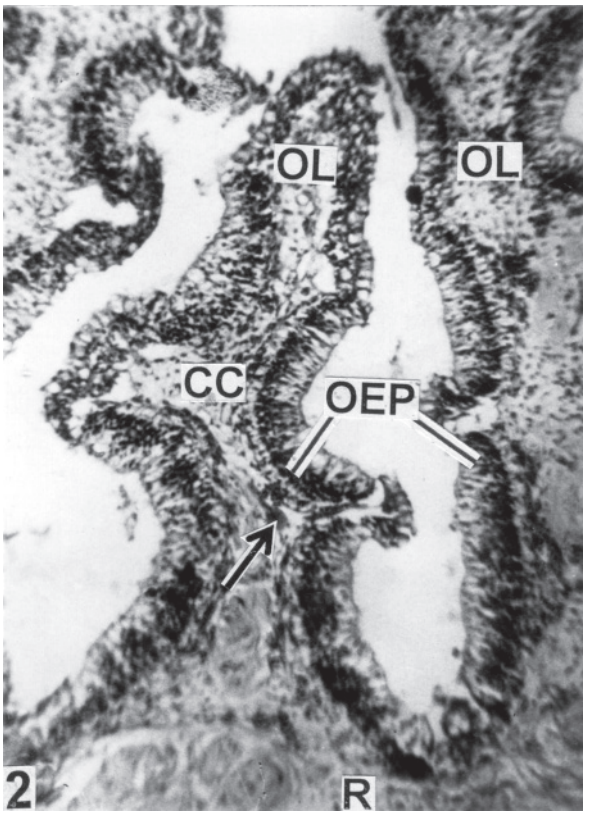

Figure 2. Olfactory lamella (OL) based on raphe (R). Note the arrangement of olfactory epithelium (OEP) separated by central core (CC). Arrow indicates blood capillaries; Mallory's Triple stain × 150.

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 mucous cells (Figs. 3, 4). However, 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. The nuclei of the primary and secondary receptor cells are more or less oval (Fig. 4). In between the receptor cells, rod cells and microvillus cells are distributed in the olfactory epithelium (Fig. 3).

Under SEM study the surface of the olfactory lamellae are also provided with restricted sensory areas surrounded by broad areas of non-sensory epithelium (Fig. 5). At higher magnification the surface of sensory epithelium can be seen to have a dense population of ciliated receptor cells and rod cells (Fig. 6). They occur together but the rod receptor cells dominate over the ciliated receptor cells, which may be distinguished on the basis of the structure of the dendrite end (Fig. 6).

Histologically, the transitional zone of the sensory and non-sensory olfactory epithelium is com-

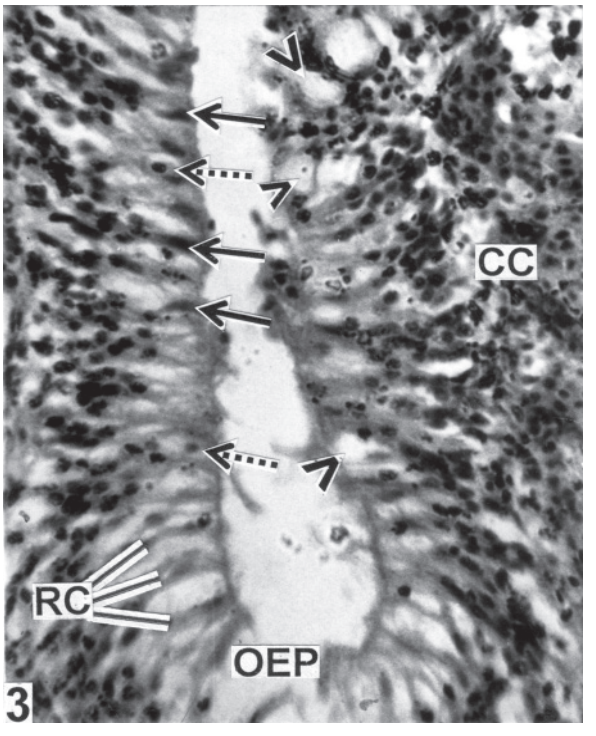

Figure 3. The sensory epithelium of olfactory lamella (OEP) showing receptor cells (RC), microvillous cells (broken arrows), rod cells (solid arrows), and mucous cells (arrow heads); CC indicates central core; Mallory's Triple stain × 400.

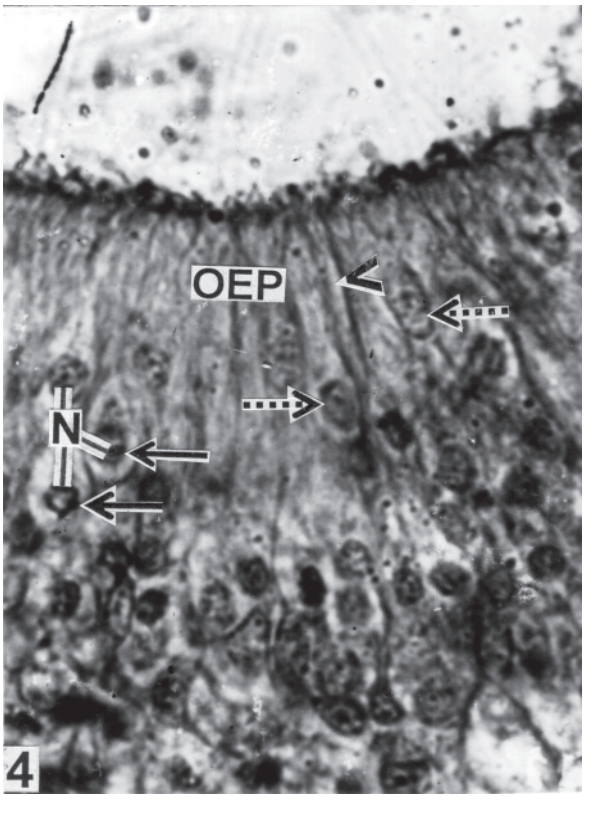

Figure 4. Sensory olfactory epithelium (OEP) in higher magnification showing primary (broken arrows) and secondary (solid arrows) receptor cells (RC). Note the cylindrical dendrite process of primary RC (arrow heads) and prominent nuclei (N) of primary and secondary RC; Mallory's Triple stain × 1000.

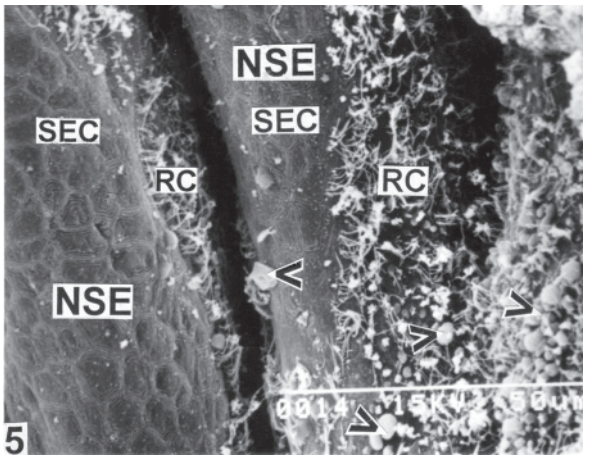

Figure 5. Restricted areas of receptor cells (RC) surrounded by non-sensory epithelium (NSE) provided with stratified epithelial cells (SEC); SEM × 3200.

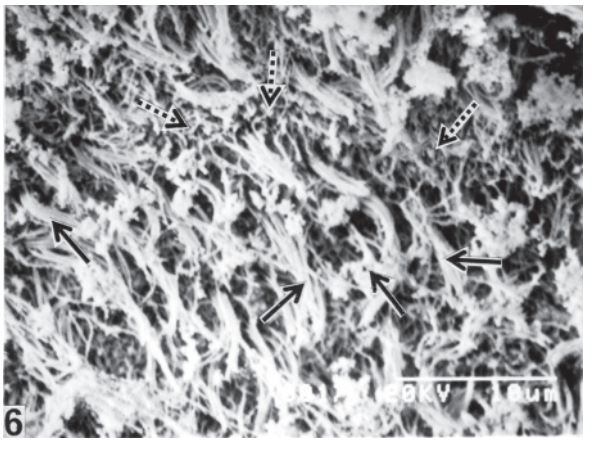

Figure 6. Sensory epithelium provided with dense population of ciliated receptor cells (broken arrows) and rod cells (solid arrows); SEM × 5000.

prised of stratified epithelial cells, mucous cells, and very few sensory receptor cells (Fig. 7). According to SEM studies this transitional zone of the sensory and non-sensory olfactory epithelial surface is provided with compactly arranged, stratified epithelial cells leaving sensory receptor cells in between (Fig. 8). In the transitional zone and crypt region of olfactory lamellae the microvillus receptor cells are somewhat submerged into the thickness of the sensory receptor cells (Fig. 9). Under SEM study the non-sensory epithelium including raphe is represented by stratified epithelial cells intercalated with the opening of mucous cells. Furthermore, the apical surface of the stratified epithelial cells are provided with a labyrinthine pattern of micro-ridges leaving shallow channels in between (Fig. 10). Secreted mucin drop-

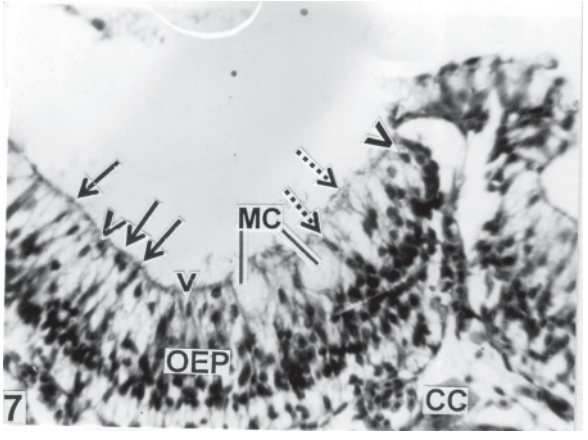

Figure 7. Transitional zone of sensory and non-sensory olfactory epithelium (OEP) showing microvillus receptor cells (solid arrows), stratified epithelial cells (arrow heads), few receptor cells (broken arrows), and mucous cells (MC); CC — central core; Mallory's Triple stain × 400.

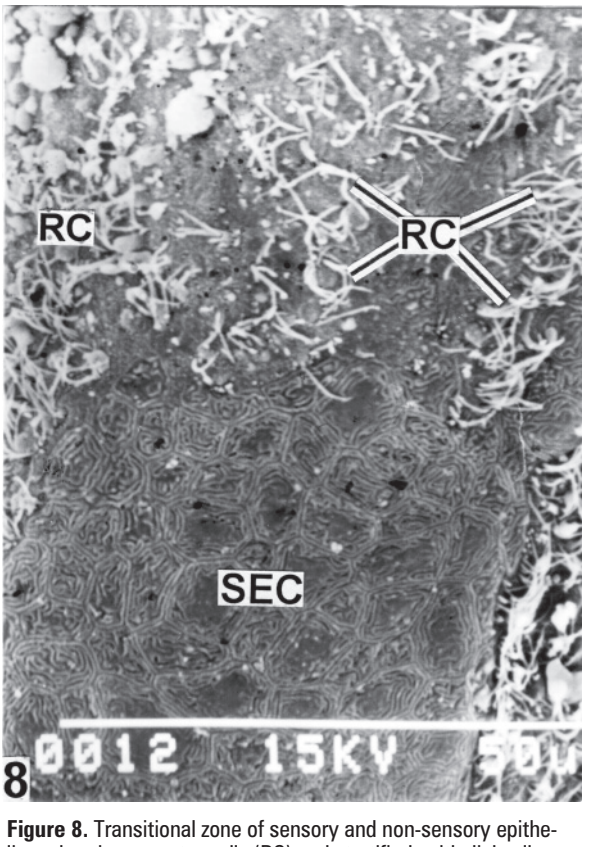

Figure 8. Transitional zone of sensory and non-sensory epithelium showing receptor cells (RC) and stratified epithelial cells (SEC); SEM × 3200.

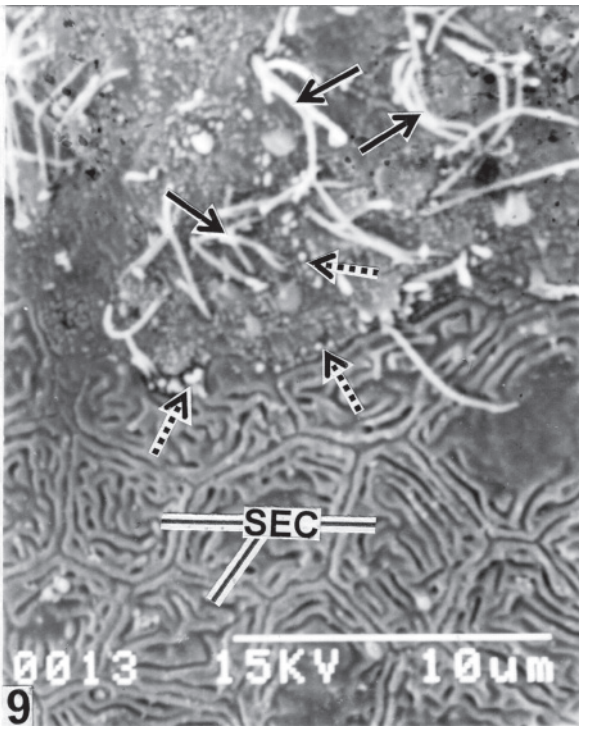

Figure 9. Higher magnification of SEM view of the transitional zone of sensory and non-sensory epithelium showing ciliated receceptor cells (RC) (solid arrows), microvillus RC (broken arrows), and compactly arranged stratified epithelial cells (SEC); SEM × 6000.

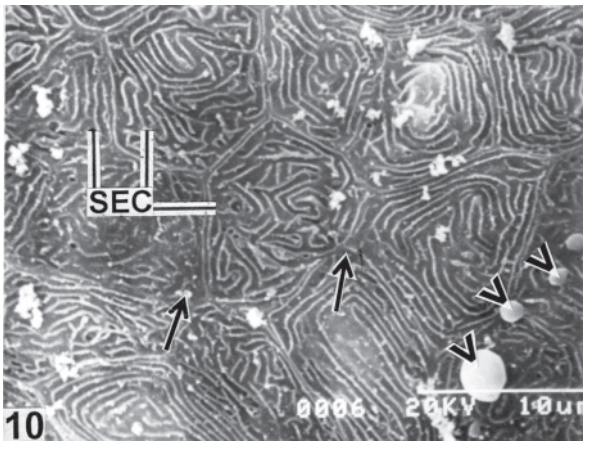

Figure 10. Surface view of non-sensory epithelium showing definite arrangement of stratified epithelial cells (SEC) and the opening of mucous cells (arrows). Note labyrinthine pattern microridges on SEC and deposition of mucin droplets (arrow heads) on SEC; SEM × 6000.

lets are deposited over the micro-ridge surface of the epithelial cells (Fig. 10).

DISCUSSION

The olfactory epithelium containing the olfactory sensory neurons is typically located on the floor of the olfactory chamber, which is often folded, forming olfactory lamellae [8]. Modifications of the olfactory system may occur in some species through adaptation to a specific environment [21]. The present study reveals that the olfactory rosette of *O. nilotica* is almost oval in shape and consists of 19–20 club-shaped

lamellae arranged radially from a central raphe. According to Teichmann [17], 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 areas in the olfactory epithelium is variable in fish [18]. In *O. nilotica* the olfactosensory epithelium occurs only in the form of islets located along the medial-lateral wall of the lamellae while broader part of the lamellae are provided with non-sensory epithelium. This is a unique feature of the non-sensory epithelium in this fish. This arrangement may be due to the fact that the islet part of the sensory epithelium faces the flow of incoming water current and hence possesses more cellular layers as well as a rich population of receptor cells as compared to the epithelium of the opposite side.

One of the most interesting features of the present study is the histological identification of primary and secondary receptor cells and their synaptic connection. Furthermore, the axons of secondary neurons extend into the central core of the lamella. In the present SEM study of the sensory epithelium of *O. nilotica* the sensory epithelium mainly consists of two morphologically distinct types of receptor cells: ciliated and rod cells. However, the receptor cells with rod shaped dendrite endings are distributed randomly in the epithelium. This type of arrangement of receptor cells is 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. In the present observation, the ciliated receptor cells correspond to the type I cells of Yamamoto and Ueda [19] indicates a more developed olfactory sensitivity and the rod cells to the type IV cells of Ichikawa and Ueda [10] represent the dendrite apical process of olfactory receptor cell. On the other hand Muller and Marc [15] reported the existence and ultrastructural integrity of rod receptor cells but advocated against their receptive nature in goldfish and catfish. However, on the basis of experimental work and developmental studies, Zielinski and Hara [24, 25] and Moran et al. [14] established that the rod shaped process of these cells represents the dendritic apical processes of olfactory receptor cells. In the present study, 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. [3] regarding the olfactory epithelium of the sturgeon. Zeiske et al. [22] and Chakrabarti and Ghosh [5] observed that the ciliated and microvillous olfactory receptor cells occur together in the olfactory organ of *Acipenser sp* and *Catla catla* but in different proportions. In *O. nilotica* the microvillous receptor cells might form a different olfactory transduction mechanism for pheromones in the regulation of reproductive activities. It is noteworthy that the sensory epithelium of *O. nilotica* also provided few mucous cells. This is perhaps an advantage for the circulation of water and for the function of receptors. In the transitional zone of sensory and non-sensory epithelium few ciliated receptor cells in between stratified epithelial cells are responsible for better monitoring of the water quality even up to this zone. Furthermore, the non-receptor epithelium and the epithelium of raphe consist of stratified epithelial cells provided with labyrinthine pattern micro-ridges on their apical surface that help to hold the mucus film over the epithelium. Therefore, such micro-ridges play a major role in protecting the sensory receptor cells from mechanical as well as chemical injury. The mucin secreted by mucous cells probably constitutes an important medium in which the odorants are diffused. In addition, the mucin secreted from the mucous cells of raphe probably helps the smooth flow of water in the olfactory chamber by binding microscopic debris which is ejected through the posterior nostril. This is in conformity with the findings of Bandyopadhyay and Datta [2] in the olfactory function of *Heteropneustes fossilis*.

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