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Ultrastructural organisation and functional aspects of the olfactory epithelium of *Wallago attu* (Bleeker)

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The topological architecture and functions of different cells of the olfactory epithelium in Wallago attu *(Bleeker) have been systematically studied using a scanning electron microscope. The elongated olfactory rosette of the fish consists of 62 to 64 primary lamellae in each left and right rosette. Each lamella is provided with apical sensory epithelium and basal non-sensory epithelium. Topological analysis reveals that sensory epithelium contains receptor cells, ciliated supporting cells, labyrinth cells, and goblet cells. The non-sensory epithelium is made up of patches of ciliated supporting cells, epidermal or stratified epithelial cells with concentrically arranged microridges, and scattered goblet cells. Different cells on the olfactory epithelium support the view that the olfactory signalling is important to the survival of this fish in an aquatic environment.* (Folia Morphol 2009; 68, 1: 40–44)

Key words: topological organisation, scanning electron microscope study, olfactory epithelium, *Wallago attu*

INTRODUCTION

The olfactory organ is the only organ in fish where nerve cells are directly exposed to the environment. In fish, olfaction and gustation are the two major chemosensory systems that enable the survival of the organism in an aquatic environment. Scanning electron microscopy was first applied to the olfactory epithelium of the gold fish [4]. Extensive information, gained by scanning electron microscope, on the topological characteristics of the olfactory epithelium of different teleosts has subsequently been reported [3, 6, 10, 14, 19]. However, there is a dearth of scanning electron microscope-derived knowledge regarding modifications of the olfactory epithelium in relation to the feeding habits of fish. The common freshwater shark *Wallago attu* is a column feeder and feeds on insects, fish fry, tadpoles, etc. Therefore, it would naturally be worthwhile to examine more closely the histology and surface architecture of the olfactory epithelium in this fish because it is essentially a chemoreceptor and plays a meaningful role not only in locating food but also in detecting the presence of odoriferous substances in aquatic ecosystems.

MATERIAL AND METHODS

Healthy, adult *Wallago attu* fish were collected from local freshwater ponds. The fish were anaesthetized directly in the test aquaria with MS 222. The heads of the *W. attu* were dissected from the dorsal side under a stereoscopic binocular microscope for removal of the olfactory rosette.

For histological study, the tissues were fixed in Bouin's fluid for 16 to 18 hours and were subsequently dehydrated properly through graded alcohols, cleaned with xylene, and embedded in paraffin.

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Sections were cut 3–4 μm thick. The deparaffinised sections were brought to water and stained with Delafield's haematoxylin-eosin stain, dehydrated, and mounted in DPX.

For scanning electron microscope (SEM) study, the olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 30 minutes. The rosettes were then dissected, and adhering mucus was removed by rinsing in Tween 40 solution. After being rinsed in 0.1 M cacodylate buffer, the tissues were infiltrated with 2.5% glutaraldehyde for 24 hours at 4°C. After fixation the tissues were rinsed in the same buffer, pH 7.4, for 10 minutes, and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer, pH 7.4, for 2 hours. The tissues were washed thoroughly in buffer and dehydrated through graded acetone followed by isoamyl acetate and subjected to the critical point drying method. After being dried the olfactory rosettes were mounted on metal stubs, coated with gold, and scanned in a Hitachi S 530 SEM.

RESULTS

The elongated olfactory rosette of *W. attu* consists of 62 to 64 primary lamellae in each left and right rosette. The elongated rosette has two rows of lamellae arranged on either side of the long raphe. The olfactory lamellae are divided into two regions: sensory and non-sensory (Fig. 1). Histologically, the surface zone of the non-sensory olfactory epithelium is basically comprised of ciliated supporting cells, labyrinth cells, goblet cells (both secretory and non-secretory), and immature basal cells. A few scattered sensory receptor cells are distributed in between the ciliated supporting cells along the surface of the olfactory epithelium (Fig. 2). The sensory epithelium occupies the apical half of the olfactory lamellae and consists of receptor cells, secondary neurons, ciliated supporting cells, labyrinth cells, mast cells, and basal cells (Fig. 3). The dendrite of each receptor cell extends as a narrow and cylindrical process up to the free epithelial surface, where it enlarges into a small knob-like structure — the olfactory vesicle (Fig. 3). According to SEM studies, the olfactory epithelial surface is provided with prominent longitudinal folds leaving long furrows between them. Some 'pock' marks representing the apical surface of labyrinth cells are also found on the mucosal folds (Fig. 4). Under SEM study, probably the dendrite process of primary receptor cells of sensory epithelium extended as a narrow flagellated process and provided the knob-like structure (Fig. 5). The tuft of

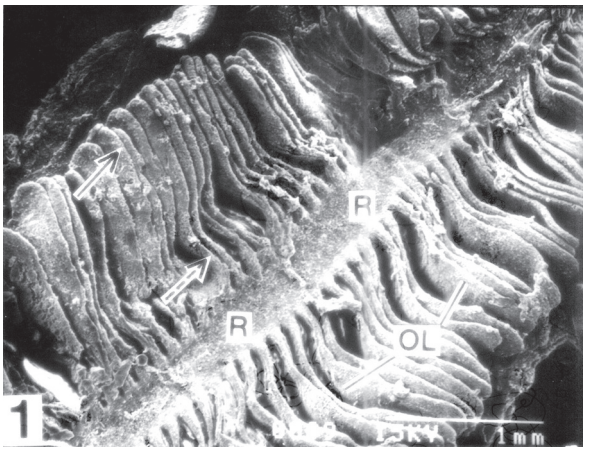

Figure 1. Elongated olfactory rosette showing different shapes of olfactory lamellae (OL) which are radiating from the median raphe (R). Note sensory epithelium (solid arrow) and non-sensory epithelium (broken arrow) of OL. SEM × 50; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

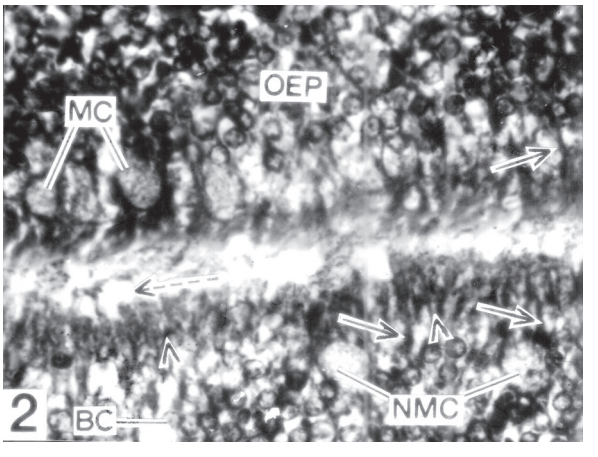

Figure 2. Section of non-sensory olfactory epithelium (OEP), showing receptor cells (solid arrows), ciliated supporting cells (arrow heads), and secretory (MC) and non-secretory (NMC) goblet cells. Note the presence of labyrinth cell (broken arrow) on the surface of the epithelium and basal cell (BC) in the sub surface epithelium. H & E × 400; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

ciliated supporting cells encircles the receptor cells, and mucin droplets are often found to be adhered to the cilia of the supporting cells (Fig. 5). However, a few stratified epithelial cells have also been detected in between receptor and ciliated supporting cells (Fig. 5). The non-sensory epithelium is made up of patches of ciliated supporting cells, among which the oval or elongated stratified epithelial cells are located (Fig. 6). The apical surfaces of the

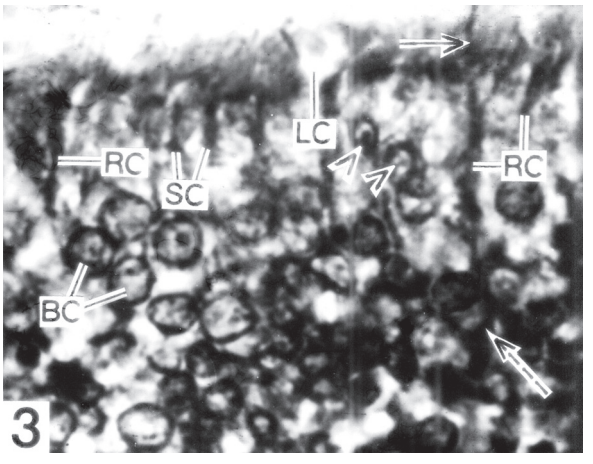

Figure 3. Section of sensory epithelium showing receptor cells (RC), supporting cells (SC), and mast cells (arrow heads) and basal cells (BC). Note the extended cylindrical part of dendrite of RC above the surface epithelium (solid arrow). Note also the secondary neuron (broken arrow) in the deeper part of the epithelium; LC — labyrinth cell. H & E × 1000; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

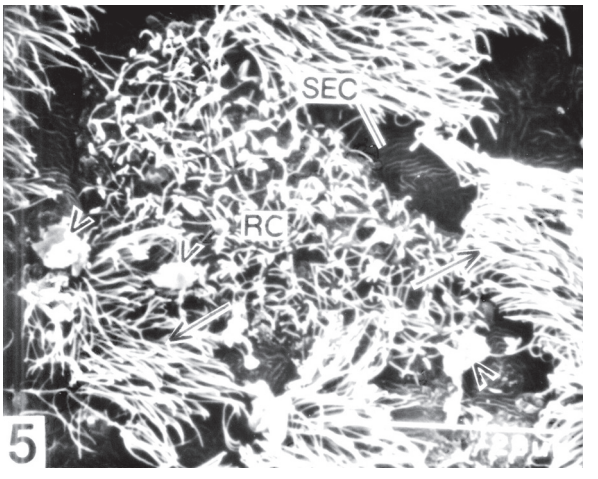

Figure 5. Dendrite process of receptor cells (RC) with terminal knob-like structure forms an islet encircled by ciliated supporting cells (SC) (arrows). Note the presence of scattered stratified epithelial cells (SEC) in between RC and SC. Note also adhering mucin droplet (arrowheads) on the SC. SEM × 3200; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

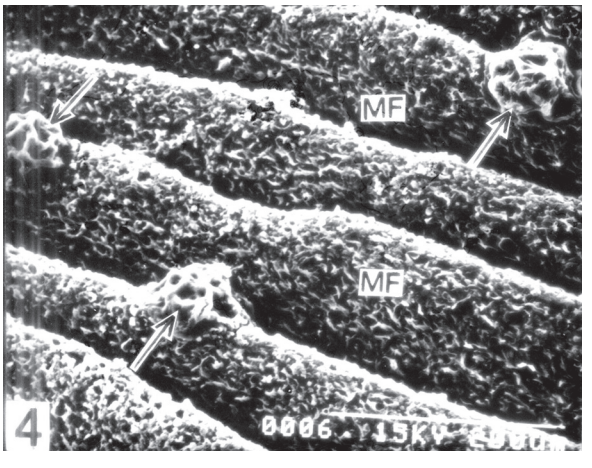

Figure 4. Longitudinal mucosal folds (MF) of the olfactory epithelium showing position of labyrinth cells (arrows). SEM × 1500; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

stratified epithelial cells (5–6 μm) are provided with unbranched microridges arranged in a concentric whorl (Fig. 6). Secreted mucin droplets are also deposited over the ciliated supporting cells (Fig. 6). Under SEM study, the transitional area between sensory and non-sensory olfactory lamellae contains patches of dendrite to form small islets. These dendrite processes of sensory receptor cells are without knob-like structure and are encircled by stratified epithelial cells (Fig. 7).

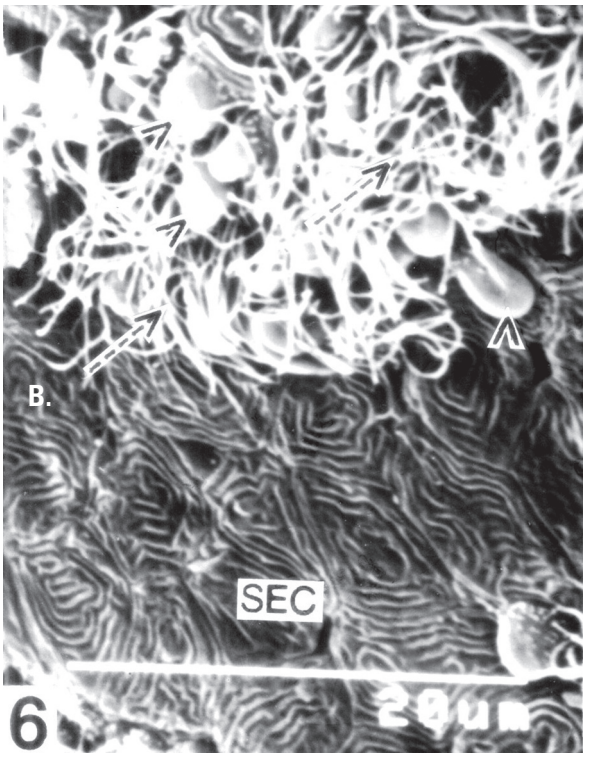

Figure 6. Surface of non-sensory epithelium showing tuft of ciliated supporting cells (SC) (broken arrows) with mucin droplets (arrowheads). Note the presence of oval or elongated stratified epithelial cells (SEC) provided with concentrically arranged unbranched microridges. SEM × 4000; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

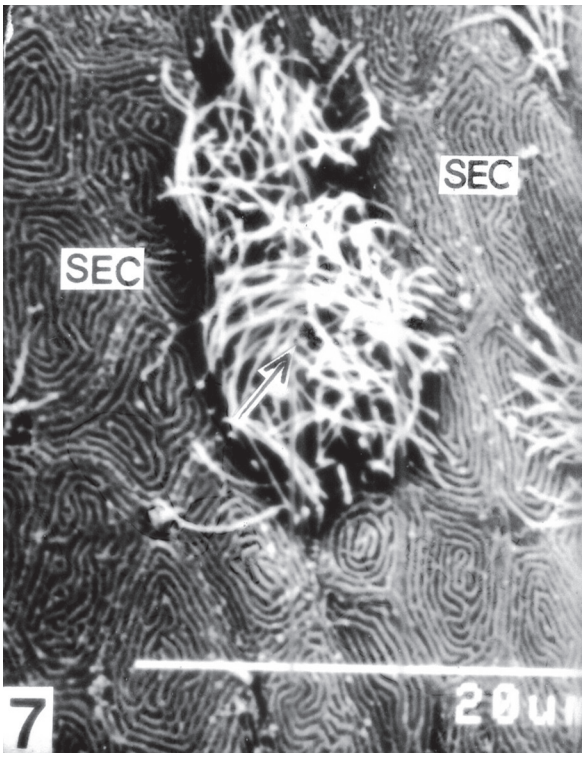

Figure 7. Transitional area of sensory and non-sensory epithelium of olfactory lamellae showing dendrite patches of receptor cells (arrows) in between stratified epithelial cells (SEC). SEM × 4000; photomicrographs of the olfactory epithelium of *Wallago attu* by scanning electron microscope (SEM) and histological technique (Haematoxylin-Eosin, H & E).

DISCUSSION

The elongated rosette of *W. attu* has two rows of lamellae arranged on either side of the long, narrow raphe. This entitles it to belong to Teichmanns [16] group of nose fishes, comprising of solitary, nocturnal predators [12]. The olfactory mucosa containing the olfactory sensory neurons is typically located on the floor of the olfactory chamber, which is often folded, forming the olfactory lamellae [9]. The distribution of the non-sensory and sensory epithelia on the surface of the lamellae shows great variety in different fish species [18]. In the present study of *W. attu*, the non-sensory epithelia is restricted to the basal portion of the lamellae up to the junction of raphe and the lamellae, and the sensory epithelia occupies the apical part of the lamellae. However, the surface of the entire lamellae is provided with ciliated supporting cells. These supporting cells on the sensory and non-sensory epithelium are there to create a slow current of water across the olfactory chambers for better monitoring of the water quality by the receptor cells. The goblet cells are distributed between the stratified epithelial cells and the ciliated supporting cells. The secreted mucin from the goblet cells probably helps in binding microscopic debris and keeps the receptor ready for new stimuli. This is in conformity with the findings of Rahmani and Khan [13] in *Anabas testudinus* and Bandyopadhyay and Datta [3] in *Heteropneustes fossilis*. In the sensory epithelium of *W. attu*, two types of sensory dendrites have been observed, the narrow flagellated process with its knob-like structure and patches of dendrites without knobs. The present study reveals that the flagellated receptor cells with knobs dominate over the receptor cells without knobs. These receptor cells are of special interest because they may form a different olfactory transduction mechanism of pheromones, or protein, amino acids. Hansen et al. [8] opined that the olfactory epithelium of channel catfish contains three intermingled types of olfactory receptor neurons: ciliated, microvillous, 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 organs to function optimally in water of different salinities. Shirai and Utida [15] speculated that the labyrinth cells may be involved in electrolyte transport because they are structurally similar to chloride cells found in fish gills. The basal cells occupy a position below the surface epithelium and have no cytoplasmic processes. The position of the basal cells may have a role as precursors of regeneration of either receptor cells or supporting cells, respectively. Using tritiated thymidine followed by autoradiography, Thronhill [17] showed that the basal cells, apart from differentiating into supporting cells, also give rise to olfactory receptor cells, which are continually replaced during life. Andres [1, 2] also suggested that the basal cells are the precursors of regenerating receptor cells. The histological peculiarities of the previously mentioned cells in the olfactory epithelium in fish having different feeding habits have been reported by various authors [7, 12, 13]. On the other hand, the apical surface of the stratified epithelial cells is provided with concentrically arranged microridges. The presence of these microridges plays a major role for the anchorage of the mucus film secreted by the neighbouring goblet cells to protect the olfactory epithelium from different hazardous substances. The mast cells are thought to cause fluctuations in the production of mucus in the olfactory mucosa. Mast cells

of the olfactory mucosa have been described in Baltic trout by Bertmar [5]. As the terminal mucus film is believed to be an important factor in the olfactory process, this may also influence variations in olfactory sensitivity [11].

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