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HISTOLOGICAL, TOPOGRAPHICAL AND ULTRASTRUCTURAL ORGANIZATION OF DIFFERENT CELLS LINING THE OLFACTORY EPITHELIUM OF RED PIRANHA, *PYGOCENTRUS NATTERERI* (CHARACIFORMES, SERRASALMIDAE)

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Histological, Topographical and Ultrastructural Organization of Different Cells Lining the Olfactory Epithelium of Red Piranha, *Pygocentrus nattereri* (Characiformes, Serrasalmidae). Ghosh, S. K., Chakrabarti, P. — The structural characterization of the olfactory epithelium in *Pygocentrus nattereri* Kner, 1858 was studied with the help of light as well as scanning and transmission electron microscope. The oval shaped olfactory rosette consisted of 26–28 primary lamellae radiated from midline raphe. The olfactory epithelium of each lamella was well distributed by sensory and non-sensory epithelium. The sensory epithelium contained morphologically distinct ciliated and microvillous receptor cells, supporting cells and basal cells. The non-sensory epithelium was made up of labyrinth cells, mucous cells and stratified epithelial cells. According to TEM investigation elongated rod emerging out from dendrite end of the receptor cells in the free space. The dendrite process of microvillous receptor cells contained microvilli. The supporting cells had lobular nucleus with clearly seen electron dense nucleolus. The apex of the ciliated non-sensory cells was broad and provided with plenty of kinocilia. Basal cells provided with oval nucleus and contained small number of secretory granules. The mucous cells were restricted to the non-sensory areas and the nuclei situated basally and filled with about two-third of the vesicles. The functional significance of various cells lining the olfactory epithelium was discussed with mode of life and living of fish concerned.

Key words: *Pygocentrus nattereri,* olfactory epithelium, cellular architecture, fine structure.

Introduction

Fishes have good sense of smell and are adept to ascertain odour with the help of a pair of olfactory organs connected to the olfactory lobes of the brain by means of olfactory nerves (Singh, 1977). Chemoreception plays a momentous role in food finding, predator-prey relationship, mother-infant recognition, reproductive behaviuour, nest finding and other behaviours (Farbman, 1994). The olfaction of fish is usually interrelated with the water ventilation by sniffing process (Nevitt, 1991). In teleost, water with dissolved chemical cues enters into the olfactory cavity through nares; resulting the receptor neurons lining the olfactory mucosa forthwith contact to water contaminants. The receptor cells of the olfactory epithelium are stimulated when they come into contact with certain chemicals carried in water and transmit signals to the nervous system (Lara, 2008).

The olfactory organs of fish exhibits extensive array of diversification relying upon systematic groups and ecological adaptations (Zeiske et al., 2009; Sarkar et al., 2014). Many reports are available on the microarchitecture of the olfactory epithelium of different fishes using light and electron microscopes (Hansen and Zeiske, 1998; Mana and Kawamura, 2002; Lazzari et al., 2007; Ma and Wang, 2010; Kuciel et al., 2011; Atta, 2013; Mokhtar and Abd-Elhafeez, 2014; Ghosh et al., 2015; Samajdar and Mandal, 2016). Studies revealed that enormous diversities exist regarding shape, number and arrangement of the olfactory lamellae, distribution of the sensory and non-sensory epithelium as well as the abundance of various receptor cell types in different teleosts depending upon systematic groups and ecological adaptations. However, there is dearth of knowledge in some aspects of these studies relating to the cellular organization of olfactory epithelium and functional significance in olfaction.

The electron microscopic investigations have shown that the surface olfactory epithelium are provided with ciliated and microvillous receptor cell cells (Yamamoto and Ueda, 1979; Hansen et al., 1999; Camacho et al., 2010).

In the present study, therefore, an attempt has been made to describe the structural organization of the olfactory epithelium of omnivorous freshwater teleost, *Pygocentrus nattereri* (Characiformes, Serrasalmidae), by histological analysis, scanning and transmission electron microscope focusing mainly on the arrangement of olfactory cells in the sensory and non-sensory epithelium.

Material and methods

Healthy, mature specimens of *P. nattereri* (ranged 29.40 ± 2.81 cm in total length; n = 12) were obtained from the local freshwater body of Burdwan (23.2333° N, 87.8667° E), West Bengal, India. Fishes were deeply anesthetized with an aqueous solution of tricaine methone-sulphonate (0.1 % MS 222, Sigma Aldrich) and sacrificed following the guidelines given by the Institutional Ethical Committee. Intact olfactory organs were attentively dissected out from the olfactory chamber and further processed for respective studies.

Histological analysis. The olfactory tissues were kept in aqueous Bouin's fixative for 16–18 hours. After that the tissues were washed thoroughly in 70 % ethanol, dehydrated with graded ascending series of ethanol and cleared in xylene. Then the tissues were infiltrated in paraffin wax of 56–58 ˚C under a thermostat vacuum paraffin-embedding bath for a period of 1 hour. Serial paraffin sections were cut at 4 µm thickness using a rotary microtome (Weswox MT-1090A). After routine histological process deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain (Mallory, 1936). The staining slides were mounted with DPX, observed and photographed under LEICA EC3 compound microscope.

Scanning electron microscopical (SEM) analysis. After dissecting the olfactory chamber, the olfactory rosettes were immersed *in vivo* with 2.5 % glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 20 minutes. Then the olfactory rosettes were carefully dissected and washed repeatedly in heparinised saline (heparin sodium salt 10 000 IU dissolved in 0.67 % NaCl solution). Then the samples were rinsed in 0.1 M phosphate buffer (pH 7.4), fixed with 2.5 % glutaraldehyde for 24 hours at 4 °C and post fixed with 1 % osmium tetroxide (OsO_4) in 0.1 M phosphate buffer (pH 7.4) for further 2 hours at room temperature. Fixed tissues were washed repeatedly in the same buffer and dehydrated in ascending series of acetone followed by isoamyl acetate. The tissues were dried with critical point drier (Hitachi 8CP2), mounted on metal stubs, coated with gold palladium (20 nm thick) and observed under scanning electron microscope, Hitachi S-530.

Transmission electron microscopical (TEM) analysis. Small pieces of olfactory rosette were fixed in a mixture of 2.5 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 6 hours at 4 °C. After washing in same buffer, the tissues were post fixed in 1 % osmium tetroxide (OsO_4) in 0.1 M sodium phosphate buffer (pH 7.3) for further 2 hours at room temperature. After fixation, they were rinsed thoroughly with same buffer to wash off the excess fixative. The samples were dehydrated through ascending series of acetone, infiltrated and embedded in Araldite-Epon mixture. Semithin sections were cut at 1 µm thickness using ultramicrotome (Leica EM UC7) and stained with Toluidine blue. Staining sections were examined under a light microscope for gross observation of the region. Then ultrathin sections of gray-silver colour interference (60–90 nm) were cut and mounted onto 300 mesh-copper grids for transmission electron microscope examination. Sections were stained with uranyl acetate and lead citrate and observed under a Tecnai G2 20S-Twin transmission electron microscope (FEI, The Netherlands) operating at 200 kV.

Results

The olfactory rosette was almost oval in shape measuring 1.5 to 2.0 mm and comprised of 26 to 28 olfactory lamellae radiated from central median raphe. The outer margin of the lamellae was attached to the wall of the olfactory chamber while the inner edges were attached to the median raphe. The apical end of each lamella was provided with tongue shaped area (fig. 1, A). Each lamella was made up of two unequal thickness of layers in the olfactory epithelium disserved by broad central lamellar space, central core contained blood vessels, connective tissue, collagen and nerve fibres. The olfactory epithelium was separated from central core by prominent basement membrane (fig. 1, B). The lamellae were characterized with mixed sensory and non-sensory epithelium having different cells in each area. The sensory epithelium was confined in the upper half of the lamella including tongue area. The non-sensory epithelium was observed at the basal part of the lamella. The sensory epithelium consisted of a large number of primary and secondary receptor cells and microvillous cells which were intermingled with supporting cells (fig. 1, C). The dendrite of each primary receptor cell extended as a narrow cylindrical process up to the free epithelial surface and in some cases it enlarged into a small knob like structure, probably the olfactory vesicles supported with sensory hairs (fig. 1, D). The dendrite of some

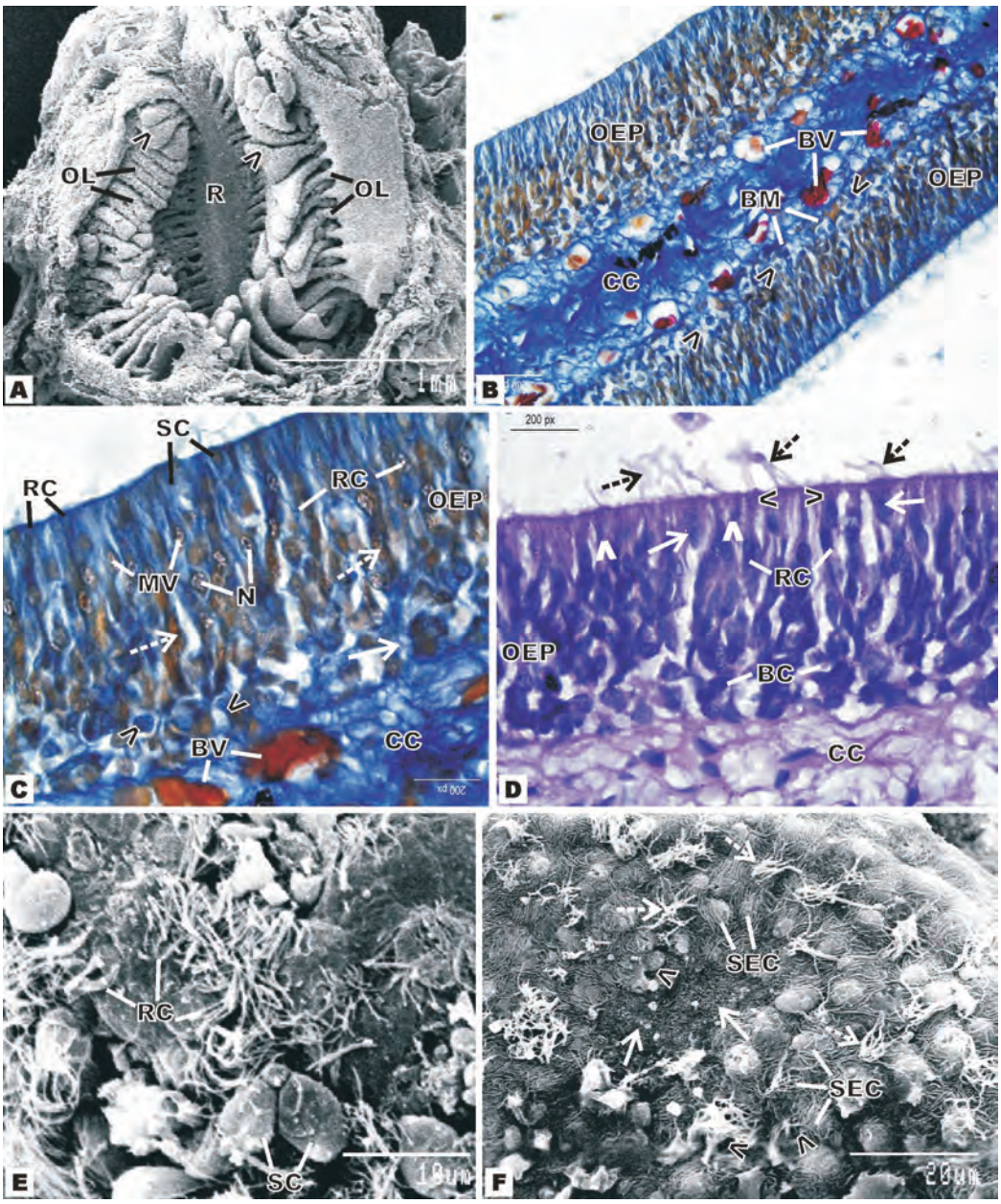

Fig. 1. Photomicrographs of the olfactory epithelium of *Pygocentrus nattereri* by scanning electron microscopy (SEM) and histological architecture stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. **A** — oval shaped olfactory rosette showing olfactory lamellae (OL) radiating from median raphe (R). Note tongue shaped structure (arrow heads) on the apical end of the lamellae (SEM) ×50. **B** — sensory olfactory epithelium (OEP) lined with receptor cells. Note the presence of blood vessels (BV) in the central core (CC) which is distinguished from OEP by basement membrane (BM). Arrow heads indicate basal cells above BM (MT) ×400. **C** — higher magnification of OEP showing a large number of primary receptor cells (RC) with conspicuous nuclei (N), secondary recptor cells (broken arrows), microvillous cells (MV) intermingled with supporting cells (SC). Note the presence of BV in CC and BC (arrow heads) near CC. Solid arrow indicates the axons of secondary RC communicate to CC (MT) ×1000. **D** — OEP exhibiting cylindrical RC with knob like vesicles (black arrow heads), ciliated supporting cells (solid arrows), non-ciliated supporting cells (white arrow heads) and BC above CC. Broken arrows mark the cilia of supporting cells on the epithelial surface (HE) ×400. **E** — tuft of receptor cells (RC) in between supporting cells (SC) (SEM) ×4000. **F** — dendrite patches of RC (broken arrows) and microvillous cells (solid arrows) in between stratified epithelial cells (SEC). Note the opening of mucous cells (arrow heads) in between SEC (SEM) ×2500.

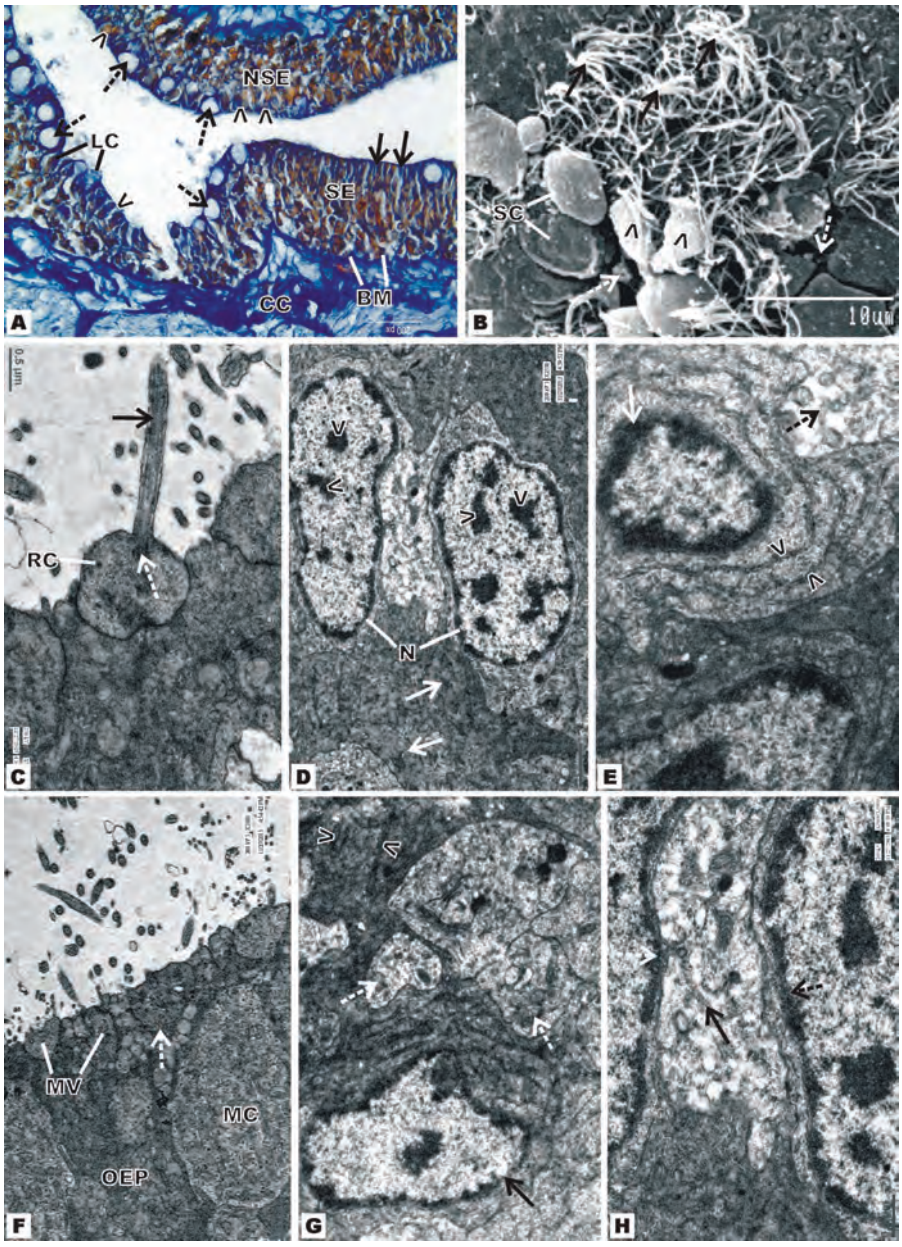

Fig. 2. Photomicrographs of the olfactory epithelium of *P. nattereri* by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and histological features stained with Mallory's triple (MT) stain. **A** — transitional zone between sensory epithelium (SE) with receptor cells (solid arrows) and non-sensory epithelium (NSE) having a series of mucous cells (MC) (Broken arrows), labyrinth cells (LC) and stratified epithelial cells (arrow heads). Olfactory epithelium separated from central core (CC) by a basement membrane (BM) (MT) ×400. **B** — surface of non-sensory epithelium showing densely arranged ciliated supporting cells (solid arrows) encircled the non-ciliated supporting cells (SC) with adhering mucin mass (arrow heads). Note the opening of MC (broken arrows) in between SC (SEM) ×4500. **C** — dendrite of receptor cell (RC) emerging out from basal body (broken arrow). Note microtubules of rod (solid arrow) parallel arranged (TEM) ×5000. **D** —nuclei of receptor cells (N) showing dispersed heterochromatin (arrow heads). Note the presence of mitochondria (solid arrows) adjacent to nucleus (TEM) ×500. **(E)** Showing cisterns of rough endoplasmic reticulum (rER) (arrow heads) encircling nucleus (solid arrow). Note Golgi apparatus (broken arrow) adjacent to rER (TEM) ×4000. **F** — OEP lined with microvillous cells (MV), mucous cell (MC) and supporting cell (broken arrow) (TEM) ×2100. **G** — microvillous cells exhibiting abundant ribosomes (broken arrows) and extended mitochondria (arrow heads). Solid arrow indicates nucleus (TEM) ×5000. **H** — axons (broken arrows) of receptor cells run parallel on both sides of basal cells (solid arrow) (TEM) ×5000.

receptor cells were bipolar and extended as a slender process reaching to the free surface. The secondary receptor cells were mainly present below the primary receptor cells and the dendrites did not extend up to the surface epithelium. The deeply stained oval nuclei of the receptor cells situated deep in the epithelium (fig. 1, C). In some areas the axonal ends of the primary receptor cells synapsed with the dendrite tips of the secondary receptor cells and the axons of the secondary receptor cells ended in the central core. Microvillous cells were confined to the surface zone of the olfactory epithelium, bearing faintly visible cilia. The nuclei of these cells were small and lightly stained (fig. 1, C). Histologically two types of supporting cells were distinguished on the surface of the olfactory epithelium of *P. nattereri*. The first type had a oval nucleus and the apical end was broad (fig. 1, B, C). The second type of supporting cells provided with cilia (fig. 1, D). The transitional zone of sensory and non-sensory epithelium contained mucous cells, supporting cells, labyrinth cells and few receptor cells (fig. 2, A).

According to the scanning electron microscopic (SEM) study the dendrite surface of the olfactory receptor cells were located in groups (fig. 1, E, F). The microvillous receptor cells were few in number and were provided with microvilli and submerged into the thickness of flagellar receptor layers (fig. 1, F). The tuft of ciliated supporting cells encircled the non-ciliated supporting cells. Mucin mass were often found to be adhered to the cilia of the supporting cells (fig. 2, B).

Under transmission electron microscopic (TEM) study ciliated receptor cells had narrow apical dendrite as elongated rod emerged out from distinct olfactory knob or basal body. The rod was 2.0 to 2.5 μm long and had a width from 0.25 to 0.27 μm. The rod had number of large microtubules or filaments to which ran parallel along the length of the rod (fig. 2, C). At the base of the rod, the subsurface cytoplasm possessed basal bodies which were the points of origin of individual microtubules (fig. 2, C). Receptor cells had characteristic appearance of the nuclei with numerous narrow invagination and small lumps of heterochromatin dispersed all around their interior. The receptor cells were rich in mitochondria adjacent to nucleus (fig. 2, D). Cisterns of rough endoplasmic reticulum (rER) were seen near Golgi apparatus (fig. 2, E). The absolute number of microvillous cells was low. The most striking characteristic of this cell was the fact that provided microvilli (fig. 2, F). The cytoplasm of this cell was electron dense, mitochondria were extended and ribosomes abundant (fig. 2, G). Axons of the olfactory receptor neurons ran parallel on both sides of basal cells (fig. 2, H). The supporting cells were full of vesicular cytoplasm at least near the free surface (fig. 3, A). Abundant electron lucent granules and microvesicles were often observed in the cytoplasm of labyrinth cells. The flat cell apex of ciliated supporting cells was broad and gave rise to plenty of kinocilia which sprout from the basal body (fig. 3, B). The cell was provided with mitochondria. 9 pairs of peripheral tubules and 2 central ones (9 + 2 arrangement) were distinctive feature of the cilia.

Histologically, the basal cells were small, almost round in shape with prominent central nucleus, scattered in the deeper part of the epithelium adjacent to central core (fig. 1, B–D). Mucous cells were located in the superior layer of the olfactory epithelium. They were round or ovoid in outline. They were few in sensory epithelium than non-sensory epithelium (fig. 2, A). The oval shaped labyrinth cells were situated in the epithelial surface with conspicuous nuclei towards the basal portions. Stratified epithelial cells were predominantly found all over the non-sensory epithelium. These cells were elliptical to columnar in shape with conspicuous nuclei (fig. 2, A). Under SEM observation, the apical surfaces of the stratified epithelial cells were provided with unbranched microridges leaving shallow channels in between (figs 1, F; 3, F).

Under TEM study, mucous cells were surrounded by supporting cells. About two-thirds of the mucous cell was filled with large granules (figs 3, A, C). The basal cells were provided with prominent lobular nucleus with the dense nucleolus. Small vesicles were also located near the nucleus and rER (fig. 3, D). In some areas the active division of basal

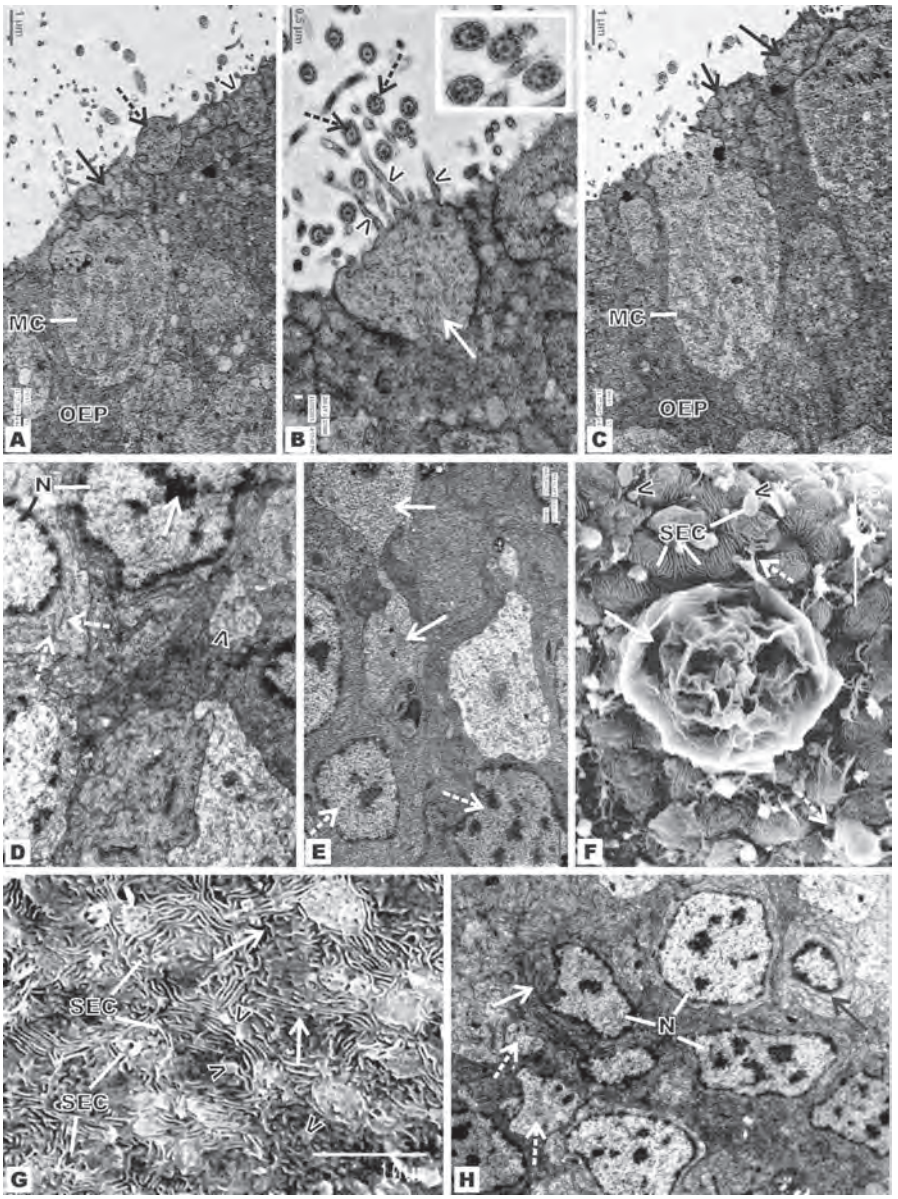

Fig. 3. Photomicrographs of the olfactory epithelium of *P. nattereri* by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). **A** — olfactory epithelium (OEP showing mucous cell (MC) having granules, microvillous cell (arrow head), labyrinth cell (broken arrow) and vesicular cytoplasm of supporting cell (solid arrow) (TEM) ×2100. **B** — flat surface ciliated supporting cell (solid arrow) provided with plenty of kinocilia (arrow heads) showing microtubular pattern (broken arrows). Note large number of mitochondria within the cytoplasm of supporting cells (TEM) ×5000. **C** — non-sensory olfactory epithelium (OEP) comprised of MC filled with large granules and supporting cells (solid arrows) (TEM) ×2100. **D** — basal cells provided with conspicuous lobular nuclei (N) having dense nucleolus (solid arrow). Note the presence of small vesicles (arrow head) adjacent the nucleus and rough endoplasmic reticulum (rER) (broken arrows) (TEM) ×2500. **E** — showing nuclear division of basal cells provided with dense nucleus (solid arrows). Broken arrows indicate mature nucleus of basal cells (TEM) ×2500. **F** — showing labyrinth cells (solid arrow) with conspicuous folding encircled by compactly arranged stratified epithelial cells (SEC). Note the presence of MC (broken arrows) in between SEC and mucin droplets (arrow heads) over SEC (SEM) ×4500. **G** — surface epithelium of raphe provided with packed SEC having labyrinth pattern microridges. Note the presence of opening of MC (solid arrows) and mucin droplets (arrow heads) over SEC (SEM) ×4500. **H** — raphe showing oval and elongated nuclei (N) of Stratified epithelial cells. Note the presence of rER (solid arrows) and vesicles (broken arrows) adjacent to nucleus (TEM) × 2500.

cells were found which were provided with dense nucleus and electron dense cytoplasm (fig. 3, E).

According to SEM study the non-sensory epithelial surface was provided with labyrinth cells encircled by stratified epithelial cells whose apical portion representing different secondary folds (fig. 3, F). The raphe was characterized by tightly packed stratified epithelial cells with labyrinth pattern of microridges and opening of mucous cells (fig. 3, G). Secreted mucins from mucous cells adhered with microridges of the stratified epithelial cells.

Under TEM investigation the stratified epithelial cells of raphe region provided with oval or elongated nuclei with lobulated electron dense nucleus. Rough endoplasmic reticulum (rER) and vesicles were located near the nucleus (fig. 3, H).

Discussion

The multilayered peripheral olfactory organ in fish permitted them an acute sense of smell in various aspects of their life history, such as feeding and reproduction, which are mediated through olfactory cues (Hara, 1992). In the present study, the oval-cup shaped olfactory rosette of *P. nattereri* consisted of 26 to 28 olfactory lamellae arranged on either side of the leaf-like median raphe and can be classified under Bateson's (1889) rosette type-3 or Burne's (1909) rosette column I. According to Teichmann (1954), the oval type of olfactory organ falls under the category of "eye-nose fish", which means that this category of fish possesses similarly developed optic and olfactory senses. Olfactory mucosa of *P. nattereri* was folded and multilamellar arrangement might increase the surface area needed for sensory activity (Zeiske et al., 1976). The ecological niche occupied by a fish has a great impact on its structural characterization and specialization of olfactory epithelium (Kuciel et al., 2011). Lamellae were parallel arranged are characterized with linguiform process at middle portion which slow down the water flow over the olfactory mucosa and facilitate better interaction of odorants particles with receptor cells. The distribution of sensory and non-sensory epithelia on the surface of the olfactory lamellae showed a great variety in different fish species (Yamamoto, 1982).

Histologically, the olfactory lamellae of *P. nattereri* comprised of two layers of olfactory epithelium. Both the epithelia were unequal thickness; the apical region of the olfactory lamellae including the free linguiform processes was thicker than basal part. The reason might be the fact that the linguiform process with receptor cells faced the flow of incoming water current and the sensory cells interacted with the water soluble chemicals during olfaction. This was a unique feature of the olfactory epithelium in this fish occupying a special ecological habitat and thus mobilizing the interaction of odorants with the receptor cells. In the present study, the sensory epithelium of *P. nattereri* was mainly consisted of two morphologically distinct types of receptor cells in different proportions: ciliated and microvillous cells. The ciliated receptor cells dominated over the microvillous receptor cells. The apical tip of the olfactory receptor cells provided with olfactory knob with dendrite. Under transmission electron microscope, presence of microtublules in dendrite of all receptor cells developed of basal body. These neurotubules might have the main role in maintaining the shape of the dendrite process as well as acting for transplantation of various substances. Mokhtar and Abd-Elhafeez (2014) clarified that high density of ciliated receptor cells in the tip of the olfactory neurons were of great importance in detection of food and were adapted to food and feeding habit of red-tail shark, *Epalzeorhynchos bicolor*. On the contrary, Theisen (1972) observed that the presence of neurotubules in dendrite of all receptor cells might have the main role for detecting of odorants. The most interesting characteristics of the olfactory epithelium of *P. nattereri* was the histological existence of secondary receptor cells in addition to primary receptor cells and the presence of synaptic connection between these two kinds of receptor cells. The axons of the secondary neurons extended into the central core of the lamellae. Therefore, it was obvious that the transduction of signals of odorants perceived at the surface of receptor cells ultimately conveyed to the

central core. In contrast to the ciliated receptor cells, the microvillous receptor cells had a slightly sunken apex and consisted of minute dendrites might have olfactory transduction mechanism for pheromones. Bhute and Baile (2007) also advocated that the microvillous receptor neurons perceive and process signals of pheromone, which is an important step of breeding in *Labeo rohita*. On the contrary, Hamdani et al. (2001) supposed that the microvillous olfactory receptor cell might have role in feeding behaviour of *Carassius carassius*, while the ciliated receptor cells were responsible for reception of pheromones.

The supporting cells were comparatively lightly stained and restricted to the sensory epithelial region of *P. nattereri*. Transmission electron microscope revealed presence of mitochondria and many secretory vesicles which may indicate a secretory function of these cells. The predominance of mitochondria might have some role in production of energy during secretion of these cells. Zeiske et al. (1992) reported that the non-ciliated supporting cells seemed to be mainly involved in secretion and the ciliated ones were identical to the ciliated non-sensory cells responsible for circulation of water and mucus.

The basal cells in *P. nattereri* occupied a position below the surface epithelium just above the basement membrane might act as stem cells for regeneration of the olfactory epithelium. The present electron microscopic observation showed that the presence of rough endoplasmic reticulum in the cytoplasm of these cells and presence of mitotic figures indicated that basal cells were dynamic group of elements in the olfactory epithelium. Moller et al. (1989) however, mentioned that the basal cells might be stem cells for regeneration of lost or damaged ciliated non-sensory and mucous cells.

The olfactory epithelium of *P. nattereri* was characterized by presence of huge number of ciliated non-sensory cells intermingling with the receptor cells. These cilia are typical kinocilia showing usual microtubular pattern with motile function. The characteristic feature of non-sensory ciliated cells might create moderate to weak current of water over the olfactory lamellae aided in spreading the mucus on epithelial surface and peripherally assisting transport of stimulant molecules to the receptor cells. Waryani et al. (2013) and Salem (2013) opined that functionally the non-ciliated supporting cells seemed to be mainly involved in the circulation of water and mucus along the sensory epithelium.

The apical surface of the non-sensory epithelium was provided with non-ciliated cells with fingerprint like microridges. The microridges might increase the surface of non-sensory epithelium and aided in holding the mucus along the olfactory epithelium and protected the receptor sensory cells from the mechanical abrasion. Similar results have been recorded in other species (Salem, 2003; Chakrabarti and Ghosh, 2009; Mokhtar and Abd-Elhafeez, 2014).

The labyrinth cells on the surface of epithelium might serve as excretory cells for osmoregulation and ion regulation. In this way they might cause the olfactory organs to function optimally in water of different salinities. Shirai and Utida (1970) reported the labyrinth cells may be involved in electrolyte transport because they were structurally similar to chloride cells found in fish gills.

The surface epithelium of median raphe contained stratified epithelial cells having labyrinth pattern microridges. These microridges might protect the olfactory mucosa from external hazards. The mucus secreted by the mucous cells lubricated the surface epithelium to smooth flow of water during ventilation and helped in binding of microscopic debris that enter with incoming water.

P. nattereri primarily scavengers and foragers, subsisted on a variety of diets which included insects, crustaceans, worms, snails, fishes, debris and plant materials; depended on olfactory sense for exploring the surrounding aquatic ecosystem in which they live. The dense aggregation of ciliated receptor cells in the olfactory epithelium was of special interest to enable the fish to detect food and perceive most of the chemical signals. The presence of microvillous cells performed a significant role in the regulation of reproduction. The microridges of stratified epithelial cells helped in holding mucus film over the epithelium

and protected from the external hazards. Further, immunohistochemical and other experimental studies on the olfactory epithelium of piranha are recommended to identify the cellular components and their proper functional significance.

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References

Atta, K. I. 2013. Morphological, anatomical and histological studies on the olfactory organs and eyes of teleost fish: *Anguilla anguilla* in relation to its feeding habits. *Journal of Basic and Applied Zoology*, **66**, 101–108.

Bateson, W. 1889. The sense organs and perceptions of fishes, with remarks on the supply of bait. *Journal of the Marine Biological Association*, **1**, 225-256.

Bhute, Y. V., Baile, V. V. 2007. Organization of the olfactory system of the Indian major carp *Labeo rohita* (Ham.): a scanning and transmission electron microscopy study. *Journal of Evolutionary Biochemistry and Physiology*, **43**, 342–349.

Burne, R. H. 1909. The anatomy of the olfactory organ of teleostean fishes. *Proceedings of the Zoological Society*, **2**, 610–663.

Camacho, S., Ostos-Garrido, M. V., Domezain, A., Carmona, R. 2010. Study of the olfactory epithelium in the developing sturgeon characterization of the crypt cells. *Chemical Senses*, **35**, 147–156.

Chakrabarti, P., Ghosh, S. K. 2009. Ultrastructural organisation and functional aspects of the olfactory epithelium of *Wallago attu* (Bleeker). *Folia Morphologica*, **68**, 40–44.

Farbman, A. I. 1994. The cellular basis of olfaction. *Endeavour*, **18**, 2–8.

Ghosh, S. K., Pan, B., Chakrabarti, P. 2015. Histoarchitectural and surface ultrastructural analysis of the olfactory epithelium of *Puntius ticto* (Hamilton, 1822). *International Journal of Aquatic Biology*, **3**, 236–244.

Hamdani, E. H., Alexander, G., Døving, K. B. 2001. Projection of sensory neurons with microvilli to the lateral olfactory tract indicates their participation in feeding behaviour in crucian carp. *Chemical Senses*, **26**, 1139–1144.

Hansen, A., Zeiske, E. 1998. The peripheral olfactory organ of the zebrafish, *Danio rerio*: an ultra-structural study. *Chemical Senses*, **23**, 39–48.

Hansen, A., Zippel, H. P., Sorensen, P. W., Caprio, J. 1999. Ultrastructure of the olfactory epithelium in intact, axotomized and bulbectomized gold fish, *Carassius auratus*. *Microscopy Research and Technique*, **45**, 325–338.

Hara, T. J. 1992. Mechanism of olfaction. *In:* Hara, T. J., ed. *Fish Chemoreception*. Chapman and Hall, London, 150–170.

Kuciel, M., Żuwala, K., Jakubowski, M. 2011. A new type of fish olfactory organ structure in *Periophthalmus barbarous* (Oxudercinae). *Acta Zoologica*, **92**, 276–280.

Lara, M. R. 2008. Development of the nasal olfactory organs in the larvae, settlement-stages and some adults of 14 species of Caribbean reef fishes (Labridae, Scaridae, Pomacentridae). *Marine Biology*, **154**, 51–64.

Lazzari, M., Bettini, S., Ciani, F., Franceschini, V. 2007. Light and transmission electron microscopy study of the peripheral olfactory organ of the Guppy, *Poecilia reticulata* (Teleostei, Poecilidae). *Microscopy Research and Technique*, **70**, 782–789.

Ma, A. J., Wang, X. A., 2010. Functional morphology of the olfactory organ of the tongue sole, *Cynoglossus semilaevis*. *Chinese Journal of Oceanology and Limnology*, **28**, 209–217.

Mallory, F. B., 1936. The aniline blue collagen stain. *Stain Technology*, **11**, 101.

Mana, R. R., Kawamura, G. 2002. A comparative study on morphological differences in the olfactory system of red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) from wild and cultured stocks. *Aquaculture*, **209**, 285–306.

Mokhtar, D. M., Abd-Elhafeez, H. H. 2014. Light-and electron-microscopic studies of the olfactory organ of red-tail shark, *Epalzeorhynchos bicolor* (teleostei: cyprinidae). *Journal of Microscopy and Ultrastructure*, **2**, 182–195.

Moller, P. C., Partridge, L. R., Cox, R. A., Pellegrini, V., Ritchie, D. G. 1989. The development of ciliated and mucous cells from basal cells in hamster tracheal epithelial cell cultures. *Tissue and Cell*, **21**, 195–198.

Nevitt, G. A. 1991. Do fish sniff? A new mechanism of olfactory sampling in pleuronectid flounders. *Journal of Experimental Biology*, **157**, 1–18.

Salem, M. H. 2003. Fine structural characterization of the olfactory epithelium in the fish *Oreochromis aureus*: a scanning and transmission electron microscopic study. *Egyptian German Society of Zoology*, **40 B**, 133–160.

Salem, M. H. 2013. Comparative study on the structure of the eye and olfactory organ in two bony fishes in relation to their feeding habits. *Egyptian Journal of Zoology*, **60**, 189–222.

Samajdar, I, Mandal, D. 2016. Histological organization and ultrastructures of the apical surface of the olfactory epithelium of a carp, *Labeo bata* (Hamilton). *International Journal of Pure and Applied Zoology*, **4**, 134–141.

Sarkar, S. K., Acharya, A., Jana, S., De, S. K., 2014. Macro-anatomical variation of the olfactory apparatus in some Indian teleosts with special reference to their ecological habitat. *Folia Morphologica*, **73**, 122–128.

Shirai, N., Utida, S. 1970. Development and degeneration of the chloride cell during seawater and freshwater adaptation of the Japanese eel *Anguilla japonica*. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **103**, 247–264.

Singh, S. P., 1977. Functional anatomy of olfactory organs in some marine teleosts. *Zoologischer Anzeiger*, **5 B**, 441–444.

Teichmann, H. 1954. Vergleichende Untersuchungen an der Nase der Fishe. *Zeitschrift. Morphologica Oekol Tiere*, **43**, 171–212.

Theisen, B. 1972. Ultrastructure of the olfactory epithelium in the Australian lungfish, *Neoceratodus forsteri*, *Acta Zoologica*, **53**, 205–218.

Waryani, B., Zhao, Y., Zhang, C., Dai, R., Abbasi, A. R. 2013. Anatomical studies of the olfactory epithelium of two cave fishes *Sinocyclocheilus jii* and *S. furcodorsalis* (Cypriniformes: Cyprinidae) from China. *Pakistan Journal of Zoology*, **45**, 1091–1101.

Yamamoto, M. 1982. Comparative morphology of the peripheral olfactory organ in teleosts. *In*: Hara, T. J., ed. *Chemoreception in fishes*. Elsevier, Amsterdam, 33–59.

Yamamoto, M., Ueda, K. 1979. Comparative morphology of fish olfactory epithelium. Perciformes, Beryciformes, Scopaeniformes and Pleuronectiformes. *Journal of the Faculty of Science, The University of Tokyo*, **14**, 273–297.

Zeiske, E., Mellinkat, R., Breucker, H., Kux, J. 1976. Ultrastructural studies on the epithelia of the olfactory organ of Cyprinodonts (Teleostei, Cyprinodontoidae). *Cell and Tissue Research*, **172**, 245–267.

Zeiske, E., Theisen, B., Breucker, H., 1992. Structure, development and evolutionary aspects of the peripheral olfactory system. *In*: Hara, T. J., ed. *Fish chemoreception*. Chapmann & Hall, London, 13–39.

Zeiske, E., Bartsch, P., Hansen, A. 2009. Early ontogeny of the olfactory organ in a basal actinopterygian fish: Polypterus. *Brain Behavior and Evolution*, **73**, 259–272.

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