Journal of Fisheries
[Early View version]
Peer Reviewed | Open Access | Online First

eISSN 2311-3111
pISSN 2311-729X

Original Article

The olfactory organ in the Gangetic catfish *Ailia coila* (Hamilton, 1822): structural and functional aspects

Saroj Kumar Ghosh

Department of Zoology, Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly-712 147, West Bengal, India

Correspondence
Dr. Saroj Kumar Ghosh; Department of Zoology, Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly-712 147, West Bengal, India

saroj.fisherylab@gmail.com

Manuscript history
Received 2 May 2020 | Revised 5 July 2020 | Accepted 9 July 2020 | Published online 17 July 2020

Citation
Ghosh SK (2020) The olfactory organ in the Gangetic catfish *Ailia coila* (Hamilton, 1822): structural and functional aspects. Journal of Fisheries. http://journal.bdfish.org/index.php/fisheries/article/view/JFish20239

Abstract

A study of the olfactory organ, with marked observation on cellular morphology of neuroepithelium was carried out in riverine catfish *Ailia coila* (Hamilton, 1822) using light and scanning electron microscopy. The elongated olfactory rosette within olfactory chamber was made up of 16 ± 2 leaf lets, the lamellae which connected laterally on the longitudinal central axis. The lamella consisted of two layers of epithelium enclosing the central core which contained connective tissue stroma along with blood vessels and nerve fibres. The epithelial lining displayed unequal compactness, was comprised of sensory receptor cells, undifferentiated basal cells, secretory mucous cells and two types of supporting cells differentiated as ciliated columnar or nonciliated oval type. Olfactory cells were identified by their staining emphasis, architecture, surface feature and distribution pattern in the mucosa. The surface of sensory epithelium was embossed with structurally distinct ciliated, microvillous and rod receptor cells for procuring olfactory stimuli from aquatic surroundings. The structural composition of the olfactory organ was dissertated with chemosensory system of the fish involved.

Keywords: *Ailia coila*; cellular feature; chemo sensation; microanatomy; olfactory epithelium.

1 | INTRODUCTION

In fish, olfaction is the most significant chemical senses which carry through olfactory apparatus. This receptor organ is better flourished or specialised in nocturnal carnivorous fishes and those remain in dark aquatic habitat or in cloudy muddy surroundings. The sense of smell is concerned with feeding, predator avoidance, sex recognition, nest finding, migration and other behaviour of teleosts (Camacho *et al.* 2010). Receptor neurons of the olfactory lining are stimulated when they are touched with chemical cues carried through water and transmit signals directly to the central nervous system by the cranial nerve-I (Lara 2008). The structure and function of the olfactory organs in teleostean fishes has been previously reported by several researchers (*e.g.* Hansen and Zielinski 2005; Kudo *et al.* 2009; Chakrabarti and Guin 2011; Sarkar *et al.* 2014; Kim *et al.* 2016; Malick *et al.* 2018; Ghosh and Das 2020). In the face of differential ecological niche inhabited by fishes, the olfactory organs show variation at least morphologically and contain identical arrangement of olfactory cells (Datta and Das 1980). Olfactory lamellae are intensified in number gradually with the age in re-

spect of increasing surface stretch of the rosette. Fishes with less number of lamellae on the rosette are termed microsmatic while those with numerous lamellae are macrosmatic (Atta 2013). Numerous sensory neurons can be speculated as various functional and anatomical entities with varied susceptibility to alien chemical stimuli (Yamamoto 1982).

The Gangetic ailia *Ailia coila*, belonging to the family Ailiidae inhabits in surface and middle layer of river and connected water bodies (Rahman 2005), carnivorous in nature (Deori *et al.* 2017). Limited attention has been endowed on the study of olfactory structure in *A. coila* (Sinha 1991, 2008). Cellular nature of the olfactory mucosa is necessary for olfactory response. Lacuna still remains on structural detail of the olfactory system in Asian schilbeids. The objective of the present work is to reveal the characteristics of the olfactory organ of adult *A. coila* (Siluriformes: Ailiidae) with the aid of light and scanning electron microscopy techniques to better understand the sensory adequacy of olfaction.

2 | METHODOLOGY

2.1 Sample collection

Adult specimens (*N* = 14) of *A. coila*, 12 ± 2.58 cm in total length were procured live from Bhagirathi-Hooghly River, a tributary channel of river Ganga near Ambika Kalna of Purba Bardhaman, West Bengal using cast net and drag net. They were identified following key to classification of fishes by Misra (2003). The specimens were euthanised with 3% urethane following the guidelines of the Institutional animal ethics committee. The olfactory rosettes were excised by dissection of olfactory pits under a Zeiss Stemi 2000-C stereoscopic binocular microscope.

2.2 Histology technique

Olfactory organs were fixed in Bouin's aqueous fluid for about 16 – 18 hours. The fixed samples were washed thoroughly in 70% ethanol, dehydrated through graded sequences of ethanol and cleared in methyl benzoate. The tissues were infiltrated in paraffin wax of 56 – 58°C for a period of 1.5 hour and ingrained in paraffin block. Serial transverse sections of tissue block were cut at 4 μm thickness using a rotary microtome (Weswox MT-1090A). Tissue sections were stretched on Mayer's albuminised glass slides. The sections were deparaffinised with xylene and brought to distilled water through descending series of ethanol and stained with Delafield's Haematoxylin-Eosin (HE) (Fischer *et al.* 2008), Mallory's Triple (MT) (Mallory 1936) and Romies Azan (RA) stain. After dehydrated through ascending ethanol series, the sections were cleared with xylene and finally mounted with dibutylphthalate polystyrene xylene. Photographs were taken under ZEISS Primo Star light microscope equipped with Tucsen 5.0 MP digital microscopy camera.

2.3 Scanning electron microscopy technique

After exposed, the olfactory rosettes were immersed in a solution containing 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4) for 20 minute. The entire olfactory rosettes were removed attentively from the olfactory pits and gently washed with 1% Tween 40 solution to eliminate the excess mucus and debris from the surface. They were bathed in the same buffer, transferred in 2.5% glutaraldehyde for 24 hour at 4°C and post-fixed in 1% 0.1 M phosphate buffered osmium tetroxide (pH 7.4) for further two hours at room temperature. Tissues were then dehydrated in a gradation of acetone changed with isoamyl acetate, dried by critical point method (Cohen *et al.* 1968) with liquid CO_2 and mounted with glue on aluminium stubs. After sputter coated (BT-150 Coater, Hind High Vacuum Co. Pvt. Ltd.) with platinum (15 nm), samples were viewed through a scanning electron microscope (ZEISS EVO 18).

3 | RESULTS

In *Ailia coila* (Figure 1) paired olfactory organs lie on the dorsolateral side of the snout in the appearance of simple pits. The olfactory rosettes are elongated in morphology containing centrally placed precise raphe; remain buried in the floor of the pits or olfactory chambers (Figure 2). It looks like a pinnately organize compound leaf. The protracted olfactory chamber together with rosette is embedded in the fossa of ethmoidal zone of skull and affixed to encompassing bones by connective tissue fibres. The long medial axis or raphe of the rosette thrusts onward the regular plane as anteroposterior support of fish body. It has 16 ± 2 sickle shaped thin lamellae disposed transversely on right and left side of the rosette, observed under scanning electron microscope. The number of lamella is not consistent; variegates according to the maturity and size of specimens. The lamellae are extent of raphe, adhered to deck of the olfactory cup by their inner margins (Figure 3). The lamellae are not equal in size; the median lamellae are the larger and moderately reduce in length on anterior and posterior margins of rosette. Laterally compressed olfactory lamellae contain sensory and non-sensory epithelium, distinguished on the basis of surface feature and cellular constituents. The olfactory cell types are recognized based on structural features, surface peculiarities and staining characteristics.

Histomicroscopically the olfactory lamella shows unequal thickness and consists of two layers of epithelium surrounding middle stromal layer, the central core. It is composed of network of blood capillaries, connective tissue stroma, nerve fibres and scanty pigment cells (Figure 4). The epithelium is stratified in nature and expands the surface area by secondary folds, separated from central core by thin basement membrane. The receptor cells having posteriorly located nuclei are intimately arranged in

the sensory mucosa. The primary receptor cell has deeply stained oval nucleus and cylindric dendron towards olfactory lining.

FIGURE 1 Photograph of *Ailia coila*.

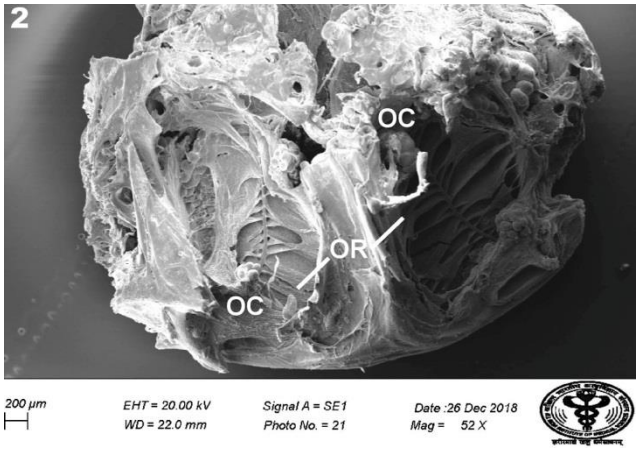

FIGURE 2 Head region displays the position of olfactory chamber (OC) holding olfactory rosettes (OR).

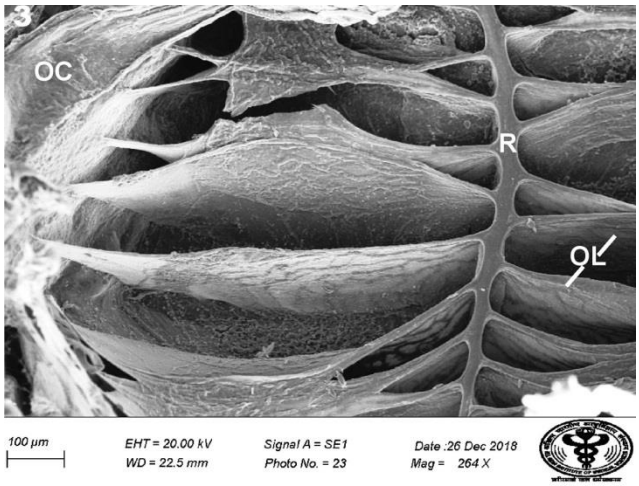

FIGURE 3 Elongated rosette bearing parallel arranged olfactory lamella (OL) from longitudinal raphe (R) occupies in the cavity of olfactory chamber (OC).

The distal end of the dendrite terminates a protuberant known as olfactory knob bearing sensory hairs on the free surface and the proximal end carry an axon which runs towards the stromal sheet (Figure 5). Microvillous receptor cells are more marginal in the epithelial lining, be endowed with moderately stained round nuclei and flat surface (Figures 4 – 5). Rod receptor cells are differentiated by their columnar alignment, bearing basally placed spindle shaped nuclei and thick alleviated length dendrite endings at the mucosal surface.

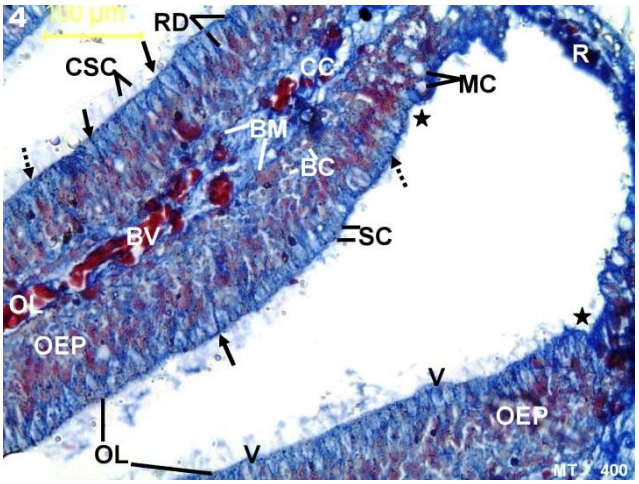

FIGURE 4 Olfactory lamella (OL) emitted from raphe (R) shows olfactory epithelium (OEP) typified with primary receptor cells (solid arrows), microvillous receptor cells (broken arrows), rod receptor cells (RD), stratified epithelial cells (SC), ciliated nonsensory cells (CSC), mucous cell (MC) and basal cells (BC). Central core (CC) containing blood vessels (BV) separate from epithelium by basement membrane (BM). Asterisks mark the folding of lamellae.

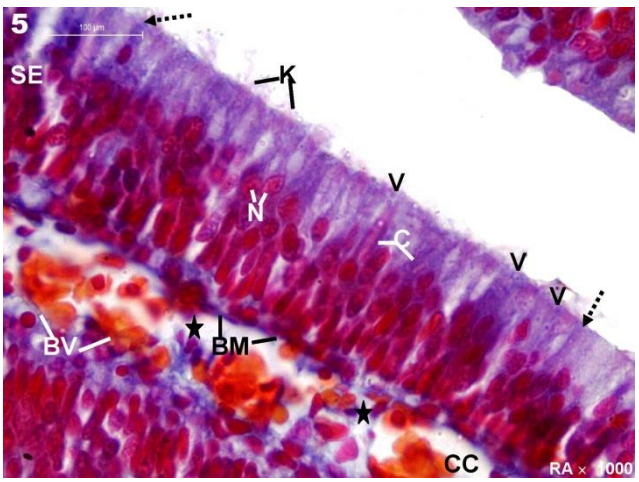

FIGURE 5 Magnified sensory epithelium (SE) illustrates primary receptor cells (C) having nuclei (N) and cylindrical dendrite process which enlarges into knob like structure (K), microvillar cells (arrow heads) and rod cells (broken arrows). Note the presence of blood vessels (BV) and pigment cells (asterisks) in central core (CC) which differentiate from epithelium by basement membrane (BM).

Scanning electron microscope observations furnish that sensory area are discontinuously distributed on both sides of the olfactory lamella. The sensory region contains three types of receptor cells: ciliated, left out cilia but with microvilli or rod shaped outline, categorised on the basis of free surface morphology (Figures 6 – 7). The microvillous receptor cells are domineer having condensed

carpet of compressed microvilli, give harsh appearing over the mucosa. Ciliated receptor cells bearing narrow hillock like apex emerging from their periphery. Sporadically occurring rod cells distend as compound tips over the epithelial surface. The rod like structure diminishes progressively forwarding the tip. The surface of nonsensory region displays firmly arranged epithelial cells with randomly arranged microridges. Copious pits are present in between epithelial cells which express the appearing of mucous cells. Mucus droplets are marked over the parts of surface epithelium. Ciliated nonsensory cells are observed in the form of scattered mass equipped with plenty of cilia (Figure 7).

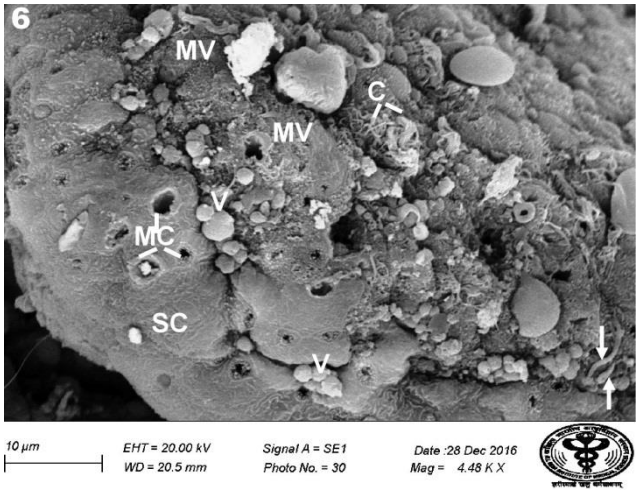

FIGURE 6 Surface of lamella shows sensory epithelium embossed with ciliated (C), microvillous (MV) and rod (solid arrows) receptor cells. Nonsensory epithelium contains numerous mucous cells (MC) along with stratified epithelial cells (SC). Note the release of mucus droplets (arrow heads) over the epithelium.

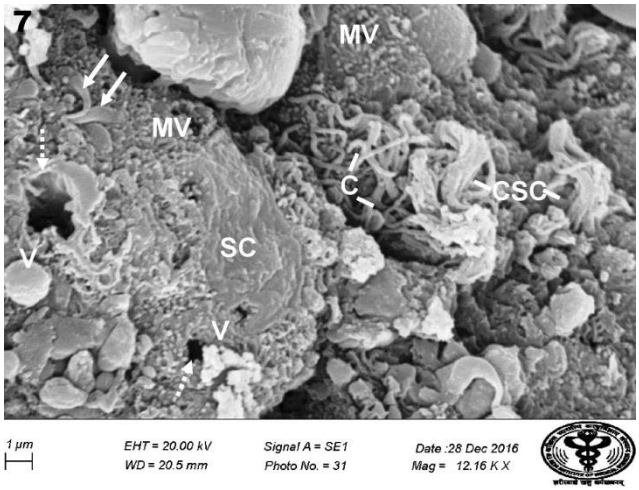

FIGURE 7 Close up view of lamella exhibits ciliated (C), microvillous (MV) and rod (solid arrows) receptor cells in sensory epithelium. Note the presence of ciliated nonsensory cells (CSC) and opening of mucous cells (broken arrows) with secreted mucin (arrow heads) neighbouring to stratified epithelial cells (SC).

Histologically the nonsensory epithelium is typified with mucous cells and two types of supporting cells, being ciliated and without cilia (Figures 4). Ciliated supporting cells are typical columnar in contour bearing distally situated nuclei, distributed near the proximal part of epithelium. The nonciliated supporting cells are interspersed among other olfactory cells forming a mosaic, contain basophilic central nuclei. They provide the structural support of the olfactory lining. Empty mucous cells are irregular in shape containing basally placed nuclei, release their content on the outermost surface (Figure 8). Undifferentiated basal cells have conspicuous large nuclei are situated adjoining to basement membrane (Figures 6 and 8).

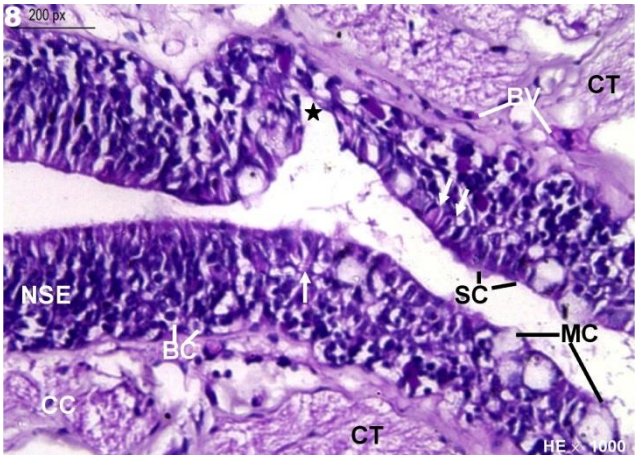

FIGURE 8 Nonsensory epithelium (NSE) furnishes stratified epithelial cells (SC), ciliated nonsensory cells (solid arrows), basal cells (BC) and mucous cells (MC) with secreted mucus. Central core (CC) contains connective tissue (CT) along with blood vessels (BV). Asterisk indicates the folding of lamellae.

A sharp difference observes in between the epithelial lining of lamella and raphe. The nonsensory raphe consists primarily of stratified epithelial cells containing microfolds and rupture mucous cells. Secretory material of mucous cells eject as lump of particulate material all over the microfolds (Figure 9). No receptor cells are observed.

4 | DISCUSSION

The epithelial surface of *A. coila* is folded to form a series of olfactory lamellae which characterized into sensory and non-sensory areas depending on ecological niche and feeding habit. Chemical cues are recognized by the organ and significant behaviours are commuted by any species (Mana and Kawamura 2002). The present study reveals that the olfactory rosette is elongated in outline, belongs to Teichmann's 3rd group of nose fishes (Teichmann 1954) which acquire greater olfactory area in comparison to retinal sphere. In *A. coila*, olfactory lamellae are arranged symmetrically from both side of medial raphe, classified as Type-H (Yamamoto 1982); *i.e.* double sided comb like appearance along a raphe. The presence of secondary folding on the lamella is an adaptation for ade-

quate usage of the available space in olfactory cavity (Bertmer 1972). Functional ability of the olfactory organ rises with the increase of its neuroepithelial surface.

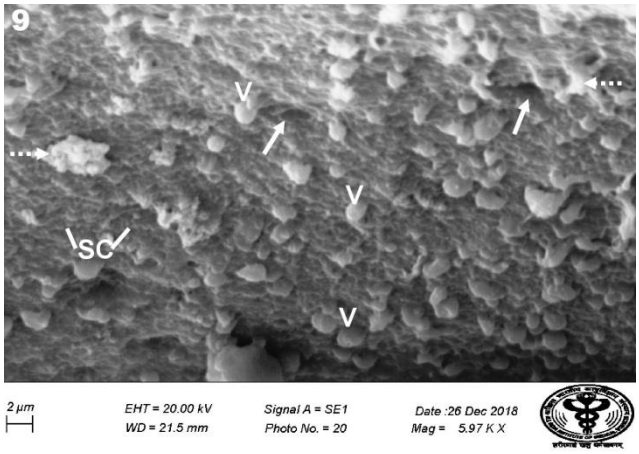

FIGURE 9 Raphe shows cluster of mucus droplets (arrow heads) and mucin mass (broken arrows) secreted from mucous cells (MC) along the microfolds of stratified epithelial cells (SC).

In sensory epithelium the apical part of receptor cells is furnished with ciliary processes or microvili or rod shaped endings which are adept to respond and judge the chemical stimuli in the surroundings. The ciliated receptor cells harmonize to type I cell of Yamamoto and Ueda (1978), though microvillous receptor cells to type II cells of Muller and Marc (1984) and rod receptor cells of those type IV cells of Ichikawa and Ueda (1977). Each neuron carries unequivocal information that brings out distinctive behaviour in response to olfactory cues associated with crucial life processes of fish (Hamdani and Døving 2007). Bannister mentioned that ciliated tips of olfactory receptor cells possibly the locus where olfaction commenced by interaction with odour bearing particles. Zippel *et al.* (1997) stated that ciliated receptor cells perform counter to amino acids while the microvillous cells mediate comeback to pheromones. While Thommesen (1982) suggested that ciliated receptor neurons are precise to bile salts whilst amino acids are recognised by microvillar neurons. The olfactory knob with sensory hairs and microvilli of receptor cells implies various functional activities and skill for recognition of chemical cues (Mokhtar and Abd-Elhafeez 2014). The microvillous receptor cells recognise and communicate with pheromone, which is a momentous proceeding of breeding in *Labeo rohita* (Bhute and Baile 2007). Datta and Bandyopadhyay (1997) reported that rod cell is not a usual subtype, the forming of rod probably due to fusion of cilia of ciliated region. Hernádi (1993) reported that the presence of the rod cell due to habitat of a new physiological climate. Existence of rod receptor cells reveals the marking of aged ciliated receptor cells (Yamamoto 1982).

The nonsensory epithelium is typified with ciliated supporting cells have no sensory function but they perhaps help in mechanical dissociation. The beating of cilia of these nonsensory cells initiates feeble current over the lamellae, aids in water discharge and guides the odoriferous molecules to contact with the tips of sensory neurons bearing receptor sites (Hara 2000). The stratified epithelial cells with microridges are thought to perform protective role in favour of supporting tissue against annoying water forces (Uehara *et al.* 1991). The microridges grip the mucin secreted from mucous cells and care for cellular components of epithelial lining. Secreted mucus helps to bind the microscopic debris coming through the water and keep the receptor cells accessible for new stimuli (Zeni and Stagni 2002). The basal cells are conceited to be the progenitor cells of the receptor and supporting cells (Zeiske *et al.* 1992). The occurrence of basal cells buried in the epithelium abets to sustain the mucosa during normal cell turn over or necrobiosis.

5 | CONCLUSIONS

Ailia coila having an elongated olfactory organ provided with chemosensory cells, acquire better sense of olfaction about the aquatic environment in which they survive. Morphologically differentiated ciliated, microvillous and rod receptor cells in olfactosensory mucosa confess different stimuli by their apical tips and are committed in odour perception. Higher studies should be performed for better understanding the functional olfactory system alliance with survival and other behaviours of fish.

ACKNOWLEDGMENTS

The author is thankful to the authority of Sophisticated Analytical Instrumentation Facility (SAIF), All India Institute of Medical Sciences (AIIMS), New Delhi-110 029, India for observing samples under scanning electron microscope.

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

REFERENCES

Atta KI (2013) Morphological, anatomical and histological studies on the olfactory organs and eyes of teleost fish: *Anguilla anguilla* in relation to its feeding habits. The Journal of Basic & Applied Zoology 66(3): 101–108.

Bertmar G (1972) Scanning electron microscopy of olfactory rosette in sea trout. Zeitschrift für Zellforschung und Mikroskopische Anatomie 128: 336–346.

Bhute YV, Baile VV (2007) Organization of the olfactory system of the Indian major carp *Labeo rohita* (Hamilton): a scanning and transmission electron microscopic study.

Journal of Evolutionary Biochemistry and Physiology 43(3): 342–349.

Camacho S, Ostos-Garrido MV, Domezain A, Carmona R (2010) Study of the olfactory epithelium in the developing sturgeon characterization of the crypt cells. Chemical Senses 35(2): 147–156.

Chakrabarti P, Guin S (2011) Surface architecture and histoarchitecture of the olfactory rosette of freshwater spiny eel, *Macrognathus aculeatus* (Bloch). Fisheries & Aquatic Life 19(4): 297–303.

Cohen A, Marlow D, Garner G (1968) A rapid critical point method using fluorocarbon ("freons") as intermediate and transitional fluids. Journal of Microscopy 7: 331–342.

Datta NC, Bandopadhyay S (1997) Ultrastructure of cell types of the olfactory epithelium in a catfish, *Heteropneustes fossilis* (Bloch). Journal of Bioscience 22: 233–245.

Datta NC, Das A (1980) Anatomy of the olfactory apparatus of some Indian gobioids (Pisces: Perciformes). Zoologischer Anzeiger 3(4): 241–252.

Deori DJ, Abujam S, Dakua S, Laishram S (2017) Investigation on feeding, growth and condition factor of certain ornamental fish from Brahmaputra River at Dibrugarh, Assam, India. Journal of FisheriesSciences.com 11(3): 43–47.

Fischer AH, Jacobson KA, Rose J, Zeller R (2008) Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harbor Protocol 3: 1–3.

Ghosh SK, Das S (2020) The olfactory mucosa of river catfish, *Eutropiichthys vacha* (Hamilton, 1822): a histochemical study. International Journal of Aquatic Biology 8(1): 50–55.

Hamdani EH, Døving KB (2006) The functional organization of the fish olfactory system. Progress in Neurobiology 82(2): 80–86.

Hansen A, Zielinski BS (2005) Diversity in the olfactory epithelium of bony fishes: development, lamellar arrangement, sensory neuron cell types and transduction components. Journal of Neurocytology 34(3–5): 183–208.

Hara TJ (2000) Chemoreception. In: Ostrander GK (Ed) The laboratory fish. Academic Press, London. pp. 471–479.

Hernádi L (1993) Fine structural characterization of the olfactory epithelium and its response to divalent cations Cd^{2+} in the fish *Alburnus alburnus* (Teleostei, Cyprinidae): a scanning and transmission electron microscopic study. Neurobiology 1(1): 11–31.

Ichikawa M, Ueda K (1977) Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*. A study of retrograde degeneration. Cell and Tissue Research 183(4): 445–455.

Kim TK, Kim HS, Park JY (2016) The anatomy and histology of the olfactory organ in the Korean sand goby *Favonigobius gymnauchen* (Pisces, Gobiidae). Korean Journal of Ichthyology 28(1): 28–34.

Kudo H, Shinto M, Sakurai Y, Kaeriyama M (2009) Morphometry of olfactory lamellae and olfactory receptor neurons during the life history of chum salmon (*Oncorhynchus keta).* Chemical Senses 34(7): 617–624.

Lara MR (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(1): 5–64.

Malick C, Chatterjee SK, Bhattacharya S, Suresh VR, Kundu R, Saikia SK (2018) Structural organization of the olfactory organ in an amphihaline migratoryfish hilsa, *Tenualosa ilisha*. Microcopy Research and Technique 81(10): 1122–1131.

Mallory FB (1936) The aniline blue collagen stain. Stain Technology 11(3): 101–102.

Mana RR, Kawamura G (2002) Olfactory organs of two pelagic teleost fish- opah (*Lampris guttatus*) and Dolphin fish (Coryphaena hippurus). South Pacific Study 22(2): 53–64.

Misra KS (2003) An aid to the identification of the common commercial fishes of India and Pakistan, Narendra Publishing House, Delhi, India.

Mokhtar DM, Abd-Elhafeez HH (2014) Light-and electron-microscopic studies of the olfactory organ of red-tail shark, *Epalzeorhynchos bicolor* (Teleostei: Cyprinidae). Journal of Microscopy and Ultrastructure 2(3): 182–195.

Muller JF, Marc RE (1984) Three distinct morphological classes of receptors in fish olfactory organs. Journal of Comparative Neurology 222(4): 482–495.

Rahman AKA (2005) Freshwater Fishes of Bangladesh. Zoological Society of Bangladesh, Dhaka, Bangladesh.

Sarkar SK, Jana S, De SK (2014) Age specific anatomy and cytological studies on unilamellar olfactory structure of a teleost (*Pseudapocryptes lanceolatus*). European Journal of Experimental Biology 4(6): 105–111.

Sinha RK (1991) Comparative study of the olfactory organs of two hill stream fishes - *Labeo boggut* (Sykes) and *Ailia coila* (Ham). Functional and Developmental Morphology 1(1): 77–80.

Sinha RK (2008) Olfaction in fishes - a vision for 21st century. In: Pandey BN (Ed) Fish research: vision for 21st century. APH Publishing Corporation, New Delhi. pp. 75–80.

Teichmann H (1954) Vergleichende Untersuchungen an der Nase der Fische. Zeitschrift für Morphologie und Ökologie der Tiere 43: 171–212 (in German).

Thommesen G (1983) Morphology, distribution and specificity of olfactory receptor cells in the salmonid fish. Acta Physiologica Scandinavica 117(2): 241–249.

Uehara K, Miyoshi M, Miyoshi S (1991) Cytoskeleton in microridges of the oral mucosal epithelium in the carp, (*Cyprinus carpio*). The Anatomical Record 230(2): 164–168.

Yamamoto M (1982) Comparative morphology of the pe-

ripheral olfactory organ in teleosts. In: Hara TJ (Ed) Chemoreception in fishes. Elsevier, Amsterdam. pp. 35–59.

Yamamoto M, Ueda K (1978) Comparative morphology of fish olfactory epithelium-III. Cypriniformes. Bulletin of the Japanese Society of Scientific Fisheries 44(11): 1201–1206.

Zeiske E, Theisen B, Breucker H (1992) Structure, development and evolutionary aspects of the peripheral olfactory system. In: Hara TJ (Ed) Fish chemoreception. Chapmann and Hall, London. pp. 13–39.

Zeni C, Stagni A (2002) Changes in the olfactory mucosa of the black bullhead *Ictalurus melas* induced by exposure to sublethal concentrations of sodium dodecylbenzene sulphonate. Diseases of Aquatic Organisms 51(1): 37–47.

Zippel HP, Sorensen PW, Hansen A (1997) High correlation between microvillous olfactory receptor cell abundance and sensitivity to pheromones in olfactory nerve-sectioned goldfish. Journal of Comparative Physiology A 180: 39–52.

SK Ghosh https://orcid.org/0000-0003-3973-8013