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Research Article
Anatomical and Histological Studies on the Olfactory Organ of Riverine Catfish, *Eutropiichthys vacha* (Hamilton, 1822)

S.K. Ghosh

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

Abstract

Background and Objective: The olfactory organ is of immense importance and plays a momentous role in various teleost behaviours. The structural detailed and functional aspects of different cells on the olfactory mucosa of *Eutropiichthys vacha* (*E. vacha*) (Hamilton, 1822) were studied morphologically as well as histologically. **Materials and Methods:** The gross morphology and the cellular composition of the olfactory epithelium in *E. vacha* were described by scanning as well as light microscopy. **Results:** The paired olfactory chambers placed on the dorsal part of the snout and communicated to surrounding environment by anterior and posterior nasal openings. The olfactory organ was lodged in the depression of the olfactory cavity and consisted of 32±2 lamellae of various sizes that inserted into both sides of narrow midline raphe, forming an oval shaped rosette. The lamella was composed of olfactory epithelium whose surfaces contained sensory and non-sensory parts. The lateral surface and linguiform process of olfactory lamella contained sensory epithelium, whereas, the rest portion of the lamella was covered with non-sensory epithelium. The sensory epithelium was embossed with three types of receptor cells distinguished on the basis of architecture on their apical part bearing cilia, microvilli or rod like processes. The non-sensory areas were comprised of supporting cells and a series of mucous cells. Basal cells were confined in the deeper region of the epithelium above the basement membrane. **Conclusion:** Role of various cells lining the epithelia of the olfactory organ related to the mode of life and living of fish concerned.

Key words: *Eutropiichthys vacha*, olfactory epithelium, morpho-histology, function

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Corresponding Author: Saroj Kumar Ghosh, Department of Zoology, Bejoy Narayan Mahavidyalaya, Itachuna, 712 147 Hooghly, West Bengal, India
Tel: +91-9474643180 Fax: +91-3213-272237

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The olfactory and gustatory systems in vertebrates include the important sensory organs for detection and recognition of chemicals in the environment. Olfaction is a major sensory modality to perceive chemical signals and implicated in variant teleost behavioural activities[1]. Olfactory information is utmost important in fish, participating in momentous life processes such as migrations, alarm situations, parental behavior, reproductive strategies, feeding and in many other ways[2]. Fish can ascertain and judge water soluble chemicals infiltrate sensory cells through cell reaction during water flows over the olfactory epithelium[3]. The olfactory organ of fish directly interacts with the surroundings and the olfactosensory epithelium is the first part to be exposed and susceptible to water contaminants. Over the past few years, the structure and function of the olfactory organs in fishes have been described by many researchers[4-11]. Teleost shows enormous diversity in olfactory organ characters including shape, magnitude, lamellar disposition, allotment of sensory and non-sensory epithelium in relation to diverse environments where they inhabit. Teichmann[12] classified fishes into three main categories: 'eye-nose fishes' have an oval rosette, 'eye fishes' with a circular rosette and 'nose fishes' with an elongated rosette. Fishes have a large number of lamellae, exhibit behavioural responses to olfactory stimulation are designated as macrosmatic (nose fish) while those with few lamellae, display lesser response to olfaction and greater to sight are termed microsmatic (eye fish). The transitional between macrosmatic and microsmatic is known as mediosmatic (eye-nose fish)[13].

However, no attempt has been made to correlate the olfactory organs of fish with their mode of life and living. *Eutropiichthys vacha* (Siluriformes; Schilbeidae) is a freshwater catfish, surface feeder and highly predaceous in nature. Adult feeds mainly on aquatic insects, crustaceans, annelids and small forage fish also[14]. An attempt has been made in the present communication to investigate the morphology of the olfactory apparatus and functional aspects of various cells lining the olfactory epithelium of the important commercial food fish, *E. vacha*.

MATERIALS AND METHODS

Sample collection: Twelve adult specimens of *E. vacha* (22±2.07 cm in total length) were procured from the river Ganga near Sahid Pally of Kalyani, Nadia district of West Bengal, India during Nonmember, 2016-July, 2017. Fishes were anaesthetized and euthanized with an overdose of 2-Phenoxyethanol (P1126, Sigma-Aldrich) approved by Institutional Ethical Committee and brought to the Laboratory of Zoology Department, Bejoy Narayan Mahavidyalaya. The olfactory rosettes were then carefully dissected out from the olfactory chamber on the dorsal side of the head under a Zeiss Stemi 2000-C stereoscopic binocular microscope and immediately processed for gross texture, histological and semithin section studies.

Scanning electron microscopic (SEM) preparation: After perfusion *in vivo* with 2.5% glutaraldehyde (Merck) solution in 0.1 M sodium cacodylate buffer (pH 7.4) for 25 min, the intact olfactory rosettes were dissected out carefully for gross anatomy. The samples were rinsed in repeatedly in 1% Tween 40 (Sigma-Aldrich) solution and again fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer for 24 h at 4°C. Thereafter, the tissues were washed in same buffer and post-fixed in 1% osmium tetroxide (Johnson Matthey) for 2 h at room temperature. The fixed tissues were washed thoroughly in buffer solution and dehydrated through graded series of acetone followed by isoamyl acetate. Samples were then desiccated by critical point drier (Hitachi 8CP2) with liquid carbon dioxide and mounted on metal stubs. Finally, they were coated with platinum (approximately 20 nm) under vacuum using AGAR sputter coater and viewed under a ZEISS EVO 18 scanning electron microscope.

Histological preparation: Olfactory rosettes were fixed in aqueous Bouin's fluid for 18 h. After fixation, the tissues were washed well in 70% ethanol, dehydrated properly through graded ethanol series, cleaned with xylene and embedded in paraffin wax of 56-58°C under a thermostat vacuum paraffin-embedding bath for a period of 1 h and 30 min. Tissues were sectioned serially at a thickness of 4 µm using a rotary microtome (Weswox MT-1090A). Deparaffinized tissue sections were stained with Delafield's Haematoxylin-Eosin (HE)[15] and Mallory's Triple (MT)[16] stain.

Semi-thin sections preparation: Small fragments of olfactory rosette were fixed in Karnovsky's fixative (a mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.4) for 4 h at 4°C. Samples were washed in same buffer and post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.4) for further 2 h at room temperature. After proper fixation, they were rinsed thoroughly with same buffer to wash off the excess fixative.

The samples were dehydrated in a growing acetone series and embedded in Araldite resin. After resin polymerization, semi-thin sections were cut at 1 µm thickness using ultramicrotome (Leica EM UC7) and stained with toluidine blue (TB)[17].

The staining sections were observed and photographed under ZEISS Primo Star compound microscope.

RESULTS

Morphology: In *E. vacha* (Fig. 1), the paired olfactory pits or olfactory chambers were almost on the dorsal side of the snout and the opening of each pit was marked by a fold of skin into an anterior inlet and a posterior outlet (Fig. 2). The olfactory chamber was not connected with the respiratory system. The olfactory pit with its rosette was located in the depression of ethmoidal region of the skull and tied to the encompassing bones by fibrous connective tissue (Fig. 3). Each olfactory rosette was followed by knob like olfactory bulb and moderate olfactory nerve or tract that finally terminated ventrally in the anterior part of telencephalic hemisphere.

The olfactory rosette was almost oval in outline with a concave outer surface and a convex mesial surface, formed of 32 ± 2 leaf-lets, the olfactory lamellae, arranged on both sides of narrow longitudinal central axis or raphe (Fig. 4). The rosette inhabited the major space of nasal chamber and was embedded in the floor of the pit. The lamellae were closely set parallel to each other in a vertical plane and their concave outer edges with linguiform processes at distal ends. The lamellae were adhered to the wall of the nasal chamber through their ventral margins and conjoined to the raphe by their proximal ends (Fig. 4, 5). The lamellae in the middle portion of rosette were the largest, while they gradually diminished in length towards the both ends. The sensory epithelium was distinguished as patches in the linguiform processes and/or blended within the non-sensory epithelium in lamellae.

Fig. 1: Photograph of *Eutropiichthys vacha*

Fig. 2: Dorsal view of the head showing nostrils (arrows)

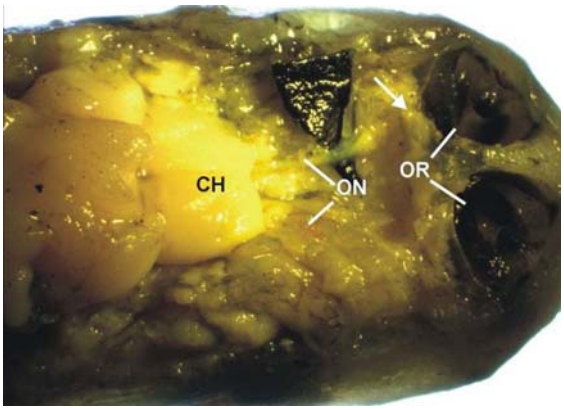

Fig. 3: Dissected portion of head shows olfactory rosettes (OR), olfactory bulbs (arrow), olfactory nerves (ON) and cerebral hemisphere (CH)

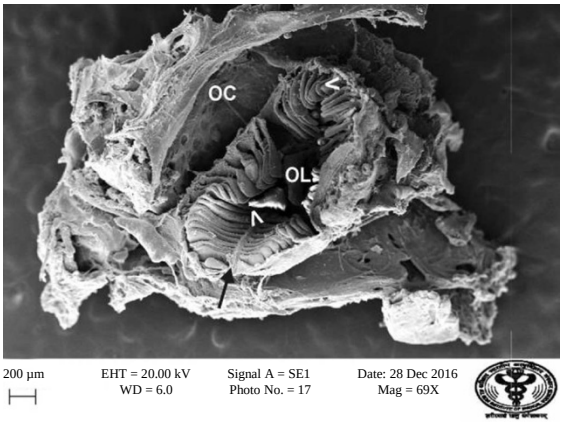

Fig. 4: Gross structure of olfactory rosette within olfactory chamber (OC) exhibits median narrow raphe (solid arrow) and the arrangement of olfactory lamellae (OL). Arrow heads mark linguiform processes of OL (SEM) × 69X

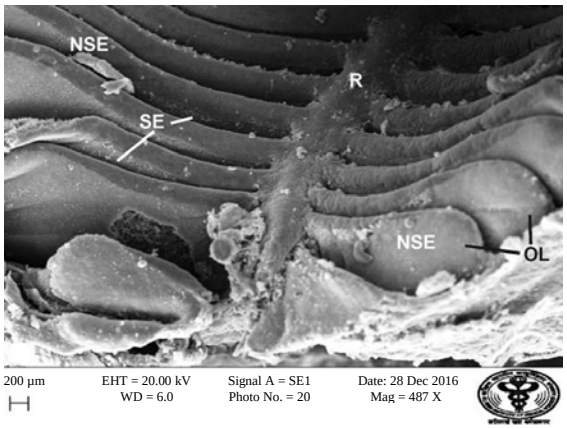

Fig. 5: Olfactory lamellae (OL) radiated from central raphe (R) showing disposition of sensory (SE) and non-sensory (NSE) epithelium (SEM)×487X

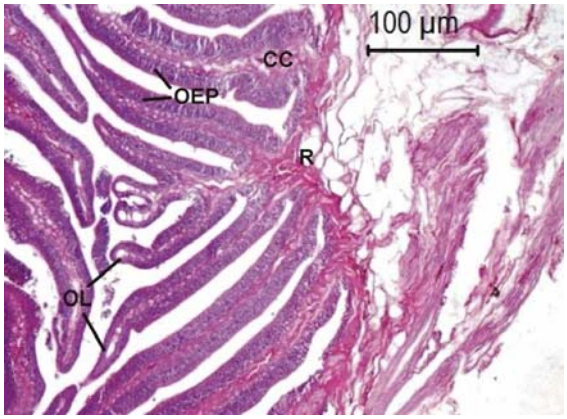

Fig. 6: Olfactory lamellae (OL) attached with raphe (R) show compact olfactory epithelium (OEP) on both sides of median central core (CC) (HE)×40X

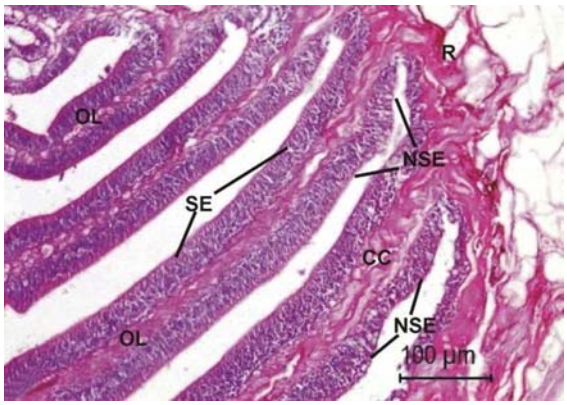

Fig. 7: Showing the arrangement of sensory (SE) and non-sensory (NSE) epithelium of olfactory lamellae (OL) emitted from raphe (R). CC indicates central core (HE)×100X

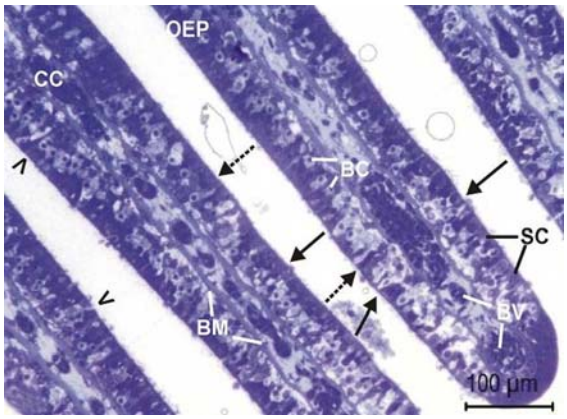

Fig. 8: Semi-thin section of olfactory epithelium (OEP) shows primary receptor cells (solid arrows), microvillous receptor cells (arrow heads), rod receptor cells (broken arrows) and supporting cells (SC). Note the presence of basal cells (BC) close to the basement membrane (BM), which separates OEP from central core (CC). BV marks blood vessels in CC (TB)×400X

Histology: Olfactory epithelium of *E. vacha* was an uninterrupted thick sheet of pseudo-stratified epithelial cells, which was folded to form lamellae. Each olfactory lamellae based on raphe consisted of an olfactory epithelium and a central lamellar space, the central core (Fig. 6). A prominent basement membrane separated olfactory epithelium from the central core. The central core was filled with loose connective tissues containing blood vessels and nerve fibres (Fig. 8-11). Each lamella was marked by sensory and non-sensory epithelium (Fig. 7). The sensory epithelium was characterized by primary, secondary, microvillous and rod receptor cells which were differentiated on the basis of architecture on their apical part (Fig. 10). The non-sensory epithelium consisted of supporting and series of mucous cells (Fig. 11).

Primary neurons or receptor cells: These cells were elongated elements of the olfactory epithelium and usually distributed in the proximal region of the olfactory mucosa. This receptor cell was differentiated by intensely stained oval nucleus that is situated deep in the epithelium and a thin long dendrite runs to the epithelial surface (Fig. 8-10). The axonal ends of primary neurons were synapsed with the dendrite tips of the secondary neurons (Fig. 9, 10).

Secondary neurons or receptor cells: The secondary neurons mainly existed underneath the primary neurons. The secondary neurons were characterized by their ovo-elongated darkly stained nuclei in the distal part of the cells (Fig. 9, 10). The axons of secondary neurons extended up to the basement membrane and passed through the central core of lamella.

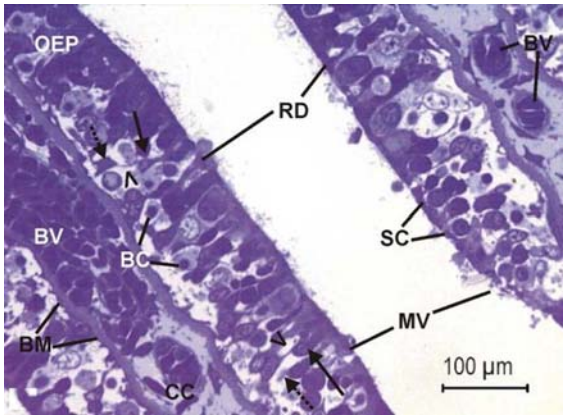

Fig. 9: Higher magnification of olfactory epithelium lined with primary receptor cells (solid arrows), secondary receptor cells (broken arrows), microvillous cells (MV), rod cells (RD), supporting (SC) and basal cells (BC). Arrow heads mark the point of contact in between primary and secondary receptor cells. Note presence of blood vessels (BV) in central core (CC), which is distinguished from OEP by basement membrane (BM) (TB)×1000X

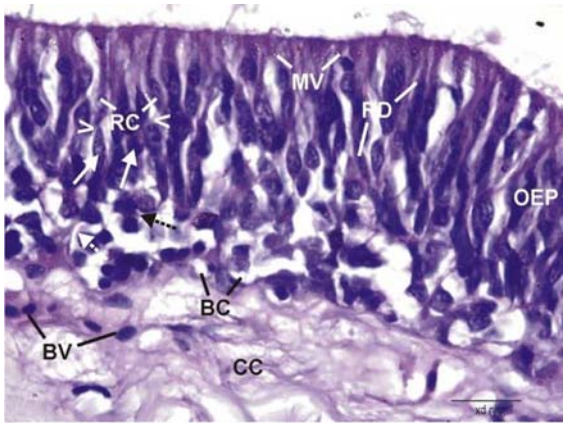

Fig. 10: Sensory olfactory epithelium (OEP) showing prominent synapse (arrow heads) in between primary (RC) and secondary (solid arrows) receptor cells. Broken arrows indicate the axons of secondary receptor cells pass to central core (CC) containing blood vessels (BV). Note the presence of microvillous cells (MV), rod cells (RD) and basal cells (BC) in OEP (MT)×1000X

Microvillous receptor cells: These receptor cells were confined to the surface zone of the olfactory epithelium and intermingled with supporting cells. They were small in size, contained lightly stained nuclei and faintly visible minute dendrites on the apical rim of the cell (Fig. 8-10).

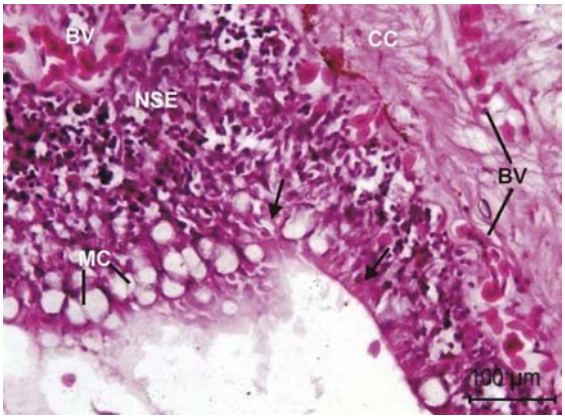

Fig. 11: Non-sensory olfactory epithelium (NSE) typified with supporting cells (solid arrows) and a series of mucous cells (MC) with secreted mucin (broken arrows). Note the presence of blood vessels (BV) in central core (CC) (HE)×400X

Rod receptor cells: Rod cells were few in number and cylindrical in shape, scattered in between supporting and other receptor cells. The apical end of the rod cells protruded as a spike like structure and distinguished by highly basophilic nuclei (Fig. 8-10).

Supporting or sustentacular cells: These cells provided the basic texture of lamella. They were columnar in shape with conspicuous central oval nuclei, situated superficially in the epithelium. The distal end the cell was broad while the tip is narrow, without any dendrite. Cytoplasm was eosinophilic and less granular (Fig. 8-11).

Mucous cells: These cells were found in the proximal region of the lamella but profusely localized in the middle and basal part of non-sensory epithelium. They were globular in shape containing secretory materials and their nuclei were placed towards basal portion (Fig. 11).

Basal cells: These cells were dissipated at the base of both sensory and non-sensory epithelium just over the basement membrane. They were irregular in outline and contain distinct round nuclei (Fig. 8-10). Cytoplasmic projection of the cell did not extend to the epithelial surface. These cells formed a reservoir for the formation of receptor and supporting cells.

DISCUSSION

Eutropiichthys vacha possesses acute sense of smell and depend on their olfactory organ for exploring the surrounding aquatic environment in which they subsist. In *E. vacha*, the

olfactory rosettes are situated in the olfactory chambers, each communicates to the exterior through anterior and posterior nostrils. Such type of morphological arrangement forwards the flow of water containing odorants over the olfactory mucosa. Passing of water flow is unidirectional, enters through the anterior nostril and passes out of posterior one after bathing the olfactory epithelium as reported also in *Anguilla anguilla*[13] and *Rita kuturnee*[18]. The anterior inlet and posterior outlet probably serve as a passage for water ventilation over the olfactory epithelium. Suitable ventilation of the olfactory chamber is needed to bring the odorants to the olfactory mucosa for perceiving the chemical signal of the aquatic ecosystem[19]. The multilamellar peripheral olfactory organs in *E. vacha* are conjoined to the olfactory lobes of the brain by means of olfactory nerves. Variation of olfactory organ may occur among teleosts due to adaptation in specific environment. The ecological niche inhabited by a fish species has an immense impact on its structural organization of olfactory apparatus and level of specialization[20].

The oval shaped olfactory rosette of *E. vacha*, with 32±2 lamella arranged on either side of the central narrow raphe is classed in Bateson's[21] rosette type 3 or belongs to Burne's[22] rosette column I. Teichman[12] reported that oval shaped olfactory organ grouped into eye-nose fishes comparing olfactory surface area to retinal area, show equal sensitivity and efficacy of olfaction and sight. The olfactory lamellae of *E. vacha* bear well developed linguiform processes which facilitate the flow of water across the olfactory rosette. Water entering the anterior opening is conducted directly over the central part of the rosette from where it flows to the interlamellar spaces. This fact has also been mentioned by Mokhtar and Abd-Elhafeez[23] in red-tail shark (*Epalzerorhynchos bicolor*). Allotment of sensory and non-sensory regions on the lamellae exhibits enormous diversity in different fish species for suitability to a particular aquatic environment[24]. In *E. vacha* the sensory epithelium is confined to the linguiform processes probably due to interact with incoming water and encode the cues achieved mainly by receptor cells.

Histologically, the olfactory lamella of *E. vacha* comprises of two layers of olfactory epithelium sandwitching a central core and the aggregation of receptor cells on the epithelial surface confirm their olfacto-sensory functions. The dendrite process of primary receptor cell contains receptor sites for olfactory stimuli and enables the fish to smell its food and exploring the surrounding in which the species live[25]. The attentive aspect of the present study is the detection of secondary neurons in addition to primary neurons and the presence of synaptic connections between these two types of neurons in the olfactory epithelium of *E. vacha*. The axons of the secondary neurons may extend into the central core of the lamellae. This clearly advocates that the impulses received by the dendrite of primary receptor cells ultimately send impulses to the central core and carried over into the brain finally.

Microvillous receptor cells are few in number and scattered in the surface zone of the olfactory epithelium. These cells probably form a different olfactory transduction mechanism for amino acids and nucleotides[26]. Bhute and Baile[27] also reported that the microvillous receptor neurons perceive and process signals of pheromone, which is an important step of breeding in *Labeo rohita*.

The present study reveals that the receptor cells with rod-shaped dendrite ending are distributed sparsely in the olfactory epithelium. Yamamoto[24] opined that appearance of rod cells as an indicator of aging of ciliated receptor cells, end with single cilium at the epithelial lining. Hernadi[28] proposed that the occurrence of the rod-shaped olfactory neuron observed in the presence of a new physiological condition.

Supporting cells provide mechanical support to other sensory cells. These cells may produce a serous secretion, which removes the remains of the stimulating substances and keeps the receptor cells ready for new stimuli. The supporting cells have been suggested to perform several functions: Secretory, absorbing and glial[28,29].

Mucous cells are globular and profusely distributed in the non-sensory area. They secrete mucin to protect the mucosa from mechanical abrasion and help in smooth flow of water during ventilation. The mucus film may also hold xenobiotics like heavy metals, salts etc. and displaces or obstructs their invading to underlying tissues[30].

The basal cells lie close to basement membrane and scatter in the deeper region of both sensory and non-sensory epithelium of *E. vacha*. These cells are assumed to be progenitors of receptor and supporting cells[31].

CONCLUSION

Riverine carnivorous catfish *E. vacha* possesses a well organized olfactory organ and turns on olfactory sensory cells in detection of food and other necessary activities surrounding in the habitat. Dense population of receptor cells on the epithelial lining mobilize different olfactory cues and assess the surrounding environment. However, further studies of transmission electron microscopy and other experimental studies will be useful in corroborating the present findings.

SIGNIFICANCE STATEMENT

This study reveals that the olfactory organ of *E. vacha* is well organized and seems to be efficient with mode of life and living. *Eutropiichthys vacha* is a carnivorous catfish which preferably feeds on aquatic insects, crustaceans, annelids and small fishes also. Large olfactory epithelial surface, presence of various receptor cells and adequate arrangement for ventilating the olfactory chamber suggests that the fish is very much dependent on its olfactory sense. With the growing interest in aquaculture the fish olfactory system can be used as an odorant receptor based biosensor for screening the chemical pollutants in the aquatic ecosystem.

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