Bulletin of Pure and Applied Sciences
Vol.39A (Zoology), No.1,
January-June 2020: P.9-16
Original Research Article

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI 10.5958/2320-3188.2020.00002.9
Available online at www.bpasjournals.com

Morphology and Surface Ultrastructure of the Olfactory Rosette of a Hillstream Fish, *Schizothorax richardsonii* (Gray)

[1]Kiran Kumari
[2]Tapan K. Ghosh*

Author's Affiliation:
[1]Assistant Professor (Guest), University Department of Zoology, Marwari College, Tilka Manjhi Bhagalpur University, Bhagalpur, Bihar 812007, India.
E-mail: kiranbgp11@gmail.com
[2]Ichthyology Research Laboratory, University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar 812007, India
E-mail: tkgzool@yahoo.co.in

***Corresponding Author:**
Tapan K. Ghosh
Ichthyology Research Laboratory, Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar 812007, India
E-mail: tkgzool@yahoo.co.in

Received on 29.12.2019
Accepted on 10.02.2020

Abstract:
The morphology and surface ultrastructure of the olfactory rosette of a cold water hill stream fish, *Schizothorax richardsonii* (Gray) has been investigated. The paired olfactory organs are situated at the anterodorsal aspect of the head in the form of olfactory chambers. Each chamber has two separate openings, the anterior and the posterior nostril. The olfactory rosette is oval and cup shaped in appearance. Each lamella of the rosette is a flat, curved and claw like structure. The olfactory lamellar epithelium of *Schizothorox richardonii* shows two distinct regions, the ciliated and non-ciliated epithelium. In this fish, the ciliated epithelium is composed mainly of four types of cells which are distinguished on the basis of their surface specialization. The non ciliated epithelial cells or the indifferent epithelium are provided with well marked microridges. The surface of the raphe shows the presence of microridged epithelial cells, mucous gland openings as well as the type-1 ciliated cells. The presence of ciliary patches on the median raphe of *Schizothorox richardonii* indicates that probably the raphe is somehow involved in olfaction.

Keywords: *Schizothorax richardsonii,* Olfactory rosette, Morphology, Surface ultrastructure.

INTRODUCTION

The study of the olfactory organ of fishes is of paramount importance because it is essentially a chemoreceptor and plays a meaningful role in not only locating food but also in detecting the presence of odoriferous substance in the aquatic ecosystem. The morphological and surface ultrastructure of the olfactory epithelium of different teleosts have been investigated by various authors including Bateson (1889), Burne (1909), Teichmann (1954),Yamamoto and Ueda (1977), Jakubowski (1981), Zielinski and Hara (1988), Singh (1994), Mana and Kawamura (2002), Kumari (2008), Chakrabarti and Guin (2011), Kumari *et. al.* (2013) and Ghosh *et. al*. (2015). Information on the olfactory rosette of hill stream fishes are few.The present study is an attempt to examine the morphology and the surface ultrastructure of the olfactory rosette as revealed by the scanning electron microscope (SEM) in the hill streamfish, *Schizothorax richardsonii*.

MATERIALS AND METHODS

Live specimens of *Schizothorax richardsonii* (Gray) were collected from Nepal. The living fishes were first anaesthetized in MS222 at the site and then preserved in formalin for further morphological

studies. The fishes were carefully dissected, olfactory rosettes were exposed and their connection with the brain was traced out. For scanning electron microscopy (SEM), the head of the anesthetized fish were directly fixed in 2.5% cold glutaraldehyde prepared in 0.1M phosphate buffer (pH 7.4), for 24 hours. The tissue was then dehydrated in ethanol, acetone and stored in anhydrous acetone. The rosettes were further critical point dried, mounted on the SEM stubs with double adhesive tapes conducted with silver dag, gold coated and observed under Quanta 200 SEM at RSIC, Bose Research Institute, Kolkata.

RESULTS AND DISCUSSION

Schizothorax richardsonii (gray) belongs to the family Cyprinidae of the order Cypriniformes. In *Schizothorax richardsonii*, the paired olfactory organs are situated at the anterodorsal aspect of the head in the form of olfactory chambers. Each chamber has two separate openings, the anterior and the posterior nostril. Both the nostrils are situated very close to each other and are more or less oval in shape and they open just above the olfactory rosette. The anterior nostrils are comparatively smaller than the posterior ones. Both the openings are separated by a nasal flap of skin (Figure 1).

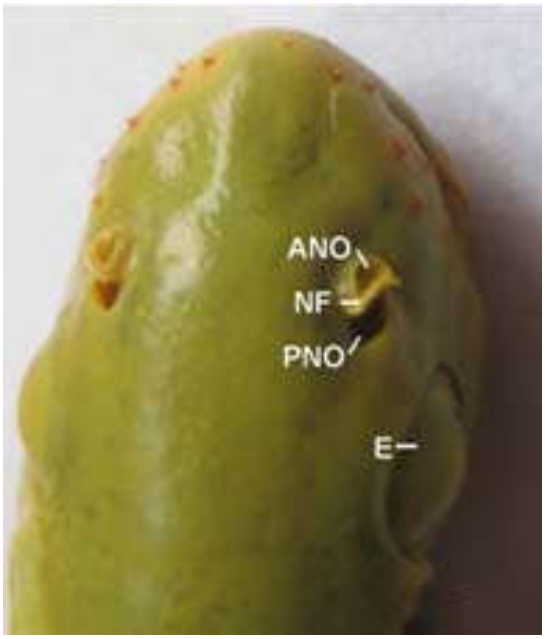

Figure 1: Dorsal view of the head region showing the position of anterior and the posterior nostrils.

The skin-bridge or the nasal flaps present between the two nasal openings are raised to form an upstanding nasal flap behind the anterior nostril. The forebrain of *Schizothorax richardsonii* is well developed. The prominent olfactory bulb is present at the base of the rosette, in the form of a vase shaped structure. Each olfactory tract arises from the base of the olfactory bulb and runs backward through the frontal region of the skull and eventually terminates in the telencephalon. The olfactory lobes are not differentiated (Figure 2).

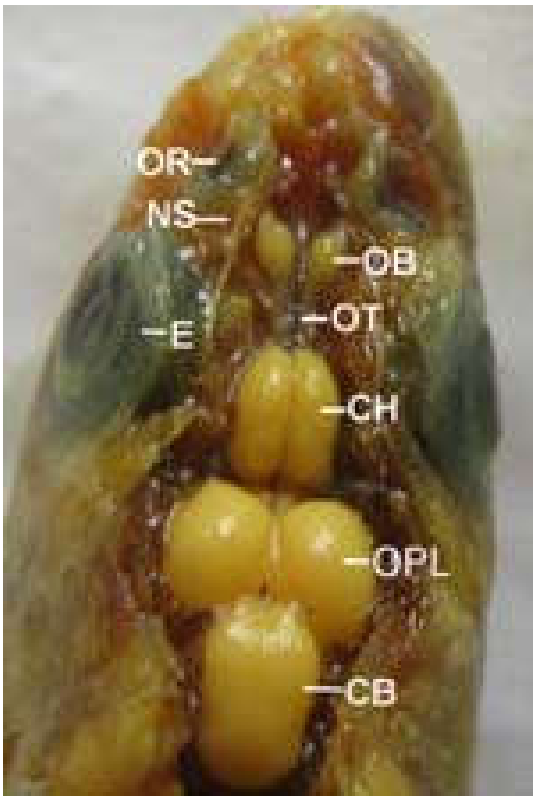

Figure 2: Showing the olfactory rosettes and their relationship with the forebrain.

Two nasal sacs are present, one at the lateral side and another at the posterior side of the rosette. The olfactory rosette is oval and cup shaped in appearance. Olfactory lamellae are arranged on both sides of the raphe. The lamellae are sparsely situated i.e. they are not compact (Figure 3) showing well marked interlamellar spaces.

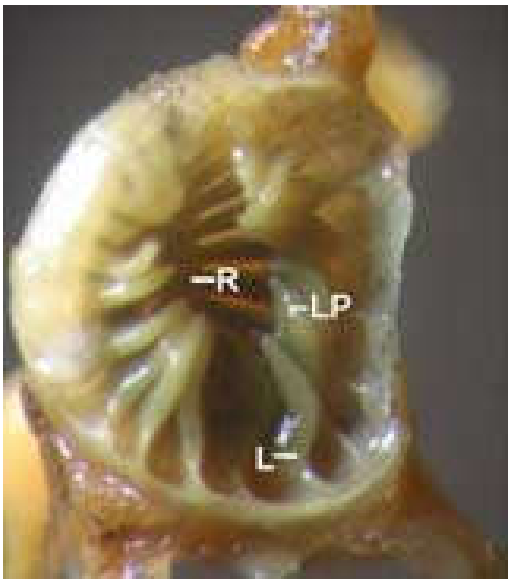

Figure 3: One Complete Olfactory Rosette.

The numbers of lamellae are much less in *Schizothorax richardsonii* as comparison to many other species. The lamellae remains attached to the wall of the olfactory rosette from its ventral side whereas the dorsal side remains free. The anterior lamellae are smaller while the posterior ones are larger. Numerous pigments have been observed on the lower surface of the lamellae. Each lamella of the rosette is a curved claw-like structure with a prominent linguiform process present on the distal end of the lamella (Figure 4).

In *Schizothorax richardsonii* the linguiform process is more prominent and pointed as compared to other fishes. The numbers of lamellae are much less in *Schizothorax richardsonii* as compared to other species viz. *Catla catla* and *Clarias batrachus*, Kumari (2008), Kumari and Ghosh (2013) respectively.

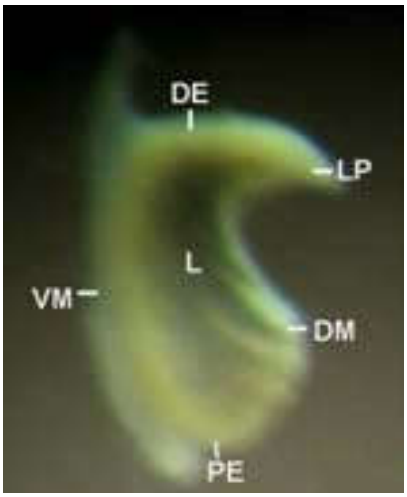

Figure 4: Structure of one complete olfactory lamella.

According to external morphology, the olfactory rosettes of this species are oval, this means they can be included in Bateson's (1889) rosette type-3, Burn's (1909) Column-1 and according to Teichmann's (1954) eye nose fishes (group-1).

The surface topography of the olfactory rosette of *Schizothorax richardsonii* was examined using scanning electron microscope (Figure 5).

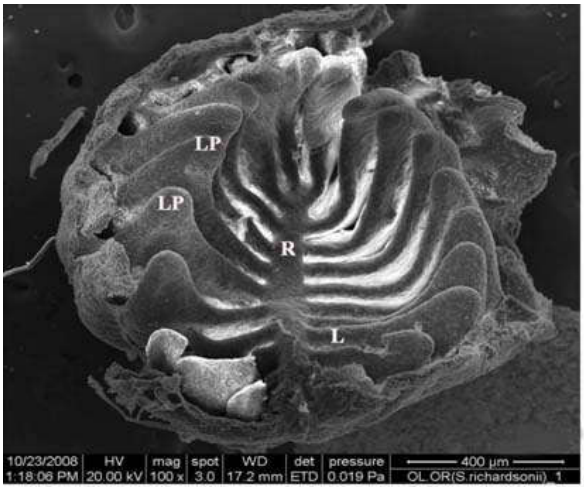

Figure 5: Full view of an olfactory rosette with its median raphe and arrangement of olfactory lamellae

The surface of the tip or linguiform part of a lamella shows numerous mucous gland openings, and a dense distribution of sensory receptor cells. The olfactory lamellar epithelium of *Schizothorax richardsonii* shows two distinct regions the ciliated and the non-ciliated epithelium. The ciliated epithelium is composed mainly of four types of cells which are distinguished on the basis of their surface specialization. The type-1 ciliated cells are provided with long cilia which are inclined in one direction (Figure 6).

Figure 6: The tip or linguiform process of a lamella showing the ciliated sensory cells and numerous mucous gland openings

Some type-II ciliated cells were also located having small hillock like cell body from which small cilia radiate on all direction. The type-III ciliated cells i.e. microvillous cells (mv) are also present in this species, which bear no cilia but only a tuft of microvilli. The type - IV ciliated cells bear neither cilia nor any microvilli but their apical ends protrude as a rod from the epithelial surface (Figure 7).

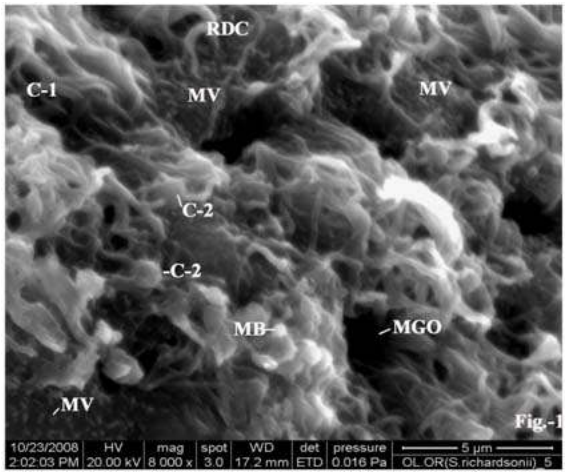

Figure 7: Part of the lamellar surface showing the ciliated type-1, type-2 cells, microvillous cells and rod cells

The cut end of lamellae shows its inner structural details (Figure 8). Each lamella is externally covered over by an epithelial layer from all sides. Externally this layer is provided with numerous openings of mucous glands. Within this a central core is easily distinguished. Rows of different types of cells are arranged between the epithelium and the central core. Of the different types of cells, mucous gland cells, tall receptor cells, supporting and basal cells were located (Figure 8).

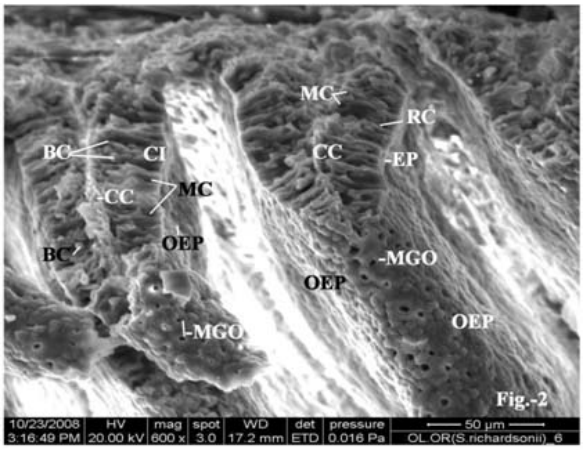

Figure 8: The inner view of an olfactory lamella showing the olfactory receptor cells, mucous cells and the central core

The surface ultrastructural details of raphe of the olfactory rosette of *Schizothorax richardsonii* revealed some interesting results. The epithelial surface of the raphe of *Schizothorax richardsonii* shows presence of ciliated cells. Although the surface does not have dense ciliary patches as seen on the lamellae but scattered ciliary areas have been observed. The ciliated cells on the surface are mostly type-I ciliated cells (Figure 9). The non ciliated cells or the indifferent epithelium is provided with well marked microridged cells. Numbers of mucous gland openings have been observed in the indifferent epithelial region of the raphe (Figure 10).

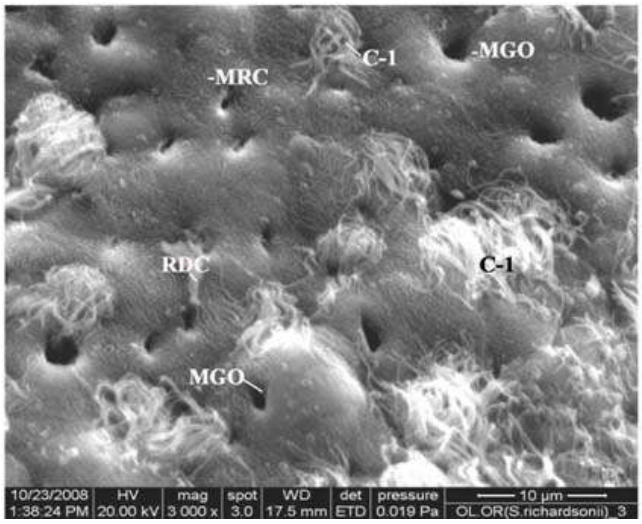

Figure 9: The surface view of the raphe showing ciliary patches of type-1 ciliated cells, mucous gland openings and the non sensory microridged epithelial cells

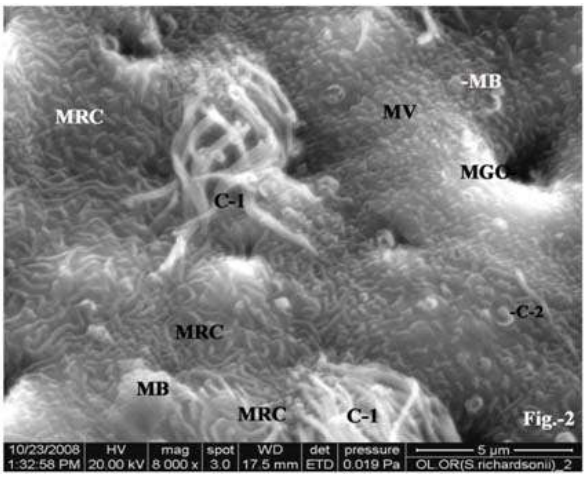

Figure 10: An enlarged view of olfactory raphe

The olfactory lamellae are covered over by an olfactory epithelium with different types of cellular components. Different workers have identified different types of cellular regions in the epithelium of olfactory lamellae of fishes. Yamamoto and Ueda (1977) identified four types of cells on the basis of their surface specialization: (i) those bearing many long cilia on the wide and flat surface (type 1 ciliated cells), (ii) those bearing several short cilia which project radially from a round cell apex (type 2 ciliated cell), (iii) those bearing no cilia but a tuft of numerous microvilli (microvillous cell) and (iv) those bearing no cilia or microvilli but protruding as a simple rod from the surface (rod cell). Besides these, he also distinguished the supporting cells having short microvilli at their free ends. According to Singh (1994) in, the olfactory epithelium of *Schizothorax richardsonii* lacks any ciliated elements either in the sensory or indifferent epithelial region. But the present observation does not corroborate the statement of Singh (1994), as dense cilia are present on the lamellar surface and even the raphe is provided with ciliary patches. In *Schizothorax richardsonii* the median raphe shows some scattered ciliary patches provided with type-1 ciliated cells (Figure 9 and 10). The presence of ciliary patches on the median raphe in olfactory rosettes of *Schizothorax richardsonii* indicates that even the raphe is somehow involved in olfaction of this fish. Patchy distribution of clusters of type-1 ciliated cells seems to be a specific feature of Cypriniformes (Yamamoto and Ueda, 1978). The ciliary type-1 cells are present in raphe, which are inclined in the same direction. Besides the ciliary epithelial cells, the raphe is covered over with non ciliated epithelium bearing epithelial cells with microvilli. The main cells covering the non ciliated epithelium or the indifferent epithelium bear finger print like pattern of microridges and numerous mucous gland openings. In the sea trout *Salmo trutta*, Bertmar (1972, 1973) has reported the presence of microvillous and ciliated cells both in the sensory and indifferent epithelium. The olfactory surface area of *Schizothorax richardsonii* is better adapted for olfaction. This is because different types of receptor cells are present over its entire lamellar surface area. Moreover, even the raphe of *Schizothorax richardsonii* are provided with type-1 ciliated cells which perhaps enhances the olfactory capacity of the fish.

ABBREVIATIONS

ANO-Anterior Nasal Opening, CB-Cerebellum, CH-Cerebral Hemisphere, DE-Distal End, DM-Dorsal Margin. E-Eye, L-Lamella, LP-Linguiform Process, NF-Nasal Flap, OB-Olfactory Bulb, OE- Olfactory Epithelium, OPL-Optic Lobe, OR-Olfactory Rosette, OT-Olfactory Tract, PE-Proximal End, PNO-Posterior Nasal Opening, R-Raphe, VM-Ventral Margin, C-1- Ciliated type-1Cell, C-2 – Ciliated type-2 Cell, CC-Central Core, IE- Indifferent Epithelium, MB- Mucous Ball, MGO - Mucous Gland Opening, MRC- Microridged Epithelial Cell, MV- Microvillous Cell, RC- Receptor Cell, RDC-Rod Cell.

ACKNOWLEDGEMENT

The first author is thankful to UGC for the financial support through Rajiv Gandhi National Fellowship No. F-16-176/SC/2006(SA-III). The authorsare indebted to Sri S.C. Maikap of RSIC, Kolkata for SEM work.

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