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**RESEARCH ARTICLE**

# DIMORPHISM IN OLFACTORY NEUROEPITHELIUM OF FISH, *ANABAS TESTUDINEUS* (BLOCH, 1792): AN ULTRASTRUCTURAL INTERPRETATION

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**ABSTRACT**

*Anabas testudineus* is an air-breathing teleost (IUCN Red List- 'Least Concern' Ver 3.1).The olfactory system of fish is a highly specialized chemosensory organ comprised of nostrils, nasal cavities, olfactory lamellae, olfactory nerves and olfactory bulbs above the brain. We conducted a study on olfactory neuroepithelium of *A. testudineus* to explore the surface topography and its dimorphism between different sexes (male and female). The olfactory rosettes of *A. testudineus* were dissected and examined under a scanning electron microscope (SEM) after fixation in 2.5% glutaraldehyde and CPD respectively. The olfactory rosette contained 8-11 numbers of lamellae and connected with an anterior raphe. The lamellae are distinguished into prominent inner sensory area bounded by outer non-sensory epithelium with different cellular components. The sensory epithelium possessed ciliated olfactory sensory neurons (CiOSNs) which remain distributed within the dense aggregations of ciliated non-sensory cells. The dispersion of non-sensory epithelium is found to vary between male and female *A. testudineus*. The non-sensory epithelium is branched and typically characterized by ciliated-microvillous cells that were more predominant in male *A. testudineus*. Hence, the olfactory neuroepithelium of *A. testudineus* possesses distinct sexual dimorphism. The uniqueness of ciliated sensory epithelium and ciliated-microvillous cells located in the non-sensory area reflects the specific adaptive nature of the species concerned.



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## INTRODUCTION

Olfaction is a prime sensory modality in vertebrates to perceive the chemical signals of the environment. In teleost, the olfactory organ is situated in the dorsal region of head. It generally comprised of paired nostrils, olfactory cavities, olfactory rosettes, olfactory nerves associated with olfactory bulb. The olfactory neuroepithelium equipped with olfactory sensory receptor neurons (OSRNs) which are the primary detector of olfactory sensation and relay the chemical informations to the brain[1].Three morphological types of OSRNs have been identified in fishes by electron microscopy, i.e., ciliated, microvillous and crypt neurons decorated with apical cilia, microvilli and both respectively [2,3,4]. The olfactory organ varied in several characters including size, shape, lamellar arrangement, distribution of sensory and non-sensory areas [5] reflecting the sensory capability of particular teleostean species. Several authors since earlier have been studied the olfactory organ of various teleosts through scanning electron microscopy (SEM)[6,7,8,9,10].

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However, the dimorphism of the olfactory neuroepithelium in fish under SEM was hardly characterized between different sexes. *Anabas testudineus* (Bloch, 1792) is an air-breathing climbing perch. The fishes are naturally inhabited in fresh and brackish water bodies mostly in rivers, ponds, canals, ditches, swamps, etc [11]. They preferred to live in shoals [12] and periodically exposed to air-water interface. They can also lead an amphibious mode of adaptation by occasional migration toward land [13]. Feeding pattern is predominantly carnivorous included protozoa, crustacea, rotifers, insects, algae, etc[14]. The present study is aimed to investigate the surface topography of olfactory neuroepithelium in *A. testudineus* by scanning electron microscopy and also addressed whether is it dimorphic in appearance between male and female *A. testudineus*?

## MATERIALS AND METHODS

Live and adult specimens of *Anabas testudineus* (total length: 120 to 160 mm, weight: 29.2 to 72.7 gm) were obtained from local fish markets of West Midnapore district, West Bengal, India.

Specimens were acclimatized in the laboratory conditions for 72 hours with natural foods. For the study of dimorphism, the male and female specimens of *A. testudineus* were collected and brought to the laboratory during breeding season (April to June) of the year. Prior to dissection, the specimens were anesthetized with tricaine methanesulfonate (ms-222) at a dosage of 100mg./l. The olfactory area of external nostrils and olfactory rosettes were carefully dissected out from the dorsal head region of *A. testudineus*. Following dissection, the olfactory tissues were immediately fixed in 2.5% glutaraldehyde solution (Sigma-Aldrich, EM Grade) in 0.1 M phosphate buffer ($Na_2HPO_4$, $NaH_2PO_4$; pH-7.4) for 2 hours at 4°C. Then samples were washed in the same buffer [PB (0.1 M); pH-7.4] thoroughly. After that dehydrated the samples through ascending grades of chilled acetone followed by isoamyl acetate. The tissue samples were critically point dried (CPD) by liquid Carbon dioxide ($CO_2$) in a critical point drier (Hitachi 8CP2). The dried specimens were mounted on metal stubs, coated with platinum (thickness-16 nm) using a sputter coater (Quorum Q150tes) and then examined under a scanning electron microscope (SEM, Zeiss EVO18) operated at 20 kV.

## RESULTS

The olfactory cavity of *Anabas testudineus* is situated on the anterodorsal region of head with two openings *viz.,* anterior and posterior nostril (Fig. 1A). The anterior nostril is tube like and the posterior nostril is oval in shape (Figs. 1B-C). The olfactory rosette is an oval structure contained 8 to 11 numbers of lamellae (Fig.1D). The olfactory lamellae are parallelly oriented and connected with a raphe in the proximal part of rosette (Figs. 1D, 3A). The raphe surface composed of stratified epithelial cells and solitary bunch of ciliated cells (Fig.1F). The stratified epithelial cells are closely arranged and characterized by micro ridges on the surfaces (Fig.1F). The ciliated cells in raphe are consisted of multiple short cilia in a cluster (Fig.1F). At the bottom of rosette, a wide shallow lumen is marked between the terminal ends of two successive olfactory lamellae (Figs. 1D-E). The surface of the lumen is lined by stratified epithelium intermingled with non-sensory ciliated cells (Fig. 1E).In both sexes of *A.testudineus*, the olfactory neuroepithelium was divisible into prominent sensory and non-sensory areas (Figs.2A, 3A). The sensory epithelium is ciliated, endowed with sensory receptor neurons and positioned in the inner lateral surface of each olfactory lamella (Figs. 2A, 3A). The sensory cells are marked as ciliated olfactory sensory neurons (CiOSNs) possess olfactory knob (OK) with shorter cilia up to 3.03 µm in length (Fig. 2B). The CiOSNs are distributed within the dense aggregation of ciliated non-sensory cells(cNSC) which possess longer cilia about a length of 8-14 µm (Fig.2B).The non-sensory epithelium is continued as a thick band from apical to basal end along the entire outer edge of olfactory lamellae (Figs.2A, 3A). The posterior tip of lamellae exclusively comprised of non-sensory epithelium with broader surface area (Figs.2A, 3C). The non-sensory epithelium is characterized by stratified epithelial cells, microvillar cells, mucous secretory pores, ciliated-microvillous cells and non-sensory ciliated cells (Figs. 1H-I, 2D, 3D-E). Mucous is secreted from the mucous cell in the form of distinct droplets at the epithelial surface (Fig. 1G).The dispersion of non-sensory region in lamella varied between male and female *A.testudineus*. In male fish, the non-sensory region at the posterior terminal end of lamellae gives rise to 3-4 branches (Fig.2A). These non-sensory branches are long, distinct and inserted within the inner sensory region of lamella (Fig. 2C). In contrast, the boundary of sensory and non-sensory olfactory epithelium across the lamella is more concise in female (Fig. 3A). The non-sensory epithelium at the posterior terminal end of lamella (female) showed a half 'U' shaped turn with no branches are visible like of male *A.testudineus* (Figs.2A, 3C).In some instances (female), a single offshoot of non-sensory epithelium is observed at the terminal end of lamella (Fig. 3F). The non-sensory region is marked with distinct ciliary aggregations in both sexes (Figs. 2C, 3D). In contrary to female, the non-sensory epithelium of male fish appeared less dense in ciliation and it is largely predominated by numerous, distinct ciliated-microvillous cells (Figs.2C-D, 3F). The sensory epithelium exhibits similar although the pattern of ciliation (non-sensory cilia) appeared more dense in female comparative to male *A.testudineus* (Figs. 2B, 3B).

## DISCUSSION

Olfactory cues are extremely important to mediate several functions in fishes *viz.,* searching of foods, avoidance of predators, spawning, migration, reproduction and parent-offspring interactions[15]. *Anabas testudineus* have a multilamellar oval shape olfactory rosette reflecting the characteristic of 'eye-nose fish' with equal sensitivity of olfaction and vision [16]. Yamamoto [17] had recognized four types of distribution pattern of sensory epithelium based on the arrangement of sensory and non-sensory cells on the surface of lamellae: Type I (continuous), Type II (large-zone), Type III (web-like) and Type IV (spotted). The sensory epithelium of *A. testudineus* is broad and resembled 'continuous type' of distribution in female and 'large-zone' in male due to non-sensory branching. Type I or Type II pattern were mostly found among predatory fishes and considered highly efficient olfactory assembly [17]. In *A.testudineus*, both sensory and non-sensory areas are ciliated, although sensory area appeared more densely ciliated. The olfactory knob of CiOSNs remains distributed within the ciliary aggregations of ciliated non-sensory cells. This is a unique feature of sensory epithelium in *A. testudineus* and may be suitable to adapt in semi aquatic mode of living. The non-sensory ciliated cells with longer cilia were the respiratory-type motile ciliated cells [18]. The unidirectional movement of cilia propel water thus creating adequate ventilation to bring odorants in the olfactory cavity for perceiving the chemical signals[19,20]. *A.testudineus* are found in waterlogged areas and can tolerate unfavourable water conditions. The ciliary aggregations of non-sensory ciliated cells provide protection to sensory receptor neurons as well as help to discriminate the favourable olfactory cues from the unwanted ones to make the olfaction more precise. The olfactory processing is initiated by the interaction of odorants with the receptor proteins present on sensory receptor cells [21]. Earlier studies suggested CiOSNs respond to a degree of odorants e.g. bile salts [22,23] and amino acids[24,25]. The position of raphe in olfactory rosette displayed variation among fishes according to the orientation of nostrils and nature of accommodation of lamellae in olfactory cavity. In present observation, the anterior raphe at the top of rosette provides the mechanical support to the parallely arranged lamellae.

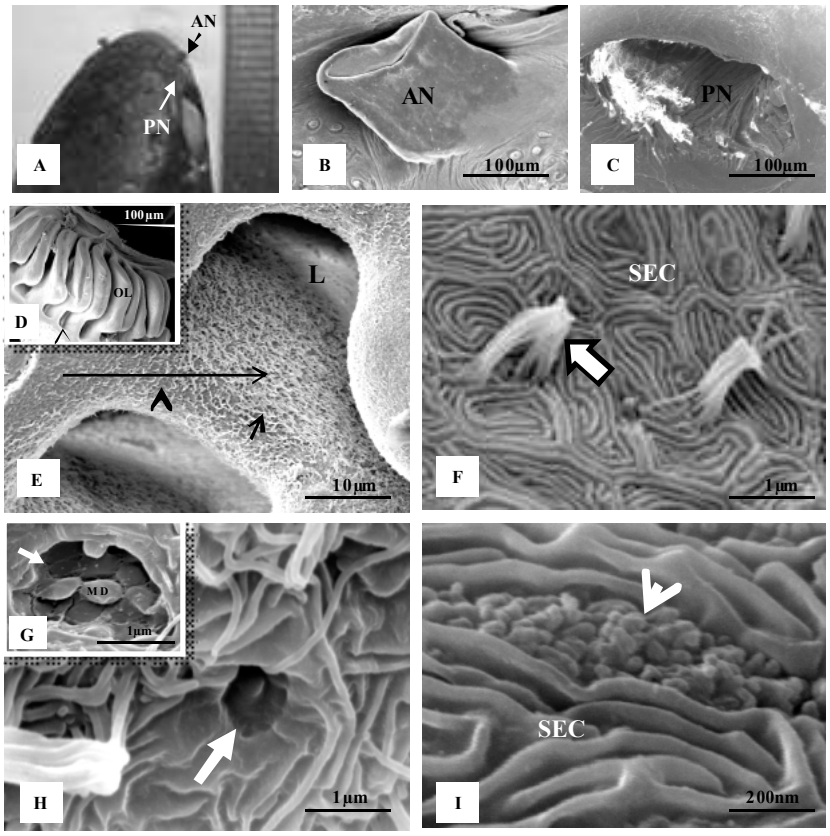


**Figure 1. (A). The photograph of a portion of head of *Anabas testudineus* is showing the location of anterior nostril (AN) and posterior nostril (PN). (B and C). The 3D structure of nostrils under scanning electron microscope (SEM). (D). Surface topography of multilamellar olfactory rosette of *A. testudineus*. Olfactory lamella (OL). (E). The lumen (L) at the bottom of rosette shows stratified epithelial cells (arrowhead) and non-sensory ciliated cells (arrow). (F). SEM micrograph of raphe surface shows stratified epithelial cell (SEC) with microridges and ciliated cells (arrow). (G and H). Arrows indicate mucous secretory pores and secreted mucin droplet (MD) at non-sensory epithelium. (I). The microvillar cells (arrowhead) at surface of non-sensory epithelium in between the stratified epithelial cell (SEC).**

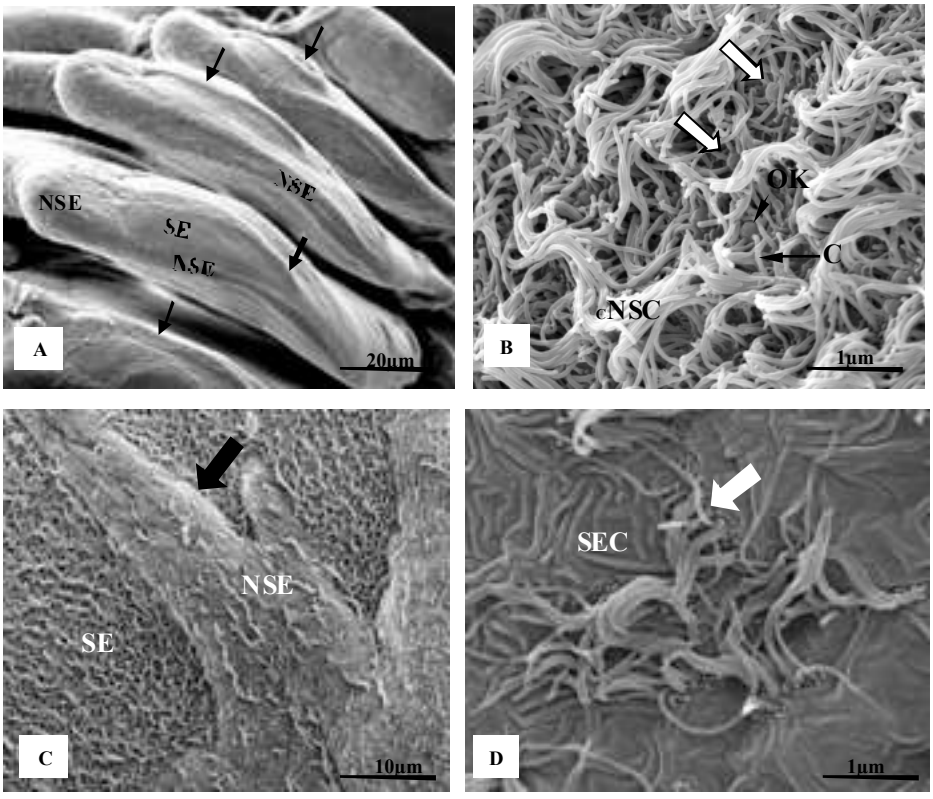


**Figure 2: Scanning electron micrographs of olfactory neuroepithelium in male *Anabas testudineus*. (A). Olfactory lamellae showing the distribution of sensory epithelium (SE) and non-sensory epithelium (NSE) with several branches (arrows). (B). Higher magnification of sensory epithelium shows CiOSNs (arrows) within the aggregations of ciliated non-sensory cells (cNSC). Ciliated olfactory sensory neurons (CiOSNs). Cilia (C). Olfactory knob (OK). (C). Olfactory epithelium covers with cilia of different density. The arrow indicates the branches of non-sensory epithelium (NSE) inserted within sensory epithelium (SE). (D). The ciliated-microvillous cells (arrow) at the non-sensory epithelium in between stratified epithelial cells (SEC).**

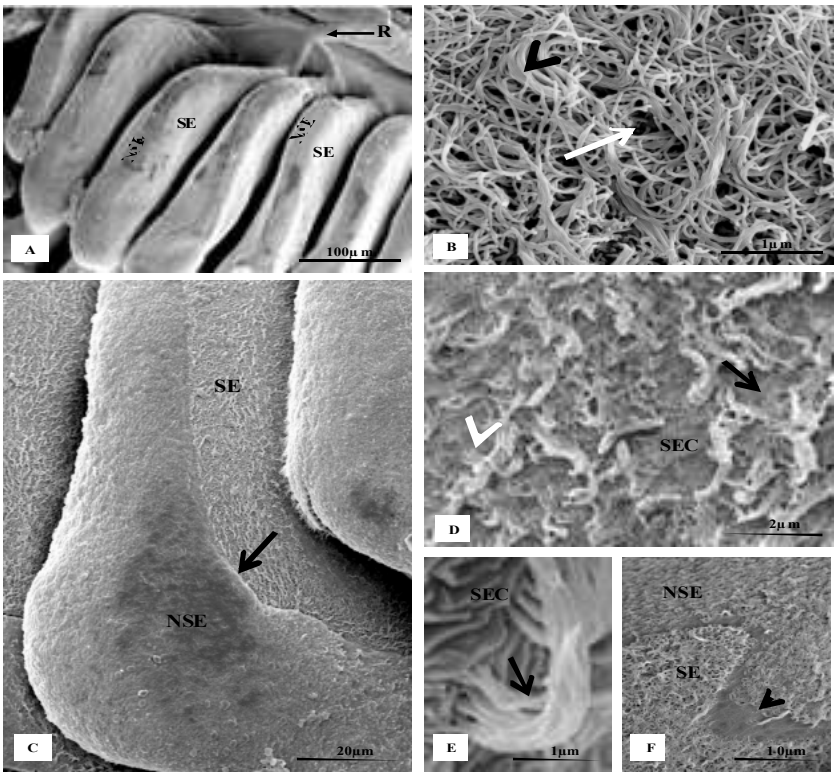


**Figure 3: Scanning electron micrographs of olfactory neuroepithelium in female *Anabas testudineus*.(A). The olfactory rosette shows anterior raphe (R) and distribution of sensory epithelium (SE) and non-sensory epithelium (NSE). (B). Sensory epithelium represents the dense ciliation of ciliated non-sensory cells (arrowhead) and CiOSN (arrow). (C). The posterior terminal end of olfactory lamella shows wide non-sensory epithelium (NSE) with no branches (arrow). (D). The non-sensory epithelial surface shows stratified epithelial cell (SEC), non-sensory ciliated cells (arrowhead) and ciliated-microvillous cells (arrow). (E). The non-sensory ciliated cell (arrow) under higher magnification at the non-sensory epithelium. (F). Dense ciliary aggregations in both sensory and non-sensory epithelium. Arrowhead marked an offshoot of non-sensory epithelium (NSE).**

The stratified epithelial cells (SEC) sculpted with microridges increased the surface area of non-sensory epithelium[26,27] and also protect the inner sensory area from abrasive thrust of water. The mucin droplets provide first line protection to olfactory epithelium as well as assist in a smooth flow of water through olfactory cavity. We observed the olfactory neuroepithelium of *A. testudineus* to exhibit dimorphism in different sexes. Goodenough et al.[28] suggested that male of most species appeared to follow a polygamous mating strategy despite costs associated with mating. In the present study, the sensory epithelium in male was less dense in ciliation resulting the olfactory sensory neurons more open to response comparative to female. This is advantageous in male *A. testudineus* for finding a mate because it mostly depends on olfactory cues together with vision. Miranda et al.[29] reported that the male of Tilapia, *Oreochromis mossambicus* are able to discriminate the reproductive status of female through olfactory sense.The sexual dimorphism of olfactory neuroepithelium has not been well documented among fishes under SEM. However, Waghray [30] in elasmobranch, *Narcine timlei* reported that the numbers of lamellar ridges were few in females as compared to the males. The deep-sea fishes, mostly bathypelagic[31] and few mesopelagic [32], dimorphism of olfactory organ is noted where males are macrosmatic than females. The non-sensory epithelium of *A. testudineus* exhibits greater sexual dimorphism and it revealed a structural peculiarity of ciliated-microvillous cells, which was rare in earlier studies among teleosts. However, the sensory receptor neurons possessed cilia as well as microvilli on their dendritic ends have observed in olfactory neuroepithelium of *Acipenser* [33] and *Ctenopharyngodon idella* [34]. In the present context, the abundant occurrence of ciliated-microvillous cells in male *A. testudineus* may be related to the reproductive repertoire of the species concerned.

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**Key points**

- Olfactory neuroepithelium of fish is directly exposed to water and detects various chemical cues to mediate many of their life activities. The sexual dimorphism of olfactory neuroepithelium in fish by scanning electron microscopy (SEM)is hardly characterized in most of the teleosts.
- The olfactory lamella of *A. testudineus* is sharply divisible into a broad inner sensory epithelium enclosed by outer margin of non-sensory areas.
- The ciliary aggregations of non-sensory ciliated cells over the ciliated olfactory sensory neurons (CiOSNs) make the sensory epithelium more effective to

descriminate the olfactory cues and provide protection to adapt in several hostile conditions.

- In male *A. testudineus*, the sensory epithelium was less dense in ciliation, the non-sensory epithelium is branched and covered with predominant ciliated-microvillous cells compared to females.
- Result shows the surface ultrastructure of olfactory neuroepithelium varied in different sexes of *A. testudineus*, suggests this trait can be use in fish identification studies.

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