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# Morphoanatomy of the Olfactory Components of *Anabas testudineus* (Bloch 1792)

**Madhabi Aich, Subrata Kumar De**

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## ABSTRACT

Olfaction is a primary type of chemoreception detecting distant chemical cues of the environment. The present study is aimed to examine the olfactory organ of *Anabas testudineus* (Perciformes, Anabantidae) by light microscopy to find out its morphocytological organization related to olfactory sense. The olfactory tissues were dissected out, histologically processed (Hematoxylin-Eosin and Nissl staining) and viewed under light microscope (LM). Macroanatomically, the olfactory components of *A. testudineus* included paired nostrils, olfactory rosette, accessory nasal sacs, olfactory nerve and olfactory bulb attached to brain. The accessory nasal sacs associated with each olfactory cavity are separate structure of larger ethmoidal sac and smaller lacrimal sac. Microanatomically, the olfactory epithelium appeared peudostratified with olfactory sensory neurons (OSNs), supporting cells, basal cells . The olfactory bulb is sessile and histologically distinguished into four concentric layers from outer to inner. The first is olfactory nerve layer, followed by glomerular layer with spherical masses of glomeruli. The third is mitral cell layer contained mitral cells and the inner core was granular cell layer with abundant granule cells. The well developed organization of the olfactory cells reflects an acute sense of olfaction of the specimen concerned.

**Keywords :** *Anabas testudineus,* Olfactory epithelium, Accessory nasal sacs, Olfactory bulb.

Madhabi Aich*, Subrata Kumar De
PhD Research Scholar
Ultrastructure and Fish Biology Research Unit, Department of Zoology, Vidyasagar University,
Midnapore (West) 721102, West Bengal, India
Email: madhabimadhabi22@gmail.com
*Corresponding author : Madhabi Aich (PhD Research Scholar)

## INTRODUCTION

Olfaction in fishes is the transport of odorants from external environment of water to the sensory surface of olfactory epithelium (Cox 2008). The olfactory cues are important in fish to mediate several behavioral functions like feeding, avoiding predation, spawning migration and reproduction, parental care and parent-offspring interactions (Zielinski and Hara 2006). The teleosts exhibit variations of olfactory organ characteristics based on the systematic groups and ecological habitats (Hansen and Reutter 2004). The olfactory sensory neurons (OSNs) are the principal components of olfactory epithelium relay the olfactory informations to the brain (Satou 1992) via the olfactory bulb. The olfactory bulb is a multilayered cellular structure in vertebrates comprised of different neuronal cell types like periglomerular cells, mitral cells, tufted cells, granule cells. Over the earlier periods, the morpho-histological architecture of the olfactory organ in different teleosts have been studied by several researchers viz., Kumari 2008, Atta 2013, Sarkar *et al*. 2014.

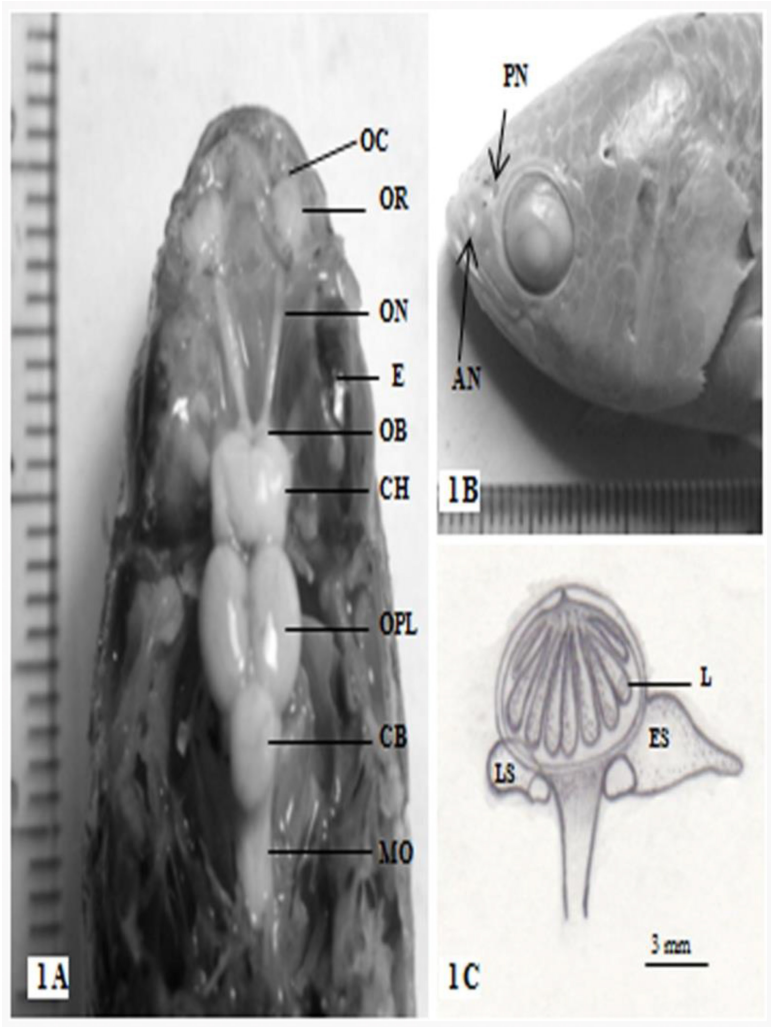


**Fig. 1.** (A) Photograph shows the dorsal view of dissected olfactory organ of *Anabas testudineus.* Olfactory cavity (OC), olfactory rosette (OR), olfactory nerve (ON), eye (E), olfactory bulb (OB), cerebral hemisphere (CH), optic lobe (OPL), cerebellum (CB), medulla oblongata (MO). Mag. 3X. (B) The portion of head is showing the location of anterior nostril (AN) and posterior nostril (PN). Mag. 1.5X. (C) Schematic representation of olfactory rosette along with lamellae (L) and accessory nasal sacs viz., ethmoidal sac (ES), lacrimal sac (LS).

*Anabas testudineus* (Bloch 1792) is a climbing perch (IUCN Red List- 'Least Concern' Ver 3.1) of Southeast Asia (Ahmad *et al.* 2019).They inhabit in waterlogged paddy fields, ponds, swamps, canals, estuaries (Menon 1999) and can tolerate extremely hostile conditions (Atack 2006). The present study is emphasized on the macro and micro-anatomical structural details and the functional aspects of different cell types of the olfactory organ in *Anabas testudineus.*

## MATERIALS AND METHODS

### Macroanatomy of olfactory apparatus

Live and healthy specimens of *A. testudineus* with variable body length (7.4 cm to 14.7 cm) and weight (6.4 cm to 52.2 g) were collected from local fisherman of Hooghly district, West Bengal. After acclimatization with the laboratory conditions, the specimens

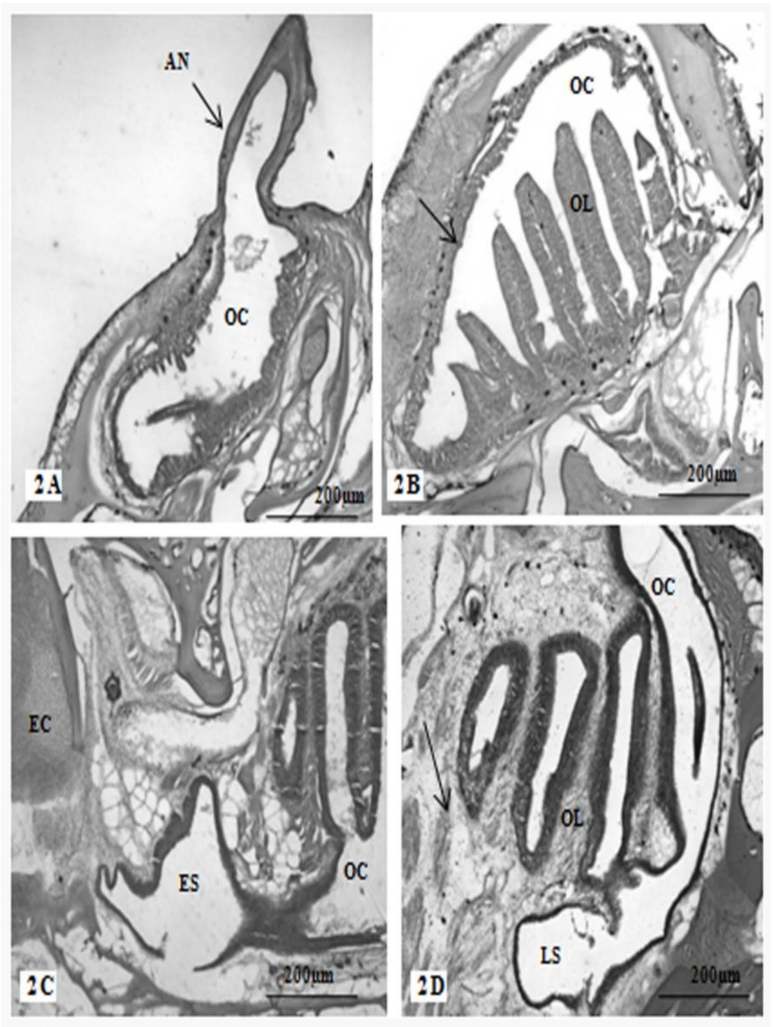


**Fig 2.** Photomicrographs of serial histological section of olfactory cavity of *Anabas testudineus.* (A) Anterior nostril (AN) at the rostral end of olfactory cavity. (B) The epithelium (arrow) borders the olfactory cavity raises into several olfactory lamella (OL). (C) The ethmoidal sac (ES) at the dorsocaudal region of olfactory cavity toward the ethmoid cartilage (EC). (D) The lacrimal sac (LS) at the ventrocaudal end of olfactory cavity in a more caudal section. Arrow shows olfactory nerve fiber. Olfactory cavity (OC).

were anaesthetized by MS-222 (*Tricaine methane-sulfonate,* dose-100 mg/L). The dissection of dorsal head region of *A. testudineus* was done to locate the olfactory organ, subsequently fixed (aqueous Bouin's solution) and then examined under binocular light microscope.

**Microanatomy of olfactory cavity**

The head of *A. testudineus* body length (5.5 cm to 7.6 cm) and weight (2.3g to 6.7g) was decapacitated and immediately fixed in aqueous Bouin's solution for 48 hours. The samples were washed in 70% ethanol, followed by decalcified in 10% aqueous solution of di-sodium EDTA by changing the solution periodically. The samples were then cryoprotected at 4°C for 24 hours in 15% sucrose solution of 0.1 M phosfate buffer (pH-7.4) and subsequently in 30% sucrose of the same buffer for overnight respectively. Serial longitudinal sections of the head (thickness: 7-10µm) were cut in a cryostat microtome (Leica CM 1850) at -22°C and glued onto gelatin coated

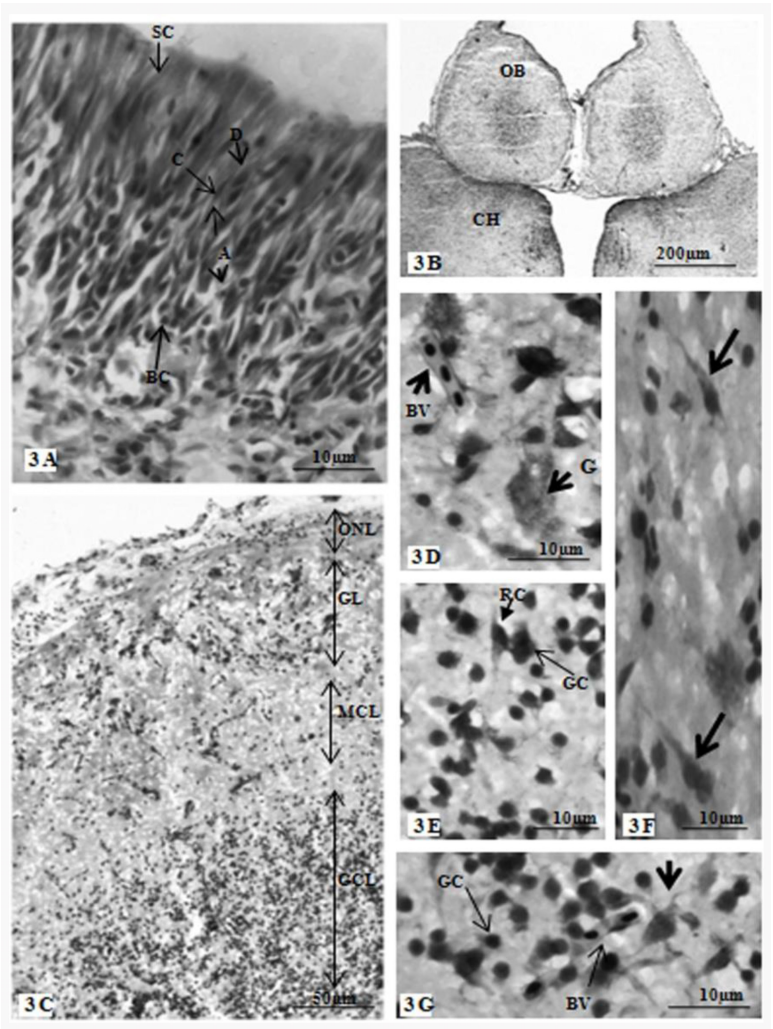


**Fig. 3**. Photomicrographs of olfactory epithelium and olfactory bulb of *Anabas testudineus* (A) Olfactory epithelium showing apical supporting cell (SC), middle olfactory sensory neurons with dendron (D), cell body (C), axon (A) and basal cells (BC) in lower part. (B) Longitudinal section of olfactory bulb (OB) along with cerebral hemisphere (CH). (C) Olfactory bulb showing four layers viz., olfactory nerve layer (ONL), glomerular layer (GL), mitral cell layer (MCL), granular cell layer (GCL). (D) Magnified part of glomerular layer shows glomeruli (G) and blood vessel (BV). (E) Arrow shows ruffed cell (RC) makes contact with granule cell (GC). (F) Arrows indicate mitral cell in the mitral cell layer. (G) Magnified part of granular cell layer showing granule cell (GC), glial cell (arrow) and blood vessel (BV).

microscope slides. The sections were stained with Hematoxylin-Eosin and examined under Trinocular Microscope (Leica DM 3000) with image analyzing software Leica Application Suit (LAS V4.1).

**Histology of olfactory lamellae and olfactory bulb**

The olfactory rosette and the olfactory bulb of *A. testudineus* were dissected out from dorsal head region and immediately fixed in 10% neutral buffered formalin (NBF) for 2 hours. Tissues were cryoprotected in 15% sucrose solution of phosfate buffer (0.1 M, pH-7.4) for 3-4 hours and subsequently in 30% sucrose of the same buffer at 4°C for overnight respectively. Longitudinal sections of the olfactory lamellae (thickness: 4-5µm) and olfactory bulb with brain (thickness: 7-10µm) were cut in a cryostat microtome (Leica CM 1850) at -22°C and were subsequently stained by

Hematoxylin-Eosin and Nissl stain respectively. The samples were visualized under Trinocular Microscope (Leica DM 3000) with a microscope imaging software Leica Application Suit (LAS V4.1).

## RESULTS AND DISCUSSION

The olfactory apparatus of *A. testudineus* is located in dorsal region of head anterior to eyes (Fig. 1A). It is a paired structure comprised of anterior and posterior nostrils, olfactory cavities, olfactory rosettes, accessory nasal sacs, olfactory nerves and olfactory bulbs terminate on the brain (Figs.1A-C). Micro anatomically, the olfactory cavity is roughly oval in shape with an anterior nostril at its rostral end (Figs. 2A, B). The epithelium lines the cavity is folded at the base to form several olfactory lamellae of different sizes (Fig. 2B). Two accessory nasal sacs i.e. ethmoidal and lacrimal sacs are noted. The ethmoidal sac is slightly elongated, appears bulbous in the middle and becomes narrow toward the posterior end (Fig. 2C). It remains connected with olfactory cavity at dorsocaudal region near to ethmoid cartilage (Fig. 2C). The lacrimal sac is occupied opposite to ethmoidal sac but at the ventrocaudal region of olfactory cavity toward lacrimal side (Fig. 2D). The lacrimal sac is small and roundish compare to ethmoidal sac (Fig. 2D). The accessory nasal sacs assist in water ventilation through the olfactory cavity by the pumping mechanism (Døving *et al.* 1977) that bring odorants toward the epithelium. The pumping action of nasal sacs (sniffing) is accomplished by the movement of skeletal elements during opening and closing of the mouth (Nevitt 1991). The olfactory epithelium is pseudostratified and divisible into three zones from apical to basal end with olfactory sensory neurons (OSNs), columnar supporting cells, basal cells (Fig. 3A). The supporting cells and the proximal projection of OSNs are located in upper region of olfactory epithelium (Fig. 3A). The cell body of OSNs is mostly prevalent at the middle as well as in lower third part of epithelium (Fig. 3A). Basal cells are round in shape and lie at the underline region of epithelium (Fig. 3A). The OSNs are thin and bipolar in appearances with apical dendron, cell body and terminal axon (Fig. 3A). Hara (1992) suggested that the OSNs in fish respond to diverse molecules like amino acids, some peptides, bile acids. The supporting cells are protective in function and found to ensheath the sensory receptor cells. The basal cells are assumed to be the progenitors involved in the continuous replacement of the sensory cells (Schwob *et al*. 2017). The olfactory nerve (ON) is raised from the base of olfactory rosette and its distal end is swollen to form a 'sessile' olfactory bulb (Fig.1A). The olfactory bulbs (OBs) are prominent ovoid structure placed at the proximal end of cerebrum (Fig. 3B). Microanatomically, OB is roughly distinguished into four layers arranged concentrically viz., 1. The outermost layer is olfactory nerve layer (ONL), 2. Glomerular layer (GL) contains glomeruli, 3. Mitral cell layer (MCL) includes mitral cells that appear to be scattered in a diffuse manner, 4. The innermost part is granular cell layer (GCL) characterized by numerous, small nucleated granular cells and few of glial astrocyte (Figs. 3C- G). The 'sessile' OBs have also been reported in *C. gachua* (Baile and Patle 2011) and *O. striatus* (Khaparde *et al.* 2012). However, in *L. rohita* (Bhute *et al.* 2007) the OB is stalk like or 'pedunculated', closed to olfactory rosette and connected to a short olfactory nerve toward the brain. In *A. testudineus,* the glomeruli are demarcated as spherical masses (Fig. 3D). The OSNs with a particular odorant receptor are converged their axons into the glomeruli of OB (Mombaerts *et al*. 1996), where the information is first processed and then transferred to other neurons. The mitral cells were specialized structure received both the excitatory input from the axons of OSNs and inhibitory input from granule cells (Isaacson and Strowbridge 1998), that released γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter. The ruffed cells have been observed in few fishes (Kosaka and Hama 1980, Zippel *et al.* 2000) but absent in mammals. In *A. testudineus,* the ruffed cells are specially located in the granular cell layer and made contact with granule cells (Fig. 3E). In Gold fish, *C. auratus* (Kosaka 1980), the ruffed cell synapses were mostly formed with granule cell dendrites. The ruffed cells and mitral cells were opposite in polarity i.e. the excitation of mitral cells activity inhibited the ruffed cells activity via the activation of lateral inhibition by granule cells (Zippel 1999). In *A. testudineus,* the large number of granule cells in OB indicates the inhibitory signals are significant. The coexistence of excitatory and inhibitory signals from different neurons in a balance state is essential for the olfactory discrimination in OB (Lledo *et al.*

2008). The glial cells are noted in close proximity of granule cells and vascular element (Fig. 3G). De Castro (1951) suggested that neuroglial cells in the olfactory bulb might release neuroactive substances and participated in neural transmission. The present study shows the olfactory sensory neurons together with other olfactory cellular components reflects the specimen possesses a good sense of olfaction. So, it could provide a platform for future ultrastructural studies to map the neuroanatomy of olfaction in fish, *Anabas testudineus*.

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