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# SURFACE CELLULAR DETAILS OF THE OLFACTORY ROSETTE: A SCANNING ELECTRON MICROSCOPIC (SEM) STUDY IN HILL STREAM CYPRINIDIAE, GARRA MULLYA (SYKES)

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## ABSTRACT

Electron microscopic study is an important tool to characterize the fine structure of olfactory cells to understand the role of olfactory system at cellular level. In this work we are reporting the structure of olfactory system in *G. mullya* by electron microscopy. Olfactory rosette in this fish is oval in shape and has a central raphe from which radiate the lamellae on both the sides in parallel fashion. The surface epithelium of median raphe having the stratified epithelial cells (SEC) which provided with inconspicuous and conspicuous labyrinthine pattern micro-ridges, opening of mucous secreting goblet cells (GC) and long ciliated non sensory cells (cNSC) in the bundles. Each lamella is composed of two distinct regions, sensory and non sensory. Sensory epithelium is composed of abundant micro-villous receptor cells (MRC), ciliated olfactory receptor cells (cORC), mucous secreting goblet cells (GC), small rod like supporting cells (SC), stratified epithelial cells (SEC) and long ciliated non-sensory cells (cNSC). Transitional region of apical sensory and non sensory region shows stratified epithelial cells (SEC), supporting cells, micro-villous receptor cells and many mucous secreting goblet cells opening. Arrangement of microridges may protect the sensory epithelium from mechanical injuries and help in holding the mucus over the epithelium.

**Key words:** - Olfactory rosette; cORC; cNSC; SEM; Hill-stream fish; *Garra mullya*

## INTRODUCTION :

Hill streams are unique aquatic ecosystems characterized by shallow, narrow channels, low temperature high altitude, different types of substratum and high current of water. In the course of evolution, nature has provided hill stream fishes with unique anchorage system to combat swift, shooting and turbulent water flow (Ojha and Singh, 1992) to successfully adapt to this unique environment. Strength of the current considerably influences the fauna. Although, the rate of water flow varies in different seasons, it is always much higher than in the rivers and streams of plain. Fishes therefore have developed adhesive organs to withstand the rapid flow of water. Fishes inhabiting hill streams can be conveniently divided into two groups. Members of one group are temporary inhabitants of the hill streams and migrate up only at certain periods of their life specifically for spawning purposes. These species move up by muscular efforts and do not exhibit special modifications. Members of the other group live permanently in the rivers and streams of hills and many of them posesses some adaptive features. These modifications are chiefly manifested in the form of adhesive structures usually located at anterior end and on the fins (Arunkumar et al., 1990).

All hill stream fishes are necessarily bottom-living forms, their head and bodies are much flattened and in highly specialized species of Bornean sucker, Balitora, Glyptosternum, Glyptothorax and Pseudoecheneis, their body becomes leaf like. Members of single genus *Garra* show all possible gradations in the shape of their bodies. While those living in the ponds and tanks have more or less cylindrical body, those living in rapid streams have distinct flattened body. In Species like *Garra abhoyai* and *G. rossicus,* dorsal surface in-front of the dorsal fin is smooth due to the absence of scales. *G. mullya* has very well developed cycloid scales over its body. Thorax on the ventral surface is scale less and smooth (Saxena, 1959).

Organs responsible for detection of chemicals in water are located on the exposed body surface of the fish. Common chemical sense was originally named by Parker way back in 1912. Chemoreception mainly depends on the sense of taste (gustation) and on smell (olfaction). These are the major pathways for detection and identification of chemical stimuli in the environment (Hara, 1994).

Olfaction is accomplished principally by the stimulation of receptor cells on neuroepithelial surface of the olfactory organ. It is innervated by the olfactory nerve (Hansen and Zeiske, 1998). Olfactory signals and cues serve a crucial role for several life functions in fish such as migration, communication, feeding, schooling, defense, and reproduction (Wilson, 2004; Camacho et al., 2010). Sensing the chemical environment is critical for all organisms. Diverse animals from insects to mammals utilize highly organized olfactory system to detect, encode and process chemo-stimuli that may carry important information critical for health, survival, social interactions and reproduction (Marc Spehr and Munger, 2009). The structural and functional link between the olfactory and reproductory systems; puberty related changes in olfactory epithelium are also reported (Schreibman et al., 1984).

In teleosts, principal organ is olfactory system consists of a pair of olfactory rosette, olfactory bulbs, olfactory tracts and olfactory lobes.

The olfactory receptor cells are located in the olfactory epithelium which covers much of the surface of olfactory rosette, a structure found within the olfactory chambers on the fish rostrum. Although the size and shape of the rosette varies greatly across different species, in most instances it has a longitudinal ridge (raphe) with two rows of olfactory lamellae radiating from it, thus increasing its surface area enormously (Zeiske et al., 1992).

Olfactory epithelium is a complex tissue comprising sensory and non-sensory epithelia. Sensory epithelium is located on the raphe and central region of the olfactory lamella in majority of the species. The sensory epithelium is present in patches in some species also (Yamamoto, 1992).

Electron microscopic study is an important tool to characterize the fine structure of olfactory cells to understand the role of olfactory system at cellular level. Scanning electron microscopic studies of the olfactory organ and bulb are carried out by some investigators (Hara, 1975; Ichikawa, 1976; Ichikawa and Ueda, 1977; Kosaka and Hama, 1979 a, b; Zeiskie et al., 1979; Kleerekoper, 1982; Schreibman et al., 1986; Hansen and Zeiske, 1998).

**MATERIAL & METHODS**

***Scanning Electron Microscopy (SEM)***

Scanning Electron Microscopy (SEM) was carried out at the SEM facility centre of division of Entomology, Indian Agriculture Research Institute Pusa, New Delhi and Department of Metallurgy, VNIT, Nagpur.

Adult female fishes were anaesthetized with 2% paraldehyde solution. The olfactory resettes were removed and fixed without any loss of the time in ice cold glutaraldehyde (PH 7.2) containing paraformaldehyde for 24 hours. After fixation, tissue was washed 3-4 times in cold PBS (PH 7.45) for 30 min. interval and dehydrated in series of acetone (from 30% acetone to 100% acetone). Tissue was dried with the help of critical point drier, coated on stub and observed under the Scanning Electron Microscope.

**RESULTS AND DISCUSSION:**

In *Garra mullya*, olfactory rosette is present in olfactory chamber on the snout. Olfactory chamber allows water in through incurrent nostrils and is expelled through excurrent nostrils (Fig-01 & 02).

***Scanning Electron Microscopy:***

In *G. mullya,* olfactory rosette is oval in shape and consists rows of linguiform lamellae on each side of median raphe (Fig-03 & 04).

The surface epithelium of median raphe having the stratified epithelial cells (SEC) which provided with inconspicuous and conspicuous labyrinthine pattern micro-ridges, opening of mucous secreting goblet cells (GC) and long ciliated non sensory cells (cNSC) in the bundles. Mucin droplets are found over the SEC (Fig-05). Each lamella is composed of two distinct regions, sensory and non sensory (Fig -06). Olfactory lamellae are radiating outward from central raphe. They also exhibit stratified epithelium with intermittent clusters of ciliated processes (Fig-07). Sensory epithelium is composed of abundant micro-villous receptor cells (MRC), ciliated olfactory receptor cells (cORC), scanty mucous secreting goblet cells (GC), small rod like supporting cells (SC), stratified epithelial cells (SEC) and long ciliated non-sensory cells (cNSC) (Fig-08,10). Apical surface of MRC is broad and consists of numerous microvilli (Fig-09), while cORC are limited in number raised in small round knobs and provided with four cilia (Fig-10). Apical surface of supporting cells having small rod like projections intermingling with microvillous cells, olfactory receptor cells and ciliated non sensory cells(Fig-09,10). Apical surface of goblet cells opening are seen and stratified epithelial cells which are flat and provided with fingerprint like micro ridges while ciliated non-sensory cells have dense bundles found in patches (Fig-08,09). Apical non sensory epithelium shows a dense mat of cilia with long ciliated non sensory cells (Fig -11). Transitional region of apical sensory and non sensory region shows stratified epithelial cells (SEC), supporting cells, micro-villous receptor cells and many mucous secreting goblet cells opening (Fiig-12 &13).

Olfaction plays a major role in fish as the olfactory organ interact with the environment and involving in various life activities include feeding, prey detection sex recognition, migration, reproduction and social interaction.

In the studied fish *Garra mullya*, the olfactory organs are paired situated dorsolaterally on the snout anterior to the eyes. Each organ present in the olfactory chamber that has two separate apertures through which water enters and leaves through it. Similar structure as also observed in *L. rohita* (Bhute and Baile, 2007); *N. notopterus* (Patle and Baile, 2014); red tail shark, *Epalzeorhynchos bicolar* (Mokhtar et al., 2014); and in other teleosts (Zeiske et al., 1992). In *G. mullya* olfactory rosette is oval in shape, has a central raphe from which radiate the lamellae on both sides in parallel fashion. Such similarities are observed in *L. rohita* (Bhute and Baile, 2007); *Catla catla* (Kumari, 2008); *Puntius sarana*

(Ghosh and chakrabarti, 2014, JR Somatkar, DS Dabhade and HV Wanjari,2017).

In *G. mullya,* olfactory epithelium is folded to form the lamellae. Each lamella is crescentic in shape and bears a linguiform process along its concave margin as in *P. sarana* (Ghosh and Chakrabarti, 2014); *Catla catla* (Kumari, 2008); *Epalzeorhy nchos bicolar* (Mokhtar et al., 2014). Foldings on the lamellae of olfactory epithelium increases the surface area of the epithelium as well as the sensitivity and efficiency of the olfactory system (Zeiske et al., 1979; Ghosh and Chakrabarti, 2014; Patle and Baile, 2014 and Mokhtar et al., 2014). These views are supported by our study as olfactory rosette in *G. mullya* is a multi-lamellar rosette comprising (18-26) number of lamellae which may provide more surface area for the binding of odorants and sensory activity.

In *G. mullya*, scanning electron microscopiy reveral the surface study of olfactory rosette. The surface epithelium of median raphe contained stratified epithelial cells with labyrinth pattern of microridges and few goblet cells. The arrangement of microridges may protect the sensory epithelium from mechanical injuries and help in holding the mucus over the epithelium. Goblet cells secrete mucin droplets probably protect olfactory epithelium from external injuries and allows smooth flow of water through the olfactory chamber. Similar findings recorded by (Ghosh and Chakrabarti, 2014) in *Puntius sarana* and (Mokhtar et al., 2014) in *Epalzeorhynchos bicolar*. The sensory epithelial surface occupied broad microvillous receptor cells, ciliated olfactory cells intermingled in different proportions. Microvillous receptor cells on lamellae may have significant role in reproduction. In the olfactory epithelium of *Cirhinus mrigala* the presence of microvillous receptor cells played a significant role in the regulation of reproduction (Biju et al., 2003; Chakrabarti et al., 2011). In *Sinocyclocheilus jii* (Waryani et al., 2013). In *S. argus* (Chakrabarti et al., 2011) Micro villous receptor cells might from a different olfactory transduction mechanism for pheromones or amino acids. Bhute and Baile (2007) also advocated that the receptor neurons perceive and process signals of pheromone, which is important step in the breeding pattern of *L. rohita.*

Ciliated olfactory receptor cells are found limited in number and form part of the olfactory transduction mechanism are stimulated by odour-bearing substances and also enable the fish to detect food. They are also responsible for detection of bile salts and amino acid odorants. Similar views are observed by (Zeiske et al, 2003., Chakrabarti et al., 2010; Ghosh and Chakrabarti, 2009). Mucous secreting goblet cells are on both sensory and non sensory surface of epithelium in *G. mullya.* Secreted mucin from the mucous cells probably helps for coagulating microscopic debris and there by keeps the receptor ready for new stimuli. This is confirmity with the findings of Bandyopadhyay and Dutta (1998) in *H. fossilis* and Ghosh and Chakrabarti (2009) in *Wallago attu.* Adequate ventilation is necessory to bring the odorants in the olfactory chamber for perceiving the chemical signals (Belanger et al., 2003). Ventilation of the olfactory chamber takes place by synchronous beating of cilia of ciliated-nonsensory cells (Hara, 1993). Since the raphe surface and sensory epithelium in *G. mullya* covered in the bundles of cilia, also is covered with a dense mat of cilia, probably the ventilation of olfactory chamber in this fish is achieved due to the beating action of cilia of ciliated non sensory cells as in Patle and Baile (2014). In the transitional zone of sensory and non sensory epithelium of *G.mullya* consists of stratified epithelial cells provided with unbranched linearly arranged microridges on their apical surface that help in holding mucous film over the epithelium and in protecting the sensory receptor cells from mechanical injuries. The goblet cells are distributed between the stratified epithelial cells of the sensory and non sensory epithelium. The mucus covering the olfactory lamellae constitutes an important medium in which odourants are diffused as in *L. bata* (Ghosh and Chakrabarti, 2011).

**CONCLUSION:**

Conclusively, olfactory rosette in this fish is oval in shape and has a central raphe from which radiate the lamellae on both the sides in parallel fashion. Under scanning electron microscope, the surface of olfactory rosette shows the following : microvillous olfactory receptor cells and ciliated olfactory receptor cells, ciliated non sensory cells in bundles and mats of cilia, mucous secreting goblet cells opening with mucin droplets, small rod like supporting cells and stratified epithelial cells with finger like microridges. Arrangement of microridges may protect the sensory epithelium from mechanical injuries and help in holding the mucus over the epithelium.

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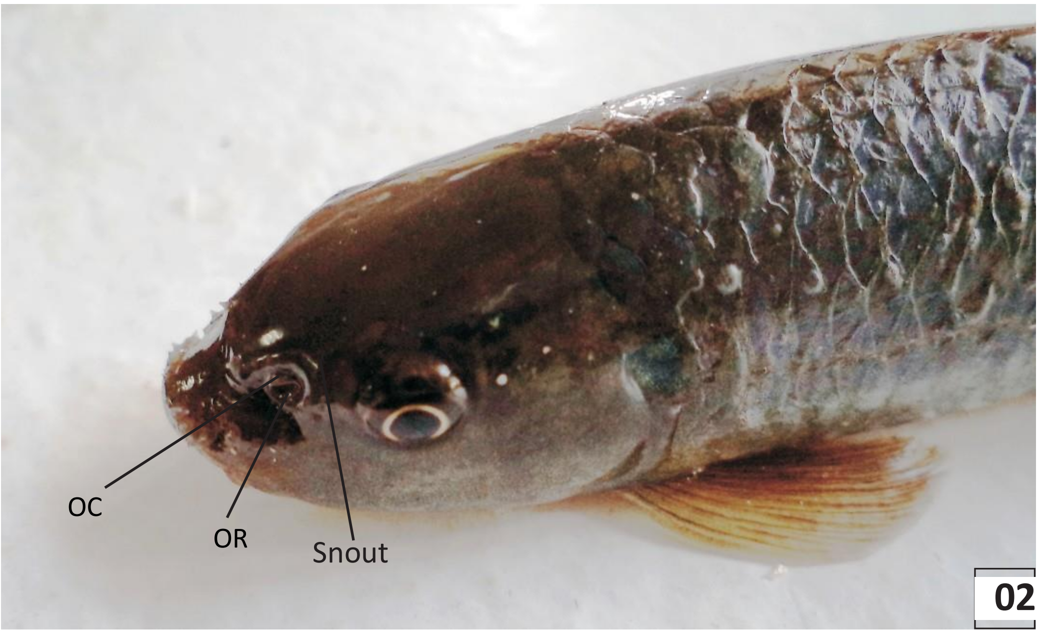


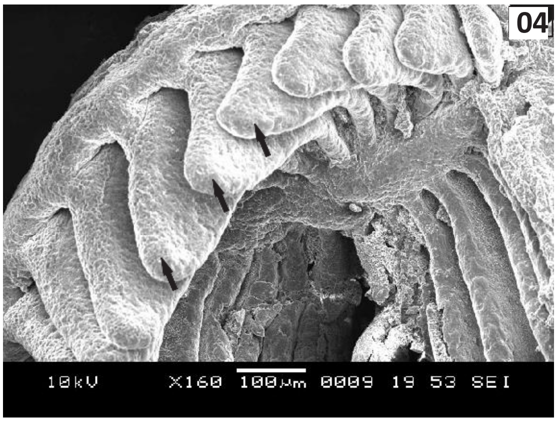
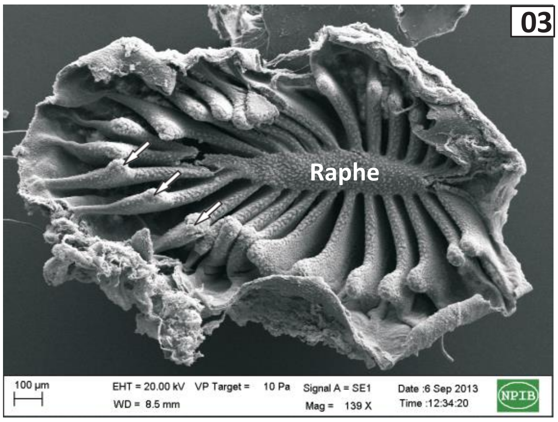
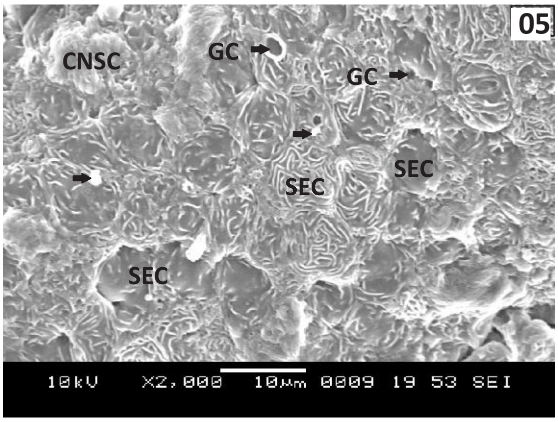
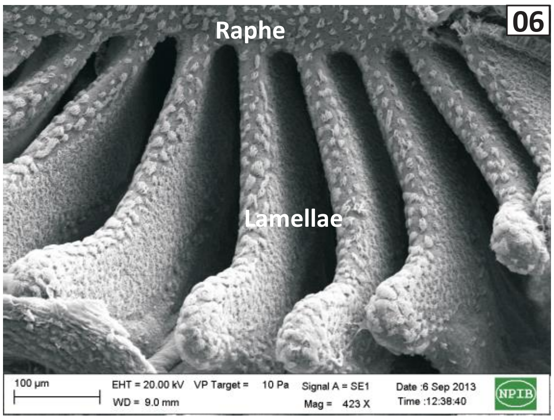
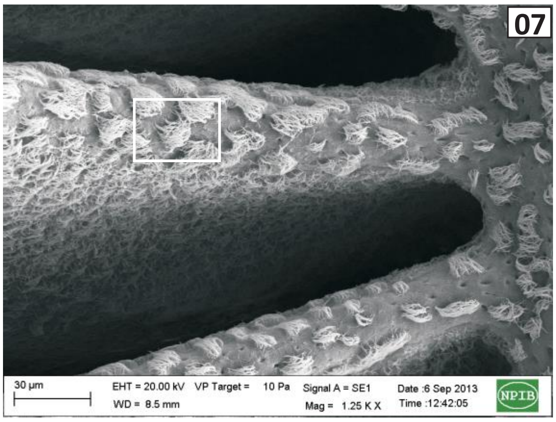



**Fig. 01:** Lateral view of fish *Garra mullya* (Sykes). **Fig. 02:** Lateral view showing olfactory rosette (OR) in the olfactory chamber (OC).



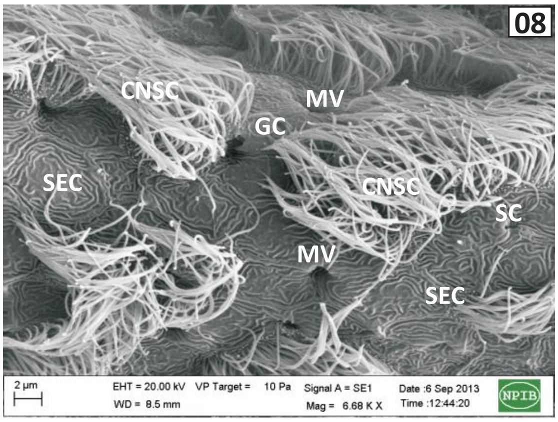



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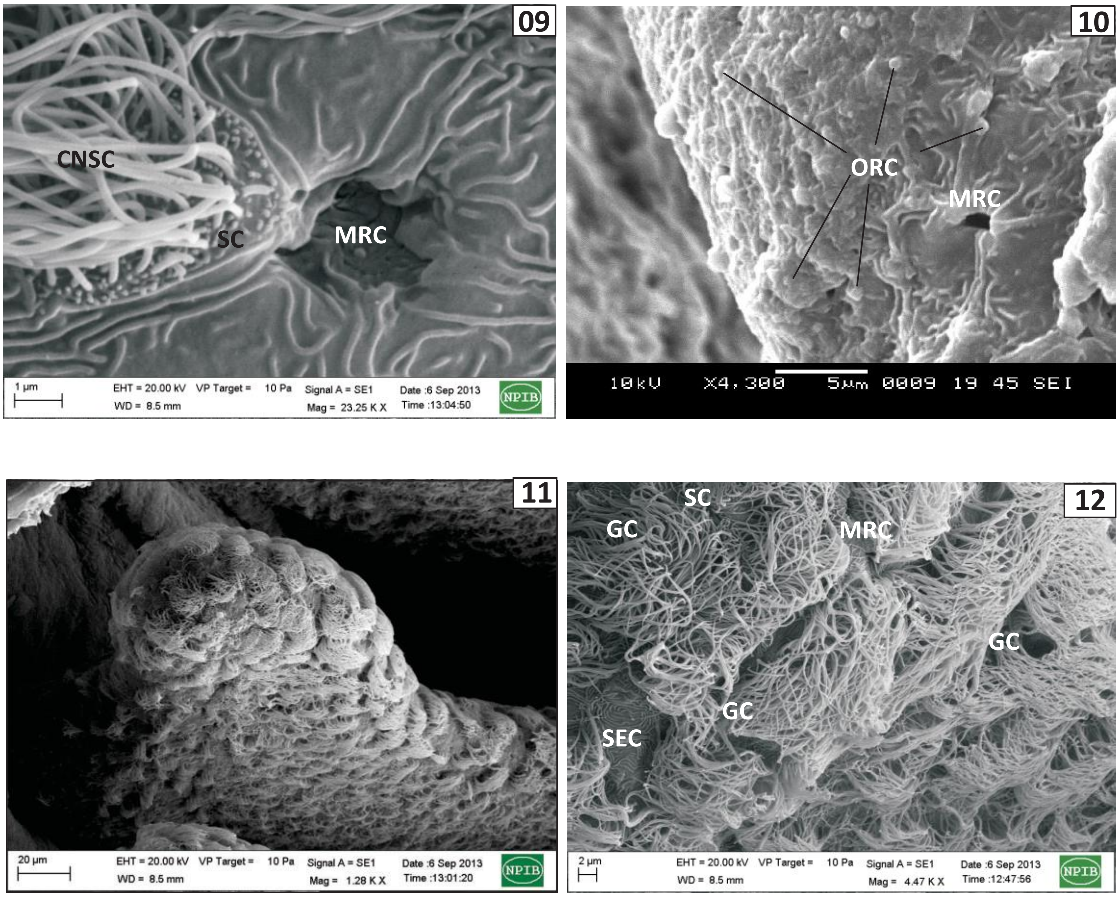
**Fig. 03 & 04:** Scanning electron micrograph (SEM) of olfactory epithelium (OE) of *Garra mullya.* oval shaped olfactory resette (OR) exhibits olfactory lamellae (OL) radiating from central median raphe (MR). Arrows indicate linguiform processes. **Fig. 05:** Scanning electron micrograph (SEM) of magnified median raphe exhibiting stratified epithelial cells (STC), opening of goblet cells (GC) and long ciliated non sensory cell (CNSC) in the bundles. **Note:** Mucin droplets are found over the SEC. **Fig. 06:** SEM of elongated olfactory lamellae (OL) shows basal sensory (OSA) and apical non-sensory areas (ONSA). **Fig. 07:** SEM of olfactory epithelium (OE) spread between median raphe (MR) with olfactory lamellae (OL) displays stratified epithelial cells (SEC) with intermittant patches of ciliated processes. Small rod like supporting cells (SC) are vesible. **Fig. 08:** SEM of magnified olfactory sensory area (OSA) shows SEC, microvillus receptor cells (MV), mucus secreting goblet cell (GC), rod like supporting cell (SC) and long ciliated non sensory cells (CNSC).



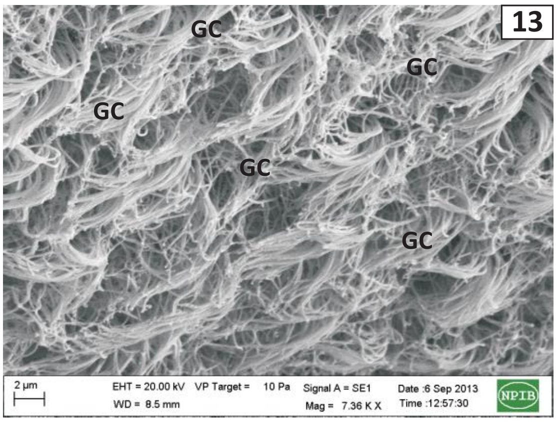



**Fig. 09:** SEM of further magnified olfactory sensory area (OSA) shows microvillus receptor cells (MV), SC and CNSC. **Fig. 10:** SEM of further magnified olfactory sensory area (OSA) shows ciliated receptor cells (CRC) and microvillus receptor cell (MV). **Fig. 11:** SEM of apical olfactory non-sensory area (ONSA) shows the fur of abundent long ciliated non sensory cells (CNSC). **Fig. 12:** SEM of magnified transitional region of olfactory sensory and non-sensory areas (OSA and ONSA) shows SEC, SC, MV and GC with abundent CNSC. **Fig. 13:** SEM of magnified olfactory non-sensory area (ONSA) shows

**a: Stratiomyidae).**