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Comparative scanning electron microscopic studies of the olfactory epithelium in two bottom feeder fishes *Anguilla vulgaris & Ctenopharyngodon idella*

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***ABSTRACT*: Our study illustrates the ultrastructure of the olfactory rosette of the Anguilla vulgaris and Ctenopharyngodon idella by using Scanning Electron Microscope. Herein, the olfactory organs are represented by two olfactory rosettes lying in two nasal chambers, one on each side of fish snout. In Anguilla vulgaris, each chamber's olfactory rosette is elongatted. There were 45 to 48 lamellae in the olfactory rosette. The sensory epithelium covers the lateral surface of the olfactory lamella, while the non-sensory region is mostly restricted to the lamellae's margins. Ciliated, micro villous olfactory receptor cells, crypt, and rodlet cells were found in the sensory epithelium. Supporting, basal and micro villous cells were also observed. Rodlet cells and stratified epithelium made up the non-sensory epithelial cells. *A. vulgaris* is a macrosmatic "nose-fish" whose eating behavior is heavily influenced by olfaction. The SEM in *Ctenopharyngodon idella* revealed that each olfactory rosette was elongated and made up of 48-50 foliar lamellae transversely arranged on both sides of a narrow median raphe. Alongside, the magnitude of lamellae differs in relation to their location on the raphe, since the larger are in the middle whereas their dimensions gradually reduced towards both ends of the rosette indicating that the number and magnitude of lamellae increase as the fish grow.**

KEYWORDS: **Olfactory rosette, olfactory epithelium, Olfactory receptors, *Anguilla vulgaris, Ctenopharyngodon idella*, SEM.**

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I. INTRODUCTION

Chemosensory processes like olfaction and gustation help fish survive. Olfaction is important in fish life because it affects a variety of activities including food search, migration, predator avoidance, and reproduction **(Hara, 1975**). Fish that live in an aquatic environment with little or no light have a highly developed chemosensory system. Olfaction in fish is of particular interest since the olfactory epithelium serves as chemical receptors not only for the sense of smell, but also for direction, feeding, reproduction, and communicating information about the surrounding environment (**Hara, 1993**).Water containing dissolved compounds passes from the nose to the olfactory organ of fish, exposing the olfactory receptor neurons to water contaminants directly. In fishes, olfaction is the highly developed sense of all vertebrates (**Kleerekoper, 1969**). The senses of smell, touch, and lateral line organs are used to capture prey, whereas nocturnal fishes use their senses of smell, touch, and lateral line organs. Fish with more olfactory lamellae display behavioural responses to olfactory stimulus, while nose fish do not (macrosmatic). However, fish with fewer lamellae respond less to olfaction and more to sight, such as eye fish (macrosmatic). Eye-nose fishes are midway between macrosmatic and mediosmatic fishes. The structure of the olfactory organs in numerous teleostean fishes has lately been extensively examined in the literature (**El-Attar *et al*., 2010; Kumari, 2008; Zeiske *et al*., 2009 and Charkrabari and Gosh, 2010**). The form, number, and arrangement of the olfactory lamellae, the distribution of the sensory and non-sensory epithelium, and the abundance of distinct receptor cell types in different teleosts have all been examined by many authors. The olfactory epithelium's folding or lamellae increase the epithelium's surface area, as well as the olfactory organ's sensitivity and efficacy (**Zeiske *et al*. 1976**). The sensitive (sensory) and non-sensory epithelium of the olfactory epithelium can be distinguished.

In teleosts, two types of olfactory receptor cells with cilia or microvilli on their apical surface are common (**Farbman 2000**), and a third type of olfactory receptor cell, crypt cell, has been described in a variety of fishes and is thought to be a common feature in bony fish (**Farbman 2000, Hansen and Finger, 2000**).

Anguilla vulgaris is one of the true freshwater eels, belonging to the order: Anguilliformes, family Anguillidae, have only one genus, *Anguilla.* All of them are migratory, found in marine, brackish, and fresh water in the tropical and temperate zones. They are bottom dwellers and hide in burrows, crevices, plant masses, and other types of obstacles **(Nandlal, 2005)**. From these nineteen species, only the European (*Anguilla anguilla*), North American (*Anguilla rostrata),* and Asian or Japanese (*Anguilla japonica*) are temperate, having more flexibility in habitat. They display a remarkable similarity in their life history traits since they are catadromous and migrate from freshwater to spawn in deep oceanic waters **(Cresci, 2020).**

Grass carp (*Ctenopharyngodon idella*, Cuvier and Valenciennes) is an bottom dweller herbivorous freshwater fish belonging to the family Cyprinidae. It inhabits backwaters of rivers, lakes, ponds of low levels (12 ppt) of salinity **(Chervinski, 1977).**They have an elongated, tubby and stout body form. Its terminal mouth is slightly inclined with steady non-fleshy lips without barbells. Body color is dark olive to brownish-yellow on the sides, with a white belly and large slightly bordered scales. Despite it tolerates cold water, it flourishes and grows at rapid rates in warm waters and its stocked young individuals of 20 cm long in spring reach over 45 cm by autumn. Grass carp lives 5-9 years, with an apparent life span of approximately 10 years **(Kirk and Socha, 2003)**. Accordingly, it was introduced in many countries around the world including several Asian, European and American countries since 1963 as an effective biological control agent for hydrilla and other aquatic plants **(Sutton *et al*., 2013, Manuel *et al*., 2013)** and so becoming the largest farmed fish globally as a food fish in China **(Subasinghe, 2017).**

II. MATERIALS AND METHODS

Five adult live fish specimens of *A. anguilla* (Family: Anguillidae) were taken from Al- Maadeyah, Edku, El Beheira Governorate. Specimens were measured in lengths ranging from 35 to 54 cm. Five adult live specimens of grass carp, *Ctenopharyngodon idella* (Family: Cyprinidae), measuring about 30±5 cm long had been collected from a private fish farm at EL-Abbasa, Abou Hammad, Sharkia, Egypt . Specimens were brought alive in oxygenated tanks to the laboratory of Experimental Zoology in the Faculty of Science, Zagazig University, Egypt. Fishes dissected for Scanning Microscope observations.

Scanning Electron Microscope (SEM)

Fish specimens were anesthetized immediately then after beheading sacrificed. From the dissected heads, olfactory rosettes were excised out from the nasal chambers and instantly immersed in saline solution in order to get rid of all the traces of stuck-on mucus. After being rinsed by 0.1 M Phosphate buffer, the rosettes were fixed in 2.5% glutaraldehyde of 0.1 M Phosphate buffer (pH 7.4) for 24 hours at 4°C. After fixation the rosettes were rinsed secondly in the same buffer, pH 7.4, for 10 minutes, and post-fixed in 1% OsO4 in 0.1 M Phosphate buffer, pH 7.4, for 2 hours. The lamellae's surface was then washed thoroughly in the same buffer and dehydrated through graded acetone followed by isoamyl acetate. The specimens after being dried to the critical point they were mounted on metal stubs, gold-coated, scanned and examined with a Joel IT200 SEM, affiliated with the Faculty of Science, Alexandria University.

III. RESULTS AND DISCUSSION

In grass carp

The peripheral olfactory organs in grass carp (*Ctenopharyngodon idella)* are pair sets in corresponding pair of nasal chambers located on dorsal-lateral sides of the snout in front of the eyes. They opened to exterior by two nostrils separated by elevated flap, anterior inlet and posterior outlet, through which water get in and out to immerse olfactory organs, rosettes (**Fig. 1.A).** The elongated rosettes of the studied fish, each holds about **48-50** leaf-like lamellae parallel oriented on both side of a narrow median raphe. The magnitude of lamellae was differed in relation to their position in rosette, since they are larger in the middle portion whereas their dimensions gradually reduced towards both ends of the olfactory rosette (**Fig. 1. B, C)**. Indicating that the number and magnitude of lamellae increase as the fish grow and so the olfactory surface epithelium. The olfactory lamellae is lined with sensory and non-sensory epithelium, both are concealed with a mucous layer and rest on a basal lamina. The sensory epithelium exhibits discontinuous distribution patterns, separated regularly by non-sensory epithelium, interspaced irregularly and scattered in islets. (**Fig.1. D, E).**

The sensory epithelium is a columnar pseudo-stratified epithelium and consists of four main cell types: receptor, supporting, mucous and basal cells. The receptor cells are bipolar neurons, their apical dendrite is in the form of elevated hillock, dendritic knob, but the other basal dendrite, the axon, show the way in the proximal direction passing through the basal lamina to aggregate forming nerve bundles. From this elevated hillock (dendritic knob) emitted up to 8 somewhat short thick primary non-motile cilia, ciliated receptor **(Fig.1.H**) or very short plentiful microvilli, microvillous receptor **(Fig.1.I**) or thick compound cilium (rod-tipped receptor), this later cells scattered in-between the other ciliated and microvillous receptor (**Fig.1. I**). The receptor cells are aggregated in separate islands in-between others of non-sensory epithelia of ciliated and non-ciliated supporting cells of motile long cilia or microridges, respectively as well as mucous goblet cells (**Fig.1. F, G, J).**

In *Anguilla*

There are two large, elongated olfactory chambers of *Anguilla vulgaris* are located just behind the eyes, one on each side of the rostrum to accommodate its large, very elongated olfactory organ (rosettes). So the two rounded or slightly oval nostrils of each chamber are remarkably far apart, not as close to each other as in other fish species (Fig. 1A, B).

In the present investigation, the olfactory organ of *Anguilla vulgaris* is symbolised by a pair of elongated, laterally constricted rosette-like structures. They occupy most of the space of the two olfactory chambers, which open externally via an anterior inlet and a posterior outlet for entering and leaving water, respectively **(Figs. 2A, B)**. Each olfactory rosette is made up of almost 90 to 100 flat radial olfactory lamellae close together and positioned perpendicularly in a plane on either side of a long, narrow support median raphe.

It is recognised that the inner side of the lamellae is adhered to the raphe while the outer comes into contact with the olfactory chamber walls. The middle lamellae of the rosette are somewhat larger and broader, with an outer concave edge Linguiform process, but they are diminished in size towards the posterior rosette's end **(Fig. 2C).** This indicates new lamellae may be added by time at the posterior ends of the rosettes, elucidating why the number of lamellae increases with the fish's age and length.

Each lamella has been walled by olfactory epithelia, which comprises a discontinuous non-sensory and sensory epithelial area. The non-sensory one is mostly restricted to the lamellae's margin, but the sensory area is apparent on the median side surfaces of each lamella **(Fig. 2D)**.The discontinuous non-sensory epithelial area is composed of indifferent stratified epithelial cells with microridges and a compact assemblage of supporting cells either of long cilia, ciliated non-sensory or non-ciliated sustentacular supporting cells interposed with mucus secretory goblet cells. So, this non-sensory area is regarded as the ciliary zone and glandular zone, respectively. The glandular zone of the non-sensory epithelial area extends around the periphery of the lamella. It is mainly built up of indifferent epithelial cells with overfull microridges mostly arranged in concentric ridges as a finger print pattern, in addition to many wide mucous cell pores (**Figs. 2 E, F).**

However, the ciliary zone of the non-sensory epithelial area is built up of ciliated non-sensory cells with a dense coat of tufted long motile cilia that cover the majority of the lamellar periphery side surfaces. These long cilia are commonly inclined in the same direction, indicating their role in water circulation in the inter-lamellar spaces. This ciliary zone is mostly distributed throughout the indifferent epithelium as a minute ciliary island or at the edge of the glandular zone bordering it from the sensory epithelium area (**Figs. 2 E, F).**

According to SEM, the free surface of the olfactory epithelium is in between receptor and supporting cells, with prominent longitudinal folds leaving long furrows in between. This represents the apical surface of the scarcely found labyrinth cells (**Fig. 2L).** The sensory epithelial area comprises mainly olfactory receptor cells, supporting cells, and a few mucus secretory goblet cells. Concerning the receptor cells, they are differentiated on the basis of their free terminal dendrites into three specific bipolar neurons: ciliated, microvillous, and scarcely rod-tipped.

Their dendritic lumps protrude slightly above the surface of the neighbouring cells, forming a terminal swelling, or dendritic knob. From each ciliated receptor cell, a varying number (4–6) of somewhat short and relatively thick primary non-motile cilia radially emitted from their dendritic knob (**Fig. 2 J, K).** However, the microvillous receptor cells release plentiful compressed microvilli-like short projections from their relatively elevated hump (**Fig. 2 I, J, and L).** Nonetheless rod-tipped cells are occasionally seen, enlarged as compound tips at the epithelium's free surface forming a middle dendritic knob that emerges out of a single thick, prominent rod-like structure of varied lengths. It is much thicker than a normal cilium of neighbouring ciliated cells and tapers progressively on the epithelial surface into the olfactory space (**Fig. 2 L).**

IV –DISCUSSION

Olfaction is an actual chemoreception plays a significant role in fishes for detecting the odoriferous substances in the aquatic ecosystem for locating food and facilitates other life activities such as, homing, reproduction and predator avoidance **(Kasumyan, 2004, Chakrabarti and Ghosh, 2011).** Grass carp (*Ctenopharyngodon idella*) possessing good sense of smell able to detect odor with two of olfactory organs (rosettes) accommodating in the corresponding olfactory chambers and connecting to the brain by the means of olfactory nerve tracts. Water bearing odorants molecules enters and leaves olfactory chambers through two nares, inlet and outlet, by the aid of forward progression of fish and activity of long cilia present in the epithelium lining the two nares as also detecting early in *Notopterus notopterus* **(Goel, 1978)**. The elongated oval-shaped rosettes of grass carp, each bears about 48-50 leaf-like lamellae on both side of a median raphe. This is very similar to the cyprinoid, *Epalzeorhynchos bicolor,* having also oval-shaped rosettes of 45-48 lamellae each and fed mainly too on plant matter and small crustaceans **(Mokhtar and Abd-Elhafeez, 2014).** In *Anguilla vulgaris* among the most crucial senses for long-distance communication is smell, especially in dimly lit areas. The fish olfactory organs are skilled at detecting water-soluble compounds for food finding, predator avoidance, social interaction, reproductive synchrony, and the pattern and direction of different migrations **(Aicardi *et al*., 2022).**

The present study aims to illustrate anatomical and ultrastructural features of the peripheral olfactory organs of a very popular and interested two fish species one of them is *Anguilla vulgaris* .It is composed of two wide olfactory chambers, each with two specialised far-away external olfactory nares, but is not connected to the buccal cavity and so performs only olfactory functions, unlike the air-breathing vertebrates, which perform both olfactory and respiratory functions **(Cox, 2008).** The two external nares, inlet and outlet, even when the fish is not actively swimming, its olfactory rosettes are in direct contact with an endless, massive volume of water that carries odorant molecules to the olfactory receptors. **(Aicardi *et al.*, 2022).**

The size, shape, and arrangement of the olfactory organs (rosettes) and their constituting olfactory lamellae are varied considerably in different fish species**.** *Anguilla vulgaris* has a keen sense of smell; it is a macrosmatic fish ''nose-fishes" since its olfactory surface area is about 59.9% of the eye retinal surface area **(Atta, 2013)**. This finding is in accordance with what that detected in *Hypophthalmichthys molitrix* **(El-Attar and Al-Zahaby, 2010).**

These multi-lamellar rosettes, save a wide surface area of olfactory epithelium, consequently the sensitivities and efficiency of the fish olfactory organ **(Cox, 2008) .** In spite of **(Hara, 1975)** doubted whether direct relation exists between the surface area of the olfactory epithelium and sensitivity to odors, since the sensory epithelium is not distributed uniformly over the surface area of the olfactory lamellae. However, **Zeiske *et al*., (1976)** reported that, the more folding of the olfactory epithelium increase the surface area of the epithelium, consequently proliferate the sensitivities and efficacy of the olfactory organ. This latest hypothesis also improved by **(Jakubowski and Kunysz, 1979),** since they stated that, the olfactory sense enhanced as the olfactory surface epithelium increases with the fish growing. **Pashchenko and Kasumyan, (2017)** affirmed a positive correlation between the number of olfactory lamellae and fish body length**.** More recently, **Aicardi *et al*., (2022)** showed that the olfactory lamellar number of fish is likely increases ontogenetically as the fish grow Furthermore **Rheinsmith *et al*., (2022**) stated that, since the olfactory surface areas increases and so gets complicated with the fish age, this complexity of course includes distribution and variation of sensory and non-sensory epithelia which may indicate increase of odor-processing capacity as the fish grow **.**

Sensory Epithelium

Almost, in all cyprinoid fish species, including the presented studied grass carp, the olfactory epithelium covering their lamellae, regardless their magnitude volume or number, comprises sensory and non-sensory areas. The first one sensory epithelium may interspaced irregularly in islets between the non-sensory epithelium as in other cyprinoids for instance, *Alburnus alburnus* **(Hara, 2000).** It holds mainly sensory receptor cells in addition to supporting cells in-between, but, the second, non-sensory areas, comprise non-sensory ciliated and non-ciliated stratified epithelial cells in addition to ovoid goblet mucous cells in-between.

The sensory receptor cells are bipolar neurons categorized by a terminal swelling or hillock (dendritic knob) from which released their peripheral naked dendritic projections (cilia or microvilli) to the site of stimulus reception. These projections are directly exposed to the ambient environment so detect well odorants **(Laing *et al*., 2012)**.

The other two minor receptor cell types are the rod-tipped and crypt receptor neurons; both are infrequently detected in grass carp. The first one, rod-tipped cells are not consistently distributed across the olfactory epithelium, solitarily or mostly clustered. They were also detected with the major cell types in cyprinoid, *Carassius auratus* by **(Ichikawa and Ueda, 1977)**, *Alburnus alburnus* by **(Hernadi, 1993),** *Hypophthalmichthys molitrix* by **(El-Attar and Al-Zahaby, 2010),** Chinese cave loaches of genus *Oreonectes* by **(Waryani *et al*., 2015)**, cave-dwelling of genus *Sinocyclocheilus* by **(Zhang *et al*., 2018),** and *Danio rerio* by **(Al-Zahaby *et al*., 2023).** These cells as in other receptors, their distal surface protrudes in a dendritic knob but bear a single actin-rich rod-like apical projection, so are known as rod-tipped cell.

The other minor receptor cell, is the crypt neuron are of distinctive pear-shaped or ovoid cells located close to the epithelial surface in at the top half of the olfactory epithelium and provided with few apical microvilli as well as occult cilia emitted from dendritic knob and extending into a crypt at apex **(Hamdani *et al*., 2008).** Crypt cells are less pronounced as other ciliated receptor neurons even unnoticeable in the SEM observation of the present studies.

The sensory cells are commonly respond to many types of olfactory stimuli: amino acids, nucleotides, bile salts and pheromones **(Hara and Zhang, 1997)**.The former two (amino acids and nucleotides) are generally feeding stimulants, whereas the latter two (bile salts and pheromones) are involved in social interactions (**Sorensen and Caprio, 1998**). Additionally, **Sato and Suzuki (2001)** outcome that, ciliated receptor neurons are generalists, respond to a wide variety of odorants, even pheromones in addition to amino acids, bile salts and other chemical cues, particularly odour-bearing food **(Hamdani and Døving, 2002).**

Subsequently, the functional role of rod-tipped cells is uncertain and may differed from both ciliary and microvilli sensory receptor cells **(Crnjar *et al*., 1992)** have well-known mechanosensory, chemosensory, and multimodal functions of the actin-rich projections of sensory cells **(Cheung *et al.*, 2021**).Concerning with the crypt neurons, in spite of their function were not fully elucidated for a long time, (**Schmachtenberg, 2006**) showed, that they display spontaneous spike activity and responded to amino acid with dose-dependent excitation and believed that they participate in both food olfaction and sex pheromones. **(Hamdani *et al.*, 2008)** also adopted the involvement of crypt neurons in fish reproduction, since their number in the olfactory epithelium of the crucian carp varies dramatically around the year.

The olfactory epithelium of *Anguilla vulgaris* possessed ciliated receptor cells and micro villous receptor cells, in addition to the infrequently present rod cell and ciliated receptor cell which are all insulated from each other by supporting cells and intermingled with supporting cell and mucus secretory goblet cells. All animals have ORNs, which are exemplar sensory neurons with strikingly similar cellular and molecular characteristics. **(Axel, 2005).** The olfactory receptor cells are bipolar neurons distinguished by their cell dendritic protrusions. From their apical free surface, a single process expands into a mound-like projection, an olfactory or dendritic knob, from which several olfactory cilia or microvilli radiate. The olfactory receptor neurons of the cilia or of the microvilli possess the necessary properties to transform an odorant-receptor stimulus into an electrical signal. Receptor elements sensing the odorants are concentrated on the dendritic protrusion, either cilia or microvilli, of the olfactory sensory neurons **(Othman *et al*., 2022).**

The microvilli are short, extremely thin protrusions of uniform length released from the cell surface, increasing its surface area and providing a critical role as a protective barrier since they are supported by parallel actin bundles **(Barr-Gillespie, 2015).** Their core is formed of microfilaments lacking (9+2) ultrastructure microtubules and do not arise from the basal granules, so they are non-motile but sense the external environment as mechanosensory **(Houdusse and Titus, 2021).** In addition to these two olfactory receptors in *Anguilla vulgaris* and even in all Anguilliformes, two other infrequently occurring forms of receptor neuron rod-tipped cell and crypt cell are detected **(Schulte, 1972).**

The rod cell is a steady rod-like or compound cilium**,** with ciliary or microvillous components partially fused and emitted from the entire free margin of a dendritic knob, so the cell is often called a rod-tipped cell.

These cells occur singly or in groups in some places where their rod-like cilia are sparse on the olfactory lamellae with a relatively long and thick rod. Despite the fact that the ciliated receptor cells and micro villous receptor cells are inclusively found practically in all fishes' olfactory epithelia, the rod cell is also detected more rarely in some fish species of Cypriniformes: e.g*: Danio rerio* **(Cheung *et al.*, 2021).**

Otherwise, **(Yamamoto and Ueda, 1977)** established that the number of rod cell increased during the smoltification period of Salmonid *Oncorhynchus masou* prior to entering saltwater from fresh water **(**anadromous fish), which may be like the present studied eel during its regular migration The fourth olfactory receptor, crypt cells have been hardly detected in the olfactory epithelium of *Anguilla vulgaris*. They have a superficially positioned, ovoid-shaped soma with an apical crypt bearing microvilli and cilia. Crypt cells also showed up in many other teleost species with the same uniform morphology **(Triana-Garcia *et al.*, 2021)** They were also detected even in elasmobranch Rajiformes **(Ferrando *et al.*, 2007).** Crypt cells are much less abundant than ciliated receptor cells or microvillus receptor cells, if they represent any fish species **(Cheung *et al*., 2021; Al-Zahaby *et al*., 2023).**

The olfactory system of fish is remarkably effective in detecting and differentiating between a wide range of water-soluble substances, including steroids, prostaglandins, amino acids, bile acids, and nucleotides. **(Yoshihara, 2014).** Every receptor neuron carries unique chemical stimuli that, when combined with olfactory cues related to fish vital life processes, produce unique behaviours. The olfactory neurons are specific receptors adjusted to detect definite odorant ligands **(Bazáes *et al*., 2013)**. This may be due to the submergence of both micro villous receptor neurons and ciliated receptor neurons in the thickness of cilia copies of both sensory and non-sensory cells **(Lazzari *et al*., 2022).**

To allow fish to detect food, bile salt and amino acid odorants (food that has an odor) are the main stimulants of ciliated receptor neurons **(Chakrabarti and Ghosh, 2011)**. They are concentrated after fish starvation, which may be performed during breeding migration **(Furne and Sanz, 2018).** While ciliated and micro villous olfactory receptor neurons are activated by amino acid odorants, ciliated receptor neurons is primarily responsible for detecting bile salt odorants. Bile salts are potent olfactory stimulants, some of which act as chemical signals for fish communication**.** Nucleotides released from food into ambient water, however, are thought to serve as feeding cues and are recognized by (**Wakisaka *et al*., 2017**).

Non-Sensory Epithelium

The non-sensory epithelial areas are made mainly of supporting, mucous and basal cells in addition to nonspecific seldom observed, rodlet and Labyrinth or chloride cells.

The supporting cells are comparable to neural glial cells; they are non-neural olfactory epithelium cells, packed in between and around the different receptor cells. In the present investigated fish, the supporting cells despite have flat top surface, haven't dendritic knobs as ciliated receptor cells. They are categorized into two morphologically distinctive cell types. They are either of plentiful long motile cilia, ciliated supporting cells, or of regular concentric micro ridges, fingerprint pattern, sustentacular indifferent epithelial cells. The ciliated ones arises from a basal cell population as do the receptor cells but the sustentacular cells are identical to the stratified epithelial cells of epidermis from which they are derived **(Goss *et al*., 2016)**.

The ciliated supporting cells were commonly detected in the olfactory epithelial surface of grass carp in the form of clustered patches as also showed in Cyprinoid *Oreonectes guananensis* that exhibited a core shaft of their cilia is formed of typical axonemal pattern (9+2) of microtubules so denoted as Kino cilium loading with dynein arms responsible of their motive force **(Waryani *et al*., 2015).** Hara early stated that, in addition to fish forward movement, water circulation occurs also with the synchronous beating action of motile Kino cilia of these supporting results in a vigorous water flow around the Olfactory lamella and attracting odors molecules that facilitate its detection by the olfactory receptor cells even in stagnant environments and this of course promotes better odorant perception **(Hara, 2000).** Moreover, the micro ridges have been proposed also serve to increasing the surface area of cells to facilitate waste product removal as **(Chakrabarti and Ghosh, 2011)** suggested that these micro ridges provide structural support anchoring the mucus secreted by the neighboring goblet cells in order to protect the olfactory epithelium from any hazard effect. So, they have sustentacular cells function as metabolic and physical support for the olfactory epithelium.

The epithelial surface of the olfactory lamellae of grass carp showed wide openings, which are the outlets of the founded mucous goblet cells. These cells are of oval shape crowded with mucin granules and open on the olfactory epithelial surface in between sensory and non-sensory cells. The plentiful secreting mucus lubricate the epithelial surface to smooth flow of water in between olfactory lamellae and it service also as a trap delays the access of external hazards like slush granules and heavy metals to the receptor neurons **(Waryani *et al.*, 2013).** In their investigation, **Al-Zahaby *et al* (2023)** found that, zinc oxide nanoparticles (ZnO-NPs) adversely upset zebrafish's olfactory epithelium, since it appeared malformed with degeneration signs of all receptor cells and hyper activation of mucous goblet cells thus, ZnO-NPs negatively affected the fish odor sensation **(Al-Zahaby *et al*., 2023).**

In the olfactory epithelium of the present studied fish, grass carp, rod let cells are pear-shaped with electron lucent cytoplasm bounded with a thick fibrillary cytoplasmic capsular wall as showed also by **(Dezfuli *et al*., 2007, Al-Zahaby *et al.*, 2023).**

In the Anguilla vulgaris of the present studies, CNC occur singly or in aggregates throughout the olfactory lamella's surface. It seems likely that the motile kinocilia of CNC are longer and more involved in the water flow through and around olfactory lamellae. The beating action of olfactory cilia was likely to favour efficient odorant transport to the olfactory epithelium **(Døving *et al.*, 1977)**. Furthermore, Cox also specified that the vigorous water flow of synchronised beating of cilia alone is sufficient both to draw water into the olfactory chambers and to circulate water within and around OL **(Cox, 2008)**. This vigorous water flow also removes foreign minute particles and microorganisms from the lamellae along with the mucus **(Reiten *et al*., 2017).**

The name "goblet" refers to the cup-shaped form of the mucus secretory goblet cells , which are unicellular glands that are specialized for the synthesis and secretion of mucus and are packed full of mucin granules as described by **(Birchenough *et al.*, 2015)**. They are abundantly scattered within the olfactory non-sensory areas in between the IEC and CNC of the studied fish, *Anguilla vulgaris*. They are found in the top layer of the olfactory epithelia and can vary widely in size. Mucigen, which is produced by their numerous rough endoplasmic reticulum and Golgi complexes, as well as mitochondria encircled in the basal dense cytoplasm around their global nuclei, are all found in their apical cytoplasm. This mucigen is converted in turn into vesicles mucins (mucus) granules at the apical cell pole to be released onto the olfactory epithelial surface**.** Since the mucus covering the olfactory lamellae surfaces constitutes an important medium in which the odorants are diffused Furthermore, the secreted mucus protects the olfactory epithelia from mechanical abrasion and possibly helps for hunting microscopic rubbish remains, which retains the receptor neurons get for new stimuli**. (Rygg *et al*., 2013)** later surmised that fish may have a higher chance of detecting an odour if the mucus layer is evenly spread across the olfactory neurons, the superficial layer of the olfactory epithelium contains labyrinth cells , which are bulbous in shape and located between sensory and non-sensory cells. They are basal nuclei with copious interconnected tubular endoplasmic reticulum intimately connected with numerous filamentous mitochondria and homogeneous fine granules. Morphologically, labyrinth cells is not modified sensory, non-sensory-supporting, or goblet cells but have quite another fine structure **(Bertmar, 1972).**

Finally, *A. vulgaris* is a migratory carnivorous teleost that feeds on small fish, worms, and other small animals. It appears that it has a keen sense of smell, as evidenced by its multi lamellar arrangement and dense population of cells, and that it relies primarily on olfactory sensory cells for food detection and possibly other vital functions. As a result, the presence of different receptor cells in the olfactory rosettes adapted to the fish's food and feeding habits. *Ctenopharyngodon idella* is a freshwater herbivorous teleost that feeds on aquatic plants called duckweeds. Its olfactory sensation is controlled by harmonizing between sensory and non-sensory cellular components in the olfactory rosette and that play a vital role in mediating physiological responses and behaviors related to food search, feeding, and sexual attraction.

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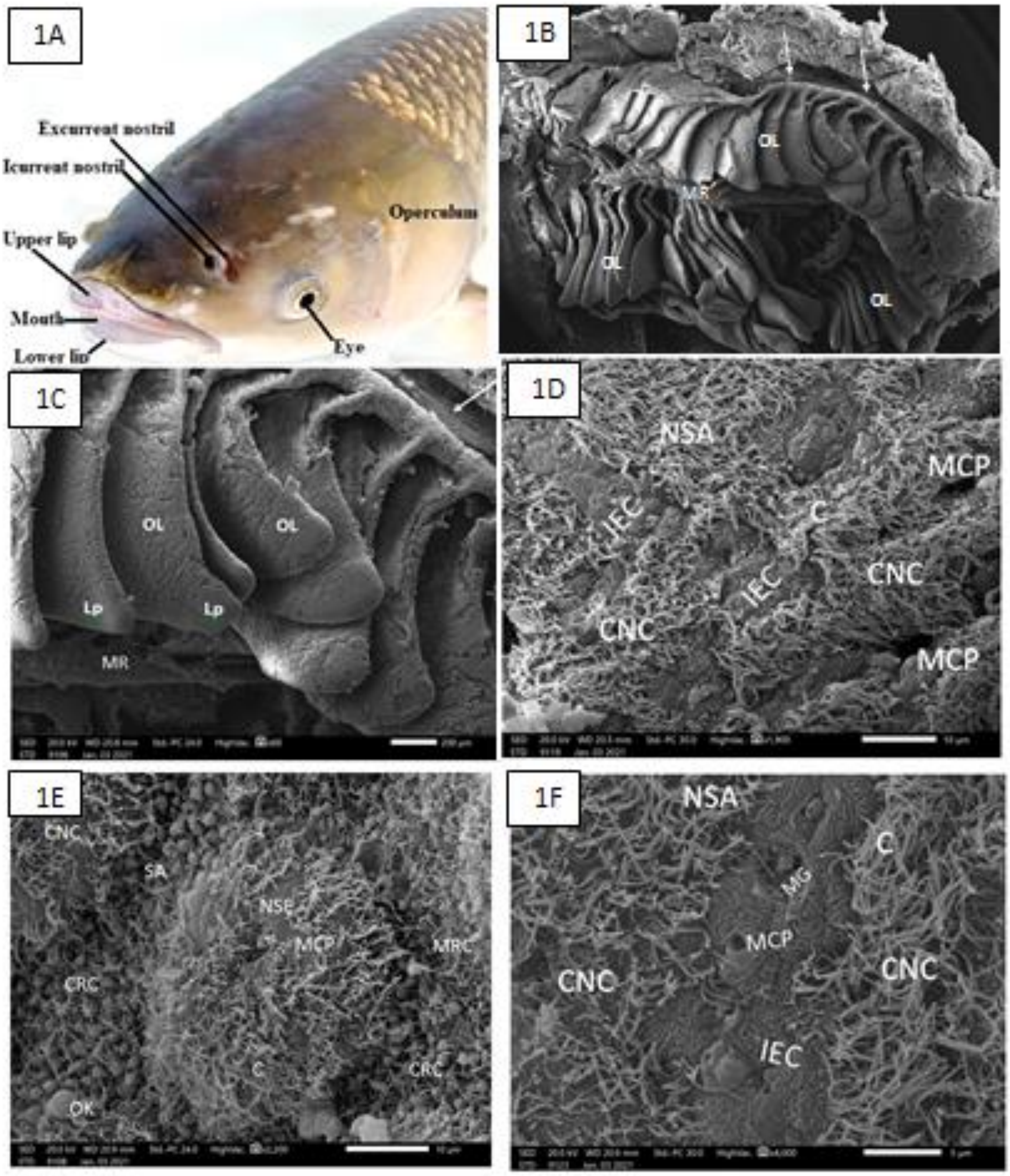
1A
Excurrent nostril
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Upper lip
Mouth
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Operculum
Eye
1B
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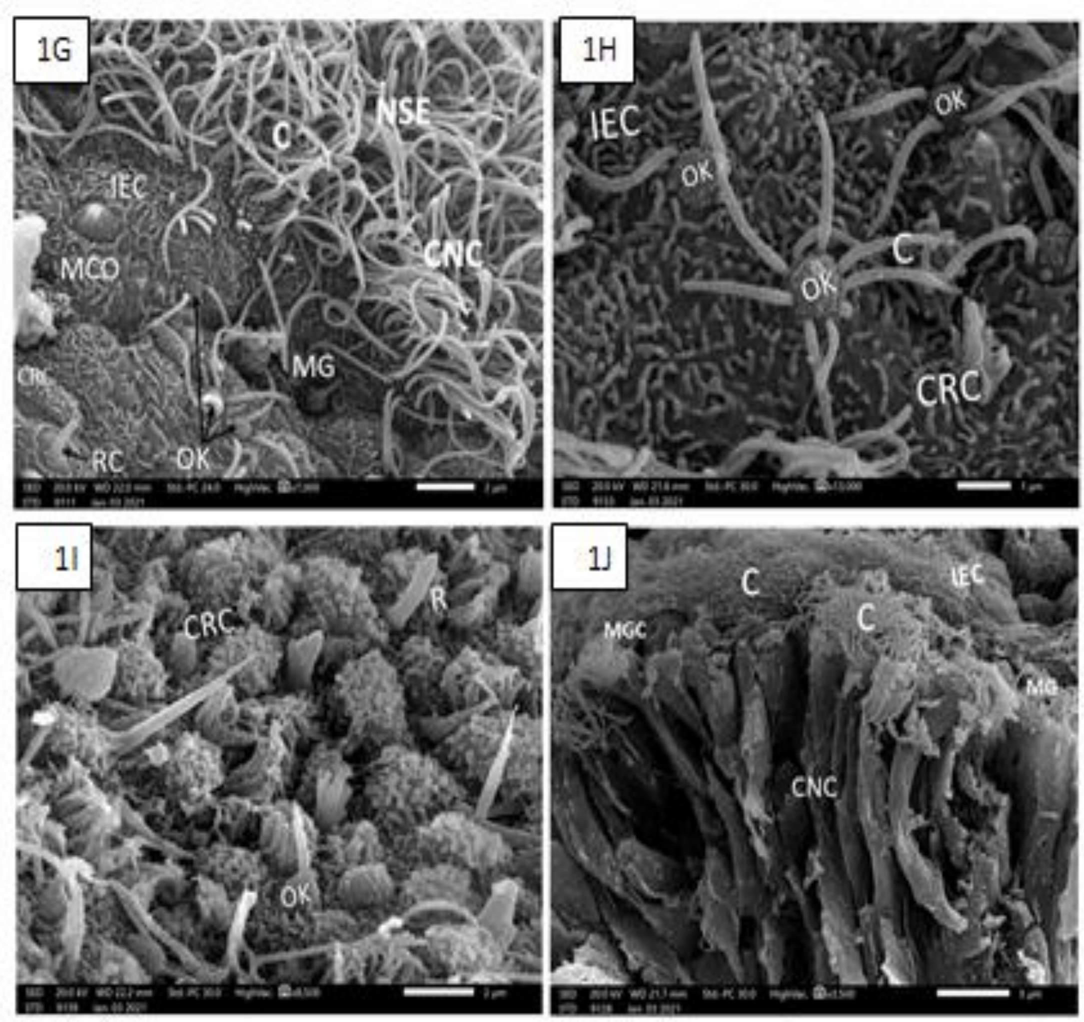


Fig.1.A. Photograph of the head of grass carp (*Ctenopharyngodon idella*, Val.) lateral view; showing the nostril with their anterior (incurrent) and posterior (excurrent) passage in the upper mid distance between eyes and mouth.

Fig.1.B. SEM micrograph of the olfactory rosette of grass carp (*Ctenopharyngodon idella*, Val.) showing; a whole olfactory rosette with their approximately (40-50) olfactory lamellae (OL) arranged on both sides of a median raphe (MR). Notice the connection between the wall's capsule and lamellae (arrows). X-19-Scale bar=500μ.

Fig.1.C. Higher magnification of the previous SEM micrograph showing; the olfactory lamellae (OL) arranged on both sides of the median raphe (MR), Notice the connection between the capsule & lamellae (Arrow). X-60-Scale bar=200µm.

Fig.1.D. SEM micrograph of grass carp's olfactory rosette showing; Nonsensory area (NSA) with strands of ciliated non-sensory cells (CNC) characterised by tufts of long motile cilia (C). Mucous cell pores (MCP) dispersed in between islands of indifferent epithelial cells (IEC) with regular microridges. X-1,900-Scale bar=10µm.

Fig.1.E. SEM micrograph of an olfactory rosette of grass carp (*C. idella*, Val.) showing; Nonsensory area (NSE) of ciliated nonsensory cells (CNC) with long cilia (C), mucous cell pores (MCP) and indifferent epithelial cells (IEC) with regular microridges. Sensory area (SA) characterised by the presences of ciliated receptor cells

(CRC) with short thick cilia (C) besides microvillous receptor cells (MRC) of short microvilli. Both cell receptor cells are with obviously overhangs dendritic knob (OK). X-2,200-Scale bar=10µm.

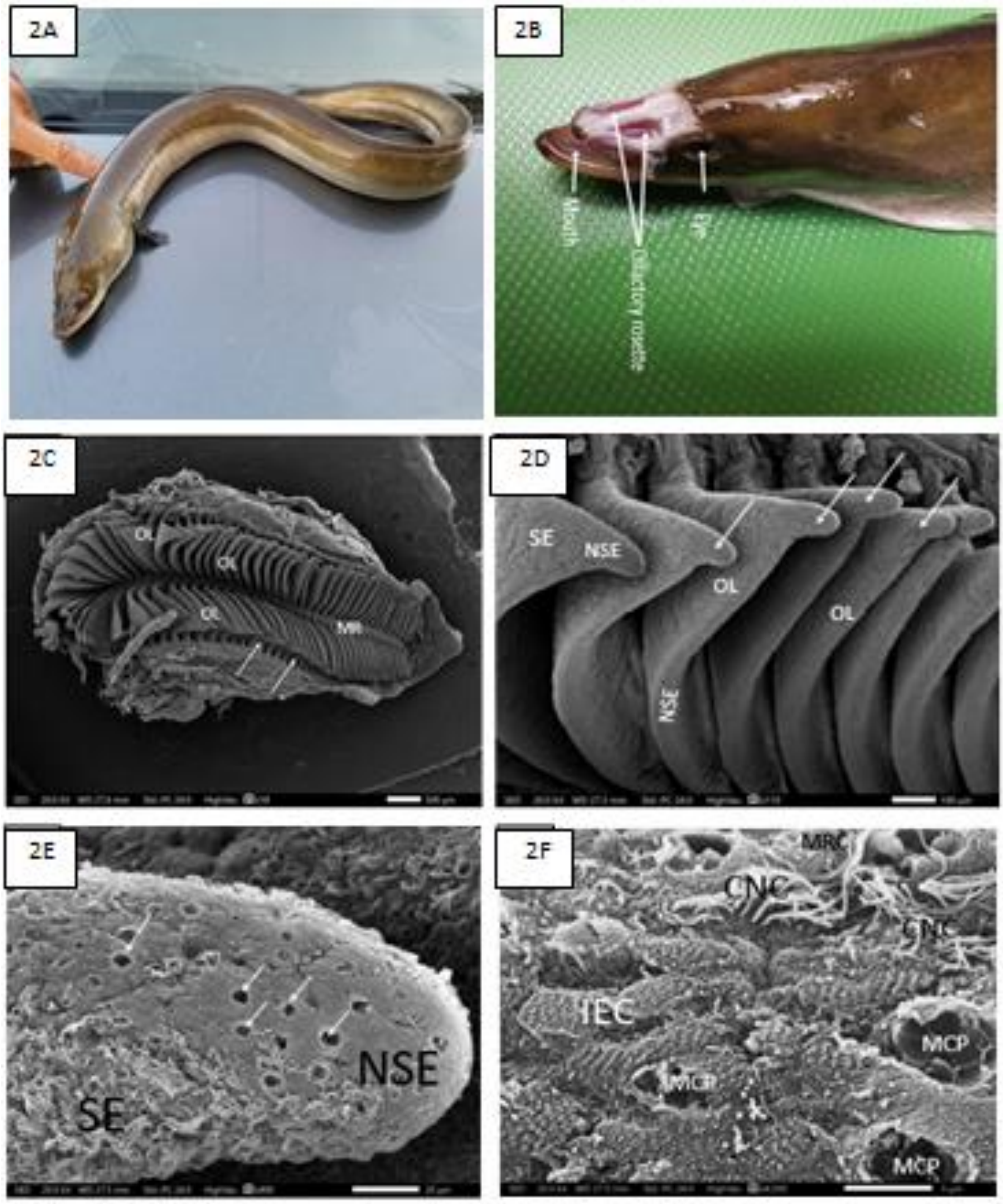
Fig.1.F. Higher magnification of the previous SEM micrograph showing; Nonsensory area (NSA) of ciliated nonsensory cells (CNC) with long cilia(C), mucous cell pores (MCP) some with secreted mucus granules (MG) within indifferent epithelial cells (IEC) with regularly arranged microridges. X-4,000-Scale bar=5µm.

Fig.1.G. Higher magnification of the previous SEM micrograph showing; Nonsensory area (NSE) of ciliated nonsensory cells (CNC) with long cilia(C), mucous cells some with opened orifice (MCO) so showing secreted mucus granules (MG) and others is not yet opened. Indifferent epithelial cells (IEC) with regularly arranged microridges. Dispersed ciliated receptors (CRC) and rod tipped cells (RC) both with dendritic knob (OK) in between indifferent epithelial cells (IEC). X-7,000-Scale bar=2µm.

Fig.1.H. Higher magnified SEM micrograph of an olfactory lamella of the grass carp showing; ciliated receptor cell (CRC) with eight short thick cilia (C) radiate from obviously overhangs dendritic knob (OK) coated with microvilli. These ciliated receptor cell (CRC) intermediate indifferent epithelial cells (IEC) of regular microridges. X-13,000-Scale bar=1µm.

Fig.1.I. High magnified SEM micrograph of an olfactory lamella of the grass carp showing; Many microvillous receptor cell (CRC) with obviously overhangs dendritic knob (OK) coated with lot of microvilli. Number of rod tipped cells with thick compound rod (R) also emitted from obviously overhangs dendritic knob (OK) also coated with microvilli. X-8,500-Scale bar=2µm.

Fig.1.J.) Higher magnified SEM V.S. micrograph in the olfactory epithelium of an o. lamella of the grass carp showing; its pseudostratified cylindrical cell body of ciliated nonsensory cell (CNC) with copious cilia (C), mucous goblet cell (MGC) with secreted mucus granules (MG), indifferent epithelial cells (IEC). X-3,500-Scale bar=5µm.


2A
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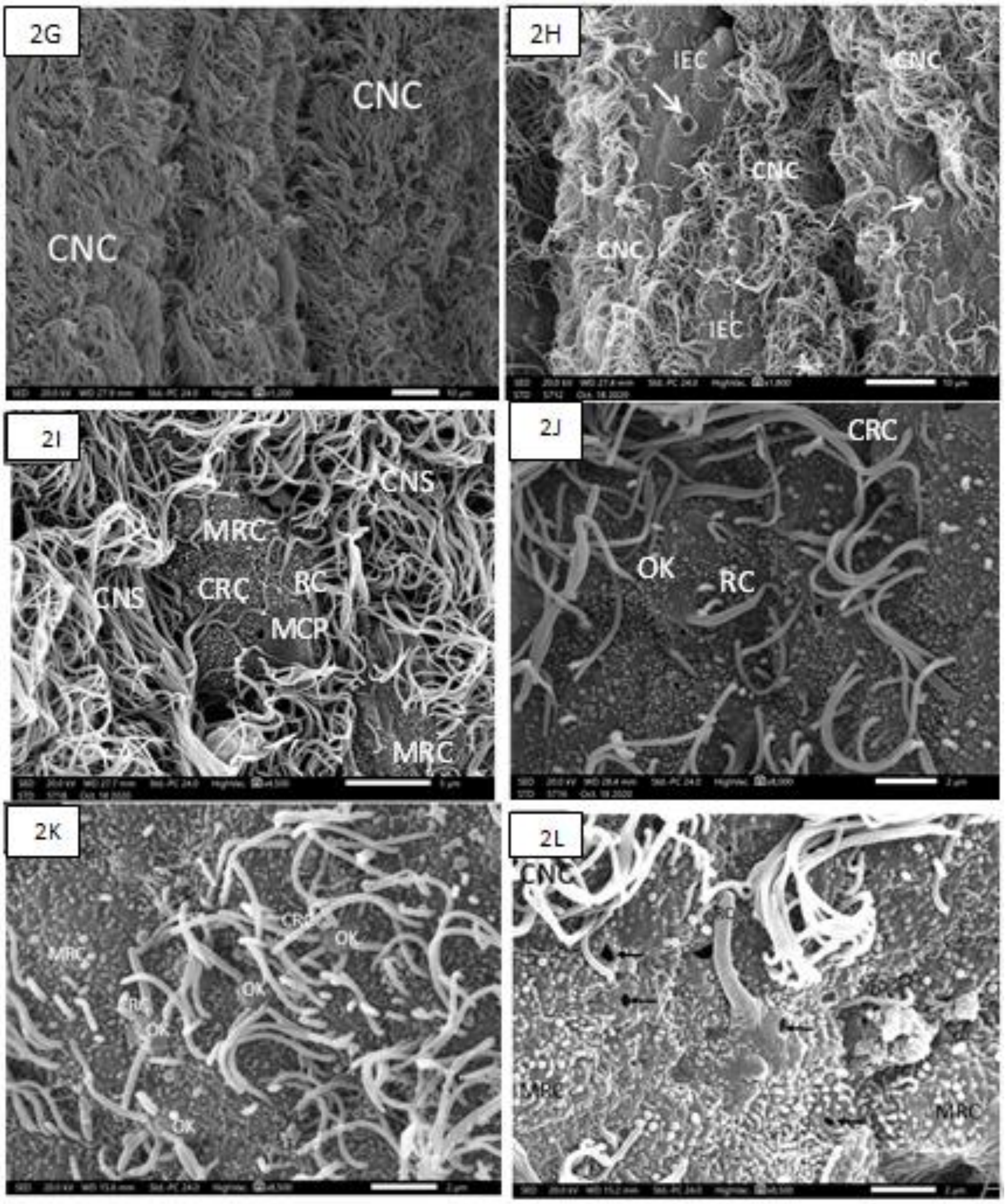

Fig.2.A. photograph of whole mount (lateral view) of the studied migratory fish stage of ***Anguilla vulgaris.***

Fig.2.B. Photograph of the head of ***Anguilla vulgaris*** (top view) with removed skin showing the two olfactory rosette and eyes.

Fig.2.C. Scanning Electron Micrograph (SEM) of the olfactory rosette of ***Anguilla vulgaris*** showing a whole olfactory rosette with their approximately (90-100) olfactory lamellae (OL) arranged on both sides of the median raphe (MR). Notice the connection between the wall's capsule and lamellae (arrows). Scale bar = 500µm.

Fig.2.D. Scanning Electron Micrograph (SEM) of the olfactory lamellae (OL) of ***Anguilla vulgaris*** showing the glandular zoon (GZ) and ciliary zoon (CZ) of nonsensory epithelium (NSE) and the sensory one (SE), linguiform process of the olfactory lamellae (arrows). Scale bar = 100µm.

Fig.2.E. Scanning electron micrograph (SEM) of the olfactory rosette of ***Anguilla vulgaris*** showing the median central glandular zoon (GZ) and the peripheral ciliary zoon (CZ) of nonsensory epithelial area (NSE) and the sensory area (SE) in-between on the linguiform process (LP) of an olfactory lamellae (OL). Scale bar = 20µm.

Fig.2.F. SEM micrograph showing; Glandular zoon (GZ) is formed of indifferent epithelial cells (IEC) with regularly arranged microridges and wide mucous cell pores (MCP) at middle center of the linguiform process (arrow) of an olfactory lamellae (OL), however the sensory area (SE) is appeared as isolated island in-between with its ciliated receptor (CRC) and microvillous receptor (MRC). Scale bar = 5µm.

Fig.2.G. SEM micrograph showing; Strands of ciliated nonsensory cell (CNS) with their copies of tufted long cilia directed in one direction on the marginal edges of an olfactory lamella. Scale bar = 10µm.

Fig.2.H. SEM micrograph showing; Sensory island of ciliated receptors (CRC), microvillous receptors (MRC) and rod receptors cell (RC) as well as other nonsensory island of indifferent (IEC) and mucous cell pores (MCP) in-between copies of tufted long cilia of ciliated nonsensory cell (CNS) on an olfactory lamella. Scale bar = 10µm.

Fig.2.I. SEM micrograph showing; two sensory islands of ciliated receptors (CRC), microvillous receptors (MRC) and rod receptors cell (RC) encountered with mucous cell pores (MCP) in-between copies of tufted long cilia of ciliated nonsensory cell (CNS) on an olfactory lamella. Scale bar = 5µm.

Fig.2.J. SEM micrograph of high magnification showing; sensory area of ciliated receptors (CRC), microvillous receptors (MRC) and rod-tipped receptors cell (RC) all of these dendrites emitted from a characteristic olfactory dendritic knob (OK) and encountered with mucous cell pores (MCP) on an olfactory lamella. Scale bar = 2µm.

Fig.2.K. SEM micrograph of high magnification showing; show; ciliated receptors cells (CRC) with 3-5 thick somewhat short sensitive cilia and microvillous receptor cells (MRC) with short sensitive microvilli (MV), both cilia and microvilli are emitted from a dendritic knob (OK). Scale bar = 2µm.

Fig.2.L. SEM micrograph of high magnification showing; rod-tipped receptors cell (RC) with very thick complex rod (R) also emitted from a dendritic knob (OK) and microvillous receptor cells (MRC) and labyrinth cells (LC) . Scale bar = 2µm.