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ORIGINAL ARTICLE

The olfactory organ of long whiskered catfish, *Mystus gulio* (Hamilton, 1822): histomorphology and ultrastructure

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Abstract
The olfactory organ of the predatory catfish, *Mystus gulio* (Siluriformes: Ailiidae) was investigated through light microscopy and electron (scanning and transmission) microscopy to elucidate its structural organization. The olfactory rosette was elongated, comprising 32 to 38 lamellae arranged along a central midline raphe. Each olfactory lamella consisted of two principal parts: the olfactory epithelium (mucosa) and the median raphe. The mucosa displayed a clear demarcation into sensory and nonsensory zones, both exhibiting distinct cellular compositions that could be identified based on histoarchitectural features, staining affinities, surface morphology, distributional arrangement and ultrastructural characteristics. The sensory mucosa contained three types of receptor cells: two classical forms bearing either cilia or microvilli, and a third characterized by rod-shaped apical specialization. The nonsensory mucosa comprised mucous cells, chloride-like cells, rodlet cells and mast cells, along with two types of supporting cells categorized as either ciliated or non-ciliated. Basal cells were situated deep within the olfactory mucosa, adjacent to the central core. Structurally, the raphe was formed by compactly arranged stratified epithelial cells exhibiting distinct microridges on the apical membrane. The study discussed the functional relevance of the diverse cell types forming the olfactory mucosa, emphasizing their specific roles in the olfactory perception of *Mystus gulio*.

Keywords: Tissue microscopy, SEM, TEM, Olfactory sensing, *Mystus gulio*

INTRODUCTION

The olfactory and gustatory systems in fish constitute the major sensory organs and are of paramount importance for the detection and identification of chemical stimuli in the surrounding water (Hara 1994). Olfaction is mediated by receptor cells located on the olfactory mucosa and usually regarded as a distance chemical sense with high sensitivity and specificity. It plays diverse roles in the life of fish, including food and mate selection, migration, breeding site recognition, parental behaviour, alarm responses and various other activities (Camacho et al. 2010). Fishes exhibit considerable variation in the morphology of the olfactory rosette which differs in the shape, number and arrangement of olfactory lamellae, as well as in the presence of various types of sensory receptor cells. The receptor neurons in the olfactosensory epithelium are directly exposed to the external aquatic environment and transmit information to the brain via cranial nerve-I. They exhibit distinct micromorphological features and are responsible for detecting a wide range of substances, such as amino acids, bile salts, sex steroids, amines, prostaglandins and nucleotides (Wakisaka et al. 2017; Dymek et al. 2024). Different sensory neurons can be regarded as distinct anatomical and functional entities, each exhibiting specific sensitivities to various olfactory cues (Yamamoto 1982). Microsmatic fish have few lamellae on their olfactory rosette, whereas macrosmatic fish have many lamellae (Gon & Fishelson 2009). The size and organization of fishes' sensory organs, especially their eyes and smell organs, reflect their feeding habits. The structural characterization of olfactory organ in teleostean fishes has been reported by several earlier researchers (Chen & Arratia 1994; Chakrabarti & Ghosh 2009; Atta 2013; Mokhtar & Abd-Elhafeez 2014; Dymek et al. 2021; Ghosh 2022; Klimenkov et al. 2023; Al-Zahaby et al. 2025). However, the olfactory structure of bagrid catfishes has not yet been investigated.

Mystus gulio is a predatory catfish and a euryphagous, voracious feeder that primarily

consumes zooplankton and various animal food materials, including rotifers, copepods, crustaceans, insect larvae, prawns and small fishes (Begum et al. 2008; Mondal & Mitra 2016). The aim of the present study is to describe the cellular organization of the olfactory epithelium and elucidate its role in olfaction in the brackishwater catfish, *Mystus gulio* (Hamilton, 1822), using light, scanning and transmission electron microscopy techniques.

MATERIALS AND METHODS

Specimen collection: Mature specimens of *M. gulio* (14±2.06cm in total length) were collected from brackish water bodies of South 24 Parganas, West Bengal. Species identification was carried out following the taxonomic keys of Misra (2003). The fishes were anesthetized with benzocaine (4mg/L) and subsequently sacrificed in accordance with the guidelines of the Institutional Animal Ethics Committee. The olfactory organs were immediately excised from the olfactory chambers and processed for subsequent histological and ultrastructural analyses.

Preparation for histology: The olfactory tissues were fixed overnight in Bouin's aqueous solution at room temperature. The fixed samples were thoroughly washed in 70% ethanol, dehydrated through an ascending ethanol series followed by acetone, cleared in benzene and embedded in paraffin wax (melting point 56-58°C). Paraffin blocks were prepared and transverse sections of 4 µm thickness were cut using a rotary microtome (Weswox MT-1090A). After routine histological processing, the deparaffinized sections were stained with Delafield's Haematoxylin-Phloxine (HP) (Suvarna et al. 2019) and Mallory's Triple stain (MT) (Mallory 1936). Following staining, the sections were dehydrated in 1, 4-dioxane ($C_4H_8O_2$), cleared in xylene and permanently mounted in DPX. The prepared slides were examined under a ZEISS Primo Star light microscope equipped with a Tucsen 5.0MP digital camera and photomicrographs were captured at various magnifications.

Preparation for scanning electron microscopy (SEM): After *in vivo* perfusion with 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4) for 15min, the olfactory organs were carefully dissected out from the olfactory chambers. The samples were gently washed with 1% Tween 40 (polyoxyethylene sorbitan monopalmitate) solution, rinsed in buffer and then fixed in 2.5% glutaraldehyde for 24hr at 4°C. Post-fixation was performed in 1% osmium tetroxide (OsO_4) for 2hr. The fixed samples were dehydrated through an ascending ethanol series, followed by acetone and isoamyl acetate ($C_7H_{14}O_2$). The samples were subsequently dried using the critical point drying method (Anderson 1951) with liquid carbon dioxide and coated with a 15nm layer of platinum using a BT-150 coater (Hind High Vacuum Co. Pvt. Ltd., India). The specimens were examined under a ZEISS EVO18 scanning electron microscope.

Preparation for semithin sections and transmission electron microscopy (TEM): A portion of the olfactory organ was cut into small tissue pieces measuring approximately 1-2mm² and fixed in modified Karnovsky's fixative (2% glutaraldehyde and 2.5% paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.4) for 4hr at 4°C. The samples were then thoroughly washed several times in buffer and post-fixed in 1% osmium tetroxide (OsO_4) for 2hr at room temperature. After multiple buffer rinses, the tissues were dehydrated through an ascending series of acetone and embedded in epoxy resin (Luft 1961). Following resin polymerization, transverse semithin sections (1µm thick) were cut with a glass knife on a Leica EM UC7 ultramicrotome, stained with Toluidine Blue (TB) (Bozzola & Russell 1992) and examined by a light microscope.

For ultrastructural observations, ultrathin sections showing silver to gold interference colours were prepared from selected regions, mounted on uncoated copper grids and stained sequentially with uranyl acetate and lead citrate (Glauert 1975). The sections were then examined under a Tecnai G2 20S-Twin transmission electron microscope (FEI, Netherlands).

RESULTS

Histoarchitecture: The lamella of the olfactory organ is composed of two principal layers: the epithelium (mucosa) and the central core (submucosa) (Figs. 1A,

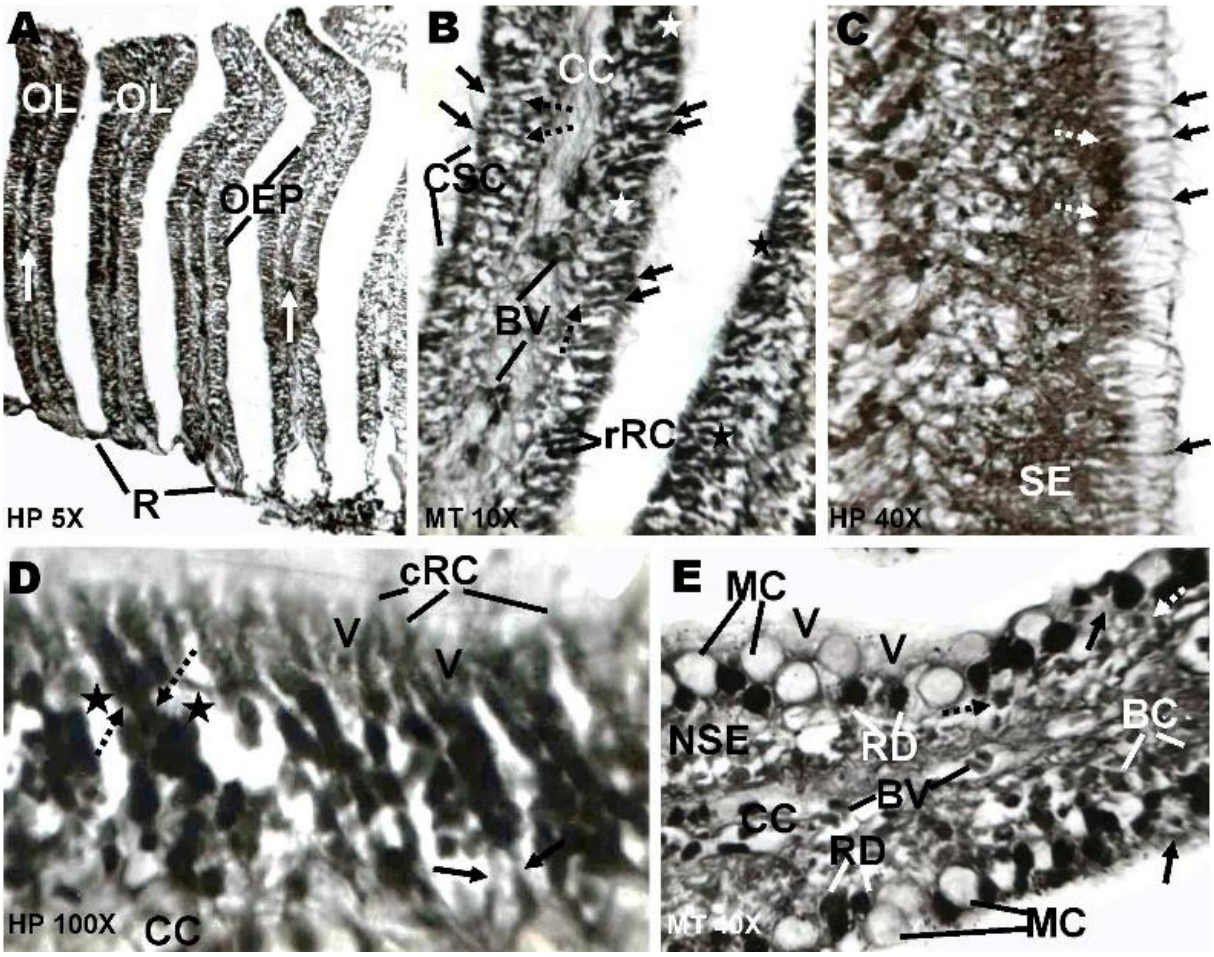

Fig.1. Photomicrographs of histological sections of the olfactory epithelium of *M. gulio* stained with Delafield's Haematoxylin-Phloxine (HP) and Mallory's triple (MT) stains. **(A)** Olfactory lamellae (OL) based on the raphe (R), showing a central core (CC) lined on both sides by the olfactory epithelium (OEP). **(B)** The OEP is characterized by ciliated receptor cells (solid arrows), rod receptor cells (rRC), chloride-like cells (asterisks) and ciliated supporting cells (CSC). Notably, the ciliated receptor cells exhibit synaptic connections (broken arrows) with spindle-shaped receptor cells. Blood vessels (BV) are observed within the CC. **(C)** The sensory epithelium contains a large number of ciliated receptor cells (solid arrows) along with scattered microvillous cells (broken arrows). **(D)** A higher magnification of the epithelium reveals ciliated receptor cells (cRC) bearing olfactory vesicles and synaptic connections (solid arrows), rod receptor cells (broken arrows), microvillous receptor cells (arrowheads) and chloride-like cells (asterisks). CC denotes the central core. **(E)** The non-sensory epithelium (NSE) exhibits a series of mucous cells (MC) with secreted mucus (arrowheads), basal cells (BC), mast cells (broken arrows), rodlet cell (RD) and stratified epithelial cells (solid arrows). The CC contains blood vessels (BV).

2A). The central core is bordered by a well-defined basement membrane that distinctly separates it from the overlying epithelium (Figs 2A-B). The central core is composed of a delicate network of connective tissues, penetrated by blood vessels, pigment cells, and nerve fibres. The lamellae, attached on both sides of the raphe, exhibit variations in mucosal thickness and comprise both sensory and non-sensory regions.

The olfactory epithelium contains three distinct types of olfactory receptor neurons: ciliated, microvillous and rod cells (Figs. 1D, 2B). The ciliated receptor cells are localized in the surface zone of the epithelium and possess spherical, darkly stained nuclei along with fibrillar dendrites (Fig. 1C). The terminal end of each dendrite forms a swollen structure known as the olfactory vesicle, which is adorned with sensory cilia. The ciliated receptor cells establish synaptic connections with spindle shaped receptor cells situated in the middle or basal region of the mucosa (Figs. 1D, 2B). The microvillous receptor cells are few in number and located superficially within the mucosa. They are faintly stained, possess round nuclei and lack distinct axons. The rod-shaped receptor cells have elongated bodies with scanty

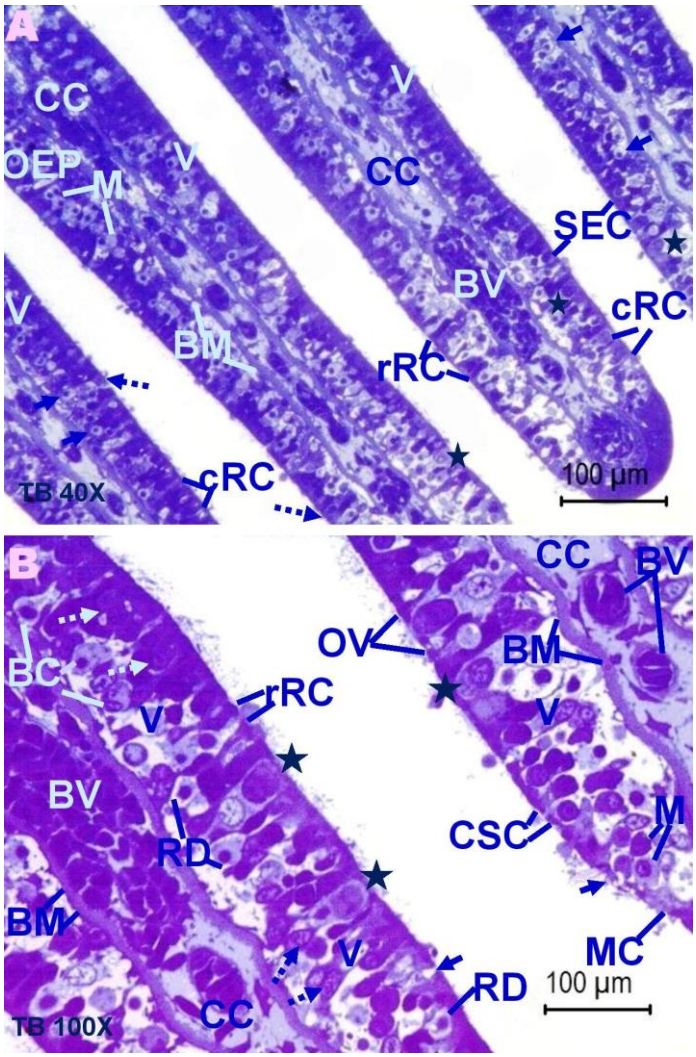

Fig.2. Photomicrographs of semithin sections of the olfactory epithelium of M. gulio stained with Toluidine Blue (TB). **(A)** The olfactory lamella exhibits olfactory epithelium (OEP), which is separated from the central core (CC) by a distinct basement membrane (BM). The OEP is characterized by ciliated receptor cells (cRC), rod receptor cells (rRC), chloride-like cells (asterisks), rodlet cells (arrow heads), stratified epithelial cells (SEC), ciliated supporting cells (CSC; broken arrows), mast cells (M) and basal cells (solid arrows). Prominent blood vessels (BV) are observed within the CC. **(B)** The olfactory epithelium shows ciliated receptor cells having olfactory vesicles (OV), along with rRC, microvillous receptor cells (solid arrows), chloride-like cells (asterisks), SEC, CSC, rodlet cells (RD), mucous cell (MC) and mast cells (M). Synaptic contacts (arrowheads) are evident between cRC and spindle-shaped cells (broken arrows). Blood vessels (BV) are present in the CC, which remains separated from the OEP by the basement membrane (BM).

cytoplasm and oval, darkly stained nuclei. They exhibit prominent dendritic extensions that project toward the interlamellar spaces (Figs. 1B, 1D, 2A-B).

Chloride-like cells are found close to the mucosal lining and spherical or pear-shaped. They are distinguished by granulated cytoplasm and globose nuclei that are faintly pigmented (Figs. 1B, 1D, 2B). Rodlet cells are often observed throughout the olfactory mucosa. Immature rodlet cells are located near the basement membrane, while developing cells occupy the middle region and mature rodlet cells are situated close to the epithelial surface (Figs. 2A-B). The mature cells are pear-shaped and possess deeply stained, vesicular nuclei (Figs. 1E, 2B). Their cytoplasm is densely packed with characteristic club-shaped inclusions. Mast cells are small in size, containing relatively little cytoplasm and polymorphic nuclei, scattered throughout the middle zone of the mucosa (Figs. 2A-B). The basal cells are uniformly distributed in the basal zone of the olfactory mucosa, just above the basement membrane (Figs. 1E, 2A-B). They are oval in shape with rounded nuclei containing distinct chromatin material. Occasional migration of basal cells into the upper and middle zones suggests that these cells are undergoing transformation into other cellular components as required by the mucosa. The supporting cells are categorized into two types: ciliated supporting cells and non-ciliated supporting cells or stratified epithelial cells (Fig. 2A). The ciliated supporting cells are tall, columnar and densely ciliated, predominantly confined to the proximal region of the lamellae (Figs. 1A, 2B). These cells are compactly arranged within the mucosa. Their nuclei are oval in shape and located in the proximal limb, whereas the distal limb is broad and elongated, bearing numerous cilia that project into the interlamellar spaces. The cytoplasm appears granular in nature. The non-ciliated supporting cells are scattered throughout the distal zone of the olfactory lamellae and surround the olfactory rosette. These cells are relatively short, with nuclei positioned in their distal limbs (Figs. 2A-B). The nuclei are deeply stained, displaying faint chromatin material and a distinct nucleolus, while the limbs exhibit strong phloxinophilic staining. The mucous cells are abundantly distributed in the upper zone of the lamella, interspersed among the supporting cells.

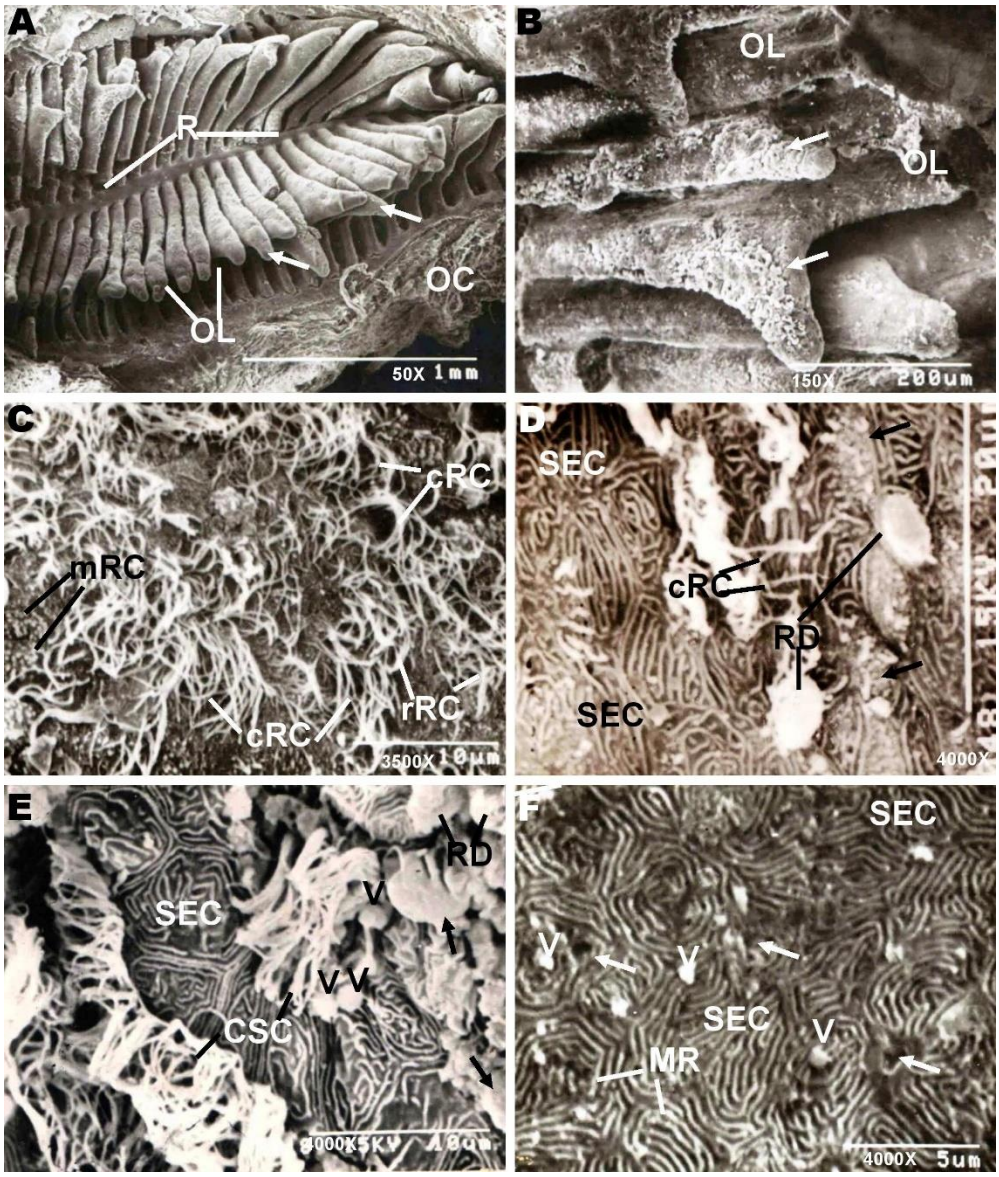

Fig.3. Photomicrographs of the surface architecture of the olfactory organ in *M. gulio* obtained by Scanning Electron Microscopy (SEM). **(A)** The olfactory rosette within the olfactory chamber (OC) exhibits multiple olfactory lamellae (OL) radiating from the median raphe (R). Note the dorsal lamellar processes (arrows) present on the lamellae. **(B)** OL displays sensory islets located within the dorsal lamellar processes (arrows). **(C)** A sensory islet reveals dendritic processes of ciliated receptor cells (cRC) and rod receptor cells (rRC), interspersed with microvillous receptor cells (mRC). **(D)** The epithelial surface shows a few cRC, stratified epithelial cells (SEC), rodlet cells (RD) and chloride-like cells (arrows). Blood cells (BC) are also evident. **(E)** The non-sensory epithelium displays diffuse patches of ciliated supporting cells (CSC) surrounded by SEC. Note the presence of mucin balls (arrow heads) and BC. **(F)** The raphe is lined with compactly arranged SEC bearing conspicuous microridges (MR). Observe the openings of mucous cells (solid arrows) and the secreted mucin masses (arrow heads) overlying the SEC.

They are elliptical in shape, lack nuclei and exhibit active muciferous secretion (Fig. 1E).

Surface architecture: The olfactory rosette is an elongated structure with a convex ventral surface and a concave dorsal surface, situated within the olfactory chamber (Fig. 3A). It comprises a midline raphe bearing two rows of lamellae, ranging from 32 to 38 in number and varying in size on each side. The lamellae are attached to the chamber wall by their convex inner margins and to the raphe by their proximal ends. The olfactory rosette rests on a horizontal platform formed by the raphe, with the largest lamellae situated medially and the smallest at both extremities. The lamellae are flattened and closely arranged, leaving narrow interlamellar spaces. Each lamella is dorsally free and bears distinct

lamellar processes that are directed towards the periphery of the rosette.

The sensory and nonsensory regions are distinctly distributed on each lamella. Receptor cells are confined exclusively to the dorsal lamellar processes (Fig. 3B) and are classically of two types-bearing either cilia or microvilli, with a third rod-shaped type distinguished by their surface morphology. The ciliated receptor cells possess an axon-like process directed toward the mucosal lining. Due to the dense aggregation of cilia, the sensory islets exhibit a hairy appearance (Fig. 3C). The microvillous receptor cells, characterized by numerous microvillus-like projections on their apical surface, display a molded appearance and are interspersed among the ciliated receptor cells. The rod receptor cells are distinguished by a single, slender cytoplasmic rod projecting from the free surface and are devoid of both cilia and microvilli.

The surface of non-sensory epithelium displays numerous ciliated non-receptor cells arranged as ciliary tufts while the supporting cells exhibit epidermal surface patterns characterized by prominent microridges (Fig. 3E). The chloride-like cells show distinct specialization, characterized by minute surface projections and a unique morphology that imparts a peculiar appearance (Fig. 3D). These cells often exhibit small apical invaginations or pits and, in some instances, convex protrusions are also visible. Rodlet cells are interspersed among the supporting cells and exhibit minute cytoplasmic protrusions that extend toward the mucosal surface (Fig. 3D). Distinct mucous cells are scattered throughout the non-sensory epithelium, with a substantial accumulation of mucin particles. In certain areas, the presence of blood cells is also noted (Fig. 3E). The raphe is composed of oval-shaped stratified epithelial cells having fingerprint-like patterns and interspersed with mucous cells (Fig. 3F).

Fine structure: The olfactosensory epithelium comprises three distinct types of receptor cells: ciliated, microvillous and rod cells (Fig. 4A). These receptor cells are differentiated by their characteristic apical dendritic specializations. The ciliated receptor cell is characterized by elongated dendrites containing longitudinally arranged microtubules that originate from a basal body (Fig. 4C). The nucleus is located in the basal region of the cell, while free ribosomes and clusters of mitochondria are concentrated in the apical portion of the dendrite. Additionally, junctional complexes are formed between adjacent microvillous receptor cells. Microvillous receptor cells possess numerous microvilli on their apical surface (Figs. 4C-E). The nucleus is located in the basal portion of the cell. The cytoplasm contains abundant ribosomes and mitochondria and is densely packed with electron opaque vesicles on the superficial end (Fig. 4E). Rod receptor cells bear a rod-shaped apical process projecting toward the mucosal surface and form junctional complexes with adjacent cells (Fig. 4D). The apical process contains longitudinally arranged microtubules along with basal bodies and centrioles are observed at its base. The cytoplasm is rich in free ribosomes, numerous mitochondria and electron-dense granules. In cross section, the microtubules of both ciliated and rod receptor cells show the classical 9 + 2 ordering, characterized by nine outer doublet microtubules encircling a central pair of single microtubules.

Chloride-like cells contain numerous microvesicles and branched tubules (Fig. 4F). They possess a spherical nucleus with dispersed heterochromatin. The perinuclear region is rich in rough endoplasmic reticulum and free ribosomes. The remaining cytoplasm is densely packed with mitochondria bearing well-developed lamellar cristae and traversed by an extensive tubular reticulum network. Supporting cells are located near the epithelial surface. They possess round to oval nuclei, and their cytoplasm contains electron-lucent granules. Supporting cells are located near the epithelial surface (Fig. 4A). They have round to oval nuclei and their cytoplasm contains electron lucent granules (Fig. 4F). Basal cells are situated adjacent to the basement membrane. They are typically small in size and possess a lobulated nucleus with uniformly distributed chromatin (Figs. 4B, 5C). Mast cells possess a dense nucleus and cytoplasm that is rich in electron density (Fig. 4B). Mucous cells,

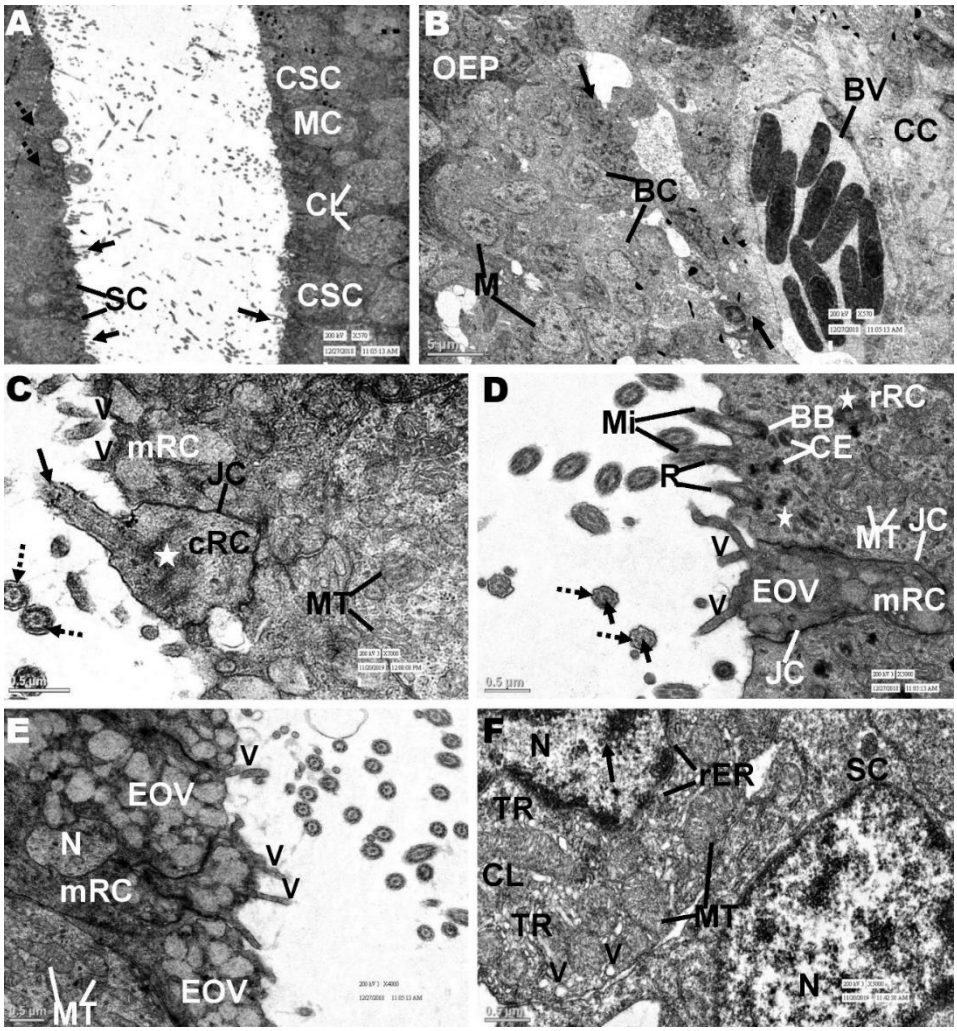

Fig.4. Photomicrographs of the olfactory mucosa of *M. gulio* by Transmission Electron Microscopy (TEM). **(A)** The olfactory epithelium is composed of ciliated receptor cells (solid arrows), rodlet cells (broken arrows), chloride-like cells (CL), ciliated supporting cells (CSC), non-ciliated supporting cells (SC) and mucous cells (MC). **(B)** OEP is delineated from the central core (CC) by the basement membrane (arrows). The existence of mast cells (M) and basal cells (BC) situated above the basement membrane, as well as the blood vessels (BV) located within CC. **(C)** The ciliated receptor cell (cRC) exhibits a cylindrical dendrite (solid arrow) that originates from the basal body (asterisk) and is densely populated with mitochondria (MT). The broken arrows indicate the cross section of the cilia. The microvillus receptor cell (mRC) adorned with microvilli (arrow heads), establishes a junctional complex (JC) with the adjacent cRC. **(D)** The rod receptor cell (rRC) displays microtubules (Mi) arranged in parallel, originating from the basal body (BB). The rRC, which contains mitochondria (MT), centrioles (CE) and electron-dense granules (asterisks), forms tight junctional complexes (JC) with the mRC that possesses microvilli (arrow heads). The cross section of the cilia reveals 9 pairs of outer microtubules (broken arrows) and 2 central microtubules (solid arrows). EOV refers to electron opaque vesicles. **(E)** Microvillous receptor cells (mRC), characterized by the presence of microvilli (arrow heads), house nuclei (N), mitochondria (MT) and electron dense vesicles (EOV). **(F)** Chloride-like cells (CL) exhibit a nucleus (N) characterized by dispersed heterochromatin (solid arrow) that is encircled by rough endoplasmic reticulum (rER), mitochondria (MT) displaying a lamellar structure, tubular reticulum (TR) and vesicular structures (arrow heads).

which are oval-shaped, can be found in the non-sensory areas and are filled with secretory granules and vesicles (Fig. 5A). Ciliated supporting cells feature an extensive apical surface embellished with a multitude of cilia (Fig. 4A). Their apical cytoplasm is filled with electron lucent vesicles. In cross-sectional views, the cilia display nine peripheral doublet microtubules encircling a central pair of microtubules (Fig. 5A).

Rodlet cells exhibit a polyhedral morphology and are filled with a multitude of electron-dense and electron-lucent granules. They possess a basally located nucleus containing both euchromatin and heterochromatin. These cells are distinguished by the

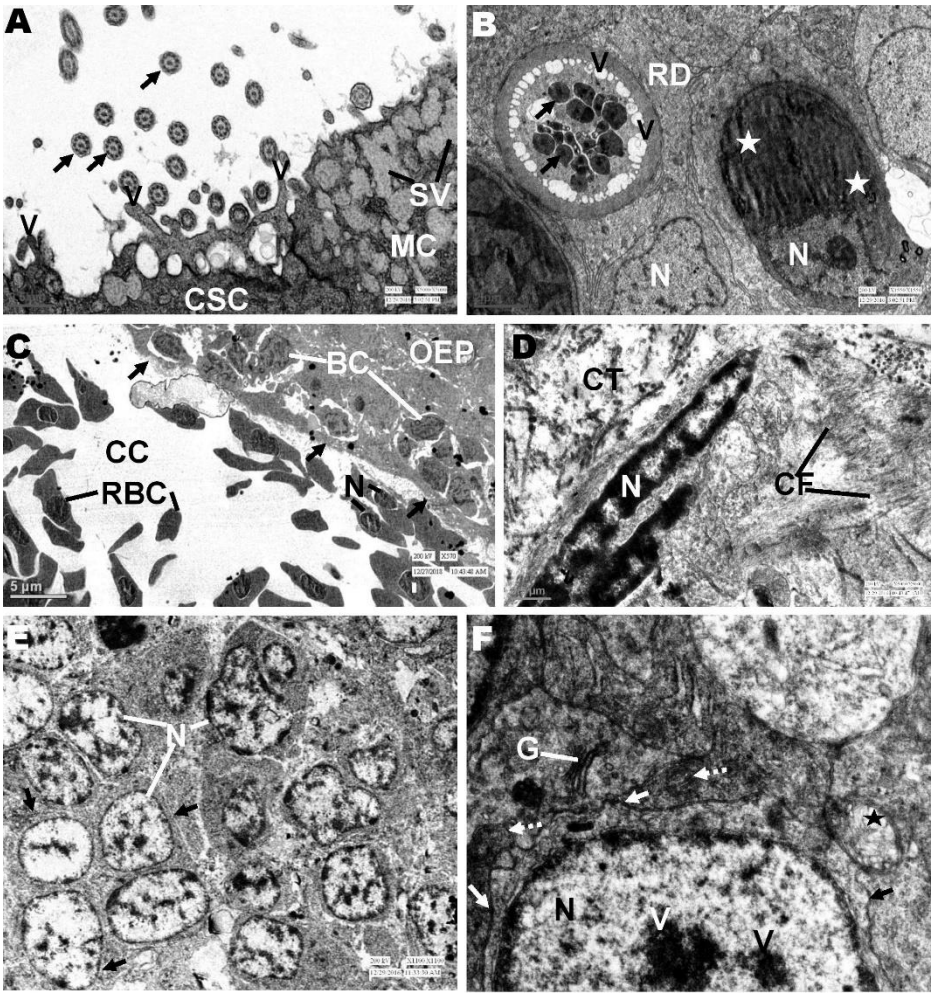

Fig.5. Photomicrographs depicting the olfactory mucosa of *M. gulio* as observed through Transmission Electron Microscopy (TEM). **(A)** The non-sensory mucosa consists of mucous cells (MC) filled with secretory vesicles (SV), alongside ciliated supporting cells (CSC) that possess non-sensory cilia (arrow heads). Solid arrows denote the transverse section of the cilia. **(B)** Rodlet cells exhibit nuclei (N) located at the base, densely packed with numerous electron-dense granules (asterisks). The presence of dark spots (solid arrows) and vesicles (arrow heads) is noteworthy. **(C)** The central core (CC) contains a cluster of red blood cells (RBC) with distinct nuclei (N), separated from the olfactory epithelium (OEP) by the basement membrane (solid arrows). Basal cells (BC) are observed near the BM. **(D)** Collagen fibers (CF) are present within the connective tissue (CT) of the CC. N represents nuclei. **(E)** The raphe displays stratified epithelial cells with nuclei (N) encircled by rough endoplasmic reticulum (arrows). **(F)** A magnified view of the stratified epithelial cells reveals prominent nuclei (N) with condensed heterochromatin (arrow heads) surrounded by rough endoplasmic reticulum (solid arrows). Additionally, the presence of the Golgi complex (G), mitochondria (broken arrows) and vesicles (asterisk) beside nucleus is noted.

presence of characteristic dark spots (rodlets) within the cytoplasm. The cytoplasm is also rich in translucent vesicles, which push the nucleus toward the deeper part of the cell (Fig. 5B).

A unique basement membrane separates the olfactory epithelium from the central core (Figs. 4B, 5C), which is composed of blood vessels, nerve fibers and connective tissues that include collagen fibers (Fig. 5D). The raphe comprises supporting cells that possess nuclei of diverse shapes, characterized by fragmented heterochromatin (Fig. 5E). The cytoplasm is enriched with free ribosomes, rough endoplasmic reticulum, mitochondria and vesicles (Fig. 5F).

DISCUSSION

Olfaction is linked to the evolution of chemosensory systems, with various teleosts releasing chemical signals into aquatic environments (Hara 2000). The apical dendritic regions of receptor cells that line the olfactory mucosa detect olfactory cues, leading to observable behaviors exhibited by organisms. The ecological niche occupied by a fish species

significantly influences its olfactory structure and degree of specialization (Kuciel et al. 2011). *M. gulio* possesses elongated olfactory rosettes and contains 32 to 38 lamellae, categorizing it under Teichmann's (1954) third group, known as nose fishes, where olfaction is superior to vision. This type of olfactory organ is classified under Pol Gerard's (1954) third category, referred to as macrosmic, indicating that the fish has a highly developed sense of smell for locating food. Based on the distribution of lamellae, it is classified as Yamamoto (1982) Type-H, characterized by a double-sided, comb-like appearance along the midline raphe. The lamella has a specialized lamellar process that facilitates the movement of water over the olfactory organ. This arrangement may be due to the fact that the sensory islets, which contain chemosensory cells, are oriented towards the flow of the incoming water current. The arrangement of sensory and non-sensory epithelium on the lamellae is not continuous and aligns with Type-II (Yamamoto 1982).

The sensory epithelium consists of three morphoanatomically distinct types of neurons: ciliated, microvillous and rod-like projections. Although these neuron types coexist, their proportions differ across various species (Zeiske et al. 2003). Ciliated receptor cells are associated with Type-I cells, as noted by Yamamoto and Ueda (1978). Microvillous receptor cells are linked to Type-II cells, as described by Müller & Marc (1984), while rod receptor cells correspond to Type-IV cells identified by Ichikawa and Ueda (1977). According to Hamdani & Døving (2007), each of these neuron types transmits unique chemical signals that provoke specific behavioral responses to olfactory cues, which are essential for various life processes in fish. The distal end of the ciliated receptor cells features an apical swelling, referred to as the olfactory knob, which has been recognized as the site of receptor locations for odorants (Hansen et al. 2004). These neurons play a crucial role in the initial detection of odors and the transduction of odorant molecules (Simpson 2018). Kuciel et al. (2011) indicated that the apical region of the receptor cell, which possesses cilia, is responsible for specific interactions with ligands. Microvillous receptor cells, distinguished by their surface microvilli, are involved in the detection of pheromones and various chemical substances present in the aquatic environment (Mokhtar & Abd-Elhafeez). Hernádi (1993) noted that the existence of rod receptor cells may be a response to a new physiological environment, while Yamamoto (1982) proposed that they could also develop due to the aging of ciliated receptor cells. Theisen (1972) indicated that microtubules within the dendrites of receptor neurons likely play a crucial role in the recognition of chemical signals. A cluster of mitochondria in the receptor cell implies that they supply the necessary energy for the movement of cilia.

The supporting cells involved in olfactory reception have not been reported to have significance beyond mere structural support and the secretion of products onto the epithelial surface (Hara 1994). According to Yamamoto & Ueda (1977), the presence of a directed water flow over the lamellae is suggested by the cilia of the supporting cells being uniformly bent in one direction across a significant portion of the mucosa. Simpson (2018) noted that ciliary clusters do not exhibit chemosensation but may serve a role in providing mechanical support. The supporting cells, characterized by microridges, are believed to assist in supporting tissues that are subjected to abrasive forces (Uehara et al. 1991). The microridges located on the apical surface of supporting cells help maintain the mucus layer across the mucosa and protect sensory cells from mechanical damage (Mandal et al. 2005). Mucous produces mucin to safeguard the mucosa against mechanical wear and facilitate the smooth passage of water during ventilation. The mucin that is secreted likely aids in binding small debris and keeps the sensory neurons ready for new stimuli (Bandyopadhyay & Datta 1996). Banerjee (1993) proposed that the mucus layer acts as an ion trap, hindering the penetration of heavy metals into the underlying cells. Chloride-like cells within the olfactory organ are likely involved in creating a specific ionic environment on the surface of the olfactory mucosa. Consequently, they are considered

a specific electrogenic mechanism that secretes potassium into the receptor media (Ruzhinskaya et al. 2001). Mast cells located in the olfactory mucosa are thought to have a significant function in the reproduction of *Labeo rohita* (Bhute & Baile 2007) as well as Baltic trout (Bertmer 1982). These cells are capable of altering the metabolic activity of receptors, which in turn affects the sensitivity of the olfactory epithelium. The basal cells appear to function as stem cells, becoming active during cell turnover and the regeneration of the cellular components of the olfactory mucosa (Frabman 1994). New olfactory receptor cells are generated by the undifferentiated basal stem cells (Fitzgerald et al. 2012). Rodlet cells represent the most intriguing feature of the olfactory mucosa observed in this study. Their nature and function remain a subject of debate; previous researchers have characterized them as modified goblet cells (Vickers 1962), secretory cells with holocrine activity (Leino 1974; Mokhtar & Abd-Elhafeez 2014), or regulators of ion transport and osmoregulation (Bielek 2005). Furthermore, they have been described as a type of eosinophilic granulocyte (Reite & Evensen 2006). Reite (2005) further suggests that rodlet cells function as non-specific immune cells, noting that their population increases significantly during parasitic infections.

CONCLUSION

M. gulio possesses a well-developed olfactory system, enabling the detection of chemical stimuli through its multilamellar olfactory organ. The presence of diverse sensory neurons within the olfactory mucosa facilitates a robust response to chemical fluctuations in the aquatic environment. To further elucidate the complexities of chemoreception and its specific influence on fish behavior, additional experimental research is suggested.

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REFERENCES

Al-Zahaby, S.A.; Al-Zahaby, A.S.; Mohamed, R.A. & Elsheikh, E.H. 2025. Ultrastructural and developmental anatomy of the peripheral olfactory organs of *Dicentrarchus labrax* inhabiting Egyptian Mediterranean water. Open Veterinary Journal 15(2): 939-953.

Anderson T.F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Transactions of the New York Academy of Sciences 13(4):130-134.

Atta, K.I. 2013. Morphological, anatomical and histological studies on the olfactory organs and eyes of teleost fish: *Anguilla anguilla* in relation to its feeding habits. Journal of Basic and Applied Zoology 66(3): 101-108.

Bandyopadhyay, S.K. & Datta, N.C. 1996. Morphoanatomy and histology of the olfactory organs of air-breathing catfish, *Heteropneustes fossilis* (Bloch). The Journal of Animal Morphology and Physiology 43(1): 85-96.

Banerjee, T.K. 1993. Response of fish skin to certain ambient toxicants. In: Singh, B.R. (eds.), *Advances in fish research.* Narendra Publishing House, Delhi. pp. 185-192.

Bertmar, G. 1972. Labyrinth cells, a new cell type in vertebrate olfactory organs. Zeitschrift fur Zellforschung und mikroskopische Anatomie 132(2): 245-256.

Begum, M.; Alam, M.J.; Islam, M.A. & Pal, H.K. 2008. On the food and feeding habit of an estuarine catfish (*Mystus gulio* Hamilton) in the south-west coast of Bangladesh. University Journal of Zoology Rajshahi University 27: 91-94.

Bhute, Y.V. & Baile, V.V. 2007. Organization of the olfactory system of the Indian major carp *Labeo rohita* (Ham.): a scanning and transmission electron microscopy study. Journal of Evolutionary Biochemistry and Physiology 43(3): 342-349.

Bielek, E. 2005. Development of the endoplasmic reticulum in the Rodlet Cell of two teleost species. Anatomical Record 283(1): 239-249.

Bozzola, J.J. & Russel, L.D. 1992. *Electron microscopy, principles and techniques for biologists*. Jones & Bartlett Learning, Burlington.

Camacho, S.; Ostos-Garrido, M.V.; Domezain, A. & Carmona, R. 2010. Study of the olfactory epithelium in the developing sturgeon characterization of the crypt cells. Chemical Senses 35(2): 147-156.

Chakrabarti, P. & Ghosh, S.K. 2009. Ultrastructural organisation and functional aspects of the olfactory epithelium of *Wallago attu* (Bleeker). Folia Morphologica 68(1): 40-44.

Chen, X. & Arratia, G. 1994. Olfactory organ of Acipenseriformes and comparison with other actinopterygians: patterns of diversity. Journal of Morphology 222(3): 241-267.

Dymek, J.; Kuciel, M. & Żuwała, K. 2021. Structural diversity of olfactory organs in Osteoglossiformes. Journal of Zoology 314(1): 43-57.

Dymek, J.; Dymek, A.M.; Kuciel, M. & Żuwała, K. 2024. Macro- and micro morphology of the olfactory organ of African bonytongue, *Heterotis niloticus* (Cuvier 1829), compared with other species of the family Osteoglossidae (Teleostei). Zoology 163: 126156.

Farbman, A.I. 1994. The cellular basis of olfaction. Endeavour 18(1): 2-8.

Fitzgerald, E.F.; Shrestha, S.; Gomez, M.I.; McCaffrey, R.J.; Zimmerman, E.A.; Kannan, K. & Hwang, S. 2012. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and neuropsychological status among older adults in New York. Neurotoxicology 33(1): 8-15.

Ghosh, S.K. 2022. Structure and function of the olfactory organ in humped featherback, *Chitala chitala* (Hamilton, 1822). In: Dey, S.; Sen, K. & Ghosh A.R. (eds.), *Sustainable Aquaculture Practices*, LAP Lambert Academic Publishing, Republic of Moldova. pp. 22-36.

Glauert, A.M. 1975. *Fixation, dehydration and embedding of biological specimens*. Elsevier and North-Holland, New York.

Gon, O. & Fishelson, L. 2009. Nasal olfactory organs and olfactory bulbs in Blennies. In: Patzner, R.A.; Goncalves, E.J.; Hastings P.H.A. & Kapoor, B.G. (eds.), *the biology of Blennies*. Science Publishers, Enfield, NH. USA. pp. 118-213.

Hamdani. E.H. & Døving, K.B. 2007. The functional organization of the fish olfactory system. Progress in Neurobiology 82(2): 80-86.

Hansen, A.; Anderson, K. & Finger, T.E. 2004. Differential distribution of olfactory receptor neurons in goldfish: structural and molecular correlates. Journal of Comparative Neurology 477(4): 347-359.

Hara, T.J. 1994. Olfaction and gustation in fish: an overview. Acta Physiologica 152(2): 207-217.

Hara, T.J. 2000. Chemoreception. In: Ostrander, G.K. (eds.), *The laboratory fish*. Academic press, London. pp. 471-479.

Hernádi. L. 1993. Fine structural characterization of the olfactory epithelium and its response to divalent cations Cd^{2+} in the fish *Alburnus alburnus* (Teleostei, Cyprinidae): a scanning and transmission electron microscopic study. Neurobiology 1(1): 11-31.

Ichikawa, M. & Ueda, K. 1977. Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*. A study of retrograde degeneration. Cell and Tissue Research 183(4): 445-455.

Klimenkov I.V.; Pyatov, S.K. & Sudakov, N.P. 2023. Structural and functional features of the olfactory epithelium in fish. Limnology Freshwater Biology 2023(6): 190-203.

Kuciel, M.; Żuwala, K. & Jakubowski, M. 2011. A new type of fish olfactory organ structure in *Periophthalmus barbarous* (Oxudercinae). Acta Zoologica 92(3): 276-280.

Leino, RL. 1974. Ultrastructure of immature, developing and secretory rodlet cells in fish. Cell and Tissue Research 155(3): 367-381.

Luft, J.H. 1961. Improvements in epoxy resin embedding methods. The Journal of Biophysical and Biochemical Cytology 9(2): 409-414.

Mallory, F.B. 1936. The aniline blue collagen stain. Stain Technology 11(3): 101.

Mandal, D.K.; Roy, D. & Ghosh, L. 2005. Structural organization of the olfactory epithelium of a spotted snakehead fish, *Channa punctatus*. Acta Ichthyologica et Piscatoria 35(1): 45-50.

Mondal, A. & Mitra, A. 2016. Growth, food and feeding habit with prey preference of long whiskered catfish, *Mystus gulio* (Hamilton, 1822) in brackishwater traditional impoundments of Sundarban, India. International Journal of Fisheries and Aquatic Studies 4(6): 49-58

Misra, K.S. 2003. *An aid to the identification of the common commercial fishes of India and Pakistan*.

Narendra Publishing House, New Delhi.

Mokhtar, D.M. & Abd-Elhafeez, H.H. 2014. Light-and electron-microscopic studies of the olfactory organ of red-tail shark, *Epalzeorhynchos bicolor* (teleostei: cyprinidae). Journal of Microscopy and Ultrastructure 2(3): 182-195.

Muller, J.F. & Marc, R.E. 1984. Three distinct morphological classes of receptors in fish olfactory organs. Journal of Comparative Neurology 222(4): 482-495.

Pol Gerard, 1954. Organe olfactif. Traite de Zoologie 12: 522-533.

Reite, O.B. 2005. The rodlet cells of teleostean fish: their potential role in host defense in relation to the mast cells/eosinophilic granule cells. Fish & Shellfish Immunology 19(3): 253-267.

Reite, O.B. & Evensen, Q. 2006. Inflammatory cells of teleostean fish: a review focusing on mast cells/eosinophilic granule cells and rodlet cells. Fish & Shellfish Immunology 20(2): 192-208.

Ruzhinskaya, N.N.; Gdovskii, P.A. & Devitsina, G.V. (2001). Chloride cells, a constituent of the fish olfactory epithelium. Journal of Evolutionary Biochemistry and Physiology 37(1): 89-94.

Simpson, K.L. 2018. Olfaction and Taste. In: Haines, D.E. & Mihailoff, G.A. (eds.), *Fundamental Neuroscience for Basic and Clinical Applications*. Elsevier, Philadelphia. pp. 334-345.

Suvarna, S.K.; Christopher, L. & Bancroft, J. D. 2019. *Bancroft's theory and practice of histological techniques*. 8th Edition. Elsevier, UK.

Teichmann, H. 1954. Vergleichende Untersuchungen an der Nase der Fische. Zeitschrift für Morphologie und Ökologie der Tiere 43(2): 171-212.

Theisen, B. 1972. Ultrstructure of the olfactory epithelium in the Australian lungfish, *Neoceratodus forsteri*, Acta Zoologica 53(2): 205-218.

Uehara, K.; Miyoshi, M. & Miyoshi, S. 1991. Cytoskeleton in microridges of the oral mucosal epithelium in the carp (*Cyprinus carpio*). Anatomical Record 230(2): 164-168.

Vickers, T. 1962. A study of the intestinal epithelium of the goldfish *Carassius auratus*: its normal structure, the dynamics of cell replacement and the changes induced by salts of cobalt and manganese. The Quarterly Journal of Microscopical Science 103(s3): 93-110.

Wakisaka, N.; Miyasaka, N.; Koide, T., Masuda, M.; Hiraki-Kajiyama, T. & 2017. An adenosine receptor for olfaction in Fish. Current Biology 27(10): 1437-1444.

Yamamoto, M. & Ueda, K. 1977. Comparative morphology of fish olfactory epithelium-I. Salmoniformes. Bulletin of the Japanese Society of Scientific Fisheries 43(10): 1163-1174.

Yamamoto, M. & Ueda, K. 1978. Comparative morphology of fish olfactory epithelium-III. Cypriniformes. Bulletin of the Japanese Society of Scientific Fisheries 44(11): 1201-1206.

Yamamoto, M. 1982. Comparative morphology of the peripheral olfactory organ in teleosts. In: Hara, T.J. (eds.), *Chemoreception in fishes*. Elsevier, Amsterdam. pp. 35-59.

Zeiske, E.; Kasumyan, A.; Bartsch, P. & Hansen, A. 2003. Early development of the olfactory organ in sturgeons of the genus *Acipenser*: A comparative and electron microscopic study. Anatomy and Embryology 206(5): 357-372.

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مقاله کامل

اندام بویایی گربه‌ماهی سبیلک‌بلند، *Mystus gulio* (Hamilton, 1822)؛ هیستومورفولوژی و فراساختار

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چکیده:

اندام بویایی گربه‌ماهی *Mystus gulio* (Siluriformes: Ailiidae) با استفاده از میکروسکوپ نوری و میکروسکوپ‌های الکترونی (روبشی و عبوری) مورد بررسی قرار گرفت تا سازمان‌یافتگی ساختاری آن آشکار شود. غنچه بویایی کشیده و شامل ۳۲ تا ۳۸ لاملا است که در امتداد یک تیغه میانی مرکزی آرایش یافته‌اند. هر لاملای بویایی از دو بخش اصلی تشکیل شده است: اپی‌تلیوم بویایی (مخاط) و تیغه میانی. مخاط دارای تمایز مشخص به نواحی حسی و غیرحسی بود که هر دو دارای ترکیب سلولی متمایزی هستند و براساس ویژگی‌های ساختار بافتی، تمایلات رنگ‌پذیری، ریخت سطحی، الگوی توزیع و ویژگی‌های فراساختاری قابل شناسایی‌اند. مخاط حسی شامل سه نوع سلول گیرنده بود: دو شکل معمول که یکی دارای مژک و دیگری دارای میکروویلی است، و نوع سوم که دارای تخصص‌یافتگی رأسی میله‌ای‌شکل است. مخاط غیرحسی شامل سلول‌های مخاطی، سلول‌های شبه کلراید، سلول‌های رودلت و سلول‌های ماست بود، همراه با دو نوع سلول پشتیبان که به‌صورت مژک‌دار و غیرمژک‌دار طبقه‌بندی می‌شوند. سلول‌های پایه در عمق مخاط بویایی و در مجاورت هسته مرکزی قرار داشتند. از نظر ساختاری، تیغه از سلول‌های اپی‌تلیالی و فشرده تشکیل شده بود که دارای ریزبرجستگی‌های مشخص در سطح رأسی بودند. این مطالعه به بررسی نقش عملکردی انواع مختلف سلول‌های تشکیل‌دهنده مخاط بویایی پرداخته و اهمیت آن ها را در فرآیند ادراک بویایی در *M. gulio* مورد تأکید قرار می‌دهد.

کلمات‌کلیدی: میکروسکوپی بافتی، SEM، TEM، حس بویایی، *Mystus gulio*