Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 5, 793-798

Olfactory epithelium organization of the grass carp (*Ctenopharyngodon idella*) at the ultrastructural level: SEM and TEM observations

Eman H. Elsheikh, Sahar S. Hassan, Sheren A. Al-Zahaby*

Zoology Department, Faculty of Science, Zagazig University, Zagazig 44519, Egypt.

ARTICLE INFO

Recieved: 24 November 2023

Accepted: 30 January 2024

***Correspondence:**

Corresponding author: Sheren A. Al-Zahaby
E-mail address: Shalahmady@yahoo.com

Keywords:

Cyprinidae
Grass Carp
Olfactory Rosette
Sensory receptor cells
Scanning Electron Microscopy
Transmission Electron Microscopy

ABSTRACT

Olfaction is the major sense of smell in teleost involved in many physiological response and habitat acclimatization including food searching, migration for spawning, predator avoidance, reproduction behavior, as well as identification of fish of the same species. Our study illustrates the ultrastructure of the olfactory rosette of the grass carp *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844) by using Scanning Electron Microscope (SEM) and transmission Electron Microscope (TEM). Herein, the peripheral olfactory organs are represented by two olfactory rosettes lying in two nasal chambers, one on each side of fish snout. Each nasal chambers opens to the exterior by two nostrils a narrow inlet and wider outlet, segregated by a somewhat elevated, nasal bridge. The two nostrils are somewhat faraway as far as length of rosette's length to permit entering and leave water flow bearing odorant molecules to the nasal cavities. The SEM revealed that each olfactory rosette is elongated oval-shaped 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. Moreover, the lamellar surface comprises sensory and non-sensory areas concealed in a mucous layer and not distributed uniformly within the epithelial surface of the olfactory lamellae. Accordingly, The TEM observations indicated that the sensory areas holds four main receptor neurons, two are major including ciliated and microvillous receptor cells bearing either cilia or microvilli, respectively emitted from a dendritic knob. Additionally, two other minor rod-tipped and crypt cells bearing a compound rod cilium, or few microvilli and occult cilia emitted also from dendritic knob were rarely observed. From the other side, the non-sensory area comprises cylindrical flat top surface; ciliated non-sensory cells with motile long kinocilium and nonciliated stratified epithelial cells with fingerprint-like microridges both are mainly of supporting in addition of and ovoid goblet mucous cells in-between and stem basal cells. Collectively, our study revealed the general organization and ultrastructure of an important economic teleost fish that affects feeding habitat and has an important influence on the fish food intake.

Introduction

Olfaction is one of the most important senses. Since, fish live collectively in water, their peripheral olfactory organs are directly exposed to the ambient aquatic environment so the detectible olfactory chemicals must be soluble in water (Hara, 1975). The importance of olfactory senses are extremely appreciated, since it involves in various life activities including food finding, homing and reproductive behavior, predator avoiding and social interaction particularly for fishes living in permanent darkness in caves (Kasumyan, 2004; Chakrabarti and Ghosh, 2011; Waryani *et al*., 2015).

Primarily, fish olfactory organs are developed from the epithelium lined nasal cavity, which is raised up into a series of folds, lamellae, to make rosettes (Hara, 1975). The arrangement, shape and degree of development of lamellae in the rosette vary considerably from species to species. At one extreme they are well developed (macrosmatic) such as in eels (Atta, 2013) and in other they are poorly developed (microsmatic) such as in pike (Esox lucius) (Garwood *et al*., 2020). However, in all cases, since the olfactory epithelium is exposed continually to the ambient water with loaded hazard ingredients, it is considered for its very sensitive structure the first affected organ even than the skin (Al-Zahaby *et al*., 2023).

The receptor cells lining the olfactory epithelium are stimulated when they come in contact with water containing odorant molecules and transmit signals to the nervous system (Lara, 2008).The olfactory organs of fish (rosettes) display extensive diversity relying upon taxonomic groups, life styles and ecological adaptations (Zeiske *et al*., 2009).

Many investigations upon the macro- and microarchitecture of the olfactory epithelium of different teleost fishes are well-documented using light and electron microscope as mentioned pre and many others and many others (Ghosh *et al*., 2015; Ghodeswar *et al*., 2020; Triana-Garcia *et al*., 2021; Aicardi *et al*., 2022; Al-Zahaby *et al*., 2023). These studies exposed to enormous varieties regarding shape, number and arrangement of the olfactory lamellae, distribution of the sensory and non-sensory epithelium as well as the abundance of various receptor cell types in different fishes. Early, Hara revealed that olfactory epithelium of fish, like other vertebrates, consists essentially of four cell types: receptor cells, supporting cells, mucous and basal cells. (Hara, 1975). Cypriniformes to which belonging the grass carp (*Ctenopharyngodon idella*), have two olfactory organs each occupies most of nasal cavity or olfactory chamber. Olfactory organs are made up of many leaf-like transversely arranged lamellae radiated bilaterally from both sides of a narrow median raphe. These lamellae have been originated from the successive folding of the olfactory mucosa located on the floor of the olfactory chamber forming multilamellar olfactory structure rosette-shape (Hara, 1975). The two wide nostrils or olfactory chambers situated on the dorsal-lateral sides of the snout in front of the eyes. Each chamber as in all teleosts is communicated with the surroundings environment through two separate nasal

ISSN: 2090-6277/2090-6269/

apertures (nostrils). The anterior narrow inlet and posterior wide outlet segregated by a somewhat elevated flashy epidermal flap, nasal bridge, as in cyprinoid *Epalzeorhynchos bicolor* (Mokhtar and Abd-Elhafeez, 2014) and *Squalus acanthias* (Timm-Davis and Fish, 2015). This nasal bridge deflecting external water flow into the nasal chamber (Garwood *et al*., 2020). Through these inlet and outlet nostrils and rhythmic ventilation movement, ambient water overflows the containing olfactory rosette, which usually is of a convex ventral surface and a concave dorsal surface.

Grass carp (*Ctenopharyngodon idella*, Cuvier and Valenciennes) is an 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).

Materials and methods

Ethics approval

This study was applied according to the guidance of ethical animal treatment for the care and use of experimental animals approved by the Institutional Animal Care and Use Committee of the Zagazig University, Zagazig, Egypt (Approval number: ZU-IACUC/1/F/29/2023 accepted in 28/1/2023).

Fish Sampling and experimental design

Adult 8 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. Four fishes were dissected for Scanning Microscope observations and the others used for Transmission Electron Microscope.

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, Egypt.

Transmission Electron Microscope (TEM)

From the other dissected heads of grass carp, *Ctenopharyngodon idella*, the excised olfactory rosettes were carefully washed and immersed in saline solution to remove 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. From these fixed rosettes, some solitary lamellae were carefully cut into small pieces which immediately post-fixed in 2.5% glutaraldehyde solution and 1% Osmium tetroxide in the same buffer (2.5% in Phosphate buffer of pH 7.4) for 2 hours, at room temperature. The post fixed lamella's pieces were then dehydrated in a graded series of ethanol and embedded in an Epon-Araldite mixture which by using a Reichert ultra-microtome, ultrathin sections of 1 µm were obtained and stained with toluidine blue contrasted in a 50% alcohol-uranyl acetate solution and lead citrate as described by Ruzhinskaya *et al*. (2001). With a transmission Philips EM 400 electron microscope at the science faculty, Alexandria University, Egypt the olfactory lamellae were examined and photographed.

Results

The peripheral olfactory organs of 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 gets in and out to immerse olfactory organs, rosettes (Fig. 1A). The elongated oval-shaped 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. 1B, C). Indicating that the number and magnitude of lamellae increase as the fish grow and so the olfactory surface epithelium. The olfactory lamellae were lined with sensory and nonsensory 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.1D,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.1H.) or very short plentiful microvilli, microvillous receptor (Fig.1I.) or thick compound cilium (rod-tipped receptor), these later cells scattered in-between the other ciliated and microvillous receptor (Fig. 1I). The receptor cells are aggregated in separate islands in-between others of nonsensory epithelia of ciliated and nonciliated supporting cells of motile long cilia or microridges, respectively as well as mucous goblet cells (Fig.1F, G, I).

The receptor cells are categorized into ciliated with long motile cilia (Fig. 2A), microvillous with copious of very short microvilli (Fig. 2B) and rod-tipped of four axonemal patterns of 9+2 united in kinocilium are understandable (Fig. 2C). The cylindrical cell soma of all receptors embracing in their electron-lucent cytoplasm; plentiful vesicular mitochondria (Mit) in their upper third, endoplasmic reticulum and Golgi complex around upper border of their basal vesicular elongated electron dense nucleoplasm (Fig. 2A, B, C). The fourth receptor neurons, crypt cells are seldom observed only in the TEM examination of the grass carp. They are of pear-shaped or ovoid soma, situated close to the epithelial surface and provided with few apical microvilli as well as occult cilia emitted also from dendritic knob and extending into a crypt at apex (Fig. 2D).

The non-sensory epithelium basically is constituted of cylindrical lightly stained cells stretched from the basal lamina up to the epithelial surface. They are scattered in-between and around the sensory receptor

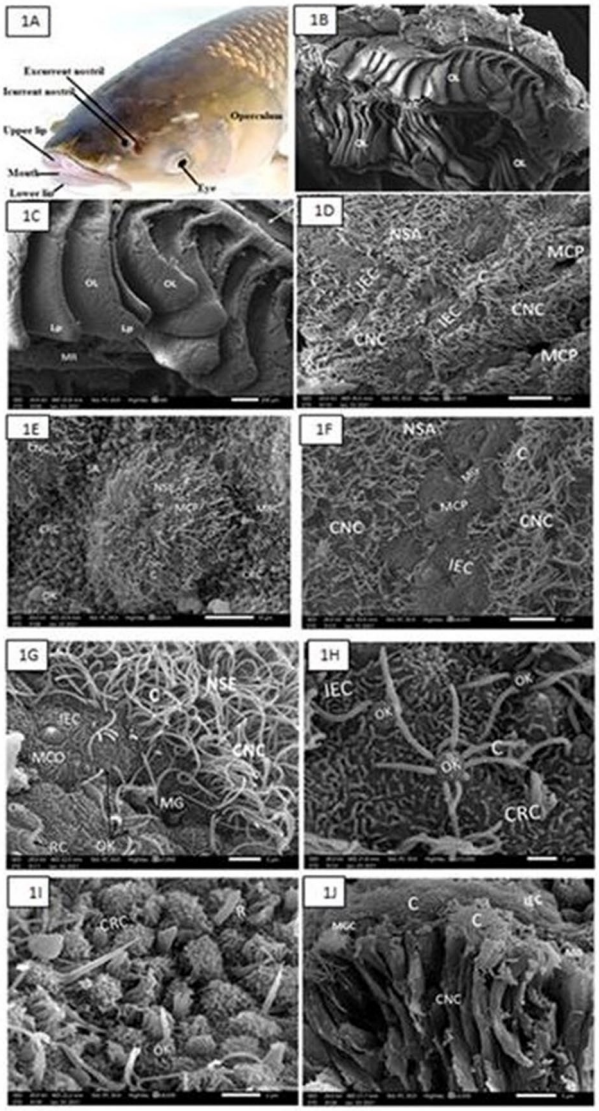

Fig.1. A) Photograph of the head of grass carp (*Ctenopharyngodon idella*, Val.) lateral view; showing the nostril with their anterior (incurrent) and posterior (excurrent) passage in the upper mid distance between eyes and mouth. B) SEM micrograph of the olfactory rosette of grass carp (*Ctenopharyngodon 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. C) Higher magnification of the previous SEM micrograph showing; the olfactory lamellae (OL) connection (Arrow) with the median raphe (MR), linguiform process (LP) of each lamella. X-60-Scale bar=200µm. D) SEM micrograph of grass carp's olfactory rosette showing; Nonsensory area (NSA) with strands of ciliated nonsensory 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. 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) characterized 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. 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. G) Highest 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. H) Highest 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. 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. 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.

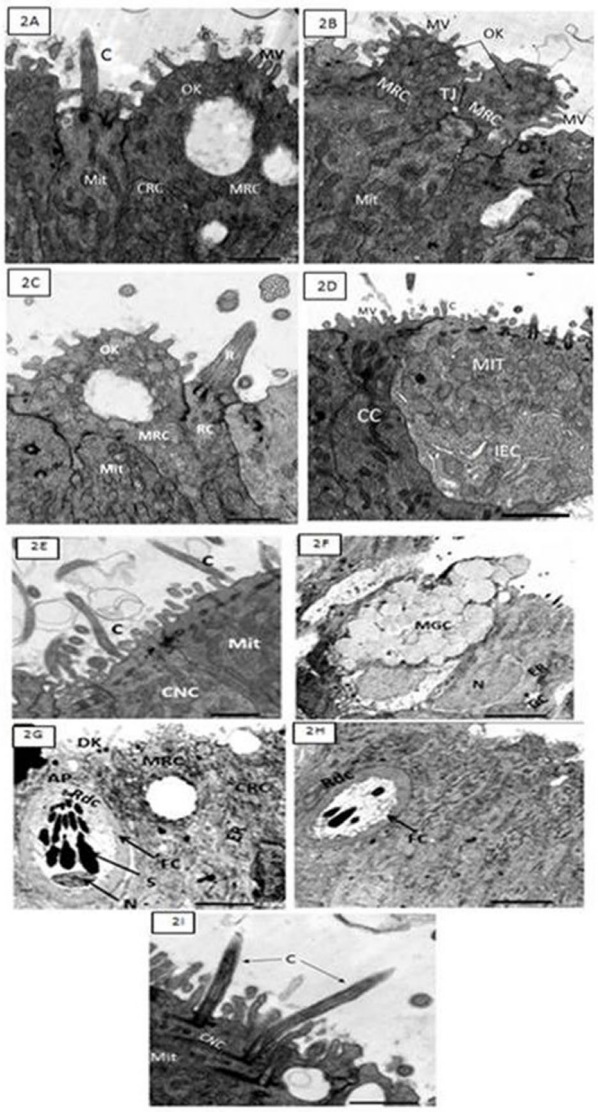

Fig. 2. A) TEM micrograph showing; ciliated receptor (CRC) cell with short thick cilia (C), Microvillous receptor cell (MRC) with microvilli (MV) Both have top mound surface, dendritic knob (OK) where their cilia and microvilli are emitted radially. Both cells have plentiful vesicular mitochondria (Mit) in the upper cell body region and laterally they exhibit zonula adherent in the form of desmosomes between the adjacent cells. X-6000, Scale bar: 1.0 µm. B) TEM micrograph showing; Microvillous receptor cell (MRC) with microvilli (MV) having top mound surface, dendritic knob (OK) where their microvilli are emitted radially, also have plentiful vesicular mitochondria (Mit) in the upper cell body region. Laterally they exhibited intercellular zonula adherent junctional (TJ) between the adjacent cells. X-6000, Scale bar: 1.0 µm. Rod-tipped (R). C) TEM micrograph showing; MRC with microvilli and RC with thick compound cilium (R) having longitudinally oriented microtubules aligned four axonemal patterns of 9+2 united in kinocilium. Both cells have dendritic knob (OK) and plentiful vesicular top surface mitochondria (Mit). Notice the adherent junctions (desmosomes) between the adjacent ciliated and rod cells. X-6000. D) TEM micrograph showing; Indifferent epithelial cell (IEC) in vicinity of crypt cell (CC) bears occult short cilia (C) surrounding apical microvilli (MV) and have plentiful vesicular top surface mitochondria (Mit). Notice the desmosomes around the crypt cell. X-4000, Scale bar: 2.0 µm. E) TEM micrograph showing; CNC with long cilia (C) emitted radially from flat top surface in addition plentiful vesicular mitochondria (Mit) in the upper cell body region. X6000, Scale bar: 1.0 µm. F) TEM micrograph showing; Mucus secretory goblet cell (MGC) with, tubular Endoplasmic reticulum (ER) Golgi complex (GC) around prominent basal nucleus (N) and realised mucus granules on the epithelial surface. X1500. G) TEM micrograph showing; Pear-shaped rodlet cell (RdC) in vicinity CRC and MRC both with dendritic knob (DK). Club-shaped rodlet sac (S) of different size are crowded in its cytoplasm of RdC with tubular rough ER, and basal nucleus (N) all are encircled with a dense fibrillar coat (FC) with narrow apical pore (AP) provided with apical part short microvilli, through which a rodlet sac (S) is released. Notice the adherent junctions (desmosomes) around fibrillar coat of the rodlet cells. X3000. H) TEM micrograph showing; Pear-shaped developing rodlet cell (RdC) intermediate setting in OE with smaller numbers of rodlet sacs and encloses in a fibrillar coat (FC). Notice the desmosomes around fibrillar coat of the rodlet cells. X3000. I) TEM micrograph showing; CNC with long cilia (C) emitted radially from flat top surface in addition plentiful vesicular mitochondria (Mit) in the upper cell body region in addition T.S. of some kinocilia showing axonemal pattern (9+2) of microtubules. X6000, Scale bar: 1.0 µm.

cells, so acting as supporting cells. Their broad distal free surface bears either cilia (ciliated non-sensory cells) or microridges (sustentacular indifferent epithelial cells).

The first ones have elongated oval nuclei with clear chromatin material, subnuclear endoplasmic reticulum and apical crowded mitochondrial vesicles, but their top surface is provided with characteristic tuft of long motile cilia established of axonemal pattern of (9+2) microfilaments so are entitled as kinocilia (Figs. 2C, E). They are scattered throughout the non-sensory and sensory epithelium as single cells or patchy clusters; probably facilitate olfactory ventilatory processes (Figs. 1F, G). Nonetheless, the second cell types possess larger almost spherical nuclei with clear chromatin material, supranuclear endoplasmic reticulum and apical crowded mitochondrial vesicles. Their comparatively broader free surface is provided with characteristic regular concentric microridges, so are sustentacular indifferent epithelial cells (Figs. 2C, D, E).

In addition to all above mentioned cells particularly in the non-sensory areas, another commonly presented cells, mucous goblet cell spreading among the supporting. They are of oval shape with weakly stained nuclei located at the cell base and are more crowded with mucus granules. They open on the olfactory epithelial surface with wide mucus openings in between the cilia and/or the microridges of both nonsensory cells, respectively (Fig. 1F, G & 2.F).

Moreover, another cell, strange controversial rodlet cell (RdC) which appeared in different cell developmental stages in between olfactory epithelium but are not specific to it (Fig. 2G, H). They are pear-shaped, settled down between the basal lamina (BL) and olfactory epithelial surface and bounded by a marked widening fibrillar cytoplasmic capsular wall (FC). The bulk of their electronlucent cytoplasm is occupied by many distinct opaque club-shaped sacs (rodlets) parallelly arranged with club heads oriented toward the cell's basal nucleus. The number of these rodlet sacs (S) increases by time at the expense of other all apical cellular constituents, endoplasmic reticulum, mitochondrial vesicles, and Golgi complex which are restricted apically far away from the nucleus. The cell's fibrillar wall (FC) is episodic by an apical narrow pore (AP) through which large amounts of club-shaped rodlet sacs (S) are extruded outside (Fig. 2G).

The inter-cell contacts, evidently observed between the different sensory and nonsensory cell's perikaryon, adherent junctions (desmosomes), are extended from the epithelial surface down along the cellular membranes (Fig. 2A, B, C, D). The junctions between different receptor cells (Fig. 2A, B, C, D) may differ from those found between receptor and supporting cells (Fig. 2E, G) by their asymmetrical and faint outline.

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 bear 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).

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) listed 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 hypophysis 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, since, 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*. (2023) 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.

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).

In grass carp (*Ctenopharyngodon idella*), two major cell types; ciliated and microvillous with cilia or microvilli, respectively, both are represented in most teleosts including cyprinodont species (Chakrabarti and Ghosh, 2010; Ghosh and Chakrabarti, 2010; Ghosh and Chakrabarti, 2016; Al-Zahaby *et al*., 2023). From the specific dendritic knob of the first one ciliated receptor cells emitted limited number (5 to 8) somewhat short thick non-motile cilia or flagella so are denoted also as flagellated or flagellar receptor cells (Chakrabarti and Ghosh, 2011). However, from the dendritic knob of the microvillous receptor cells release plentiful minute microvilli, instead of cilia characterize ciliated receptor cells. Both cell types are categorized by their well-developed rough endoplasmic reticulum and Golgi complex (Bhute and Baile, 2007).The microvillous receptor cells are considered as progenitor of ciliated receptor ones (Bannister, 1965; Bakhtin, 1977).

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.

Rod-tipped cell were detected in olfactory epithelium of many cyprinoid fish species such as; *Phoxinus phoxinus* (Bannister, 1965), *Carassius auratus* (Ichikawa and Ueda, 1977); *Alburnus alburnus* (Hernadi, 1993), silver carp (El-Attar and Al-Zahaby, 2010), cave-dwelling fish of *Sinocyclocheilus* genus (Zhang *et al*., 2018), and Chinese cave loaches of genus *Oreonectes* (Waryani *et al*., 2015), and Zebrafish, *Danio rerio* (Al-Zahaby *et al*., 2023).

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 ORNs 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 microvillar 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 non-sensory epithelial areas are made mainly of supporting, mucous goblet 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 microridges, 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 kinocilium 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 kinocilia 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 microridges 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 microridges provide structural support anchoring the mucus secreted by the neighbouring 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 nonsensory 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 hyperactivation of mucous goblet cells thus, ZnO-NPs negatively affected the fish odor sensation.

In the olfactory epithelium of the present studied fish, grass carp, rodlet cells are pear-shaped with electron lucent cytoplasm bounded with a thick fibrillar cytoplasmic capsular wall as showed also by Dezfuli *et al*. (2007) and Al-Zahaby *et al*. (2023). These rodlets which are increased in number by time as showed in figure (2 E&F) of mature and immature cells in grass carp, are extruded outwards into the surrounding water through the episodic apical narrow pore existing at the epithelial surface and this done under the force contraction of the fibrillar capsular wall bound the whole cell (Dezfuli *et al*., 2022).

Conclusion

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.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aicardi, S., Bozzo, M., Amaroli, A., Gallus, L., Risso, B., Carlig, E., Di Blasi, D., Vacchi, M., Ghigliotti, L., Ferrando, S., 2022. The arrangement of the peripheral olfactory system of Pleuragramma Antarcticum: A Well-Exploited Small Sensor, An Aided Water Flow, and A Prominent Effort in Primary Signal Elaboration. Animals 12, 663.

Al-Zahaby, S.A., Farag, M.R., Alagawany, M., Taha, H.S., Varoni, M.V., Crescenzo, G., Mawed, S.A., 2023. Zinc Oxide Nanoparticles (Zno-Nps) Induce Cytotoxicity in The Zebrafish Olfactory Organs Via Activating Oxidative Stress and Apoptosis at The Ultrastructure and Genetic Levels. Animals 13, 2867.

Atta, K., 2013. Morphological, Anatomical and Histological Studies on The Olfactory Organs And Eyes of Teleost Fish: *Anguilla anguilla* in Relation To Its Feeding Habits. The Journal of Basic & Applied Zoology 66, 101-108.

Bakhtin, E., 1977. Peculiarities of Fine-Structure of Olfactory Organ of Scualus-Acanthias. Tsitologiya 19, 725.

Bannister, L. 1965. The Fine Structure of The Olfactory Surface of Teleostean Fishes. Journal of Cell Science 3, 333-342.

Bhute, Y.V., Baile, 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, 342-349.

Chakrabarti, P., Ghosh, S., 2010. Histoarchitecture And Scanning Electron Microscopic Studies of The Olfactory Epithelium in The Exotic Fish (Bleeker). Fisheries & Aquatic Life 18, 173-177.

Chakrabarti, P., Ghosh, S.K., 2011. The Structural Organization and Functional Aspects of The Olfactory Epithelium of Tigerperch, *Terapon jarbua* (Forsskål, 1775)(Perciformes: Terapontidae). Turkish Journal of Zoology 35, 793-799.

Chervinski, J., 1977. Note On The Adaptability Of Silver Carp-Hypophthalmichthys Molitrix (Val.)-And Grass Carp-*Ctenopharyingodon Idella* (Val.)-to Various Saline Concentrations. Aquaculture 11, 179-182.

Cheung, K.Y., Jesuthasan, S.J., Baxendale, S., Van Hateren, N.J., Marzo, M., Hill, C.J., Whitfield, T.T., 2021. Olfactory Rod Cells: A Rare Cell Type in The Larval Zebrafish Olfactory Epithelium With A Large Actin-Rich Apical Projection. Frontiers in Physiology 12, 626080.

Cox, J.P., 2008. Hydrodynamic Aspects of Fish Olfaction. Journal of The Royal Society Interface 5, 575-593.

Crnjar, R., Slcalera, G., Bigiani, A., Tomassini Barbarossa, I., Magherini, P., Pietra, P., 1992. Olfactory Sensitivity To Amino Acids in The Juvenile Stages of The European Eel Anguilla Anguilla (L.). Journal of Fish Biology 40, 567-576.

Dezfuli, B.S., Capuano, S., Simoni, E., Previati, M., Giari, L., 2007. Rodlet Cells and The Sensory Systems in Zebrafish (*Danio rerio*). The Anatomical Record: Advances In Integrative Anatomy and Evolutionary Biology: Advances In Integrative Anatomy And Evolutionary Biology 290, 367-374.

Dezfuli, B.S., Pironi, F., Maynard, B., Simoni, E., Bosi, G., 2022. Rodlet Cells, Fish Immune Cells and A Sentinel of Parasitic Harm in Teleost Organs. Fish & Shellfish Immunology 121, 516-534.

El-Attar, A., Al-Zahaby, S.A., 2010. Structural organization of the chemoreceptor organs in silver carp. In: Proceedings of International Congress of Biological Science and Zoology 103-118.

Garwood, R.J., Behnsen, J., Ramsey, A.T., Haysom, H.K., Dalby, L.J., Quilter, S.K., Maclaine, J.S., Wang, Z., Cox, J.P., 2020. The functional nasal anatomy of The Pike, Esox Lucius L. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 244, 110688.

Ghodeswar, P., Gorghate, N., Masram, S., 2020. Light and Transmission Electron Microscopic Study of Olfactory Organ of Spotted Snakehead, Channa Punctata (Bloch). Advances in Zoology and Botany 8, 483-490.

Ghosh, S., Chakrabarti, P., 2016. Histological, Topographical and Ultrastructural Organization of Different Cells Lining The Olfactory Epithelium of Red Piranha, Pygocentrus Nattereri (Characiformes, Serrasalmidae). Vestnik Zoologii 50, 447.

Ghosh, S.K., Chakrabarti, P., 2010. Histological and scanning electron microscopic organization and functional aspects of the surface olfactory epithelium of the freshwater minor carp, puntius sophore (hamilton). In: Proceedings of The Zoological Society, 2010. Springer, pp. 115-119.

Ghosh, S.K., Pan, B., Chakrabarti, P., 2015. Histoarchitectural And Surface Ultrastructural Analysis of The Olfactory Epithelium of Puntius Ticto (Hamilton, 1822). International Journal Of Aquatic Biology 3, 236-244.

Goel, H., 1978. The Structure And Functions of The Olfactory Organs in The Fresh Water Teleost *Notopterus notopterus* (Ham.). Folia Morphologica 26, 392-396.

Goss, G.M., Chaudhari, N., Hare, J.M., Nwojo, R., Seidler, B., Saur, D., Goldstein, B.J., 2016. Differentiation Potential of Individual Olfactory C-Kit+ Progenitors Determined Via Multicolor Lineage Tracing. Developmental Neurobiology 76, 241-251.

Hamdani, E.H., Døving, K.B., 2002. The Alarm Reaction In Crucian Carp Is Mediated By Olfactory Neurons With Long Dendrites. Chemical Senses 27, 395-398.

Hamdani, E.H., Lastein, S., Gregersen, F., Døving, K.B., 2008. Seasonal Variations in Olfactory Sensory Neurons—Fish Sensitivity To Sex Pheromones Explained? Chemical Senses 33, 119-123.

Hara, T., Zhang, C., 1997. Topographic Bulbar Projections And Dual Neural Pathways of The Primary Olfactory Neurons In Salmonid Fishes. Neuroscience 82, 301-313.

Hara, T.J., 1975. Olfaction In Fish. Progress In Neurobiology 5, 271-335.

Hara, T.J., 2000. Chemoreception. The Laboratory Fish. Elsevier.

Hernadi, L., 1993. Fine Structural Characterization of The Olfactory Epithelium And Its Response To Divalent Cations Cd2+ in The Fish *Alburnus alburnus* (Teleostei, Cyprinidae): A Scanning and Transmission Electron Microscopic Study. Neurobiology (Budapest, Hungary) 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, 445-455.

Jakubowski, M., Kunysz, E., 1979. Anatomy And Morphometry Of The Olfactory Organ Of The Wels *Silurus glanis* L. (Siluridae, Pisces). Zeitschrift Fur Mikroskopisch-Anatomische Forschung 93, 728-735.

Kasumyan, A., 2004. The Olfactory System In Fish: Structure, Function, And Role In Behavior. Journal Of Ichthyology 44, S180.

Kirk, J.P., Socha, R.C., 2003. Longevity And Persistence of Triploid Grass Carp Stocked Into The Santee Cooper Reservoirs Of South Carolina.

Laing, D.G., Doty, R.L., Breipohl, W., 2012. The Human Sense of Smell, Springer Science & Business Media.

Lara, M.R., 2008. Development of The Nasal Olfactory Organs In The Larvae, Settlement-Stages And Some Adults of 14 Species Of Caribbean Reef Fishes (Labridae, Scaridae, Pomacentridae). Marine Biology 154, 51-64.

Manuel, K.L., Kirk, J.P., Barwick, D.H., Bowen, T.W., 2013. Hydrilla Management In Piedmont Reservoirs Using Herbicides And Triploid Grass Carp: A Case Study. North American Journal Of Fisheries Management 33, 488-492.

Mokhtar, D.M., Abd-Elhafeez, H.H., 2014. Light-And Electron-Microscopic Studies of Olfactory Organ of Red-Tail Shark, *Epalzeorhynchos bicolor* (Teleostei: Cyprinidae). Journal Of Microscopy And Ultrastructure 2, 182-195.

Pashchenko, N., Kasumyan, A., 2017. Development of The Olfactory Organ In The Ontogeny of Carps (Cyprinidae). Journal of Ichthyology 57, 136-151.

Rheinsmith, S.E., Quinn, T.P., Dittman, A.H., Yopak, K.E., 2023. Ontogenetic Shifts In Olfactory Rosette Morphology of The Sockeye Salmon, Oncorhynchus Nerka. Journal of Morphology 284, E21539.

Ruzhinskaya, N., Gdovskii, P., Devitsina, G., 2001. Chloride Cells, A Constituent of The Fish Olfactory Epithelium. Journal of Evolutionary Biochemistry and Physiology 37, 89-94.

Sato K., Suzuki N., 2001. Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to amino acids, pheromone candidates and urine in rainbow trout. Chem. Senses. 26, 1145-1156.

Schmachtenberg, O., 2006. Histological and Electrophysiological Properties of Crypt Cells From The Olfactory Epithelium of The Marine Teleost Trachurus Symmetricus. Journal of Comparative Neurology 495, 113-121.

Sorensen P.W., Caprio J., 1998. Chemoreception. In: The physiology of fishes (ed. Evans D.H.,), CRC Press, Boca Raton. FL, USA, pp. 375-405.

Subasinghe, R., 2017. World Aquaculture 2015: A Brief Overview. Fao Fisheries And Aquaculture Report. https://www.fao.org/documents/card/en/c/ef016bab-8323-4797-881e-e1bde714b973/

Sutton, D.L., Vandiver Jr, V.V., Hill, J.E., 2013. Grass Carp: A Fish For Biological Management Of Hydrilla And Other Aquatic Weeds In Florida: Bul867/Fa043, 11/2012. Edis, 2013.

Timm-Davis, L., Fish, F.E., 2015. Flow Through The Nasal Cavity Of The Spiny Dogfish, Squalus Acanthias. The European Physical Journal Special Topics 224, 3407-3417.

Triana-Garcia, P.A., Nevitt, G.A., Pesavento, J.B., Teh, S.J., 2021. Gross Morphology, Histology, And Ultrastructure Of The Olfactory Rosette Of A Critically Endangered Indicator Species, The Delta Smelt, Hypomesus Transpacificus. Journal Of Comparative Physiology A 207, 597-616.

Waryani, B., Dai, R.-J., Abbasi, A.R., 2013. Anatomical Studies Of The Olfactory Epithelium Of Two Cave Fishes *Sinocyclocheilus* Jii and S. Furcodorsalis (Cypriniformes: Cyprinidae) From China.

Waryani, B., Zhao, Y., Zhang, C., Abbasi, A., Ferrando, S., Dai, R., Soomro, A., Baloch, W., Abbas, G., 2015. Surface architecture of the olfactory epithelium of two chinese cave loaches (cypriniformes: nemacheilidae: *Oreonectes*). Italian Journal of Zoology 82, 179-185.

Zeiske, E., Bartsch, P., Hansen, A., 2009. Early ontogeny of the olfactory organ in a basal actinopterygian fish: polypterus. Brain Behavior and Evolution 73, 259-272.

Zeiske, E., Melinkat, R., Breucker, H., Kux, J., 1976. Ultrastructural studies on the epithelia of the olfactory organ of cyprinodonts (teleostei, cyprinodontoidea). Cell And Tissue Research 172, 245-267.

Zhang, X.-Y., Huang, Z.-Q., Ning, T., Xiang, X.-H., Li, C.-Q., Chen, S.-Y., Xiao, H., 2018. Microscopic and submicroscopic gradient variation of olfactory systems among six *Sinocyclocheilus* species living in different environments. Zoological Science 35, 411-420.