Applied Microscopy
Korean Society of Microscopy

pISSN 2287-5123 · eISSN 2287-4445
http://dx.doi.org/10.9729/AM.2016.46.1.51

Regular Article

The Anatomy and Histoarchitecture of the Olfactory Organ in the Korean Flat-Headed Goby *Luciogobius guttatus* (Pisces; Gobiidae)

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The histology and anatomy of the olfactory organ in *Luciogobius guttatus* was investigated using a light microscopy and scanning electron microscopy. The paired olfactory organs in the dorsal part of the snout are situated in between the upper lip and the eyes. They consist of two nostrils, one anterior and the other posterior openings, and a single olfactory cavity. The anterior nostril, an incurrent opening, forms a short tubular structure from the skin. The posterior nostril, an excurrent opening, forms a circular structure opened to the exterior. The distributional pattern of the sensory epithelium is a continuous type. The sensory epithelium with numerous-motile cilia is made up of receptor cells, supporting cells, basal cells, and mucous cells. In contrast, the non-sensory epithelium is comprised of stratified epithelial cells and two types of mucous cells, acidic and neutral cells. The cilia number of the receptor cell is in range of 3 to 4 units. Such results in *L. guttatus* may reflect its ecological habit and microhabitat in the tidal zone with a periodic tide.

Key Words: Tubular structure, Single cavity, Cilia, Receptor cell, Mucous cell

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Received March 7, 2016
Revised March 21, 2016
Accepted March 21, 2016

INTRODUCTION

A flat-headed goby *Luciogobius guttatus* is distributed in the temperate coast and tidal zone of the Korean Peninsula, Japan, and the Northwest Pacific of China (Nelson, 1994). In general, the coastal region where such an intertidal fish lives has turbulent environments: a fluctuating water amount, a variety of salinity, and wave forces caused dramatically by periodic physical and chemical conditions (Horn et al., 1999). In particular, the periodic tidal cycle not only applies a physical effect directly to the breaking of boulders, the movement of gravels, the floating of sand and mud particles but also exposes the land of intertidal zone in the air during the low tide period (Van Straaten & Kuenen, 1958). Therefore, the intertidal fishes exposed at the low tide are faced with a grave stress by physical impacts, a desiccant with emerging partial skin in tide pool and an underwater with low oxygen level (Congleton, 1980; Richards, 2011). To overcome these severe-repeated hard aquatic environments, they came to have special organs to perform various functions. Among them, the olfactory organ has been known as a good apparatus to recognize unstable and extreme environments.

Olfaction in fishes is a biologically essential chemoreception. It provokes important behaviors for the survival of fish such as feeding, reproduction, avoiding predator and intraspecific communication (Hara, 1975). In teleost, the olfactory organ responsible for this reception generally consists of peripheral organs, olfactory tract nerves and olfactory bulbs, conducting signal process and transduction with appropriate cells (Hara, 1986). It also varies in not only gross morphology, position, but also the number and morphology of the receptor cell, and the distributional pattern of the epithelium between species (Yamamoto, 1982). Kuciel et al. (2013) in the recent study reported that the olfactory organ of the intertidal fishes

displays a specialized structure as adapted to the microhabitat and inherent ecological habit. For *L. guttatus*, however, a study of the olfactory organ related closely to the fish's unique microhabitat and ecological habits is not yet undertaken. Thus, the aim of this study is to investigate anatomical and histological structure of the olfactory organ and then analyze any relation between environments and habits with the olfactory structure.

MATERIALS AND METHODS

Mature specimens (5 male and 8 female) of *L. guttatus* (41.0 to 53.7 mm standard length) (Fig. 1A) were caught in between April and August 2015 in rock pools and gravelly fields of Gyeokpo-ri, Byeonsan-myeon, Buan-gun, Jeollabuk-do, South Korea, 35°01' 56"N, 126°09' 02"E (Fig. 1B) and Yerae-dong, Seogwipo-si, Jeju-do, South Korea, 33°14'26"N, 126°23'48"E. During the low tide, the total 8 specimens collected using an aquarium landing net (10 cm diameter) were fixed in 10% neutral buffered formalin solution (3 in male and 5 in female for each sex). Living 5 fishes for each sex were taken to the laboratory for serial fixing process (explained below). To investigate the anatomical structure of the olfactory organ, after the measurement, the olfactory tissue of the fixed specimens was dyed using stock solution of Harris hematoxylin devised by Jakubowski (1967, 1975), anatomized under a stereoscopic microscopy (SM, Stemi DV4; Carl Zeiss, Germany) and then filmed with digital camera (TG-3; Olympus, Japan). To investigate the histological architecture of the olfactory epithelium, the dyed olfactory tissue (above identical method) were resected from the head of specimen, were dehydrated properly though an ascending series of ethanol, were cleaned with xylene, and then were embedded in paraffin wax (Paraplast; Oxford, USA) for 24 hours. Sections with olfactory tissue were cut at five micrometer, deparaffinized, stained with Harris H&E (Gurr, 1956) for a general histology and then observed under a light microscopy (LM, LE REL 4.4; Carl Zeiss). The tissues were also combined alcian blue (pH 2.5)-periodic acid Schiff to ascertain a mucous cell (Mowry, 1956). The epithelial thickness of the olfactory cavity was measured using Axio vision (Carl Zeiss). For the study of scanning electron microscopy (SEM) observation, the living specimens were anaesthetized with MS-222 (Sigma-Aldrich, USA), and fixed firstly in 2.5% glutaraldehyde (G.A. solution) in 0.1 M phosphate buffer (pH 7.4) for 24 hours, after that, and the olfactory fragments were dissected out from the head, and once again fixed secondly in 2.5% G.A. solution (EMS, USA) for 24 hours, and are post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, and dehydrated gradually by ethanol series, and dried by critical point drier with liquid CO_2 (VFD-21S; Vacuum Device Co., Ltd., Japan) and coated with osmium tetroxide by ion sputtering (HPC-1SW; Vacuum Device Co., Ltd.), and then were examined under a SEM (SUPRA40VP; Carl Zeiss). A cell type of determination was referred to Hara (1975) and Yamamoto (1982).

RESULTS

Gross Morphology

The olfactory organ of *L. guttatus* is paired on the dorsal snout of the head. The each peripheral part of the olfactory organ has externally two openings, anterior and posterior nostrils. The two openings are placed in between the upper lip and the eye (Fig. 2). The anterior nostril (0.15 to 0.30 mm major axis diameter) forms a tubular structure projected from the skin. It exhibits an elongated oval entrance. The posterior nostril (0.21 to 0.30 mm major axis diameter) forms a circular opening. It is at the terminal part of the olfactory cavity and just below the eye (Fig. 2). Above two nostrils are commonly opened to the exterior without a flap (inserted photograph in Fig. 2). The olfactory nerves connect from the epithelium of

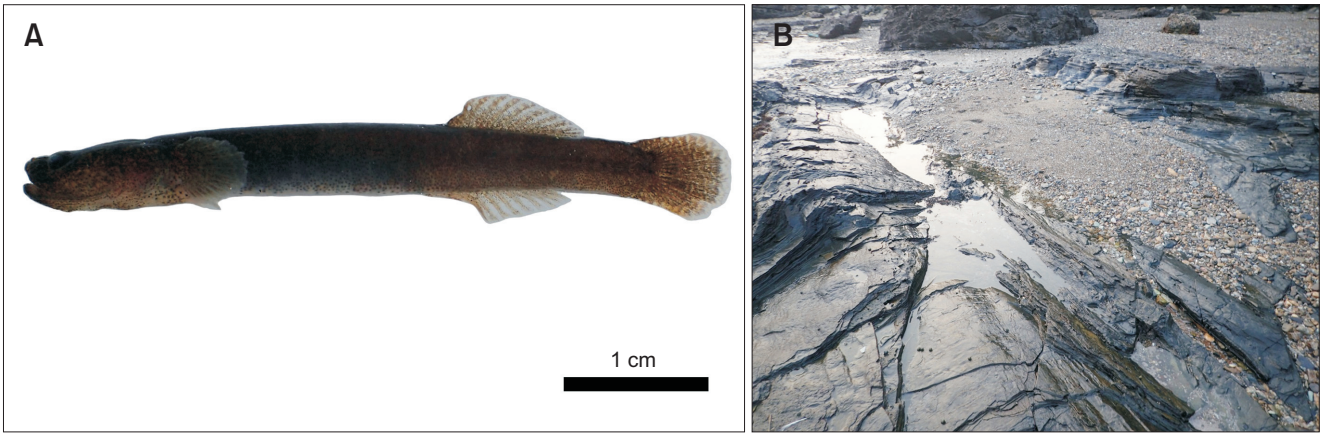

Fig. 1. The photograph (A) and habitat (B) of *Luciogobius guttatus*. This goby is characterized by a small body size and flat snout with the olfactory organ.

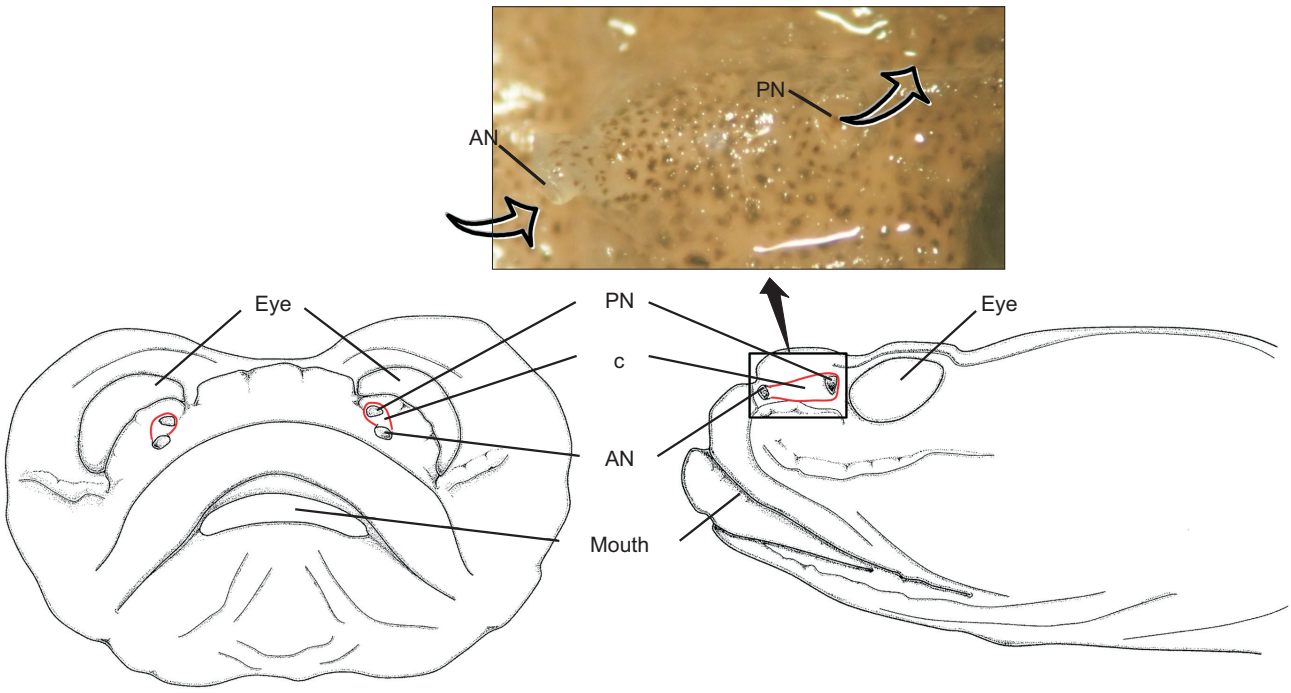

Fig. 2. Diagrams of the front (left) and side (right) view in the head of *Luciogobius guttatus.* The solid red lines indicate the external outline of the olfactory organ. Arrows in the above photograph indicates the flowing of the water via the olfactory cavity. AN, anterior nostril; PN, posterior nostril; c, olfactory cavity.

the olfactory cavity to the olfactory bulbs. The olfactory bulbs are adjacent tightly to the cerebrum, which is the one of brain contents (cerebellum, cerebrum, and optic lobe) (Fig. 3).

Histological Structure

The histological study of the olfactory epithelium was investigated using a LM. In the epithelium of the olfactory cavity, the initial part with the anterior nostril is made up of the sensory epithelium at the ventral floor and the non-sensory epithelium at the lateral and dorsal floors, and is an irregular shape in outline. Its non-sensory epithelium occupies larger proportion than the sensory epithelium (Fig. 4A). The central part is built entirely of the sensory epithelium, and forms an elongated ellipse (Fig. 4B). The terminal part is made up of the sensory epithelium at the lateral and ventral floors and the non-sensory epithelium at the partial dorsal floor, and forms an overturned heart in shape (Fig. 4C). In the occurred region, the sensory epithelium occupies most of the ventral and lateral regions whereas the non-sensory epithelium occupies only the partial region of the dorsal floor. The distributional pattern of the sensory epithelium as a multi-cell layer is a continuous type unseparated by the non-sensory epithelium. It is composed of receptor cell, supporting cell, basal cell and mucous cell. The receptor cells, which have prominent oval nuclei, are bipolar neurons. These nuclei are situated in between the basal cells and the supporting cells

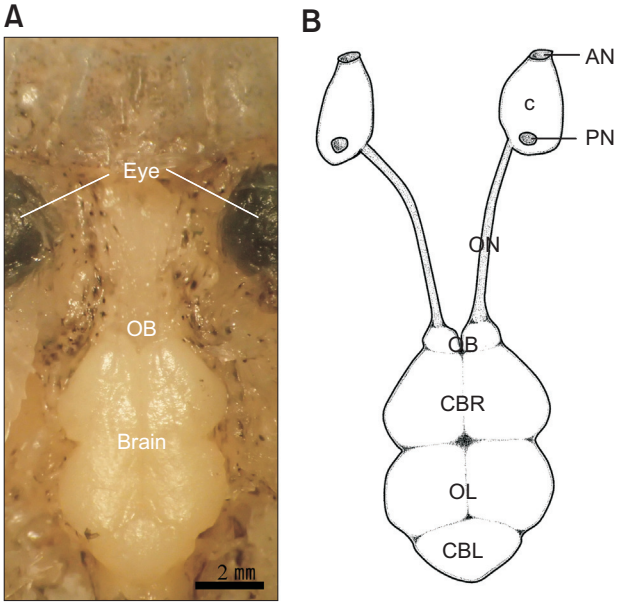

Fig. 3. The gross morphological photograph (A) and diagram (B) of the olfactory organ in *Luciogobius guttatus*. The olfactory organ is mainly divided into three contents, a single olfactory cavity, olfactory nerve, and olfactory bulb. OB, olfactory bulb; AN, anterior nostril; PN, posterior nostril; c, olfactory cavity; ON, olfactory nerve; CBR, cerebrum; OL, optic lobe; CBL, cerebellum.

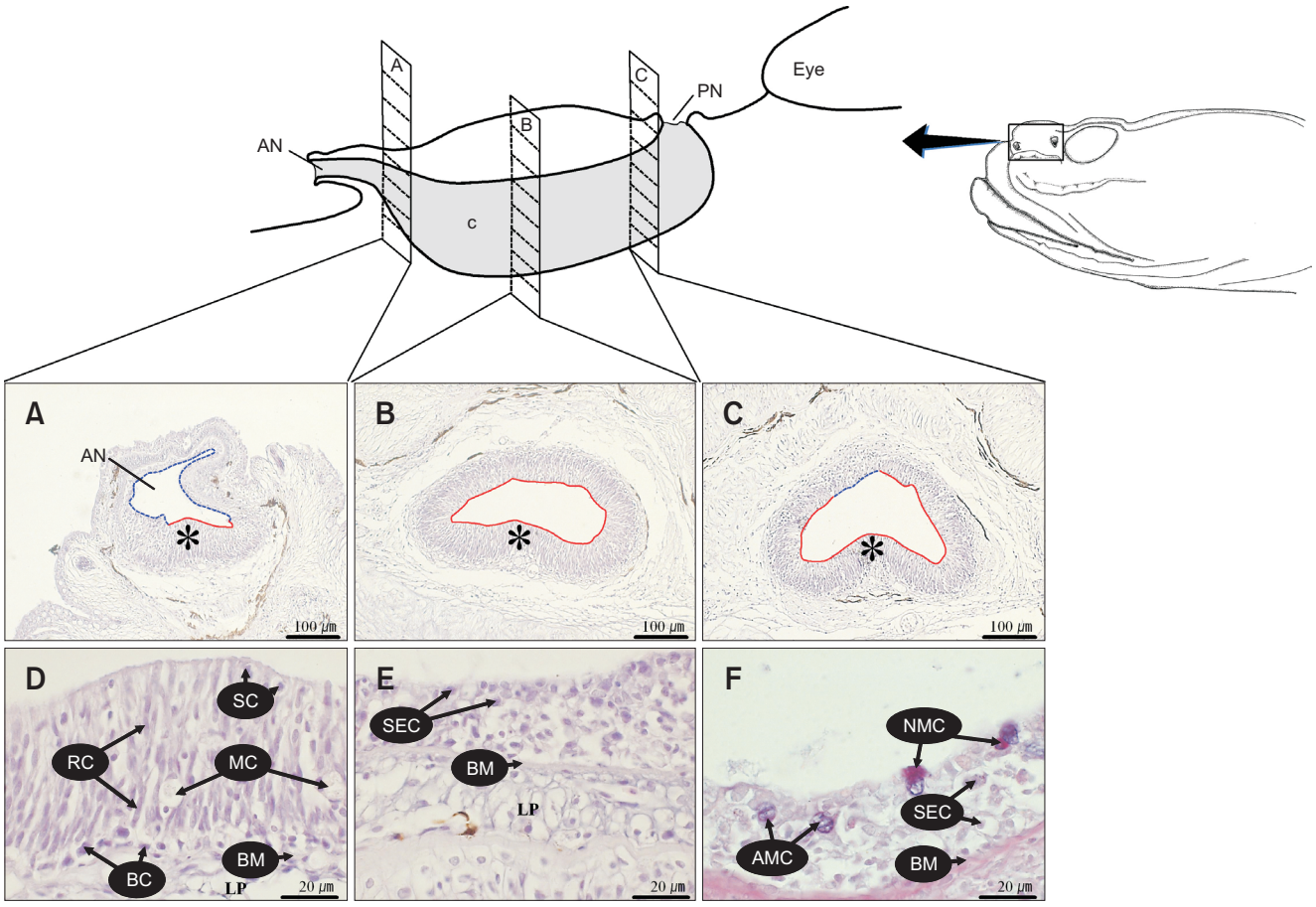

Fig. 4. The anatomical diagram of the side view (above) and the photographs (below) of its cross-serial sections stained with H&E (A-E) and alcian blue (pH 2.5)-periodic acid Schiff (F) in the olfactory organ of *Luciogobius guttatus*. (A) The initial part with the sensory epithelium at the ventral floor and the non-sensory epithelium at the lateral and dorsal floor in the anterior nostril. (B) The central part with the sensory epithelium in the entire floors of the olfactory cavity. (C) The terminal part with the sensory epithelium at the lateral and ventral floors and the non-sensory epithelium at the partial dorsal floor in the olfactory cavity. (D) The sensory epithelium which is made up of receptor cell, supporting cell, basal cell and mucous cell. (E) The non-sensory epithelium built of mainly stratified epithelial cell. (F) The non-sensory epithelium has both acidic mucous and neutral mucous cells. AN, anterior nostril; PN, posterior nostril; c, olfactory cavity; asterisk, sensory epithelium; broken blue line, outline of the non-sensory epithelium; solid red line, outline of the sensory epithelium; RC, receptor cell; SC, supporting cell; MC, mucous cell; BC, basal cell; BM, basement membrane; LP, lamina propria; SEC, stratified epithelial cell; AMC, acidic mucous cell; NMC, neutral mucous cell.

of the epithelial layer. The supporting cells are placed near to apical part of the epithelium, and surround the receptor cells. Its nuclei also are a round shape. The basal cells are just above the basement membrane, and arrayed along the membrane. These cells also contain flat round nuclei (Fig. 4D). The non-sensory epithelium consists of stratified epithelial cells (Fig. 4E) and two types of mucous cells, acidic and neutral cells (Fig. 4F). These mucous cells, which are stained positively for alcian blue (pH 2.5)-periodic acid Schiff, are highlighted in different colors. The acidic mucous cells stained as a light violet color whereas the neutral mucous cells stained as a dark red color (Fig. 4F). In the mean thickness, the sensory epithelium is 68.7±13.3 μm in the initial part (vs 25.5±11.1 μm in the non-sensory epithelium) and in the terminal part (vs 30.7±7.7 μm in the non-sensory epithelium). The thickness of the sensory epithelium is about two times thicker than the non-sensory epithelium (Table 1).

Table 1. Measurement of epithelial thickness in the olfactory cavity of *Luciogobius guttatus* (n=6)

	Sensory epithelium (μm)	Non-sensory epithelium (μm)
Initial part	68.7±13.3 (53.4~87.3)	25.5±11.1 (13.8~45.2)
Central part	63.1±6.5 (55.2~72.3)	-
Terminal part	54.8±7.9 (45.1~64.1)	30.7±7.7 (19.4~42.2)

Values are presented as mean±standard deviation (range).

In the observation of SEM, the cytological and histological characteristics of the epithelial surface are as follows. The initial part displays the ciliated and microvillous receptor cell, supporting cell, and the mucous cell (Fig. 5A). The central

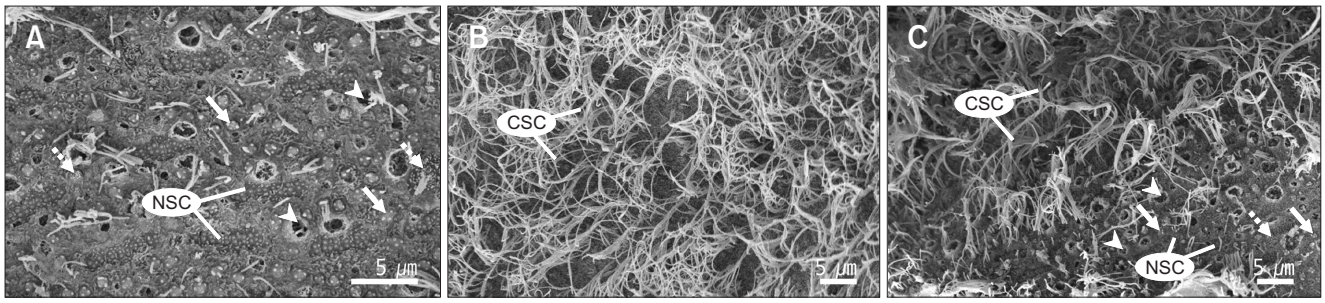

Fig. 5. Scanning electron micrographs showing the surface of the sensory epithelium in the floor of olfactory cavity. (A) The initial part have two types of the receptor cells (the ciliated and microvillous cells), non-ciliated supporting cells, and the secretory opening of the mucous cells. (B) The central part have mainly the long cilia bundles of ciliated supporting cells. (C) The terminal part have two types of the receptor cells (the ciliated and microvillous cells), the ciliated supporting cells with the long cilia, and the secretory opening of the mucous cells. Broken arrow, microvillous receptor cell; solid arrow, ciliated receptor cell; arrowhead, mucous cell; NSC, non-ciliated supporting cell; CSC, ciliated supporting cell.

part displays long cilia bundle of the ciliated supporting cells (Fig. 5B). The terminal part displays the ciliated and microvillous receptor cell, ciliated and non-ciliated supporting cell, and mucous cell (Fig. 5C). The initial and terminal parts have diverse types of cells.

Commonly in the initial and terminal parts, the ciliated receptor cells are scattered on the surface, and equip extremely short cilia, as dendritic processes, projecting from the round knob. A few of microvillous receptor cells are distributed in between supporting cells, and contain microvilli, growing directly from the surface (Fig. 5A and C). The supporting cells are divided into two types of cells, ciliated and non-ciliated cells, and surround other cells. The ciliated cells with long cilia occur in the central and terminal part (Fig. 5B and C). In contrast, the non-ciliated supporting cells with fine projections occur in the initial and terminal part (Fig. 5A and C). The secretory openings of the mucous cell with an irregular size are dispersed on the epithelial surface of the initial and terminal parts (Fig. 5A and C).

DISCUSSION

In cartilaginous fishes, sharks have paired two nostrils in the ventral position (Zeiske et al., 1987), whereas lamprey has a only single nostril dorsally at the dorsal position of the head (Ren et al., 2009). In contrast, teleost typically displays paired two nostrils, anterior and posterior openings, at the dorsal part of the snout, and equips a nasal flap or bridge with varying length between two openings (Yamamoto, 1982). Such the external morphologies of the olfactory organ across species vary in position and size of two openings and nasal flap (Zeiske et al., 1992). It is caused directly by evolutionary degree, ecological habit and habitat condition of species (Lauder, 1982).

L. guttatus has two nostrils that are situated at the dorsal part of the snout. According to Doving (1986), this structure of two openings can be categorized as ditermous (vs monotermous of a single opening). Also, these openings are opened to the exterior. It is similar to other intertidal gobies. Unlike *L. guttatus*, however, some amphibious fishes as the genera *Periophthalmus* and *Boleophthalmus* own a closed posterior nostril (Kuciel et al., 2013). These differences may be related with turbidity difference of the water in the microhabitats they live in. The above amphibious fishes inhabit mudflat with murky water (Murdy, 1989), whereas *L. guttatus* is active in the moist space between gravel, sand or rock and tidal pool.

The ventilation of water via the olfactory organ occurs naturally situation of which fish moves forward and in the aquatic environment where water flows (Kasumyan, 2004). On the other hand, the other fishes which are active in the stagnant water system should possess specific mechanisms for the ventilation (Pipping, 1927). Thus, they have largely two distinctive mechanisms, the performances by accessory nasal sacs and motile cilia (Doving et al., 1977). Although *L. guttatus* does not possess nasal sacs, this species has the well-developed non-sensory cilia bundle in the olfactory cavity. The motile cilia on the olfactory epithelium have known to help water flow (Doving et al., 1977). So, it might allow a complicated inner structure and a spare space between epithelial tissues to transfer water in the olfactory cavity (Teichmann, 1959).

Compared to other teleost's groups, the olfactory organ of the gobies is organized with more complicated subdivisions: olfactory canal, chamber or cavity, and sac. In the gobies, they can be varied significantly in its location and distributional pattern with a various spaces (Kuciel et al., 2013). In regard to its location of the sensory epithelium, *N. melanostomus* develops in the olfactory chamber (Belanger et al., 2003) whereas *B. boddarti*, *P. barbarus*, and *S. gigas* occurs in the olfactory canal (Kim et al., 2014; Kuciel et al., 2013). Unlike the above fishes, however, the sensory epithelium of *L. guttatus*, exists in the single olfactory cavity. The distributional

pattern of the sensory epithelium can be categorized largely into four types: continuous (*B. boddarti*, *S. gigas*, *Parapocryptes rictuosus*), large-regional, mixed irregularly, and small-dispersed patterns (*P. barbarus*, *P. variabilis*, *P. chrysospilos*) (Yamamoto, 1982; Yamamoto & Ueda, 1978). In this study, it was confirmed that *L. guttatus* is a continuous type.

Generally in teleost, the sensory epithelium is made up of receptor cell, supporting cell, and basal cell (Hara, 1975). In particular, the receptor cell is directly responsible for the recognition of the environmental condition change (Hara, 1986). Functionally, by contact with the odor, this cell provokes a signal transduction that is carried over into the brain finally (Hara, 1994). According to the morphology of the dendritic process in the apical part of the epithelium, the receptor cell of teleost is categorized into three distinct types: ciliated, microvillous and rod cells, (Hara, 1975). In some fish, the combination or composition of these cell types is used as part of the taxonomic purposes. In two sympatric congeners, *Schizothorax plagiostomus* has ciliated and microvillous receptor cells whereas *S. richardsonii* has rod and microvillous receptor cells (Singh, 1994). *L. guttatus* has two types, ciliated and microvillous receptor cells. Even if these receptor cells have known as a general type in the family gobiidae, there are difference in composition compared with other gobiid fishes (vs only ciliated receptor cell in *Scartelaos gigas* and giant cell in *B. boddarti*) (Kim et al., 2014; Kuciel et al., 2013).

The mucus secreted by mucous cells exerts significant roles, i.e., in respiration, ionic and osmotic regulation, reproduction, excretion, disease resistance, communication, feeding, nest building, and protection (Jakowska, 1963; Shephard, 1994). Especially, when fishes are in adverse conditions influenced factors as acid rain, ions at low pH, high level of ultraviolet light and silt-laden water, they contain a thick epithelial layer and have lots of the mucous cells (Muniz & Leivestad, 1980; Roberts & Bullock, 1980). In *L. guttatus*, the mucous cell contains two types, acidic and neutral cells in nature, in both sensory and non-sensory epithelium. This character may be related to an adaptation to protect the olfactory cell and epithelium against exposed to the air and occasionally to the sun light.

Such an olfactory organ of *L. guttatus*, therefore, is likely related with its ecological habitat in the tidal zone undergoing a periodic tide.

CONCLUSIONS

The paired olfactory organ in *L. guttatus* is comprised of two nostrils (one anterior and the other posterior openings) and a single olfactory cavity. The sensory epithelium (vs non-sensory epithelium with stratified epithelial and mucous cells) with cilia bundles shows a continuous type in its distributional pattern and consists of four types of cells: receptor cell (3~4 cilia), supporting cell, basal cell, and mucous cell. This flat-headed goby inhabits moist bottoms beneath boulders or between gravels at the low tide, which is subject to drought and exposed to the air periodically. Consequently, the olfactory organ may be considered as a well-adapted organ reflecting the above ecological habitats in the tidal zone.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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