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A Study on the Structure of Peripheral Olfactory Organ in the Korean Mudskipper, *Scartelaos gigas* (Pisces, Gobiidae)

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ABSTRACT An olfactory organ in *Scartelaos gigas*, so-called mudskipper known as adaptation to an amphibious lifestyle, was investigated anatomically and histologically. *S. gigas* possessed the paired olfactory organ comprising respectively the one elongated canal and two nasal sacs, lacrimal and ethmoidal nasal sac. The sensory epithelium developed partly in the canal contained four distinct types of cells: (1) receptor cell with 3 to 4 cilia in number, (2) supporting, (3) basal, (4) mucus cell. The sensory epithelium was also of transitional layer as multi cellularity structure. The non-sensory epithelium had no sensory elements. The two nasal sacs possessed typically a lot of mucin droplets. These results might be considered that anatomical structure and histological characters of the olfactory organ showing in *S. gigas* is adapted to semi-aquatic life associated with its ecological habit and habitat.

Key words : *Scartelaos gigas*, olfactory organ, ecological habit, mudskipper

INTRODUCTION

In fish, the olfaction exerts a functional role in an aspect of homing migration, feeding, reproduction, and fright reaction (Hara, 1986). In teleost, the olfactory organ responsible for olfaction has mainly rosette-like structure within a pair of nasal chamber or sac; this structure is comprised of lamella from only one to dozens which is covered by sensory epithelium (Yamamoto, 1982). The receptor cells which develop in sensory epithelium perceive the odorant released by exterior environment; the chemical characteristic of this receptor cells is closely related to habitat and food of fish (Yamamoto, 1982; Singh and Singh, 1986; Singh, 1994). Accordingly, the structural variation of the olfactory organ, including the shape, arrangement and distributional pattern of receptor cells and epithelium, probably vary considerably from species to species, and has been used in taxonomic character (Hara, 1975; Yamamoto, 1982; Singh and Singh, 1986).

Scartelaos gigas, known as the mudskipper, is distributed in intertidal zone of Korea, China and Taiwan (Murdy, 1989; Lin *et al*., 1994; Randall and Lim, 2000; Park *et al*., 2008), and has been known to inhabit in mudflats with more than 99% mud (Kim *et al*., 2011). This species which has much commercial value may is being endangered by restricted habitat compared with *Boleophthalmus pectinirostris* (Park *et al*., 2008). Additionally, the conservation of *S. gigas* is getting more and more important, because of decrease in number caused not only by environmental pollution by human activities but also overfishing (Kim *et al*., 2005; Park *et al*., 2008; Kim *et al*., 2011). Unfortunately, a few of previous studies on this species has yet been reported in Korea, comparison with *B. pectinirostris* to be abundantly studied in extensive fields (Chung *et al*., 1989; Chung *et al*., 1991; Ryu *et al*., 1995; Jeong *et al*., 2004; Kim and Jeong, 2007). The purpose of this study was to investigate the characteristics of olfactory organs focusing on the relation between its ecological habits linked with food and habitat.

MATERIALS AND METHODS

Scartelaos gigas, ranging from 160.1 to 175.5 mm standard length were collected by fishermen between

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August and October 2013 in Dangchon-ri, Shinan-gun, Jeollanam-do, Korea, 35°01′56″N, 126°09′02″E (Fig. 1). Across twenty specimens collected by casting a net, ten were fixed in 10% neutral buffered formalin solution; the living remainders were taken to the laboratory. To examine the anatomical structure of the olfactory organ by stereoscopic microscope (SM), the organs were dyed using a stock solution of hematoxylin, devised by Jakubowski (1967, 1975). A light microscope (LM, Carl Zeiss, Germany) and a scanning electron microscope (SEM, Carl Zeiss, SUPRA40VP, Germany) were used for the histological and morphological characteristics of the olfactory epithelium. For histological examination, the olfactory organ resected from the head, was dehydrated through a standard ethanol series to 100%, cleared in xylene, and then embedded in wax (Paraplast, Oxford). Five-micrometer sections were deparaffinized and stained with hematoxylin and eosin (Gurr, 1956) for general histology. The sections were also combined alcian blue-periodic acid Schiff (AB-PAS, pH 2.5) to ascertain a mucus cell. For study of observation through SEM, living specimens were anaesthetized with MS-222, and olfactory organ of specimens were dyed using a stock solution of hematoxylin for 1 hour. After dyeing, olfactory tissues were resected from head of specimen and prefixed in 2.5% glutaraldehyde solution with 0.1 M phosphate buffer (pH 7.4) for 24 hours, and postfixed in 1% osmium tetroxide solution with 0.1 M phosphate buffer, and dehydrated gradually by ethanol series, and dried by critical point drier with liquid CO_2 and coated with osmium tetroxide by ion sputtering, and then were examined under a SEM. A cell type of determination was referred to Yamamoto (1982).

Fig. 1. Habitats of *Scartelaos gigas* (Dangchon-ri, Shinan-gun, Jeollanam-do, Korea).

RESULTS

1. External morphology

The paired olfactory organs set anteriorly in the head are made up of the canal and two nasal sacs. The elongated canals which are connected from anterior nostril (0.62～1.07 mm diameter) at upper lip to lacrimal sac are located in each left and right side of snout, and have the sensory epithelium partly developed inside the wall. The end part of the anterior nostril situated at upper lip forms the short tentacle, and the posterior that is a slit form. The nasal sacs fall into ethmoidal and lacrimal nasal sac. The ethmoidal nasal sac is located just below the eye and has the posterior nostril (1.25～1.76 mm diameter) where water is drained outside, whereas lacrimal nasal sac is ventrally arranged and is linked with the end of canal (Fig. 2).

2. Light microscopy

Histologically, in the canal which forms the closely

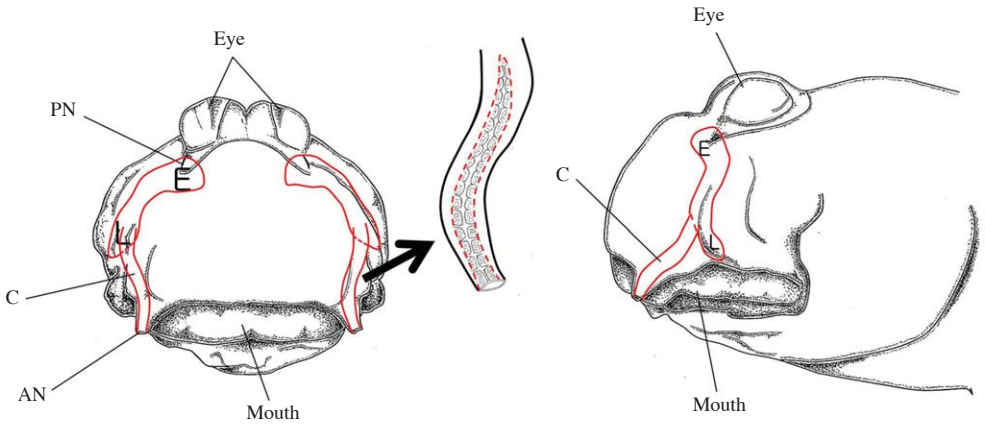

Fig. 2. The anatomical structure of olfactory organ in *Scartelaos gigas*. The red broken line within the canal indicates distributional pattern of the sensory epithelium. AN, anterior nostril; C, canal; E, ethmoidal nasal sac; L, lacrimal nasal sac; PN, posterior nostril.

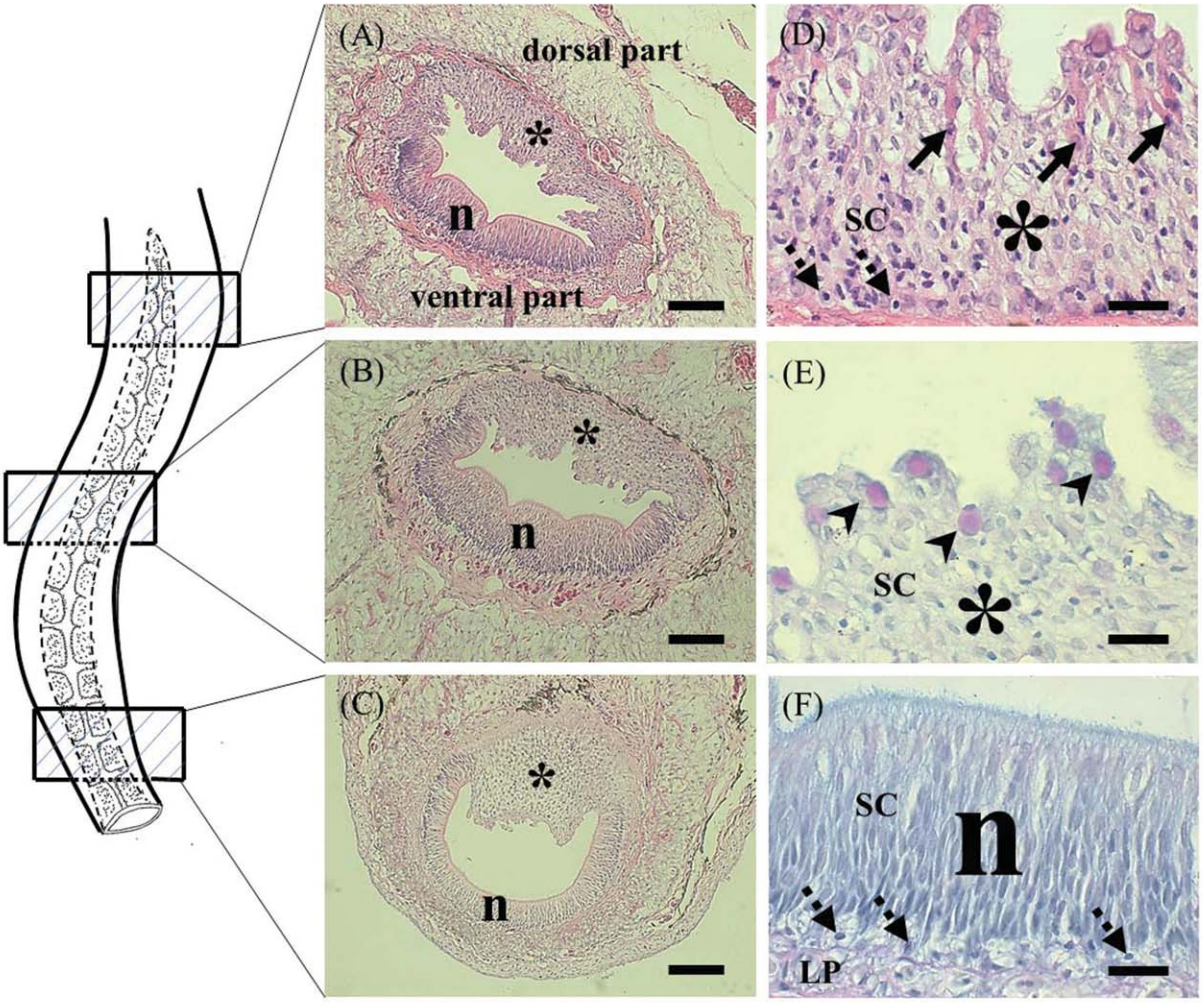

Fig. 3. Histological characteristics of each part of the canal, sensory and non-sensory epithelium stained with AB-PAS (pH 2.5) (E, F) and hematoxylin-eosin (A-D). (A), the terminal part is the more elongated form and each part are typically the sensory at dorsal and the non-sensory epithelium at ventral. (B), the central part is the elongated form; (C), the initial part is the closely circle form. (D), the sensory epithelium with a unequable surface structure is multi cell structure consisting of the receptor cell, the supporting cell and basal cell. (E), the apical part in sensory epithelium has the neutral mucus cell. (F), the non-sensory epithelium comprises supporting and basal cell. Star, sensory epithelium; n, non-sensory epithelium; solid arrow, receptor cell; arrowhead, mucus cell; broken arrow, basal cell; LP, lamina propria; SC, supporting cells. Bars indicate 100 μm in (A), (B), (C) and 20 μm in (D), (E), (F), respectively.

circle and the elongated-oval in the cross-sectional morphology of each part, the olfactory epithelium falls into two types, sensory at the dorsal and non-sensory epithelium at the ventral and is getting increase of the sensory and decrease of the non-sensory from initial to terminal in ratio covering a total area (Fig. 3A-C). The thickness of sensory epithelium in the canal is 210 μm (vs 60 μm in non-sensory), 131 μm (vs 100.7 μm), 93.2 μm (vs 78.6 μm), respectively (Table 1). Accordingly, the sensory epithelium is the thickest at the initial part while the non-sensory is at the central. The sensory epithelium is of transitional layer and multi-cellularity structure which is made of the receptor, supporting, basal and mucus cells. The receptor cells ruddily stain with eosin is situated in between supporting cells and is being elongated vertically. The supporting cells which are a major component of the epithelium show irregularly and relatively small cell body in the sensory epithelium but the vertically long cell body in the non-sensory epithelium. The basal cells as cuboidal form being arranged side by side at the basement membrane exhibit a big cell body relatively than other type of cells (Fig. 3D).

The apical part in sensory epithelium has mucus cells that possess the nucleus situated at the bottom. Through AB (pH. 2.5)-PAS staining method, the mucus cells showed a strong positive reaction as a reddish-purple tint (Fig. 3E). The non-sensory epithelium comprises only supporting and basal cells (Fig. 3F).

Table 1. Measurements of the olfactory epithelial thickness in the canal. N=number of determinations

Thickness (μm)	Non-sensory epithelium			Sensory epithelium		
	n	Mean ± SD	Range	n	Mean ± SD	Range
Initial part	20	60.0 ± 14.7	34 ~ 85	20	210.0 ± 34.1	150 ~ 261
Central part	20	100.7 ± 18.5	65 ~ 141	20	131.2 ± 10.1	113 ~ 154
Terminal part	20	78.6 ± 8.6	66 ~ 96	20	93.2 ± 15.0	46 ~ 115

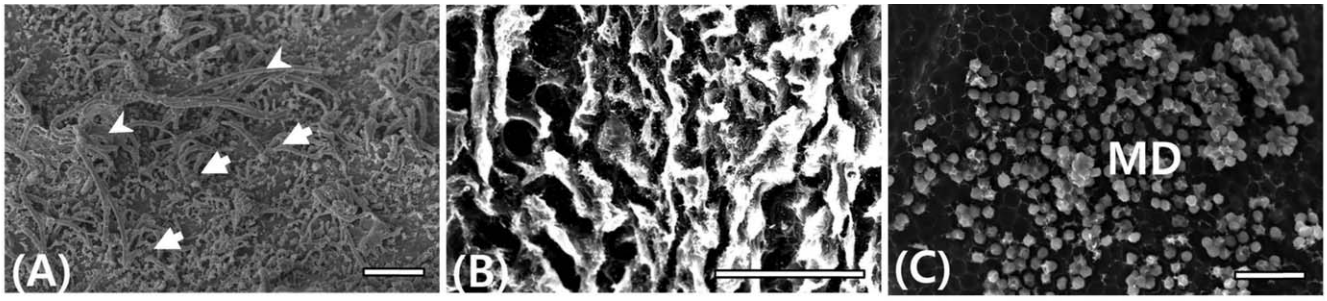

Fig. 4. Scanning electron micrographs of the surface of epithelium in olfactory organ of *Scartelaos gigas*. (A), the long kinocilias (arrowhead) and receptor cells with cilia (solidarrow) showing on the surface of sensory epithelium in the canal. (B), the surface of non-sensory epithelium in the canal is furrowed and have no sensory elements. (C), there are a lot of mucin droplets over the epithelium in nasal sacs. MD, mucin droplet. Bars indicate 2 μm, 30 μm, 50 μm, respectively.

3. Scanning electron microscopy

In study of SEM observation, the sensory epithelium developed in the canal is not only the elongated corniform entirely in distributional type (Fig. 2), but is also characterized by presence of receptor cell as only ciliated. In receptor cell, the cilia arising in apical part of the cell are 3 or 4 in number and the distance between this cells is 2 ~ 2.5 μm, roughly. A few long kinocilias are also distributed on the surface (Fig. 4A). On the other hand, on non-sensory epithelium the surface is furrowed and sensory elements were not observed (Fig. 4B). On the epithelial surface of two nasal sacs, the mucin droplets occur commonly in both of the sacs (Fig. 4C).

DISCUSSION

Scartelaos gigas possesses the paired olfactory organ comprising respectively the one elongated canal and two nasal sacs, lacrimal and ethmoidal nasal sac. The feature in the anatomical structure of the olfactory organ in *S. gigas* resemble that of *Boleophthalmus boddarti* and *S. histophorus* as a congener with the *S. gigas*, but is different from two species above, having lacrimal and ethmoidal nasal sac in the position of nasal sac. In addition, until now, most of species in *Periophthalmus* known as fish more terrestrial than the *S. gigas* have only one nasal sac (Horn *et al*., 1999). Thus, the degree of adaption from sea to land environment may be causative of extensive variation on place of nasal sac. But, the hypothesis in the point of this view is needed further comparative studies with olfactory organ of each other species in Gobiidae. In teleost, the nasal sac expanded from olfactory cavity not only provides the environment of interaction between odorant and receptor cell, but also may be evolutionally correlated with structural variation of morphology (Hara and Zielinski, 2007). Most of species in Perciforms have only two nasal sacs, except for a few species belonging to Oxudercinae, amphibious fish (Kuciel *et al*., 2013).

Yamamoto (1982) suggested the category of olfactory organ classified by arrangement of the sensory epithelium, number of lamella, their shape and organization and type of receptor cells, and opined that this organ in fish is relation with their ecological habit or olfactory ability. *S. gigas* is characterized by absence of rosette-like structure and the occurrence of sensory area in olfactory canal, similarly to previous studies in Oxudercinae, but there are evident differences in view from arrangement of sensory epithelium and type of receptor cells (Kuciel *et al*., 2013). *S. gigas* is entirely similar to *B. boddarti* and *S. histophorus* as a cuneiform in distributional pattern of sensory epithelium, but is different in aspect of that arrangement of sensory field shows no continuous in detail. Continuously, *Periophthalmus barbarus*, *P. variabilis* and *P. chrysospilos* is of form of islet. In morphology of receptor cell, *B. boddarti* has giant cell whereas *S. gigas* has only receptor cell with cilia (Kuciel *et al*., 2013). It may be expected that ability of olfaction in fish is closely related on the size of olfactory organ with distribution of sensory epithelium and

receptor cell (Yamamoto, 1982). In fish, lamella folded on inner wall in olfactory cavity or rosette built of one or several lamella increase surface of olfactory epithelium; this structure is related to odorant sensitivity (Meredith and Kajiura, 2010). Moreover, fish having high olfactory sensitivity, *Oncorhynchus mykiss* and *Salmo gairdneri* possess 4 or more cilia radiating a receptor cell (Hara, 1986; Zielinski and Hara, 1988), but *P. barbarus*, congener with *S. gigas*, known as low sensitivity fish does not exceed 4 in number of cilia (Kuciel *et al*., 2011).

The mucus secreted over olfactory epithelium provides function of intermediate in between receptor cells and environments to help in binding chemical odorants, and protect the cell against hazard materials in habitat (Shephard, 1994). As the mucus droplets in *S. gigas* is unique, and were observed extensively only in two nasal sacs that does not have sensory elements, but not in canal. Presence of substances like a mucin mass in olfactory organ was reported in *Sperrata aor* and *Labeo bata* (Chakrabarti and Ghosh, 2011; Ghosh and Chakrabarti, 2011). It is considered that the presence of the mucin droplet and mucus cell in our result may be a distinctive form to adapt to its dry and environment extremely and changeably with hazardous materials, as well as mudflat in intertidal zone.

In epithelium of canal in *S. gigas*, the layers of two distinctive types, sensory and non-sensory epithelium, occur dorsally and ventrally, respectively, and receptor cell is only in epithelium at dorsal. Especially, among the structures above, it is considered that the transitional layer as multi-cell comprising sensory epithelium in the canal may be a role in keeping the cells of olfactory epithelium through changing the size of space in canal, affected by force letting in water (Kuciel, 2013). Mudskippers in Gobiidae have been known to do ventilation by sniffing; this performance improves circulation of water or gas exchange in nasal sac (Nevitt, 1991; Murphy and Stacey, 2002; Belanger *et al*., 2006; Meunier *et al*., 2013).

Mudskippers belonging to Gobiidae have a special and prominent morphology in eyes for surviving life, including avoiding predator and feeding out of water (Sayer, 2005). So, Peripheral olfactory characters of *S. gigas* is concluded to rely less on detecting environmental chemistry by olfaction than vision, and to protect epithelial tissue against dry in olfactory organ during ebb tide; the mudskipper is included as eye fish in category of Teichmann (1954), and to support this result further study on vision of *S. gigas* is needed. From this study, we confirmed that the characteristic of olfactory organ in *S. gigas* is adapted to semi-aquatic life associated with its ecological habits.

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한국산 남방짱뚱어 *Scartelaos gigas*의 후각기관 구조에 관한 연구

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요 약 : 수중과 육상의 이중적인 생활을 하는 남방짱뚱어 *Scartelaos gigas* (amphibious mudskipper)의 후각기관을 해부학과 조직학적으로 연구하였다. 그 결과 후각기관은 좌우 한 쌍이 존재하며 각 기관은 한 개의 가느다란 긴 관 (an elongated canal)과 사비강과 누비강의 두 개의 비강 (ethmoidal and lacrimal nasal sacs)으로 구성되어 있었다. 각 관의 내벽에 발달한 감각 상피는 3～4개의 섬모를 가지는 감각 수용기 세포 (receptor cell), 지지세포 (supporting cell), 기저세포 (basal cell) 그리고 점액세포 (mucus cell)들로 구성되는 다세포성 구조로 이루어져 있었다. 반면에 비감각 상피는 감각기를 갖지 않는 주름진 표면만을 가지고 있었다. 또한 두 개의 비강은 상피 표면에 점액 방울 (mucin droplet)을 가지고 있었다. 따라서 남방짱뚱어의 이러한 후각기관의 특징은 생태적 습성과 서식처에 관련된 반수중 생활 (semi-aquatic life)에 적응한 특징으로 사료된다.

찾아보기 낱말 : *Scartelaos gigas*, 후각기관, 생태적 습성, 서식처