Comparative Morphology and Morphometry of the Olfactory Organ in the Five Korean Torrent Catfishes, Genus *Liobagrus*, with a Taxonomic View

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The morphology and morphometry of the olfactory organ of Korean torrent catfishes, genus *Liobagrus*, consisting of only five endemic species, were investigated by stereo microscopy, scanning electron microscopy, and statistical analysis. They showed the same morphological structure, externally and internally, including a tubular anterior nostril, a slit posterior nostril, and a rosette structure with several linguiform lamellae. Interestingly, however, the lamellar number (LN) revealed specific characteristics useful to identify the five species anatomically: 16–19 in *Liobagrus andersoni* (with standard length, SL, 96.8 ± 5.5 mm, mean ± SD), 14–16 in *Liobagrus obesus* (86.9 ± 13.4 mm), 22–27 in *Liobagrus mediadiposalis* (99.8 ± 14.7 mm), 19–24 in *Liobagrus somjinensis* (90.1 ± 6.7 mm), and 14–18 in *Liobagrus hyeongsanensis* (74.0 ± 6.7 mm). Regarding SL, that of *L. andersoni* was longer than those of *L. somjinensis* and *L. hyeongsanensis* (21.1 ± 1.4%) were greater than that of *L. andersoni* (18 ± 1.2%). These differences might be considered to reflect an interspecific morphological adaptation to micro-habitat according to olfactory importance and can be used as a taxonomic characteristic for this genus.

Key words: olfactory structure, standard length, lamellar number, ratio, micro-habitat adaptation, taxonomic character

INTRODUCTION

In fish, diverse sensory systems such as hearing, mechanosensation, taste, vision, and olfaction, give critical information for survival in their surrounding environment, and accordingly show diverse sensory capabilities by species (Hara, 1994). Among them, olfaction enables fishes to conduct specific ecological behaviors, such as feeding, reproduction, fright reaction, and homing migration, and occurs physiologically by a neuronal signal transduction of the olfactory organ, which has a rosette structure with lamellae (Hansen et al., 2003; Hara, 1986). There are some fishes that possess no lamellae, such as mudskipper amphibious fishes of the subfamily Oxudercinae (Kuciel et al., 2013), and the family Ostraciidae (Yamamoto and Ueda, 1979) and Adrianichthyidae (Kim et al., 2016). However, in most teleost fishes, such sensory organs generally possess a rosette structure consisting of one to numerous lamellae (Yamamoto, 1982; Zeiske et al., 1992; Hamdani and Doving, 2007). The transformation or variation of the rosette structure is affected by the habitat, environmental conditions, and dependence on olfactory sense, of a fish and sometimes reveals the species to which a fish belongs (Yamamoto, 1982; Zeiske et al., 1992; Atta, 2013; Howard et al., 2013; Ferrando et al., 2015, 2017).

Considering the local geological properties and consequently geographic isolation, the Korean torrent catfishes of the genus Liobagrus were divided into five endemic species from South Korea: Liobagrus andersoni Regan, 1908; Liobagrus obesus Son et al., 1987; Liobagrus mediadiposalis Mori, 1936; Liobagrus somjinensis Park and Kim, 2010; and Liobagrus hyeongsanensis Kim et al., 2015. Although some of these species, L. obesus and L. mediadiposalis, have overlapping areas of distribution, their microhabitats such as water velocity and depth, as well as bottom structures, are different, thus clearly supporting speciation (Son and Choo, 1988; Kim and Park, 2002). In the overlapping area, L. obesus prefers a pool zone of slower current with sandy bottom, whereas L. mediadiposalis prefers a rapids zone of faster current with rocky and pebbly bottom (Son and Choo, 1988). Such differences of microhabitats may cause selection for morphological variations in fish organs, especially when these differences are greater (Datta-Munshi and Dutta, 1996; Anker and Dullemeijer, 2017). Using such a morphological approach, no previous anatomical study has been carried out. A comparative study on the structure of egg membranes of four of the above five species, excluding L. hyeonganesis, was performed, and demonstrated the same result by evaluating a specific structure that reveals intrinsic characteristics of the egg membrane of each species, which is considered another key trait useful to identify related and distinct species (Baek et al., 2007). Meanwhile, expanding similar studies to other sensory organs influenc-

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ing the survival and adaptation of fishes in aquatic ecosystems is required. Until now, there has not been a sufficient understanding of catfish's olfactory organ as related to habitat differentiation, although one report described something regarding this for *L. somjinensis* (Kim and Park, 2018). Therefore, this paper focuses on a comparative anatomical study of the olfactory organ for five Korean endemic catfishes, with reference to a taxonomic view.

MATERIALS AND METHODS

Sample preparation

A total of 75 specimens of the five Korean catfishes of genus Liobagrus (L. andersoni, L. obesus L. mediadiposalis, L. somjinensis, and L. hyeongsanensis) were caught with a hand net (7 ×7 mm mesh) in a riffle region at nighttime from March to April 2019, and were close to the maximize size of these species. (Hubbs and Lagler, 1958; Table 1). Among them, L. obesus was caught after obtaining written permission (Number 2020-4) from the Ministry of the Environment of South Korea. For this study, the specimens used were large adults that had reached two-thirds of the maximum size of their species, and that were large enough to identify a sexual dimorphism (Kim and Park, 2002). The collected specimens were anesthetized with 0.1% m-aminobenzoic acid ethylester methanesulfonate (MS222, Sigma, St Louis, MO) immediately in the field. Three specimen per species were fixed with 2.5% glutaraldehvde solution with 0.1 M phosphate buffer (GA solution), and the rest were fixed with 10% neutral formalin solution (pH 7.4). All procedures performed with the fishes strictly obeyed the rules of the Jeonbuk National University Institutional Animal Care and Use Committee.

Microscopic investigation

For anatomical observation, the olfactory organ was dissected from each fish's head, and at the same time it was observed, it was filmed with a digital camera (TG-3, Olympus, Japan) under a stereo microscope (SM; Stemi DV4, Carl Zeiss, Germany). For scanning electron microscopy, the olfactory rosette of the specimens prefixed in GA solution were dissected from the olfactory chamber under the SM, and then re-fixed with GA solution due to the low infiltration capacity. Subsequently, the sample was immersed in 1.0% osmium tetroxide-phosphate buffer (pH 7.4) (1:1) mixed solution at room temperature, dehydrated in solutions of an ascending alcohol series (50%, 60%, 70%, 80%, 90%, 95%, and 100%), transferred to tert-butyl alcohol, dried using a freezing dryer (Vfd-21S, Vacuum Device Co., Ltd., Ibaragi, Japan), coated with osmium via an ion sputtering coater (HPC-1SW, Vacuum Device Inc., Tokyo, Japan), and filmed with a scanning electron microscope (SEM, Carl Zeiss, SUPRA40VP, Germany).

Statistical analysis

SPSS, the statistical software from IBM (SPSS version 18.0, USA), was used for statistical analysis of the SL, lamellar number (LN), and the interspecific ratio between them. The Kruskal-Wallis test was applied to the SL and LN for non-parametric estimation determined by the Levene test, and the ANCOVA test was used for analysis of the ratio of LN to SL. Pearson's correlation coefficient was calculated to check for an intraspecific interaction between the SL and LN.

RESULTS

The external morphology and rosette structure of the olfactory organ

All of the Korean torrent catfishes share the same olfactory organ morphology. Their external structure consists of the anterior and posterior nostrils. The anterior nostril forms a tube-like hole protruding outward, and is located at the front of the nasal barbel. The posterior nostril forms a narrow slit with a bent outline, and is at the posterior base of the nasal barbel (Fig. 1).



Fig. 1. Schematic diagram of the external view of the heads of the five Korean torrent catfishes, with the anterior nostril (**A**) and the posterior nostril (**B**) at the base of the nasal barbel. The blue curved arrows indicate a water flow. AN; anterior nostril; NB, nasal barbell; PN, posterior nostril.

Table 1.	Measurements of Ko	prean torrent catfishes	(genus Liobagrus)	and collection localities
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	SL (mm)	HL (mm)	Collection locality (GPS)
L. andersoni (n = 20)	96.8 ± 5.5	21.2 ± 1.7	Hwayang-ri, Cheongcho-myeon, Goesan-gun, Chungcheongbuk-do
	(85.8~112.1)	(17.6~24.7)	(36°41'01"N, 127°47'08"E)
L. obesus (n = 15)	86.9 ± 13.4	19.1 ± 3.5	Eupnae-ri, Gosan-myeon, Wanju-gun, Jeollabuk-do
	(67.8~111.4)	(14.5~24.4)	(35°58′27″N, 127°12′57″E)
L. mediadiposalis (n = 20)	99.8 ± 14.7	21.7 ± 2.8	Shincheon-ri, Sicheon-myeon, Sancheong-gun,
	(80.5~136.8)	(17.9~29.5)	Gyeongsangnam-do (35°14′30″N, 127°48′32″E)
L. somjinensis (n = 20)	90.1 ± 6.7	18.4 ± 2.2	Sikjeong-dong, Namwon-si, Jeollabuk-do
	(78.2~101.1)	(12.7~21.1)	(35°27'00"N, 127°26'03"E)
L. hyeongsanensis (n = 20)	74 ± 6.7	15.6 ± 1.4	Yongdong-ri, Yangbuk-myeon, Gyeongju-si, Gyeongsangbuk-do
	(66.1~86.2)	(13~17.9)	(35°49′55″N, 129°24′43″E)

Mean \pm SD (Min~Max); SL, standard length; HL, head length; n = number

posterior direction



anterior direction

Fig. 2. Scanning electron micrographs showing the rosette structure of genus *Liobagrus* including *L. andersoni* (A), *L. obesus* (B), *L. mediadiposalis* (C), *L. somjinensis* (D), and *L. hyeongsanensis* (E). Asterisk, lamellae; R, medium raphe. All scale bars indicate 200 µm.



Fig. 3. Interspecific differences of genus *Liobagrus* in standard length (A) and lamellar number (B). LN, lamellar number; SL, standard length (mm).

Internally, the olfactory rosette of the Korean torrent catfishes is composed of several lamellae and a middle raphe, set compactly within the olfactory chamber. Each lamella shows consistent morphological characters as follows (Fig. 1): i) a linguiform process radiating from the lateral and ventral inner walls of the chamber, ii) units leaning posteriorly, and iii) posterior units larger than the anterior ones.

Numerical analysis of the olfactory lamellae

In contrast to the external morphology, the LNs were different among the five Korean torrent catfishes: L. mediadiposalis had the highest range (23.3 ± 1.7, 22-27; Mean ± SD, Range); L. somjinensis, 21.9 ± 1.4, 19–24; L. andersoni, 17.4 \pm 1.0, 15–19; and L. obesus and L. hyeongsanensis showed the lowest range, 14.9 ± 0.8 , 14-16; 15.6 ± 0.9 , 14-18, respectively. There was a highly significant difference in the LN measurements (Kruskal-Wallis test, $\chi^2 = 80.668$, df = 4, P < 0.001; Fig. 3B). The SL measurements were the highest in L. mediadiposalis (80-136 mm) and the lowest in L. hyeongsanensis (66-86 mm). There was a highly significant difference in the SL between species (Kruskal-Wallis test, χ^2 = 46.941, df = 4, P < 0.001; Fig. 3A). The LN/SL ratio showed meaningful differences: L. somjinensis (24.4 ± 2.1%); L. mediadiposalis $(23.6 \pm 2.1\%);$ L. hyeongsanensis $(21.1 \pm$ 1.4%); L. andersoni (mean \pm SD, 18 \pm 1.2%); L. obesus (17.5 \pm 2.6%). There was a highly significant difference in LN/SL ratio (ANCOVA, F = 158.472, df = 4, P < 0.001). Meanwhile, the two factors, LN and SN, had no significant correlation in L. andersoni (Pearson's correlation coefficient, r = 0.335, P = 0.149), L. obesus (r = 0.242, P = 0.384), and L. somjinensis (r = 0.358, P = 0.122), but had a correlation in L. mediadiposalis (r = 0.858, P < 0.001) and L. hyeongsanensis (r = 0.358, P =0.001) (Fig. 4).

DISCUSSION

The diurnal catfishes of the Loricariidae or Callichthyidae are known for using their large eyes to achieve good sight (George and Atakpa, 2015), whereas nocturnal catfishes possess remarkably small eyes relative to the head size (Kim and Park, 2002), like the five Korean torrent catfishes in this study. Such nocturnal catfishes commonly have a strong tendency to rely much more on gustation (or taste) and olfaction rather than other senses (vision, lateral line, electroreception, and mechanoreception) for a behavioral advantage in ecology (Caprio et al., 1993; Hara, 1994; Morais, 2017; Ikenaga and Kiyohara,



Fig. 4. A dispersion diagram showing the relationship between standard length (x-axis) and lamellar number (y-axis) of genus *Liobagrus*.

2018).

Through our study, it was revealed that the five Korean torrent catfishes display the same structure in their olfactory morphology: tubular anterior and slit posterior nostrils, and a rosette structure consisting of several lamellae inside the olfactory chamber. The morphology of the anterior nostril in teleost fishes can be classified into some types of shape: i) semicircle with wide opening flat to the surface, ii) semicircle with an anterior arch structure, iii) circle with small hole flat to the surface, and iv) tube (Zeiske et al., 1992; Kim, 2018; Kim et al., 2019). Among them, a tube-like nostril was well known mainly in Perciformes and Siluriformes (Zeiske et al., 1992; Kasumyan, 2004). Cox (2008) opined that a tubular nostril protruding beyond the skin surface helps offset a "boundary layer," a barrier occurring due to friction in the contact area between the skin and fluid that interrupts the transport of an odorant into the olfactory chamber. Such a boundary layer is generally thicker in slow swimming than in fast swimming fish (Cox, 2008). Denny (1993) also documented that the action of a boundary layer causes a delay in detecting a food near fish. Regarding this matter, Cox (2008) noted that a laterally protruding nostril beyond the nose surface also helps locate odorant sources of food.

In correlation to fish ecology, tubular or pipe-types of the nostril tend to be present in mostly bottom-dwelling, inactive, and burrowing species, and would aide in their living on the bottom (Kasumyan, 2004). Thus, we assumed that a such tubular nostril is a morphological adaptation to reduce the physical disturbance of the boundary layer, for the inactive fish's bottom-dwelling life.

The olfactory rosette of the five Korean torrent catfishes consists of several linguiform lamellae, similarly to those in other catfishes, including *Heteropneustes fossilis* (Bloch) (95–165 mm in total length) with 25–54 units (Goel, 1978), and *Plotosus lineatus* (140–150 mm in total length) with 10 units (Theisen et al., 1991)

Although our five species are very closely related, interestingly, the LN differed by species. Such differences have been considered in some spikefishes of Paratriacanthodes (7-8 in Paratriacanthodes abei: 10-13 in Paratriacanthodes retrospinis; 13-14 in Paratriacanthodes herrei) as taxonomic evidence (Tyler, 1997) and in some cave-dweller fishes of Sinocyclocheilus as an ecological adaption (12-14 in Sinocyclocheilus jii with functional eyes; 11-13 in S. furcodorsalis with functionless eyes or loss of eyes) (Waryani et al., 2013). In regard to catfishes at least, the difference in the LN can become an important characteristic for classifying more complicated taxa and serve as an indicator adaptation for the ecology and micro-habitat where they live. Although the five catfishes studied here are basicallv nocturnal and bottomdwelling fishes that prefer to live in flowing water with a sandy or

rocky bottom, their geographic distribution and microhabitats, including velocity or depth, and bottom structure are different for each species (Kim and Park, 2002; Kim et al., 2015). Finally, we suggest that the olfactory characters, such as LN and LN/SL ratio, can be new taxonomic criteria for confused taxa such as the genus *Liobagrus*.

As the body size (the SL or the head length) increases, eventually the LN may increase. Based on this hypothesis, we compared interspecific differences in the SL, LN, and LN/SL ratio, and also analyzed whether there was an interaction between intraspecific LN/SL ratios. As a result, L. andersoni (96.8 \pm 5.5 mm, Mean \pm SD) was found to have greater SL measurements than L. somjinensis (90.1 \pm 6.7 mm) and L. hyeongsanensis (mean 74.0 \pm 6.7 mm). In contrast to SL, however, the LN/SL ratio was higher in L. somjinensis (24.1 \pm 2.1%) and L. hyeongsanensis (21.1 \pm 1.4%) than in L. andersoni (18 \pm 1.2%). Thus, an intraspecific correlation between LN and SL seems to be less significant for Korean catfishes. Nevertheless, it is known that LN is correlated with body size (head length, body weight, SL), and that the lamellae increase as body size gets larger, for Carcharhinid and Sphyrnid sharks (Kajiura et al., 2005), genus Catla (Kumari, 2008), and Chum salmon Oncorhynchus keta (Kudo et al., 2009). Kumari (2008), in particular, reported that a higher value for LN relative to body size is more common for efficient olfaction of fish. Based on previous reports on two species, L. somjinensis and L. mediadiposalis, a higher LN/SL ratio may be responsible for effective olfactory ability.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

H-T Kim wrote the manuscript and made figures and the table. J-Y Park edited and approved the manuscript for publication.

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