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Comparative Morphology and Morphometry of the Olfactory Organ of *Carassius auratus* and *Carassius cuvieri* (Cypriniformes, Cyprinidae)

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ABSTRACT The olfactory organ of *Carassius auratus* and *C. cuvieri* was compared morphologically and morphometrically using stereomicroscopy and statistical program (SPSS version 18.0). The external morphology of the olfactory organ consists of the open semicircular anterior and posterior nostril, and nasal flap. The internal structure showed the oval rosette consisting of several lamellae arranged radiately. In statistical analysis of standard length (SL), olfactory lamellar number (LN), and SL/LN ratio between two species using independent two sample t-test and Pearson's correlation and coefficient, *C. cuvieri* is the longer SL than that of *C. auratus* and *C. auratus* LN (14~20) is lager than that of *C. cuvieri* (14~16) ($P<0.001$) and *C. auratus* LN/SL ratio (12.7±0.7%) is larger than that of *C. cuvieri* (8.2±0.6%). These results suggest that i) open semicircular nostrils is functional morphology to offset the boundary layer and ii) the lager LN of smaller *C. auratus* may be a morphological adaptation to reflect its higher olfactory dependence than *C. cuvieri* and iii) such interspecific difference in LN and LN/SL ratio could be applied as a new taxonomic trait for identification.

Key words: *Carassius auratus*, *C. cuvieri*, olfactory organ, olfactory rosette, olfactory lamellae, taxonomic trait

INTRODUCTION

The olfactory organ in teleost fishes detects various chemical odors occurring in aquatic ecosystems, triggering essential ecological behaviors for survival, such as food searching, predator avoiding, parental caring, reproduction, social communication, migration, and habitat recognition (Hara, 1986; Kasumyan, 2004; Camacho *et al*., 2010; Daghfous *et al*., 2012). Adapting to reflect these diverse ecological habits, the shape and structure of the olfactory organ enable individuals to effectively and efficiently intake and expel water in their habitats (Cox, 2008). Unique olfactory organ characteristics suitable for habitat and ecology are expressed in various ways depending on species, including the size, shape, and distribution of rosettes, the arrangement and number of lamellae, the distribution and structure of sensory epithelium, and the number and shape of olfactory neurons' dendrites (Yamamoto, 1982; Zeiske *et al*., 1992). Over the recent years, many fish anatomists and tissue researchers have evaluated the degree of olfactory dependence and the taxonomic position among species using distinguishable traits such as rosette arrangement and lamellae number as criteria (Teichmann, 1954; Singh, 1994; Sarkar and De, 2011; Paschenko and Kasumyan, 2015). Specifically, the arrangement of rosettes and the number of lamellae have been utilized as taxonomic characteristics for closely related species, indicating subtle differences in habitat environment and ecological habits (Kim and Park, 2020; Matsuura *et al*., 2021).

The crucian carp *Carassius auratus* belonging to the genus *Carassius* of Cyprinidae is distributed throughout China, Japan, Eurasia, including South Korea, and typically exhibits a golden or greenish-brown coloration, although regional variations in coloration are common depending on the habitat (Kim and Park, 2002). The white crucian

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carp *C. cuvieri* was introduced to South Korea as part of an internal aquatic resource development project in 1972, and has spread throughout Korean rivers nationwide. *C. cuvieri* is very similar to *C. auratus* in terms of habitat type, morphology, and overall coloration but can be distinguished by its higher body height compared to body length and the presence of 84~114 gill rakers (vs. 44~52 in *C. auratus*). Comparative studies between *C. auratus* and *C. cuvieri* include genetic comparisons of genomic DNA (Yoon and Park, 2006), analysis of cytochrome C oxidase I gene for genetic differences (Kang *et al*., 2014), and phylogenetic analysis using the Cytochrome B (MTCYB) goldfish and crucian carp (Kim *et al*., 2020). Additionally, since intermediate phenotypic characteristics in artificially hybridized goldfish and crucian carp can serve as important classification indicators for identifying hybrid individuals in natural populations (Liu *et al*., 2018), a research on distinct and abundant classification traits and criteria between these two species is considered highly significant (Lee *et al*., 2009; Kwak *et al*., 2020). This study aims to describe and compare the morphology and morphometric differences of the olfactory organ between *C. auratus* and *C. cuvieri* discovered during the investigation of fish's morphological characteristics inhabiting Jeollabuk-do and Chungcheongnam-do provinces, and to present their relationship with ecological habits as well as other taxonomic traits beyond body height and gill rakers.

MATERIALS AND METHODS

1. Specimen preparation

From March to May 2023, using a scoop net (4 × 4 mm mesh size) and a casting net (6 × 6 mm mesh size), we collected 20 adult specimens each of *C. auratus* (106.5~170.8 mm in ST, standard length) and *C. cuvieri* (146.0~203.3 mm in ST) at the Gokgyocheon Stream of Namgwan-ri, Pungse-myeon, Cheonan-si, Chungcheongnam-do (36°45′ 7.41″N, 127°7′30.92″E), Silok2-dong, Asan-si, Chungcheongnam-do (36°47′44.22″N, 126°59′31.72″E), Cheongho-ri, Haseo-myeon, Buan-gun, Jeollabuk-do (35°44′25″N, 126°39′52″E). The adults of two species were identified based on their size during the spawning (Park and Han, 2021). The collected specimens were immediately anesthetized on-site using a 0.1% m-aminobenzoic acid ethyl ester methanesulfonate solution (MS222, Sigma, USA), transfered to 10% neutral buffered formalin solution, and then transported to the fish laboratory. The experimental procedure was conducted in strict compliance with the "Guidelines for the management and use of experimental animals" by Jeonbuk National University's Institutional Animal Care and Use Committee (License Number: 2016-12ET-0097).

2. Microscopic and statistical investigation

To check the structure of the olfactory organs in *C. auratus* and *C. cuvieri*, tissue samples were anatomized using a stereo microscope (Stemi DV4, Carl Zeiss, Germany), and detailed structures were described by capturing photographs with a digital camera (TG-3, Olympus, Tokyo, Japan). For statistical analysis, independent two-sample t-tests using SPSS (SPSS version 18.0, IBM, USA) were used to compare and analyze the standard length (SL), olfactory lamellar number (LN), and LN/SL ratio of two species. ANCOVA (analysis of covariance) analysis was used to examine the correlation of LN change with SL increase. Additionally, Pearson's correlation analysis (Pearson's correlation coefficient) was used to assess the degree of allometric association between SL and LN.

RESULTS AND DISCUSSION

1. Anatomy

The olfactory organs of *C. auratus* and *C. cuvieri* are located as a pair on the snout. Its external structure consists of a semicircular anterior nostril, a posterior nostril, and a nasal flap. Internally, it exhibit a rosette structure, which is composed of several olfactory lamellae and a medium raphe (Fig. 1). The bentho-pelagic Cypriniforms species like two fishes show such external configuration with open semicircular anterior and posterior nostrils separated by the nasal flap (Kasumyan, 2004). In a view of functional morphology to have such morphology and location of nostrils, the boundary layer hindering or delaying the influx of external chemical odorants into the olfactory organ typically occurs on the body surface of fish which inhabit a highly flowing water region (Vogel, 1994). Hence, counteracting or reducing the disturbance caused by this boundary layer is significant for a good olfaction so that teleost species have adapted selectively with three main types in nostril morphology and location (Denny, 1993). The first adaptation involves an active swimming forward to reduce the thickness of the boundary layer. The second is positioning the nostrils at the foremost part of the snout where the boundary layer is somewhat thinner than other body part. The third possesses a tube-like nostril structures to mini-

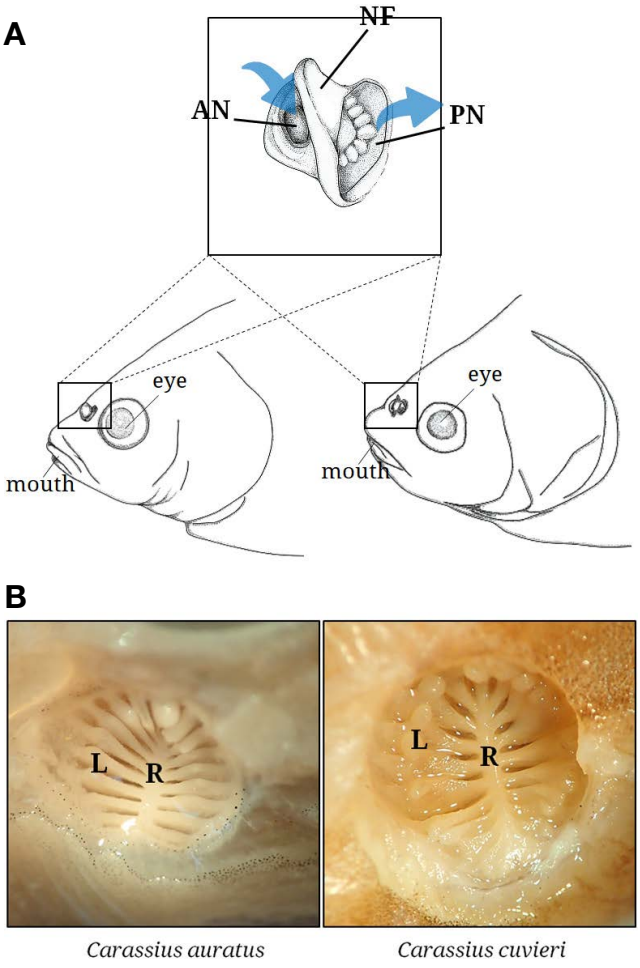

Fig. 1. The external morphology (A) and inner rosette (B) of the olfactory organ of *Carassius auratus* and *C. cuvieri*. The blue arrows indicate water flow. AN, anterior nostril; L, olfactory lamellae; NF, nasal flap; PN, posterior nostril; R, medium raphe.

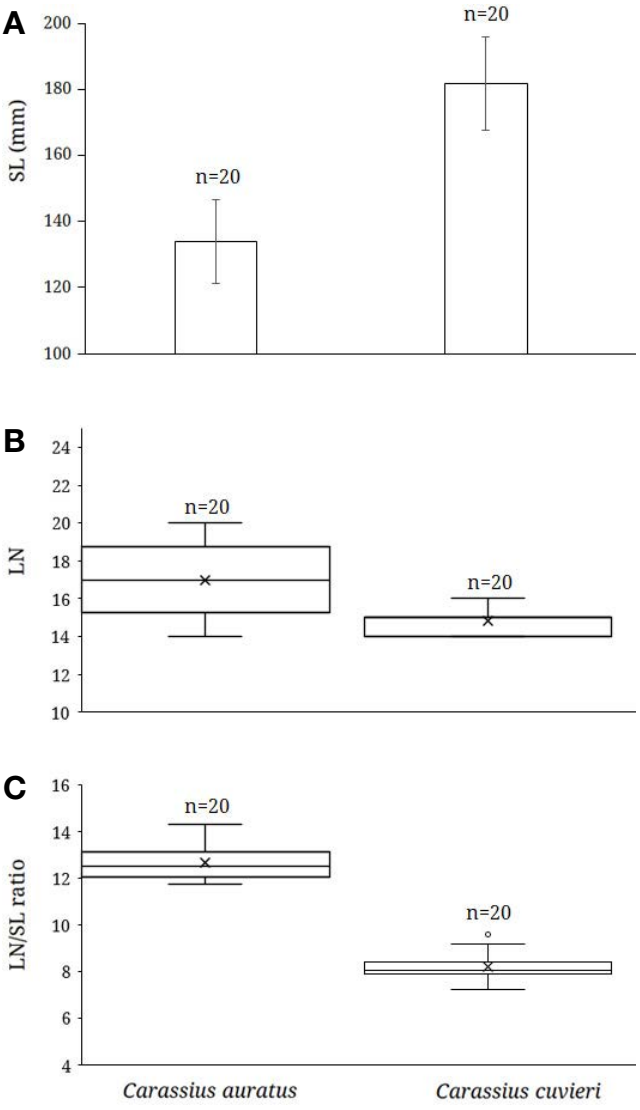

Fig. 2. Interspecific comparison of standard length (A), olfactory lamellar number (B), olfactory lamellar number/standard length ratio (C) of *Carassius auratus* and *C. cuvieri*. LN, olfactory lamellar number; SL, standard length.

mize the influence of the boundary layer (Denny, 1993). In this regard, the open semicircular formation of the nostrils separated by the nasal flap and located on the snout in *C. auratus* and *C. cuvieri* is considered a morphological adaptation that allows them to rapidly move forward, facilitating the influx of water containing external chemical odorants into the olfactory organs with the assistance of the nasal flap. This adaptation is thought to reflect their evolutionary position among many Cypriniform fish species (Zeiske *et al*., 1992).

2. Morphometric analysis of olfactory lamellar number

The statistical analysis comparing the SL between two species (n = 20, 134 ± 12 vs. n = 20, 181.8 ± 14.2) revealed a significant difference, indicating that *C. cuvieri* was larger than *C. auratus* (independent two-sample t-test; $t = -11.244$, $df = 38$, $P < 0.001$; Fig. 2A). The LN between the two species (*C. auratus*, n = 20, 17.0 ± 1.7 vs. *C. cuvieri*, n = 20, 14.8 ± 0.8) showed a significant difference, that *C. auratus* has more the LN than *C. cuvieri* ($t = 5.153$, $df = 38$, $P < 0.001$; Fig. 2B). The LN/SL ratio (n = 20, 12.7 ± 0.7 vs. n = 20, 8.2 ± 0.6) indicated that *C. auratus* also had a higher value than the *C. cuvieri* with significant difference ($t = 22.666$, $df = 38$, $P < 0.001$; Fig. 2C). In both species, there was a significant positive correlation between the SL and the LN (Pearson's correlation coefficient; n = 20, $r = 0.840$, $P < 0.001$, *C. auratus*; n = 20, r =

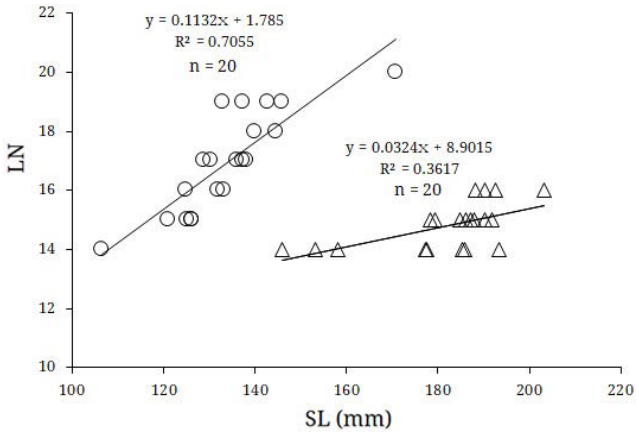

Fig. 3. Dispersion diagram of relationship between standard length (x-axis) and olfactory lamellar number (y-axis) of *Carassius auratus* and *C. cuvieri*. Circle, *C. auratus*; triangle; *C. cuvieri*.

0.601, $P<0.05$, *C. cuvieri*; Fig. 3). Utilizing such interspecific differences, Karna *et al.* (2018) reevaluated the taxonomic position of the silver tripodfish (genus *Triacanthus*) based on several anatomical structural differences, including 24~27 vs. 24~40 in the LN of two closely related species, *Triacanthus nieuhofii* and *T. biaculeatus*, which share very similar morphology when reaching their adult stage. Silva-Junior and Zanata (2022) reported a new species, *P. pukuixe*, inhabiting the rio Pardo basin in Brazil, based on the analysis of differences in the LN with the SL value among the seven species of *Parotocinclus* genus that exhibit varying sizes but similar forms. Therefore, differences of the LN (14~20 in *C. auratus* vs. 14~16 in *C. cuvieri*) and LN/SL ratio (11.7~14.3% in *C. auratus* vs. 7.2~9.6% in *C. cuvieri*)) are considered as new taxonomic characteristics distinguishing these two species, which show morphological ecological similarities, compared to other cyprinid fish species (LN: 10 in *Pseudogobio esocinus*, 21 in *Zacco platypus*, 17 in *Misgurnus anguillicaudatus*, 14~15 in *Rhodeus uyekii* (Yamamoto, 1982; Kim *et al*., 2019). Additionally, the development of a greater LN is generally associated with a high olfactory dependence (Kumari, 2008). Therefore, the higher LN and LN/SL ratio in *C. auratus* is considered as a morphological adaptation reflecting a greater degree of olfactory dependence crucial for survival than *C. cuvieri* at least in Cypriniformes.

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붕어 *Carassius auratus*와 떡붕어 *Carassius cuvieri* 후각기관의 형태 및 형태계측학적 비교 연구

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요 약 : 본 연구에서는 붕어 *Carassius auratus*와 떡붕어 *C. cuvieri* 후각기관의 형태 및 형태계측학적 특징을 실체현미경과 통계프로그램 SPSS를 이용하여 묘사하고 분석하였다. 형태에서 두 종의 후각기관은 외부적으로 반원형의 개방된 전비공, 후비공, 그리고 비경계판으로 구성하며 다수의 후판으로 이루어진 로제트를 보유한 비강 내 구조를 나타냈다. 독립표본 t-검정과 Pearson 상관계수를 이용한 두 종간 체장과 후판의 통계적 분석에서 후판의 개수는 붕어(14~20)가 떡붕어(14~16)보다 더 많았고($P<0.001$), 체장대비 후판의 비율 또한 붕어(12.7±0.7%)가 떡붕어(8.2±0.6)보다 더 높은 값을 나타냈다($P<0.001$). 결과적으로, 주둥이 위쪽의 개방형 비공의 위치와 모양은 외부의 후각 물질의 유입을 방해하는 경계층(boundary layer)의 감소와 연관되는 형태적 적응이며 떡붕어보다 더 작은 체장에도 불구하고 더 많은 수의 붕어 후판은 생존을 위해 더 높은 후각의존도를 반영하는 형태적 전략으로 간주되며 후판 수 차이와 체장대비 비율 차이는 두 종의 새로운 분류학적 형질로 사료된다.

찾아보기 낱말 : 붕어, 떡붕어, 후각기관, rosette 구조, 후판, 분류학적 형질