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Skeleton and Scale Characteristics of Smallscale Blackfish, *Girella leonina* and Largescale Blackfish, *Girella punctata*

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벵에돔, *Girella punctata*과 긴꼬리벵에돔, *Girella leonina*의 골격 및 비늘 특징

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Abstract

Two Girellidae species, smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata* were compared using meristic traits, scale characteristics, and X-ray approaches. Meristic counts revealed three significant differences out of seven meristic characteristics ($P < 0.05$). *Girella leonina* had more numbers of dorsal soft rays, caudal fin rays, and pectoral fin rays while *G. punctata* had more numbers of gill rakers ($P < 0.05$). X-ray photographs indicated that *G. punctata* (45.1 ± 2.34°) had 8.4% more curved vertebral column than *G. leonina* (38.4 ± 1.82°). However, *G. leonina* (24.5 ± 2.51°) had more curved caudal fin than *G. punctata* (18.2 ± 2.16°) in crescent shaped tail. Regarding the results of scale comparison between *G. leonina* and *G. punctata*, scales of five sites displayed different sizes. The overall scale size of *G. punctata* (2.5 ± 0.56 cm^2) was 1.8 times larger than *G. leonina* (1.4 ± 0.35 cm^2). However, samples with the same ctenoid scale of ctenii showed different number of pored lateral line scales and primary apical groove ($P < 0.05$). The morphological differences between *G. leonina* and *G. punctata* were primarily the caudal part of truss dimension, x-ray observations, and scales. Our results confirmed that the two species could be adequately distinguished by their external body shape. The results of our study could be used to identify them in the family of Girellidae through taxonomical parameters.

Key words: Caudal fin, *Girella leonina*, *G. punctata*, Meristic, Scale, Vertebrae

Ⅰ. Introduction

Smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata* are commercially valuable for the aquaculture industry, especially in countries around the East Sea of Korea. However, the two share similar external traits. Only a few studies have reported their differentiation (Okuno, 1962; Yagishita & Nakabo 2003; Haruo et al., 2007).

In the past, meristic traits of fish have been reported (Tortonese, 1975). Meristic characteristics

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are discrete, serially repeated, and countable structures fixed in the embryos or larvae of fish (Turan, 2004). Meristic variations may also include skeletal anomalies. These elements of taxonomic identification are under the control of genotype. However, they can be modulated by environmental conditions (Winans, 1987). Sufficient differences of habitat may result in notable morphological, meristic, and shape differences among stocks of a species. These might be recognizable as a basis for identifying stocks (Winans, 1987; Turan, 2004; Mazlan et al., 2010).

Measurements of fin rays, caudal fin, scales, gill rakers, and vertebral column are morphological characteristics of teleost fish population (Bainbridge, 1963; Nag, 1967). The shape of caudal fin can have biological and evolutional variations. Scales are skeletal elements that cover and protect the skin of fishes (Gritsai, 2002). Fish scales have characteristics shared by other structures such as bones, teeth, and mineralized tendons (Yoshihiro et al., 1996). Vertebrae, other bones, and spines of fish could be successfully used for interspecific identification (Granadeiro & Silva, 2000), although they are much less used than otoliths in taxonomic classification. X-ray images of vertebrae and crescent shaped tail of fishes are shown to have definite differences in morphological characters (Ford, 1937; Grandadeiro & Silva, 2000). Measurements of the angle of curved vertebral column and crescent tail have not been reported yet. However, delicate differences of tail and vertebrae column angles can influence the velocity of fish swimming (Imre et al., 2002; Iosilevskii & Weihs, 2008).. However, comparison of scales has been used for species classification. In addition, shapes and annuli of scales have been used to estimate fish age and fish population (Gritsai, 2002; Park et al., 2006).

Therefore, the aim of this study was to determine the external and internal differences of *G. leonina* and *G. punctata* using different methods, including comparison of scales size, meristic characteristics and x-ray. The results of this study will be useful for the differentiation of two similar species, smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata* belonging to family Girellidae.

Ⅱ. Materials and methods

1. Fish sampling

Both smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata* were obtained fromby fishing in the offshore of Jeju. The samples were rapidly frozen in Future Aquaculture Research Center (FARS), National Institute of Fisheries Science (NIFS) in Jeju Island, Korea, and then they were transported to a mariculture facility at Fishery Genetics and Breeding Sciences Laboratory, Korea Maritime and Ocean University, Busan, Korea.

A total of 50 specimens of each species were adequately defrosted for meristic analysis and staining scales. Standard length and body weight of specimens were measured to the nearest 0.1 g and 0.1 cm, using electric balance (AX 200, Shimadzu Corp., Japan) and digital vernier caliper (CD-20 CP; Mitutoyo, Japan), respectively. The average standard length (SL) of *G. leonina and* G. *punctata* were 34.3 ± 2.36 cm and 31.5 ± 2.78 cm, respectively. The average weights of *G. leonina* and *G. punctata* were 944 ± 84.7 g and 910 ± 87.2 g, respectively. To compare colors of the two Girella fish species, digital images of each species were taken with a digital camera (Coolpix 4500, Nikon, Japan).

2. Meristic counts

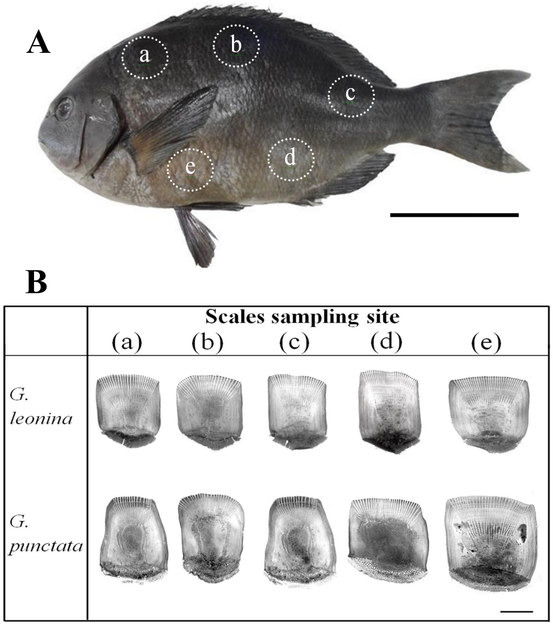
In order to compare meristic characteristics, seven parts in fin rays, gill rakers, and vertebrae of a total of 50 *G. leonina* and *G. punctata* were measured. The numbers of fin rays, vertebrae, and gill raker were also counted. Spinous rays included dorsal fin spine (DFR1), anal fin spine (AFR1), and ventral fin spine (VFR1). Soft rays included dorsal soft ray (DFR2), anal soft ray (AFR2), caudal soft ray (CFR2), pectoral soft ray (PFR2), and ventral soft ray (VFR2). Gill raker (GR) was then observed under a stereoscopic microscope (Carl Zeiss, Germany). All meristic characteristics were counted three times by the same observer.

3. X-ray photograph

In order to visualize the vertebrae, skeleton, and swim bladder of whole fish by radiographic X-ray photographs (Bailey & Gosline, 1955), images were taken at lateral view of overall body shape of 10 defrosted samples of each species. Images were stored on an X-ray machine (Fire CR, 3 Disc Imaging, Korea). We described a left angled triangle between a straight line and the highest abdominal vertebrae to measure the angle of curved vertebrae. Angle of crescent shaped tail was measured by drawing a left angled triangle. The triangle of tail was made of a straight line and the highest one in crescent shaped tail. Through X-ray photographs, the total number of vertebrae (TV) was counted three times by the same observer.

4. Comparison of scalesn

To compare the scales of *G. leonina* and *G. punctata,* we collected 50 scales at 5 sites for each species. As shown in [Fig. 1], designated scale sites were divided by lateral line and fin ray with the following positions: (a) frontward of dorsal fin and above the lateral line, (b) medium of dorsal fin and above the lateral line, (c) rearward of dorsal fin and above the lateral line, (d) rearward of anal fin and below the lateral line, and (e) frontward of ventral fin and below the lateral line.



[Fig. 1]. Description of scale collection site in smallscale blackfish, *G. leonina* (A) and shape of scales of smallscale blackfish and largescale blackfish, *G. punctata* (B). Scales sampling site predicated, (a) positioned: frontward of dorsal fin and upper the lateral line; (b) positioned: medium of dorsal fin and upper the lateral line; (c) positioned: rearward of dorsal fin and upper the lateral line; (d) positioned: rearward of anal fin and below the lateral line; (e) positioned: frontward of ventral fin and below the lateral line. Scale bars indicate (A): 6 cm and (B): 4 mm.

The collected 50 scales were processed using method of Park et al. (2006). Briefly, samples were fixed in 10% formalin followed by washing with tap water. Scales were then placed in 5 ml of 0.5% KOH solution and 1-3 ml of 3% H_2O_2 solution and stained with 0.01% alcian blue (Sigma, USA) for 1 hour. They were then stained with 0.1% alizarin red S (Sigma, USA) for 2 hours. To clean the scales, samples were transferred to absolute glycerol and stored for 3 days. Stained scales were observed under a stereoscopic microscope (Carl Zeiss, Germany) and measured with vernier calipers. The number of primary apical groove (NPAG) and the number of pored lateral line scales (NPLLS) were counted for the 50 specimens of each species. NPAG was also stained with the method of Park et al. (2006) and counted under a stereoscopic microscope (Carl Zeiss, Germany).

5. Statistical analysis

The study was performed in triplicates. Results are reported as the means ± standard deviations (n=10) unless otherwise stated. Data were analyzed with Student's t-test using SPSS statistical package (SPSS 9.0, SPSS Inc., Chicago, IL, USA). Statistical significance was considered when *P* value was less than $P < 0.05$.

Ⅲ. Results

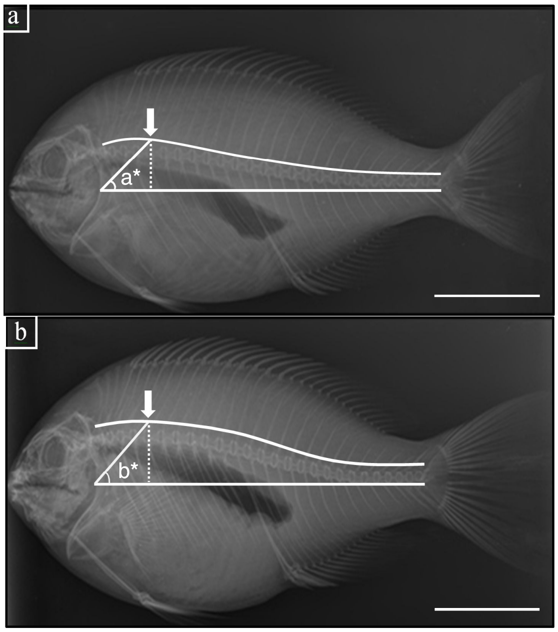
Results of meristic characteristics are summarized in <Table 1>. For both smallscale blackfish, *Girella leonina* and largescale blackfish *G. punctata*, seven measurements were measured. The meristic counts of DFR1, AFR, and VFR were not significantly different in individual ray ($P > 0.05$).

<Table 1> Meristic characteristics of smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata**

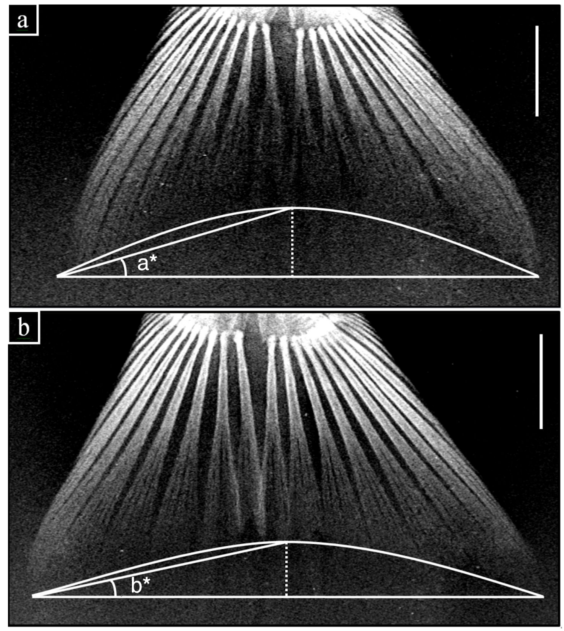
Meristic characteristics		*G. leonina*	*G. punctata*
Fin rays			
Dorsal	Spinous rays	14.5 ± 0.69^{a} (XIII~XV)	13.8 ± 0.45^{a} (XIII~XV)
	Soft rays	13.8 ± 0.54^{b} (13~15)	11.6 ± 0.58^{a} (10~12)
Anal	Spinous rays	3.5 ± 0.67^{a} (III~IV)	3.7 ± 0.45^{a} (III~IV)
	Soft rays	13.2 ± 0.21^{a} (13~14)	13.3 ± 0.18^{a} (13~14)
Caudal	Soft rays	19.1 ± 0.60^{b} (18~20)	17.2 ± 0.54^{a} (16~17)
Pectoral	Soft rays	19.5 ± 1.05^{a} (18~20)	18.6 ± 1.32^{a} (17~21)
Ventral	Spinous rays	0.6 ± 0.53^{a} (0~I)	0.7 ± 0.54^{a} (0~I)
	Soft rays	4.3 ± 0.29^{a} (4~5)	4.5 ± 0.32^{a} (4~5)
Gill rakers		27.9 ± 1.24^{a} (26~30)	31.8 ± 1.43^{b} (29~34)
Vertebrae		25.3 ± 0.21^{a} (25~26)	25.7 ± 0.25^{a} (25~26)

*The values are means ± SD (*n*=10) of triplicate groups. Data were using t-test on data transformed to the arcsine of the square root. Different capital letters on the values indicate statistical significance among meristic characteristics ($P < 0.05$). Refer to the [Figs. 1, 2 and 3].

Descriptive X-ray images of curved vertebral column, swim bladder, and crescent shaped tail are shown in [Figs. 2 and 3]. Regarding the angle of vertebral column, *G. leonina* (38.4 ± 1.82°) was more curved than *G. punctata* (45.1 ± 2.34°, $P < 0.05$) ([Fig. 2]). As shown in [Fig. 3], lunate tail was evidently different between the two. The degrees of deeply forked and slightly forked were measured by analyzing the angles of caudal fin. X-ray images of caudal fin are shown in [Fig. 3].



[Fig. 2] X-ray image of skeleton in smallscale blackfish, *G. leonina* (a) and largescale blackfish, *G. punctata* (b). Arrow point: highest one in abdominal vertebrae; a left angled triangle: the angle between straight line and highest one in abdominal vertebrae Angle: a*=38.4± 1.82° ; b*=45.1±2.34° . Scale bars indicate 6 cm.



[Fig. 3] X-ray image of caudal fin in smallscale blackfish, *G. leonina* (a) and largescale blackfish, *G. punctata* (b). Arrow point: the highest one in crescent shaped tail. (a): left angled triangle; the angle between straight line and the highest one in crescent shaped tail. Angle: a*= 24.5±2.51° ; b*= 18.2±2.16° . Scale bars indicate 3 cm.

The angles of caudal fins of *G. leonina* and *G. punctata* were 24.5 ± 2.51° and 18.5 ± 2.16° ($P < 0.05$), respectively. As shown in [Fig. 2], the total number of vertebrae was the same between the two species (both 25~26, $P > 0.05$). The shapes and sizes of scales of *G. leonina* and *G. punctata* were different. There were also differences between individual fish of each fish species (<Table 2>, [Fig. 1]). Results of scale comparison are shown in <Table 3>. They were significant differences in the number of NPLLS (*G. leonina*, 63.5 ± 1.99; *G. punctata,* 53.2 ± 2.47) and the number of NPAG (*G. leonina,* 33.7 ± 1.43; *G. punctata,* 38.3 ± 0.75) ($P < 0.05$).

As shown in [Fig. 1], both *G. leonina* and *G. punctata* had the same ctenoid scale with ctenii. However, their scale sizes were different. Results of scale sizes of each species are shown in <Table 2>. For the five different scale positions, G. leonina had the following results: (a) 1.1 ± 0.22; (b) 1.1 ± 0.29; (c) 1.5 ± 0.25; (d) 1.8 ± 0.41; and (e) 2.3 ± 0.47 cm^2, while *G. punctata* had the following results: (a) 2.0 ± 0.34; (b) 2.3 ± 0.38; (c) 2.6 ± 0.54; (d) 2.6 ± 0.37; and (e) 3.3 ± 0.42 cm^2 ($P < 0.05$).

<Table 2> Comparison of the number of primary apical groove, number of the scale in lateral line, and size of scales at five sizes of smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata*[*1]

	No. of primary apical groove[*2]	No. of pored lateral line scales	Scale sampling site (cm^2)				
			a	b	c	d	e
G. leonina	$33.7±0.43^a$ (32~35)	$63.5±0.99^b$ (61~65)	$1.1±0.22^a$	$1.1±0.29^a$	$1.5±0.25^a$	$1.8±0.41^a$	$2.3±0.47^a$
G. punctata	$38.3±0.75^b$ (37~39)	$53.2±1.67^a$ (50~55)	$2.0±0.34^b$	$2.3±0.38^b$	$2.6±0.54^b$	$2.6±0.37^b$	$3.3±0.42^b$

[*1] The values are means ± SD (n=50). Data were analyzed using one-and two-way ANOVA after data were transformed to the arcsine of the square root. Different capital letters on the values indicate statistical significantly different (P < 0.05). Refer to the [Fig. 1].

[*2] Number of primary apical groove counted lateral line scales of each species.

Ⅳ. Discussion

Haruo et al. (2007) have found that smallscale blackfish, *Girella leonina* and largescale blackfish *G. punctata* have several differences in meristic characters, operculum flap, shape of caudal fin, and nucleotide identity. To supplement for the lack of information due to few studies in this area, we compared the two species with meristic, vertebrae, and scale analyses.

Meristic variables of fin rays demonstrated that DFR2, CFR2, PFR2, and GR showed differences between the two species. They could be used for species identification.

The total vertebral number (VN) has systematic significance in meristic characteristics of fish population (Bailey & Gosline, 1955). Both G. *leonina and* G. *punctata* had 25~26 TV. We also determined the VN counts and vertebral column flexibility [Fig. 2] because they could be used for species identification in Girellidae species (Ford, 1937; Bailey & Gosline, 1955; Braninerd & Patek, 1998). Braninerd & Patek (1998) have found intervertebral joint angles in Tetraodoniform fishes. Therefore, we induced a description of the highest placed vertebrae, abdominal 5th vertebrae, among the vertebral column to demonstrate the differences in curvature of vertebral column. Results of curvature of vertebral column showed that *G. leonina* was 12% more curved than *G. punctata.*

Developmental differences of caudal fin can results in various shaped tails in accordance with body movement and swimming propulsion (Bainbridge, 1963; Iosilevskii & Weihs, 2008). Therefore, caudal fin is a reliable external morphological difference for comparing these species. Imre et al. (2002) have investigated the phenotypic plasticity of caudal fin of brook charr. Therefore, morphological caudal fin analysis is a useful tool for discriminating similar fishes in a fish population (Nag, 1967). Our results revealed that *G. leonina* had 32% more curved caudal fin than *G. punctata*. The crescent shape of tails might help fish swim faster over long distance. It has been reported that the stiffness and crescent shape of tails make it easier for the fish to change direction suddenly (Iosilevskii & Weihs, 2008).

Various measurements in this study confirmed that *G. leonina* and *G. punctata* had different velocities of water flow. The curvature of vertebral column and crescent tail might have influenced the velocity of water flow. Phenotypic plasticity with multivariate morphological methods are needed to cluster similar species for species identification and estimate size-adjustment (Turan, 2004). The observed meristic and scale differences in this study are probably helpful for differentiating species of Girellidae. The investigated information from this experiment could be a better understanding for differentiation of Girellidae species.

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