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Comparison of Morphological Characteristics between Smallscale Blackfish, *Girella leonina* and Largescale Blackfish, *G. punctata*

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벵에돔, *Girella punctata*과 긴꼬리벵에돔, *G. leonina*의 외부계측형질 비교

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Abstract

In order to discriminate the morphologically analogous smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata,* various methods were used by using biometric and geometric approaches. Morphometric analysis showed considerable 19 differences of total 44 morphometric dimensions: classical dimension 1 part; truss dimension 12 parts; and head part dimension 6 parts (P < 0.05). Some differences of morphological features primarily involved in caudal part of truss dimension. Our results of this study confirmed that two species adequately can distinguish with external body shape, and we hope that the results of our study could be used to identify in Girellidae family as taxonomical parameters.

Key words : *Girella leonina, G. punctata*, Morphological characteristics

Ⅰ. Introduction

Smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata* belonging to Percifomes: Kyphosidae and Osteichthyes, 15 species of *Girella* were known throughout the world (Okuno, 1962). These species widely distributed in the tropical and sub-tropical waters from Indopan Pacific to Korea's Jeju Island, China, and Southern parts of Japan (Yagishita and Nakabo, 2003). Among 15 species, 3 species of *G. punctata*, *G. leonina* and *G. mezina* were coexisted and closely collerated each other (Yagishita and Nakabo, 2003). *G. leonina* and *G. punctata* were herbivorous species and having similar morphological characters in juvenile (Okuno, 1962), and *G. leonina* having a crescent shaped tail and dark colored opercular flap while *G. leonina's* tail is characterized by round shaped tail (Haruo et al., 2007). These two species look alike externally, but there are differences in their industrial applicability, so it is necessary to investigate and classify the characteristics of the biometric traits of *G. leonina* and *G. punctata* in detail. Previous studied Haruo

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et al. (2007) showed differences of meristic counts (dorsal fin spines, soft rays and anal fin soft rays) and mitochondrial DNA of *G. leonina* and *G. punctata,* but conducted little research from the morphological aspect. Our study studied the research, focusing on the characteristics of the morphometric and meristic differences, on which there was a lack of research in Haruo et al. (2007). Phenotypic plasticity changes according to the growth and surrounded environment, confirmation of the species classified through the morphological differentiation. A better understanding of compared the similar species, identified the uncertain species and determined the hybrid is identified multivariately morphological approach analysis (Winans, 1987; Cardin, 2000). Phenotypic variations were completely not appertain to genetic factors and subjected to surrounding aquatic environment, while fish were grown and developed, themselves were modified body shape and form, but unaffected organs and structures are evidence of stock identification (Todd et al., 1981; Currens et al., 1989).

Therefore, phenotypic characters estimated morphological differences in the principally attributed to external influences (Todd et al., 1981; Straüss, 1985; Currens et al., 1989). Morphometric investigations were measured the changed plasticity of species and discover the modification of body shape and they were composed of convergent distances and anatomical network system (Straüss and Bookstein, 1982; Turan, 1999; Cardin, 2000; Albertson et al., 2001).

Truss network system was made by interconnected distances between various digitized landmarks, which was constructed the analytical tools in body shape (Straüss, 1985; Winans, 1987). Pointed landmarks in classical and truss dimension selected the representative feature of fish (fin rays and operculum), it means more easily analyzed of samples. Morphometric characteristics analysis were used other studies, such as measuring the changes in early growh period of Korean rose bitterling, *Rhodeus uyekii* and cyprinid loach, *Misgurnus anguillicaudatus* (Han et al., 2013; Goo et al., 2014). According to the Park et al. (2004), a closely associated fish have a morphological differences. Thus, morphometric analysis used for many purpose.

In the present study, we used the multivariate morphological methods including morphemoetric analysis, meristic characteristics, comparison of scales and X-ray images of caudal fin, those methods were induced in order to diversely compare the *G. leonina* and *G. punctata*. We provide the further evidence for the morphological studies and hope to make a various compared methods.

Ⅱ. Materials and methods

1. Fish sampling

The smallscale blackfish, *Girella leonina*, and largescale blackfish, *G. punctata* were obtained from Future Aquaculture Research Center (FARS), National Fisheries & Development Institute (NFRDI) in Jeju island, Korea. A total of, *G. leonina* and *G. punctata* were randomly collected from the neighboring sea Jeju, Korea, by fishing from March to July, 2015. Fish were quick frozen in FARS, NFRDI, Jeju, Korea and transported to a mariculture facility at the Fishery Genetics and Breeding Sciences Laboratory of the Korea Maritime and Ocean University in Busan, Korea.

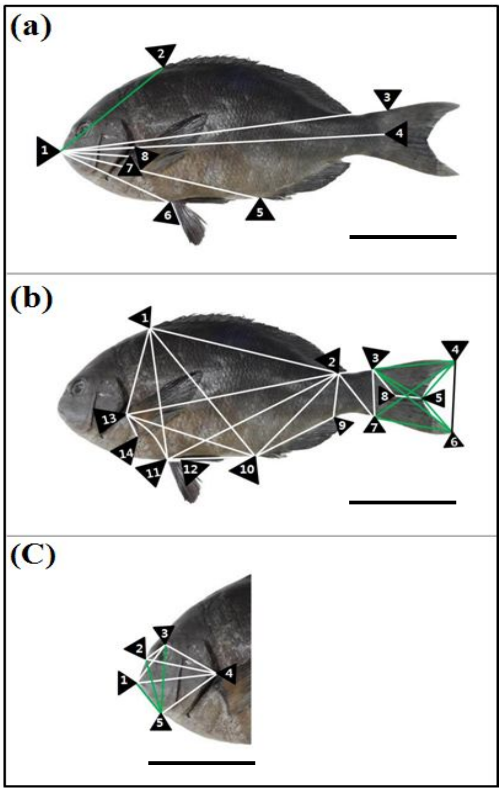
G. leonina and *G. punctata* were distinguished by operculum flap coloring. In total, 100 specimens of each species were defrosted before to use for the morphometric analysis, respectively.

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 calipers (CD-20 CP; Mitutoyo, Japan), respectively. Weighing on average, *G. leonina* had 977±90.6 g, *G. punctata* had 912±93.7 g and measuring standlard length (SL) on average, *G. leonina* had 38.6±3.17 cm, *G. punctata* had 36.5±3.22 cm. We used the camera for comparison of coloring between *Girella* fishes, digital images of each species were taken by digital camera (Coolpix 4500, Nikon, Japan).

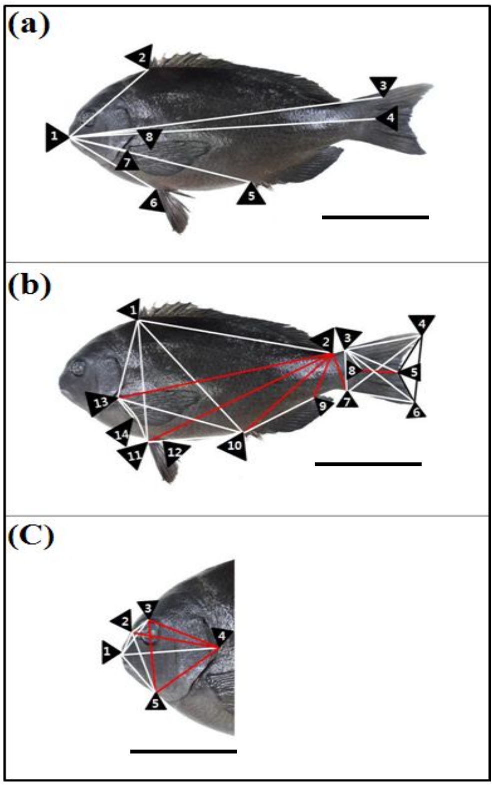
2. Landmark-based morphometrics

Analysis were conducted by the methods of Straüss and Bookstein (1982), Turan (1999), Cardin (2000) and Albertson et al. (2001). The multivariately linear dimensions of pictures [Figs. 1 and 2] were taken to the nearest 0.1 cm using digital vernier calipers. We designed the 3 parts of morphometric analysis, classical dimension (CD), truss dimension (TD) and head part dimension (HD), in total, 26 landmarks and 43 distances were measured, respectively.

As shown in <Table 1>, CD consisted of 8 landmarks and 7 distances [Figs. 1a and 2a], TD consisted of 13 landmarks and 27 distances [Figs. 1b and 2b] and HD consisted of 5 landmarks 9 distances [Figs. 1c and 2c], and all CD dimensions and TD dimensions were divided into standard length **1**X**4** (SL). As shown in <Table 1, and Figs. 1a and 2a>, horizontal and transversal CD distances were made a convergent shape, **1**X**2**: most anterior extension of the head (MAEH) to origin of dorsal fin base **1**X**3**: MAEH to origin of caudal fin base **1**X**5**: MAEH to origin of anal fin base **1**X**6**: MAEH to origin of ventral fin base **1**X**7**: MAEH to origin of pectoral fin base and **1**X**8**: MAEH to operculum.



[Fig. 1] Morphometric measurements of classical dimension (a), truss dimension (b) and head part dimension (c) in smallscale blackfish, *Girella leonina*. Each landmarks for morphometric measurements are numbered. Scale bars indicate 6 cm. Details of measured distances of morphometric values are described in Table 1. Green lines: *G. leonina* has greater distances more than *G. punctata*; White lines: 2 fishes were not found significantly correlated distances.



[Fig. 2] Morphometric measurements of classical dimension (a), truss dimension (b) and head part dimension (c) in largescale blackfish, *Girella punctata*. Each landmarks for morphometric measurements are numbered. Scale bars indicate 6 cm. Details of measured distances of morphometric values are described in Table 1. Red lines: *G. punctata* has greater distances more than *G. leonina*; White lines: 2 fishes were not found significantly correlated distances.

As shown in <Table 1, and Figs. 1b and 2b>, truss system constructed the network system on a fish body and anatomically described. By interconnecting landmarks measured the distances: **1X2**: origin of dorsal fin base (ODFB) to insertion of dorsal fin base (IDFB) **1X10**: ODFB to origin of anal fin base (OAFB) **1X11**: ODFB to origin of ventral fin base (OVFB) **1X13**: ODFB to origin of pectoral fin base (OPFB); **2X3**: insertion of dorsal fin base (IDFB) to origin of above caudal fin base (OACFB); **2X7**: IDFB to origin of bottom caudal fin base (OBCFB) **2X9**: IDFB to insertion of anal fin base (IAFB); **2X10**: IDFB to OAFB **2X11**: IDFB to OVFB **2X13**: IDFB to OPFB **3X4**: origin of above caudal fin base (OCFB) to above of caudal fin base (ACFB) **3X5**: OCFB to center of caudal fin base (CCFB); **3X6**: OCFB to bottom of caudal fin base (BCF); **3X7**: OCF to origin of bottom caudal fin base (OBCFB) **3X8**: OCFB to center of anterior caudal fin base (CACFB); **4X5**: above of posterior caudal fin base (APCFB) to center of posterior caudal fin base (CPCFB); **4X6**: APCFB to bottom of posterior caudal fin base (BPCFB); **4X7**: APCFB to OBCFB **5X6**: CPCFB to BPCFB **5X8**: CPCFB to CACFB **6X7**: BPCFB to OBCFB **9X10**: IAFB to OAFB **10X11**: OAFB to OVFB **10X13**: OAFBto OPFB **11X12**: OVFB to insertion of ventral fin base (IVFB); **11X13**: OVFBto OPFB and **13X14**: OPFB to insertion of pectoral fin base (IPFB).

As shown in <Table 1, and Figs. 1c and 2c>, the HD dimensions divided into distance of head length **1X4** (HL), it convergently constructed the distances and involved eye diameter (ED) and interorbital width (IW), **1X4**: most anterior extension of the head (MAEH) to posterior aspect of operculum (PAO); **1X2**: MAEH to above of nostril (AN) 1X3: MAEH to above of eye (AE); **1X5**: MAEH to snout; **2X3:** AN to AE; **2X4**: AN to posterior aspect of operculum (PAO); **2X5**: AN to snout; **3X4**: AE to PAO; **3X5**: AE to snout; **4X5**: PAO to snout.

<Table 1> Dimensions of body shape for smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata*

Classical dimension	
Standard length (*SL*)	1×4
Most anterior extension of the head – origin of dorsal fin base	1×2
Most anterior extension of the head – origin of caudal fin base	1×3
Most anterior extension of the head – origin of anal fin base	1×5
Most anterior extension of the head – origin of ventral fin base	1×6
Most anterior extension of the head – origin of pectoral fin base	1×7
Most anterior extension of the head – posterior aspect of operculum	1×8
Truss dimension	
Origin of dorsal fin base – insertion of dorsal fin base	1×2
Origin of dorsal fin base – origin of anal fin base	1×10
Origin of dorsal fin base – origin of ventral fin base	1×11
Origin of dorsal fin base – origin of pectoral fin base	1×13
Insertion of dorsal fin base – origin of above caudal fin base	2×3
Insertion of dorsal fin base – origin of bottom caudal fin base	2×7
Insertion of dorsal fin base – insertion of anal fin base	2×9
Insertion of dorsal fin base – origin of anal fin base	2×10
Insertion of dorsal fin base – origin of ventral fin base	2×11
Insertion of dorsal fin base – origin of pectoral fin base	2×13
Origin of above caudal fin base – above of posterior caudal fin base	3×4
Origin of above caudal fin base – center of caudal fin base	3×5
Origin of above caudal fin base – bottom of posterior caudal fin base	3×6
Origin of above caudal fin base – origin of bottom caudal fin base	3×7
Origin of above caudal fin base – center of anterior caudal fin base	3×8
Above of posterior caudal fin base – center of posterior caudal fin base	4×5
Above of posterior caudal fin base – bottom of posterior caudal fin base	4×6
Above of posterior caudal fin base – origin of bottom caudal fin base	4×7
Center of posterior caudal fin base – bottom of posterior caudal fin base	5×6
Center of posterior caudal fin base – center of anterior caudal fin base	5×8
Bottom of posterior caudal fin base – origin of bottom caudal fin base	6×7
Insertion of anal fin base – origin of anal fin base	9×10
Origin of anal fin base – origin of ventral fin base	10×11
Origin of anal fin base – origin of pectoral fin base	10×13
Origin of ventral fin base – insertion of ventral fin base	11×12
Origin of ventral fin base – origin of pectoral fin base	11×13
Origin of pectoral fin base – insertion of pectoral fin base	13×14
Head part dimension	
Head length *(HL)*	
Most anterior extension of the head – above of nostril	1×2
Most anterior extension of the head – above of eye	1×3
Most anterior extension of the head – snout	1×5
Above of nostril – above of eye	2×3
Above of nostril – posterior aspect of operculum	2×4
Above of nostril – snout	2×5
Above of eye – posterior aspect of operculum	3×4
Above of eye – snout	3×5
Posterior aspect of operculum – snout	4×5

3. Statistical analysis

The study was performed in triplicate, and the results are reported as means±SD (n=100), unless otherwise stated. The data were analyzed with Student's *t*-test using the SPSS statistical package (SPSS 9.0, SPSS Inc., Chicago, IL, USA), and were considered significantly different at $P < 0.05$.

Ⅲ. Results

Body coloration of smallscale blackfish, *Girella leonina* typically composed of charcoal gray color and having a dark colored opercular flap. The largescale blackfish, *G. punctata* had generally dark brown color in body and gray and transparent color in opercular flap [Figs. 1 and 2]. The measured results of CD dimensions and TD dimensions were divided into distance of standard length **1**X4 (SL).

<Table 2> Classical dimension results of smallscale blackfish, *Girella leonina* and largescale *blackfish, G. punctata**

Morphometric measurements	*G. leonina* (%)	*G. punctata* (%)	*t*-test
1×2/*SL*	35.1±1.35	31.8±1.41	*
1×3/*SL*	98.0±2.73	96.9±2.14	NS
1×5/*SL*	64.1±1.66	62.6±3.08	NS
1×6/*SL*	35.9±1.84	36.5±1.76	NS
1×7/*SL*	25.4±0.65	24.2±0.58	NS
1×8/*SL*	22.3±1.22	24.7±1.47	NS

*The values are means±SD (n=100). Data of each experimental group were analyzed using *t*-test on data transformed to the arc sine of the square root. NS: not significant; *: indicate statistical significance between morphometric distances ($P < 0.05$). Refer to the landmarks in Table 1 and, Figs. 1a and 2a for the dimension numbers in Table 2.

<Table 2> shows morphometric formula (dimensions/SL), and *G. leonina* has greater value in **1**X**2** ($P < 0.05$). The remaining measurements were not found significantly correlated ($P > 0.05$). Results of CD analyses were not displayed significant differences between the two species ($P > 0.05$) <Table 2, Figs 1a and 2a>.

The truss dimension anatomically described external fish body by digitized and interconnected landmarks in *G. leonina* and *G. punctata* [Figs. 1b and 2b]. <Table 3> shows morphometric formulas (dimensions/SL), comparison of interconnected fin ray distances, *G. punctata* has greater values in distance of dorsal fin ray base, **1**X**2** and other fin distances were not found significantly correlated ($P > 0.05$). On the basis of origin of dorsal fin base (ODFB), **1**X**11**, **1**X**13** and **1**X**10** has not found significant difference ($P > 0.05$). On the basis of insertion of dorsal fin base (IDFB), *G. punctata* has greater values than *G. leonina* in 4 dimensions ($P < 0.05$), except **2**X7 dimensions was not found significant difference ($P > 0.05$).

Two species had showed conspicuously different crescent shaped tail shape [Figs. 1b and 2b], therefore we constructed 11 distances and 6 landmarks to completely analyze. As shown in <Table 3>, on the basis of origin of above caudal fin base (OACFB), *G. leonina* had greater values in **3**X**4** and **3**X**6** ($P < 0.05$) and **3**X**5**, **3**X**7** and **3**X**8** were not found significantly correlated ($P > 0.05$). In succession, above of posterior caudal fin base (APCFB) parts, *G. leonina* had greater values in **4**X**5** and **4**X**7**, and **4**X**6** were not found difference. Followed by, center of caudal fin base (CCFB) had 2 results, *G. leonina* had greater value in **5**X**6** ($P < 0.05$), while *G. punctata* has greater values in **5**X**8** ($P < 0.05$).

<Table 3> Truss dimension results of smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata**

Morphometric measurements	*G. leonina* (%)	*G. punctata* (%)	*t*-test
1×2/*SL*	52.9±2.59	55.8±1.17	*
1×10/*SL*	49.1±0.60	48.4±1.19	NS
1×11/*SL*	38.2±2.74	37.0±0.84	NS
1×13/*SL*	25.4±1.71	26.5±1.82	NS
2×3/*SL*	6.8±0.41	6.3±0.77	NS
2×7/*SL*	15.2±1.17	16.1±1.29	NS
2×9/*SL*	12.3±0.69	15.7±1.04	*
2×10/*SL*	31.4±0.93	34.5±0.91	*
2×11/*SL*	50.1±0.78	53.4±1.14	*
2×13/*SL*	52.5±1.64	56.2±1.90	*
3×4/*SL*	24.2±1.15	20.3±1.22	*
3×5/*SL*	17.3±1.15	16.2±1.24	NS
3×6/*SL*	24.7±1.26	21.5±1.38	*
3×7/*SL*	17.3±1.84	16.1±2.67	NS
3×8/*SL*	7.6±1.13	7.3±1.34	NS
4×5/*SL*	14.8±1.37	11.2±1.26	*
4×6/*SL*	23.5±1.24	24.3±1.33	NS
4×7/*SL*	28.5±1.15	25.4±1.34	*
5×6/*SL*	16.6±0.81	13.7±1.04	*
5×8/*SL*	13.8±1.06	16.6±0.78	*
6×7/*SL*	24.7±0.75	19.5±1.16	*
9×10/*SL*	27.5±1.44	26.1±2.66	NS
10×11/*SL*	29.8±0.71	30.2±1.17	NS
10×13/*SL*	22.9±0.86	22.1±1.63	NS
11×12/*SL*	4.9±1.19	4.5±0.65	NS
11×13/*SL*	9.7±0.47	9.2±0.42	NS
13×14/*SL*	5.0±0.54	4.8±0.71	NS

*The values are means±SD (*n*=100). Data of each experimental group were analyzed using *t*-test on data transformed to the arcsine of the square root. NS: not significant; *: indicate statistical significance between morphometric distances ($P < 0.05$). Refer to the landmarks in Table 1 and, Figs. 1b and 2b for the dimension numbers in Table 3.

In the last part of the caudal fin, bottom of caudal fin base (BCFB), *G. leonina* had greater value in **6**X7 ($P < 0.05$). On the basis of origin of anal fin base (OAFB), origin of ventral fin base (OVFB) and origin of pectoral fin base (OPFB) were not found significantly correlated ($P > 0.05$). Results of TD dimensions showed that *G. leonina* had greater values than *G. punctata* in overall caudal parts ($P < 0.05$).

The HD dimensions divided into distance of head length **1X4** (*HL*), those were enlargely described the head of fish [Figs. 1c and 2c], HD composed of divided into 9 morphometric measurements <Table 4>.

<Table 4> Head part dimension results of smallscale blackfish, *Girella leonina* and largescale blackfish, *G. punctata**

Morphometric measurements	*G. leonina* (%)	*G. punctata* (%)	*t*-test
1×2/*HL*	19.1±2.65	17.8±2.01	NS
1×3/*HL*	41.0±2.73	42.9±2.14	NS
1×5/*HL*	62.5±1.76	58.9±1.84	*
2×3/*HL*	20.4±0.75	19.8±0.88	NS
2×4/*HL*	85.7±1.47	88.3±1.22	*
2×5/*HL*	66.3±0.89	63.2±0.93	*
3×4/*HL*	69.3±1.26	73.8±1.14	*
3×5/*HL*	84.6±1.14	81.8±1.26	*
4×5/*HL*	79.7±1.26	83.4±1.19	*

*The values are means±SD (*n*=100). Data of each experimental group were analyzed using *t*-test on data transformed to the arcsine of the square root. NS: not significant; *: indicate statistical significance between morphometric distances ($P < 0.05$). Refer to the landmarks in Table 1 and, Figs. 1b and 2b for the dimension numbers in Table 4.

<Table 4> shows morphometric formula (dimensions/*HL*), on the basis of most anterior extension of the head (MAEH), two species had not significant differences in **1**X**2** and **1**X**3** ($P > 0.05$) and *G. leonina* had greater value than the other in **1X5** ($P < 0.05$). On the basis of above of nostril (AN), **2X3** was not found significantly

correlated (P > 0.05), *G. punctata* had greater value than the other in **2**X**4** and *G. leonina* had greater value than the other in **2**X**5** (P < 0.05). On the basis of above of eye (AE), *G. punctata* had greater value than the other in **3**X**4** (P < 0.05) and *G. leonina* had greater value than the other in **3**X**5** (P < 0.05). Posterior aspect of operculum (PAO) to posterior aspect of snout (PAS): **4**X**5**, *G. punctata* had greater value than the other (P < 0.05). Results of HD cluster analysis showed that *G. leonina* has greater values in longitudinal distances and *G. punctata* has larger horizontal distances (P < 0.05).

Ⅳ. Discussion

Phenotypic variations in this study were appreciably observed that morphological differences between smallscale blackfish, *Girella leonina,* and largescale blackfish, *G. punctata,* were discriminately analyzed by multivariate measurements. In general, other studies were demonstrated that the morphological variances in external body shape and traits within fish populations were determined by vulnerably environmental change and various situation adaptability (Todd et al., 1981; Currens et al., 1989; Bronte and Moore, 2007).

According to the previous studies, such as Turan (2004), Tzeng (2004), Bronte and Moore (2007), and Mazlan et al. (2010) have definitely classified morphological variation among populations of some certain species, sibling species and congeneric species. Haruo et al. (2007) found that *G. leonina* and *G. punctata* have several differences about meristic characters, operculum flap, shape of caudal fin and nucleotide identity. To supplement for the lack of research of Haruo et al. (2007), we tried to compare these species with multivariate morphological approaches and scale analysis.

Results of morphometric investigation were apparently different individual traits, transverse and convergent CD method shows the greatly nonsignificant, TD was recorded significant differences between those samples, truss network systems were used to construct a anatomical distance for accurately analysis. So, it was definitely showed that discriminant results of TD is an effective method for identifying fish populations of closely related species. *G. punctata* had longer distances than *G. leonina* in fish body of TD (11 parts), and caudal fin of *G. leonina* had longer distances than *G. punctata* in caudal fin of TD, it means ordinary people adequately classified the these species with the discernible external traits.

Measurement values of HD were comfirmed differences, *G. leonina* has graeater values in distance of eye to snout and nostril to snout, however *G. punctata* has greater values in distance of eye to operculum and nostril to operculum. It means that two species can be distinguished by observation of the head part. Through the different morphometric characteristics, it is possible to infer the habitats and prey of both fish (Todd et al., 1981; Albertson and Kocher, 2001; Tzeng, 2004; Bronte and Moore 2007). In *Girella* species morphological studies, the morphometric measured factors may account for 43.2% (CD, TD and HD).

As these samples showed through the variable measurements, it is essential to establish which morphometric characteristics in which species do not change as a function of variations in feed and environmental conditions (Todd et al., 1981; Currens et al., 1989; Bronte and Moore, 2007). An understanding of the morphometric characteristics of fish is limited because they can be modified by the environment, the general factor of fish is mainly determined by genetic factors (Currens et al., 1989).

Measurement values of morphometric characteristics were difficult to distinguish the these species, therefore, the classification of *G. leonina* and *G. punctata* need to be translated into action with methods of in our study.

In conclusion, the present study provided that phenotypic discrimination in body shape differentiation suggest the apparently evidence to demonstrate a comparison between *G. leonina* and *G. punctata*. We induced the basic morphological research about the *Girella* species and hope to the further investigations are supplemented.

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