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Intraspecific variations in morphology of swamp barb (*Puntius brevis*)

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ABSTRACT

Understanding inter-population variation and sexual differences in morphology is a fundamental part of taxonomic and biological research. External morphology of swamp barb (Puntius brevis) was compared between sexes and among three populations in the Mekong Delta, including O Mon-Can Tho (n=86), U Minh Thuong-Kien Giang (n=49) and U Minh Ha-Ca Mau (n=77). The results illustrate that color and countable parameters are similar in all populations. Nonetheless, 9 out of 20 morphometric indices are significantly different among three populations (P<0.05), where 6 indices are more important criteria to distinguish among swamp barb populations. Furthermore, the sexual dimorphism of this species is indicated in 16/20 morphometric indices consisting of 5 indices relating egg-carrying characteristics. In particular, the females have higher values of indices relating to abdomen and head parts while most of fin length indices are larger in males than in females. In sum, intraspecific variation in morphometrics of swamp barb is mainly due to sexual dimorphism rather than populations.

1. INTRODUCTION

Swamp barb (*Puntius brevis* Bleeker, 1849) is one of common cyprinids in the Mekong Delta, which inhabits in benthopelagic zones (floodplains, canals, ditches, and sluggish streams) in freshwater areas of the tropical regions. The distribution of *Puntius brevis* is widely in Mekong and Chao Phraya basins, Malay Peninsula, and Java (Froese & Pauly, 2020; Tran et al., 2013). Swamp barb usually prefers swimming to newly inundated land during flood seasons for spawning (Rainboth, 1996; Vidthayanon, 2002). It is an important species, contributing a part of food and economic income to local fishermen in the Mekong river basin due to its proliferative capability and high flesh quality (Rainboth, 1996). Until now, the yield of this species mainly comes from capture in the wild. However, the pesticide abuse was harmfully influencing production of rice field fish species in general and *Puntius brevis* in particular (Ngo To Linh & Nguyen Van Cong, 2009; Pham Van Toan, 2013).

Based on external morphology, *Puntius brevis* is easily differentiated from the other cyprinids by smooth dorsal fin spine (no serration and discrete dark particles), a pair of maxillary barbels and one black blotch at caudal peduncle (Rainboth, 1996; Tran et al., 2013). However, under the impact of ecological changes, many organisms including fish have their own adaptive mechanisms in morphology, especially morphometrics (measurable traits), resulting in intraspecific variations (Agrawal, 2001; West-Eberhard, 2003). In Eurasian perch (*Perca fluviatilis*), for example, Svanbäck and Eklöv (2002) reported that the fish inhabited in the littoral zone had a deeper body, larger head and mouth, longer fins than the ones in pelagic zone. Besides, differences in morphology between males and females of fish species are primarily originated from natural and sexual selection (Kelly et al., 2013; Spoljaric & Reimchen, 2008; Székely et al., 2004), which are related to secondary sexual characters (Eakin et al., 2006; Kitano et al., 2007), sex-biased dispersal (Gunawickrama, 2008) and sexual growth performances (Saillant et al., 2001). Because of such plastic changes in morphology, the use of morphological characteristics is an effective method in distinguishing populations, which plays an important role in taxonomic identity, species evolution and efficient enhancement of fisheries resources management (Gunawickrama, 2008; Ukenye et al., 2019).

As a result of a wide range in distribution, *Puntius brevis* can exhibit changing in external structure as a result of adaptive responses to various habitats. This hypothesis has not been tested so far. Hence, the present study was aimed to assess variations in morphological characteristics of *Puntius brevis* among three different populations in two habitat types of opened water in Can Tho and closed water systems in two National Parks in Ca Mau and Kien Giang. This study result would provide imperative information for the domestication and conservation strategies of the species in the Mekong Delta.

2. MATERIALS AND METHOD

2.1. Fish sampling

The specimens were collected from three locations of the Mekong delta comprising one in the Hau River at O Mon-Can Tho (n=86) and two in conservation areas in U Minh Thuong-Kien Giang (n=49) and U Minh Ha-Ca Mau (n=77). These sampling sites were chosen as a comparison between an opened water system (representing by the Hau River in Can Tho) and closed water systems (representing by blocked canals in conservation areas of Ca Mau and Kien Giang). Samples were collected by local fishermen using long fence trap net and cast net in 2020. The collected specimens were preserved freshly in ice boxes and then transported to a laboratory of College of Aquaculture and Fisheries, Can Tho University for morphological analyses.

2.2. Morphological analyses

Species identification of *Puntius brevis* from collected specimens was based mainly on the guidelines of Rainboth (1996) and Tran et al. (2013). Then, these identified specimens were weighed and taken photos with a code and a ruler. After that, they were operated to observe the gonad for sex information. The total weight (TW) and 23 morphometric parameters (Fig. 1) in the body and the head were recorded based on the guideline of Kottelat (2001), modified by Lumbantobing (2010). These morphometric parameters were measured using "ImageJ" method, in which each measurement was transformed into a pixel unit from fish photos into a length unit (Schneider et al., 2012).

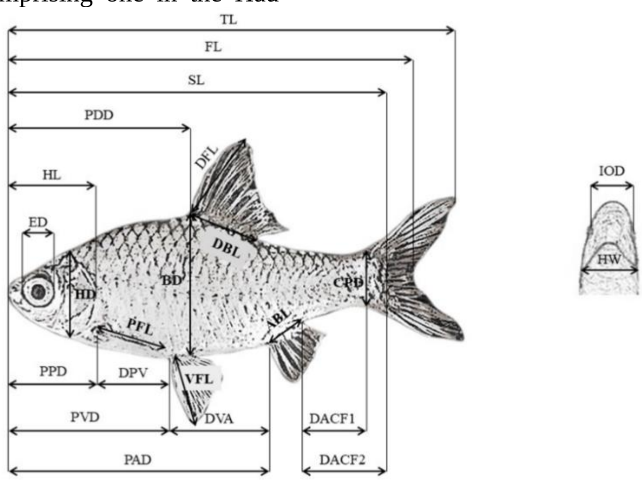


Figure 1. Morphometric parameters of Puntius brevis

Notes: Body parts include total length (TL), standard length (SL), fork length (FL), caudal peduncle depth (CPD), distance between anal and caudal fin 1 (DACF1), distance between anal and caudal fin 2 (DACF2), body depth (BD), predorsal distance (PDD), pre-pectoral distance (PPD), pre-ventral distance (PVD), pre-anal distance (PAD), distance between pectoral and ventral fin (DPV), distance between ventral and anal fin (DVA), dorsal fin base length (DFB), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin base length (ABL). Head parts consist of eye diameter (ED), head length (HL), head depth (HD), head width (HW), inter-orbital distance (IOD).

2.3. Data analysis

Countable parameters were compared with a previous study of Truong Thu Khoa and Tran Thi Thu Huong (1993) using mean and ranges. Fish size (total length and total weight) and 20 morphometric indices (ratios of morphometric parameters to standard length and head length) were compared between two sexes and three populations using two-way ANOVA. Significant differences in morphometric indices among populations were further analyzed using Duncan multiple-range tests.

Because size ranges of fish are not similar among populations, morphometric parameters were adjusted to eliminate size effects by using the method of Elliott et al. (1995):

$$M_{adj} = M^*(L_S/L_O)^b$$

In which: Madj: adjusted morphometric parameter

M: original morphometric paramter

L_S: overall average standard length of all individuals

Lo: standard lenght of each individual

b: regression coefficient of the equation $\log M = a + b^*\log L_o$

The morphometric indices and adjusted data were then analyzed by using principal component analysis (PCA) to find out important parameters showing variations among populations. PCA results were compared with ANOVA results to identify important parameters for population differentiation and sexual dimorphism. Results of discriminant analysis (DA) using stepwise method were compared between morphometric indices and adjusted data to test the classification among populations. The SPSS 22 software was used for all analyses.

3. RESULTS

3.1. External appearance of *Puntius brevis* from different populations

Generally, the appearance of this fish looks the same in three different populations (Fig. 2). They have all specific characters of *Puntius brevis* including a black spot in caudal peduncle, smooth (not serrated) last simple dorsal-fin ray, silvery scales covered the whole body (except head part) and a broad reddish-orange stripe on midlateral body. Likewise, the male and female fish have no remarkable differences in external morphology except the appearance of a big belly in a female (Fig. 3).

Figure 2. Figures of *Puntius brevis* caught in three populations Ca Mau, Can Tho, and Kien Giang

Figure 3. External appearance of female (left) and male (right) *Puntius brevis*

3.1.1. Countable parameters of Puntius brevis

Countable parameters of *Puntius brevis* are similar among three populations. The number of spines and rays of this species are III,8 in dorsal fin; I,11 in pectoral fin and 8 in anal fin. The result of countable parameters is consistent with a previous finding of Truong Thu Khoa and Tran Thi Thu Huong (1993).

3.1.2. Variation in morphometric indices between the sexes and among three populations

The weight and length of fish are significantly different among three populations (p<0.01). Specimens from Ca Mau have the smallest size (both weight & length) while the largest ones are observed in Kien Giang population. In the same population, females are larger than males (Table 1).

Table 1. Weight, length and morphometric indices of *Puntius brevis* in three populations and the two sexes

Indices	Can Tho (N=86)	Ca Mau (N=77)	Kien Giang (N=49)	Female (N=139)	Male (N=73)	P values		
						Pop.	Sex	PopxSex
Weight (g)	10.09±4.21^{b}	5.75±3.15^{a}	13.22±7.36^{c}	11.7±5.45^{y}	4.6±1.41^{x}	<0.01	<0.01	<0.01
Length (cm)	8.53±1.28^{b}	7.30±1.06^{a}	9.23±1.89^{c}	8.98±1.41^{y}	6.85±0.6^{x}	<0.01	<0.01	<0.01
Ratios to standard length (%)								
CPD	13.9±0.7^{a}	14.7±0.8^{b}	14.7±0.8^{b}	14.2±0.9^{x}	14.7±0.7^{y}	<0.01	<0.01	<0.01
DACF1	13.7±1.4^{a}	13.2±1.6^{a}	13.1±1.8^{a}	13.3±1.6^{x}	13.5±1.6^{x}	0.24	0.11	0.18
DACF2	19.0±1.2^{a}	19.0±1.7^{a}	18.4±1.7^{a}	18.7±1.5^{x}	19.3±1.6^{y}	0.85	<0.01	0.16
BD	38.3±2.1^{a}	38.0±2.6^{a}	39.1±2.3^{a}	39.1±2.2^{y}	37.1±2.1^{x}	0.75	<0.01	0.46
PDD	48.1±1.8^{a}	49.0±2.3^{a}	49.6±2.0^{a}	48.8±2.1^{x}	48.7±2.1^{x}	0.08	0.41	<0.01
PPD	24.5±2.2^{a}	26.1±2.0^{b}	25.0±2.2^{a}	24.7±2.3^{x}	26.1±1.8^{y}	0.04	0.02	0.21
PVD	46.6±1.5^{a}	46.3±1.9^{a}	46.7±2.1^{a}	46.9±1.8^{y}	46.0±1.8^{x}	0.94	<0.01	0.90
PAD	71.1±1.7^{a}	70.1±1.9^{a}	71.4±2.4^{a}	71.5±1.8^{y}	69.6±1.7^{x}	0.72	<0.01	0.12
DPV	22.3±1.9^{a}	20.9±1.8^{a}	21.9±1.7^{a}	22.3±1.7^{y}	20.5±1.7^{x}	0.09	<0.01	0.97
DVA	24.4±1.8^{a}	23.8±1.7^{a}	24.4±2.1^{a}	24.6±1.8^{y}	23.5±1.7^{x}	0.75	<0.01	0.04
DFB	20.0±1.2^{a}	19.9±1.7^{a}	19.7±1.6^{a}	20.0±1.4^{x}	19.8±1.6^{x}	0.63	0.21	0.40
DFL	22.6±1.6^{a}	25.2±1.8^{b}	23.1±2.0^{a}	23.1±1.9^{x}	24.9±1.9^{y}	<0.01	<0.01	0.55
PFL	20.2±1.6^{a}	21.1±1.5^{b}	20.2±1.7^{a}	20.3±1.6^{x}	21.0±1.5^{y}	0.02	0.05	0.19
VFL	18.1±1.5^{a}	21.3±1.8^{b}	20.9±1.5^{b}	19.4±2.2^{x}	21.0±1.9^{y}	<0.01	0.01	0.02
ABL	11.3±1.0^{a}	11.5±0.8^{a}	11.3±1.2^{a}	11.3±1.1^{x}	11.5±0.7^{x}	0.34	0.92	0.36
HL	24.4±1.7^{a}	26.1±1.8^{b}	24.9±2.1^{a}	24.8±1.9^{x}	26.1±1.7^{y}	<0.01	<0.01	<0.01
Ratios to head length (%)								
HD	94.0±8.3^{a}	91.6±7.2^{a}	94.2±7.2^{a}	94.9±7.6^{y}	90.0±6.9^{x}	0.72	<0.01	0.10
HW	70.4±6.0^{b}	66.4±6.8^{a}	67.5±8.0^{a}	69.8±6.9^{y}	65.3±6.3^{x}	0.05	<0.01	0.82
ED	33.7±2.2^{a}	34.8±3.0^{b}	34.7±2.7^{b}	33.9±2.6^{x}	35.1±2.6^{y}	0.04	0.01	0.21
IOD	60.3±5.9^{b}	52.6±5.0^{a}	53.8±6.5^{a}	58.3±6.5^{y}	51.6±4.8^{x}	<0.01	<0.01	<0.01

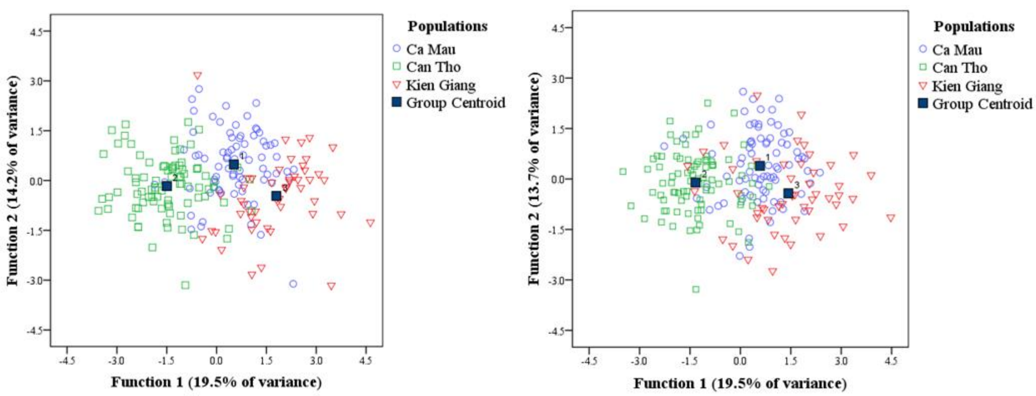
Note: Values in the same row with different characters (Where a, b, c for populations and x, y for sexes) are significantly different (P<0.05). Abbreviations are shown in Figure 1.

Morphological analyses using 20 morphometric indices illustrate that 9/20 indices (6 indices compared to standard length and 3 ratios to head length) significantly differ among three populations (Table 1). On body indices, the fish individuals in Can Tho have the smallest ratios of caudal peduncle depth (CPD) and ventral fin length (VFL) to standard length compared to the other two wild populations. In addition, Can Tho and Kien Giang fish have smaller pre-pectoral distance (PPD), dorsal fin length (DFL) and pectoral fin length (PFL) than fish in Ca Mau. On head characteristics, three populations are similar in head depth (HD) to head length (HL) ratio (P=0.72). However, Can Tho fish has a bigger inter-orbital distance (IOD) and head width (HW) but smaller eye diameter (ED) compared to individuals from Ca Mau and Kien Giang populations (P<0.05). These indices are similar between fish in Ca Mau and Kien Giang populations.

Furthermore, 80% of indices (16/20) significantly vary between males and females (Table 1). Females have larger values than males in 5 abdomen indices (ratios to standard length, P<0.01) including body depth (BD), pre-ventral distance (PVD), pre-anal distance (PAD), distance between pectoral and ventral fin (DPV), distance between ventral and anal fin (DVA). Meanwhile, males have bigger values than females (P<0.01) at CPD, DACF2, DFL, PPD, PFL, VFL, HL. In the head measurements, females have HW and HD but shorter HL (except in CM population, Fig. 4A), and larger IOD but ED than those of males.

The principal component analysis (PCA) based on adjusted morphometric data (removing size effects) reveals that the first 3 essentially principal components explaining 44.3% of total variation. In which, the first principal component (PC1) explained 19.5% of variation with important morphometric parameters of HW, BD, IOD, HD and DPV (arranging by the order of importance). The PC2 explained 14.2% of variation, mainly relied on HL, PPD, ED and DPV. The important parameters contributing to PC3 are VFL, CPD and DFL explaining 11.6% of variation. The highly important parameter contributing to both PC1 and PC2 is DPV. Nevertheless, combining result of PCA and ANOVA analyses (Table 1) reveals that among 11 parameters mentioned above, three parameters (BD, HD, DPV) are not significantly different among populations but different between two sexes ($P_{Pop.}>0.05$, $P_{Sex}<0.01$) (Table 1).

Therefore, the remaining eight important parameters were used for discriminant analysis (DA). The result shows that individuals from Ca Mau and Kien Giang are relatively close to each other and both populations are more separated from Can Tho population (Fig. 5). Based on these eight adjusted morphometric parameters, individuals of Can Tho and Ca Mau populations can be classified into their original groups with high correct assignments of 84.9% and 70.1%, respectively. Conversely, a large portion of individuals in Kien Giang population was misclassified, in which 32.7% individuals were assigned to Ca Mau and 10.2% to Can Tho populations (Table 3). Individual classifications based on 22 indices and 22 adjusted measurements show similar trends as the result mentioned above (data not shown).



(A) **(B)**

Figure 5. Canonical discriminant analyses based on 20 adjusted morphometric data (A) and 8 important parameters (B) for population identification of *Puntius brevis*

Table 3. The percentage (%) of individual classification into three populations based on 8 important parameters for population identification of *Puntius brevis*

Original populations	Assigned populations			
	Can Tho	**Ca Mau**	**Kien Giang**	**Total (N)**
Can Tho	**84.9**	14	1.2	100 (86)
Ca Mau	14.3	**70.1**	15.6	100 (77)
Kien Giang	10.2	32.7	**57.1**	100 (49)

4. DISCUSSION

Overall, the external morphology of *Puntius brevis* from three populations is not remarkably different under visual observation, but they have significant differences in their morphometric parameters. The causes of this variation may originate mainly from environmental conditions (Albert et al., 2008; Beldade et al., 2011). The morphometric variation among populations of this present study indicates that individuals in Can Tho are differentiated from those in Ca Mau and Kien Giang while fish in these two populations are more similar, resulting in high ratios of incorrect assignment. This result can be explained by differences in habitat, feeding niche, and other factors relating to the environment. In the Can Tho population, the fish is exposed to an opened water system with fresh and current water (in the Hau River), whereas the ones from Ca Mau and Kien Giang are in conservation areas with slow water velocity, alum soil and sometimes infected brackish water due to salinity intrusion. Additionally, U Minh Ha-Ca Mau and U Minh Thuong-Kien Giang used to be in the same forest, then they were separated into two regions by two rivers: Trem and Cai Tau rivers (Vietnam Forestry, 2019). Therefore, the ecological conditions of Ca Mau and Kien Giang sampling sites are nearly similar.

Morphological variations among populations are also reported in other species. For instance, a study on striped dwarf catfish (*Mystus mysticetus*) in the Mekong Delta showed that 22/23 morphometric indices were significantly different among four populations collecting from three different types of environments (closed water system with alum soil, large river with fast water velocity and rice field with slow velocity) (Nguyen Thi Ngoc Tran & Duong Thuy Yen, 2020). Three *Puntius brevis* populations differ from each other in 8 important parameters, especially CPD, ED, DFL, etc. Likewise, freshwater blenny (*Salaria fluviatilis*) among three different populations (1 river and 2 lakes) are considerably different in CPD, ED, PFL, and jaw length, which is explained by water velocity, feeding adaptation and predatory rate (Neat et al., 2003). However, Gunawickrama (2008) comparing among 6 populations of blackspot barb (*Puntius singhala*), the same genus with the species in this present study) declared that there was no correlation between morphometric variation and geographic distances, but sexual dimorphisms were confirmed as an intraspecific variation.

This present study also demonstrates sex dimorphisms in 16 out of 20 morphometric indices, in which 10 indices different between two sexes are not dependent on populations (no significant interactions between population and sex, P>0.05). Females of *Puntius brevis* have higher values of morphometric indices in abdomen and head parts. In which, abdomen indices are those related to egg-carrying characteristics, including BD, PVD, PAD, DPV, DVA as well as HD, HW and IOD from head part. On the contrary, males are greater than females in the length of fins (PFL, DFL and VFL) and CPD. Investigations on sexual dimorphisms in cyprinid species have been carried out for a long time, typically a study of Kim et al. (2008), who found out 12/37 dissimilarly morphometric indices between the sexes of Korean chub (*Zacco koreanus*) in the spawning seasons, where most of indices of males were bigger than those of females. Similarly, the males also gain greater indice values than females in other cyprinids, namely Danube barbel (*Barbus balcanicus*) with 9/28 morphometric indices of sexual dimorphism (Petar et al., 2014). Especially, a congeneric species (*Puntius singhala*) with this present study occurs sexually dimorphic characters in the length of pectoral fin, caudal peduncle and dorsal fin base with a larger value in males (Gunawickrama, 2008). In the same trend, *Puntius brevis* males have predominant value in pectoral fin and caudal peduncle length except dorsal fin base length, which is not noticeably different between the sexes in this present study (P=0.21).

5. CONCLUSIONS

Swamp barb (*Puntius brevis*) varied among populations in 8 important morphometric indices, including pre-pectoral distance, ventral fin length, caudal peduncle depth, dorsal fin length, eye diameter, head width, inter-orbital distance, and head length. These indices can be used to assign individuals into their original populations with the correct probabilities of 57.1 to 84.9%. Sexual dimorphisms were displayed in 16 indices, where females have larger indices relating to abdomen and head parts, and males have longer fins and higher caudal peduncle depth.

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