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Comparison of morphological characteristics of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids

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Abstract: Various biometric and geometric measures were used to discriminate between the morphologically similar river puffer, *Takifugu obscurus*, tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids. The hybrids and triploid hybrids had greater anal fin width, nostril width, and snout length than the parental species ($p<0.05$). However, they had less caudal peduncle depth, inter-orbital width, head length, and head width ($p<0.05$). The morphometric and meristic characteristics of the hybrids and triploid hybrids were either intermediate between the parental species or more similar to those of one parental species. However, the external morphology of the hybrids and triploid hybrids was predominantly maternal. The triploid hybrids had asymmetry in the fin rays and gill raker numbers. This study identified phenotypic characteristics by distinguishing the morphological variables of river puffer, tiger puffer, their hybrids, and their triploid hybrids.

Keywords: hybrids, triploid hybrids, morphometric characteristic, river puffer (*Takifugu obscurus*), tiger puffer (*T. rubripes*)

INTRODUCTION

Morphological differences between species or populations are usually understood and compared in terms of general or specific anatomical shapes (Straüss and Bond 1990; Park *et al.* 2015; Lim *et al.* 2016; Park 2020a). The morphometric characteristics of fish can be measured in millimeters, unlike countable meristic characteristics. Although our understanding of the morphometric characteristics of fish is limited because they can be readily modified by the environment, the general shape of a fish is often determined by genetic factors (Park *et al.* 2006, 2015). Morphometric characteristics are primarily used to discriminate sexes and species and to identify confusing species such as crossbred hybrids (Park *et al.* 2006, 2015, 2017).

Traditional morphometrics have been used to study the characteristics of fish for over 30 years, and are focused on lengths, widths, and heights of fish, in addition to axes of fish bodies, including their tails and heads (Straüss and Bond 1990; Park *et al.* 2006; Caillon *et al.* 2018). Consequently, the original shape cannot be unequivocally reconstructed from the measurements (Caillon *et al.* 2018). To avoid this drawback of traditional measurements, the truss network system may be used as an alternative (Rawat *et al.* 2017). The truss network consists of a systematically arranged set of distances that are measured between a set of preselected anatomic landmarks, which are points identified based on local morphological features and are chosen to divide the body into functional units (Straüss and Bookstein 1982; Straüss and Bond 1990). The truss network, which includes components of body depth and length along the longitudinal axis, covers the entire fish body with no loss of information, and is therefore more sensitive to change (Park *et al.* 2006; Mojekwu and Anumudu 2015; Park 2020a).

The Korean domestic consumption of puffer fish is 10,000 tons year^{-1}, most of which is imported from China and Japan; puffer fish is a high-value fish in Japan and Republic of Korea (Kang *et al.* 2007; Park *et al.* 2019). The river puffer, *Takifugu obscurus* (Tetraodontiformes, Tetraodontidae) inhabits rivers during the fry stage, brackish water zones during juvenile stage, coastal zones during adult stage, and migrate back up the rivers during the spawning season (Park *et al.* 2019). The tiger puffer, *T. rubripes* (Tetraodontiformes, Tetraodontidae), inhabits the coastal waters of Korea and China (Kotaro and Takeshi 2007; Park *et al.* 2017). River puffers can reach up to 45 cm in length and are highly priced owing to their high-quality meat; however, it takes more than 30 months for fish to reach maturity (Yang and Chen 2003; Kotaro and Takeshi 2007). Tiger puffers, in contrast, reach up to 75 cm and grow faster than river puffers (Park *et al.* 2017).

Hybridization is a method of combining desirable genotypes and phenotypes from two different species and can be used to generate sterile fish. Interspecific and intergeneric hybrids may be functionally sterile because of genetic incompatibility; however, several hybrids showing fertile gonad development have been obtained (Chevassus 1983). Furthermore, hybrid fish offer opportunities to improve production characteristics, including flesh quality, disease resistance, morphology, and growth. Many variables affect the early survival, hatching, and abnormality rates of interspecific hybrids (Chevassus 1983). Heterosis is demonstrated when artificially-induced hybrid offspring have higher performance than the parental fish, and have high overall vigor and resistance to environmental change (Chevassus 1983; Kim *et al.* 1995; Park *et al.* 2017; Yoo *et al.* 2018). In addition, hybrids may have greater tolerance to low-oxygen conditions, increased resistance to many diseases, and high tolerance to sulfide concentrations, low temperatures, and water pH variations (Chevassus 1983; Kim *et al.* 1995). The hybrid of the river puffer (♀) and the tiger puffer (♂), which was produced by artificial hybridization experiments, has large potential economic value because of the combination of their individual advantageous characteristics (Park *et al.* 2017; Dou *et al.* 2019). Furthermore, the toxicity of the ovaries, liver, and intestines from hybrid puffer fish has been reported to be lower than that of cultured and wild puffer fish, resulting from their infertility (Kim *et al.* 2006; Nunez-Vazquez *et al.* 2012; Hamasaki *et al.* 2013).

Triploidy induction in fish has been used to generate sterility for applications in commercial farming and fisheries management (Benfey 1999). Triploid fish have impaired gametogenesis, so investment in somatic growth is not reduced by the metabolic costs of sexual maturation. Triploid sterility can also be used to prevent the decline in flesh quality associated with sexual maturation, and to address concerns regarding the environmental impact of farm escapees (Peruzzi *et al.* 2004; Park 2019; Park *et al.* 2019; Park 2020b). In addition, growth rates tend to slow or cease in maturing fish; a growth advantage of triploid fish over diploids is most likely to be observed during the later stages of sexual maturation (Gray *et al.* 1993; Benfey 1999). This growth advantage is most likely to be demonstrated by species that have diploids with high or complete mortality associated with sexual maturation and spawning, such as ayu, *Plecoglossus altivelis*, and twice-spawned rainbow trout, *Oncorhynchus mykiss* (Benfey 1999). One cause of unsuccessful interspecific fish hybridization is high mortality rates during the hybrids' early life stages (Chevassus 1983; Yoo *et al.* 2018). This problem has been partially solved in interspecific hybrid fish by inducing triploidy (Scheerer and Thorgaard 1983; Gray *et al.* 1993; Park *et al.* 1997). Therefore, hybrid triploidy in fish provides advantageous synergistic effects for commercial farming and fisheries management applications (Park *et al.* 2006, 2017; Yoo *et al.* 2018).

Several studies on the characteristics of hybrids, triploid hybrids, and their parental species have been studied to compare their biological characteristics and to identify their abnormal deformities and growth advantages (Park *et al.* 1997, 2006, 2017). Triploid animals generally have similar, if not identical, morphological and meristic characteristics to those of their diploid counterparts (Bonar *et al.* 1988; Kim *et al.* 1995; Park 2020a). However, several morphological differences and abnormalities have been associated with triploidy in fish. A variety of deformities have been reported in the triploid pejerrey, *Odontesthes bonariensis* (Strüssmann and Takashima 1993), but it is unclear whether the examined fish were triploid or aneuploid (i.e., having a chromosome number other than a complete multiple of the haploid genome). In this study, we investigated and compared the external traits of the river puffer, the tiger puffer, their hybrids, and their triploid hybrids using morphological methods, including morphometric analysis and meristic characteristics.

MATERIALS AND METHODS

1. Fish sampling

Hybrids and triploid hybrids between the river puffer, *Takifugu obscurus*, and the tiger puffer, *T. rubripes* were induced using the methods of Park *et al.* (2017) and Yoo *et al.* (2018). Two-hundred river puffers and 200 tiger puffers were injected with human chorionic gonadotropin (Sigma, USA). After 24 hours, the eggs and sperm of the river puffer and the tiger puffer were combined to induce interspecific hybrids (river puffer ♀×tiger puffer ♂). A total of 3,000 fertilized interspecific hybrid eggs were subjected to cold shock treatment (4°C) to prevent extrusion of the second polar body. Three-thousand untreated fertilized eggs were used as the hybrid group.

One-hundred individuals from each experimental group (river puffer, tiger puffer, hybrids, and triploid hybrids) were reared over a period of 5 months at a water temperature of 25°C and 30 ppt salinity in an aquarium system. They were fed a commercial feed (Cheonhajaeil Feed Corp., Republic of Korea) twice daily. Following the methods of Yoo *et al.* (2018), to distinguish hybrids and triploid hybrids flow cytometry analysis was used to estimate the average cellular DNA content of each group. Tissue was collected from each experimental fish's caudal fin and fixed in 10 mL of cold 70% ethanol. Cells (10^6) were collected and stained using a high-resolution DNA staining kit (Partec GmbH, Germany) under dark conditions for ~15 min. The stained cells were analyzed using a PA-II flow cytometer (Partec GmbH, Germany) to determine the relative DNA content.

All experiments performed in this study complied with the current laws Republic of Korea (Ordinance of Agriculture, Food and Fisheries, No. 1: The Law Regarding Experimental Animals, No. 9932) and the Ethical Guidelines of

Table 1. Body shape measurements of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids

Traditional measurements	
Standard length (*Ls*)	1×4
The most anterior extension of the head - origin of the dorsal fin base	1×2
The most anterior extension of the head - origin of the caudal fin base	1×3
The most anterior extension of the head - origin of the anal fin base	1×5
The most anterior extension of the head - most posterior aspect of the operculum	1×6
Truss measurements	
Origin of dorsal fin base - origin of the anal fin base	2×5
Origin of dorsal fin base - the most posterior aspect of the operculum	2×6
Dorsal fin width	2×7
Dorsal origin of the caudal fin - insertion of the dorsal fin	3×7
Dorsal origin of the caudal fin - ventral origin of the caudal fin	3×8
Dorsal origin of the caudal fin - insertion of the anal fin	3×9
Origin of the anal fin base - the most posterior aspect of the operculum	5×6
Anal fin width	5×9
Pectoral fin width	6×10
Insertion of the dorsal fin - ventral origin of the caudal fin	7×8
Ventral origin of the caudal fin - insertion of the anal fin	8×9
Head part measurements	
Head length (*HL*)	1×14

Upper side		Lateral side	
Nostril width	11×17	The most anterior extension of the head - Above of the nostril	1×11
Inter-orbital width (IW)	12×16	The most anterior extension of the head - Above of the eye	1×12
Head width	13×15	The most anterior extension of the head - The posterior end of the supraoccipital	1×13
		Eye diameter (ED)	18×19

Hybrids and triploid hybrids from river puffer (♀)×tiger puffer (♂).

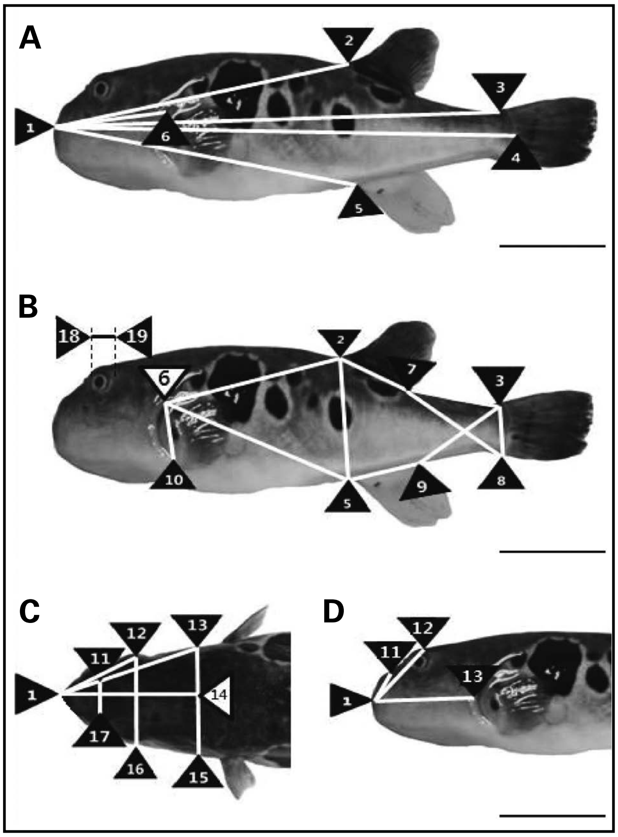

Fig. 1. Morphometric characteristic landmarks for obtaining (A) traditional measurements, (B) truss measurements, (C) the dorsal view of the head part measurements, and (D) the lateral view of the head part measurements of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids. Scale bars indicate 3 cm. The landmark numbers are referred to in Table 1.

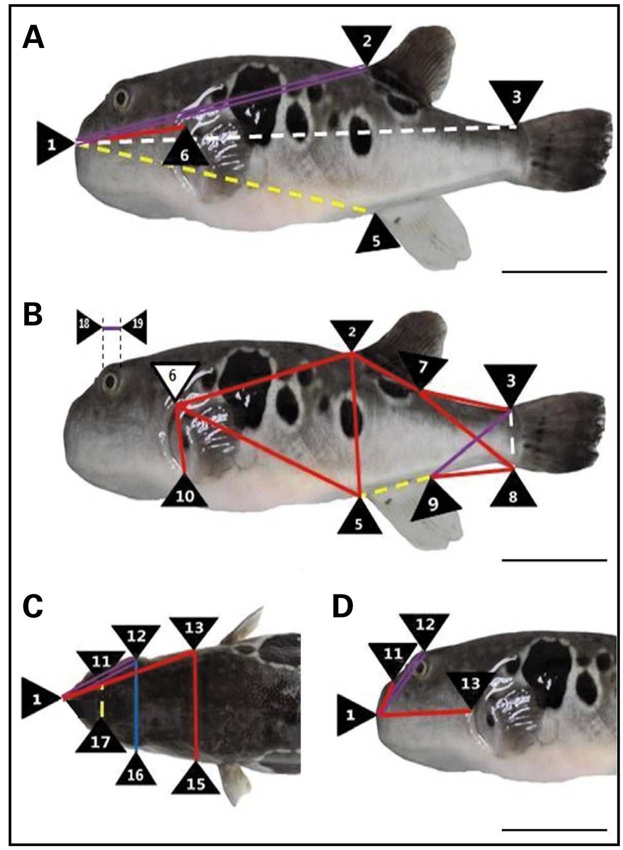

Fig. 2. Morphometric characteristic results using landmarks to obtain traditional measurements, truss measurements, and head part measurements in the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids. The panels indicate (A) traditional measurements, (B) truss measurements, (C) the dorsal view of the head part measurements, and (D) the lateral view of the head part measurements. The longest measurement in the river puffer is indicated by a blue line, in the tiger puffer by a red line, in the hybrid by a violet line, and in the hybrid triploid by a violet double line. The white dotted lines indicate the longest measurements of both the river puffer and tiger puffer. The yellow dotted lines indicate the longest measurements of both the hybrids and triploid hybrids. Scale bars indicate 3 cm. The landmark numbers are referred to in Table 1.

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2. Landmark-based morphometrics

Morphometric analyses were conducted using the methods reported by Turan (1999), Cardin (2000), Albertson and Kocher (2001), and Park *et al.* (2006). The linear dimensions (Table 1 and Fig. 1) of 30 fish from each group were measured to the nearest 0.1 cm using a digital Vernier caliper. Traditional measurements (TRAs), truss measurements (TRUs), and head part measurements (HDs) were obtained using a total of 19 landmarks and 24 distances. TRAs were obtained using 6 landmarks and 5 distances (Fig. 1A), TRUs using 8 landmarks and 11 distances (Fig. 1B), and HDs using 10 landmarks and 8 distances (Fig. 1C, D). In the following text, the bold numbers separated by "×" refer to landmarks (the first number), from which distances were measured to a specific point on the body (the second number) (Table 1; Figs. 1 and 2). All TRAs and TRUs were divided by standard length measurements (1×4: *Ls*). To ensure that variations in this study were only attributed to body shape differences and not to the fish's relative sizes, size effects were eliminated by standardizing the morphometric parameters (Normala *et al.* 2017).

The TRAs included 1×2 (the most anterior extension of the head (MAEH) to the origin of the dorsal fin base);

Table 2. Results of traditional measurements, truss measurements, and head part measurements of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids

Morphometric measurement	River puffer	Hybrids	Triploid hybrids	Tiger puffer
		Traditional measurements		
Ls (cm)	8.3 ± 0.61^{a}	10.6 ± 0.46^{c}	9.6 ± 0.58^{b}	16.2 ± 1.33^{d}
1×2/*Ls*	67.6 ± 4.88^{b}	69.1 ± 3.61^{bc}	74.1 ± 3.61^{c}	66.1 ± 1.12^{a}
1×3/*Ls*	97.2 ± 1.74^{c}	95.3 ± 2.56^{a}	96.5 ± 1.48^{b}	97.2 ± 2.36^{c}
1×5/*Ls*	69.7 ± 2.15^{b}	70.3 ± 2.38^{c}	70.1 ± 3.47^{c}	68.5 ± 1.88^{a}
1×6/*Ls*	33.9 ± 1.52^{b}	32.3 ± 3.04^{a}	32.9 ± 1.17^{a}	35.2 ± 2.08^{c}
		Truss measurements		
2×5/*Ls*	20.6 ± 1.42^{a}	19.2 ± 2.71^{a}	20.3 ± 1.86^{ab}	24.1 ± 0.92^{b}
2×6/*Ls*	36.4 ± 2.81^{a}	39.2 ± 2.14^{b}	38.8 ± 1.97^{b}	41.9 ± 1.57^{c}
2×7/*Ls*	12.1 ± 0.47^{a}	13.1 ± 1.52^{b}	14.1 ± 1.64^{c}	14.9 ± 2.03^{d}
3×7/*Ls*	18.3 ± 3.42^{b}	17.8 ± 1.61^{a}	17.1 ± 2.34^{a}	21.2 ± 0.42^{c}
3×8/*Ls*	9.2 ± 0.51^{b}	7.5 ± 0.83^{a}	7.1 ± 0.61^{a}	9.7 ± 0.91^{b}
3×9/*Ls*	22.1 ± 1.74^{a}	24.9 ± 1.03^{c}	23.9 ± 0.98^{b}	22.3 ± 1.36^{a}
5×6/*Ls*	37.7 ± 2.45^{a}	41.6 ± 2.27^{b}	40.5 ± 1.93^{b}	42.4 ± 1.83^{bc}
5×9/*Ls*	11.6 ± 1.25^{a}	14.6 ± 2.19^{b}	14.1 ± 1.75^{b}	11.9 ± 0.83^{a}
6×10/*Ls*	9.2 ± 1.36^{a}	9.2 ± 2.15^{a}	9.3 ± 1.88^{a}	10.6 ± 1.95^{b}
7×8/*Ls*	21.8 ± 1.93^{a}	20.6 ± 1.25^{a}	20.5 ± 1.54^{a}	24.6 ± 1.85^{b}
8×9/*Ls*	16.1 ± 1.83^{a}	19.2 ± 2.65^{b}	19.0 ± 2.27^{b}	21.2 ± 1.67^{c}
		Head measurements		
HL (cm)	2.3 ± 0.24^{a}	3.3 ± 0.35^{b}	2.8 ± 0.87^{ab}	4.5 ± 1.15^{c}
1×11/*HL*	22.6 ± 3.27^{a}	26.6 ± 2.56^{b}	27.4 ± 1.42^{c}	32.1 ± 4.12^{d}
1×12/*HL*	53.9 ± 7.52^{b}	56.3 ± 7.18^{c}	58.1 ± 4.48^{d}	48.7 ± 6.08^{a}
1×13/*HL*	116.7 ± 6.15^{b}	105.3 ± 3.27^{a}	104.9 ± 4.89^{a}	120.1 ± 7.88^{c}
11×17/*HL*	24.6 ± 8.24^{b}	26.0 ± 2.61^{c}	26.7 ± 1.94^{c}	22.6 ± 6.35^{a}
12×16/*HL*	75.6 ± 5.31^{c}	53.0 ± 4.73^{a}	54.8 ± 3.81^{a}	62.0 ± 2.53^{b}
13×15/*HL*	86.8 ± 4.73^{b}	75.2 ± 5.84^{a}	76.8 ± 6.60^{a}	90.8 ± 3.28^{c}
18×19/*HL*	15.7 ± 2.33^{a}	16.9 ± 1.42^{b}	16.0 ± 1.11^{a}	15.9 ± 1.79^{a}

Hybrids and triploid hybrids from river puffer (♀)×tiger puffer (♂). Each value (mean of triplicate ± SD) with a different superscript indicates significant difference ($p < 0.05$). Refer to the landmarks in Fig. 1 and Table 1 for morphometric measurement definitions.

1×3 (MAEH to the origin of the caudal fin base); 1×5 (MAEH to the origin of the anal fin base); and 1×6 (MAEH to the most posterior aspect of the operculum). The TRUs included 2×5 (the origin of the dorsal fin base to the origin of the anal fin base); 2×6 (the origin of the dorsal fin base to the most posterior aspect of the operculum); 2×7 (dorsal fin width); 3×7 (dorsal origin of the caudal fin to the insertion of the dorsal fin); 3×8 (the dorsal origin of the caudal fin to the ventral origin of the caudal fin); 3×9 (the dorsal origin of the caudal fin to the insertion of the anal fin); 5×6 (the origin of the anal fin base to the most posterior aspect of the operculum); 5×9 (anal fin width); 6×10 (pectoral fin width); 7×8 (insertion of the dorsal fin to the ventral origin of the caudal fin); and 8×9 (ventral origin of the caudal fin to the insertion of the anal fin). The HDs were separated into upper and lateral sides, and the measurements were divided by the head length measurement (1×14: *HL*). They were convergently constructed with distances including the eye diameter (18×19: ED) and the inter-orbital width (12×16: IW). The upper side HDs included: 11×17 (nostril width), 12×16 (IW), and 13×15 (head width). The lateral side HDs included: 1×11 (MAEH above the nostril), 1×12 (MAEH above the eye), 1×13 (MAEH to the posterior end of the supra-occipital), and 18×19 (the ED).

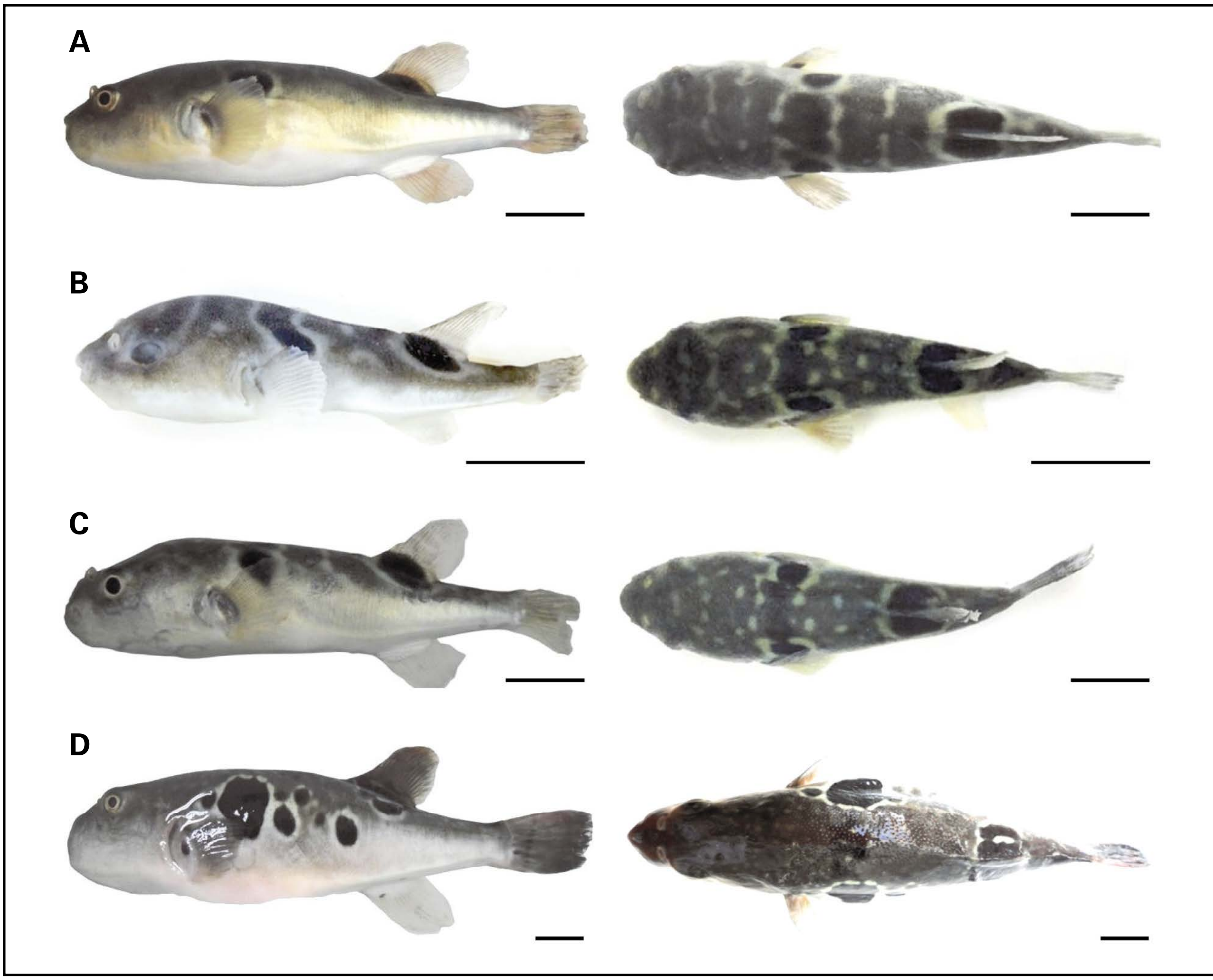

Fig. 3. External morphology of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids. Left side: lateral view of external morphology; right side: dorsal view of external morphology. The panels indicate (A) river puffer, (B) hybrids, (C) triploid hybrids, and (D) tiger puffer. Scale bars indicate 3 cm.

3. Meristic characteristics

Measurements of 6 meristic characteristics were repeated on 100 fish from each group. The meristic characteristics included the number of fin rays (including the anal fin, caudal fin, dorsal fin, and pectoral fin), vertebrae, and gill rakers. Gill rakers were observed using a stereomicroscope (Carl Zeiss, Germany); each meristic measurement was performed three times by the same observer.

4. Statistical analysis

All measurements of morphometric and meristic characteristics were performed in triplicate; unless otherwise stated, the results are reported as means of triplicate ± SD. Data were analyzed using the SPSS statistical package v 9.0 (SPSS Inc., Chicago, IL, USA) using one-way ANOVA and Duncan's multiple range test (Duncan 1955). Differences were considered statistically significant when $p < 0.05$.

RESULTS

The standard length of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids were 8.3 ± 0.61 cm, 16.2 ± 1.33 cm, 10.6 ± 0.46 cm, and 9.6 ± 0.58 cm, respectively (Table 2). The TRAs and TRUs of the experimental fish were divided by the standard length (1×4; *Ls*), and the HDs were divided by the *HL* (1×14). The HDs were enlarged to describe the head of the fish (Fig. 1). Morphometric data for TRAs/*Ls*, TRUs/*Ls*, and HDs/*HL* are listed in Table 2. The maximum dimensions for each group are shown in Fig. 2. The *Ls* and *HL*, beginning with the greatest values, were tiger puffer, hybrids, triploid hybrids, and river puffer ($p<0.05$). The 1×2 and 1×5 measurements (linked to head and trunk regions) for river puffer were greater than those of tiger puffer and lower than those of the hybrids and triploid hybrids ($p<0.05$). The 2×5 and 6×10 measurements for tiger puffer were the largest of all groups ($p<0.05$). The 2×6, 5×6, and 8×9 measurements for the hybrids and triploid hybrids were greater than those of the river puffer, but less than those of the tiger puffer ($p<0.05$). The 3×9 and 5×9 linked to tail region, and the 3×8 measurements of the hybrids and triploid hybrids, were greater and smaller, respectively, than those of their parental species ($p<0.05$). Measurements 3×9, 5×9, and 3×8 were not significantly different between the river puffer and the tiger puffer ($p>0.05$). The 12×16, 13×15, and 1×13 measurements of the river puffer and the tiger puffer were greater than those of their hybrids and triploid hybrids ($p<0.05$). The 12×16 measurements of river puffer were greater than those of the tiger puffer, whereas the opposite was true for measurements 13×15 and 1×13 ($p<0.05$). The hybrids' 18×19 measurements were the greatest among all groups ($p<0.05$); the other groups did not significantly differ ($p>0.05$).

The external morphologies of the experimental groups are shown in Fig. 3. The river puffer had black spots behind the pectoral fin and at the insertion of the dorsal fins and had a white dotted line in the upper part (Fig. 3A). The river puffer had yellow anal and caudal fins but yellow-brown dorsal fins (Fig. 3A). The tiger puffer had large black spots outlined in white behind the pectoral fin and at the insertion of the dorsal fins; on each side, round black spots and white patterns were observed until the caudal peduncle (Fig. 3D). The tiger puffer had white anal fins but black caudal and dorsal fins (Fig. 3D). The hybrids and triploid hybrids had black spots back of the pectoral fin, at the insertion of the dorsal fins, and on each side (Fig. 3B, C). They also had many more white spots on the upper part, and their fin colors were more like those of the river puffer (Fig. 3B, C).

The meristic characteristics of the various experimental groups are listed in Table 3. The river puffer and triploid hybrids had a higher mean number of dorsal fin rays than the other groups ($p<0.05$), while those in river puffer and tiger puffer were not significantly different from the triploid hybrids and the hybrids, respectively ($p>0.05$). The mean numbers of hybrids' anal fin rays, caudal fin rays, gill rakers, and vertebrae were higher than those for the river puffer and triploid hybrids, but lower than those of tiger puffer ($p<0.05$). The mean numbers of left and right pectoral fin rays of the hybrids were higher than those of the tiger puffer, but lower than those of the river puffer and triploid hybrids ($p<0.05$). The mean numbers of pectoral fin rays and gill rakers in the hybrids and triploid hybrids were not significantly different between the left and right sides (Table 3). The asymmetry in the number of pectoral fin rays and gill rakers was greater in the triploid hybrids than in the hybrids (Table 3).

Table 3. Meristic characteristics of the river puffer, *Takifugu obscurus*, the tiger puffer, *T. rubripes*, their hybrids, and their triploid hybrids

Meristic characteristics		River puffer	Hybrids	Triploid hybrids	Tiger puffer
Fin rays					
Dorsal		16.2 ± 0.35^{b} (15–17)	14.9 ± 0.62^{a} (14–17)	15.7 ± 0.34^{b} (15–16)	15.1 ± 0.67^{a} (14–16)
Anal		11.0 ± 0.76^{a} (10–12)	12.1 ± 0.60^{b} (12–13)	11.9 ± 0.88^{a} (10–14)	13.3 ± 0.18^{c} (13–14)
Caudal		10.4 ± 0.24^{a} (10–11)	11.6 ± 0.58^{b} (10–12)	10.8 ± 0.37^{a} (10–12)	12.6 ± 0.51^{c} (12–13)
Pectoral	Left	15.4 ± 0.67^{c} (15–16)	13.8 ± 0.54^{b} (13–16)	14.9 ± 0.90^{c} (13–17)	12.9 ± 0.98^{a} (12–14)
	Right	15.1 ± 0.21^{c} (15–16)	13.1 ± 0.88^{b} (13–15)	14.4 ± 0.49^{c} (11–15)	12.5 ± 0.71^{a} (12–14)
Gill rakers	Left	27.9 ± 1.24^{a} (26–30)	30.9 ± 1.84^{b} (27–33)	28.4 ± 1.13^{a} (26–33)	32.8 ± 1.43^{c} (29–34)
	Right	28.0 ± 1.01^{a} (26–30)	30.4 ± 2.01^{b} (27–32)	28.1 ± 1.86^{a} (24–32)	32.6 ± 1.39^{c} (29–34)
Vertebrae		20.9 ± 0.21^{a} (20–22)	22.3 ± 0.20^{b} (21–24)	21.2 ± 0.30^{a} (21–23)	24.7 ± 0.28^{c} (23–26)

The values (mean of triplicate ± SD) with different superscripts are significantly different ($p<0.05$). Data were transformed to the arcsine of the square root and analyzed using a one-way ANOVA. Different letters on the values indicate statistical significance among each species ($p<0.05$).

DISCUSSION

In this study, the standard lengths (*Ls*) of the hybrids were longer than those of the triploid hybrids. Given that this study's experimental groups contained fish in the pre-spawning period, the results of this study are consistent with those of Park and Zhang (1994). Their morphometric data report on diploid and induced triploid cherry salmon, *Oncorhychus masou*, revealed that the body length of diploid cherry salmon was greater than that of the triploid salmon. They noted that the lower growth rate in triploids was due to genetic instability from ploidy, and decreased metabolism was caused by the reduction of red blood cells. Similarly, Bonnet *et al.* (1999) reported that morphometric traits (especially, mean body weight and condition factor) were significantly higher in diploids freshwater rainbow trout, *O. mykiss*, and seawater brown trout, *Salmo trutta*, than in triploids before male and female spawning period. The body weight of the rainbow trout diploid was likewise heavier than that of the triploid during the 48 weeks after hatching (Thorgaard *et al.* 1982; Solar *et al.* 1984).

We observed that some morphometric and meristic characteristics of the hybrids and triploid hybrids were intermediate between those of the parental species; however, they were generally more similar to one of the parental species. Other characteristics were more or less pronounced than those of their parental species. Park *et al.* (1997) reported that the morphometric characteristics of hybrids and triploid hybrids between rainbow trout and cherry salmon were intermediate for their parental species. For some parameters though, there was a slight tendency for the hybrids to be more similar to the maternal species rather than to the paternal species. However, the hybrids of the channel catfish, *Ictalurus puntatus*, and congenerics were more similar to the paternal parent (Dunham *et al.* 1982). The results of Park *et al.* (2006) suggest that the morphological traits of hybrids may be paternally influenced, maternally influenced, or out of the parental species' range. Therefore, no consistent trait inheritance occurs during hybridization (Chevassus 1983; Park *et al.* 2017; Yoo *et al.* 2018). However, it is possible to identify hybrids using morphometric traits that differ from those of the parental species.

The body color of the hybrids and the triploid hybrids tended to be maternally dominated. The upper parts of the hybrids and triploid hybrids were yellow-brown, while that of the river puffer and the tiger puffer were yellow-brown and black, respectively. In addition, we observed many white spots on the upper part of the hybrids and triploid hybrids. In a separate study, hybrids formed between the willow shiner, *Gnathopogon elongatus elongatus* (♀), and the crucian carp, *Carassius carassius* (♂), resembled the willow shiner in body color and color pattern but had a black spot near the caudal fin, which is uniquely found in the crucian carp, and a tentacle, which occurs in the willow shiner (Kasama and Kobayasi 1991). Likewise, loach hybrids have resembled the mud loach, *Misgurnus mizolepis* (♀), in flatness and mud-yellow color, but resembled the cyprinid loach, *M. anguillicaudatus* (♂) in body shape, which was cylindrical (Kim *et al.* 1995). Despite the many factors suggested by the aforementioned studies, phenotypic characteristics are largely determined by genotype, or the interaction between genes and the environment (Normala *et al.* 2017; Dou *et al.* 2019).

The meristic traits investigated in this study showed elevated fluctuating asymmetry in the hybrids, which increased in triploid hybrids. The morphometric traits showed no elevated fluctuating asymmetry in the hybrids, and the triploid hybrids were relatively unaffected. These findings differ from those of previous studies by Leary *et al.* (1985) and Park and Gil (2018). Wilkins *et al.* (1995) have reported that triploid Atlantic salmon, *S. salar*, does not differ significantly in its fluctuating asymmetry from its diploid counterparts, although their overall values were lower. Therefore, the presence of the extra chromosome set had no significant effect on the developmental stability of triploid Atlantic salmon. In these fish, duplication of the maternal set of Atlantic salmon chromosomes restores the relational balance that is absent in the diploid hybrids, resulting in a statistically significantly reduced fluctuating asymmetry and presumably greater developmental stability. Leary *et al.* (1985) also observed a significant reduction in the fluctuating asymmetry attributable to triploidy. Therefore, future studies of asymmetry in hybrids and triploid hybrids of river puffer and tiger puffer should focus on the causes of increased fluctuating asymmetry during triploidization.

This study used external morphological traits, including morphometric and meristic characteristics, to distinguish among river puffer, tiger puffer, their hybrids, and their triploid hybrids. We observed significantly different parameters among the four groups; Additional investigations in the future should focus on comparatively analyzing various characteristics for the purpose of improving commercial aquaculture of river puffer, tiger puffer, their hybrids, and their triploid hybrids.

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