

Research

Evaluation of Growth Performance and Morpho-Meristic Characteristics in The Progenies of *Barbonymus gonionotus* and *Barbonymus schwanenfeldii*

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ABSTRACT

Recognizing the potential benefits of combining the favorable traits of both species, this study was designed to evaluate the culture performance and morphometric variations of F1 hybrids derived from artificial reciprocal hybridization between *Barbonymus schwanenfeldii* and *Barbonymus gonionotus*. Four crosses were successfully produced, namely *B. gonionotus* ♀ × *B. gonionotus* ♂ (GG), *B. gonionotus* ♀ × *B. schwanenfeldii* ♂ (GS), *B. schwanenfeldii* ♀ × *B. schwanenfeldii* ♂ (SS) and *B. schwanenfeldii* ♀ × *B. gonionotus* ♂ (SG). Uniform-sized fingerlings of pure GG, hybrid GS, pure SS, and hybrid SG were stocked at a rate of 50 fingerlings per fiberglass 1-tonne tank. The fingerlings were fed a commercial tilapia pellet (Dinding Malaysia) with 35% crude protein, the feeding was done twice a day, at 9:00 and 16:00, with equal portions given at each feeding. After 8 weeks of culture, the mean weight gained by GG, GS, SS, and SG was 7.09 ± 0.06 , 5.75 ± 0.15 , 4.69 ± 0.21 , and 5.60 ± 0.17 g, respectively. The growth performance of the hybrid GS 5.75 ± 0.15 was significantly higher compared to the slow-growing SS (4.69 ± 0.21) but lower than the value recorded in GG (7.09 ± 0.06) under identical rearing conditions. A similar trend was observed in the final weight and specific growth rate (SGR) (%/day). In terms of FCR value, hybrid GS had the best value (1.10 ± 0.10) compared to hybrid SG (1.31 ± 0.13) or pure species (1.29 ± 0.09 , 1.39 ± 0.13). Reciprocal hybrids showed intermediate morphometric traits between the two species; The reciprocal hybrids displayed intermediate morphometric traits that fell between the two parental species. The results of the discriminant function analysis confirmed that there were significant differences in the characters of all four species.

Key words: *Barbonymus gonionotus*, *Barbonymus schwanenfeldii*, hybridization, morpho-meristic

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INTRODUCTION

Barbonymus is a member of the Cyprinidae family, which is the most important group in Asia. This species is a true freshwater fish that is omnivorous but tends to be herbivorous (Batubara *et al.*, 2018). *Barbonymus* species is widespread across tropical and subtropical areas, particularly in Asia; the Mekong and Chao Phraya rivers, Borneo, and Sumatra, and all rivers and lakes in Peninsular Malaysia (Isa *et al.*, 2012; Zakaria *et al.*, 2018).

Barbonymus gonionotus (Bleeker) locally known as Lampam Jawa (Zakaria *et al.*, 2018) is a commercially significant fish species in Southeast Asia's capture fisheries and aquaculture (Kamonrat *et al.*, 2002). It is a major tropical fast-growing freshwater fish species that may easily breed in captivity and adapt to a broad range of cultural conditions (Chaudhary *et al.*, 2008; Rahman *et al.*, 2009). However, the natural population of this species is declining due to irresponsible fishing, habitat destruction, and other ecological changes in their habitat (Jasmine & Begum, 2016). Meanwhile, *B. schwanenfeldii* is referred to as Lampam Sungai in Peninsular (Kamarudin & Esa, 2009),

which is a slower-growing species (Dewantoro *et al.*, 2018). But it has gained economic importance as a result of its tasty meats. Its meat is highly valued and sells for a reasonable price, which makes it vital for the aquaculture sector (Isa *et al.*, 2012). Hybridization has been widely used in genetic enhancement projects for aquaculture production, particularly in fish species. It has been successful and influential in various fish species, including crayfish, carp, cichlids, moronids, salmonids, sparids, sunfish, oysters, and catfish. Hybridization occurs more frequently in fish than in any other vertebrate groups (Hulata, 1995; Harrell, 1997; Scribner *et al.*, 2001; Bartley *et al.*, 2001). Artificial hybridization is commonly employed to produce hybrid progenies with improved performance (Rahman *et al.*, 2013). These progenies often exhibit heterosis, also known as hybrid vigor, due to the combination of valuable traits from both parent species (Gray *et al.*, 1993). This can result in several benefits, such as a higher growth rate, improved food conversion, increased disease resistance, better flesh quality, enhanced productivity, and greater environmental tolerance (Rahman *et al.*, 2013; Okomoda *et al.*, 2018).

Several genetic improvement programs in some important aquaculture species have been successfully applied in hybrids to improve the growth performance and productivity of aquacultural strains (Basavaraju *et al.*, 1995; Scribner *et al.*, 2001). For example, the Lemon fin barb hybrid stock was carefully selected and hybridized by the Department of Fisheries in Malaysia by crossing (*B. gonionotus*) and Kerai (*Hypsibarbus wetmorei*) with the aim of rapid growth rate and ease of reproduction (Zakaria *et al.*, 2018). A hybrid between *B. gonionotus* and *T. tambroides* was successfully produced by Azfar-Ismail *et al.* (2020) to enhance the commercial aquaculture of tropical carp for the aquaculture industry. Okomoda *et al.* (2018) produced a cross between *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) to assess the possibility of combining desirable traits from both pure species, such as increased growth rate, improved productivity, better fillet quality, disease resistance, increased environmental tolerance, better food conversion, sexual dimorphism, and increased harvesting rate in culture systems.

The economic benefits of hybrid fish have increased massively over the last few decades. Some of the hybrids become the most important part of aquaculture industries and produce significant production. When a new hybrid is developed and commercialized, it is highly of concern since the fish can be identified and distinguished from its parentages. It is problematic if a hybrid introduced into aquaculture cannot be distinguished and lose its distinctiveness, especially with pure species (Begg *et al.*, 1999). As a result, it is important to establish a method for diagnosing and categorizing this hybrid. Morphometric and meristic analysis has been the most frequently used and cost-effective method for determining inter-specific hybrids in fish stocks and were used before molecular work to differentiate morphological characters (Baharuddin *et al.*, 2014; Solomon *et al.*, 2015; Siddik *et al.*, 2016; Duong *et al.*, 2017). However, hybrids might show features differently, leading to a violation of the intermediate assumption. More advanced multivariate statistical techniques, such as discriminant function analysis and principal component analysis, have been used to analyze morphological data to detect hybrids (Duong *et al.*, 2017).

Nevertheless, hybridization between *B. schwanenfeldii* and *B. gonionotus* is a relatively recent attempt. The proper matching of growth data with morphological and genetic characteristics may help design the future and it might have garnered farmers' support for devising more effective ways of managing the species and conserving them in their native habitats. We successfully produced a novel aquaculture candidate from *Barbonymus gonionotus* (Bleeker, 1849) and *Barbonymus schwanenfeldii* (Bleeker, 1854) to combine desirable traits from both species. The purpose of this study is to evaluate the breeding and growth performance, and morpho-meristic variation, of reciprocal hybrids to their pure species.

MATERIALS AND METHODS

Broodstock selection and conditioning

To produce the first filial hybrid generation *B. schwanenfeldii* and *B. gonionotus* were artificially hybridized (male & female). For the breeding program, broodstock (0.4 - 0.6 kg) free of diseases and abnormalities (i.e., deformed body unusual scale patterns, body color changes) was selected. Healthy mature males and females broodstock for the crossing of pure SS and hybrid SG were bought from a local fish farmer in Temerloh, Pahang, and pure GG and hybrid GS were from the brood-rearing tanks of the "Aquaculture Experimental Station" at Puchong Farm, Universiti Putra Malaysia.

Acclimatization process

To facilitate acclimatization and prepare the broods, both male and female fish were kept in one-tonne holding tanks for two weeks. Adequate aeration was ensured by maintaining a continuous

water flow. The water parameters, including temperature, pH, and dissolved oxygen (DO) levels, were carefully monitored and maintained within the ranges of 26.61 - 28.73 °C, 6.5 - 7.9, and 6.50 - 8.05 mg.L⁻¹, respectively. During this conditioning period, the fish were provided with Tilapia commercial (R7001, 35% crude protein) feed.

Hybrid production from reciprocal crosses of *B. gonionotus* and *B. schwanenfeldii*

The complete diallel crosses were performed with the following combinations: *B. gonionotus* ♀ × *B. gonionotus* ♂ (GG), *B. gonionotus* ♀ × *B. schwanenfeldii* ♂ (GS), *B. schwanenfeldii* ♀ × *B. schwanenfeldii* ♂ (SS) and *B. schwanenfeldii* ♀ × *B. gonionotus* ♂ (SG). Each group has three replications originating from 3 pairs of males and 3 pairs of females (Table 1).

In this experiment, commercial Ovaprim hormone (Syndel, Asia) was used as an induced agent to prepare the fish for artificial fertilization. The fish was carefully held and weighed. A calculated amount of a single dose of Ovaprim injection to both sexes of *B. gonionotus* and *B. schwanenfeldii* was given intramuscularly at an angle of 30 - 45° at the dorsal fin. Females were injected with a dose of 0.5 m.L kg⁻¹ body weight of the fish, and males were injected with half of that volume respectively, which was recommended by Epasinghe *et al.* (2016).

The fish were removed from the tank after six hours of injection when the ovulation was completed. Then the fish was subjected to strip spawn; cross-fertilizations between the sperm and eggs of the two species were conducted at room temperature 25.5 - 28.7 °C. Then, the fertilized eggs were transferred to 120 L hatching aquaria for incubation. A continuous flow of water was maintained through air stones. The larvae were stocked in a glass aquarium until they reached the juvenile stage. After reaching the juvenile stage 2.24 - 2.81 g, the fingerlings were reared in fiberglass tanks for 8 weeks to observe their growth performance.

Pure and reciprocal hybrids culture and growth performance

The growth performance experiment was conducted for 8 weeks, in twelve rectangular 1-tonne fiberglass tanks at the Universiti Putra Malaysia Aquaculture Experimental Station in Puchong. The experiment was designed as pure GG, hybrid GS, pure (SS), and hybrid SG and had three replications (R1, R2 & R3) respectively. All other conditions during the experiment were maintained in similar ways.

Uniform-sized fingerlings of pure GG, hybrid GS, pure SS, and hybrid SG weight 2.81 ± 0.31, 2.37 ± 0.31, 2.24 ± 0.34, and 2.50 ± 0.10 g, respectively were stocked at the rate of (*n*=50) fingerlings in a fiberglass 1 tonne tank. The progenies were fed with a commercial tilapia pellet (Dinding Malaysia) with 35% crude protein, as recommended by Dewantoro *et al.* (2018). Furthermore, the fish were fed in equal proportions twice a day (9:00 & 16:00) throughout the experiment period. A record of supplied feed was maintained to determine the food conversion ratio. Every morning (09:00), the fecal output and leftover feed were siphoned from the tank.

Table 1. Diallel breeding protocol between *B. gonionotus* and *B. schwanenfeldii* in fiberglass tanks

	Diallel Crosses	Combination
1	<i>B. gonionotus</i> ♀ × <i>B. gonionotus</i> ♂	GG (Control)
2	<i>B. gonionotus</i> ♀ × <i>B. schwanenfeldii</i> ♂	GS (Hybrid)
3	<i>B. schwanenfeldii</i> ♀ × <i>B. schwanenfeldii</i> ♂	SS (Control)
4	<i>B. schwanenfeldii</i> ♀ × <i>B. gonionotus</i> ♂	SG (Hybrid)

A 30% water exchange was carried out twice a week to maintain water conditions. Water quality parameters were measured between 7:00 and 8:00 on that day before each water exchange. Total Ammonia-N was tested using a test kit (TAN, API® freshwater master test kit, Chalfont, PA, USA). Water temperature (°C), pH, and dissolved oxygen mg.L⁻¹ were 26.60 - 27.75°C, 7.33 - 7.90, and 6.35 - 6.90 mg.L⁻¹, respectively, and were recorded with a YSI (Yellow Spring, Ohio, USA).

Growth of the fingerlings was evaluated at fortnightly intervals by 20% of the stocked fish. Sampling was done using a seine net to observe the growth performance like survival rate, SGR, CF, and FCR of the fish. This measurement was also done to adjust the feeding rate for the following observation periods. The weight of the fish was measured with a Precision Electronic scale model LT5001E to the nearest gram of 0.1 g. The tanks were aerated for 24 hr each day, during cleaning, any mortality of fish was observed and recorded. Sampling was usually done before the feed to avoid the biases of weight due to the presence of excessive feed. Throughout the culture period, general and fish health conditions were monitored regularly. The performance index was calculated using the following equations:

The following formula was used to estimate the specific growth rate (Liu *et al.*, 2015):

$$\text{SGR (\%/day)} = 100 \times (\text{Ln } W_f - \text{Ln } W_i) / \Delta t.$$

where: SGR = specific growth rate (% day⁻¹);

W_i = the average weight of fish at the beginning of the study (g);

W_f = the average weight of fish at the end of the study (g);

T = duration of the experiment (day).

α. The body weight gain was calculated as follows:

$$\text{Weight gain (g)} = \text{Average final weight} - \text{Average initial weight}.$$

The conditional factor of the tested fish was calculated following the methods recommended by Froese (2006):

$$\text{CF} = W/L^3 \times 100$$

where: CF = conditional factor;

W = body weight (g);

L = standard length (cm).

The Feed conversion ratio (FCR) was determined using the formula of Abowei & Ekubo (2011):

$$\text{FCR} = \text{FI} / (W_f - W_i) \times 100$$

where: FCR = feed conversion ratio (%);

W_i = the weight of fish biomass at the beginning of the study (g);

W_f = the weight of fish biomass at the end of the study (g);

FI = the weight of feed consumed (g dry weight).

The survival rate was estimated using the following formula recommended by (Rivas-Vega *et al.* (2013):

$$\text{SR} = N_f / N_i \times 100$$

where: SR = survival (%);

N_f = the number of live fish at the end of the study (individual);

N_i = the number of fish at the beginning of the study (individual).

The data were examined using a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. These statistical analyses were performed using computer software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA)

Morphometric comparisons of reciprocal hybrids and pure crosses

In the current study, ($n=48$) fish samples from each of the pure GG, hybrid GS, pure SS, and hybrid SG were used for morphological characterization. All samples were collected from our breeding collection and were reared for approximately 35 g weight before morphological analysis was done.

The test fishes were transferred to the laboratory and kept in a glass flow-through aquaria filled with 150 L of dechlorinated stock water. The measurements were carried out at the minimum and maximum atmospheric temperatures of 30.5 - 33.5 °C, respectively. All procedures involving the care and use of animals in this study adhered to institutional, national, and international guidelines.

A total of 12 morphometric characters were measured according to Batubara *et al.* (2018). Apart from dimensions, taxonomic traits were ascertained using 8-point meristic counts according to Jaffar *et al.* (2019) which include lateral lines, scales in lateral series, dorsal fin rays, anal fin rays, pectoral fin rays, and pelvic fin rays. Before measurements, selected fish were anesthetized with clove oil at 0.05 mL of clove oil per 500 mL water according to Fernandes *et al.* (2016); later, it was gently placed on the board, and the traits of the fish were counted using a digital caliper to the nearest 0.01 mm (Mitutoyo 500-197-20/30, Japan) after then the tested fish were placed in a recovery tank filled with fresh aerated dechlorinated water. The fish in recovery tanks were monitored. All meristic characteristics (average values) were subdivided according to their stocking premises. The same person did measurements and counting to avoid artificial error, using the buss truss protocol as shown in (Supplementary Table 1).

To make sure that the variations observed in this experiment were due to differences in body shape rather than relative fish sizes, size effects were removed from the data set by standardizing the morphometric parameters, using the allometric formula proposed by Elliott *et al.* (1995):

$$M_{\text{adj}} = (Ls/Lo)^b$$

where M is the original measurement, M_{adj} is the size-adjusted measurement, Lo is the standard length of the specimen, and Ls is the overall mean of the standard length for all fish from all samples. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all fish in all groups.

The analysis of variance (ANOVA) for all the quantitative traits was performed using computer software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA). The least significant difference (LSD) at a 5% level was used to test differences among species for each character and to compare the significant difference of each characteristic among pure and reciprocal hybrids.

Multivariate analysis, part of the discriminate function analysis (DFA), was used to discover variables that enable differentiating between various species or groups and classifying the fish with better than a probability of correctness. The SPSS program version 25.0 was used to perform DFA analysis (AnvariFar *et al.*, 2013; Park *et al.*, 2015). Sample sizes that are several times larger than the number of variables are preferable. This helps to avoid overfitting, which can produce results that are not generalizable to the population (Elliott *et al.*, 1995).

RESULTS

Growth performance of pure and reciprocal hybrids

The broods were ovulated six hours after injection, and the brooders were found to be "ready to spawn". Fertilized egg hatching was observed from 13.5-14 hours (Temperature 25.70 – 28.85 °C). There was successful breeding in all four trials. After three days, the yolk sac was completely absorbed, and the larvae began exogenous feeding.

The detailed growth parameters such as survival rate, weight gain, specific growth rate, feed utilization, and condition factors of the reciprocal hybrids, and parental species are summarized in (Table 2). In contrast, the growth trend is shown in (Figure 2). It was observed that the growth of the reciprocal hybrids was significantly higher compared to the slow-growing pure SS ($P < 0.05$), but less than the pure GG under identical rearing conditions. On the other hand, the hybrids had higher values when compared to their pure SS but did not significantly differ from each other.

Table 2. Growth performance of *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

Parameters	GG	GS	SS	SG
	Pure	Hybrid	Pure	Hybrid
Survival rate (%)	98.67 ± 2.30 ^a	100 ± 0.00 ^a	98 ± 2.00 ^a	100 ± 0.00 ^a
Initial mean weight (g)	2.81 ± 0.31	2.37 ± 0.31	2.24 ± 0.34	2.50 ± 0.10
Final mean weight (g)	9.9 ± 0.36 ^a	8.12 ± 0.18 ^b	6.93 ± 0.53 ^c	8.1 ± 0.20 ^b
weight gain (g)	7.09 ± 0.06 a	5.75 ± 0.15 ^b	4.69 ± 0.21 ^c	5.60 ± 0.17 ^b
weight gain (%)	254.50 ± 24.27 ^a	245.71 ± 36.2 ^a	211.79 ± 27.33 ^a	224.24 ± 11.59 ^a
Specific growth rate (% day ⁻¹)	2.11 ± 0.12 ^a	2.06 ± 0.18 ^{ab}	1.89 ± 0.14 ^b	1.96 ± 0.06 ^{ab}
Feed conversion ratio	1.29 ± 0.09 ^a	1.10 ± 0.10 ^{ab}	1.39 ± 0.13 ^b	1.31 ± 0.13 ^b
Condition factor	3.45 ± 0.22 ^a	3.55 ± 0.16 ^a	2.92 ± 0.57 ^b	2.90 ± 0.45 ^b

Means ± SD, different letters within the same row indicate statistical difference by Duncan's Multiple Range Test ($P < 0.05$) for the growth parameters raised in the tanks.

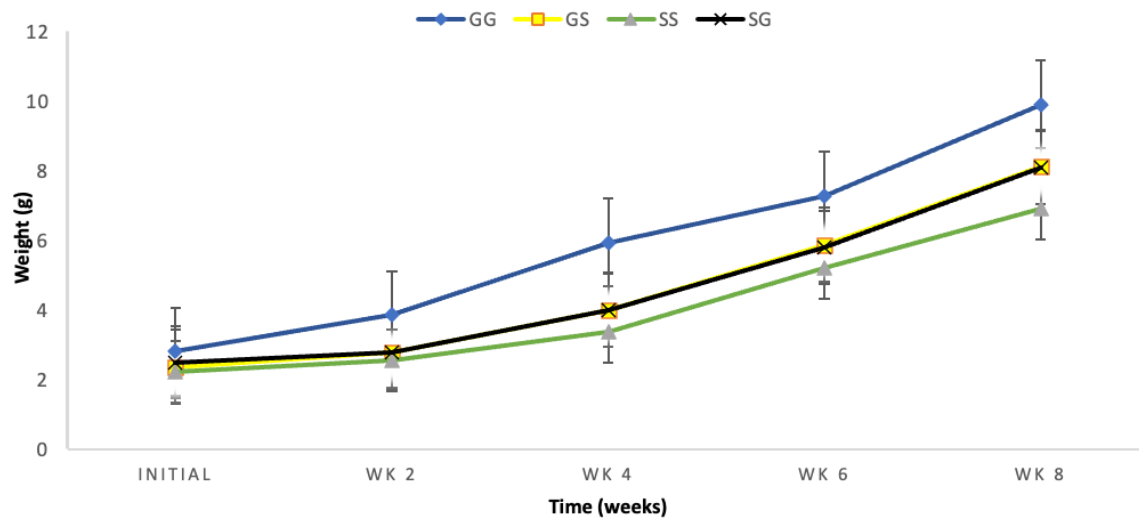


Fig. 1. Mean weights attained by *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

After culture for 8 weeks, the mean weight gained by pure GG, hybrid GS, pure SS, and hybrid SG was 7.09 ± 0.06 , 5.75 ± 0.15 , 4.69 ± 0.21 , and 5.60 ± 0.17 g, respectively. Of the two reciprocal hybrids, the GS hybrid recorded faster growth than pure SS and SG hybrid, but slightly less than pure GG (Table 2). Other growth parameters such as final weight, weight gain (%), and specific growth rate (SGR) (%/day) had the same trends as weight gain. In contrast, there was no significant difference between reciprocal hybrids, and the lowest value was recorded in pure SS.

The results obtained from this study on all treatments revealed that GS hybrid and pure GG provided the best FCR, which was 1.10 ± 0.10 and 1.29 ± 0.09 , followed by hybrid SG, and pure SS, respectively. The best value was recorded in GS hybrid among all four groups (Table 2). Condition factor of the fingerlings in pure GG, hybrid GS, pure SS, and hybrid SG, were 3.45 ± 0.22 , 3.55 ± 0.16 , 2.92 ± 0.57 , and 2.90 ± 0.45 g.cm⁻¹, respectively, where the highest value was found in GS hybrid and lowest in pure SS. The condition factors of pure GG, hybrid GS, pure SS, and hybrid SG differed significantly ($P < 0.05$) from each other (Table 2). The survival rate of the fingerlings was 98.67 ± 2.30 , $100 \pm .00$, 98 ± 2.00 , and 100 ± 0.00 % in pure GG, hybrid GS, pure SS, and hybrid SG respectively after 8 weeks of the experimental period where the highest survival rate was in both reciprocal hybrids followed by GG, SS in that order, which was statistically not significant (Table 2).

Morphometric and meristic variations of pure *B. gonionotus*, pure *B. schwanenfeldii*, and reciprocal hybrids

The body shape of pure GG was found to be slender, whereas SS had a wider body dept. Their hybrid GS had an intermediate shape of the body. The body colors of reciprocal hybrids GS and SG were slightly different. Fresh specimens of GG are silvery-white in color, with a gold tint on occasion. The dorsal and caudal fins are grey to grey-yellow, the anal and pelvic fins are pale orange with reddish tips, and the pectoral fins are pale yellow. Furthermore, pure SS is known for its red pectoral, pelvic, and anal fins, as well as black blotched dorsal fin tips. Moreover, on its caudal side, white and black edges fin. The merely light color of the body, light red pectoral, pelvic, and anal fins, and black blotched dorsal fin tips for hybrids GS and SG. The body and fin color of reciprocal hybrids were found to be a distinction from their parents and tended to follow SS (Figure 4).

The discriminate function analysis revealed that all four species' characters were significantly different ($P < 0.05$). The mean size of every character in (Table 3 & 4) was varied among pure species and hybrids, in general, the hybrids were found to have characters closer to pure GG. Pure GG recorded the highest value in total length followed by hybrid GS, SG, and pure SS respectively. Body depth of SS recorded the highest, followed by hybrid (GS and SG), and GG, respectively. The meristic count of F1 hybrids was statistically different from their parental species. In the case of dorsal fin number, hybrid GS recorded the highest number followed by pure GG, SS, and hybrid SG.

Discriminant analysis

Discriminant analysis showed that three significant eigenvalues of 22.620, 0.499, and 0.150 in three functions for morphometric traits accounted for 97.20% of the observed variation among the traits loaded in function 1. Meanwhile, the eigenvalue in function 3, the most negligible value of 0.150, was found to be 0.60%. The eigenvalue of 12.209, 2.218, and 0.789 of the meristic values was elucidated at the rate of 80.2 %, 14.6 %, and 5.2% in the first, second, and third functions. The eigenvalue of a function is the ratio of between to within-group variability. The greater the values, the better at accounting for group distinction; it indicates that Function 1 made a significant contribution to the discrimination of these four groups. The value of canonical correlation was used to quantify the degree of group variability in each Function. Function 1, 2, and 3 recorded canonical correlations of 0.979, 0.577, and 0.361 for the morphometric component, respectively, and 0.961, 0.830, and 0.664 were recorded for the meristic component; function 1 showed a high correlation among the score of its Function as compare to Function 2 and group differences (Table 5).

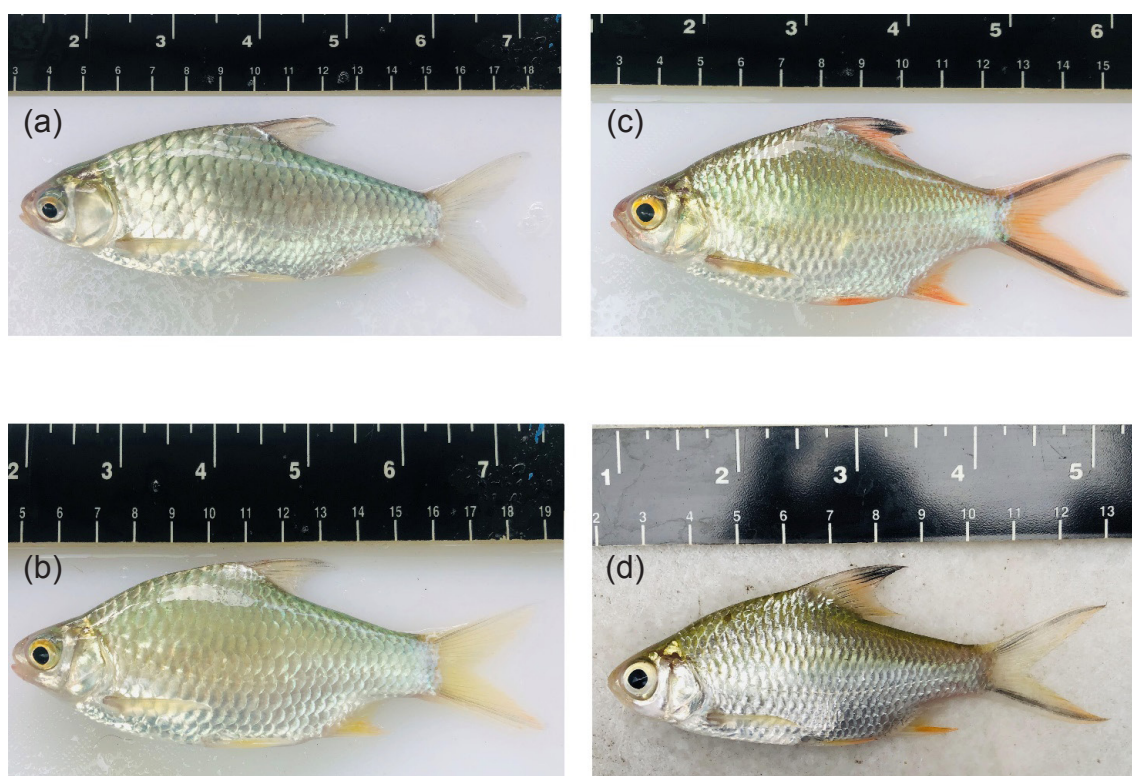


Fig. 4. Morphological appearance of *B. gonionotus*♀ × *B. gonionotus*♂ (a), *B. gonionotus*♀ × *B. schwanenfeldii*♂ (b), *B. schwanenfeldii*♀ × *B. schwanenfeldii*♂ (c), and *B. schwanenfeldii*♀ × *B. gonionotus*♂ (d)

Table 6 shows the coefficient of the canonical values of the components, and this could be used to calculate the score or the discriminate function of the predictor. Morphometric distance of total length with a discriminate coefficient score of 0.908 was the highest among the other discriminating factors of standard length, head length, dorsal fin base length, body depth, and pelvic fin length with a score of 0.273, 0.246, 0.506, 694 and 0.137 respectively. The meristic count of anal fin rays with a discriminate coefficient score of 0.751 was the highest among the other discriminant component of dorsal fin rays, pectoral fin rays, pelvic fin rays, caudal-fin rays, scale along the lateral line, and scale in lateral line series with a score of 0.748, 0.557, 0.377, 0.683, 0.422 and 0.593 respectively.

Table 7 presents the test of Wilk's Lambda. The significance of the morphometric and meristic traits differences was also indicated by the Wilks' Lambda principle based on the ratio of different variables between the species and the total variability for the discriminating variables. For morphometric and meristic components, Wilks' lambda was near 0 for Functions 1 - 3, indicating that most of the variables captured by Function 1 were due to species differences. In function 2, Wilks' lambda was 0.580 for morphometric and 0.174 for meristic, implying that Function 2 caught minor variability, leading to variations across species. In addition, chi-square values indicated a statistically significant variability at ($P < 0.05$) levels for the group differences before extraction.

Table 3. Morphometric traits of *B. gonionotus* (GG), *B. schwanenfeldii* (SS) and their hybrids (GS, and SG)

Morphometric Characters	Pure (GG)	Hybrid (GS)	Pure (SS)	Hybrid (SG)	F-value	P-value
Total length (TL)	138.68 ± 4.31 ^a	114.34 ± 2.71 ^b	105.45 ± 1.97 ^d	109.88 ± 2.33 ^c	1225.12	0
Standard length (SL)	106.58 ± 12.48 ^a	84.66 ± 10.94 ^b	76.36 ± 6.57 ^c	82.11 ± 6.82 ^b	93.451	0
Head length (HL)	27.61 ± 2.10 ^a	22.65 ± 2.95 ^b	22.07 ± 0.93 ^b	22.85 ± 0.67 ^b	88.843	0
Caudal peduncle length (CPL)	21.6 ± 16.50 ^a	16.07 ± 1.60 ^b	13.68 ± 1.29 ^b	14.80 ± 1.30 ^b	9.103	0
Snout length (SNL)	7.08 ± 0.67 ^a	6.14 ± 0.68 ^c	6.28 ± 1.51 ^c	6.68 ± 0.73 ^b	9.664	0
Dorsal fin depth (DFD)	25.79 ± 1.65 ^a	23.25 ± 2.51 ^b	21.10 ± 1.42 ^c	21.55 ± 1.33 ^c	68.691	0
Dorsal fin base length (DFBL)	13.49 ± 1.35 ^c	13.66 ± 1.17 ^{bc}	15.26 ± 1.43 ^a	14.16 ± 1.40 ^b	17.154	0
Eye diameter (ED)	10.35 ± 0.52 ^a	9.56 ± 0.50 ^b	9.23 ± 0.41 ^c	9.36 ± 0.43 ^c	55.704	0
Caudal peduncle depth (CPD)	14.62 ± 0.99 ^a	13.24 ± 3.16 ^b	11.66 ± 0.41 ^c	12.58 ± 2.38 ^b	17.757	0
Body depth (BD)	34.03 ± 7.61 ^d	36.32 ± 2.72 ^b	40.90 ± 7.24 ^a	35.29 ± 1.74 ^{bc}	14.505	0
Pectoral fin length (PFL)	23.61 ± 1.83 ^a	20.13 ± 1.62 ^b	17.33 ± 0.97 ^d	18.71 ± 1.25 ^c	167.492	0
Pelvic fin length (VFL)	21.93 ± 1.96 ^a	19.59 ± 1.61 ^b	17.32 ± 0.97 ^d	18.10 ± 1.04 ^c	94.845	0

The values (mean ± SD, mm) with a different letter are significantly different at ($P=0.05$).

Table 4. Meristic Component of *B. gonionotus* (GG), *B. schwanenfeldii* (SS) and their hybrids (GS & SG)

Meristic Characters	Pure (GG)	Hybrid (GS)	Pure (SS)	Hybrid (SG)	F-value	P-value
Dorsal fin rays	9.20 ± 0.407 ^b	9.42 ± 0.499 ^a	9.00 ± 0.000 ^c	8.78 ± 0.418 ^d	25.2	0
Anal fin rays	7.61 ± 0.492 ^a	7.00 ± 0.000 ^b	6.50 ± 0.505 ^c	7.00 ± 0.000 ^b	82.17	0
Pectoral fin rays	14.00 ± 0.000 ^a	13.86 ± 0.639 ^a	14.00 ± 0.000 ^a	13.48 ± 0.707 ^b	12.97	0
Pelvic fin rays	9.00 ± 0.000 ^a	8.40 ± 0.926 ^a	8.63 ± 0.489 ^b	9.00 ± 0.000 ^a	15.77	0
Caudal fin rays	18.80 ± 0.407 ^a	18.16 ± 0.370 ^{ab}	18.63 ± 0.489 ^a	18.52 ± 0.505 ^b	17.99	0
Scale along the lateral line	29.98 ± 0.901 ^d	29.58 ± 1.401 ^d	35.00 ± 0.505 ^a	33.46 ± 0.767 ^b	377.9	0
Scale in lateral line series	13.00 ± 0.000 ^c	13.16 ± 0.370 ^b	14.00 ± 0.000 ^a	13.16 ± 0.370 ^b	142.49	0

Means ± SD, with a different letter, are significantly different at ($P<0.05$).

Table 5. Summary of canonical discriminant functions of morphometric and meristic characters of *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

Morpho-meristic	Function	Eigen value	Percentage Variance	Cumulative percentage	Canonical Correlation
	1	22.620	97.20	97.20	0.979
Morphometric	2	0.499	2.1	99.40	0.577
	3	0.150	0.60	100.0	0.361
Meristic	1	12.209	80.2	80.2	0.961
	2	2.218	14.6	94.8	0.830
	3	0.789	5.2	100.0	0.664

Table 6. Summary of standardized canonical discriminant function coefficients of *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

Components	Canonical Coefficient
Total length	0.908
Standard length	0.273
Head Length	0.246
Dorsal fin base length	0.506
Body Dept	0.694
Pelvic fin length	0.137
Dorsal fin rays	0.748
Anal fin rays	0.751
Pectoral fin rays	0.557
Pelvic fin rays	0.377
Caudal fin rays	0.683
The scale along the lateral line	0.422
Scale in lateral line series	0.593

Table 7. Results from Wilks' lambda test for verifying difference among reciprocal hybrids and their parents using discriminant function analysis

	Test of Function(s)	Wilks' Lambda	Chi-square	Df	Sig.
Morphometric	1 through 3	0.025	696.840	36	0.000
	2 through 3	0.580	102.362	22	0.000
	3	0.870	26.221	10	0.000
Meristic	1 through 3	0.013	825.048	21	0.000
	2 through 3	0.174	333.390	12	0.000
	3	0.559	110.765	5	0.000

When the discriminating score is compared with each centroid group, the results revealed that each species was well discriminated against as the values were not closely related (Table 8).

A significant role in this interpretation is to evaluate the efficacy of a set of Functions based on their ability to classify the species into a different group accurately. It was observed that for morphometric GG 100 %, hybrid GS 86 %, SS 91.7 %, and hybrid SG 84 %, and for meristic GG 100 %, hybrid GS 100 %, SS 100 %, and hybrid SG 84 % were correctly classified using the Function generated in the analysis (Table 9).

Table 8. Function at group centroids *B. gonionotus* (GG), *B. schwanefeldii* (SS), and their hybrids (GS & SG)

	Fish	Function		
		1	2	3
Morphometric	<i>B. gonionotus</i>	7.88	0.322	0.010
	Hybrid GS	-0.879	-1.072	0.289
	<i>B. schwanefeldii</i>	-4.425	0.838	0.339
	Reciprocal SG	-2.602	-0.490	-0.621
Meristic	<i>B. gonionotus</i>	3.899	1.231	0.902
	Hybrid GS	2.746	-1.913	-0.695
	<i>B. schwanefeldii</i>	-4.546	-0.936	0.867
	Reciprocal SG	-2.202	1.605	-1.021

Unstandardized canonical discriminant functions evaluated at group means

The scatterplot successfully differentiated the individuals into different groups; however, for the morphometric, the hybrids slightly overlapped with SS, whereas there was a negative relationship between the species' characters; additionally, function two successfully differentiated the individuals into different groups (Figure 5 & 6).

Table 9. Classification of predicted group membership for original and cross-validated *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

	Fish	Predicted Group Membership						Total
		Pure (GG)	Hybrid (GS)	Pure (SS)	Hybrid (SG)	Total		
Morphometric	Pure GG	100.0	0.0	0.0	0.0	100.0	100.0	
	Hybrid GS	0.0	86.0	0.0	14.0	100.0	100.0	
	Pure SS	0.0	0.0	91.7	8.3	100.0	100.0	
	Hybrid SG	0.0	8.0	8.0	84.0	100.0	100.0	
	Pure GG	100.0	0.0	0.0	0.0	100.0	100.0	
	Hybrid GS	0.0	86.0	0.0	14.0	100.0	100.0	
Cross-validated (%)	Pure SS	0.0	0.0	89.6	10.4	100.0	100.0	
	Hybrid SG	0.0	8.0	8.0	88.0	100.0	100.0	
	Pure GG	100	0	0	0	100	100	
	Hybrid GS	0	100	0	0	100	100	
	Pure SS	0	0	100	0	100	100	
	Hybrid SG	0	0	16	84	100	100	
Meristic	Pure GG	100	0	0	0	100	100	
	Hybrid GS	0	84.0	0	0	100	100	
	Pure SS	0	0	85.4	0	100	100	
	Hybrid SG	0	0	16	82.0	100	100	

a. 90.9% of original grouped cases were correctly classified.

b. Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

c. 87.8% of cross-validated grouped cases are correctly classified.

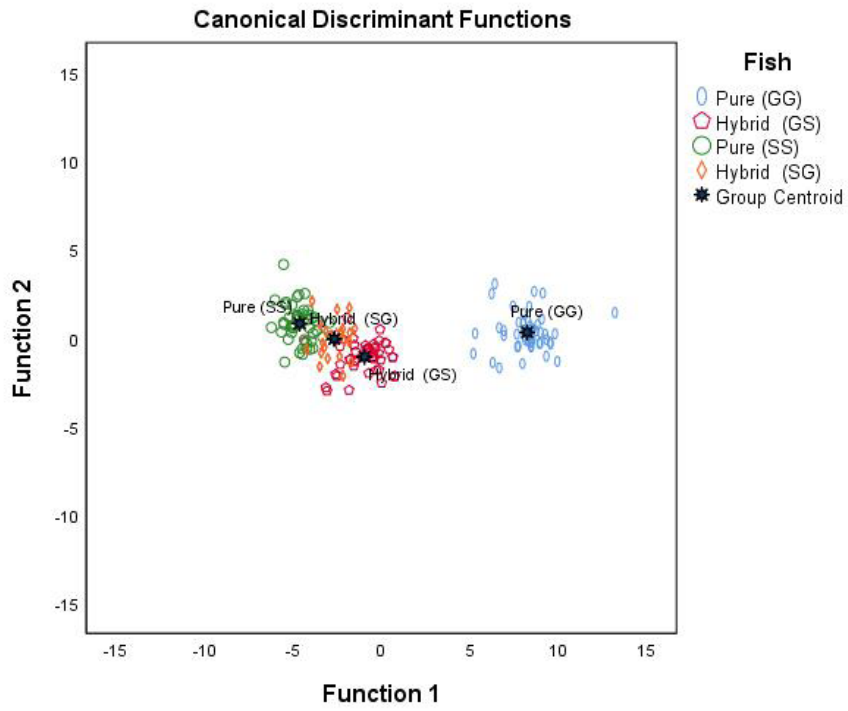


Fig. 5. Scatterplot of Function 1 against 2 of the morphological characters of *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

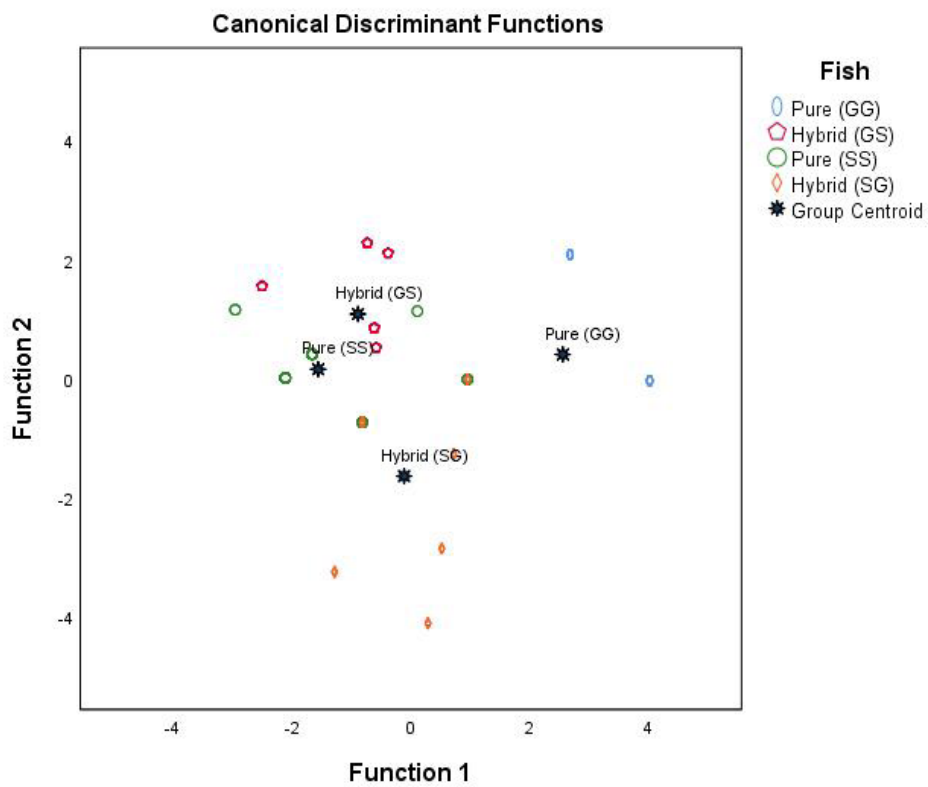


Fig. 6. Scatterplot of Function 1 against 2 of the meristic characters of *B. gonionotus* (GG), *B. schwanenfeldii* (SS), and their hybrids (GS & SG)

DISCUSSION

The physiochemical parameter observed and considered during this work indicates uniformity, making it possible to see growth differences among strains and hybrids due to feeding intake and the differences related to the genetic makeup. The modified culture environment in the tanks created a suitable habitat throughout the study. The effect of physio-chemical parameters like temperature is essential to the general well-being of fish at culture as it may affect food consumption, fecal production, growth, and the metabolic rate of fish (Sun & Chen, 2014). The physicochemical parameters of water quality have a significant impact on the survival, and growth of fish. Water temperature, in particular, plays a crucial role in the life of fish, which are poikilotherms. Dissolved oxygen (DO) levels in water are also considered a critical factor in water quality, as instances of hypoxia can lead to fish mortality worldwide. The fish species are influenced by physicochemical parameters such as temperature and DO levels. Different fish species have specific temperature ranges within which they thrive, and deviations from these optimal ranges can impact their survival and growth (Nyanti *et al.*, 2018). According to Xu and Boyd (2016), it is recommended to standardize the number of water quality parameters measured during fish culture to avoid unnecessary resource wastage in monitoring parameters that can be inferred from the observation of other related parameters. This approach ensures uniformity in the observed mean temperature among the test fish and optimizes the allocation of resources for water quality monitoring.

The water temperature, DO, and pH within all experimental tanks varied from 26.60 - 26.75 °C, 6.35 - 6.90 mg.L⁻¹ and 7.33 - 7.90 is consistent with prior research published by Boyd, (1982) who found a temperature range from 26.06 to 31.97 °C, Moniruzzaman *et al.* (2015) recorded 6.3 to 7.3 mg.L⁻¹. pH range from 6.4 to 8.3 is acceptable for tropical fish production (Roberts *et al.*, 1940).

The hatching period of pure GG, SS, and both reciprocal hybrids was 13.5 - 14 hr after fertilization, which was similar to those reported 14 hr for (*Hypsibarbus wetmorei*♂ × *Barbonymus gonionotus*♀) hybrid (Zakaria *et al.*, 2018), and 13 - 14 hr for *B. gonionotus* in lab-reared conditions at 30.5°C (Basak *et al.*, 2014). Other tropical carp species, like *Puntius sarana*, *Hypsibarbus malcolmi*, *Tor tambroides*, and *T. douronensis* hatched at 15 - 20 hr at room temperature (Chakraborty, 2003; Ingram *et al.*, 2005; Ogata *et al.*, 2010; Udit *et al.*, 2014), whereas *Neolissochilus hexagonolepis* hatched in 22 hr after fertilization at 22 ± 2°C (Sarma *et al.*, 2015). The development of the embryo and hatching time in the fertilized eggs of most fishes are usually influenced by water temperature (Basak *et al.*, 2014).

The hybrid and their parent's sib started eating three days before the yolk absorption. Similarly, Basak *et al.* (2014) for *B. gonionotus* and Azfar-Ismail *et al.* (2020) for mahseer hybrid (*Tor tambroides*♂ × *B. gonionotus*♀) observed a similar phenomenon of commencing initial feeding before yolk sac absorption. Udit *et al.* (2014) revealed that the size of the yolk sac in fish larvae, as well as the temperature of the rearing water, affect the time of yolk sac absorption. The yolk sac of larvae was fully reabsorbed three days after hatching in hybrids and pure species respectively. The results of this study were also in line with previous studies, which revealed that the yolk sac of *B. gonionotus* completely disappeared 72 hr after hatching at a water temperature of 30 °C (Basak *et al.*, 2014). Moreover, the yolk sac of Malaysian mahseer was almost totally reabsorbed three days after hatching (Ramezani-Fard *et al.*, 2011). Meanwhile, other research found that the yolk sac is completely disappeared by the larvae of lemon fin barb hybrid (Zakaria *et al.*, 2018), *Scopthalmus maximus* (Cunha & Planas, 1999), *H. malcolmi* (Ogata *et al.*, 2010), *Cyprinus carpio* (Haniffa *et al.*, 2007), and *Mystus nemurus* (El Hag *et al.*, 2012) within 2 - 3 days after hatching. According to Jafari *et al.* (2009), it takes 20 days for *Rutilus frisii kutum* to completely reabsorb its yolk sac.

Hybridization has been proven to have a significant impact on production-related characteristics (particularly growth), with some studies revealing F₁ hybrids growing intermediate to the parent species (Pallipuram, 2020). Hybridization has been used extensively in fishes, plants, and other vertebrates to increase growth rate, transmit desirable characteristics between species, combine desirable traits from two species into a single species, reduce undesirable reproduction by producing sterile or mono-sex progeny, increase environmental tolerances, exploit sexual dimorphism, improve harvestability, enhance the productivity of aquacultural strains or enhance recreational angling opportunities and increase total strength under culture conditions (Xu *et al.*, 2019; Bartley *et al.*, 2001; Scribner *et al.*, 2001).

In Hungary, Bakos *et al.* (1978) conducted a comprehensive crossbreeding experiment involving five cyprinid species: *Cyprinus carpio*, *Hypophthalmichthys nobilis*, *H. molitrix*, *Ctenopharyngodon idella*, and tenchs. The researchers found that culturing these hybrids was suitable for natural waterways and stocking ponds. All hybrids showed improvement, except the *C. idella* × *C. carpio* hybrid, which did not hatch successfully. The hybrid between *C. carpio* and *H. molitrix* was viable, while the reciprocal combination was not. These hybrids reached maturity at the age of four, but viable F₂ or backcross progeny could not be obtained. Hybridization between common carps and grass carps (but not in the

reciprocal direction) was also feasible, although post-fry stage growth was not evaluated. The hybrid of *C. carpio* and *H. nobilis* showed viability and promising development in the first and second years, but reciprocal crosses were not attempted. Successful reciprocal crossings were observed between big-head carps and silver carps, with their growth rates being similar to those of their parent species. However, when big-head carps were crossed with grass carps reciprocally, poor viability and gross abnormalities were observed. Hybrids resulting from the cross between *C. carpio* and *Tinca tinca* were viable, while the reciprocal cross was not feasible.

Furthermore, evidence for superior performances and hybrid vigor has been reported in a wide range of finfish and catfish (Varghese *et al.*, 1984; Basavaraju *et al.*, 1995; Hulata, 1995; Rahman *et al.*, 1995; Rahman *et al.*, 2005; Owodeinde & Ndimele, 2011; Okomoda *et al.*, 2018; Yusoff, 2019). Hybrids between several species of tilapia also demonstrated positive heterosis in terms of production, growth performance, and other desirable qualities (Bhowmick *et al.*, 1987; Hulata *et al.*, 1993; Lim *et al.*, 1993; Head *et al.*, 1994; Verdegem *et al.*, 1997). The DOF studied the lemon fin barb hybrid produced by crossing male *Hypsibarbus wetmorei* with female *B. gonionotus*, with the aim of a fast growth rate and good taste (Zakaria *et al.*, 2018). Mia *et al.* (2005) reported the successful production of a hybrid between *Aristichthys nobilis* and Chinese carp, *Hypophthalmichthys molitrix*. According to Rahman *et al.* (1995) hybrid between *C. gariepinus*♂ and *Clarias batrachus*♀ is not only successful, but additionally, resulted in higher growth performance, fertilization, and hatching.

The growth and nutritional utilization factors of two separate species of *B. gonionotus*, *B. schwanenfeldii*, and their hybrids fed on a 35% crude protein diet showed that the fish could utilize the diet successfully under identical rearing conditions. The hybrid GS had intermediate growth and better feed utilization over both parents, followed by pure GG, SG hybrid, and pure SS. This finding is consistent with the findings of Adewolu *et al.* (2008) who concluded that hybrid catfish grew faster and converted feed to fish flesh more efficiently than pure *Clarias gariepinus* and *H. longifilis*. In terms of growth rate, feed conversion, and disease resistance, hybrids were often superior to their parental line (Adewolu *et al.*, 2008; Okomoda *et al.*, 2018; Rahman *et al.*, 2005). On the other hand, the hybrid vigor in this study recorded an increasing quantity of deleterious traits introduced at hybridization. This is ameliorated by the increase in traits (growth) beneficial to the culturist, as observed in Table 2. Although the wholesome modification of phenotypic traits differs from growth initiated from feed intake because there are genes responsible for craving food and the final conversion of food to flesh (Francis, 2018). The geneticists opined that the most interesting characteristics are survival, feed conversion, and quality in the growth rate (Bakos & Gorda, 1995; Ninh *et al.*, 2014; Khaw *et al.*, 2016).

The hybrid's best performance in terms of growth rate, feed conversion ratio, and disease resistance can be attributed to the increased hybrid vigor they possess. These findings are consistent with previous studies by Basavaraju *et al.* (1995), Dada & Madu (1996), Rahman *et al.* (1995), and Rahman *et al.* (2006), which also reported that hybrids outperformed parental strains and exhibited intermediate growth performance compared to their parental species. The current study further supports the opinion that hybridization can enhance growth rates and overall performance due to hybrid vigor, as suggested by Thodesen *et al.* (2013) and Neira *et al.* (2016).

Crossing experiments revealed that different combinations of parents exhibited positive heterosis, manifested in growth rate, survival, feed conversion, slaughter value, and flesh quality (Bakos & Gorda, 1995). For example, a hybrid between fimbriatus (*Labeo fimbriatus*) and catla (*Catla catla*) was discovered to combine desired characteristics such as the small head of the *Labeo fimbriatus* and the wide-body shape of the (*C. catla*) Indian major carps. It revealed heterosis and had meat with higher flesh quality than both parental species (Basavaraju *et al.*, 1995). This study indicated that the hybrids between *B. schwanenfeldii* and *B. gonionotus* were fertilizable, viable, positive heterosis, intermediate growth with a low feed conversion ratio and the development of larvae showed no abnormalities. Similar results have been reported by Rahman *et al.* (2000), Chevassus (1983), Basavaraju *et al.* (1995), and Varghese *et al.* (1984).

The feed conversion ratio (FCR) of fish was assessed next to determine their ability to convert feed to flesh. The hybrid GS has the best FCR of 1.10, making it a better feed-to-flesh converter. This means that the hybrid was able to convert a substantial quantity of feed to the required flesh at a rate that indicates that the consumption rate is near the actual quantity of feed given. Although the rearing period of 8 weeks in the tank might not have given the fish time to reach full potential because the growth cycle concerning strain differs; some are fast growers at the initial stage while others pick up midterm, this, still provides a baseline for understanding growth rate of test fish in the tank.

Genetic enhancement is an important part of improving farm fish stocks and is becoming increasingly important in aquaculture industries. Even a slight improvement in body weight and size would be a

significant advance since body size at a certain age is a common performance feature in breeding programs (Liu *et al.*, 2005; Neira *et al.*, 2006). Geneticists and fish culturists use repeated hybridization and selection processes to project characteristics of interest (color, growth rate, fillet consistency & quantity). It is a recognized method of improving the stock of interest and introducing strain (Bentsen *et al.*, 2012). Proper genetic selection and management have the potential to prevent inbreeding and genetic deterioration, as well as to make fish more disease-resistant. Schom & Bailey (1986) stated that improving stock performance is an essential priority of aqua culturists. Therefore, the improved stock of the present fish species will certainly benefit aquaculture production in this region. The development of one of the most successful hybrids (GIFT & lemon fin barb hybrid) resulted from careful breeding, a selection from an array of available genes from strains that have the potential of performing well at culture (Khaw *et al.*, 2016; Zakaria *et al.*, 2018). This is a bold step because it involves pooling genes together and selecting the one that can outperform the existing strain for developmental purposes, aquaculture importance, and minimizing poor harvest. The genetic traits of the test fish in these studies were hybrids at different selection levels with the hope of producing fish with a good growth rate and expressed FCR of interest.

When a new hybrid is produced and commercialized, it is highly of concern since the fish can be identified and distinguished from its parentages. It is problematic if a hybrid introduced into aquaculture cannot be distinguished and loses its distinctiveness, especially with the original species (Begg *et al.*, 1999). Morpho-meristic characteristics were the most often used method of identifying hybrids (Scribner *et al.*, 2001). When the issue of identification arises, external morphological characteristics are always given top priority. The present study showed the morphological variation of hybrids and their parental species based on morphometric and meristic characters, as well as phenotypic traits. Furthermore, it is essential to measure the traits on different parts of the fish body using a combination of conventional and truss networks, as this can assist in searching for the most differential characters to discriminate hybrid. It is worth noting that some morphological characteristics observed in juvenile fish can change as they grow and mature. While certain characteristics may vary at different life stages, certain morphological characteristics generally remain the same throughout all life stages of a fish. In this study, body shape, color, and fin characteristics remain constant throughout the study, corresponding to the study by McPhail (1984), who examined the morphological characteristics of sticklebacks and their hybrids in Enos Lake, British Columbia. He found that certain morphological characteristics, such as body shape and fin structure, remained constant across life stages, suggesting that these characteristics are heritable and not just influenced by environmental factors or growth.

Furthermore, studies on other fish species, such as cichlids (Kocher *et al.*, 1993; Duftner *et al.*, 2007) have also shown that certain morphological traits can persist throughout different life stages, supporting the idea that these traits can be reliable indicators of species identity and hybridization. These studies provide evidence that certain morphological traits in fish species, including hybrids, can remain consistent across various life stages. However, it is important to note that individual variation, environmental influences, and specific species characteristics can also contribute to some degree of variation in morphological traits. Therefore, it is crucial to consider multiple factors and consult species-specific studies for a comprehensive understanding of morphological consistency in fish across different life stages. This research provides evidence that, despite some changes in certain traits, there are morphological characteristics that can be used to identify fish throughout their entire life cycle. It suggests that these consistent traits can be utilized in distinguishing hybrids from their parent species, even as they grow and develop.

Numerous researches have confirmed the inheritance of hybrids' phenotypic features. Bhowmick *et al.* (1981) found that a hybrid between *C. catla* (female) and *L. rohita* (male) exhibited characteristics of both parents but tended more toward the (*L. rohita*) parent in terms of body proportions and fin ray counts. The hybrid's body color was similar to a catla, while the shape of its mouth was similar to a rohu. The hybrids produced by Basavaraju & Varghese (1981) were a cross between *L. rohita* (male) and *C. mrigala* (female). The hybrid produced by Ibrahim (1977) was a cross between *C. mrigala* (female) and *C. catla* (male), both hybrids were observed to have intermediate characteristics between the parents.

Univariate and multivariate analyses were used in this study to discriminate and classify hybrids based on morphological variation to identify possible clusters from GG, SS, and hybrids (GS & SG) based on morphometric and meristic characteristics. Most of the measured characteristics were statistically significant for the sampled species. It is expected that high phenotypic differences between *B. gonionotus* and *B. schwanenfeldii* as these two species belong to different taxa as Yusoff (2019) observed in *P. hypophthalmus* and *P. nasutus*. Reciprocal hybrids GS and SG had intermediate/shared features of both parents. In terms of body size, head size, and fin size, reciprocal hybrids appeared

more resemble GG, which has meant significant values closer to pure GG species. Whereas, the body and fin color of the hybrids were almost near to pure SS species. This is confirmed by the discriminant function analysis of morphometrics, which clustered the hybrids to their parental species with slight or no overlapping (Figure 5 & 6), This opinion was also expressed by (Yusoff *et al.* (2019) and Okomoda *et al.* (2018).

According to Hubbs (1955), hybrid vigor is expected to exhibit the features of its parental figures or intermediate in the first generation (F_1), and backcrossing is considered when the hybrid species are highly diversified. Several hybrids were identified to exhibit traits that were intermediate between their parents. For example, a hybrid between *C. macrocephalus* × *C. gariepinus* showed intermediate phenotypes compared to their parental species in most morphometric and meristic parameters except, the distance from dorsal to caudal fins (DDCF) comparable to *C. macrocephalus* and the shape of the occipital process similar to *C. gariepinus* (Duong *et al.*, 2017). A hybrid from yellow flounder *Limanda ferruginea* (♀) × Winter flounder, *Pseudopleuronectes americanus* (♂) showed intermediate morphological differences between the maternal and paternal species (Park *et al.*, 2003). Bhowmick *et al.* (1981) stated that the hybrid between *Gibelion catla* (♀) × *Labeo rohita* (♂) showed intermediate morphometric characteristics of the parents. Basavaraju *et al.* (1995) produced a hybrid from *Catla catla* (♀) × *Labeo fimbriatus* (♂), that demonstrated intermediate traits of its parental species. Morphological characteristics of *P. hypophthalmus*, *P. djambal*, and their reciprocal hybrids showed intermediate phenotypic features and have substantial similarity with them the latter than the former (Gustiano & Kristanto, 2007). This agrees with our finding that reciprocal hybrids between *B. gonionotus* and *B. schwanenfeldii* exhibited intermediate morphological characters and had a strong similarity in body color to their SS pure species.

According to the findings of the current study, the researchers conducted a multivariate analysis to identify the most effective morphometric traits for distinguishing hybrids from their parental species. They found that six specific morphometric traits, including Total length, Standard length, Head length, Dorsal fin base length, Body depth, and Pectoral fin length, were the strongest predictors for differentiation. These traits were predominantly observed on the body, fins, and head portion of the fish.

The results of the multivariate analysis, presented in Table 7, revealed high Eigenvalues of 22.620 with a variance of 97.20% for the morphometric traits and 12.209 with a variance of 80.20% for the meristic characters. This indicates that these components have a significant discriminating ability and can effectively place strains of fish into their respective groups. The high Eigenvalues make these traits particularly valuable for identification purposes, as emphasized by previous studies conducted by Samaradivakara *et al.* (2012) and González *et al.* (2016).

These findings highlight the importance of using a combination of morphometric and meristic characters to accurately identify and distinguish hybrids from their parental species. By considering these specific traits, researchers and aqua culturists can effectively differentiate between different strains of fish and maintain the integrity and distinctiveness of the original species. Reciprocal hybrids, on the other hand, showed relatively larger morphometric and meristic characteristics compared to their SS pure species. This means that these hybrids not only perform in the expected intermediate shape but their traits also showed that they were larger or smaller than their parents. The morphometric variability among the four groups in this study was primarily due to the variation of traits associated with fins, head, and body features. This is because the allometric transformation successfully eliminated the effect of size, as demonstrated by univariate proportion and multivariate analysis, which was favorable in comparison to the previous study; based on the standard-length ratio, the size factor may create a more significant variance in the collection of variable (Baharuddin *et al.*, 2014). According to Haddon & Willis (1995), morphometrics of the head and body depth are the most important characters for distinguishing fish populations, for example, *Lophius vomerinus*, *Clupea pallasii pallasii*, and *Hoplostethus atlanticus*.

CONCLUSION

In conclusion, our study demonstrates the feasibility of reciprocal hybrids (GS & SG) with higher breeding, growth performance, and survival than SS. In terms of FCR however, only GS showed positive FCR. It is reasonable to conclude that hybridization is not only a favored way of genetic enhancement but also a possible tool for stock improvement through transferring decent characteristics to their offspring. The morphological aspect of the body color and size, fin color and size, and head size differed between hybrids and their parents. These distinguishing features may be utilized to assist in the quick and straightforward identification and discrimination of hybrids. However, to improve this identification process, it is possible to integrate this method of characteristic assessment with the molecular identification system such as AFLP (Amplified Fragment Length Polymorphism) or DNA

barcoding. More research is needed to evaluate disease resistance and to assess the sustainability conservation, and future management of this hybrid for mass production.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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