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Characterization of Best Growing Line of the Minnow, *Gymnostomus ariza* (Hamilton 1807) through Landmark-based Morphometric Analysis

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ABSTRACT

A great decline has been witnessed in the minnow, *Gymnostomus ariza* (Hamilton, 1807) in the natural water bodies; a phenomenon that requires high consideration. Hence, the present research has been organized to focus on the quality seed production of this fish. Specimens of *G. ariza* from three distinct rivers (the Kangsha, the Atrai and the Jamuna Rivers in Bangladesh) were collected and subjected to form six breeding lines with15 species in each line. The progeny from these lines has been inspected phenotypically to assess the structure and shape variation of the population based on landmark and morphometric and meristic characters. After checking normality, One-way ANOVA disclosed that all morphometric, meristic and truss system measurements were dissimilar among the six lines, while line-4 exhibited significantly higher growth in all aspects. For the morphometric and truss measurements, the discriminant functions graph revealed a well-detached group of six lines indicating that the values differed significantly among the lines. The dendrogram constructed by means of morphometric and landmark data displayed one leading cluster of line-4 connected with all other lines combined. Considering the best growing line identified in the current research, the outcomes would be beneficial to promote the culture of this species, maintain proper conservation, attain successful management, and in addition, support further research with informative data.

INTRODUCTION

Bangladesh, one of the leading emerging fish producing countries of the world, is blessed with huge open water body having a diversified aquatic life. Along with 4.34 million ha productive water body (**DoF, 2020**), it is also enriched witha variety of fishes. This ranked the country the 3rd in Asia, with approximately 293 native freshwater fishes (**Hossain *et al*., 2012; DoF, 2016**). The fisheries sector of Bangladesh is playing an increasingly significant role in the economy having a contribution of 3.61% to our GDP

and around 25.30% to agricultural GDP (**DoF, 2020**). Bangladesh ranked the third in global inland water capture production just after China and India and the fifth in the inland aquaculture production (**DoF, 2020**). These inland fisheries of Bangladesh consist mainly of carps, minnows, catfishes and 40-50 small indigenous fish species. These species are popularly known as SIS that grows up to 25-30 cm length depending on the species. (**Felt *et al.*, 1996; Hossain *et al.*, 2012**). Prior to 1970s, numerous numbers of small native fishes, e.g. climbing perch, meni, snakeheads, bata, minnow, butter catfish, mystus catfish, walking catfish, olive barb and others were plentiful in all water bodies of Bengladesh(**Ahamed *et al.*, 2012; Hossain, 2014**). The afore- mentioned native fishesare commonly captured, and a big portion of the capture is kept for the consumption of a huge number of subsistent fishers(**Wahab, 2003**). These fishes comprise great quantity of calcium, zinc and iron (**Thilsted *et al.*, 1997**). Compared to big carps, these indigenous fishes contain more calcium and phosphorus (**Hossain *et al.*, 1999**). Moreover, they are most favored for their taste and the extra income they provide for local fishers. However, for conservation and management of the small indigenous species, their culture systems should be introduced in Bangladesh (**Mazid & Kohinoor, 2003; Wahab, 2003**). In this regard, minnow (*G. ariza*) can be a good choice to meet the challenge.

G. ariza comprises of tinny bands dorsally to its lateral line; bigger fish frequently has an extensive mid-lateral band. The fish can be simply identifiable by its silvery scale of intermediate size, profound blue or dark dorsal part of the body and silvery shiny abdomen. It has 38 or 39 scales on its lateral line, almost all of them seem to have a black spot extended from the boundary of operculum to the tail in its early life (**Rahman *et al.*, 2009**). However, in adult fish, this black spot is turned shorter and can be seen between the operculum and the abdomen (**Talwar & Jhingran, 1991; Rahman, 2005**). The minnow is spread over Indo-pacific counties (**Jhingran, 1991; Rahman, 2005; Hogan, 2012**) and inhabits crystal clear rivers (**Naser, 2015**). In Bangladesh, it is extensively spread over the Karnafuly River and water basins in Chittagong hilly areas (**Roberts, 1997; Kohinoor *et al.*, 1998; Hogan, 2012**).Remarkably, the fish spreads in almost all the minor rivers, valleys and creeks of Bangladesh (**Hussain & Mazid, 2001; Ahammad *et al.*, 2015**). The omnivorous fish can reacha length of 30 cm in natural water body, and notably, females are larger than males (**Felts *et al.*, 1996; Akhteruzzaman e*t al.*, 1998; Rahman *et al.*, 2009**). Both sexes obtain adulthood in the 1st year; usually by the end of the 1st year, and they breed in inundated water bodies from June to September (**Roberts, 1997**). Spawning period of this fish is from April to August reaching a peak during the month drizzling period (**Akhteruzzaman *et al.*, 1998; Hussain & Mazid, 2001**). Each female of this prolific breeder lays about 3 million eggs (**Roberts, 1997**). Complete fecundity of female ranges from 2,00,000 to 2,50,000 for one kg of body weight (**Hussain & Mazid, 2001**).

This specieshas been declining significantly owing to fishing pressure and numerous anthropogenic events, such as aquatic pollution, siltation and damage of natural

habitation for reproduction and development (**Akhteruzzaman *et al.*, 1998; Hussain & Mazid, 2001; Sabbir *et al*., 2021**). These issues not only devastated the spawning ground but also instigated stress to the brood fish, fry and fingerlings living in the water body (**Hussain & Mazid, 2001; Mawa *et al*., 2021**). The consequences have beenrecently documented as one of the most vulnerable and threatened species in Bangladesh (**DoF, 2007; DoF, 2014; IUCN, 2015; Naser, 2015**). Even though they are vulnerable, a small quantity of fish is available in some rivers, haors, baors and beels (**DoF, 2014**). That is why it is essential to preserve and rehabilitate this fish through artificial propagation and culture in captivity.Correspondingly, to protect the minnow from annihilation, the need to develop the artificial breeding and culture practice, and the urge to generate statistical information for effective management and developing conservation strategy are highly recommended (**Siddik *et al*., 2014**). *G. ariza* has great nutritious value withsatisfactory quantity of calcium, protein and low fatty acid (**Gupta, 1975**). However, protein, fat and carbohydrate calories of the minnow (*G. ariza*) are relatively higher than those in the Indian major carps (**Ahammad *et al*., 2015**). Due to its good demand among the consumers and its initial quick growth, the *G. ariza* is considered a candidate species for artificial culture in ponds (**Ahammad *et al*., 2018**). In addition, the potential value of its culture in ponds by co-stocking with Indian Major Carps has also been reported earlier. Though *G. ariza* is used to attain full maturity in ponds though it does not spawn there. Hence, the induced breeding is the only measure followed to solve this problem (**Ahammad *et al*., 2018)**. In this regard, good quality fish seed can be produced through various line breeding programs of wild sources of minnow (*G. ariza*) populations. A base population of *G. ariza* should provide quality fish seed resulting in economic and nutritional benefits.

Measurable characters common to all fishes are known as morphometric characters (**Rahman *et al*., 2019; Hossen *et al*., 2020; Islam *et al*., 2020**). Some points selected arbitrarily on a fish body called landmarks help the shape of the fish to be scrutinized. The learning of morphometric features in fish is vital for the distinction of taxonomic units and taxonomic identification that constitute the initial stage in species' study (**Langer *et al*., 2013**). Meristic, on the other hand, is a part of ichthyology which narrates quantitative characters of fish, like the fins or scale number. Meristic or countable trait is used to define a specific fish species, or detect an unidentified species. Meristic characters are described in a shorthand notation known as a meristic formula. These are the characters most frequently used for distinction among species or population. In case of salmonids, a number of scales have been extensively used for the distinction of population within species. In case of rainbow trout and steelhead trout, the utmost notable variation among population occurring in scale counts is used (**Fishbase, 2020**).

The minnow, *G. ariza* is avital and highly valuable food fish, but it is decreasing in natural water reservoirs of Bangladesh, and thats why it has currently become the most expensive freshwater fish food. Therefore, aagreat interest has been drawn in the biology,

culture and conservation of this fish. Due to its substantial economic and cultural status, a successful development of sustainable aquaculture production systems of these species might be enormously supportive for the protection, conservation and rehabilitation of minnow from this vulnerable condition. No previous research has yet been undertaken on seed production through line breeding technique under captive condition for this species. Therefore, the current study was conductedto find out an economically viable and practical procedure for mass seed production of *G. ariza* in captive condition and identify the best cross-bred line using the powerful growth measurement tools known as morphometric and landmark analysis.In this context, this study was designed to determine the best cross-breeding population line of *G. ariza* using landmark-based morphometric analysis which wouldhelp in the better culture and conservation of the minnow.

MATERIALS AND METHODS

2.1. Minnow sample collection and domestication

Wild minnow populations were collected from three geographically distinct areas, such as Atrai River, Dinajpur; Kangsha River, Mymensingh; and Jamuna River, Sirajganj inBangladesh. Total 300 fingerlings from each location were collected and transported live to the Fisheries Faculty Field Laboratory Complex, Bangladesh Agricultural University, Mymensingh, Bangladesh. Fish were stocked and acclimatized in fiber tanks at water temperature of 26-27°C.

2.2. Improved nursery and grow-out management of minnow in earthen ponds

Then, fingerlings were reared for 6 months in the separate rectangular ponds (dimensions 18×14m^2 and average depth of 1.3m) prepared earlier. Supplementary feed and formulated feed were provided during the nursing period. After 6 months, adult minnows were reared for another 4 months in grow-out ponds until their gonadal maturation. All facilities including water supply, inlet and outlet were provided. A distinct feed rich in protein and vitamin-E, were provided at those 4 months which enhances the gonadal maturation in fishes (**Mollah *et al.*, 2009**). Feed was applied at 5% of their body weight twice a day during the whole 10 months period.

2.3. Line breeding trials of three populations of minnow

Line breeding trials were performed with PG (pituitary gland) extract. Six lines are designed as presented in Table (1). In this regard, free oozing broods of 1:1 (male:female) sex ratio were selected for artificial propagation. Females were inoculated with PG of 4 $mgkg^{-1}$ body weight (1^{st} dose) and 8 $mgkg^{-1}$ body weight (2^{nd} dose), and males were inoculated with a single dose of 4 mgkg-1 body weight at the time of second jab of female. During inoculation, broods were kept on a water-logged foam and wrapped with a soft wet cloth. The intramuscular inoculation of PG was applied at the ventral part

of the body beneath the pectoral fin of brood. The broods were ovulated after 7-8 hours of injection. Then, the fish were subjected to strip, and the inseminated eggs were relocated into a circular hatching tank supported with nonstop water flow for proper aeration. Each line was treated as a treatment in this experiment, and the variation among the lines was observed and evaluated during the total experimental period.

Table 1. Schematic representation of six breeding line of Bhagna, *G. ariza*

SL. No.	Group	Breeding line	Pattern
1	Conspecific group	Line-1	Kangsha♀×Kangsha♂
2		Line-2	Jamuna♀×Jamuna♂
3		Line-3	Atrai♀×Atrai♂
4	Heterospecific group	Line-4	Kangsha♀×Atrai♂
5		Line-5	Kangsha♀×Jamuna♂
6		Line 6	Atrai♀×Kangsha♂

2.4. Rearing larvae of six different lines in glass aquaria and pond condition

Subsequently, newly hatched larvae were reared in glass aquaria under different stocking densities with temperature-controlled system using thermostat up to 21 days after hatching. After that, the fry of minnow of six different lines were transferred into six different ponds for grow-out culture up to twelve months. Here, regular aeration and siphoning were done. Feed was provided to the larvae at 5% of their body weight. At this stage, water pH and DO were measured by pH and DO meters, respectively. After rearing the larvae in glass aquarium for three weeks, they were raised in isolated rectangular ponds (dimensions: 18×14 m^2, mean depth: 1.3 m) with supplementary and formulated feed. Regular fertilization with urea and TSP @ 200 g/decimal were applied. Subsequently, 10% from each line of the adult minnow were selected based on individual selection for landmark analysis.

2.5. Morphometric, meristic and landmark-based analysis

All morphometric measurements were recorded by using centimeter scale and are presented in Fig. (1). For landmark analysis, 10 landmark points weredrawn (Fig. 2) and twenty-two different distances were defined by joining these points on the fish body (Fig. 2). This was done by putting minnow on a paper. For meristic counts, 9 meristictraits;dorsal fin rays (DFR), pectoral fin rays (PCFR), caudal fin rays (CFR), scale above lateral line (SaLL), branchiostegal rays (BSR), scale on lateral line (SabLL), scale below the lateral line (SbLL) were investigated. For counting meristic traits, fish were set counter to light course in a room, and traits were counted by the aid of a fine needle. Ten landmark points describing 22 truss distances were constructed on the body of minnow (Fig. 2). Each point was obtained by employing a fish on a white paper.Then, the landmark points were spotted with color signature pen on the paper to construct

precise and consistent truss network. Lastly, the points on the paper were connected and measured by a centimeter scale.

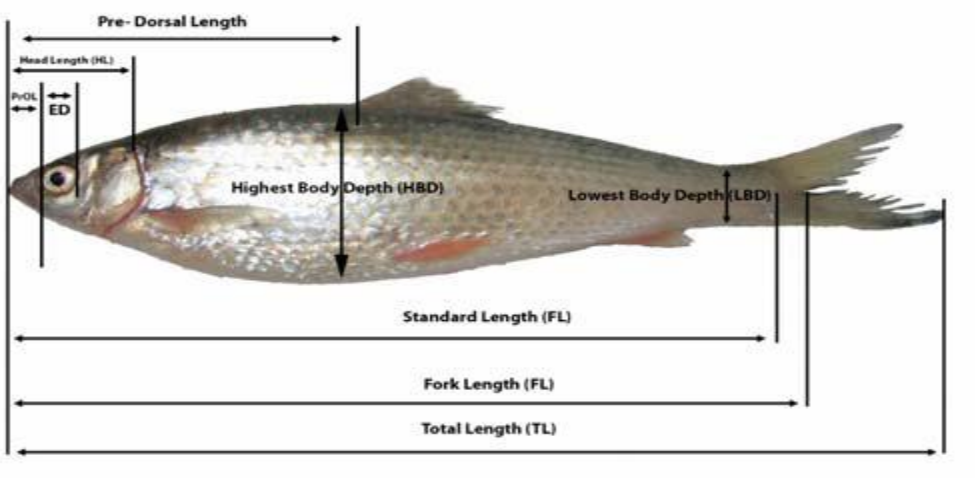

Fig 1. Morphometric measurement *G. ariza*

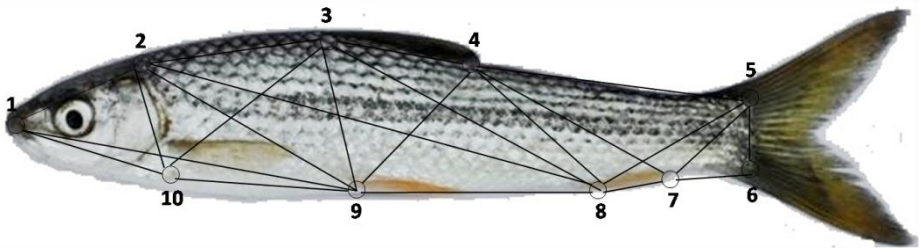

Fig. **2**. Location of the 10 landmarks for constructing the truss network on fish body illustrated as small circle and morphometric distance measures between the circles as lines. Landmarks refer to (1) anterior tip of snout at upper jaw, (2) most posterior aspect of neurocranium (beginning of scaled nape), (3) origin of dorsal fin, (4) insertion of dorsal fin, (5) anterior attachment of dorsal membrane from caudal fin, (6) anterior attachment of ventral membrane from caudal fin, (7) insertion of anal fin, (8) origin of anal fin, (9) insertion of pelvic fin and (10) insertion of pectoral fin.

2.6. Statistical analysis

To identify grouping of variables that reveal the best separate *L ariza* species, a multivariate discriminant function analysis of morphometric data wasperformed. Before analysis, size effects of the data set were minimized. Dissimilarities were mainly attributed to shape differences of fish body, rather than relative fish size. The following allometric formula given by **Elliott *et al.* (1995)** wasused to eliminate the size effect of the data.

$M_{adj} = M\ (L_S/L_o)^b$

Where, M denotes original measurement, M_{adj} denotes size after adjustment, L_o refers to the total length of fish, and Ls represents over-all mean of the total length for samples from six lines. Parameter b was estimated for every trait from the experimental data as the slope of the regression of log M on log L_o, using all samples from six lines. All statistical analyses were done by SPSS 22.0 version (SPSS, Chicago, IL, USA) and MS Excel 2007.

RESULTS

3.1. Morphometric measurement

The average lengths of the six lines of *G. ariza* after sampling are described in Table (2). A significant variation in the mean total length between line 4 (heterospecific group) and the rest wasobserved from the obtained results. Eight morphometric measures of six lines and their mean values are presented in Table (3). Among these, the total length (TL) is related with the SL, FL, LBD, HBD, MG, ED and PDL. From the data, it is clear that, the highest average length was observed in line 4. There is little difference between line 4 and line 6 in morphometric counts, but there are significant differences from line 4 to line 1, line2, line 3, line 5 in morphometric counts.

Table 2. Average length (cm) of the samples insix lines

Group name	Sample size	Total length (cm)
Line 1	15	17.31 ± 0.78^{b}
Line 2	15	17.04 ± 0.66^{c}
Line 3	15	16.97 ± 0.21^{c}
Line 4	15	18.67 ± 0.48^{a}
Line 5	15	17.09 ± 0.86^{c}
Line 6	15	18.03 ± 0.77^{b}

**different superscript in the same column indicates significant difference.*

3.2. Landmark distances and meristic measurements

Twenty-two landmark distances with their average value for six lines are shown in Table (4). These data show a little difference among the six lines, while line 4 has the significantly highest value. Nine meristic counts and their median for six lines aredisplayed in Table (5). The DFR was found to correlate with CFR with significant difference ($P<0.05$) and extremely correlated with SaLL, SabLL and SbLL ($P<0.01$). The CFR was correlated with SabLL ($P<0.05$). No significant differences were observed among average number of PCFR, PVFR, AFR, BSR. The effectiveness of the allometric method in eliminating the size effect from the data was validated by using correlations between the total length and adjusted traits. Among the 8 changed morphometric traits and 22 truss network measurements, no significant correlation was found ($P>0.05$). Therefore, the data were subjected to a next analysis. ANOVA of 10 morphometric traits

and 22 truss network distances revealed a highly significant difference (P<0.001) among the morphometric and truss measurements of six lines (Tables 5 & 6).

Table 3. Morphometric measurement (mean in cm) of minnow (*G. ariza*) of six different lines (in the parenthesis indicates minimum & maximum counts)

Characters	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
TL (cm)	17.31^{b} (15.2-20.4)	17.04^{c} (15.1-20.2)	16.97^{c} (15.1-19.6)	18.67^{a} (15.2-20.4)	17.09^{c} (15-20)	18.03^{b} (15.3-20.6)
SL (cm)	12.31^{bc} (11.4-15.5)	12.91^{b} (11.2-15.2)	12.75^{b} (11.1-14.7)	14.23^{a} (13.5-15.5)	12.99^{b} (11.1-15.2)	14.12^{a} (11.7-15.7)
FL (cm)	14.97^{b} (13-17)	14.74^{b} (13-17.2)	14.63^{b} (12.8-16.9)	16.09^{a} (13-17.6)	14.78^{b} (13-17.2)	16.04^{a} (13.1-17.7)
HBD (cm)	2.98^{b} (2.3-3.7)	2.91^{b} (2.3-3.7)	2.93^{b} (2.1-3.7)	3.44^{a} (2.7-3.8)	2.94^{b} (2.3-3.8)	3.35^{a} (2.7-3.8)
LBD (cm)	1.37^{b} (1.1-1.7)	1.34^{b} (1.1-1.5)	1.25^{c} (1-1.5)	1.46^{a} (1.1 1.7)	1.37^{b} (1.2-1.8)	1.45^{a} (1.1-1.8)
MG (cm)	0.40^{ab} (0.3-0.5)	0.32^{c} (0.3-0.5)	0.37^{b} (0.3-0.5)	0.43^{a} (0.3-0.5)	0.43^{a} (0.3-0.6)	0.43^{a} (0.3-0.5)
ED	0.89^{a} (0.7-1.8)	0.81^{b} (0.7-1)	0.78^{b} (0.6-1)	0.92^{a} (0.7-1)	0.81^{b} (0.7-0.9)	0.87^{ab} (0.7-1)
PDL	5.67^{b} (4.7-6.4)	5.53^{c} (4.7-6.5)	5.48^{c} (4.6-6.5)	5.88^{a} (4.8-6.6)	5.53^{c} (4.7-6.3)	5.87^{a} (4.9-6.6)

*different superscript in the same row indicates significant difference

Table 4. Landmark distance counts (average in cm) of minnow (*G. ariza*) of six different lines (in the parenthesis indicates minimum & maximum counts)

Characters	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
Dist 1-2	2.51^{b} (2.2-2.8)	2.50^{b} (2.2-3.1)	2.52^{b} (2.1-3.1)	2.71^{a} (2.3-3.1)	2.42^{c} (1.3-3.2)	2.77^{a} (2.2-3.2)
Dist 2-3	3.63^{a} (3-4.3)	3.50^{b} (2.9-4.2)	3.53^{b} (3.1-4.1)	3.62^{a} (2.8-4.3)	3.46^{bc} (2.9-4.1)	3.63^{a} (2.8-4.4)
Dist 3-4	2.12^{a} (1.7-2.6)	2.0^{b} (1.7-2.4)	2.17^{a} (1.5-2.5)	2.13^{a} (1.4-2.5)	1.97^{b} (1.5-2.5)	2.18^{a} (1.6-2.6)
Dist 4-5	5.02^{bc} (4.3-6)	5.09^{bc} (4.3-6.30	4.57^{c} (4.3-6.3)	5.76^{a} (4.8-6.5)	5.21^{b} (4.2-6.4)	5.66^{a} (4.5-6.6)
Dist 5-6	1.55^{b} (1.1-2)	1.49^{b} (1.1-1.7)	1.50^{b} (1.1-1.7)	1.71^{a} (1.3-2.2)	1.53^{b} (1.1-1.8)	1.71^{a} (1.3-2.3)
Dist 6-7	1.53^{c} (1.3-1.8)	1.63^{bc} (1.3-2.4)	1.68^{b} (1.3-2.6)	2.18^{a} (1.7-2.1)	1.79^{b} (1.2-2.5)	2.07^{a} (1.3-2.7)
Dist 7-8	1.16^{b} (0.9-1.4)	1.16^{b} (0.9-1.9)	1.17^{b} (1-1.5_	1.09^{c} (0.8-1.9)	1.07^{c} (0.7-1.4)	1.23^{a} (1.1-1.9)
Dist 8-9	3.55^{b} (3-4.8)	3.69^{b} (3-4.7)	3.58^{b} (3-4.4)	4.22^{a} (3.2-4.9)	3.63^{b} (3.1-4.5)	4.13^{a} (3.1-4.8)
Dist 9-10	3.67^{a} (3.1-4.2)	3.63^{a} (2.9-4.1)	3.60^{a} (3.1-4.3)3	3.61^{a} (2.9-4.4)	3.55^{a} (3-4.3)	3.63^{a} (2.9-4.2)
Dist 1-10	3.35^{b}	3.22^{c}	3.24^{c}	3.55^{a}	3.34^{b}	3.63^{a}

	(2.8-3.6)	(2.7-3.5)	(2.6-3.7)	(2.9-4.1)	(2.1-3.8)	(2.8-4.3)
Dist 2-10	2.22^{b} (1.8-2.6)	2.13^{c} (1.7-2.5)	2.22^{b} (1.9-2.6)	2.33^{ab} (1.8-2.8)	2.19^{b} (1.6-2.5)	2.43^{a} (1.8-2.9)
Dist 3-10	3.70^{b} (3.3-4.4)	3.68^{b} (3.2-4.5)	3.67^{b} (3.3-4.2)	3.95^{a} (3.1-4.6)	3.51^{c} (3-4.1)	3.96^{a} (3.3-4.6)
Dist 1-9	6.92^{b} (6-7.8)	6.81^{b} (5.9-7.6)	6.86^{b} (6.2-7.5)	7.13^{a} (5.8-7.8)	6.86^{b} (6-7.4)	7.17^{a} (6.1-7.9)
Dist 2-9	5.17^{ab} (3-5.6)	5.06^{b} (4.1-6)	5.09^{b} (4.5-5.6)	5.19^{a} (4.1-5.8)	5.10^{b} (4.1-5.8)	5.33^{a} (4.3-5.9)
Dist 3-9	3.14^{b} (2.8-3.8)	3.21^{b} (2.7-3.7)	3.35^{ab} (3-3.8)	3.43^{a} (2.6-3.8)	3.22^{b} (2.6-3.7)	3.49^{a} (2.7-3.8)
Dist 4-9	3.41^{ab} (2.9-4.2)	3.33^{b} (2.8-4.2)	3.30^{b} (2.7-3.9)	3.59^{a} (2.8-4.2)	3.23^{b} (2.9-3.9)	3.63^{a} (2.8-4.3)
Dist 2-8	8.44^{ab} (7.4-10.2)	8.24^{b} (10.1-7.2)	8.19^{b} (7.1-9.2)	8.81^{a} (6.9-9.7)	8.27^{b} (7.3-9.7)	8.65^{a} (6.9-9.8)
Dist 3-8	5.36^{b} (4.5-6.4)	5.09^{c} (4.4-6.3)	5.19^{bc} (4.3-5.9)	5.73^{a} (4.5-6.3)	5.25^{b} (4.5-6.2)	5.75^{a} (4.5-6.3)
Dist 4-8	3.73^{b} (3.2-4.5)	3.63^{b} (3.1-4.4)	3.67^{b} (3.1-4.2)	3.97^{a} (3-4.3)	3.68^{b} (3.1-4.2)	3.99^{a} (3.1-4.4)
Dist 5-8	3.09^{c} (2.5-3.3)	3.31^{b} (2.8-3.8)	3.19^{c} (2.8-3.9)	3.55^{a} (2.9-4.1)	3.16^{c} (2.5-3.9)	3.55^{a} (2.9-4.2)
Dist 4-7	4.45^{ab} (3.9-5.3)	4.22^{c} (3.6-5.2)	4.32^{b} (3.8-4.9)	4.65^{a} (3.5-5.3)	4.34^{b} (3.7-4.8)	4.69^{a} (3.5-5.4)
Dist 5-7	2.20^{b} (2-2.4)	2.24^{b} (2-2.9)	2.25^{b} (2-2.9)	2.65^{a} (2-3.3)	2.32^{b} (2-3.1)	2.67^{a} (2.1-3.4)

*different superscript in the same row indicates significant difference.

Table 5: Meristic counts (median) of minnow (*G. ariza*) of six different lines (in the parenthesis indicates minimum and maximum counts)

Line	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
DFR	8^{c} (8-9)	10^{b} (9-10)	7^{c} (7-7)	12^{a} (11-12)	11^{a} (11-12)	9^{b} (1-10)
PCFR	10^{c} (10-11)	12^{b} (11-13)	9^{c} (9-9)	14^{a} (13-14)	13^{a} (11-13)	11^{bc} (11-12)
PVFR	7^{c} (7-9)	9^{b} (8-10)	6^{c} (6-7)	11^{a} (11-12)	11^{a} (10-11)	9^{b} (9-10)
AFR	7^{a} (6-7)	6^{b} (6-7)	6^{b} (6-7)	7^{a} (6-7)	7^{a} (5-7)	7^{a} (6-7)
CFR	20^{a} (20-20)	20^{a} (20-22)	20^{a} (20-20)	20^{a} (18-20)	20^{a} (20-20)	20^{a} (18-22)
BSR	6^{a} (6-6)	6^{a} (6-6)	6^{a} (6-6)	6^{a} (6-6)	6^{a} (6-6)	6^{a} (6-6)
SaLL	379^{a} (311-395)	379^{a} (351-392)	369^{b} (343-386)	379^{a} (365-417)	368^{b} (365-417)	378^{a} (353-401)
SabLL	70^{b}	67^{b}	64^{b}	87^{a}	70^{b}	79^{ab}

	(65-83)	(61-78)	(57-73)	(41-73	(66-81)	(68-93)
SbLL	572[b] (401-623)	561[bc] (395-603)	551[c] (384-587)	587[a] (517-623)	557[c] (404-611)	551[c] (241-607)

*different superscript in the same row indicates significant difference.

Table 6: Univariate analysis of variance (ANOVA) of morphometric measurements in six lines

Tests of Equality of Group Means					
Characters (cm)	Wilks' Lambda	F	df1	df2	Sig. (*)
TL	.061	257.613	5	84	.000
SL	.028	573.536	5	84	.000
FL	.030	547.745	5	84	.000
HBD	.217	60.743	5	84	.000
LBD	.502	16.694	5	84	.000
MG	.906	1.744	5	84	.133
ED	.892	2.042	5	84	.081
PDL	.907	1.728	5	84	.137

Discriminant function analysis formed two discriminant functions (DF_1 and DF_2) for both morphometric and truss measurements (Table 6 & Table 7). For morphometric and truss measurements, the two discriminant functions mutually explicated 100% of the total variability of six lines. Among the six lines, line 4was found dissimilar and clearly disconnected from the other five lines that are shown in the discriminant graph from the morphometrical point of view (Fig. 3). There was a virtual overlapping between line 2 and line 6. Line 5 and line 3 werealso morphometrically different and separated from each other as well as from the other lines. There was also virtually no overlapping between line 3 and line 5. Again line 1, line 6 and line 2 are not visibly detached from each other according to the discriminant graph inFig. (3). The canonical graph of line 5, then line 3, then line1, then line 6, then line 2 and finally line 4 were chronologically distributed in a form of cluster round to their centroid values. For the truss measurements, six lines are also varied in the discriminant graph ofFig. (4). This indicates that there was a slight mixing among the lines and the lines were not completely detached.

Table 7: Univariate analysis of variance (ANOVA) of Landmark distances in six lines

Landmark Distance	Wilks' Lambda	F	df1	df2	Sig. (*)
1-2	.007	2307.492	5	84	.000
2-3	.049	324.901	5	84	.000
3-4	.005	3330.682	5	84	.000
4-5	.004	4725.453	5	84	.000
5-6	.010	1680.296	5	84	.000
6-7	.015	732.89	5	84	.000
7-8	.932	1.234	5	84	.300
8-9	.796	4.297	5	84	.002
9-10	.989	.179	5	84	.970
1-10	.839	3.223	5	84	.010
2-10	.866	2.592	5	84	.031
3-10	.841	3.178	5	84	.011
1-9	.925	1.364	5	84	.246
2-9	.956	.780	5	84	.567
3-9	.901	1.847	5	84	.113
4-9	.865	2.631	5	84	.029
2-8	.956	.773	5	84	.572
3-8	.827	3.509	5	84	.006
4-8	.855	2.847	5	84	.020
5-8	.708	6.930	5	84	.000
4-7	.875	2.395	5	84	.044
5-7	.733	6.106	5	84	.000

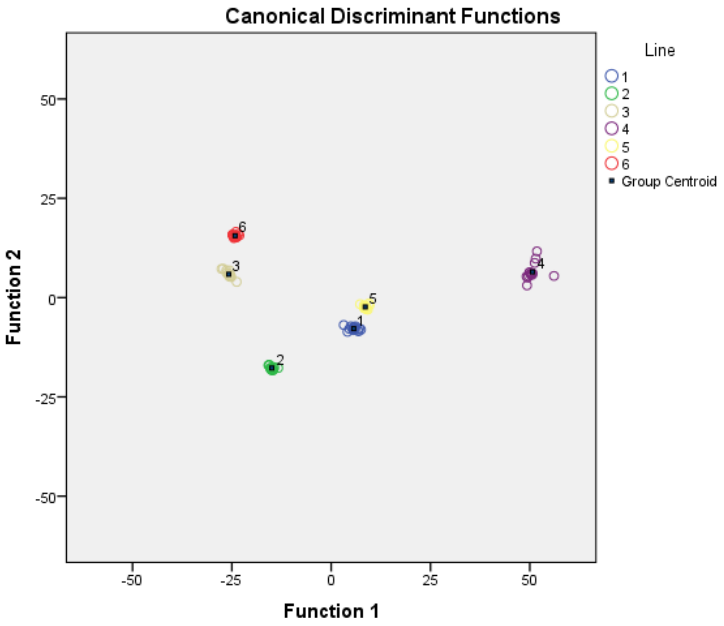

Fig 3. Sample centroids of discriminant function scores based on morphometric measurements. Samples referred to, 1: Line-1, 2: Line-2, 3: Line-3, 4; Line-4, 5: Line-5, 6: Line 6

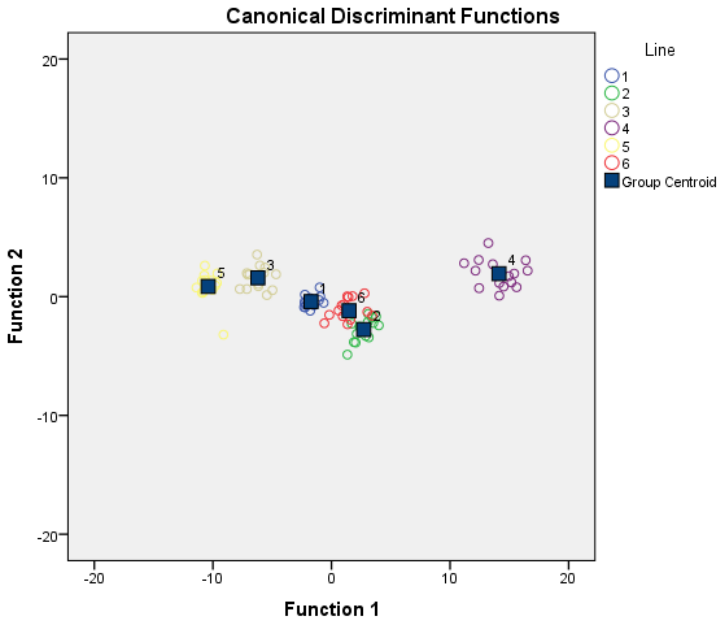

Fig 4: Sample centroids of discriminant function scores based on landmark distance. Sample referred to, 1: Line-1, 2: Line-2, 3: Line-3, 4; Line-4, 5: Line-5, 6: Line 6.

Pooled within group correlations between discriminant variables and the five functions exposed that total length (TL),standard length (SL) and fork length (FL) among eight morphometric trait dominantly contributed to function 1; one measurement of the highest body depth (HBD) had significant contribution to function 2; one measurement of the lowest body depth (LBD) dominantly contributed to function 3;two measurements of

pre-dorsal length (PDL) and eye diameter (ED) haddominant contribution to function 4 and other trait, and eye diameter (ED) contributed to the function 5 (Table 8). For the 22 truss network measurements, 16 measurements viz: dist 1-2, dist 7-8, dist 8-9, dist 1-10, dist2-10, dist 5-7, dist 3-10, dist 1-9, dist 2-9, dist 3-9, dist 4-9, dist 2-8, dist 3-8, dist 4-8, dist 5-8, dist 4-7 dominantly contributed to function 5; one character dist 4-5 contributed to function 1; one measurement dist 5-6 contributed to function 2; one character dist 3-4 contributed to function 3, and finally two characters dist 2-3 and dist 9-10 contributed to function 4 (Table 9).

Table 8: Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions (Variables ordered by absolute size of correlation within function). (*) denotes the largest absolute correlation between each variable and any discriminant function.

Character (cm)	Discriminant Function (DF)				
	1	2	3	4	5
SL	.725*	-.391	.094	.131	-.144
FL	.708*	-.398	.071	-.081	.555
TL	.485*	-.312	.008	.124	-.374
HBD	.150	.866*	.016	.130	.283
LBD	.084	.088	.870*	.209	.200
ED	.033	.073	.153	.576*	.496
PDL	.030	.021	.198	.523*	.523
MG	.010	.044	.361	-.099	.436*

Table 9: Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions (Variables ordered by absolute size of correlation within function). (*) denotes the largest absolute correlation between each variable and any discriminant function

Landmark Distance	Discriminant Function (DF)				
	1	2	3	4	5
4-5	.605*	-.082	-.410	.143	-.414
5-6	.169	.760*	.339	-.341	-.281
3-4	.471	-.314	.636*	.033	.269
2-3	.143	-.089	-.026	.877*	.316
9-10	-.001	-.001	.011	.025*	.006
1-2	.416	.012	-.388	-.411	.575*
3-8	.004	.029	.013	-.033	.246*
1-10	.005	.026	.008	-.037	.244*
8-9	.009	.026	.022	-.057	.234*

5-8	.006	.044	.019	-.128	.217*
4-8	.002	.027	.011	-.036	.211*
5-7	.008	.041	.002	-.116	.206*
4-9	.004	.021	.026	-.017	.197*
3-10	.003	.024	.036	-.042	.166*
4-7	.005	.026	.010	.017	.159*
2-10	.001	.031	.008	-.003	.148*
2-9	.000	.014	.004	.016	.121*
1-9	.003	.020	.008	-.024	.114*
3-9	.001	.025	.019	.020	.085*
7-8	-.008	.006	.017	.015	.081*
2-8	.007	.003	.009	.003	.017*

A dendrogram (Fig 5) constructed using morphometric and truss data was illustrated based on six lines. Line-2 and line 6 population formed one cluster. Then, line 1 and line 2 formed another cluster which was little deviated from line-2 and line 6 populations' clusters. Line 3 made a cluster combined with line 1 and line 2. Line 5 made a cluster with line 3. Line 4 formed a separated cluster with line 3 and combined line 1 and line 2. Line 4 population again formed a separate group based on distance of square Euclidean dissimilarity method which verified a close distance amid line 1, line 2, line 3, line 5 and line 6 and the distance for line 4 population was more deviated.

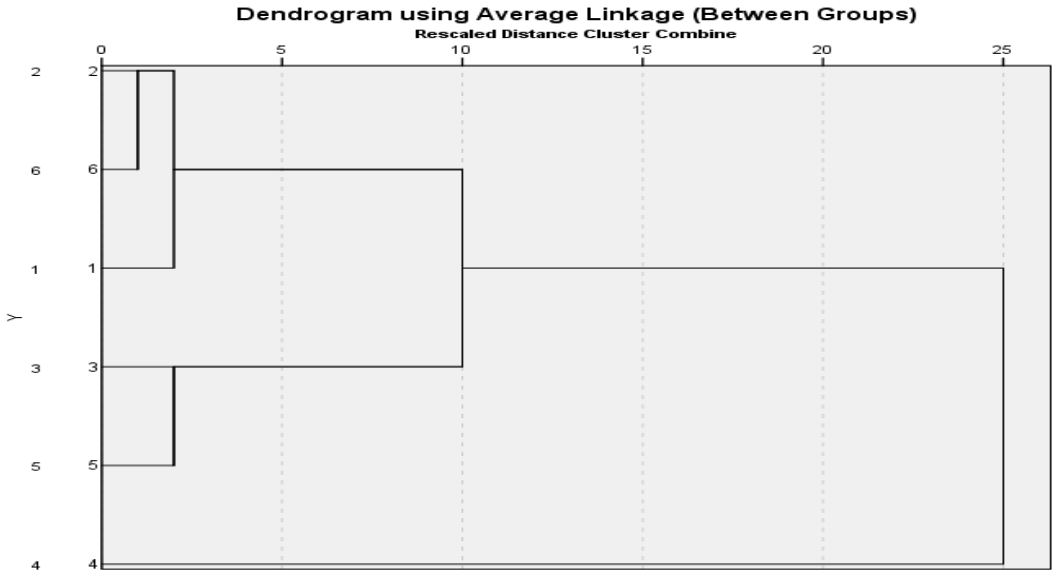

Fig 5. Dendrogram based on morphometric characters and landmark distances of six lines.

DISCUSSION

In the present study, line-4 is a heterospecific group (Atrai and Kangsha) which showed higher performance in growth. Among the eight morphometric measurements, line 4 was better than the other five lines. The growth (length) was superior in the heterospecific groups to the conspecific groups. This may be due to the heterozygosity present in the heterospecific groups for the crossing between two different populations or due to heterosis. According to **Ahammad *et al.* (2018),** phenotypic variations among population of three river (The Atrai, the Jamuna and the Kangsha) as well as genetic variation may be due to their distinct habitat and isolated location. Thus, the heterospecific groups attain a high level of heterozygous alleles compared to the conspecific groups. Again, line 4 (Atrai♂×Kangsha♀) showed highest growth (morphometric, meristic, landmark distances) among the six lines. According to **Wohlfarth (1993),** heterosis are seen usually in young carp, specially in their first summer. According to the findings of **Ali *et al*. (2017),** better growth can be obtained through selective breeding between different parent populations with genetic differences due to geographical differences as happened in the case of the Nile tilapia. The results of **Bentsen *et al.* (2017)** on the selection for improved body mass at the harvesting time in *Oreochromis niloticus* are also supportive with the present findings. Commonly, fish express more variation in morphological trait equally within and between populations than other vertebrates and are extra susceptible to environmentally induced morphological differences (**Wimberger, 1992**). The landmark distances were also not significantly different among the six lines, but line 4 showed the highest result.

Twenty two truss distances in the present experiment were dissimilar among the six lines ($p<0.001$). **Hossain *et al.* (2010)** detected significant variances ($p<0.001$) in 4 of 9 morphometric (HBD, PrOL, PL and MxBL) and 4 of 22 truss network measurements in *L. calbasu* from the Jamuna, the Halda and a hatchery in Bangladesh. Alternatively, **Rahman *et al.* (2014)** revealed that 16 morphometric traits and 23 truss measurement were differ significantly ($p<0.001$) in the Old Brahmaputra river, the Tanguar haor and a private fish hatchery in Mymensingh. This result coincides with the present findings of present research. In case of Anchovy (*Engraulis encrasicolus L.*), out of 25 truss measurements, 16 were different significantly ($p<0.05$) from Aegean and Northeastern Mediterranean Sea (**Turan *et al.*, 2004**). A significant variation ($p<0.001$) was reported by **Parvej *et al.* (2014)** in 4 of 17 morphometric characters and only 1 truss measure out of 22 in *Eutropiichthv vacha* from Meghna River, Kaptai Lake & Tanguar Haor of Bangladesh. In the present research, discriminant function (DF) disclosed well variation among 6 lines and significant variances were detected between size, and truss characteristics among the six lines of *G. ariza* which were parallel to the conclusions of **Turan *et al.*, (2004)**. The dendrogram formed in the present experiment gave rise to 6 different clusters. In addition, difference among the clusters might be due to the

difference in environment, geography, food and genetics. DFs reported a moderate segregation of the six lines in the case of morphometrics which is dissimilar to the outcome of **Hossain *et al.* (2010)**. **Hossain *et al.* (2010)** reported the Jamuna River and a hatchery stocks of *L. calbasu* in one cluster and *L. calbasu* from the HaldaRiver in another cluster. They concludedthat morphological variation between hatchery and wild stocks were perhaps a result of discrete environment, isolated habitat and genetic variations. In case of *Glossogobius guiris*, a dendrogram depending on the data of the meristic and morphometric traits revealed that, the fish stockfrom pond in Mymensingh region were detached from the fish stock of haor and estuary stocks possibly as a result of environmental influences or isolated habitat in addition to genetic variability amid the stocks described by **Mollah *et al.* (2012)**. In their study, the DFs exposed segregation in morphology among the stocks from various habitat of Bele, *G. guiris* which is similar to the finding of the present study.

CONCLUSION

Landmark-based morphometric and meristic dissimilarities of *G. ariza* collected from six lines displayed little variations in all morphometric and meristic traits. The current study determined the best line of minnow through landmark-based morphometric and meristic analysis. Line breeding of minnow through the establishment of a founder stock can help provide quality fish seed for aquaculture. As the quality of hatchery produced seeds are often questionable, this technique provides an alternative for restoring the genetic variance for different life history traits. The outcomes of this experiment would help producing quality seed of *G. ariza* in the hatchery condition and commercial seed production, and hence, culture of this fish in captive condition may prompt. The knowledge obtained through the present experiments on the GSI data, and the induced breeding and muscle fiber histology of *G. ariza* may be a good choice for further research to save this species from possible threat of extinction.

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