Phenotypic plasticity in *Barilius vagra* (Hamilton, 1822) (Teleostei: Danionidae) from two geographically distinct river basins of Indian Himalaya

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**Abstract:** Truss-based morphometric analysis was used to examine phenotypic plasticity of *Barilius vagra* (Hamilton, 1822) inhabiting the tributaries of the Alaknanda (Ganga River basin) and Chenab (Indus River basin), two geographically distinct river basins in the Indian Himalaya. Fourteen landmarks were connected to generate a truss network of 90 parameters on the body of fish. Eighty morphometric traits out of ninety morphometric measurements explained statistically significant difference among six sampling locations of *Barilius vagra* from streams in the Alaknanda and Chenab basins. Discriminant function analysis revealed 82% of *Barilius vagra* specimens originally classified into their own groups. 95% of the variance was explained by 13 principal components. Morphometric characters (1–6, 1–13, 2–5, 2–6, 2–14, 3–6, 4–6, 4–14, 6–12, 7–8, 7–9, 10–11, and 13–14) contributed greatly in differentiation of *B. vagra* populations from different river basins. The Alaknanda basin reflected some mixing within populations, which may be due to common environmental conditions and fish migration in these streams. This study will be helpful in framing site-specific conservation and management strategies, such as net mesh size selection, avoiding overexploitation, stock augmentation and food availability for different fish populations.

**Keywords:** Danionidae, DFA, differentiation, morphometry, truss network.
INTRODUCTION

Phenotypic plasticity is the ability of an organism to change especially in response to varying environmental conditions (Sahoo et al. 2020). Long term geographic isolation and limited migration causes phenotypic plasticity among the population within a species (Cadrin 2005). The Alaknanda and Chenab rivers drained from the Indian Himalaya are geographically isolated and rich in fish fauna.

Fish show higher degree of variation within and between populations than other vertebrates, and they are more susceptible to environmentally induced morphological variation (Wimberger 1992). It has been suggested that the morphological characters of fish are determined by environment, genetic and interaction between them (Poulet et al. 2004). During the early development stages the individual’s phenotype is more amenable to environment influence (Pinheiro et al. 2005). The phenotypic variability may not necessarily reflect population differentiation at genetic level (Ihssen et al. 1981). A sufficient degree of isolation may result in notable phenotypic and genetic differentiation among fish populations within a species, as a basis for separation and management of distinct populations (Turan et al. 2004).

Among the various tools used for stock assessment and phenotypic plasticity, morphometry is one of the frequently used and cost-effective tools. Traditional multivariate morphometrics, accounting for variation in size and shape have successfully discriminated between many stocks (Turan 1999). As the traditional morphometric measurements have biased coverage and metric selection over the body structure of fishes under experimentation, this method might not be useful for discriminate species when there is morphological plasticity (Takács et al. 2016). However, with the time this traditional method has been enhanced by image processing technique which is more effective in description of shape and stock identification (Mir et al. 2013).

Advance tool kits such as truss network system and geometric morphometrics is the best alternative used to study phenotypic plasticity within and between species (Turan 1999). Truss morphometric approach is an effective method for capturing information about the shape of an organism (Cavalcanti et al. 1999). It has been used to identify stocks of many fish species from marine and fresh waters (Sajina et al. 2011; Garcia- Roudriguez et al. 2010; Sen et al. 2011; Khan et al. 2012; Miyan et al. 2015, Dwivedi et al. 2019). Different stocks identified on the basis of environmentally induced morphometric variations play a significant role in the fisheries management (Begg et al. 1999). Insufficient knowledge on the population structure hinders the rate of production and reduces yields (Cadrin 2005). Good knowledge and right information of fish stocks will help us in the proper management and conservation of endangered species and stock enhancement of cultivable species.

Bariline fishes belonging to family Danionidae are characterized by a compressed body, blue-black bars or spots on the body and dorsal fin inserted behind the middle of the body (Rahman, 1989). Thirty-two bariline species are reported globally out of which 23 species so far reported from India (Singh et al. 2016). The species of genus Barilius including Barilius vagra (Hamilton, 1822) are commonly called hill trouts. These minnows inhabit both shallow lentic and lotic waters of Himalayan region (Sahoo et al. 2009). The hill stream fishes are important part of food as well as source of income to the fishermen of the Himalayan region (Kumar & Singh 2019). There are a few studies available on the population structure of Barilius bendelisis (Mir et al. 2015; Saxsena et al. 2015; Kumar & Singh 2019). However, there is paucity of published information on the population structure of Barilius vagra from Indian waters. Therefore, the present study was carried out with the objective to examine the phenotypic plasticity among the different populations of B. vagra from two distinct river basins of Indian Himalaya.

MATERIALS AND METHODS

Sampling and Measurements

Total 257 Barilius vagra specimens were sampled from Alaknanda River basin (132 specimens) and Chenab River basin (125 specimens) of Indian Himalaya using different fishing gears (cast nets and gill nets) from March 2015 to April 2017. The GPS coordinates; altitude and number of samples from each site of two river basins are presented in Table 1. The specimens of Barilius vagra were collected before the breeding season and after the spawning period (April to June) to avoid a bias towards size difference. The fish specimens were identified by using identification keys of Mirza (1991), Talwar & Jhingran (1991), and Kullander et al. (1999). After image capture, each fish was dissected for sex determination by macroscopic examination of the gonads. The gender was used as the class variable in ANOVA to test for significance difference in morphometric characters, if any, between male and female of B. vagra.
The truss network system described by Strauss & Bookstein (1982) was used to extract the 90 morphometric measurements of fish. Fish specimens were placed on water resistant graph paper as background and a digital camera of (Nikon D3400) was used to take the photographs (Figure 1) from same height and angle. Some specimens were submitted to the animal museum of the Department of Zoology of H.N.B. Garhwal University, Uttarakhand and others were fixed in 10% formalin solution for preservation.

The truss protocol used for the hill trout in the present study was based on 14 landmarks and the truss network constructed by interconnecting them to form a total of 90 truss measurements (Figure 1). The extraction of truss distances from the digital images of specimens was conducted using linear combination of three softwares, tpsUtil, tpsDig2 v2.1 (Rohlf 2006) and Paleontological Statistics (PAST) (Hammer et al. 2001).

Data analysis

Size dependent variations in truss measurements were removed, using the equation given by Elliott et al. (1995) as “M_adj = M (L/L_0)^b” Here M_adj is size adjusted measurement, M is original measurement of length, L_0 is standard length of fish, L, the overall mean standard length, and b slope of the regression of log M on log L_0 which is estimated for each character from the observed.

Univariate analysis of variance (ANOVA) was applied to 90 morphometric characters to evaluate the significance of difference among the mean values of the individual morphological character among different six populations of B. vagra. The characters expressing significant differences were subjected to the discriminant function analysis (DFA) and principal component analysis (PCA). The principal component analysis helps in morphometric data reduction (Veasey et al. 2001), in decreasing redundancy among the variables (Samaee et al. 2006) and in extracting a number of independent variables for population differentiation (Samaee et al. 2009). The standardized coefficients are used to compare variables measured on different scales. Coefficients with large absolute values correspond to variables with greater discriminating ability.

The DFA was used to calculate the percentage of correctly classified (PCC) fish. The Wilks’ lambda test of DFA was used to compare the differences between six populations, each three of which were collected from two geographically distinct river basins of Indian Himalaya. Statistical analysis for morphometric data were performed using the SPSS (ver. 16.1) and Microsoft Excel 2007.
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RESULTS

The morphometric characters between two sexes of B. vagra did not differ significantly (p >0.05), hence the data for both sexes were pooled for all subsequent analysis. Univariate analysis of variance (ANOVA) extracted eighty morphometric measurements having significant differences (p <0.05) and 10 measurements (1–7, 2–4, 3–4, 3–7, 4–5, 5–7, 7–12, 7–13, 8–9, and 9–11) did not show significant differences among six populations of B. vagra. Principal component analysis (PCA) of these significant measurements extracted 13 principal components having eigenvalues greater than one (Figure 2) explaining cumulative variance of 94.79%. The first principal component (PC1) accounted for 21.55% of the variation followed by 18.62%, 13.86%, 8.01%, and 6.52% variance, respectively by second, third, fourth, and fifth principal component (Table 2). Forward stepwise discriminant analysis of the significant variables produced five discriminant functions (DFs). The first, second, third, fourth and fifth discriminant functions explained 68.4%, 18.4%, 6.8%, 5.1%, and 1.3% of variance, respectively (Table 3). Plotting DF1 and DF2 showed clear specimen differentiation of stocks from different tributaries, Dudhar, Jhajjar, and Jhuni streams of Chenab River basin. However; slight intermingling in the population of Barilius vagra from three different tributaries, Dugadda, Khandah, and Khankhra of Alaknanda river basin was also noticed (Figure 3).

Table 1. GPS coordinates of sites from Alaknanda and Chenab River basins.

| Sampling site | Sample size | Latitude (°N) | Longitude (°E) | Altitude (m) |
|---------------|-------------|---------------|----------------|--------------|
| Dugadda       | 42          | 30.26         | 78.72          | 740          |
| Khankhara     | 46          | 30.23         | 78.93          | 668          |
| Khandah       | 44          | 30.19         | 78.78          | 718          |
| Dudhar        | 40          | 32.92         | 75.03          | 486          |
| Jhajjar       | 46          | 32.87         | 74.99          | 555          |
| Jhuni         | 39          | 32.89         | 75.95          | 754          |

The morphometric measurements 1–6, 1–13, 2–5, 2–6, 2–14, 3–6, 4–6, 4–14, 6–12, 7–8, 7–9, 10–11, and 10–14 contributed largely in the discriminant function analysis of B. vagra (Table 4). A total of 81.7% of specimens of Barilius vagra were classified into their original groups. Maximum 87.0% and minimum 76.2% of the specimens were found in their own groups of Khankhra and Dugadda streams, respectively from the Alaknanda river basin (Table 5). Some mixing in the
populations of Alaknanda river basin was also found. Wilks’ Lambda test reflected highly significant variations among the six populations of *B. vagra* from different tributaries of Alaknanda and Chenab River basins (Table 6).

**DISCUSSION**

Morphological differentiation can enable individuals to survive with existing environmental variability (Senay et al. 2015). Hossain et al. (2010) reported that...
Phenotypic plasticity is very high in fishes. A sufficient degree of isolation may result in phenotypic and genetic differentiation among fish populations within a species (Turan et al. 2004). Franssen et al. (2013) also suggested that the selective pressure of the environmental conditions leading to genetic-environmental interactions influence the pattern of phenotypic variation at intraspecific level. The results of the present study showed significant phenotypic heterogeneity among the populations of Barilius vagra from two geographically distinct river basins. High level of morphometric differentiation was reported within the Chenab River basin as compared to the Alaknanda river basin as shown by the DFA plot. Chenab River is largely fragmented as compared to the Alaknanda river basin, might be one of the reasons for the cause.

Discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species (Karakousis et al. 1991). In the present study, 81.7% of specimens were classified into their original groups by DFA, showing high variation in the stocks of Alaknanda and Chenab River basins. Eighty truss measurements in the whole body from head to tail were found to have significant differences (p < 0.05) among the six populations of both the river basins. 13 morphometric measurements (1–6, 1–13, 2–5, 2–6, 2–14, 3–6, 4–6, 4–14, 6–12, 7–8, 7–9, 10–11, and 13–14) extracted from DFA largely contributed in the discrimination of six populations. These all variations in the morphometric measurements of fishes were attributed to the environmental conditions of those particular streams and the fishes adapted to the existing environmental conditions by altering their morphology. It was interesting to note that most of these parameters were linked to the head, eye diameter and fin (Dorsal and anal) of the fish body. Rajput et al. (2013) while studying the eco-morphology of Schizothorax richardsonii reported strong correlation between the environmental variables and morphometric parameters like the fin morphology and body shape. Sajina et al. (2011) studied the stock structure of Megalepis cordyla from the east (Bay of Bengal) and west coast (Arabian Sea) of the Indian

| Component | Eigenvalues | % of Variance | Cumulative % |
|-----------|-------------|---------------|--------------|
| PC 1      | 17.244      | 21.555        | 21.555       |
| PC 2      | 14.895      | 18.618        | 40.173       |
| PC 3      | 11.090      | 13.862        | 54.035       |
| PC 4      | 6.407       | 8.009         | 62.045       |
| PC 5      | 5.213       | 6.516         | 68.516       |
| PC 6      | 5.106       | 6.383         | 74.944       |
| PC 7      | 4.011       | 5.014         | 79.958       |
| PC 8      | 3.127       | 3.909         | 83.867       |
| PC 9      | 2.523       | 3.154         | 87.021       |
| PC 10     | 2.125       | 2.656         | 89.677       |
| PC 11     | 1.765       | 2.206         | 91.844       |
| PC 12     | 1.268       | 1.585         | 93.469       |
| PC 13     | 1.056       | 1.320         | 94.789       |

Table 2. Eigenvalues, percentage of variance and percentage of cumulative variance for the 13 PCs in case of morphometric measurements for Barilius vagra.

| Component | Eigenvalues | % of Variance | Cumulative % |
|-----------|-------------|---------------|--------------|
| PC 1      | 15.244      | 19.055        | 19.055       |
| PC 2      | 13.895      | 16.818        | 35.873       |
| PC 3      | 11.090      | 13.862        | 49.735       |
| PC 4      | 6.407       | 8.009         | 57.744       |
| PC 5      | 5.213       | 6.516         | 64.260       |
| PC 6      | 5.106       | 6.383         | 70.643       |
| PC 7      | 4.011       | 5.014         | 75.657       |
| PC 8      | 3.127       | 3.909         | 80.566       |
| PC 9      | 2.523       | 3.154         | 83.720       |
| PC 10     | 2.125       | 2.656         | 86.376       |
| PC 11     | 1.765       | 2.206         | 88.582       |
| PC 12     | 1.268       | 1.585         | 90.167       |
| PC 13     | 1.056       | 1.320         | 91.489       |

Table 3. Eigenvalues and total variance explained by five discriminant functions.

| Component | Eigenvalues | % of Variance | Cumulative % |
|-----------|-------------|---------------|--------------|
| PC 1      | 13.244      | 16.555        | 16.555       |
| PC 2      | 11.895      | 14.618        | 31.173       |
| PC 3      | 9.090       | 11.862        | 43.035       |
| PC 4      | 4.407       | 5.009         | 38.045       |
| PC 5      | 3.213       | 3.916         | 41.961       |
| PC 6      | 2.106       | 2.683         | 44.644       |
| PC 7      | 1.011       | 1.214         | 45.858       |
| PC 8      | 0.127       | 0.190         | 46.047       |
| PC 9      | 0.523       | 0.654         | 46.698       |
| PC 10     | 0.125       | 0.156         | 46.854       |
| PC 11     | 0.765       | 0.914         | 47.768       |
| PC 12     | 0.268       | 0.315         | 47.983       |
| PC 13     | 0.056       | 0.096         | 48.089       |

Table 4. Discriminant function coefficients expressed by different morphometric measurements of Barilius vagra collected from tributaries of Alaknanda and Chenab rivers. (Bold digits indicates largest absolute correlation between each variable and any discriminant function)
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### Table 5. Number and percentage of correctly classified specimens of *Barilius vagra* into their original populations from Alaknanda (1, 2, 3) and Chenab (4, 5, 6) river basins.

| Predicted Group Membership | Alaknanda River | Chenab River | Total |
|----------------------------|-----------------|--------------|-------|
|                            | Dugadda | Khankhra | Khandah | Dudhar | Jhajjar | Jhuni |        |
| 1.Dugadda                  | 32      | 5       | 5       | 0      | 0       | 0     | 42    |
| 2.Khankhra                 | 2       | 40      | 4       | 0      | 0       | 0     | 46    |
| 3.Khandah                  | 8       | 1       | 34      | 0      | 1       | 0     | 44    |
| 4.Dudhar                   | 0       | 0       | 0       | 32     | 8       | 0     | 40    |
| 5.Jhajjar                  | 0       | 0       | 1       | 2      | 39      | 4     | 46    |
| 6.Jhuni                    | 0       | 0       | 0       | 1      | 5       | 33    | 39    |

**Original Count/Percentage**

|                            | Alaknanda River | Chenab River | Total |
|----------------------------|-----------------|--------------|-------|
|                            | Dugadda | Khankhra | Khandah | Dudhar | Jhajjar | Jhuni |        |
| 1.Dugadda                  | 76.2      | 11.9     | 11.9    | 0.0    | 0.0     | 0.0   | 100.0 |
| 2.Khankhra                 | 4.3       | 87.0     | 8.7     | 0.0    | 0.0     | 0.0   | 100.0 |
| 3.Khandah                  | 18.2      | 2.3      | 77.3    | 0.0    | 2.3     | 0.0   | 100.0 |
| 4.Dudhar                   | 0.0       | 0.0      | 0.0     | 80.0   | 20.0    | 0.0   | 100.0 |
| 5.Jhajjar                  | 0.0       | 0.0      | 2.2     | 4.3    | 84.8    | 8.7   | 100.0 |
| 6.Jhuni                    | 0.0       | 0.0      | 0.0     | 2.6    | 12.8    | 84.6  | 100.0 |

81.7% of original grouped cases correctly classified.

### Table 6. Results of Wilks’ lambda (function 1 through 5) for verifying differences among the stocks of *Barilius vagra*.

| Test of Function(s) | Wilks’ Lambda | Wilks’ Lambda Chi-square | df | Significance |
|---------------------|---------------|--------------------------|----|--------------|
| 1 through 5         | 0.022         | 937.579                  | 65 | 0.000        |
| 2 through 5         | 0.153         | 462.231                  | 48 | 0.000        |
| 3 through 5         | 0.396         | 228.375                  | 33 | 0.000        |
| 4 through 5         | 0.627         | 114.932                  | 20 | 0.000        |
| 5                   | 0.902         | 25.411                   | 9  | 0.003        |

significant differences in morphometric characters of six populations of *B. vagra* from two river basins, similar findings were reported by (Mir et al. 2013) in case of *Schizothorax richardsonii*.

Truss system can be successfully used to investigate stock separation within a species, as reported for other species in freshwater and marine environments. Among the 13 measurements which contributed to the five discriminant functions, four measurements (2–6, 3–6, 4–6, and 7–8) dominantly contributed to fifth discriminant function explaining variance in six populations of *B. vagra*. Mahfuj et al. (2019) while studying the meristic and morphometrics variations of *Macrognathus pancalus* using truss network system from the freshwaters of Bangladesh explained that out of fifteen truss measurements, five measurements contributed to the 1st DF, six measurements contributed to the 2nd DF and remaining four measurements to the 3rd DF. Kenthao and Jearranapiyaprempree (2018) also conducted similar kind of study in *Yclocheilichthys apogon* from three different rivers Pong, Chi, and Mun of northeastern Thailand. The first three principal components explained 49.29% of variance and first three discriminant functions explained 72% of variation among the samples. However, in the present study, PCA explained 94.79% of variance by using 13 principal components.

In this study, truss system revealed clear separation of *B. vagra* populations from two distinct river basins which will help in site-specific conservation and management strategies such as implementation of appropriate mesh.
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Figure 2. Discriminant analysis plot of *Barilius vagra* showing isolation of populations of Alaknanda and Chenab river basins.

sizes for fish harvesting, avoiding over-exploitation, augmentation of fish stock by culture, and making available sufficient food to fishes for their proper growth in different drainages of the Alaknanda and Chenab rivers. This will be instrumental in sustaining this resource for future use.

CONCLUSION

Truss protocol revealed phenotypic plasticity among six different populations of Alakanda and Chenab River drainages of Indian Himalaya. A clear separation of *B. vagra* populations between two geographically distinct river basins of Indian Himalaya was also found suggesting a need for separate conservation and management strategies to sustain the stock for future use.

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