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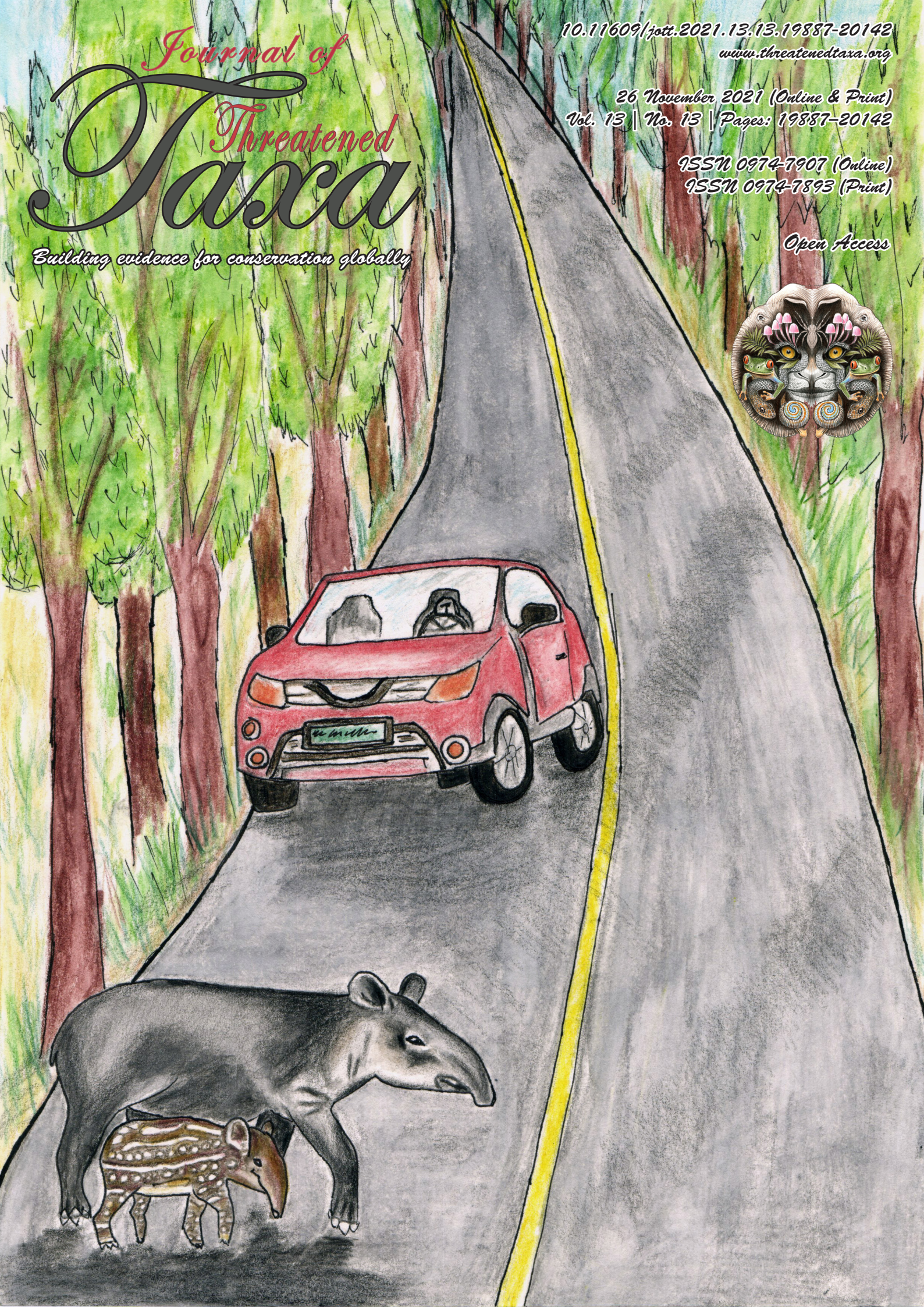
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Caption: Lowland Tapir *Tapirus terrestris* (Medium—watercolours on watercolour paper) © Aakanksha Komanduri.



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.

Fishes 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-Rodriguez 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; Saxena 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 " $M_{adj} = M (L_s/L_0)^b$ " Here M_{adj} is size adjusted measurement, M is original measurement of length, L_0 is standard length of fish, L_s 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.

List of extracted 90 truss generated morphometric measurements of *Barilius vagra*.

	Landmark No.	Particulars of Truss distance
1	1–2	Tip of snout to the anterior border of eye
2	1–3	Tip of the snout to the posterior border of eye
3	1–4	Tip of snout to the posterior border of operculum
4	1–5	Tip of snout to end of frontal bone
5	1–6	Tip of snout to pectoral fin origin
6	1–7	Tip of snout to dorsal fin origin
7	1–8	Tip of snout to pelvic fin origin
8	1–9	Tip of snout to dorsal fin termination
9	1–10	Tip of snout to origin of anal fin
10	1–11	Tip of snout to termination of anal fin
11	1–12	Tip of snout to dorsal side of caudal peduncle
12	1–13	Tip of snout to ventral side of caudal peduncle
13	1–14	Tip of snout to termination of lateral line
14	2–3	Anterior border of eye to posterior border of eye
15	2–4	Anterior border of eye to posterior border of operculum
16	2–5	Anterior border of eye to end of frontal bone
17	2–6	Anterior border of eye to pectoral fin origin
18	2–7	Anterior border of eye to dorsal fin origin
19	2–8	Anterior border of eye to pelvic fin origin
20	2–9	Anterior border of eye to dorsal fin termination.
21	2–10	Anterior border of eye to origin of anal fin
22	2–11	Anterior border of eye to termination of anal fin
23	2–12	Anterior border of eye to dorsal side of caudal peduncle
24	2–13	Anterior border of eye to ventral side of caudal peduncle
25	2–14	Anterior border of eye to termination of lateral line
26	3–4	Posterior border of eye to posterior border of operculum
27	3–5	Posterior border of eye to end of frontal bone
28	3–6	Posterior border of eye to pectoral fin origin
29	3–7	Posterior border of eye to dorsal fin origin
30	3–8	Posterior border of eye to pelvic fin origin
31	3–9	Posterior border of eye to dorsal fin termination
32	3–10	Posterior border of eye to origin of anal fin
33	3–11	Posterior border of eye to termination of anal fin
34	3–12	Posterior border of eye to dorsal side of caudal peduncle
35	3–13	Posterior border of eye to ventral side of caudal peduncle
36	3–14	Posterior border of eye to termination of lateral line
37	4–5	Posterior border of operculum to end of frontal bone
38	4–6	Posterior border of operculum to pectoral fin origin
39	4–7	Posterior border of operculum to dorsal fin origin
40	4–8	Posterior border of operculum to pelvic fin origin
41	4–9	Posterior border of operculum to dorsal fin termination
42	4–10	Posterior border of operculum to origin of anal fin
43	4–11	Posterior border of operculum to termination of anal fin
44	4–12	Posterior border of operculum to dorsal side of caudal peduncle.

	Landmark No.	Particulars of Truss distance
45	4–13	Posterior border of operculum to ventral side of caudal peduncle
46	4–14	Posterior border of operculum to termination of lateral line
47	5–6	End of frontal bone to pectoral fin origin
48	5–7	End of frontal bone to dorsal fin origin
49	5–8	End of frontal bone to pelvic fin origin
50	5–9	End of frontal bone to dorsal fin termination
51	5–10	End of frontal bone to origin of anal fin
52	5–11	End of frontal bone to termination of anal fin
53	5–12	End of frontal bone to dorsal side of caudal peduncle
54	5–13	End of frontal bone to ventral side of caudal peduncle
55	5–14	End of frontal bone to termination of lateral line
56	6–7	Pectoral fin origin to dorsal fin origin
57	6–8	Pectoral fin origin to pelvic fin origin
58	6–9	Pectoral fin origin to dorsal fin termination
59	6–10	Pectoral fin origin to origin of anal fin
60	6–11	Pectoral fin origin to termination of anal fin
61	6–12	Pectoral fin origin to dorsal side of caudal peduncle
62	6–13	Pectoral fin origin to ventral side of caudal peduncle
63	6–14	Pectoral fin origin to termination of lateral line
64	7–8	Dorsal fin origin to pelvic fin origin
65	7–9	Dorsal fin origin to dorsal fin termination
66	7–10	Dorsal fin origin to origin of anal fin
67	7–11	Dorsal fin origin to termination of anal fin
68	7–12	Dorsal fin origin to dorsal side of caudal peduncle
69	7–13	Dorsal fin origin to ventral side of caudal peduncle
70	7–14	Dorsal fin origin to termination of lateral line
71	8–9	Pelvic fin origin to dorsal fin termination
72	8–10	Pelvic fin origin to origin of anal fin
73	8–11	Pelvic fin origin to termination of anal fin
74	8–12	Pelvic fin origin to dorsal side of caudal peduncle
75	8–13	Pelvic fin origin to ventral side of caudal peduncle
76	8–14	Pelvic fin origin to origin of anal fin
77	9–10	Dorsal fin termination to origin of anal fin
78	9–11	Dorsal fin termination to termination of anal fin
79	9–12	Dorsal fin termination to dorsal side of caudal peduncle
80	9–13	Dorsal fin termination to ventral side of caudal peduncle
81	9–14	Dorsal fin termination to termination of lateral line
82	10–11	Origin of anal fin to termination of anal fin
83	10–12	Origin of anal fin to dorsal side of caudal peduncle
84	10–13	Origin of anal fin to ventral side of caudal peduncle
85	10–14	Origin of anal fin to termination of lateral line
86	11–12	Termination of anal fin to dorsal side of caudal peduncle
87	11–13	Termination of anal fin to ventral side of caudal peduncle
88	11–14	Termination of anal fin to termination of lateral line
89	12–13	Dorsal side of caudal peduncle to ventral side of caudal peduncle
90	13–14	Ventral side of caudal peduncle to termination of lateral line

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
Jhajar	46	32.87	74.99	555
Jhuni	39	32.89	75.95	754

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, Jhajar, and Jhuni streams of Chenab River basin. However, slight intermingling in the population of *Barilius vagra* from three different tributaries, Dugadda, Khandah, and Khankhara of Alaknanda river basin was also noticed (Figure 3).

Thirteen truss 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 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 Khankhara and Dugadda streams, respectively from the Alaknanda river basin (Table 5). Some mixing in the

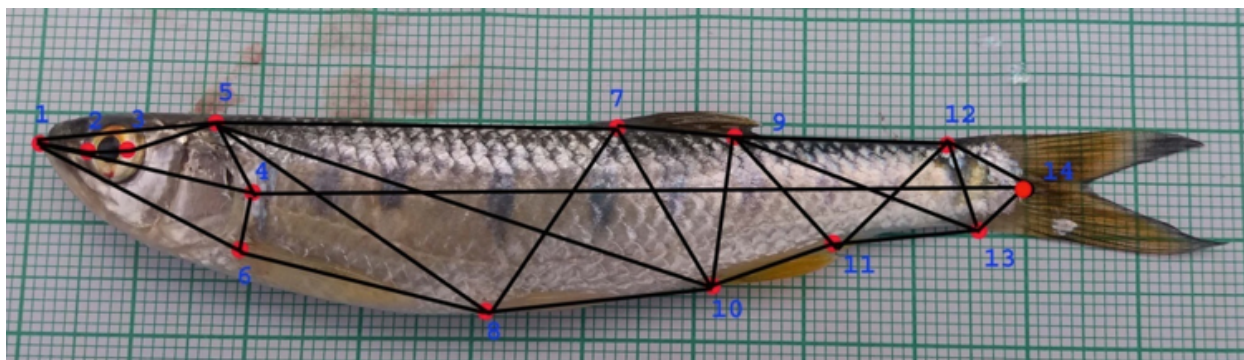


Image 1. *Barilius vagra* showing 14 morphometric landmarks and truss network: 1—Tip of snout | 2—end of eye towards mouth | 3—end of eye towards tail | 4—end of operculum | 5—forehead (end of frontal bone) | 6—dorsal origin of pectoral fin | 7—origin of dorsal fin | 8—origin of pelvic fin | 9—termination of dorsal fin | 10—origin of anal fin | 11—termination of anal fin | 12—dorsal side of caudal peduncle | 13—ventral side of caudal peduncle | 14—end of lateral line.

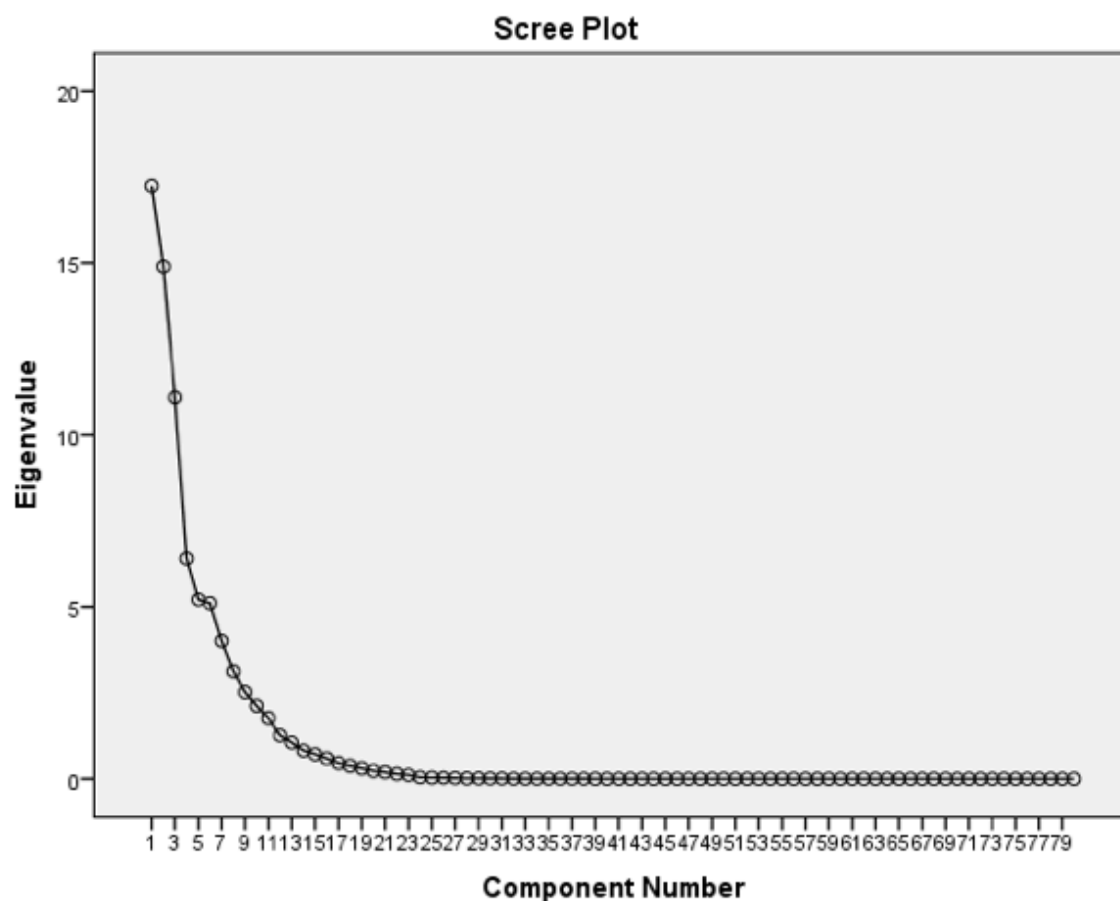


Figure 1. Principal component analysis plot showing maximum variance explained by 13 significant truss morphometric measurements of *Barilius vagra* collected from tributaries of Alaknanda and Chenab rivers.

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

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		
	Total	% 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	68.561
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
PC11	1.765	2.206	91.884
PC 12	1.268	1.585	93.469
PC 13	1.056	1.320	94.789

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

Function	Eigenvalues			Canonical Correlation
	Eigenvalue	% of Variance	Cumulative %	
DF 1	5.878 ^a	68.4	68.4	0.924
DF 2	1.582 ^a	18.4	86.8	0.783
DF 3	0.584 ^a	6.8	93.6	0.607
DF 4	0.438 ^a	5.1	98.7	0.552
DF 5	0.109 ^a	1.3	100.0	0.313

^a First 5 canonical discriminant functions were used in the analysis.

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 *B. 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.

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)

Standardized canonical discriminant function coefficients					
Variables	Function				
	DF 1	DF 2	DF 3	DF 4	DF 5
VAR 1-6	0.550	1.044	-0.003	0.556	-0.896
VAR 1-13	-0.310	-0.046	0.652	-0.447	0.112
VAR 2-5	0.033	-0.026	0.197	0.702	0.418
VAR 2-6	1.895	-0.705	0.779	-1.366	2.319
VAR 2-14	0.040	1.232	-0.664	-1.299	-0.139
VAR 3-6	-1.515	-0.409	-0.730	1.021	-1.842
VAR 4-6	0.183	0.594	-0.098	0.578	0.606
VAR 4-14	1.195	-0.482	1.176	1.388	0.386
VAR 6-12	-0.798	-0.342	-0.640	-0.080	0.299
VAR 7-8	0.237	-0.063	-0.438	-0.151	-0.457
VAR 7-9	-0.201	0.453	0.316	0.177	-0.152
VAR 10-11	-0.148	0.035	0.374	-0.337	-0.084
VAR 13-14	-0.649	0.141	0.304	-0.526	0.048

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

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								
Variables		Alaknanda River			Chenab River			Total
		Dugadda	Khankhra	Khandah	Dudhar	Jhajjar	Jhuni	
Original Count/Percentage	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
	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*.

Wilks' Lambda				
Test of Function(s)	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

peninsula using truss morphometric analysis and found significant heterogeneity among the stocks, attributed it to the uncommon hydrological conditions of habitats. Mir et al. (2013) investigated phenotypic variation in *Schizothorax richardsonii* from four rivers Jhelum, Lidder, Alaknanda, and Mandakini by using DFA and PCA and reported morphological discrimination among the stocks due to environmental factors.

Intermingling was noticed in three populations of Ganga River basin, which may be due to some common environmental conditions, migration and similar genetic origin at earlier period. Dwivedi et al. (2019) observed low level of morphometric differentiation among wild populations of *Cirrhinus mrigala* from ten different tributaries of Ganges and attributed it to the migration of individuals within the basin and common ancestry in the prehistoric period. In the present investigation Wilks λ test of discriminant function analysis indicated

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 *Macroglyptus 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 Jearanaiprepame (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

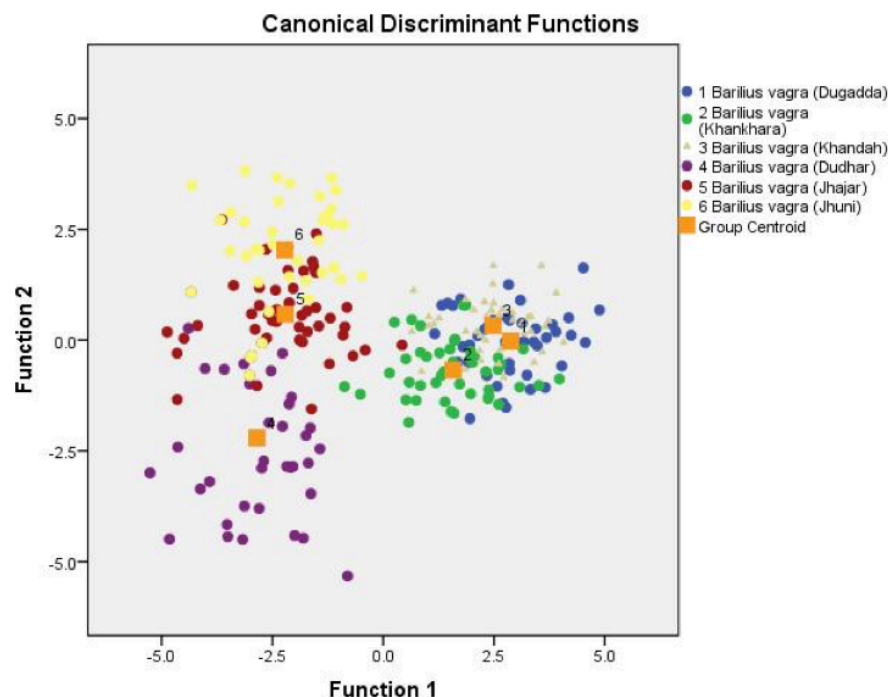


Figure 2. Discriminant analysis plot of *Barilius vagra* showing isolation of populations of Alaknanda and Chenab river basins.

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 Alaknanda 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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