Phenotypic differentiation of *Barilius bendelisis* (Cypriniformes: Cyprinidae) in four rivers from Central Indian Himalaya

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Abstract: *Barilius bendelisis*, commonly known as Indian Hill Trout is an upland water fish of South East Asia. It belongs to the family Cyprinidae and dwells in shallow, clear and cold water. In this study, the intraspecific variation of *Barilius bendelisis,* on the basis of morphometric characters, was investigated. Altogether, 402 specimens were collected from four rivers in the Central Indian Himalaya. A truss network was constructed by interconnecting 12 landmarks to yield 30 distance variables that were extracted from digital images of specimens using tpsDig2 and PAST software. Allometric transformed truss measurements were subjected to univariate analysis of variance, factor analysis and discriminant analysis. All variables exhibited significant differences between the populations. Altogether 88% of the specimens were classified into their original populations (81.98% under a 'leave-one-out' procedure). With factor analysis measurements of the head region, the middle portion and the caudal region had high loadings on the first and second axis. The results indicated that *B. bendelisis* has significant phenotypic heterogeneity between the geographically isolated regions of Central Indian Himalaya. We hypothesize that the marked interspecific variation in *B. bendelisis* is the result of local ecological conditions. Rev. Biol. Trop. 63 (1): 165-173. Epub 2015 March 01.

Key words: discriminant function analysis, shape, truss box, fishes, Cyprinidae.

Morphological characters are commonly used in fisheries and systematics to measure discreteness and relationships among various taxa and have long been used to delineate stocks of fish. Morphometry are continuous characters describing the body shape, which have provided evidence for stock discreteness and for species identification (Murta, 2000; Costa, Almeidam, & Costa, 2003). Meristic are the number of discrete serially countable structures, and often being used for species identification. Morphometric and meristic analyses can thus be a first step in investigating the stock structure of species with a large sized population (Daud, Mohammad, Siraj, & Zakaria, 2005).

However, there is a major limitation in using morphological characters at the intra specific level, in which phenotypic variation is not directly controlled by genetic factors but rather subject to environmental changes (Mir, Mir, & Chandra, 2013). To improve the use of morphometric analysis, truss morphometry has been developed especially for stock differentiation (Mir et al., 2013). Truss morphometry has proven to be more powerful in describing morphological variation between closely related fish taxa than conventional morphometry (Strauss, & Bookstein, 1982).

Barilius bendelisis (Hamilton), commonly known as Indian Hill Trout is an upland water fish of South East Asia. It belongs to the family Cyprinidae and dwells in shallow, clear and cold water. In Northeastern India, it is commonly distributed in hilly streams and rivers of Himalayan region. The fish plays significant role in the capture fishery in several parts of the Himalayan region of Uttarakhand, inhabiting shallow lotic and seasonal lentic water bodies where Indian major carps and exotic carps cannot be raised successfully (Sahoo, Saikia, & Das, 2009). This species is distributed in Brahmaputra and Ganga Basins along the Himalayan foot hills of India, Bangladesh and Nepal (Talwar, & Jhingran, 1991) and is also recorded as present in Myanmar, Pakistan, Thailand and Sri Lanka (Eschmeyer, & Fricke, 2011). The fish is characterized by its relatively elongated compressed body, blue black bars or spots on the body and dorsal fin inserted behind the middle of the body. The maximum length of fish is 22.7cm (Rahman, 1989). Adults of this fish occur in streams and rivers along the base of hills (Talwar, & Jhingran, 1991) with pebbly and rocky bottom (Menon, 1999). As per IUCN (2012) this fish has been categorized as of least concern but in the future the major threat to this species is over exploitation as well as habitat destruction due to human activities. *B. bendelisis* is a popular ornamental species among aquarists as well as a food fish for local population, but is poorly studied; as a result this species has suffered a drastic reduction in the recent past (Sah, Barat, Pande, Sati, & Goel, 2011). Therefore, the present study is a first step to determine the stock structure of this species based on morphometry, using truss network system with the goal of fomenting a management plan to guarantee a sustainable harvest of this species throughout the Indian Himalayas.

MATERIAL AND METHODS

Study area: The Indian Himalayas are drained by 19 main rivers, including three major river systems; the Indus, Ganga and Brahmaputra. The Indus Basin system is the longest river system which originates from Western Indian Himalayas (160 000km^2) and consists of five rivers. The Ganga basin system consists of nine rivers and originates from Central Indian Himalayas (150 000km^2) and the Brahmaputra Basin is the second longest river system which starts in Eastern Himalayas (150 000km^2) having five rivers (Hora, 1954). This study includes four major and lesser drainages of Ganga basin from Central Indian Himalayas (Gaula, Kosi, Alaknanda and Mandakini). River Gaula and Kosi are the main water sources of Kumaon region of Uttarakhand and River Alaknanda and Mandakini flow in Garhwal region of Uttarakhand, both regions are geographically isolated (Hora, 1954). The Gaula River is approximately 500km long. It originates in the Sattal lakes of Uttarakhand state and joins the Ramganga River (Major tributary of Ganga basin) about 15km. The river Kosi is one of the major tributaries of Ramganga River. It originates from village Budha Peenath of Kausani region of Uttarakhand (Kumar, & Bahadur, 2009). River Alaknanda is a major source of water in Garhwal region of Uttarakhand; it originates from Satopanth Glacier in Garhwal Himalaya (Menon, 1954). It is about 190km long. River Mandakini is a major tributary of river Alaknanda and originates from the Chorabari Glacier near Kedarnath in Garhwal region of Uttarakhand. It joins river Alaknanda at Rudraprayag (Mir et al., 2013). These are the four least explored rivers of Central Indian Himalaya and are an important fishery resource for the daily living of local people (Mir et al., 2013).

Sampling: A total of 402 specimens of *Barilius bendelisis* were collected from four different rivers across Central Indian Himalaya viz. Gaula, Kosi, Alaknanda and Mandakini, by using different fishing gear (cast nets and gill nets) between November 2012 and February 2013. The specimens of *B. bendelisis* were obtained before the breeding season and after the spawning period to avoid a bias toward size difference; every sampling station was visited six times during this period. The brooders and other small sized specimens obtained from the natural habitat during fishing were kept for breeding purpose and voucher specimens were kept in the museum at the Directorate of Coldwater Fisheries Research, India for possible future comparison studies. The mesh size of the fishing gear (cast nets: 9m length, 9m breadth and 1/2cm mesh size and drag nets: 100m length, 20m breadth, 1⁄2cm mesh size) was designed for the large sized specimens to avoid any fingerling and fry capture. The specimens were measured by using digital caliper and the total length range of the fish was 7.8-18.6cm. GPS coordinates, altitude (m.a.s.l), number of samples, min-max length and weight of *B. bendelisis* across the Central Indian Himalaya are included in Table 1.

Specimen digitization and truss distance measurements: The sampled specimens were first cleaned in running water, drained and placed on a flat platform with graph paper as a background to equilibrate scaling information to the digital images. The fins were erected to make the origin and insertion points visible. Each individual was labeled with a specific code to identify it. A Sony Cybershot DSC-W300 digital camera was used for capturing the digital images. To avoid errors in image capture, all photos were taken by a single person from same angle and height at every shot. After image capture, some specimens were dissected to identify the sex by macroscopic examination of the gonads. Gender was used as the class variable in ANOVA to test for significant differences in the morphometric characters, if any, between males and females of *B. bendelisis*.

The extraction of truss distances from the digital images of specimens was conducted using a linear combination of three software platforms, tpsUtil, tpsDig2 v2.1 (Rohlf, 2006) and Paleontological Statistics (Hammer, Harper, & Ryan, 2001). The truss protocol used for the *B. bendelisis* was based on 12 landmarks (Fig. 1A, Fig. 1B). A box truss of 30 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Strauss & Bookstein, 1982; Mir et al., 2013). All the measurements were transferred to a spreadsheet file (Excel 2007), and the X-Y coordinate data transformed into linear distances by computer (using the Pythagorean Theorem) for subsequent analysis.

Multivariate data analysis: Size dependent variation was corrected by adopting an allometric method as suggested by Elliott, Haskard, & Koslow (1995):

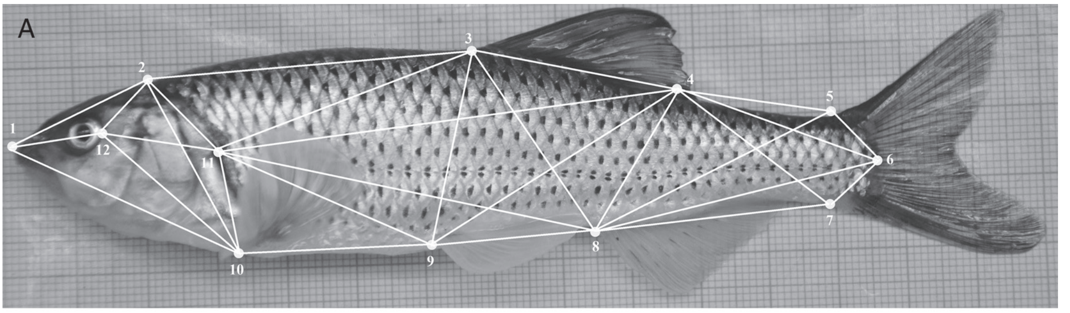
$$M_{adj} = M\,(L_s/L_0)^b$$

where M is the original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, L_s the overall mean standard length, and *b* was estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish from every group. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Turan, 1999). Univariate analysis of variance (ANOVA) was performed for the 30 morphometric characters to evaluate the significant difference between the four locations. These 30 transformed truss measurements were subjected to FACTOR analysis, to explain these variables in terms of their common

TABLE 1

Variable	Rivers			
	Gaula	Kosi	Alaknanda	Mandakini
Latitude °N	29°17’25”	29°27’48”	30°13’16”	30°44’02”
Longitude °E	79°32’43”	79°28’81”	78°48’34”	79°03’56”
Altitude (m.a.s.l.)	595	1 033	536	3 533
Number of samples	120	115	85	82
Min-Max TL (cm)	8.1-17.6	8.5-15.7	7.8-19.9	8.0-18.5
Min-Max BW (g)	5.04-39.7	4.21-37.8	5.2-48.7	5.0-44.6

Some data of sites: altitude (m.a.s.l; meters above sea level), number of samples, min-max length (cm) and weight (g) of *Barilius bendelisis* across Central Indian Himalaya.



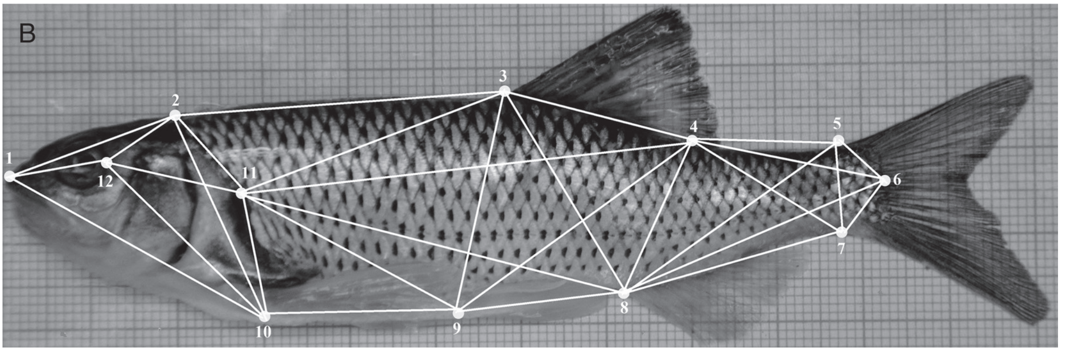


Fig. 1 A.B. Locations of 12 landmarks used for shape analysis. Land marks refer to 1. Anterior tip of snout at upper jaw; 2. Most posterior aspect of neurocranium (beginning of scaled nape); 3. Origin of dorsal fin; 4. Posterior end of dorsal fin; 5. Anterior attachment of dorsal membrane from caudal fin; 6. Posterior end of vertebrae column; 7. Anterior attachment of ventral membrane from caudal fin; 8. Origin of anal fin; 9. Origin of pelvic fin; 10. Insertion of pectoral fin; 11. End of operculum; 12. Posterior end of eye (Adapted from truss box, after Strauss and Bookstein (1982) and Bookstein (1991). Fig. 1A (Kumaon region), Fig. 1B (Garhwal region).

underlying dimensions. A maximum likelihood method was used to extract the factors. With the assistance of Scree plot, the cumulative variance explained by the factors and the meaningful biological groupings of the traits loading on each factor were taken into consideration to retain the number of factors for a rotation procedure. The retained factors were subjected to a Varimax rotation procedure and to identify the variables demonstrating high loadings for a given factor, the rotated factors were subjected to a scratching procedure, as described by Hatcher (2003). The Wilks' λ was used to compare the difference between all groups. The discriminant function analysis (DFA) was used to calculate the percentage of correctly classified (PCC) fish and a cross-validation using PCC was done to estimate the expected actual error rates of the classification functions. Statistical analyses for morphometric data were performed using the SPSS vers. 16.1.0 and Microsoft Excel 2007.

RESULTS

None of the size-adjusted truss measurements showed a significant correlation with the standard length of the fish, indicating that the variation in body length had been successfully removed by the allometric transformation. Among four selected rivers, means of all the truss measurements of *B. bendelisis* were found to be significantly ($p<0.001$) different in univariate analysis of variance. The morphometric characters between two sexes did not differ significantly ($p>0.05$) hence; the data for both sexes were pooled for all subsequent analysis.

Wilks' lambda tests of DFA indicated significant differences in morphometric characters of all populations ($p<0.001$). In discriminant function analyses, three discriminant functions (DFs) were formed. The first discriminant function (DF I) accounted for 98.9% of the total variation. The second discriminant function (DF II) and third discriminant function (DF III) accounted for 0.6 and 0.5%, respectively, between group variability among the populations. The morphometric measurements, 1-2, 1-12, 1-10, 4-11, 3-11, 6-8, 4-6, 4-7, 3-8, 5-8, 4-5 and 4-8 contributed to DF I, 5-6, 9-11, 2-3, 8-11 and 2-12 contributed to DF II and 6-7, 3-4, 10-12, 11-12, 5-7, 4-9, 2-11, 8-9, 10-11, 2-10, 7-8 and 3-9 contributed to DF III (Table 2), showing that these characters were the most important in distinguishing the population.

The DF I vs. DF II plot explained 99.5% of total variance among the samples and formed two separate groups. The first group formed from the samples of River Gaula and Kosi. The second group formed from the samples of the River Alaknanda and Mandakini (Fig. 2). All the samples from the rivers of Central Indian Himalayan regions were clearly separated from each other in discriminant space. However, Alaknanda River showed overlapping with Mandakini River and river Gaula showed intermingling with Kosi River.

The classification of individuals into their original population varied between 83.8% and 72.8% by discriminant analysis and 88.0% of individuals could be classified into their original a priori grouping (Table 3). The proportion of Gaula River samples into their original population was the highest (83.8%). A cross-validation test using leave one out procedure was also performed by which 81.98% of the samples were classified into their original populations. An overt intermingling was observed between rivers of Kumaon region (Gaula and Kosi; 16.2% misclassification) and between rivers of Garhwal region (Alaknanda and Mandakini; 27.2 and 28.8% misclassification; Table 3).

TABLE 2
Discriminant functions (DFs) of morphometric variables of *Barilius bendelisis* collected from four rivers across Central Indian Himalaya

Truss measurements	Function		
	DFI (98.9%)	DFII (0.6%)	DFIII (0.5%)
1-2	-.727(*)	.115	.078
1-12	-.617(*)	.113	.088
1-10	-.553(*)	.046	.067
4-11	-.065(*)	.010	.043
3-11	-.085(*)	.024	-.034
6-8	-.073(*)	.035	.043
4-6	-.075(*)	.030	.022
4-7	-.071(*)	.026	-.004
3-8	-.068(*)	.011	.053
5-8	-.058(*)	.015	.034
4-5	-.059(*)	.022	.011
4-8	-.047(*)	.010	.043
5-6	.016	.247(*)	-.060
9-11	-.087	.030(*)	.005
2-3	-.087	.084(*)	-.020
8-11	-.098	.046(*)	.025
2-12	.012	.030(*)	.007
9-10	-.088	.025	-.032
6-7	.045	.041	-.155(*)
3-4	-.048	.083	.161(*)
10-12	-.088	.045	.132(*)
11-12	-.032	.067	.182(*)
5-7	.022	-.033	-.110(*)
4-9	-.037	.018	.080(*)
2-11	-.010	.084	.099(*)
8-9	-.066	.067	.078(*)
10-11	.011	.050	.087(*)
2-10	-.053	.035	.088(*)
7-8	-.052	.012	.050(*)
3-9	-.038	.039	.067(*)

*Indicates largest absolute correlation between each variable and any discriminant function.

DISCUSSION

The results of present study indicated that the *B. bendelisis* showed significant phenotypic heterogeneity between the two geographically isolated Central Himalayan regions. Discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species (Karakousis, Triantaphyllidis, & Economidis, 1991). In the present paper, 78% of individuals were correctly classified into their respective groups by DFA, indicating high

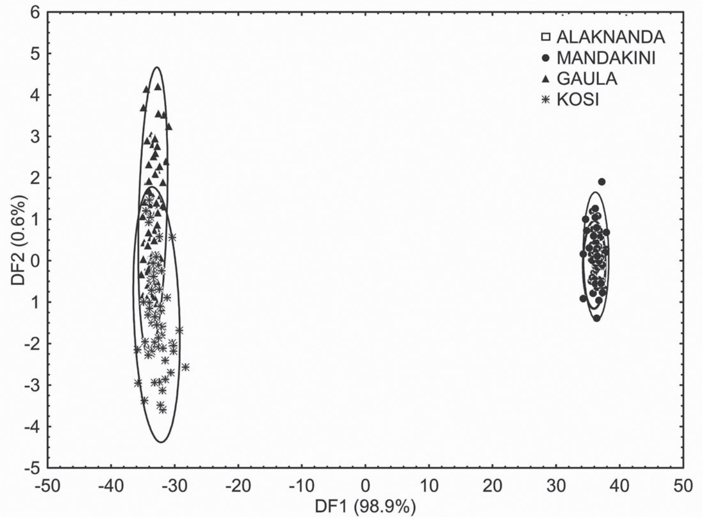


Fig. 2. Discriminant analysis plot for *B. bendelisis* collected from four rivers across Central Indian Himalaya.

TABLE 3
Percentage of specimens in each group and after cross validation for morphometric measurements of *Barilius bendelisis* from four rivers across Central Indian Himalaya (88.0% of original grouped cases correctly classified, 81.98% of cross-validated grouped cases correctly classified)

Rivers	Gaula	Original group (%)			Total
		Kosi	Alaknanda	Mandakini	
Gaula	83.8	16.2	.0	.0	100
Kosi	16.2	83.8	.0	.0	100
Alaknanda	.0	.0	71.2	28.8	100
Mandakini	.0	.0	27.2	72.8	100
	Cross-validated (%)				
Gaula	72.3	27.7	.0	.0	100
Kosi	21.1	78.9	.0	.0	100
Alaknanda	.0	.0	89.7	10.3	100
Mandakini	.0	.0	15.0	85.0	100

variation between two stocks. Mir et al. (2013) studied snowtrout, *Schizothorax richardsonii* from different regions of Indian Himalaya, finding significant morphometric heterogeneity among different populations by applying DFA and it to migration of fish and environmental factors. The DFA confirmed that the variation in morphological measurements was evident in the whole body, contributed mainly by middle portion, head region, body depth and caudal peduncle, between these morphologically distinct populations of *B. bendelisis*. Hossain, Nahiduzzaman, Habiba, Mst & Alam (2010) applied DFA and PCA on three populations of *Labeo calbasu* from river Jamuna, Halda and hatchery, and reported morphological discrimination among them due to the environmental factors and local migration of the fish. Similar observations were noticed by Khan, Miyan & Khan (2012) in case of *Channa punctatus* from three Indian rivers and lead the conclusion that environmental conditions play an important role in spatial distribution, movement and isolation of fish stocks. Mir, Mir & Chandra

(2014) observed similar inferences in *Schizothorax curvifrons* from Indus river basin, and attributed to changing physical and ecological conditions of water bodies.

These morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted by allometric transformation. On the other hand, size-related traits play a predominant role in morphometric analysis and the results may be erroneous if not adjusted for statistical analyses of data (Tzeng, 2004). In the present study, the size effect was removed successfully by allometric transformation, and the significant differences between the populations are due to the body shape variation when tested using ANOVA and multivariate analysis.

The causes of morphological differences among different populations are often quite difficult to explain (Poulet, Berrebi, Crivelli, Lek, & Argillier, 2004). It has been suggested that the morphometric differentiation of a fish can be influenced by genetic, environment and the interaction between them (Mir et al., 2013). Apparently, the fragmentation of river impoundments can lead to an enhancement of pre-existing genetic differences, providing a high interpopulational structuring (Esguicero, & Arcifa, 2010). The influences of environmental parameters on morphometric characters have been well discussed by several authors in the course of fish population segregation (Swain, & Foote, 1999). It is well known that morphological characteristics can show high plasticity in response to differences in environmental conditions (Wimberger, 1992). The separation of the stocks within the basin may be due to different biotic and abiotic factors such as food availability, salinity, temperature, which are affecting the morphometry of fish (Rohfritsch, & Borsa, 2005). Differentiation between samples from adjacent stations may be due to the geographic isolation of stations by artificial obstacles from each other allowing morphological differentiation to proceed independently at each station (Samaee, Mojazi-Amiri, & Hosseini-Mazinani, 2006).

The water fragmentation obstructs the migration of fish populations resulting in an ecological trap (Jager, Chandler, Lepla, & Winkle, 2001). A series of barrages have been commissioned in the upper segment of river Ganges from Rishikesh to Narora (Rao, 2001). The river Gaula is regulated by a large dam at Kathgodam and a century pulp and paper mill at Lalkuan area of Uttarakhand (Valdiya, 1991). The effluent after discharge from this industry falls to river Gaula which affects the aquatic biodiversity (Valdiya, 1991). The river Kosi has a masonry dam at Lalpur, and most of the canals, which irrigate the major portion of the district, are dependent on its water. During dry periods, no discharge is released downstream of Lalpur dam, and in stretches the river becomes nearly dry which in turn effects the local migration of the fishes (Kumar, & Bahadur, 2009). The water of river Alaknanda and Mandakini is fragmented due to construction of a number of dams and hydroelectric power projects (Mir et al., 2013). The blockage of fish movements can have a very significant impact on fish stocks by obstructing the genetic exchange (McAllister, Craig, Davidson, Delany, & Seddon, 2001). Also, the construction of a dam can lead to dramatic changes in the environment of a river and particularly affect fish communities. Dams can also alter the feeding habits of the species, availability of food items, growth pattern and reproductive strategy of fish species of a river (AnvariFar et al., 2011). The importance of such factors on producing morphological differentiation in fish species is well known (Akbarzadeh et al., 2009).

The inferences of this study leads to the conclusion that *B. bendelisis* has distinct stocks in the two geographically isolated regions of Central Indian Himalaya, while the samples of two rivers of Kumaon region did not exhibit discrimination in the fish stock and similar was the case within the rivers of Garhwal region. The misclassification rate within the rivers of two isolated regions was insignificant. This additional examination would provide further confirmation of the stock structure resolved in this study with the truss analysis. However, further management measures have to be taken by the enforcement of mesh size regulation and imposition of a closed season during the breeding of some commercially important fish species in Himalayan regions to sustain this resource for the future use.

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RESUMEN

Diferenciación fenotípica de *Barilius bendelisis* (Cypriniformes: Cyprinidae) en cuatro ríos del Himalaya central de la India. *Barilius bendelisis*, comúnmente conocido como trucha "Indian Hill", es un pez de tierras altas del sudeste de Asia. Pertenece a la familia de los ciprínidos y habita en aguas poco profundas, claras y frías. En este estudio se investigó la variación intraespecífica de *Barilius bendelisis* basado en caracteres morfométricos. En total se obtuvieron 402 muestras de cuatro ríos en el centro del Himalaya hindú. Se construyó una red "truss" mediante la interconexión de 12 puntos de referencia para producir 30 variables de distancia que fueron extraídas de las imágenes digitales de las muestras, utilizando el software tpsDig2 y PAST. Las mediciones alométricas de la red truss se transformaron mediante un análisis univariado de varianza, análisis factorial y discriminante. Todas las variables mostraron diferencias significativas entre las poblaciones. En total, el 88% de los especímenes se clasificaron en sus poblaciones originales (81.98% con el procedimiento "leave-one-out"). Las mediciones del análisis factorial de la región de la cabeza, la parte media y la región caudal mostraron altas concentraciones en el primer y segundo eje. Los resultados indicaron que *B. bendelisis* tiene heterogeneidad fenotípica significativa entre las regiones geográficamente aisladas del centro del Himalaya hindú. Nuestra hipótesis es que hay una marcada variación interespecífica de *B. bendelisis* como resultado de las condiciones ecológicas locales.

Palabras clave: función de análisis discriminante, forma, caja truss, peces, Cyprinidae.

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