

## Interrelationships of Five Species of the Genus *Labeo* by Morphometric Analysis

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**Abstract:** The genus *Labeo* under Cyprinidae family is of much importance as many species under this genus are ornamental species, some food species, some used for extracting oil, some considered to be of medicinal value etc. Morphometric studies were conducted using eleven quantitative body parameters of five species of *Labeo* genus – *Labeo bata*, *L. calbasu*, *L. rohita*, *L. pangusia* and *L. dyocheilus* occurring from Assam, India, in order to identify the morphometric variation and taxonomic relationship among these species. All measurements were taken on a continuous scale using digital vernier caliper parallel to the anterior-posterior body axis except for the body depth that was taken perpendicular to the body axis between dorsal and ventral margins. The means of all measurements were standardized and multivariate cluster and principal component analysis were conducted using *bdpro32* software. A dendrogram has been prepared showing the relatedness among the species. The results obtained on the basis of morphometric variation among the species using cluster analysis showed that *L. bata* is most distantly related whereas *L. rohita* and *L. calbasu* are most closely related with a similarity of 98.3965 (distance = 1.6035) followed by *L. pangusia* and *L. dyocheilus*. The scores of PC1, PC2 and PC3 are also most similar between *L. rohita* and *L. calbasu*. The findings of this study will help in developing new strategies for conservation and breeding programmes of these species.

**Keywords:** Cluster analysis, dendrogram, *Labeo* genus, morphometric variation, principal component analysis, taxonomic relationship.

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### I. Introduction:

The state of Assam, India, which lies in two biodiversity hotspot regions of the world (The Himalayas and the Indo-Burma), harbours a large variety of threatened and endemic flora and fauna including a large variety of fishes. Since fishes are the most ancient group of the vertebrates, their diversity and taxonomic studies is very necessary. Cyprinids are the major component of Indian freshwater fish fauna with respect to the number both of individuals and of species. The role of this family within freshwater ecosystem is therefore central. The genus *Labeo* under Cyprinidae family is of much importance as many species under this genus are ornamental species, some food species, some are used for extracting oil and some are considered to be of medicinal value etc. Morphometric characters have been commonly used in fisheries biology as powerful tools for measuring discreteness and relationships among various taxonomic categories [1].

Understanding the origins, maintenance and consequences of variation is a fundamental part of biological research and requires that variation be both precisely and accurately estimated. Complex variation associated with body form is one of the most difficult types of variation to quantify and the methods used to access it are collectively referred to as morphometrics [2]. These methods are concerned with quantifying shape variation within and among samples usually to address developmental and evolutionary questions relating to shape change during growth. Morphometrics is a field concerned with studying variation and change in the form (size and shape) of organisms or objects [3]. There are several methods for extracting data from shapes, each with their own benefits and weaknesses. These include measurement of lengths and angles, landmark analysis and outline analysis. Morphometrics adds a quantitative element to descriptions, allowing more rigorous comparisons. It enables one to describe complex shapes in a rigorous fashion, and permits numerical comparison between different forms [4] and when combined with multivariate statistical methods (e.g. Principal Component Analysis, Cluster Analysis etc) they offer powerful tool for testing and displaying differences in shape [5] [6]. All landmark based morphometric methods face the fundamental challenge of removing variation in size from variation in shape. Traditional morphometrics uses one of three general approaches to try to isolate shape from size variation: ratios, regression and multivariate factor or component analysis [7] [8].

Application of morphometrics in *Labeo* genus for study of taxonomic relationship is limited and is still an open issue in this region. Thus, the present study had been undertaken with the main objective to analyze possible morphometric variations using various measurements of the body parts and identify the interrelationship among the selected species.

**II. Materials & Methods:**

A total of fifty specimens, ten for each of the five species of fish of *Labeo* genus under Cyprinidae family: *Labeo bata*, *L. calbasu*, *L. rohita*, *L. pangusia* and *L. dyocheilus* were collected from the water bodies of Assam by random sampling. The species were identified by the characters described by [9] [10]. No significant sexual dimorphism with respect to the selected morphometrics was observed; therefore the data analyses were performed without taking the sex of the individual into consideration.

Eleven measurements were taken from the lateral side of the fish on a continuous scale using digital vernier caliper. All lengths were taken parallel to the anterior-posterior body axis except for the body depth that was taken perpendicular to the body axis between dorsal and ventral margins [11]. The mean of the data for each species were calculated and also the standard deviation. The mean values have been used for the analysis (Table 2). Besides effects from the environment and evolutionary history, morphometric characters may contain growth and/or allometric trends. To correct for (relative) differences in size all measurements have been standardized (expressed as proportions of total length Vs other measurements (Fig: 1, Table 2)). Moreover, selecting specimens from a specific size range may also contribute in the elimination of growth trends. Multivariate cluster analysis and principal component analysis were conducted using the standardized morphometric data with the help of bdpro32 software [12].

**Table 1: Data of the measurements of the mean body parameters.**

Mean body parameters (cm)	Species				
	<i>Labeo bata</i>	<i>Labeo calbasu</i>	<i>Labeo rohita</i>	<i>Labeo pangusia</i>	<i>Labeo dyocheilus</i>
<b>Total length</b>	32.003	28.883	32.000	31.261	25.355
<b>Standard length</b>	26.720	23.337	27.904	25.759	21.475
<b>Fork length</b>	28.992	25.272	29.696	28.041	23.123
<b>Pre-anal length</b>	19.968	17.763	22.304	18.850	17.520
<b>Pre-dorsal length</b>	11.168	10.744	12.736	11.097	10.420
<b>Pre-pelvic length</b>	13.184	12.968	14.336	12.754	12.119
<b>Pre-pectoral length</b>	5.536	5.689	5.920	5.752	4.817
<b>Body depth</b>	7.360	6.816	7.456	5.533	7.124
<b>Head length</b>	5.728	5.632	6.304	6.314	5.172
<b>Eye diameter</b>	1.747	1.188	1.280	1.161	0.848
<b>Pre-orbital length</b>	1.145	1.582	1.708	2.052	2.363

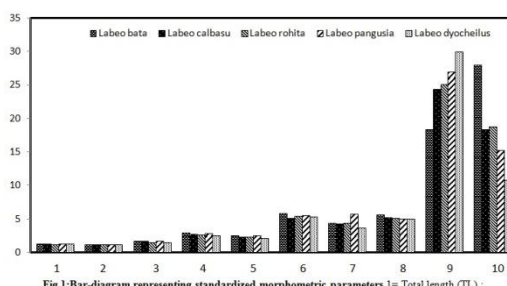


Fig 1: Bar-diagram representing standardized morphometric parameters. 1= Total length (TL) : Standard length (SL); 2 = TL : Fork Length; 3 = TL : Pre-anal Length; 4 = TL : Pre-dorsal Length; 5 = TL : Pre-pelvic Length; 6 = TL : Pre-pectoral Length; 7 = TL : Body Depth; 8 = TL : Head Length; 9 = TL : Eye diameter; 10 = TL : Pre-orbital Length.

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**Table 2: Data of the standardized body parameters.**

Standardized body parameters:	Species				
	<i>Labeo bata</i>	<i>Labeo calbasu</i>	<i>Labeo rohita</i>	<i>Labeo pangusia</i>	<i>Labeo dyocheilus</i>
<b>Total length (TL) : Standard length (SL)</b>	1.197	1.237	1.146	1.213	1.180
<b>TL : Fork Length</b>	1.103	1.142	1.077	1.114	1.096
<b>TL : Pre-anal Length</b>	1.602	1.626	1.434	1.658	1.447
<b>TL : Pre-dorsal Length</b>	2.865	2.688	2.512	2.817	2.433
<b>TL : Pre-pelvic Length</b>	2.427	2.227	2.232	2.451	2.092

<b>TL : Pre-pectoral Length</b>	5.780	5.076	5.405	5.434	5.263
<b>TL : Body Depth</b>	4.348	4.237	4.291	5.649	3.559
<b>TL : Head Length</b>	5.587	5.128	5.076	4.951	4.902
<b>TL : Eye diameter</b>	18.318	24.312	25.000	26.925	29.899
<b>TL : Pre-orbital Length</b>	27.950	18.257	18.735	15.234	10.730

**III. Results And Discussion:**

The results of the multivariate cluster analysis of the present study have been summarized in Table 3. Similarity matrix values were calculated for all standardized morphometric parameters. The similarity was found to be highest between *Labeo rohita* and *Labeo calbasu* (98.3965), between *L.rohita* and *L.pangusia* the similarity was 94.2019, between *L.calbasu* and *L.pangusia* (93.9869), between *L.pangusia* and *L.dyocheilus* (91.7007), between *L.rohita* and *L.dyocheilus* (89.0069), between *L.calbasu* and *L.dyocheilus* (88.4254), between *L.calbasu* and *L.bata* (87.2793), between *L.rohita* and *L.bata* (87.2303), between *L.bata* and *L.pangusia* (82.8593). The least similarity was found between *L.bata* and *L.dyocheilus* (76.2756).

**Table 3: Summary of the results of the multivariate cluster analysis based on the variation in the standardized morphometric parameters of the selected species.**

Step	Clusters	Distance	Similarity	Joined 1	Joined 2
1	4	1.603456616	98.39654338	2	3
2	3	5.798114777	94.20188522	2	4
3	2	8.299308777	91.70069122	2	5
4	1	12.7207222	87.2792778	1	2
<b>Similarity Matrix</b>					
	<i>Labeo bata</i>	<i>Labeo calbasu</i>	<i>Labeo rohita</i>	<i>Labeo pangusia</i>	<i>Labeo dyocheilus</i>
<i>Labeo bata</i>	*	87.2793	87.2303	82.8593	76.2756
<i>Labeo calbasu</i>	*	*	98.3965	93.9869	88.4254
<i>Labeo rohita</i>	*	*	*	94.2019	89.0069
<i>Labeo pangusia</i>	*	*	*	*	91.7007
<i>Labeo dyocheilus</i>	*	*	*	*	*

A single-link bray-curtis cluster analysis dendrogram was constructed from the combined data for all standardized morphometric parameters showing the relationship among the selected species of *Labeo* (Fig: 2). The most closely related species were found to be *Labeo rohita* and *L.calbasu* followed by *L.pangusia* and *L.dyocheilus* while *L.bata* was found to be most distantly related.

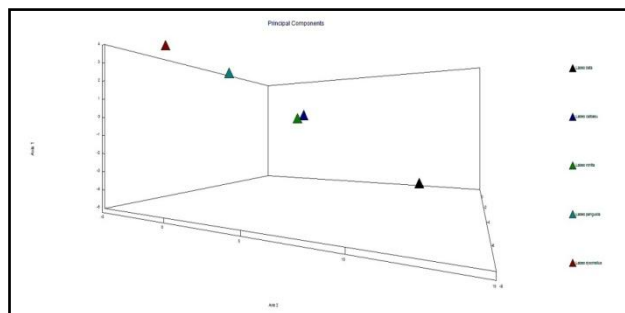


**Fig 2: Bray-Curtis Cluster Analysis (Single Link) dendrogram**

Details of the results of the principal component analysis have been listed in Table 4. Three principal components were extracted where the Eigen value of PC1 is 5.38779, PC2 = 2.98844 and PC3 = 1.42567. The scores of PC1, PC2 and PC3 of *L. calbasu* (PC1 = 4.74959, PC2 = 0.23272 and PC3 = -4.88328) and *L. rohita* ( PC1 = 4.58594, PC2 = 0.00993 and PC3 = -5.11625) are most similar and the relative positions of these two species in a projection of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> principal components in Fig:3 indicates that they tend to group together . The PC1, PC2 and PC3 scores of *L. bata* are the most different from the rest indicating this species to be most distantly related.

**Table 4 : Summary of the results of the principal component analysis**

<b>Proportions</b>			
<b>Eigenvalue</b>	5.38779	2.98844	1.42567
<b>Components</b>			
<b>Variable</b>	PC1	PC2	PC3
<b>Total length (TL) : Standard length (SL)</b>	0.21065	0.46436	0.26246
<b>TL : Fork Length</b>	0.16152	0.4778	0.35233
<b>TL : Pre-anal Length</b>	0.34581	0.34323	-0.0293
<b>TL : Pre-dorsal Length</b>	0.42084	0.09686	-0.09671
<b>TL : Pre-pelvic Length</b>	0.38682	0.00385	-0.36859
<b>TL : Pre-pectoral Length</b>	0.26421	0.39187	-0.23699
<b>TL : Body Depth</b>	0.24145	0.21411	-0.60102
<b>TL : Head Length</b>	0.32754	-0.2926	0.33964
<b>TL : Eye diameter</b>	-0.35582	0.24994	-0.27442
<b>TL : Pre-orbital Length</b>	0.34816	0.28529	0.22991
<b>Scores</b>			
<b>Variable</b>	PC1	PC2	PC3
<i>Labeo bata</i>	10.74909	4.44466	-1.20191
<i>Labeo calbasu</i>	4.74959	0.23272	-4.88328
<i>Labeo rohita</i>	4.58594	0.00993	-5.11625
<i>Labeo pangusia</i>	3.28729	1.96189	-7.4011
<i>Labeo dyocheilus</i>	-0.28819	3.48909	-7.81209



**Fig 3: Result of Principal Component Analysis.**

The species of *Labeo* genus are identified conventionally based on morphological and meristic characters, relying mainly on the meristic counts, pigmentation pattern and colouration of the skin. The morphological approach cannot be used to establish the similarity/dissimilarity among the species i.e. taxonomic relationship among the species. The morphological approach is beset with problems including wide variation in the colour pattern between mating and non mating seasons of the same individuals of the same species. Thus, supportive techniques like the one we have used in this study are needed to ratify the taxonomic status and relationship of these species which are very important from both fisheries and aquaculture points of views.

The results of the present investigation clearly showed the relationships among the species and have grouped them into clusters on the basis of their morphometric variations. *Labeo rohita* and *L. calbasu* which are morphologically different are grouped together in one cluster, inferring that these two species are most similar to each other and are the descendents of a very near common ancestor. The present study provides the pioneering report on the application of morphometric analysis of the selected species from this region. Morphometric studies have been widely used to discriminate the populations of various fish species [13] and have been able to identify differences between fish taxa [14]. [15] identified different variants in a fish species *Etroplus maculatus* by morphometric analysis. [16] identified the relationships among six species of *Puntius* on the basis of morphometric variation among them.

#### IV. Conclusion

The use of morphometry is an easy to implement method, relatively rapid and also inexpensive. Since the connectivity between species and their taxonomic relationship is a major point for conservation and management of species, the use of morphometry to this purpose appears to be very promising and the results of the present study may be a useful reference for further investigations and developing new strategies for conservation and breeding programmes of these species. A definite confirmation of the taxonomic relationships of these species has to wait till an extensive set of characters, such as DNA sequences, become available.

#### V. Acknowledgement:

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