



# Phylogenetic relations of the cyprinid fishes (Cyprinidae) in Bangladesh inferred from morphological traits and cytochrome b gene sequences

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**Abstract.** The evolutionary relationships of 28 cyprinid fishes were inferred using the morphological traits and nucleotide sequences of mitochondrial genes Cytochrome b (Cytb) for the first time in Bangladesh. The morphological traits were observed, recorded, and analyzed using Mesquite (v.2.6) software. The nucleotide sequences of the Cytb gene were retrieved from the NCBI genebank database and analyzed with the Molecular Evolutionary Genetic Analysis (MEGA ver. 6.01) software. The GC content (42.92%) was lower than the AT content (58.08%) in the nucleotide composition of Cytb genes. The transition/transversion rate was  $K_1 = 2.894$  for purines and  $K_2 = 4.126$  for pyrimidines, whereas the transition/transversion bias was  $R = 1.842$ . The transitional substitutions (64.24) were much higher than the transversional substitution (35.76). The highest evolutionary divergence was found between *Rasbora daniconius* and *Securicula gora* (0.359), but the lowest was found between *Catla catla* and *Labeo rohita* (0.065). In phylogenetic trees, Leuciscinae subfamily showed monophyletic lineage - where Garrinae, Schizothoracinae, Rasborinae, Cultrinae, Cyprininae subfamilies were polyphyletic. This study revealed a complex evolutionary relationship of cyprinid fishes in Bangladesh which would be useful in crossbreeding and hybridization programs. Future studies through sequencing of mitochondrial and nuclear genes of the cyprinids in Bangladesh will provide better insights on their phylogeny.

**Key Words:** Cypriniformes, cytochrome b, evolutionary divergence, phylogenetic relationship.

**Introduction.** Cyprinidae belongs to the order Cypriniformes, being the largest family of fishes with more than 2000 species (Bănărescu & Coad 1991). Species from the Cyprinidae family are distributed all over Asia, but the highest species richness is reported in Southeast Asia (Ismail 1990). In Bangladesh, the Cyprinidae family contains 67 species under 23 genera which is the highest number of species in a single family (Rahman 2005). Considerable morphological diversity exists among the species of the Cyprinidae family, and phylogenetic analysis is relatively challenging.

The molecular and morphological traits are used to infer the phylogenetic and evolutionary relationship among the taxa (Egge & Simons 2009). Morphological traits serve as the fundamental tools for systematic and phylogenetic studies of fishes (Wiens 2004; Krabbenhoft et al 2009). However, molecular techniques produce relatively accurate data to better understand the phylogenetic and evolutionary relationship among different taxa (Mayden & Chen 2010; Tang et al 2011). Single mitochondrial gene has widely been used to assess population or low-level phylogenetic relationships among animals (Rocha-Olivares et al 1999; Lovejoy & Araujo 2000). Among the mitochondrial genes, the structural and functional properties of the cytochrome b (Cytb) gene are well known (Esposti et al 1993), and considered one of the most useful genes for phylogenetic

reconstruction and evolutionary analyses. Published literature indicates that phylogenetic studies have been conducted on cyprinid fishes in Europe (Brito et al 1997; Zardoya & Doadrio 1999; Gilles et al 2001), and in Asian countries such as in China (He et al 2004) in Malaysia (Esa et al 2012), and in Middle east countries (Durand et al 2002). However, the phylogenetic relationship of the species belonging to the Cyprinidae family in Bangladesh remains unknown. This study aimed to fill the research gap by phylogenetic and evolutionary analyses of cyprinid fishes from Bangladesh based on the morphological traits and Cytb gene sequences.

## Material and Method

**Sample collection.** A total of 28 taxa of the Cyprinidae family were included in this study. All the taxa except *Diptychus maculatus* and *Lepidopygopsis typus* were collected from the Atrai River, located in Dinajpur districts of Bangladesh. *D. maculatus* is native to India, Pakistan, China, Nepal, and *L. typus* is native to India in the Periyar River and lake Kerala (Talwar & Jhingran 1991); these two species were included in the study to understand the Cyprinidae evolution. *Botia lohachata* belongs to the same order but to a different family (Botidae) and was included in the phylogenetic reconstruction as outgroup taxa. The scientific names and subfamilies of the studied taxa are shown in Table 1.

Table 1  
Selected taxa of Cyprinidae fishes used in this study

Serial number	Subfamily	Scientific names
01		<i>Barbonymus gonionotus</i>
02		<i>Catla catla</i>
03		<i>Cirrhinus mrigala</i>
04		<i>Cirrhinus reba</i>
05		<i>Cyprinus carpio</i>
06	Cyprininae	<i>Labeo bata</i>
07		<i>Labeo calbasu</i>
08		<i>Labeo dyocelius</i>
09		<i>Labeo gonius</i>
10		<i>Labeo rohita</i>
11		<i>Puntius sarana</i>
12	Danioninae	<i>Laubuka laubuca</i>
13		<i>Securicula gora</i>
14		<i>Amblypharyngodon mola</i>
15		<i>Barilius barila</i>
16		<i>Barilius bendelisis</i>
17	Rasborinae	<i>Cabdio morar</i>
18		<i>Esomus danricus</i>
19		<i>Raiamas bola</i>
20		<i>Rasbora daniconius</i>
21		<i>Ctenopharyngodon idella</i>
22	Xenocyprininae	<i>Aristichthys nobilis</i>
23		<i>Hypophthalmichthys molitrix</i>
24		<i>Mylopharyngodon piceus</i>
25	Schizothoracinae	<i>Diptychus maculatus</i>
26		<i>Lepidopygopsis typus</i>
27	Garrinae	<i>Crossocheilus latius</i>
28		<i>Garra gotyla</i>
29	Cobitidae	<i>Botia lohachata</i>

**Morphological traits selection and data collection.** Thirty morphological traits and four osteological characters were observed, counted, and recorded from the collected

specimen. Morphological data of *D. maculatus* and *L. typus* belongs to the subfamily Schizothoracinae were collected from the literature (Wiley et al 1998) as these are not distributed in Bangladesh. These two species were included to understand the phylogenetic relationship of non-native vs native cyprinid in Bangladesh. The morphological and osteological traits and the criteria used for each of the traits are presented in Table 2.

Table 2

Morphological and osteological traits of the cyprinid fishes

No.	Characters	Character states
01.	Caudal-fin shape	Pointed = 0; not pointed = 1
02.	Epural shape (O)	Straight = 0; curved = 1
03.	Parhypural and hypural (O)	Separate = 0; fused = 1
04.	Head shape	Laterally compressed = 0; dorsoventrally flattened = 1; others = 2
05.	Gill membrane location	Below pectoral fin = 0; anterior to pectoral fin=1
06.	Snout length	Short = 0; long = 1
07.	Dorsal-fin spines	Absent = 0; present = 1
08.	Dorsal fin gradation	None = 0; graded = 1
09.	Number of dorsal-fin rays	Fewer than or equal to 20 = 0; more than 20 = 1
10.	Dorsal-fin number	Single = 0; double = 1
11.	Dorsal fins covered in flesh	Uncovered = 0; covered = 1
12.	Anal-fin spine	Absent = 0; present = 1
13.	Number of anal-fin rays	≤ 9 = 0; > 9 and < 14 = 1; ≥ 14 = 2
14.	Number of pectoral-fin rays	≤ 15 = 0; > 15 = 1
15.	Number of lateral lines	Single = 0; multiple lateral line = 1
16.	Lateral line	Incomplete = 0; complete = 1
17.	The shape of the lateral line	Curved = 0; straight = 1
18.	Body scales	Absent = 0; fully scaled = 1
19.	Pharyngeal teeth	≤ 2 rows = 0; > 2 rows = 1
20.	Number of total vertebrae (O)	≤ 29 = 0; > 29 = 1
21.	Number of abdominal vertebrae (O)	< 13 = 0; > 13 = 1
22.	Mouth gap	Narrow = 0; wide = 1
23.	Dorsal profile	Not convex = 0; convex = 1
24.	Gill membrane	Separate = 0; united = 1
25.	Preoperculum	Thin = 0; thick = 1
26.	Pore on the snout	Absence = 0; presence = 1
27.	Band on the body	Absence = 0; presence = 1
28.	Body shape	Elongated = 0; flattened = 1; laterally compressed = 2
29.	Barbel	Absence = 0; Presence = 1
30.	Upper lip	Absence = 0; present fringed = 1; present entire = 2

O = osteological character.

**Retrieval of cytochrome b sequences from the NCBI gene bank databases.** The nucleotide sequences of the cytochrome b gene of 28 cyprinid species and one outgroup taxa were retrieved from the NCBI nucleotide databases (<https://www.ncbi.nlm.nih.gov/nucleotide/>) by using Basic Local Alignment Search Tools (BLAST). The retrieved nucleotide sequence data were sorted manually and the coding sequences (CDS) were selected for the analyses. The cytochrome b sequences ranged from 1120-1150 base pairs were used

for phylogenetic reconstruction and related evolutionary analyses which were shown in Table 3.

Table 3

Accession number of the retrieved Cytb gene of selected taxa

Taxa	Accession number	Taxa	Accession number
1. <i>Amblypharyngodon mola</i>	NC_043864.1	15. <i>Esomus danricus</i>	HM224287.1
2. <i>Aristichthys nobilis</i>	AF051855.1	16. <i>Garra gotyla</i>	KF574711.1
3. <i>Barbonymus gonionotus</i>	KP712204.1	17. <i>Hypophthalmichthys molitrix</i>	MT002871.1
4. <i>Barilius barila</i>	HM224257.1	18. <i>Labeo bata</i>	JX074260.1
5. <i>Barilius bendelisis</i>	KF574527.1	19. <i>Labeo calbasu</i>	KF574600.1
6. <i>Botia lohachata</i>	AY887789.1	20. <i>Labeo dyocelius</i>	JX074262.1
7. <i>Cabdio morar</i>	BAK23003.1	21. <i>Labeo gonius</i>	KF574558.1
8. <i>Catla catla</i>	JX074273.1	22. <i>Labeo rohita</i>	KF574617.1
9. <i>Cirrhinus mrigala</i>	KF574574.1	23. <i>Laubuka laubuca</i>	HM224291.1
10. <i>Cirrhinus reba</i>	KF574681.1	24. <i>Lepidopygopsis typus</i>	KF113569.1
11. <i>Crossocheilus latius</i>	KF574665.1	25. <i>Mylopharyngodon piceus</i>	AF051870.1
12. <i>Ctenopharyngodon idella</i>	JN673556.1	26. <i>Puntius sarana</i>	HM010726.1
13. <i>Cyprinus carpio</i>	DQ868871.1	27. <i>Raiamas bola</i>	HM224329.1
14. <i>Diptychus maculatus</i>	AY463514.1	28. <i>Rasbora daniconius</i>	KF574538.1
		29. <i>Securicula gora</i>	HM224381.1

**Evolutionary analyses and phylogenetic tree reconstruction.** The morphological trait-based phylogenetic tree was constructed according to Clardy (2014) using NEXUS data editor (NDE) and Mesquite software (v.2.6). For each taxon, data matrix was coded based on character-by-taxon. Where assessment criteria were unable to determine coding for the taxon due to a lack of information or inapplicability, missing entries (coded as "?") were used. Character polarity was determined through outgroup comparison with *Botia lohachata* (Botidae, Cypriniformes) as the root. To validate the morphological trait-based tree, the phylogenetic tree was reconstructed based on Cytb gene sequences. Nucleotide composition, genetic distances, phylogenetic tree were constructed by using Molecular Evolutionary Genetic Analysis software (MEGA ver. 6.01).

**Results.** Among the 30 morphological traits observed, 13 traits showed uniformity, and 17 traits showed variation (Table 4). All the taxa showed uniqueness in caudal fin shape, epural shape, parhypural and hypural shape, absence of dorsal fin spine, dorsal fin gradation, number of dorsal fin rays, dorsal fin number, embedded dorsal fin, anal fin spine, scales of body, number of total vertebrae, number of abdominal vertebrae (Table 4). Head shape was laterally compressed (0) in the majority of the fishes, however different head shapes (2) were also observed. In most of the taxa, the location of the gill membrane located below the pectoral fin (0), snout length was short among ten taxa (0) and long among the rest of the taxa (1). In most of the taxa, the numbers of anal fin rays were < 9 (0), between 9 to 13 in *M. piceus*, *C. idella*, *B. bendelisis*, *B. barna* and *R. bola* (1) and  $\geq 14$  in *C. labuca*, *S. gora*, *H. molitrix* and *H. nobilis* (2). A variation on the number of pectoral-fin rays was also observed, i.e.  $\leq 15$  and  $> 15$ . The number of the lateral line was mostly single (0), and the pattern was complete (1) with curve (0) or straight (1) in shape. Mouth gape was narrow in most taxa but wide mouth gape was found in *C. carpio*, *C. catla*, *C. idella*, *R. bola*. Dorsal profile was convex (0) in 12 taxa. Gill membrane was united in most taxa but gill membrane was separated in *L. calbasu* and *R. bola*. The band was present in seven taxa including *L. laubuca*, *B. barna*, *R. bola*, *R. daniconius*, *L. typus*, *D. maculatus*, and *C. latius* (Table 4).

Table 4

## Data matrix from morphological traits

Taxa	Characters																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>A. mola</i>	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1	0	1	1	0	1	0	2	0	0	
<i>A. nobilis</i>	0	0	0	0	1	1	0	1	0	0	1	0	2	1	0	1	0	1	0	1	1	0	0	1	1	0	0	2	0	2
<i>B. barila</i>	0	0	0	2	1	0	0	1	0	0	1	0	1	0	0	1	1	1	1	1	0	0	1	0	1	1	2	0	2	
<i>B. bendelisis</i>	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	1	1	1	1	0	0	1	0	1	0	2	0	2
<i>B. gonionotus</i>	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	0	1	0	2
<i>B. lohachata</i>	0	1	1	0	1	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	2	1	0
<i>C. catla</i>	0	0	0	2	1	1	0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0	2	0	2
<i>C. carpio</i>	0	0	0	2	0	0	0	1	1	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	2	1	2
<i>C. idella</i>	0	0	0	0	1	1	0	1	0	0	1	0	1	1	0	1	0	1	0	1	1	1	0	1	1	1	0	0	0	2
<i>C. latius</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	1	0	0	1	0	1	2
<i>C. morar</i>	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	1	0	?	?	0	0	1	?	1	0	0	0	?
<i>C. mrigala</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	?
<i>C. reba</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	2
<i>D. maculatus</i>	0	0	0	0	1	?	1	1	0	0	1	0	0	0	0	1	?	1	1	1	1	0	1	1	?	1	1	0	1	?
<i>E. danricus</i>	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	1	0	1	0	2	1	2
<i>G. gotyla</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1	1	1	0	1	1	?	1	?	0	1	2
<i>H. molitrix</i>	0	0	0	0	1	1	0	1	0	0	1	0	2	1	0	1	0	1	0	1	1	0	0	1	1	0	0	2	0	2
<i>L. bata</i>	0	0	0	2	1	1	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	0	1	1	0	1	0	0	1	1
<i>L. calbasu</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1	0	0	0	1	1
<i>L. dyoceilus</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	2
<i>L. gonius</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	0	1	1	0	1	0	0	1	1
<i>L. rohita</i>	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	0	1	0	1	0	0	1	1
<i>L. laubuca</i>	0	1	0	2	1	0	0	1	0	0	1	0	2	0	0	1	0	1	1	1	1	0	0	1	0	1	1	0	0	0
<i>L. typus</i>	0	0	0	0	1	?	0	1	0	0	1	0	0	1	0	1	0	1	1	?	?	0	0	1	?	1	1	2	0	2
<i>M. piceus</i>	0	0	0	2	1	1	0	1	0	0	1	0	1	1	0	1	0	1	0	1	1	1	0	1	1	1	0	0	0	2
<i>P. sarana</i>	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	1	1	0	0	0	1	2
<i>R. bola</i>	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	1	1	1	1	1	0	0	1	1	1	2	0	2
<i>R. daniconius</i>	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	1	1	0	1	1	0	0	1	0	1	1	2	0	2
<i>S. gora</i>	0	0	0	0	1	0	0	1	0	0	1	0	2	0	0	1	0	1	?	1	1	0	0	1	0	1	0	2	0	0

Note, characters: 1 = caudal-fin shape; 2 = epural shape, 3 = parhypural and hypural, 4 = head shape, 5 = gill membrane location, 6 = snout length, 7 = dorsal fin spine, 8 = dorsal fin gradation, 9 = number of dorsal fin rays, 11 = dorsal fins covered in flesh, 12 = anal fin shape, 13 = number of anal fin rays, 14 = number of pectoral fin rays, 15 = number of lateral line, 16 = lateral line pattern, 17 = the shape of lateral line, 18 = scales on body, 19 = pharyngeal teeth, 20 = number of vertebrae, 21 = number of abdominal vertebrae, 22 = mouth gape, 23 = dorsal profile, 24 = gill membrane, 25 = preoverculum, 26 = pore on the snout, 27 = band on the body, 28 = body shape, 29 = barbell, 30 = upper lip.

The average nucleotide frequencies were 28.50% (A), 28.3% (T/U), 27.87% (C), and 15.32% (G) where the GC content (43.2) was lower than AT content (56.8) (Figure 1). The transition/transversion biases of Cytb gene sequences substitution were estimated using a Kimura 2 parameter (K2P) model (Kimura 1980). The overall transition/transversion bias was  $R = 2.10$ , where  $R = [A * G * k_1 + T * C * k_2] / [(A + G) * (T + C)]$ . The rates of different transitional substitutions are shown in bold and transversional substitutions are shown in italics (Table 5). The rate of transitional substitutions from A to G, T to C, C to T, G to A were 15.47, 21.99, 21.74, and 8.11, respectively. On the other hand, the rate of transversional substitution from A to T, A to C, T to A, T to G, C to A, C to G, G to T, G to C were 4.71, 4.71, 4.61, 4.61, 14.56, 4.56, 2.47 and 2.47 respectively (Table 5). The rate of transitional substitutions among the Cytb nucleotide sequences of studied cyprinid taxa was found higher (67.31) than the transversional substitution (32.69).

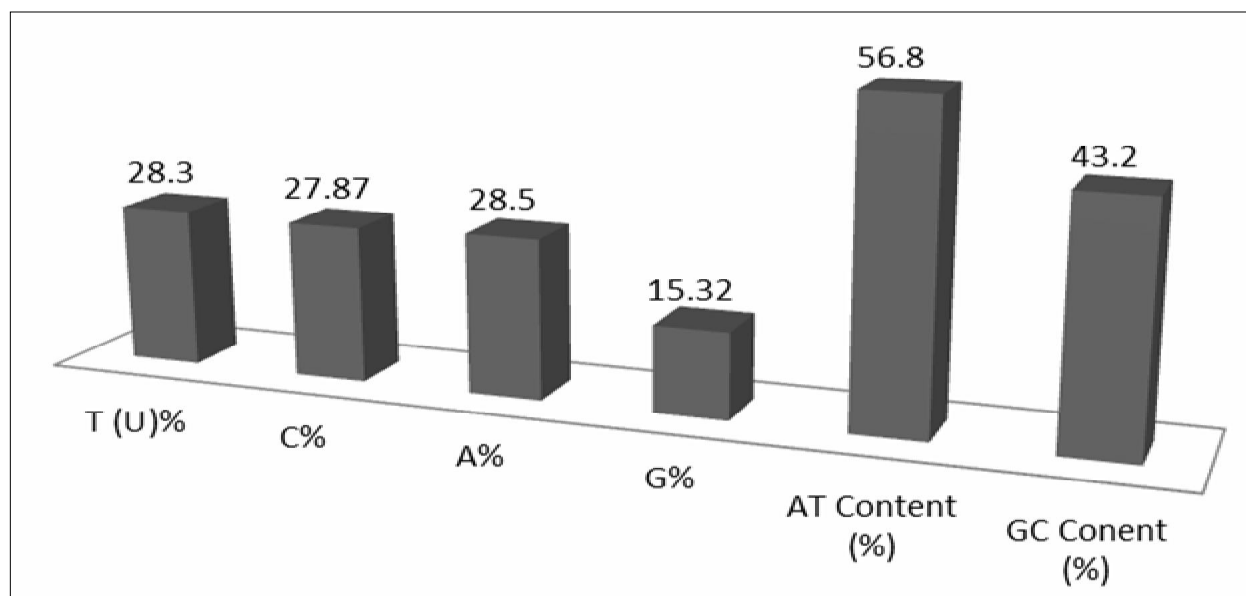


Figure 1. Average nitrogen bases and AT-GC content of the cytochrome b genes: A = Adenine, T = Thymine, C = Cytosine, G = Guanine.

The estimates of evolutionary divergence between sequences of the cytochrome b both at species and genus level were determined using Kimura's two-parameter model of nucleotide substitution. The highest and lowest estimates of evolutionary divergence both at the species and genus level were found between *Catla catla* and *Esomus danricus* (0.773), and the lowest between *Labeo rohita* and *Catla catla* (0.062) (Table 6).

The constructed phylogenetic tree from the data matrix of morphological traits is shown in Figure 2 and from retrieved cytochrome b molecular sequences in Figure 3. Both trees were inferred using the maximum parsimony method (Nei & Kumar 2000). Both phylogenetic trees divided cyprinid fishes into 6 clades and revealed a complex relationship. The taxa of the Cyprininae subfamily showed complex lineage in both the trees (Figures 2 and 3). In both trees, the taxa of the Rasborinae subfamily formed clades with the taxa of Danioninae subfamily, the *Securicula gora*, *Cabdio morar*, *Esomus danricus*, *Rasbora daniconius* formed Rasborinae I and *Barilius bendilisis*, *Barilius barila*, *Riamas bola*, *Laubuka labuca*, *Amblypharyngodon mola* formed the clade Rasborinae II. In the cytochrome b based phylogenetic tree, *Lepidopygopsis typus* (Schizothoracinae) formed cluster Rasborinae II. The taxa of Cyprininae subfamily formed two clades, *Labeo rohita*, *Cirrhinus reba*, *Labeo gonius*, and *Labeo bata* formed Cyprininae-I, and *Labeo dyochelius*, *Barbonymus gonionotus*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Catla catla* formed Cyprininae-II. The other taxa of

Schizothoracinae, the *Diptychus maculatus* found to form clade with the Cyprininae-II subfamily. Four taxa those are exotic in Bangladesh such as *C. idella*, *M. piceus*, *H. molitrix* and *A. nobilis* belong to the Xenocyprininae subfamily showed monophyletic relationship in both trees. Taxa of Garrinae subfamily such as *Garra gotyla* and *Crossochelius latius* formed a separate clade with the few taxa of Cyprininae subfamily and *D. maculatus* of Schizothoracinae subfamily, where in Cytb gene based tree, the *Garra gotyla* and *Crossochelius latius* formed clade without mixing with any taxa of other subfamilies. In the morphological trait based tree *A. testudineus* was included as outgroup taxa and *Botia lohachata* (Cobitidae) was added to understand the evolutionary relationship of Cyprinidae family with the other family of the order cypriniformes. As, in the morphological tree the *Botia lohachata* (Cobitidae) placed as separate taxa, it was included as outgroup in the cytochrome cytochrome b based tree. The outgroup taxa *A. testudineus* in morphological based tree and the *B. lohachata* in cytochrome based tree was placed outside of the other group that formed the root of these trees.

Table 5

Maximum likelihood estimate of substitution matrix

	A	T/U	C	G
A	-	<i>4.61</i>	<i>4.56</i>	<b>8.11</b>
T/U	<i>4.71</i>	-	<b>21.74</b>	<i>2.47</i>
C	<i>4.71</i>	<b>21.99</b>	-	<i>2.47</i>
G	<b>15.47</b>	<i>4.61</i>	<i>4.56</i>	-

Note: The rates of different transitional substitutions are shown in **bold** and transversionsal substitutions are shown in *italics*.

Table 6

## Genetic distances using the Kimura 2-parameter model at species level

**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1																												
2	0.18																											
3	0.18	0.14																										
4	0.23	0.20	0.20																									
5	0.33	0.32	0.32	0.33																								
6	0.18	0.14	0.11	0.21	0.32																							
7	0.22	0.20	0.20	0.24	0.29	0.21																						
8	0.17	0.14	0.11	0.21	0.33	0.09	0.20																					
9	0.20	0.14	0.13	0.22	0.33	0.13	0.20	0.13																				
10	0.18	0.11	0.14	0.22	0.32	0.13	0.21	0.15	0.15																			
11	0.18	0.14	0.10	0.21	0.33	0.09	0.20	0.10	0.12	0.13																		
12	0.24	0.21	0.17	0.24	0.31	0.18	0.23	0.20	0.19	0.21	0.18																	
13	0.71	0.72	0.71	0.73	0.77*	0.71	0.72	0.71	0.70	0.72	0.71	0.72																
14	0.19	0.14	0.12	0.21	0.33	0.11	0.21	0.12	0.11	0.15	0.11	0.20	0.71															
15	0.18	0.07	0.13	0.22	0.32	0.14	0.21	0.15	0.15	0.10	0.13	0.22	0.72	0.14														
16	0.17	0.14	0.11	0.21	0.33	0.09	0.20	0.11	0.12	0.13	0.10	0.18	0.70	0.11	0.14													
17	0.16	0.14	0.11	0.21	0.33	0.08	0.21	0.10	0.12	0.13	0.10	0.19	0.70	0.11	0.13	0.10												
18	0.19	0.15	0.12	0.21	0.33	0.08	0.21	0.10	0.11	0.14	0.11	0.19	0.71	0.11	0.15	0.10	0.07											
19	0.19	0.15	0.11	0.21	0.33	0.06*	0.20	0.09	0.13	0.13	0.10	0.18	0.71	0.10	0.14	0.10	0.08	0.08										
20	0.18	0.15	0.11	0.21	0.33	0.07	0.20	0.10	0.12	0.14	0.10	0.18	0.71	0.11	0.15	0.10	0.08	0.08	0.08									
21	0.75	0.74	0.75	0.74	0.77	0.74	0.76	0.75	0.75	0.74	0.75	0.76	0.74	0.74	0.74	0.75	0.74	0.75	0.75	0.74								
22	0.22	0.21	0.21	0.24	0.34	0.18	0.22	0.20	0.22	0.19	0.19	0.26	0.72	0.20	0.21	0.20	0.20	0.19	0.19	0.19	0.74							
23	0.18	0.09	0.13	0.20	0.32	0.13	0.21	0.13	0.14	0.09	0.12	0.21	0.72	0.13	0.08	0.13	0.13	0.14	0.14	0.13	0.74	0.20						
24	0.18	0.17	0.14	0.25	0.34	0.14	0.22	0.14	0.14	0.17	0.13	0.21	0.70	0.13	0.16	0.13	0.14	0.14	0.14	0.14	0.76	0.21	0.16					
25	0.23	0.21	0.20	0.24	0.27	0.21	0.18	0.20	0.21	0.20	0.21	0.24	0.72	0.20	0.21	0.21	0.22	0.22	0.22	0.22	0.76	0.21	0.21	0.22				
26	0.21	0.23	0.22	0.26	0.35	0.22	0.26	0.22	0.22	0.23	0.22	0.25	0.70	0.22	0.21	0.21	0.21	0.23	0.22	0.23	0.77	0.26	0.21	0.22	0.26			
27	0.18	0.15	0.13	0.22	0.33	0.12	0.20	0.12	0.15	0.14	0.12	0.20	0.71	0.13	0.15	0.11	0.12	0.12	0.11	0.12	0.75	0.18	0.14	0.13	0.21	0.22		
28	0.22	0.22	0.21	0.24	0.28	0.22	0.19	0.21	0.22	0.21	0.22	0.25	0.73	0.21	0.21	0.21	0.21	0.22	0.21	0.23	0.75	0.23	0.21	0.23	0.19	0.27	0.22	

\* indicates the highest and lowest genetic distance.

\*\* Number of respective species: 1 = *Amblypharyngodon mola*, 2 = *Aristichthys nobilis*, 3 = *Barbonymus gonionotus*, 4 = *Barilius bendelisis*, 5 = *Cabdio morar*, 6 = *Catla catla*, 7 = *Barilius barila*, 8 = *Cirrhinus mrigala*, 9 = *Crossocheilus latius*, 10 = *Ctenopharyngodon idella*, 11 = *Cyprinus carpio*, 12 = *Diptychus maculatus*, 13 = *Esomus danricus*, 14 = *Garra gotyla*, 15 = *Hypophthalmichthys molitrix*, 16 = *Labeo bata*, 17 = *Labeo calbasu*, 18 = *Labeo gonius*, 19 = *Labeo rohita*, 20 = *Labeo dyocheilus*, 21 = *Lepidopygopsis typus*, 22 = *Laubuka laubuca*, 23 = *Mylopharyngodon piceus*, 24 = *Puntius sarana*, 25 = *Raiamas bola*, 26 = *Rasbora daniconius*, 27 = *Cirrhinus reba*, 28 = *Securicula gora*.



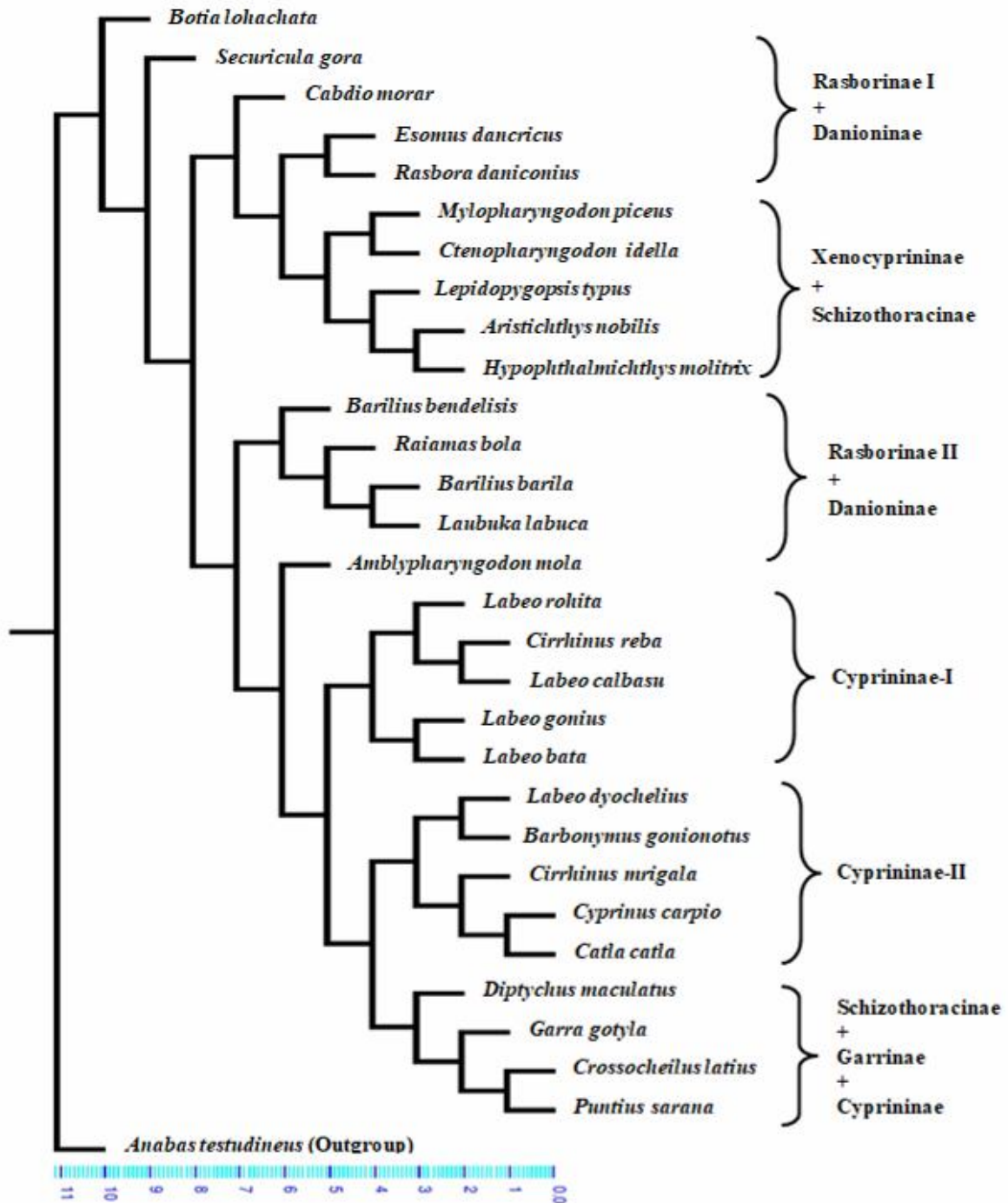


Figure 2. The most-parsimonious trees of 3129 steps with CI = 0.375 and RI = 0.589 using MESQUITE software.

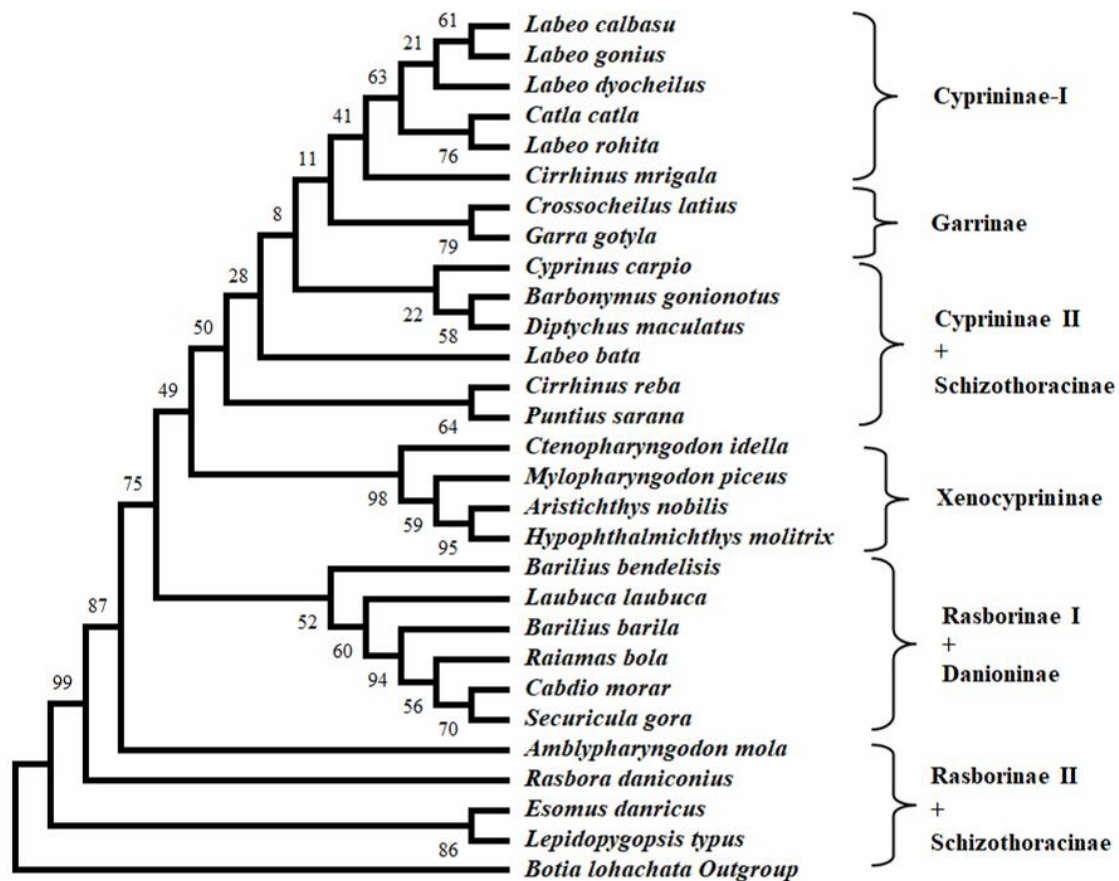


Figure 3. Phylogenetic relationship of Cyprinidae family fishes in Bangladesh inferred from Cytb sequences using the Maximum Parsimony (MP) method.

**Discussion.** We found that forked caudal fin with tapering sharp point in cyprinid fishes and round caudal fin in outgroup *B. lohachata* which similar to the findings of Talwar & Jhingran (1991). Our findings of straight epurals in most of the cyprinid fishes were congruent with the results described by Irfan & Gunawickrama (2011) and Raghavan et al (2013). The slightly curved epural found in *L. laubuca* was similar to the results described by Jinadasa & Kotalawala (1991). Separate parhypural and hypural found in *L. laubuca*, *P. sarana*, *C. latius*, *A. nobilis*, *B. bendelisis* was similar to the results described by Kotalawala & Jinadasa (1991) and Raghavan et al (2013). Dorsal fin gradation without any spine was observed in all fishes of the Cyprinidae family except *D. maculatus* and outgroup taxa of the Botidae family the *B. lohachata* were congruent with Talwar & Jhingran (1991). The numbers of dorsal fin rays, anal fin rays, pectoral fin rays observed in this study were similar to Ahmed et al (2007) and Rahman (2005). The number of total vertebrae (30-39) and abdominal vertebrae (19-22) were higher than previously reported vertebrae in some taxa. Khatun (2014) reported 28 vertebrae in *L. rohita*, 37-38 in *L. rohita*. Kotalawala (1992) mentioned 18-21 abdominal vertebrae in Cyprinidae. The findings on lateral line completeness, curved or straight pattern were similar to the previously reported findings of Ahmed et al (2007) and Rahman (2005). The presence of pharyngeal teeth and the number of rows of pharyngeal teeth found in this study was similar to observations of Zhang (2005), Ahmed et al (2007), and Talwar & Jhingran (1991). The mouth gape types, the position of lips, dorsal profile, joining of gill membranes to isthmus in this study were similar to the past observation by Ahmed et al (2007) and Talwar & Jhingran (1991). The presence of bands, stripes, blotches, barbels in some taxa in this study was similar to the previous study of Talwar & Jhingran (1991), Rahman (2005), and Ahmed et al (2007).

The length of Cytb nucleotide sequences was reported within the ranges by several authors (Esposti et al 1993; Cantatore et al 1994; Sarmasik et al 2008; Wei et al

2014; Devi et al 2014). The nitrogen bases thymine/uracil (T/U), cytosine (C), guanine (G), adenine (A) composition of the Cytb sequences were T (U) 28.3%, C 27.87%, A 28.5%, and G 15.32%. According to Ketmaier et al (2004) Cytb sequences were globally G deficient (17.52%); the frequencies of other nucleotides were A 25.84%; C 27.26%; T 29.38% (Ketmaier et al 2004). The nucleotide composition has been frequently reported in Cytb based studies on a variety of fishes, including cyprinids (Cantatore et al 1994; Brito et al 1997; Briolay et al 1998; Durand et al 2002). Li et al (2016) discussed the full genome *Sinilabeo rendahli* (Cyprinidae) and found the nucleotide composition was T 24.77%; C 27.77%; A 31.36%, G 16.09% similar to this study. The GC content (43.2%) was lower than that of AT content (56.8%), which fell within the range of the GC content typical for vertebrates (Nei & Kumar 2000; Yang et al 2006). The transition/transversion bias in this study was (R=2.10), which is similar to other studies such as R=1.7 or 2.0) Esa et al (2012), Zhu et al (1994). The genetic distances based on the Cytb nucleotide divergence among the species were estimated with the Kimura two-parameter model (Kimura 1980). The lowest genetic distance (0.067%) was found between the closely related taxa *L. rohita* and *C. catla* that was congruent with Esa et al (2012) and Briolay et al (1998) where they reported the lowest genetic distance between two closely related cyprinid species were 0.05% and 0.07%, respectively. The value of genetic distance varies from species to species, but the value is relatively lower among the closely related species.

The subfamilies of Cyprinidae showed complex relationship in their placement and relationship (Conway et al 2010). The taxa of the Cyprininae subfamily showed complex lineage in both the trees (Figures 2 and 3). Complex lineage among the taxa of the Cyprininae subfamily was found with the formation of two clades and split position of single taxa which was similar to the previously described taxonomic literature (Talwar & Jhingran 1991; Rahman 2005). The polyphyly of the Cyprininae subfamily supported by the Zardoya et al (1999) from molecular studies, previous morphological studies by Karakousis et al (1993), and allozymic Karakousis et al (1995). The taxa of Danioninae and Rasborinae formed clades together. Conway et al (2008) reported in a study that the clade of Rasborinae was very close to Danioninae. Based on the presence of barbels Danioninae and Rasborinae subfamily formed different clades (Coburn & Cavender 1992). We found that Rasborinae is not a monophyletic subfamily of cyprinid because there are no synapomorphies to support the group. These results are consistent with other findings from molecular phylogenetic analyses by Mayden et al (2007) and He et al (2008). All of the current classifications using Rasborinae have been inferred from tradition and designated for convenience. Danioninae is recognized as a mixed clade by Thai (2007) and Saitoh et al (2006). Multiple, separate lineages were identified for species of the Rasborinae subfamily (He et al 2008), which was consistent with the polyphyletic origin found by Saitoh et al (2006), Mayden et al (2007). Only the Xenocyprininae subfamily observed as a monophyletic group with the sister taxa which are exotic to Bangladesh, the monophyletic relationship of this subfamily was found by Cunha et al (2002).

**Conclusions.** The vast majority of investigations of these species are pre-Hennigian revisionary and systematic studies, faunal works, and comparative taxonomic studies. The current understanding on the phylogenetic relations is primarily based on the phenetic studies. Therefore, it is expected that many of the forth-coming systematic studies of this family will result in changes in the taxonomy of the group. This study preliminarily revealed the complex phylogenetic relationship of Bangladeshi and Asian cyprinids based on the morphological trait and molecular sequences. Results from this study would be useful for gene pool conservation and breeding programs. Future studies should consider a combination of mitochondrial and nuclear gene sequences for a better insights into the phylogenetic relations of cyprinids in Bangladesh.

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