Molecular approach for identification of *Labeo bata* based on COI gene sequence from Pakistan

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**ABSTRACT**

DNA barcoding is a rapid, precise and effective way of species identification. A short and standard target gene/genetic marker is used to create sequence profile of identified species against unidentified. Specific tag or marker is used, which is derived from mitochondrial COI for identification. Effectiveness of this method axes the degree of divergence among species. Compared to method of visible or external morphology, barcoding is more reliable.

*Labeo* genus constitutes an imperative group of organisms with intense diversification and has a potential viable aquaculture importance in subcontinent. Identification is necessary for their representation. The genetic distances were computed, and neighbourhood-joining tree was constructed based on the Kimura 2 Parameter method. GenBank and BOLD revealed definitive identity matches. Conspecific and congeneric K2P nucleotide divergence was estimated. Evolutionary tree was analysed clearly by relating their species to phylogenetic tree, as same as species were bunched under same tree node, while species were differently clustered under distinct nodes. These finding concluded that the gene sequence may serve as milestone for identification and phylogenetic history of related species at molecular level.

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*Labeo bata*; COI; DNA barcoding; Identification

**Introduction**

Fishes are the largest and most diverse class, estimated 30,000 in number (Eschmeyer et al. 2010). Identification of fish species is one of the most important tasks of taxonomy (Ward et al. 2009). Fish identification is traditionally based on external morphological features (Strauss and Bond 1990). Scientific debate concerning molecular method in taxonomy emerged as a more viable tool for identification (Teletchea 2010). Molecular identification is challenging, and DNA barcoding provides us a new perspective in fish systematic and ecology (Hubert et al. 2008). DNA sequence for identification gained global support as an applicable tool for identification, in particular fishes coordinated using FISH-BOL (Ward et al. 2009). Molecular approach involves sequencing and amplification of short universal mitochondrial COI for identification (Jenkins et al. 2012).

To the best of our knowledge, molecular identification has not been carried out from this region. Thus, the aim of this study was to identify species in proper representation, its divergence and lineage.

**Materials and methods**

**Sampling of fish**

*Labeo bata* samples were collected from River Chenab, Muzaffargarh (Lat: 30° 04’ 31.33”N, Long: 71° 11’ 31.67” E), Punjab, Pakistan. Samples were stored at fisheries laboratory, IP & AB, BZU, Multan.

**Species identification**

Samples were identified with the help of standard taxonomic key (Mirza and Sandhu 2007) on the basis of their morphometric characteristics.

*Labeo bata* (Hamilton 1822), Dorsal profile is slightly more convex than abdomen. Lips which is thin and continuous and lower one is reflected from off mandible and with shallow groove along its hind edge. Pair of maxillary barbels, Pharyngeal teeth, Dorsal fin is as high as head with concave upper edge and pectoral fin, deeply forked caudal fin. Fin Formula, B.iii, D. 10 (2/8–3/10), P. 16, V. 7, A. 7, C. 18, L. I. 35, L. tr. 6. (Sarma et al. 2017).

**DNA isolation, quantification and PCR**

DNA isolation was done by the modified phenol chloroform extraction method and DNA was stored at −20°C until further analyses. DNA was quantified by measuring the absorbance at 260/280 nm with Nanophotometer (IMPLEN). PCR for amplification of genomic DNA and region of mitochondrial COI was performed. The most clarified samples were selected for the purpose of sequencing (Table 1).
**Analysis of the data**

Sequence was analysed using BioEdit (7.0.5.3) following Hall (1999) and K2P following Kimura (1980). NJ trees (Saitou and Nei 1987) were constructed through MEGA version 5 software (Tamura et al. 2011). Sequence accession no#BOLD (AAV6679) and GenBank (YP_004285842.1) are as shown in Table 1.

**Results**

Molecular identification revealed similarity for consensus sequence. The average read lengths were 639 bp. Minimum, maximum and average K2P distances were found to be 0, 1.41 and .2, respectively, within species, while they were .11, 1.82 and .34, respectively, among same genus (Table 1).

Labeo genus evolutionary analyses were conducted through Mega5 following Tamura et al. (2011), and evolutionary history by maximum-likelihood method, based on the Tamura and Nei (1993) (Figure 1).

**Discussion**

A total of 650 base pairs of COI were used for specie identification (Lohman et al. 2009). Basic purpose for molecular analysis is to define the unidentified individual species to be identified, but, occasionally relatively surprisingly, can also central for detection of unpredicted diversity and unnoticed species (Kerr et al. 2009).

Absorbance comparison (260/280 nm) of 1.6–2, for pure samples (Cawthorn et al. 2011), as revealed in present study is in general agreement.

Mean intraspecific K2P distance was found to be .2% and intragenerically .34%, revealing more divergence among different species as freshwater Indonesian fishes of .15% and 2.53 (Hubert et al. 2015), Canadian freshwater fishes of .27% and 8.37% (Hubert et al. 2008), Australian freshwater fishes of .39% and 9.93% (Ward et al. 2005) and Indian freshwater fish than 0–90% and .10–15.00% (Lakra et al. 2015) are in general agreement.

Molecular identification of genus Labeo by COI is used in molecular taxonomy marker for animal species (Luo et al. 2011). Primer COI is used for species and population divergence and identification (Khaliq 2012). Molecular identification is used to distinguish different species having higher resemblance morphologically.

**Conclusion**

Molecular identification is not a familiar practice in Pakistan. Study enlightens work importance for identification. Molecular approach is one of the useful identification techniques compared to various traditional identification methods. Subsequent identification method used for decades has many limitations as it does not work during the fish developmental body stages, and for processed fish, fish fillet and damaged specimen. Molecular approach also estimates the nucleic divergence among species, genus and family.
Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure 1. Neighbourhood-joining tree representing genetic relationship among Labeo species.