# Bioinformatics Study of Operational Taxonomic Units of Fish *Rasbora daniconius*

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**Abstract**
Among various genes involved in DNA taxonomy, DNA barcoding is related to COI gene related taxonomy. MEGA is one of the software used to align DNA and protein sequences. Present study deals with study of the operational taxonomic units of the native fish *Rasbora daniconius* with other closely related species *Rasbora wilpita*. Of the evaluated data eleven Operational Taxonomic Units were found out of the present latest data available on NCBI mostly located at the beginning and end part of the sequences. The initial part of the gene was occupied with SNPs and the end part included triplet codons.

**Keywords:** Operational Taxonomic Units, SNPs, Triplet codons.

## 1. Introduction

Habitat selection by fishes especially those living in littoral zone is affected by predation pressure, plant density and macrophyte type [1]. The fishes when placed form natural to cultured environment random genetic drift and domestication effects change gene frequency, reducing genetic variation [2]. Among freshwater fishes low altitude fishes show greater diversity than those at high altitudes as various isolating mechanisms act on those causing spatial isolation; temporal isolation [3].

Of the different tools being used for DNA taxonomy COI gene based taxonomy is considered to be a reliable method for species identification of fishes and fish products [4-5] also for all the life stages [6]. Considering large numbers of characteristics in classifying many phenomena, notably living organisms, fossil organisms and even imaginary organisms is possible because of computers [7].

In the present study we have found the Operational Taxonomic Units (OTUs) working in one of the native fish *Rasbora daniconius* in comparison with its closest related species *Rasbora wilpita* on NCBI data base. The black-line Rasbora or *Rasbora daniconius* is a species of ray finned fish inhibiting a variety of habitats like ditches, ponds canals streams, rivers and inundated fields [8]. Rasbora fish is mostly freshwater fish but also found in brackish water and benthopelagic region. Fish reaches its maturity up to maximum length of 15cm. Rasbora having 9 dorsal soft rays and 7 anal soft rays, black stripe running from eye to caudal fin; nearly complete lateral line, with only last few scales lacking pores [9-10]. The fish feeds mainly on aquatic insects and detritus matter as it is a surface feeder [8]. The fish is hardy found in the studies done in the rivers near the study area [11-12] adaptable in the aquarium (National Institute of Malaria Research, 2009) [13].

## 2. Material Methods

DNA barcoding is one of the emerging sciences in species identification and can broaden understanding of both phylogenetic signal and population level variation [14]. But the region of 650 bases in not easily obtained [15].

The COI gene sequences of the fish *Rasbora daniconius* and *Rasbora wilpita* were downloaded from NCBI and were aligned using the software Molecular Evolutionary Genetic Analysis (MEGA) version 5.05 [16]. The alignment system used for the DNA sequences was Clustal W.

The accession numbers of the fishes used for the study are as follows:

| No | Fish | Accession number |
|---|---|---|
| 1 | *Rasbora daniconius* | JX260290 |
| 2 | *Rasbora daniconius* | KJ476780 |
| 3 | *Rasbora daniconius* | JX983478 |
| 4 | *Rasbora daniconius* | JX983476 |
| 5 | *Rasbora daniconius* | JX260960 |
| 6 | *Rasbora wilpita* | JX260962 |
| 7 | *Rasbora wilpita* | JX260962 |

Fish *Rasbora wilpita* was chosen as it had same length uploaded to NCBI like of *Rasbora daniconius* and is most coinciding morphospecies of the same. Although the COI gene measures 652bp present length aligned in the study has been 513 bases only.

## 3. Result

The output of the sequence alignment has been exported in the results showing different point mutations suggesting the sites of coevolution of DNA SNPs with species.

**Table 1:** Nucleotide composition of the reference sequence and sequences of fishes undertaken.

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JX260960.1 *Rasbora daniconius* voucher RDGA03 cytochrome oxidase subunit 1 (COI) gene partial cds mitochondrial | G | A | A | C | T | T | A | G | C | C | A | A | C | C | G | G | G | A | T | C | A |
| KJ476780.1 *Rasbora daniconius* isolate NBFGRRD-003 cytochrome c oxidase subunit I (COI) gene partial cds mitochondrial | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478.1 *Rasbora daniconius* voucher NF446 cytochrome oxidase subunit 1 (COI) gene partial cds mitochondrial | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476.1 *Rasbora daniconius* voucher NF780 cytochrome oxidase subunit 1 (COI) gene partial cds mitochondrial | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| gi\|399932588\|gb\|JX260960.1\| *Rasbora daniconius* voucher RDGA03 cytochrome oxidase subunit 1 (COI) gene partial cds mitochondrial | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962.1 *Rasbora wilpita* voucher PMUM01 cytochrome oxidase subunit 1 (COI) gene partial cds mitochondrial | . | . | . | G | . | . | . | . | . | . | T | G | . | . | . | . | . | . | . | . | . |
| JX260960 | C | T | A | C | T | A | G | G | A | G | A | C | G | A | T | C | A | A | A | T | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | T | A | T | A | A | T | G | T | C | A | T | T | G | T | A | A | C | T | G | C | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | A | T | G | C | T | T | T | T | G | T | A | A | T | G | A | T | T | T | T | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | T | T | T | A | T | A | G | T | T | A | T | A | C | C | A | A | T | A | C | T | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | A | T | C | G | G | A | G | G | A | T | T | T | G | G | G | A | A | C | T | G | A |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | T | T | G | G | T | C | C | C | A | C | T | G | A | T | A | A | T | T | G | G | G |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | G | C | A | C | C | A | G | A | C | A | T | A | G | C | A | T | T | C | C | C | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | G | A | A | T | A | A | A | C | A | A | T | A | T | A | A | G | C | T | T | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

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| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . |
| JX260960 | T | G | A | C | T | T | C | T | C | C | C | C | C | C | A | T | C | A | T | T | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | T | T | C | T | T | T | T | A | T | T | A | G | C | C | T | C | C | T | C | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | G | G | T | G | T | T | G | A | A | G | C | A | G | G | T | G | C | C | G | G | G |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | A | C | A | G | G | A | T | G | A | A | C | A | G | T | T | T | A | T | C | C | A |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | C | A | C | T | T | G | C | A | G | G | A | A | A | C | C | T | C | G | C | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | A | C | G | C | A | G | G | A | G | C | A | T | C | A | G | T | A | G | A | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | T | A | A | C | A | A | T | T | T | T | C | T | C | T | C | T | C | C | A | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | T | T | A | G | C | A | G | G | T | G | T | G | T | C | A | T | C | A | A | T | C |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | T | T | A | G | G | G | G | C | T | A | T | T | A | A | T | T | T | C | A | T | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | A | C | A | A | C | A | A | T | T | A | T | T | A | A | T | A | T | G | A | A | G |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

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| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | C | T | C | C | A | G | C | C | A | T | T | A | C | T | C | A | A | T | A | T |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | A | A | A | C | T | C | C | T | T | T | A | T | T | T | G | T | C | T | G | A |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | G | C | C | G | T | A | C | T | A | G | T | A | A | C | A | G | C | C | G | T | G |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | C | T | T | C | T | A | C | T | A | C | T | A | T | C | C | C | T | A | C | C | A |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | C | T | A | C | ? | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | G | T | C | C | T | A | G | C | T | G | C | T | G | G | A | A | T | C | A | C | A |
| KJ476780 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983478 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX983476 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260960 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| JX260962 | . | . | . | . | . | . | . | G | . | . | A | . | . | . | . | . | . | . | . | . | . |
| JX260960 | A | T | A | C | T | C | C | T | C | | | | | | | | | | | | |
| KJ476780 | . | . | . | . | . | . | . | . | . | | | | | | | | | | | | |
| JX983478 | . | . | . | . | . | . | . | . | . | | | | | | | | | | | | |
| JX983476 | . | . | . | . | . | . | . | . | . | | | | | | | | | | | | |
| JX260960 | . | . | . | . | . | . | . | . | . | | | | | | | | | | | | |
| JX260962 | . | . | . | . | . | . | . | . | T | | | | | | | | | | | | |

Comparison of nucleotide sequences in table 1 shows the species specific SNPs at sites 4, 11, 12, 180, 491, 494, 513. Whereas a continuous series of mutations for 468, 469, 470, 471, 472 bases.

## 4. Conclusion

It is concluded from the present study that the Single Nucleotide Polymorphism exist in this species at the initial part of the COI gene, whereas the continuous mutations were at the end part of gene. Thus the mutations which co-evolved with species and speciation have been pointed out in the present study.

## 5. References

1. Chick JH, Mlvor CC. Habitat selection by three littoral zone fishes: effects of predation pressure, plant density and macrophyte type. Ecology of Freshwater Fish, 1997; 6:27-35. doi: 10.1111/j.1600-0633 tb00139.x
2. Lucian Gorgan. Environmental Effects and Genotypic Variability of Fishes. Scientific Annals of University AlexandruIoan Cuza Genetics and Molecular Biology Section, TOMIX, 2008, 47-54.
3. Lowe-Mcconnell RH. Speciation in tropical freshwater fishes. Biological Journal of the Linnean Society. 1969; 1:51-75. doi: 10.1111/j.1095-8312.1969.tb01812.x
4. Espineira M, Gonzalez-Lavin N, Vieites JM, Santaclara FJ. Development of a Method for the Genetic Identification of Flatfish Species on the Basis of Mitochondrial DNA Sequences. J. Agric. Food Xhem. 2008; 56:8954-8961.
5. Ward RD, Zemlak TS, Innes BH, Last PR. DNA barcoding Australia’s fish species. Phil. Trans. R. Soc. B. 2005, 360:1847-1857.
6. Ahrens D, Monaghan MT, Vogler AP. DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). Molecular Phylogenetics and Evolution. 2007; 44:436-449.
7. Sokal Robert R. Numerical Taxonomy WH. Freeman and Company California. 1966, 1059.
8. Rahman AKA. Freshwater fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka.1989, 364.
9. Jayaram KC. The fresh water fishes of Indian Region. Narendra Publication, New Delhi, India, 1999, 551.

10. Jhingran AG, Talwar PK. Inland fishes of India and Adjacent Countries, TYK publications. Dhaka. 1991, 1158.
11. Shinde SE, Pathan TS, Raut KS, Bhandare RY, Sonawane DL. Fish Biodiversity of Pravara River at Pravara Sangam District Ahmednagar, (M.S.) India. World Journal of Zoology. 2009; 4(3):176-179.
12. Kalyankar VB, Nalage DN, Dhondage TB, Akhade SS. and Jamdar SV. Study of Fish Biodiversity from Lower Dudhana Project at Parbhani District, India, World Journal of Zoology. 2012; 7(4):320-322.
13. National Institute of Malaria Research: A Profile of National Institute of Malaria Research, Indian Council of Medical Research, New Deli. 2009, 269.
14. Hajibabaei Mehrdad, Singer Gregory AC, Hebert Paul DN, Hickey Donal A. DNA barcoding: how it complements taxonomy, molecular phylogenetic and population genetics, Trends Genet. 2007. doi:10.1016/j.tig.2007.02.001
15. Hajibabaei M, Smith Alex, Janzen Daniel H, Rodriguez Josephine J. A minimalist barcode can identify a specimen whose DNA is degraded. Mol. Ecol. Notes. 2006; 6:959-964.
16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. 2011; 28:2731-2739.