Bioinformatics Study of Operational Taxonomic Units of fish *Rasbora daniconius*.

[1]Milindkumar M. Kharat1[st], [2]Kapil T. Patil 2nd,.
[1]Assisstant Professor1[st], [2]Assisstant Professor2[nd]
[1]Department of Zoology1[st]
1[st]Rajiv Vidnyan Va Vanijya Mahavidyalay1[st], Dist. Yavatmal, State Maharashtra, India.

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.

IndexTerms- Operational Taxonomic Units, SNPs, Triplet codons.

Introduction:

Habitat selection by fishes especially those living in littoral zone is affected by predation pressure, plant density and macrophyte type (Chick and Mlvor, 2006). The fishes when placed form natural to cultured environment random genetic drift and domestication effects change gene frequency, reducing genetic variation (Lucian Gorgan, 2008).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 (Lowe-McConnell R H 1969). 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 (Espineira M., et. al. 2008, Ward R. D. et. al. 2005) also for all the life stages (Ahrens D. et. al. 2007). Considering large numbers of characteristics in classifying many phenomena, notably living organisms, fossil organisms and even imaginary organisms is possible because of computers (Sokal R. R. 1966).

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 (Rahaman, 1989). 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 (Jayaram, 1999, Jhingran and Talwar, 1991). The fish feeds mainly on aquatic insects and detritus matter as it is a surface feeder (Rahaman, 1989). The fish is hardy found in the studies done in the rivers near the study area (Shinde et. al. 2009, Kalyankar et. al 2012) adaptable in the aquarium (National Institute of Malaria Research, 2009).

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 (Hajibabaei, M. et al., 2007). But the region of 650 bases in not easily obtained (Hajibabaei, M. et al., 2006).

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(Tamura et. al. 2011). 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.

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.

Table1: Nucleotide composition of the reference sequence and sequences of fishes undertaken.

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	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		

Sequence																					
JX983476	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JX260960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JX260962	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Sequence																					
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	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

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

Sequence																					
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	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

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

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

Sequence																					
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	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

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

Sequence																					
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	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	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	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	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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	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	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.

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.

References:

[1] **Ahrens D., Monaghan M. T., Vogler A. P. (2007):** DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). Molecular Phylogenetics and Evolution 2007, 44 436-449.

[2] **Chick, J. H. and Mlvor, C. C.:** 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**Espineira M., Gonzalez-Lavin N., Vieites J. M. and Santaclara F. J. :** Development of a Method for the Genetic Identification of Flatfish Species on the Basis of Mitochondrial DNA Sequences. 2008 J. Agric. Food Xhem. 56, 8954-8961.

[3] **Hajibabaei Mehrdad, Singer Gregory A.C., Hebert Paul D.N. and 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

[4] **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

[5] **Jayaram, K.C. :** The fresh water fishes of Indian Region. Narendra Publication, New Delhi, India, 1999, 551pp.

[6] **Jhingran, A.G and Talwar P.K. :** Inland fishes of India and Adjacent Countries, TYK publications. Dhaka, 1991, 1158pp.

[7] **Kalyankar, V.B., Nalage, D.N., Dhondage, T. B., Akhade S. S. and Jamdar S.V. :** Study of Fish Biodiversity from Lower Dudhana Project at Parbhani District, India, World Journal of Zoology 2012, 7 (4): 320-322.

[8] **Lowe-Mcconnell, R. H.:** Speciation in tropical freshwater fishes. Biological Journal of the Linnean Society, 1969, 1: 51–75. doi: 10.1111/j.1095-8312.1969.tb01812.x

[9] **Lucian Gorgan:** Environmental Effects and Genotypic Variability of Fishes. Scientific Annals of University "Alexandru Ioan Cuza" Genetics and Molecular Biology Section, 2008, TOM IX, 47-54.

[10] **National Institute of Malaria Research :** A Profile of National Institute of Malaria Research, Indian Council of Medical Research, New Deli. 2009, 269p.

[11] **Rahman, A.K.A., :** Freshwater fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka. 1989, 364 p.

[12] **Shinde, S.E. , Pathan, T.S., Raut, K.S., Bhandare R.Y. and Sonawane D.L. :** Fish Biodiversity of Pravara River at Pravara Sangam District Ahmednagar, (M.S.) India. World Journal of Zoology 2009, 4 (3): 176-179.

[13] **Sokal Robert R. :** Numerical Taxonomy. W. H. Freeman and Company California 1966, 1059.

[14] **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S** : MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 2011, 28: 2731-2739.

[15] **Ward R. D., Zemlak T. S., Innes B. H., Last P. R. (2005):** DNA barcoding Australia's fish species. Phil. Trans. R. Soc. B 2005, 360, 1847-1857.