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Bioinformatics study of Operational Taxonomic Units of fish *Wallago attu*

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Abstract:
Taxonomy based of Cytochrome Oxidase 1 Gene is a novel technique in the field of animal identification. DNA sequences to be aligned for the taxonomic process are aligned in software MEGA. Alignment of different sequences world over for the fish *Wallago attu* have been worked out in the present study with its taxonomically close individual *Ompok bimaculatus*. Different nucleotide sequences in the alignment are pointed out which vary within the species as well as between the species. Interspecific variations are designated as Molecular Operational Taxonomic Units. Thus comparison of both morphological characters and Molecular Operational Taxonomic Units in the present study helps to identify the effectiveness of both the methods together giving a better insight in coevolution of sequences with altering morphology.

Keywords: Molecular Operational Taxonomic Units, MEGA.

Introduction:

Biodiversity and its conservation are one of the major issues to enable sustainable use of natural resources (Kar D. et. al. 2006). Freshwater fishes are getting declined rapidly because of their high sensitivity to qualitative and quantitative changes of aquatic habitats (SenGupta S. and Homechaudhuri S. 2015). Basic scientific information on biodiversity is important for sustained exploitation and simultaneous conservation of fisheries resources (Ahirrao and Mane 2000). Habitat loss, pollution, introduction of exotic species (Yede S. W. et al. 2016), over exploitation cause aquatic biodiversity change and depletion (Biju Kumar A. 2000).

COI gene based molecular taxonomy is an efficient method for species identification of fishes and fish products (Espineira M., et. al. 2008, Ward R. D. et. al. 2005) for different life stages (Ahrens D. et. al. 2007). Bioinformatics has made it possible to consider large numbers of characteristics in classifying many phenomena, notably living organisms, fossil organisms and even imaginary organisms (Sokal R. R. 1966).

Present study the Operational Taxonomic Units (OTUs) working in one of the native fish *Wallago attu* in comparison with its closest related species *Ompok bimaculatus* on NCBI data base.

Wallago attu species has been described from Malabar Southern India and is widely distributed eastward to the Mekong river drainage and south word to Java, Indonesia. The conspecific existence of the population of Indian subcontinent, Myanmar and South East Asia await verification and require further studies. The fish is cat fish which inhabits large rivers, tanks and lakes. It is a sluggish, bottom dwelling, voracious feeder and invasive not letting other fishes grow in the habitat (Ng, H.H. 2010).

Material Methods:

DNA barcoding is one of the newer developing sciences in species identification and help to broaden understanding of both phylogenetic signal and population level variation (Hajibabaei, M. et al., 2007). It is tough to obtain a complete gene of 650 bases (Hajibabaei, M. et al., 2006).

The COI gene sequences of the fish *Wallago attu* and *Ompok bimaculatus* were downloaded from NCBI and were aligned using the software Molecular Evolutionary Genetic Analysis (MEGA) version 5.05(Tamura et. al. 2011). The sequences were aligned using Clustal W alignment method.

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

No	Fish	Accession number
1	*Wallago attu*	JX260823
2	*Wallago attu*	JX983510
3	*Wallago attu*	FJ170769
4	*Wallago attu*	KX657717
5	*Ompok bimaculatus*	JX887604
6	*Ompok bimaculatus*	JX260923
7	*Ompok bimaculatus*	JN628878

Ompok bimaculatus stood close to *Wallago attu* in BLAST and being morphologically very much similar, has been chosen as a part of this study. Although the COI gene measures 652bp present length aligned in the study has been 516 bases only.

Result:

The output of the sequence alignment exported in the results is showing no mutations within both *Wallago attu* and *Ompok bimaculatus* species.

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

JX260993.1 Wallago attu voucher WAPA04 cytochrome oxidase subunit 1	G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
JX983510.1 Wallago attu voucher NF674 cytochrome oxidase subunit 1	G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
FJ170769.1 Wallago attu voucher NBFGR:WA8089C cytochrome c oxidase subunit I	G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
KX657717.1 Wallago attu voucher DUZM130 cytochrome oxidase subunit I	G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
JX887604.1 Ompok bimaculatus voucher OKPA01 cytochrome oxidase subunit 1	G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C

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JX260923.1      G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
Ompok
bimaculatus
voucher
OBND03
cytochrome
oxidase
subunit 1
JN628878.1      G A G C T G G C C C A A C C T G G C G C C C T T C T A G G C
Ompok
bimaculatus
voucher KCF3
cytochrome
oxidase
subunit I

JX260993.1      G A C G A C C A A A T T T A C A A C G T T A T T G T T A C C
JX983510.1      G A C G A C C A A A T T T A C A A C G T T A T T G T T A C C
FJ170769.1      G A C G A C C A A A T T T A C A A C G T T A T T G T T A C C
KX657717.1      G A C G A C C A A A T T T A C A A C G T T A T T G T T A C C
JX887604.1      G A T G A C C A A A T T T A T A A T G T T A T T G T C A C C
JX260923.1      G A T G A C C A A A T T T A T A A T G T T A T T G T C A C C
JN628878.1      G A T G A C C A A A T T T A T A A T G T T A T T G T C A C C

JX260993.1      G C C C A C G C T T T G T A A T A A T T T C T T T A T A
JX983510.1      G C C C A C G C T T T G T A A T A A T T T C T T T A T A
FJ170769.1      G C C C A C G C T T T G T A A T A A T T T C T T T A T A
KX657717.1      G C C C A C G C T T T G T A A T A A T T T C T T T A T A
JX887604.1      G C C C A C G C C T T C G T A A T A A T T T C T T T A T A
JX260923.1      G C C C A C G C C T T C G T A A T A A T T T C T T T A T A
JN628878.1      G C C C A C G C C T T C G T A A T A A T T T C T T T A T A

JX260993.1      G T A A T G C C C A T T A T G A T C G G G G G C T T C G G G
JX983510.1      G T A A T G C C C A T T A T G A T C G G G G G C T T C G G G
FJ170769.1      G T A A T G C C C A T T A T G A T C G G G G G C T T C G G G
KX657717.1      G T A A T G C C C A T T A T G A T C G G G G G C T T C G G G
JX887604.1      G T A A T A C C A A T C A T G A T T G G G G G C T T T G G A
JX260923.1      G T A A T A C C A A T C A T G A T T G G G G G C T T T G G A
JN628878.1      G T A A T A C C A A T C A T G A T T G G G G G C T T T G G A

JX260993.1      A A T T G A C T A G T G C C T C T A A T G A T T G G G G C C
JX983510.1      A A T T G A C T A G T G C C T C T A A T G A T T G G G G C C
FJ170769.1      A A T T G A C T A G T G C C T C T A A T G A T T G G G G C C
KX657717.1      A A T T G A C T A G T G C C T C T A A T G A T T G G G G C C
JX887604.1      A A T T G A C T C G T G C C C C T T A T G A T T G G A G C A
JX260923.1      A A T T G A C T C G T G C C C C T T A T G A T T G G A G C A
JN628878.1      A A T T G A C T C G T G C C C C T T A T G A T T G G A G C A

JX260993.1      C C A G A C A T A G C A T T C C C C C G A A T A A A T A A C
JX983510.1      C C A G A C A T A G C A T T C C C C C G A A T A A A T A A C
FJ170769.1      C C A G A C A T A G C A T T C C C C C G A A T A A A T A A C
KX657717.1      C C A G A C A T A G C A T T C C C C C G A A T A A A T A A C
JX887604.1      C C A G A T A T A G C A T T C C C C C G A A T A A A T A A C
JX260923.1      C C A G A T A T A G C A T T C C C C C G A A T A A A T A A C
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JN628878.1      C C A G A T A T A G C A T T C C C C C G A A T A A A T A A C

JX260993.1      A T A A G C T T C T G A C T C C T T C C C C C A T C C T T T
JX983510.1      A T A A G C T T C T G A C T C C T T C C C C C A T C C T T T
FJ170769.1      A T A A G C T T C T G A C T C C T T C C C C C A T C C T T T
KX657717.1      A T A A G C T T C T G A C T C C T T C C C C C A T C C T T T
JX887604.1      A T A A G C T T C T G A C T T C T G C C T C C A T C A T T C
JX260923.1      A T A A G C T T C T G A C T T C T G C C T C C A T C A T T C
JN628878.1      A T A A G C T T C T G A C T T C T G C C T C C A T C A T T C

JX260993.1      C T T C T C T T G C T A G C C T C A T C T G C C G T T G A A
JX983510.1      C T T C T C T T G C T A G C C T C A T C T G C C G T T G A A
FJ170769.1      C T T C T C T T G C T A G C C T C A T C T G C C G T T G A A
KX657717.1      C T T C T C T T G C T A G C C T C A T C T G C C G T T G A A
JX887604.1      C T C C T T C T A T T A G C A T C T T C T G G A G T T G A A
JX260923.1      C T C C T T C T A T T A G C A T C T T C T G G A G T T G A A
JN628878.1      C T C C T T C T A T T A G C A T C T T C T G G A G T T G A A

JX260993.1      G C A G G A G C A G G A A C A G G A T G A A C T G T T T A T
JX983510.1      G C A G G A G C A G G A A C A G G A T G A A C T G T T T A T
FJ170769.1      G C A G G A G C A G G A A C A G G A T G A A C T G T T T A T
KX657717.1      G C A G G A G C A G G A A C A G G A T G A A C T G T T T A T
JX887604.1      G C A G G G G C A G G C A C A G G G T G A A C T G T T T A T
JX260923.1      G C A G G G G C A G G C A C A G G G T G A A C T G T T T A T
JN628878.1      G C A G G G G C A G G C A C A G G G T G A A C T G T T T A T

JX260993.1      C C T C C C C T T G C A G G G A A T C T T G C A C A C G C A
JX983510.1      C C T C C C C T T G C A G G G A A T C T T G C A C A C G C A
FJ170769.1      C C T C C C C T T G C A G G G A A T C T T G C A C A C G C A
KX657717.1      C C T C C C C T T G C A G G G A A T C T T G C A C A C G C A
JX887604.1      C C C C C A C T T G C A G G A A A T C T T G C A C A C G C A
JX260923.1      C C C C C A C T T G C A G G A A A T C T T G C A C A C G C A
JN628878.1      C C C C C A C T T G C A G G A A A T C T T G C A C A C G C A

JX260993.1      G G G G C T T C T G T A G A T T T A A C A A T C T T T T C A
JX983510.1      G G G G C T T C T G T A G A T T T A A C A A T C T T T T C A
FJ170769.1      G G G G C T T C T G T A G A T T T A A C A A T C T T T T C A
KX657717.1      G G G G C T T C T G T A G A T T T A A C A A T C T T T T C A
JX887604.1      G G G G C C T C T G T A G A C T T A A C A A T T T T C T C A
JX260923.1      G G G G C C T C T G T A G A C T T A A C A A T T T T C T C A
JN628878.1      G G G G C C T C T G T A G A C T T A A C A A T C T T C T C A

JX260993.1      C T A C A T C T T G C A G G T G T G T C C T C T A T T C T T
JX983510.1      C T A C A T C T T G C A G G T G T G T C C T C T A T T C T T
FJ170769.1      C T A C A T C T T G C A G G T G T G T C C T C T A T T C T T
KX657717.1      C T A C A T C T T G C A G G T G T G T C C T C T A T T C T T
JX887604.1      C T A C A T C T T G C A G G G G T A T C A T C C A T T C T A
JX260923.1      C T A C A T C T T G C A G G G G T A T C A T C C A T T C T A
JN628878.1      C T A C A T C T T G C A G G G G T A T C A T C C A T T C T G

JX260993.1      G G G G C C A T C A A T T T T A T T A C A A C A A T T A T T
JX983510.1      G G G G C C A T C A A T T T T A T T A C A A C A A T T A T T
FJ170769.1      G G G G C C A T C A A T T T T A T T A C A A C A A T T A T T
KX657717.1      G G G G C C A T C A A T T T T A T T A C A A C A A T T A T T
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JX887604.1      G G G G C A A T T A A C T T C A T T A C A A C A A T T A T T
JX260923.1      G G G G C A A T T A A C T T C A T T A C A A C A A T T A T T
JN628878.1      G G G G C A A T T A A C T T C A T T A C A A C A A T T A T T

JX260993.1      A A C A T A A A A C C T C C A G C C A T C T C A C A A T A T
JX983510.1      A A C A T A A A A C C T C C A G C C A T C T C A C A A T A T
FJ170769.1      A A C A T A A A A C C T C C A G C C A T C T C A C A A T A T
KX657717.1      A A C A T A A A A C C T C C A G C C A T C T C A C A A T A T
JX887604.1      A A C A T A A A A C C C C C A G C C A T C T C A C A A T A T
JX260923.1      A A C A T A A A A C C C C C A G C C A T C T C A C A A T A T
JN628878.1      A A C A T A A A A C C C C C A G C C A T C T C A C A A T A T

JX260993.1      C A A A C A C C C T T G T T T G T G T G A G C T G T A C T A
JX983510.1      C A A A C A C C C T T G T T T G T G T G A G C T G T A C T A
FJ170769.1      C A A A C A C C C T T G T T T G T G T G A G C T G T A C T A
KX657717.1      C A A A C A C C C T T G T T T G T G T G A G C T G T A C T A
JX887604.1      C A A A C A C C A T T A T T T G T A T G A G C C G T C C T A
JX260923.1      C A A A C A C C A C T A T T T G T A T G A G C T G T C C T A
JN628878.1      C A A A C A C C A C T A T T T G T A T G A G C C G T C C T A

JX260993.1      A T C A C A G C A G T A C T G C T T C T A C T A T C C C T A
JX983510.1      A T C A C A G C A G T A C T G C T T C T A C T A T C C C T A
FJ170769.1      A T C A C A G C A G T A C T G C T T C T A C T A T C C C T A
KX657717.1      A T C A C A G C A G T A C T G C T T C T A C T A T C C C T A
JX887604.1      A T T A C A G C A G T T C T C C T A C T A T T G T C T C T C
JX260923.1      A T T A C A G C A G T T C T C C T A C T A T T G T C T C T C
JN628878.1      A T T A C A G C A G T T C T C C T A C T A T T G T C T C T C

JX260993.1      C C T G T C C T A G C C G C A G G C A T T A C A A T G C T G
JX983510.1      C C T G T C C T A G C C G C A G G C A T T A C A A T G C T G
FJ170769.1      C C T G T C C T A G C C G C A G G C A T T A C A A T G C T G
KX657717.1      C C T G T C C T A G C C G C A G G C A T T A C A A T G C T G
JX887604.1      C C T G T A C T A G C C G C A G G T A T C A C A A T G C T T
JX260923.1      C C T G T A C T A G C C G C A G G T A T C A C A A T G C T T
JN628878.1      C C T G T A C T A G C C G C A G G T A T C A C A A T G C T T

JX260993.1      T T A A C A
JX983510.1      T T A A C A
FJ170769.1      T T A A C A
KX657717.1      T T A A C A
JX887604.1      C T A A C A
JX260923.1      C T A A C A
JN628878.1      C T A A C A
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Observing nucleotide sequences aligned it can be conclude that there are no intraspecific variations in the sites where as there are inter-specific variations existing while changing from *Wallago attu* to *Ompok bimaculatus*.

Conclusion:

Although variation in fishes is reduced when they are shifted from natural to cultured environment (Lucian Gorgan, 2008) it has not been observed in this fish on COI gene basis during the present study which controversial to our previous observations in *Rasbora daniconius* (Kharat M. M. et. al. 2017). Although fishes at low altitude show greater diversity than those at high altitudes because of different isolating mechanisms acting on those causing spatial isolation; temporal isolation (Lowe-McConnell R H 1969) no any changes in any nucleotide implied lack of selection pressure on different sites of occurrence of the fish.

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