Molecular characterization of selected *Mystus* species using COI gene as DNA barcode.

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Abstract - *Mystus* (Scopoli, 1777) is a genus of small to medium-sized edible riverine catfish found across South Asia. 18 species are present in Indian water bodies, out of 44 species worldwide. Traditionally, *Mystus* species are identified using morphological traits, but significant ambiguity exists due to morphological similarity *Mystus* and overlapping meristic counts. Especially the morphometric character of the three species *Mystus gulio (Hamilton), Mystus montanus (Jerdon), Mystus cavasius (Hamilton)* are similar and it leads to further study in DNA barcodes for resolving their taxonomic ambiguity. The cytochrome c oxidase I (COI) gene was used to assess the levels of genetic diversity among those three species. The sequence patterns were different among the three species. The Total genomic DNA was isolated using phenol chloroform method. Amplification and sequencing of COI gene was done. Phylogenetic tree was constructed for *Mystus* species using COI gene sequence to check whether DNA barcoding can be used to discriminate *Mystus* species (Lashari Punhal et al., 2018; Achom Dharshan et al., 2019). The sequence data was aligned and Neighbour joining (NJ) analysis was implemented, to build tree-type representations of the molecular divergences of COI utilizing CLUSTALX and MEGA 3 softwares. The sequence patterns clearly indicate that the three species slightly vary from each other species, *Mystus gulio* and *Mystus cavasius* are similar in sequence pattern and in their morphometric characters also and *Mystus montanus* slightly differ from other two species.

Keywords: cytochrome c oxidase I (COI) gene, DNA barcoding, Mitochondrial DNA, *Mystus* species

I INTRODUCTION

DNA sequence analysis is one of the global standards for species identification. Various gene sequences have been employed to identify species for over three decades. Hebert and his colleagues proposed DNA barcoding techniques in2003, claiming that a single mtDNA (Mitochondrial DNA) gene, Cytochrome c oxidase I (COI), would be enough for species identification in the animal kingdom (Khaliq 2012, Khedkar et al. 2014). The 645 bp mitochondrial COI gene mutates swiftly enough to identify closely similar species. Following that, other investigations using the COI gene sequence were undertaken, demonstrating its efficacy in species identification (Ward et al. 2008; Hubert et al. 2008).

Mystus (Scopoli, 1777) is a genus of small to medium-sized edible riverine catfish found across South Asia. 18 species are present in Indian water bodies, out of 44 species worldwide (Talwar &Jhingran 1991; Jayaram 2010; Jayaram and Sanyal, 2003). Traditionally, *Mystu s*species are identified using morphological traits, but significant ambiguity exists due to morphological similarity *Mystus* and overlapping meristic counts. Thus, proper identification method is important for morphologically similar species for aquaculture practices and conservation.

The molecular approach can be a valuable tool in species identification to complement the taxonomic data and validation of systematic positions and phylogeny. A few studies have been carried out on *Mystus* species using RAPD markers (Garg et al. 2009; Saini et al. 2009) and the DNA sequence-based analysis has not yet been used extensively in this genus as systematic tool to study phylogeny and species identification. In order to address the taxonomic discrepancy among the *Mystus* species, a comprehensive investigation based on COI gene DNA sequencing was conducted.

II MATERIALS AND METHODS

Fish sampling

The Live samples of all three species of *Mystus gulio, Mystus montanus and Mystus cavasius*(Fig. 1) were collected from Maruthur check dam (8°74'30" N, 77°80'66"E) in river Tamiraparani of Tirunelveli district, Thayarthoppu (8°93'39" N, 77°43'68" E) in chittar river which is one of the major sub basin of river Tamiraparani in Tenkasi district and Bhavani Sagar dam (11°47'92" N, 77°13'41"E), Which is situated in the confluence of river Moyyar and river Bavani in Erode district, respectively (Fig. 2). Five to ten samples of the fishes were collected from the selected locations using mono filamentous, multi filamentous gill nets and cast nets of different mesh sizes from 8-29 mm. The fish samples were identified with the help of standard taxonomic key (Jayaram KC, 2010) on the basis of morphological characters. The blood

samples were obtained through puncturing in the caudle vein and stored in 95% ethanol at 4ºC. The specimens were kept preserved in formalin in Freshwater Fish Ecology lab of Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University Alwarkurichi.

Total genomic DNA extraction

The total genomic DNA was extracted from the blood samples by following phenol chloroform method (Naeem et al., 2019; Garg et al 2013). The purity and concentration of the total genomic DNA was determined by optical density value in spectrophotometer measured at 260 and 280 nm. Samples showing 1 OD equivalents to 50µg/ml and purity1.8 alone were taken for subsequent analysis.

Total amount of DNA =

(Absorbance at 260nm)x50x(Dilution factor)µg/ml

$$\text{Purity of DNA} = \frac{\text{Absorbance at 260nm}}{\text{Absorbance at 280nm}}$$

After RNAase treatment the quality and concentration of DNA was checked in 0.7% Agarose gel.

PCR amplification of Cytochrome C Oxidase I gene

Standard polymerase chain reaction (PCR) procedures were applied to amplify cox1 fragments. Primers complementary to the cox1 region encoding for the cox1 subunit of the cytochrome c oxidase, were designed. The following primer pairs

F-5' TCAACCAACCACAAAGACATTGGCAC 3'
R-5'TAGACTTCTGGGTGGCCAAAGAATCA 3'

were used to amplify bp COI gene (Mohammed Quyash Khan et al 2021). PCR reactions were carried out in an eppendorf thermal cycler as described by Kumar et al. (2008). The resulting PCR products were resolved on 2% agarose gels, and stained with ethidium bromide and visualised on UV transilluminator and the amplified product were used for COI gene sequencing and it was sequenced using Big Dye on ABI 3700 automated DNA sequencer.

Statistical analysis

The CLUSTALX programs were used for sequence editing and alignment purpose respectively. Haplotype diversity, AMOVA and pair wise differences between species (FST) was be calculated using the program ARLEQUIN. The maximum likelihood phylogenetic tree was constructed using the program MEGA3 which reflects the phenotypic similarities between sequences.

III RESULTS AND DISCUSSION

Most morphometric and meristic characters of the three *Mystus* species- *Mystusgulio*, *Mystus montanus* and *Mystus cavasius* are similar however there are few distinguishing character among the species. Variability between species (both across populations and between individuals within a population) is critical for their ability to survive and adapt to environmental changes (Nagarajan 2014).

The standard "barcode" region of COX1 has been bidirectionally sequenced for six experimental individuals belonging to three *Mystus* species. The dendogram tree (Fig. 1) highlights the interspecific generic variation, and illustrates that it is possible to discriminate between the species using the COX 1 marker. Neighbour joining (NJ) analysis was implemented, to build tree-type representations of the molecular divergences of COI utilizing MEGA 3 (Kumar et al, 2004). The variation observed within *M. gulio* and *M. cavasius* to a lesser extent. *H. fossils* was used as tree root for dendrogram analysis.

Among the three species, the highest genetic identity was found between *M. gulio* and *M. cavasius*, whereas the lowest was observed between *M. montanus* and *M. cavasius*. On the basis of genetic identity, clustering analysis showed the genetic relationship of the three populations through a dendogram (Fig.3). It appears that *M. gulio* is genetically closer to the *M. cavasius* species rather than the *M. montanus* species. The present study showed a significant correlation between genetic identity and geographical distance. The distributions of river systems affect the population structure of freshwater creatures (Yang et al 2020, Kanthimathi et al 2016, Shandanu kundu et al., 2019).

This study has strongly validated the efficacy of COI barcodes for confirming the differentiation between fish species. There was high congruence between morphological and molecular classification, and COI provided effective species-level discrimination for nearly all *Mystus* species. Based on the morphometric characters and genome sequence analysis the three species, *M. gulio*, *M. cavasius* are almost similar and *M. montanus (Jerdon)* is slightly dissimilar from the other two.

Based on the genetic identity values, it is possible to conclude that *M. gulio* and *M. cavasius* species were closer to each when compared with the *M. montanus* species. COI divergences can serve as an effective tool in species recognition

(Smith *et al*. 2008). Moreover, the fact that some 'species' show higher divergences does not compromise the use of COI sequences for their identification.

The current work established that the DNA barcoding may be used to efficiently identify freshwater fish species, particularly the species complex of small-sized species, and that the current COI barcoding can be brought to use in ecology and systematics in the future.

IV CONCLUSION

This study supports the view that the use of COI barcodes is a powerful tool for species identification (Kerr *et al*. 2007). Using this method would clearly allow the identification of individually isolated freshwater fishes, larvae, etc.,

hence providing new tools useful for the practice of conservation and forensics genetic in these freshwater fishes. From a systematic perspective, COI barcodes provide a new and fast approach for screening the real number of species characterized by private sets of diagnostic characters (Raja et al 2017). We conclude that cox1 sequencing, or 'barcoding', can be used for resolving the taxonomic ambiguity of fish species.

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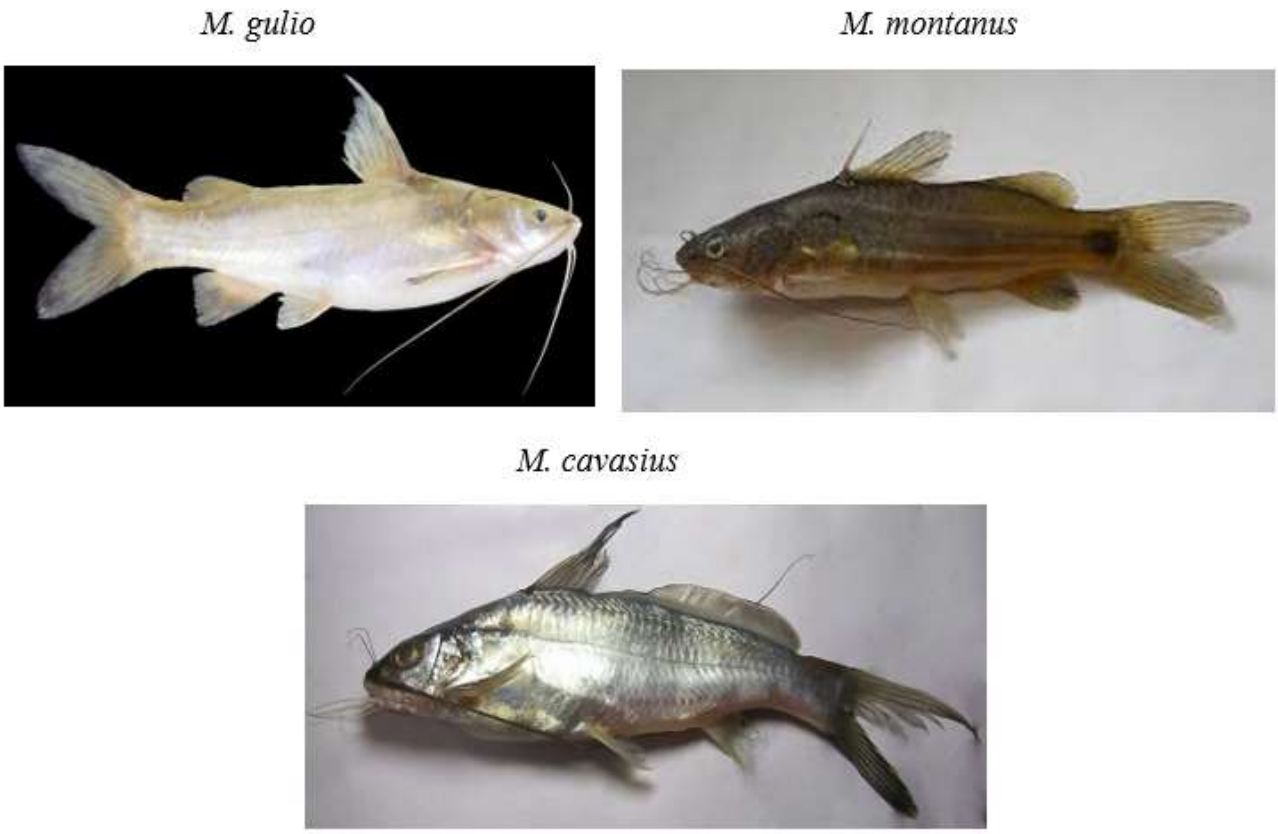
Fig: 1 Experimental fishes of the genus *Mystus*



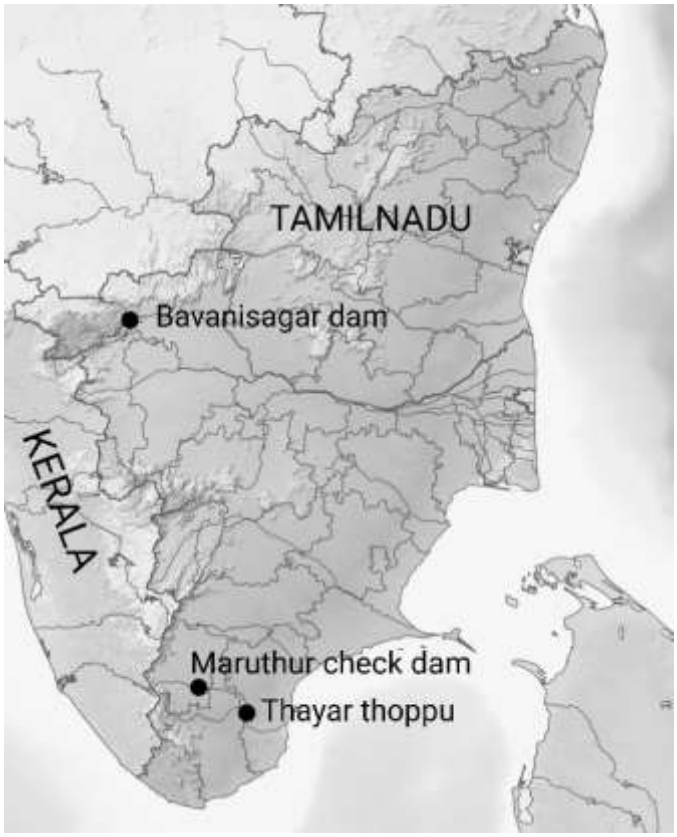


Fig: 2 Sampling sites of *Mystus* species used in this study

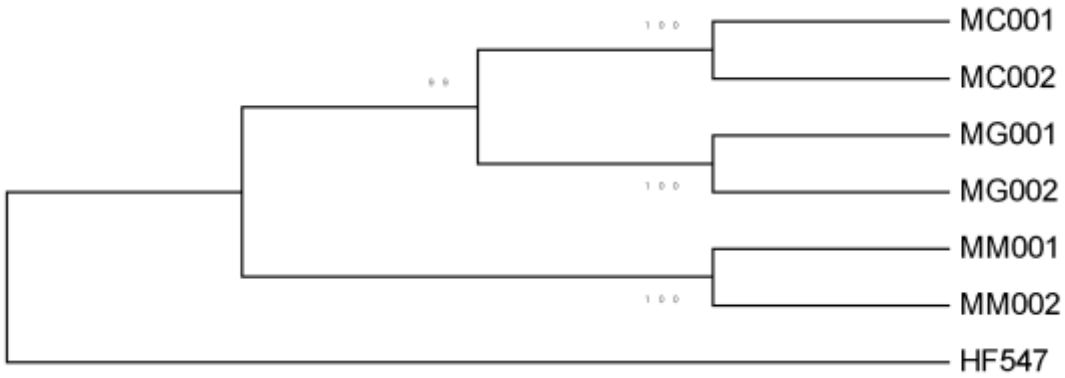


Fig: 3 Dendogram results of three species of *Mystus*
MM – *Mystus montanus*, MG – *Mystus gulio*, MC – *Mystus cavasius*