Genetic variation in natural population of *Mystus gulio*

Rajkumar Guchhait[1]* Sukhendu Maity[2], Atanu Meyta[1], Subhamoy Das[1]

[1]Department of Zoology, Mahishadal Raj College, Purba Medinipur, WB

[2]Integrative Biological Research Unit, Department of Life Sciences,

Presidency University, Kolkata

***E-mail**: r.guchhait001@gmail.com

Abstract: *Long-whiskered catfish, Mystus gulio is one of the important estuarine as well as freshwater fish. It was distributed large geographical area. Due to its delicious taste, it is very popular in eastern India and Bangladesh. Genetic variation was estimated among 29 sequences of three populations of* Mystus gulio *i.e., Tamilnadu (*n = *15), Bangladesh (*n = *13), and West Bengal (n = 2) using COI gene sequencing.In population 1, the total alignment length was 696 base pairs (including sites with gaps /missing data) containing 577 monomorphic and 45 polymorphic sites with eight haplotypes. In population 2, COI sequence total alignment length was 846 base pairs (including sites with gaps /missing data) containing 487 monomorphic and 21 polymorphic sites with nine haplotypes.Haplotype diversity (Hd) and nucleotide diversity (π) of population 1 were 0.8901±0.063 and 0.02043±0.0062 respectively. Haplotype diversity (Hd) and nucleotide diversity (π) of population 2 were 0.8718±0.052 and 0.01023±0.0025 respectively. Genetic distances among the three populations were varying from 0.00 to 7.3%. The results of haplotype networks can help in conservation decisions and management of the fishery of this species.*

***Keywords*:** *Mystus gulio, COI, population structure, genetic diversity*

1. Introduction:

Mystus gulio is also known as the long-whiskered catfish. It was reported that this fish dwells predominantly in brackish water but can also be found in freshwater (Talwar and Jhingran, 1991). It was documented that they were also found in beels, haors, canals, oxbow lakes, rivers and estuaries of Bangladesh (Shafi and Quddus, 2001). It has been reported that this fish is distributed in India, Sri Lanka, Indonesia, Bangladesh, Java, Pakistan, Nepal, Myanmar and Malaysia (Day, 1878; Talwar and Jhingran, 1991; Roberts, 1993; Jhingran, 1997; Kottelat, 2001; Senarathne and Pathiratne, 2007; Weber and de Beaufort, 1913). Due to its delicious taste, it is very popular in eastern India and Bangladesh (Tripathi, 1996; Sarker et al., 2002). This fish is also reported as an ornamental fish (Ng, 2010; Gupta and Banerjee, 2014).

Analysis of genetic variability has been helpful for the proper conservation and management of any natural population (Mukhopadhyay and Bhattarcharjee, 2014). Measurement of

population structure and genetic variability could be analyzed by molecular markers (Deepak and Harikrishnan, 2016). Cytochrome c oxidase I (COI), a mitochondrial gene acts as an important molecular marker for the identification of organisms and their closely related species (Abbas et. al., 2018). This is an important method for the management of fisheries (Shen et al., 2016).

This species is distributed in large geographical areas. So, we assume that there should be the presence of different subgroups. This study aims to describe the population structure and genetic diversity of *M. gulio* using cytochrome c oxidase I (COI) from three different geographical locations. This study will provide basic information about decision-making for fisheries management and conservation strategies for this species.

2. Method:

For this study, we have selected cytochrome c oxidase I (COI) gene. A total of 29 COI gene sequences of *Mystus gulio* were retrieved from Gene Bank database (Table 1). Out Of the 30 gene sequences, 15 were from Tamil Nadu, 13 were from Bangladesh, and 2 were from the Bengal *Mystusgulio* population. Multiple sequence alignment was done by Clustal X2 software (Thompson et al., 1997). Genetic diversity indices (number of haplotypes, haplotype diversity and nucleotide diversity) were analysed by DnaSP6 (Librado & Rozas, 2009; Jose et al., 2021) software. Population structure (haplotype network) was analysed by PopArt. Maximum Likelihood (ML) analysis with MEGA 11 was used to estimate the phylogenetic relationships between haplotypes and 1000 bootstrap pseudoreplicates were performed to determine node support values for the resultant phylogeny (Kumar, Stecher, & Tamura, 2016). Intraspecific divergence was measured using K2P distance matrix (Jose et al., 2021)

Table 1. Accession number of COI gene sequence of *Mystusgulio* retrieved from NCBI(Gene Bank).

Area	Accession number
Tamilnadu	MK902732, MK902728, KF824815, KC595988, KF824814, KF824813, KF574791, KF574789, FJ384684, KF574790, KF574792, KP316241, MK681762, MK359910

Bangladesh	MN458398, MF611594, MK572350, MK572346, MK572348, MK572347, MK572345, MK572349, KX455905, MN083111, MK995086, MF611595, KX455898
West Bengal	KF511564, KJ959643

3. Results:

Ten mtDNA haplotypes were found in 29 samples from Tamilnadu, West Bengal, and Bangladesh (fig. 2). Haplotype 1 was shared in all three geographical areas such as Tamilnadu, West Bengal, and Bangladesh. Haplotype 4 was found in Tamilnadu and Bangladesh populations. Haplotypes 7, 8, 9 and 10 were unique haplotypes found only Bangladesh population. Haplotypes 2, 3, 5, and 6 were also unique haplotypes found only in Tamil Nadu mystus population.

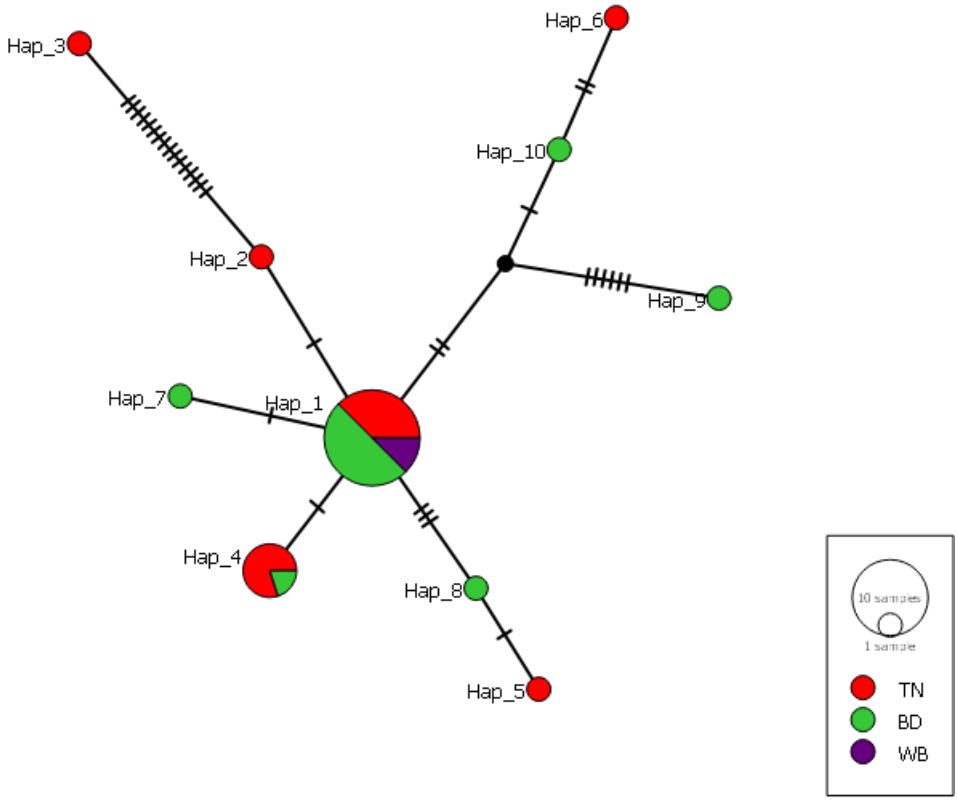

Fig 1. Median-joining network of the mitochondrial haplotypes sampled from *Mystus gulio* in the Tamilnadu (TN), West Bengal (WB), and Bangladesh (BD). The haplotypes are colour–coded by sampling population, with size being proportionate to the number of individuals

sampled with that haplotype. Each hatch mark or black circle between the haplotypes corresponds to a nucleotide substitution.

In Tamil Nadu, Bangladesh and West Bengal mystus population, 14, 13 and 2 COI sequences representing populations 1, 2, and 3 respectively. In population 1, the total alignment length was 696 base pairs (including sites with gaps / missing data) containing 577 monomorphic and 45 polymorphic sites with eight haplotypes. Haplotype 1 contains 3 individuals (MK572350, MK572346, and MK572349). Four individuals (KF574792, KF574790, KF574789, and KF574791) were found in haplotype 2. Haplotype 3 contained two individuals (MK572347 and MK572348). Haplotypes 4, 5, 6, 7, and 8 were represented by one individual of each (MK572345, KF214302, KF824813, KX455905, and MN458398) respectively. Haplotype diversity (Hd) and nucleotide diversity (π) of population 1 were 0.8901±0.063 and 0.02043±0.0062 respectively. In population 2, COI sequence total alignment length was 846 base pairs (including sites with gaps /missing data) containing 487 monomorphic and 21 polymorphic sites with nine haplotypes. Haplotype 3 contained five individuals (MK359910, KX455898, MK995086, MK902728, and MK902732). Haplotypes 1, 2, 4, 5, 6, 7, 8, and 9 were represented by one individual of each(MN083111, MK681762, KC595988, KF824815, FJ384684, KF824814, MF611594, and KJ959643) respectively. Haplotype diversity (Hd) and nucleotide diversity (π) of population 2 were 0.8718±0.052 and 0.01023±0.0025 respectively (Table 2). In population 3, one haplotype was found with two individuals (KP316241 and KF511564). Due to a lack of samples, a detailed analysis was not performed in population 3.

Taxon	*Mystus gulio*	
	Tamil Nadu (Population 1)	Bangladesh (Population 2)
Number of sequences	14	13
Alignment length	696	846
Number of monomorphic sites	577	487
Number of polymorphic sites	45	21
Number of haplotypes (h)	8	9
Haplotype diversity (Hd)	0.8901±0.063	0.8718±0.052
Nucleotide diversity (π)	0.02043±0.0062	0.01023±0.0025

Table 2. Genetic characteristics of populations of *Mystus gulio* found in Tamil Nadu, West Bengal, and Bangladesh as inferred from COI sequences.

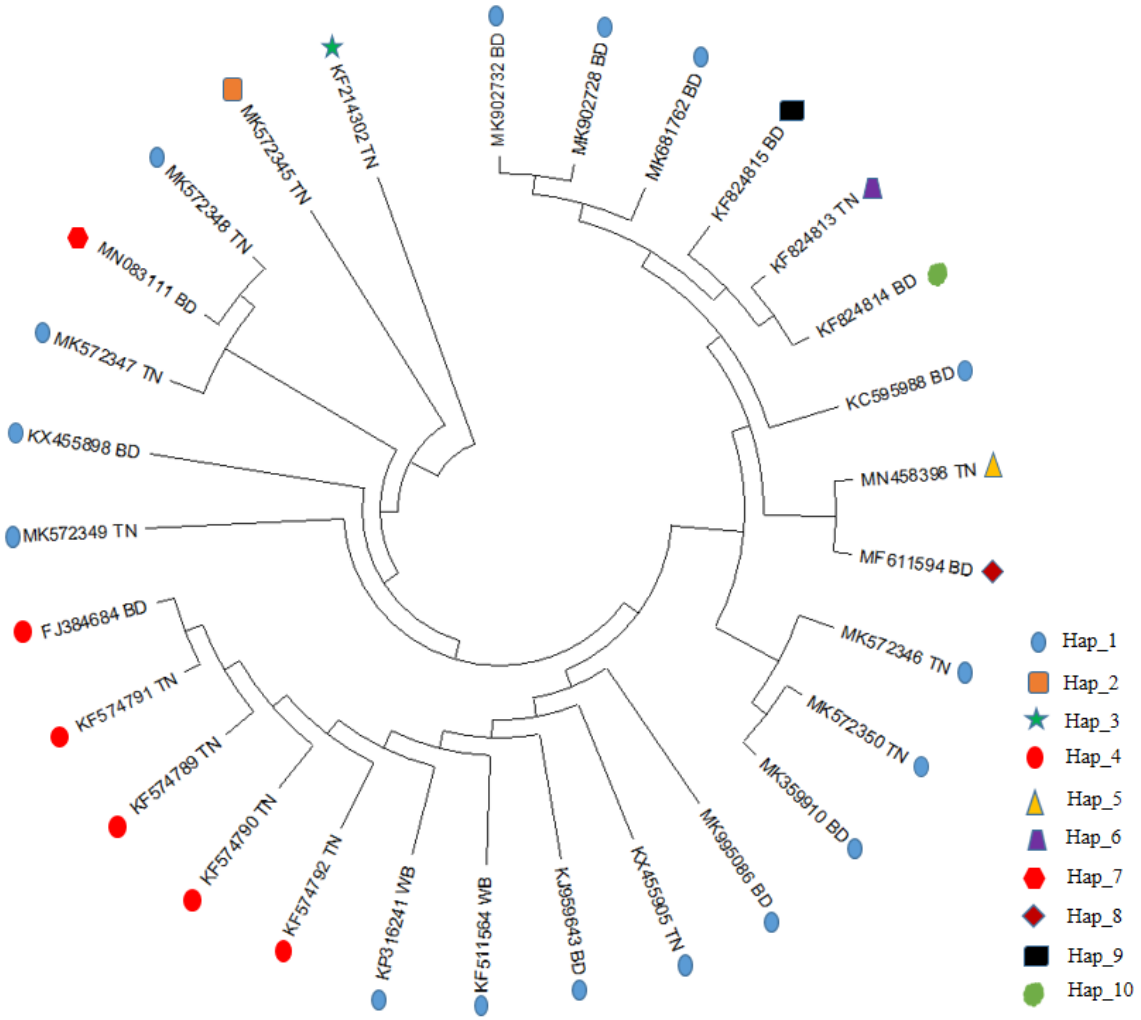

Fig. 2 Intra-specific phylogram based on neighbour joining of *Mystus gulio* in Tamil Nadu, West Bengal, and Bangladesh population. Different shapes and colour symbols indicate different haplotypes.

Intra-specific phylogenetic tree generated using NJ method of a total of 29 samples was not shown any clear relationship among the different haplotypes. So, the intra-specific divergence among the different populations was very complex. All individuals within haplotype 4 were monophyletic but in haplotype 1, all individuals were shown in polyphyletic relation. Individuals in haplotypes 5 and 8 & 6 and 10 were monophyletic (Fig. 2).

4. Discussion:

Genetic diversity regulates a vital role in the adaptation capability of a population in the face of fluctuating environmental conditions (Markert et al., 2010). The genetic distance was calculated using pairwise distance with Kimura 2 Parameter model. The genetic distances varied from 0.00 to 5.4% in individuals of populations 1 and 2 separately. But in population 1, more than 2% of genetic distances were more frequent than in population 2. Genetic distance among the three populations (populations 1, 2 and 3) were varying from 0.00 to 7.3%. This information revealed that the population and among the population contained different subgroups of the population. The population of *C. travancoricus* from two different geographical locations was different among the haplotypes (Jose et al., 2021). This study has reported that genetic divergence within and among the natural inhabiting natural population *M. gulio* has present. The high number of haplotypes were indicate that *M. gulio* can adapt according to its changing environment (Ciftci and Okumus, 2002; Jose et al., 2021).

A haplotype network is a widely used method for analysing and visualizing the relationships among DNA sequences within a population. The haplotype circle size is proportionate to the number of individuals sampled with that haplotype and the length of the lines is proportional to the number of mutations (Paradis, 2018). Higher frequency of the haplotypes are older and it's present in the interior side of the network and newer haplotypes are peripheral. Older haplotypes show a broad geographical distribution because their carriers have had a long time to disperse. Haplotypes with only one connection are connected to haplotypes from the same population because they are recently evolved and their carriers haven’t had time to disperse. Haplotype networks can help conservation decisions (Wilson et al., 2015). Our results show that haplotype 1 consists of 16 samples from all three geographical locations and were an older haplotype. Haplotype 4 contained five individual samples from populations 1 and 2. Haplotypes 3, 5, 4, 6, 7, and 9 were peripheral and they were recently evolved (Fig. 1).

References:

1. Abbas, E. M., Megahed, E. T., Hemeda, S. A., & ElNahas, A. F., 2018. DNA barcoding and molecular population structure of two species from genus Diplodus based on COI gene in the Egyptian Mediterranean Sea. International Journal of Fisheries and Aquatic Studies, 6(1), 1–8.

2. Çiftci, Y., & Okumuş, İ., 2002. Fish population genetics and applications of molecular markers to fisheries and aquaculture: I-Basic principles of fish population genetics. Turkish Journal of Fisheries and Aquatic Sciences, 2(2), 145–155.
3. Day F., 1878. The fishes of India; being a natural history of the fishes known to inhabit the seas and fresh waters of India, Burma, and Ceylon. William Dowson and Sons, London. 778 p.
4. Deepak, J., & Harikrishnan, M., 2016. Non-homologous COI barcode regions: A serious concern in decapod molecular taxonomy. Mitochondrial DNA Part A, 28(4), 482–492. https://doi. org/10.3109/19401736.2015.1137902
5. Gupta, S., Banerjee S., 2014. Indigenous ornamental fish trade of West Bengal. Narendra Publishing House, New Delhi. Pp 63.
6. Jhingran V.G., 1997. Fish and fisheries of India. Hindustan Publishing Corporation, Delhi, India. xxiii, 727 p.
7. Jose, D., Mahadevan, H. & Kalathil Mukundan, A., 2021. Morphological and Genetic variations in the natural populations of Carinotetraodon travancoricus. Journal of Applied Ichthyology, *37*(2), pp.246-257.
8. Kottelat M., 2001. Freshwater fishes of northern Vietnam. A preliminary check-list of the fishes known or expected to occur in northern Vietnam with comments on systematics and nomenclature. The World Bank, Environment and Social Development Unit, East Asia and Pacific region, Washington D.C. 123 p
9. Kumar, S., Stecher, G., & Tamura, K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33, 1870–1874.
10. Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, *25*(11), 1451–1452. https://doi.org/10.1093/bioin forma tics/btp187
11. Mukhopadhyay, T., & Bhattacharjee, S., 2014. Study of the genetic diversity of the ornamental fish Badis badis (Hamilton-Buchanan, 1822) in the Terai region of sub-Himalayan West Bengal, India. International Journal of Biodiversity, 2014, 1. https://doi.org/10.1155/2014/791364
12. Ng H. H., 2010. *Mystus gulio*. The IUCN Red List of Threatened Species. Version 2014.2.
13. Paradis, E., 2018. Analysis of haplotype networks: The randomized minimum spanning tree method. Methods in Ecology and Evolution, *9*(5), pp.1308-1317.
14. Roberts T.R., 1993. The freshwater fishes of Java, as observed by Kuhl and van Hasselt in 1820-1823 Zoologische Verhandelingen, 285(1): 1-94.
15. Sarker, P. K., Pal, H. K., Rahman, M. M., & Rahman, M. M., 2002. Observation on the fecundity and gonado-somatic index of *Mystus gulio* in brackishwater of Bangladesh. Journal of Biological Sciences. 2(4): 235-237.
16. Senarathne P., &Pathiratne K.A.S., 2007. Accumulation of heavy metals in a food fish, Mystus gulio inhabiting Bolgoda Lake, Sri Lanka. Sri Lanka Journal of Aquatic Sciences, 12: 61-75.
17. Shafi M., & Quddus M.M.A., 2001. Bangladesher Matsho Shampad. Fisheries of Bangladesh. Kabir Publication, Dhaka, Bangladesh. pp: 186-187

18. Shen, Y., Guan, L., Wang, D., & Gan, X., 2016. DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. Ecology and Evolution, 6(9), 2702–2713. https://doi. org/10.1002/ece3.2060
19. Talwar P.K., &Jhingran A.G., 1991. Inland fishes of India and adjacent countries. Vol-1 and Vol-2. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, Bombay and Calcutta. 1063 p.
20. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, *25*(24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876
21. Tripathi S.D., 1996. Present status of breeding and culture of catfishes in south Asia. In: M. Legendre, J.P. Proteau (ed.). The biology and culture of catfishes. Aquatic Living Resources. 9, Hors Serie. pp: 219-228
22. Weber M.C.W. &de Beaufort L.F., 1913. The fishes of the Indo-Australian archipelago. II. Malacopterygii, Myctophoidea, Ostariophysi: I Siluroidea. E.J. Brill Ltd. Leiden. 404 p.
23. Wilson, A.G., Chan, Y., Taylor, S.S. &Arcese, P., 2015. Genetic divergence of an avian endemic on the Californian Channel Islands. PLoS One, *10*(8), p.e01344