

The Identity and Distribution of Striped Bagrid Catfish, *Mystus Tengara* (Hamilton 1822) Revealed Through Integrative Taxonomy

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Research Article

Keywords: bagrids, genetic distance, *Mystus tengara*, phylogeny

Posted Date: August 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-746005/v1>

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Version of Record: A version of this preprint was published at Molecular Biology Reports on November 1st, 2021. See the published version at <https://doi.org/10.1007/s11033-021-06880-2>.

Abstract

In the present study, an integrated taxonomic approach has been applied to clarify the taxonomic status, identity and distribution of bagrid catfish, *Mystus tengara*. Comparative morphometric evaluation of *M. tengara* identified in the present study from distant geographical location revealed variations of the traits in response to body length and environment, without significant genetic distance. The observed morphometric traits of *M. tengara* were found to be overlapping with available morphometric traits of *M. tengara*, *M. carcio* and *M. vittatus*. Maximum likelihood phylogenetic analysis based on mitochondrial cytochrome c oxidase (*COI*) gene also could not resolve their identity, and five paraphyletic clades comprising of *M. tengara*, *M. vittatus* and *M. carcio* from India, Nepal and Bangladesh were observed. Morphological and genetic evidence along with comparative evaluation of *M. tengara*, from its type locality, we consider *M. tengara* identified in the present study to be true, with its distribution extending from North East India to West Bengal, North India, Central India, Northern peninsular India and Bangladesh. The observation of paraphyletic subclade and evaluation of genetic distance between subclades reveals, there could be at least four cryptic species in this group. Further confirmation on the identity of *M. vittatus* and *M. carcio*, by integrated taxonomic approach based on fresh specimens collected from type locality, is required.

1. Introduction

Genus *Mystus* Scopoli 1777 (Teleostei: Bagridae) comprises of small to medium-sized freshwater and estuarine catfishes distributed from the Middle East to South, and South East Asia [1]. Currently, 42 species are considered valid within the genus, of which, 15 species are reported in India. The taxonomic validity of an additional six species, described from India, requires confirmation as they have been published in 'predatory journals' and are considered 'unavailable' [2]. The taxonomy of members of the genus *Mystus* is in flux, as many species are morphologically similar, and subtle diagnostic characters have been used to delimit the species [1]. Therefore, accurate species-level identification using morphological characters alone is problematic [3]. Further, as the monophyly of the genus has been considered doubtful [4], several studies continue to be carried out on the molecular phylogenetics and genetic based resolution of species level identities [5, 6].

Mystus tengara, *M. vittatus* and *M. carcio*, three common 'striped' bagrid catfishes distributed on the Indian subcontinent, are used as both food fish and in the aquarium trade [7]. The three species have ambiguous taxonomic history, and thus their identity is confusing as they share similar and often overlapping morphological characters [7, 8]. Initially, *M. vittatus* was described from Tranquebar (Tamil Nadu), India [9] and subsequently described *M. tengara* and *M. carcio* from the erstwhile Bengal Presidency [10]. As the original description of *M. tengara* and *M. carcio* were based on limited number of diagnostic characters [11], many subsequent authors considered *M. carcio* as a junior synonym of either *M. tengara* [3, 12] or *M. vittatus* [12–17]. Some researchers also considered *M. tengara* as a junior synonym of *M. vittatus* [14–16, 18].

Several authors attempted to clarify the long-standing confusion in the literature by re-describing *M. carcio* and *M. tengara* [11, 19]. They also confirmed that *M. tengara*, *M. carcio* and *M. vittatus* are distinct species. Nevertheless, the molecular phylogeny and geographical distribution of these three species have not been studied. Further, studies reported the occurrence of these species far away from their type locality. For example, *M. vittatus*, described from south-eastern part of the India (Tamil Nadu) was subsequently recorded from North-East India [20–22]. Similarly, various authors have recorded *M. tengara*, a species described from Bengal, in Southern peninsular India [23]. Though, the validity of these records has been debated [11, 24] several genetic sequences presumably of the three species collected from distinct geographical regions are available; thus, necessitating a study to understand and clarify the identity and distribution of *M. tengara*, *M. carcio* and *M. vittatus*. In the present study, we have attempted to fill this knowledge gap using an integrated taxonomic approach.

2. Materials And Methods

2.1. Study area and sampling

Specimens of *M. tengara* were collected from the Sodepur fish market ($n = 5$), West Bengal, and from Nath Sagar ($n = 9$), Godavari River ($19^{\circ}32'05.9''N$, $75^{\circ}20'09.7''E$), Maharashtra, India. For molecular analysis, fin clips along the left side of the specimens were stored in 95% ethanol. All samples were preserved in 10% formalin for morphological studies.

2.2. Morphometrics and meristics

The morphometric characters were measured with an automated digital caliper (to the nearest 0.1 mm), and counts were recorded from left side of the fish, following the standard literature [25]. Measurements were reported as percentages of standard length (SL), whereas subunits along the head region were presented as percentages of head length (HL). Species level identification was confirmed by using available taxonomic literature given by eminent taxonomists [3, 10–11, 17, 19, 26–27].

2.3. Deoxyribonucleic acid (DNA) extraction, Polymerase chain reaction (PCR) amplification and sequencing

Genomic DNA was isolated from the muscle tissue ($n = 6$) using Phenol-Chloroform method [28] PCR amplification of mitochondrial cytochrome *c* oxidase (*COI*) gene was carried out using the primers FishF1: 5'-TCAACCAACCACAAAGAC ATTGGCAC3' and FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' [29]. PCR amplified product was analyzed on 1.5 % agarose gel and both sense and antisense strands were sequenced by Xcelris Lab Limited (Gujarat, India). The generated sequences have been deposited in GenBank with accession numbers MT928144 to MT928148 and MT928150.

2.4. Data analysis

The dataset was prepared including sequences generated in the present study (Five *COI* sequences of *Mystus tengara* and one *COI* sequence of *Mystus cf. tengara*) and those reported in GeneBank (*M. tengara*-27, *M. vittatus*-41, *M. carcio*-8 and other species of the genus *Mystus* - 22) (Supplementary Table 1). Sequences of *Hemibagrus menoda* and *H. punctatus* were used as outgroup. All the sequences were aligned using Clustal W program [30] (Supplementary Table 2). Phylogenetic tree was built using the maximum likelihood (ML) approach employing PhyML plugin in Genious Prime v 2019.1.3. The most appropriate model was selected employing jModeltest v2.1 (31) under the Akaike information criterion (AIC), as recommended [32]. Best-fit model of sequence evolution was HKY + I + G. The gamma distribution parameter was obtained using jModeltest v2.1, and the robustness of tree topology was estimated by bootstrap analysis based on 1000 replicates. Intra and inter-specific genetic distance values were estimated using the Kimura 2-parameter model using MEGA7 software [29, 33].

3. Results

3.1. Description

A comparative description of morphometric characters of *M. tengara* (those determined from the present study and published literature), *M. carcio* and *M. vittatus* are presented for differentiation (Table 1). *Mystus tengara* (Fig. 1.) can be distinguished from all other congeners by the following combination of characters: Eye diameter 20.06–26.8% of HL; dorsal spine length 11.44–17.79% of SL; length of adipose fin base 22.62–35.31% of SL; post adipose distance 11.92–15.80% of SL; 12–17 serrae along the posterior margin of pectoral fin; tympanic spot present; presence of four longitudinal stripes separated by three pale interspaces.

Table 1
Morphological features of *M. tengara*, *M. vittatus* and *M. carcio*

	Present study				Darshan et al. 2013		Sudasinghe et al. 2016			Darshan et al. 2010		
	<i>M. tengara</i> (n = 5) West Bengal		<i>M. tengara</i> (n = 9) Maharashtra		<i>M. tengara</i> (n = 36)		<i>M.tengara</i> (n = 6)		<i>M. vittatus</i> (n = 6)		<i>M. carcio</i> (n = 32)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean± S.D.	Range	Mean ±S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
Standard length	60.77– 67.95	64.48 ± 2.95	81.06– 98.58	90.61± 5.38	-	-	59.5– 85.2	-	85.9– 115	-	-	-
<i>In percentage (%) of SL</i>												
Pre-dorsal length	37.95– 41.37	39.71 ± 1.28	34.87– 40.52	37.42± 2.20	37.6– 40.8	40.2 ± 1.5	36.5– 39.5	38.2 ± 1.1	37.8– 39.7	38.8 ± 1.0	41.5– 45.8	43.9 ± 1.4
Pre-anal length	69.63– 77.09	74.28 ± 3.18	70.29– 73.00	71.6± 1.02	71.2– 72.6	71.9± 0.7	68.6– 73.8	70.5 ± 1.9	70.4– 72.5	71.3 ± 1.0	67.3– 72.0	69.1 ± 1.8
Pre-pelvic length	51.20– 53.03	53.03 ± 1.29	52.70– 56.64	54.65± 1.44	49.1– 55.4	52.2± 1.9	48.6– 53.4	50.6 ± 1.7	50.5– 54.4	53.0 ± 1.9	52.9– 58.0	55.2 ± 1.7
Pre-pectoral length	21.46– 25.00	22.67 ± 1.46	19.01– 24.13	21.57± 2.04	22.3– 25.4	23.6± 0.8	22.9– 25.4	24.1 ± 1.2	22.4– 24.4	23.3 ± 1.0	23.2– 31.5	26.1 ± 2.7
Length of dorsal-fin base	14.24– 15.49	14.91 ± 0.59	13.34– 16.14	14.75± 0.96	12.2– 15.2	13.9± 1.2	15.7– 17.4	16.7 ± 0.7	15.0– 15.7	15.3 ± 0.4	10.8– 14.4	13.1 ± 1.3
Dorsal spine length	14.23– 17.79	15.78 ± 1.43	11.44– 13.31	12.54± 0.68	12.3– 17.2	14.1± 1.8	11.6– 15.2	13.3 ± 1.2	10.7– 12.8	12.1 ± 1.0	16.3– 23.5	19.5 ± 2.3
Anal fin length	18.75– 20.26	19.45 ± 0.56	16.36– 18.84	17.58± 0.98	16.9– 20.4	18.2± 1.0	-	-	-	-	19.8– 22.6	21.4 ± 1.0
Pelvic fin length	14.03– 15.53	14.76 ± 0.60	12.70– 15.78	14.31± 1.18	13.9– 17.8	15.7± 1.0	14.8– 17.3	20.8 ± 1.3	13.2– 16.1	14.5 ± 1.3	13.2– 16.7	16± 1.3
Pectoral fin length	20.89– 24.67	22.29 ± 1.41	17.61– 21.77	19.07± 1.56	17.9– 23.2	20.1± 1.4	19.4– 21.8	15.6 ± 1.4	18.9	-	22.8– 32.1	26.1 ± 3.4
Pectoral spine length	18.76– 20.19	19.68 ± 0.55	16.55– 17.77	17.08± 0.41	15.0– 20.4	17.6± 1.6	-	-	-	-	22.1– 31.1	24.8 ± 3.3
Caudal fin length	22.31– 31.89	28.86 ± 3.80	24.00– 28.44	25.75± 1.73	26.6– 30.7	28.3± 1.5	29.0– 29.5	29.3 ± 0.4	28.5– 31.4	29.6 ± 1.3	27.8– 30.8	29.3 ± 0.9
Length of adipose-fin base	22.62– 30.51	27.46 ± 3.42	27.40– 35.31	31.7± 3.32	24.0– 31.7	28.8± 2.2	20.8– 29.5	26.8 ± 3.1	18.2– 27.2	22.9 ± 4.6	8.5– 11.9	10.3 ± 1.2
Adipose maximum height	4.18– 6.13	5.18 ± 0.70	4.87– 6.84	5.5± 0.74	4.0– 5.7	5.3± 0.5	4.6– 6.1	5.3 ± 0.6	4.2– 6.0	5.1 ± 0.8	3.9– 6.1	4.7 ± 0.7
Post adipose distance	12.06– 15.80	14.59 ± 1.51	11.92– 15.18	13.36± 1.21	13.6– 17.1	15.4± 1.0	10.7– 16.7	15.0 ± 2.3	15.6– 17.3	16.3 ± 0.8	17.9– 20.5	19.0 ± 1.0
Caudal peduncle length	14.72– 16.62	15.74 ± 0.79	14.95– 18.29	16.67± 1.2	16.3– 19.9	17.9± 1.2	16.6– 19.2	18.2 ± 1.0	15.5– 19.8	18.1 ± 1.9	15.4– 17.2	16.6 ± 0.6

	Present study				Darshan et al. 2013		Sudasinghe et al. 2016				Darshan et. al. 2010	
Caudal peduncle depth	9.46–11.69	10.76 ± 0.96	10.37–11.80	11.07 ± 1.17	9.8–11.6	10.8 ± 0.6	10.4–11.7	10.8 ± 0.6	10.6–13.5	11.8 ± 1.3	11.5–13.6	12.1 ± 0.7
Body depth at anus	17.48–19.44	18.53 ± 0.82	19.15–23.98	21.64 ± 1.94	20.7–24.3	21.6 ± 1.4	18.2–22.6	19.9 ± 1.6	17.8–23.2	20.2 ± 2.4	24.4–28.2	26.8 ± 1.6
Head length	25.92–29.94	28.00 ± 1.62	28.25–31.79	30.29 ± 1.16	26.9–28.9	28.0 ± 0.7	27.0–29.0	27.8 ± 0.8	26.2–27.5	26.9 ± 0.6	28.5–35.8	30.9 ± 2.4
Head width	19.18–22.65	21.00 ± 1.50	19.47–21.09	20.1 ± 0.64	16.5–19.6	17.9 ± 0.9	16.7–19.6	18.3 ± 1.0	18.1–21.7	19.7 ± 1.6	15.7–17.7	16.9 ± 0.8
Head depth	17.05–21.27	18.17 ± 1.75	16.21–19.04	17.53 ± 1.02	16.2–19.8	17.9 ± 2.4	17.3–20.3	18.2 ± 1.1	16.4–18.4	17.5 ± 0.8	21.9–25.9	23.9 ± 1.4
<i>In percentage (%) of HL</i>												
Snout length	31.06–33.56	32.06 ± 0.95	26.29–32.85	29.4 ± 2.47	32.7–36.5	34.8 ± 1.6	34–37	35.8 ± 1.3	34–37	35.8 ± 1.3	29.3–35.2	32.1 ± 2.1
Inter-orbital distance	29.76–35.21	32.06 ± 2.30	26.24–31.95	29.5 ± 1.95	32.3–37.5	35.5 ± 1.7	34–38	36.2 ± 1.4	35–38	36.4 ± 1.5	39.3–42.3	40.8 ± 0.9
Eye diameter	25.05–26.8	25.73 ± 0.69	20.06–26.00	22.2 ± 2.22	19.0–23.8	21.1 ± 1.4	22–24	23.7 ± 0.7	17–19	17.9 ± 0.9	25.6–30.7	27.2 ± 1.9
Nasal barbel length	56.61–71.06	64.88 ± 5.51	52.77–69.76	59.15 ± 6.15	55.3–84.8	66.1 ± 7.9	68	-	48–60	53.4 ± 5.0	49.1–85.0	63.9 ± 11.5
Maxillary barbel length	299.77–322.20	313.6 ± 9.91	245.20–262.13	252.63 ± 7.00	254.5–360.5	297.6 ± 37.8	311	-	150–244	190.0 ± 39.0	151.9–195.8	174.4 ± 18.1
Inner mandibular barbel length	66.99–83.48	75.42 ± 6.67	62.80–72.82	66.06 ± 3.59	62.3–94.9	79.1 ± 11.2	63–100	81.8 ± 26.3	63–77	69.3 ± 6.5	46.2–72.1	64.2 ± 9.4
Outer mandibular barbel length	113.00–138.69	130.4 ± 10.19	107.04–17.55	111.11 ± 4.23	110.4–151.3	135.3 ± 10.7	139–141	140. ± 1.6	95–104	99.0 ± 4.3	97.5	102.5 ± 5.5

Body moderately compressed. Dorsal profile rising evenly from tip of snout to origin of dorsal fin and sloping ventrally from origin of dorsal fin to end of caudal peduncle. Ventral profile more convex up to anal fin base, then sloping slightly dorsally to end of caudal peduncle. Bony elements of dorsal surface of head covered with thin skin. Anterior cranial fontanel extending from level of posterior nasal opening to posterior orbital margin. Posterior cranial fontanel long, invading the region of supraoccipital bone, and reaching base of the occipital process in juvenile specimens. Occipital process reaching basal bone of dorsal fin (West Bengal specimens), and in some cases a considerable gap seen between occipital process and basal bone of dorsal fin (Maharashtra specimens). Eyes located on dorsal half of head. Gill membranes free from isthmus. Mouth sub terminal, with moderately fleshy lips. Teeth small and villiform. Barbels 4 pairs; maxillary barbel reaching anal fin, sometimes extending just beyond anal fin or reaching caudal fin base in juvenile specimens; nasal barbel reaching base of occipital process; inner mandibular barbel reaching pectoral fin base, and outer mandibular barbel reaching posterior tip of pectoral fin.

Skin smooth. Lateral line complete and mid lateral in position. Dorsal fin with a spinelet, one spine and 7 branched rays; dorsal fin spine moderately long (11.44–17.79% SL) with 7 serrations on its posterior edge. Pectoral fin with stout spine, sharply pointed at its tip with 7(3)–8(11) rays. Anterior spine margin smooth; posterior spine margin with 12 (4), 14(4) or 15 (6) serrations along its entire length. Distal margin of pectoral fin straight. Pelvic fin short, slightly convex with 5 rays. Adipose fin not reaching base of last dorsal fin ray, length of its base

about 22.62–35.31% of SL. Anal fin with ii,8 (5); iii 8 (9) rays. Caudal fin forked with i,7,7,i (8) ; i,7,8,i (3); i,8,8,i (3) rays, upper lobe slightly longer than lower.

3.2. Coloration:

In fresh condition, body greenish to bright yellow with dark brown to black stripes on either side of the body along with a dark tympanic spot above the pectoral fin. In 10% formalin, dorsal surface of the head and body pale brown; ventral surface of the head and body dirty white. Dark spot in tympanic region present. Four pale brown lateral stripes separated by pale interspaces on both sides.

3.3. Phylogenetic and genetic distance analysis

In the phylogenetic tree, sequences labelled as *M. tengara*, *M. vittatus* and *M. carcio* formed four paraphyletic clades with significant bootstrap values (Fig. 2–4). However, these values were not high to signify relationship between clades.

Clade I comprises of *Mystus tengara* (samples collected from Maharashtra, Western India and West Bengal, Eastern India, as a part of present study; published sequences from Assam, North-eastern India and Bangladesh); *M. vittatus* (reported from Assam, Tripura, Manipur, Meghalaya - North-east India; West Bengal, Andhra Pradesh-Eastern India; Madhya Pradesh, Telangana - Central India; Maharashtra - Western India and Korea), *M. carcio* (Assam) and *M. horai* (Uttar Pradesh). Clade II includes species of *M. carcio* (Bangladesh) and *M. tengara* (North-east India, Bangladesh and Korea). *Mystustengara*, recorded from Assam (MH156942), formed a separate branch in the tree, which was observed to be a sister group to clade II. Clade III comprised exclusively of various populations of *M. vittatus* recorded from northern India (Uttarakhand), north-east India (Arunachal Pradesh), central India (Madhya Pradesh) and Nepal. Clade IV comprised of specimens of *Mystus cf. tengara* (eastern India: West Bengal) collected in the present study, and *M. vittatus* (reported from north-east India). Clade III and Clade IV were observed to be sister groups with significant bootstrap values. The published sequences of *M. tengara* from Uttar Pradesh formed a distinct clade which occupied the basal position in the phylogenetic tree (Clade V).

The average genetic distance values within and between clades are provided in Table 2. The genetic divergence value among clades ranged from 9.0–11.3 (Clade I-II), 12.1–18.0 (Clade I-III), 12.6–13.8 (Clade I-IV), 17.8–19.0 (Clade I-V), 9.1–16.3 (Clade II-III), 8.4–9.6 (Clade II-Clade IV), 16.2–17.4 (Clade II- V), 3–17.1 (Clade III-IV), 3.0–3.8 (clade III-V) and 16.2–17.4 (Clade IV-V) (Supplementary Table 2).

Table 2
Pair-wise average genetic distance values within and among the clades in the phylogenetic tree

Clades	Clade I	Clade II	Clade III	Clade IV	Clade V
Clade I	0.003				
Clade II	10.6	0.004			
Clade III	12.5	12.3	0.001		
Clade IV	13.1	8.8	10.1	0.009	
Clade V	18.2	16.0	3.0	16.0	0.002
Note: Values in diagonal represents within clade genetic distance values					

4. Discussion

4.1. Comparative morphometric evaluation

The present study used an integrated taxonomic approach to resolve the identity and distribution of *Mystus tengara*. Comparative morphological evaluation of freshly collected specimens of *M. tengara*, from West Bengal, showed close similarity to the original description [10], in having four longitudinal stripes separated by 3 pale interspaces, presence of large tympanic spot above pectoral fin, length of four barbels longer than head, and occipital process reaching basal bone of dorsal fin. Specimens of *M. tengara* collected from Maharashtra also match with the description of Hamilton for *M. tengara*, except with the presence of small interspace between occipital process and dorsal fin base.

M. tengara is differentiated from *M. vittatus* (10) by the absence of serrations in dorsal spine (vs. presence), a character which suggested to be an error [27], based on Gunther's description of "*Macrones tengara*". *M. tengara* further differentiated from *M. vittatus* [26] by median longitudinal groove reaching to base of occipital process and occipital process reaching basal bone of dorsal fin vs. median longitudinal groove reaching midway behind the hind edge of the eye and base of the occipital process and short interspace between occipital process

and basal bone of dorsal fin. In the present study, we observed variations in median longitudinal groove with size and geographical locations.

The re-description of *M. tengara* [11] to establish and confirm its taxonomic identity and differentiated this species from *M. vittatus* in having a longer maxillary barbel length (254.5–360.5% HL vs. 214.3–244.9% HL), dorsal spine length (12.3–17.2% SL vs. 10.7–12.2) and length of median longitudinal groove (reaching base of occipital process in juvenile or reaching anterior one third of supraoccipital bone in adult vs. terminating at the anterior border of supraoccipital bone not invading the supraoccipital region). In the present study, specimens of *M. tengara*, from Eastern India (West Bengal), showed variations from specimens collected from Western India (Maharashtra) in maxillary barbel length, dorsal spine length (14.23–17.79% SL vs. 11.44–13.31) and extent of occipital process (reaching dorsal fin base vs. not reaching) but, without much overall variations from the morphometric description of *M. tengara* and *M. vittatus* [11, 24], which shows that the effects of environmental variations in this trait cannot be ruled out.

4.2. DNA barcoding and phylogenetic study

DNA barcodes have been used for confirming identity of species and their distribution [35]. Previous studies have shown that genetic divergence value of 2–3% at DNA barcoding gene (COI) could be used as threshold value to discriminate species [36, 37]. Accordingly, conspecific individuals show genetic divergence value of < 3%, while congeneric species > 3%. During the present study, in Clade I, sequences identified as *M. vittatus*, *M. carcio* and *M. horai* from different geographical locations, were clustered with '*M. tengara*' (collected in the present study) having a genetic distance of < 3%. This observation suggests that the sequences identified and labelled as *M. carcio*/*M. vittatus*/*M. horai* in GenBank could be misidentifications of *M. tengara*. The original description of *M. carcio* (10) which distinguishes it from *M. tengara* in the length of maxillary barbel (extending beyond pectoral vs. reaching to end of caudal and serrations on dorsal spine-presence vs absence).

The redescription of *M. carcio* [19] where it is further distinguish it from *M. tengara* and *M. vittatus* based on shorter adipose-fin base length (8.5–11.9% SL vs. 24.0–31.7 and 21.5–26.9% SL respectively) and posterior fontanel length (reaching base of supraoccipital process vs. not reaching middle of supra occipital bone vs. terminating at the anterior tip of supraoccipital respectively). The description of median longitudinal groove in *M. vittatus*-terminating at the anterior border of supraoccipital bone, not invading the supraoccipital region [19], is not in agreement with [26] whose description of median longitudinal groove in adult specimens of *M. tengara* not reaching beyond the middle of supra occipital bone, is also not in agreement to the present study. However, *M. carcio*, re-described by the authors [19], was distinct in other characters from *M. tengara* identified in the present study. The re-description of *M. carcio* [19] was based on specimens collected from Assam, Tripura and Bangladesh but without any molecular evidence. In our phylogenetic analysis, specimens identified as *M. carcio* from Assam was grouped together with *M. tengara* sensu stricto, whereas, specimens identified as *M. carcio*, from Bangladesh, grouped together with *M. tengara* [1] clades with significant genetic variation to be considered distinct.

Further, specimens collected from West Bengal and Maharashtra could be distinguished in morphometric characters such as dorsal spine length (14.23–17.79 vs. 11.44–13.31), pectoral spine length (18.76–20.19 vs. 16.55–17.77), eye diameter (25.05–26.8 vs. 20.06–26.00), maxillary barbel length (reaching posterior tip of anal fin base or to caudal fin base in smaller specimens vs reaching anal fin base) and occipital process (reaching basal bone of dorsal fin vs. a considerable gap present), which reveals geographical variations in these diagnostic phenotypic characters. Similar to our observations, there is a geographical variation in maxillary barbel length [26] in *M. tengara* from Punjab and Assam (reaching to middle of the pectoral fin vs. reaching to the base of pelvic fin). These finding clearly indicates that these characters may be influenced by size of the fish and the environment, in which they inhabit and cannot be considered as good diagnostic characters for these species [3].

4.3. Molecular evidence reveals cryptic species

In clade II, sequences identified and labelled as *M. tengara* could likely be misidentifications as the genetic distance of these sequences with those in clade I are higher than 3%. *M. tengara* recorded from Assam (MH156942) also showed higher genetic distance with sequences in clade I and could be a distinct species. Clade III comprised of *M. vittatus* and this species was confirmed to be distributed in northern, north-eastern, western and central India. Interestingly, specimens of *Mystus* cf. *tengara*, the focus of the present study clustered with *M. vittatus* recorded from north-east India, and formed clade IV. Though a sister group relationship was observed between clade III and clade IV, the average genetic distance between these two clades was 10.1% suggesting the occurrence of cryptic species in this group. Species names and identities in clade V are also likely to be erroneous due to morphological ambiguities. Studies on generating reference DNA barcodes without morphological taxonomy could often lead to species misidentification [38–39], has been demonstrated recently in hillstream loaches of the Western Ghats [40]. Due to overlapping diagnostic characters and morphological similarities, various authors could have misidentified *M. tengara*, *M. vittatus* and *M. carcio* resulting in the deposition of erroneous sequences in NCBI GenBank.

Based on morphological and genetic evidence of freshly collected *M. tengara*, from its type locality, we consider sequences that form part of clade I to be *M. tengara sensu stricto*, with the distribution extending from North East India to West Bengal, North India, Central India, Northern peninsular India and Bangladesh. Further confirmation on the identity of *M. vittatus* and *M. carcio*, by integrated taxonomic approach based on freshly specimens collected from type locality, is required. The observation of paraphyletic subclade and evaluation of genetic distance between subclades reveals that there could be at least four cryptic species in this group, opening up avenues for future research on the group.

Declarations

Acknowledgements

The authors are grateful to the Dr. Gopal Krishna, Director, ICAR-Central Institute of Fisheries Education (CIFE), Mumbai for encouragement and the Indian Council of Agricultural Research for financial support in the form of a fellowship to Sangeetha M. Nair. The authors also express their sincere thanks to Madhavi Umesh Bhise, Research Fellow, ICAR-CIFE for the technical support during barcoding studies.

Declaration of competing interest

The authors declare no conflicts of interest in authorship and publication

Ethical statement

All the fish species caught for the research work are belonging to the food fish category and are not protected under The Wildlife Protection Act, 1972 (Last amended in 2017), Ministry of Environment and Forest, Government of India.

Funding

ICAR-Central Institute of Fisheries Education (CIFE) provided financial assistance for the work.

Authors' Contribution

Sangeetha M. Nair: Conceptualization, Investigation, Resources, Laboratory analysis, Writing-original draft, Writing-reviewing and editing. **Kavita Kumari:** Data curation, Software analysis, Writing-original draft, Writing-reviewing and editing. **Annam Pavan Kumar:** Software analysis, Writing-reviewing and editing. **Rajeev Raghavan:** Formal analysis, Writing-reviewing and editing. **A.K. Jaiswar:** Supervision and over all guidance

Consent to participate

Not applicable

Consent to publication

All the authors agreed to give the consent for publication of the content in the manuscript

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Figures



Figure 1

Mystus tengara collected from West Bengal (67.95 mm SL)

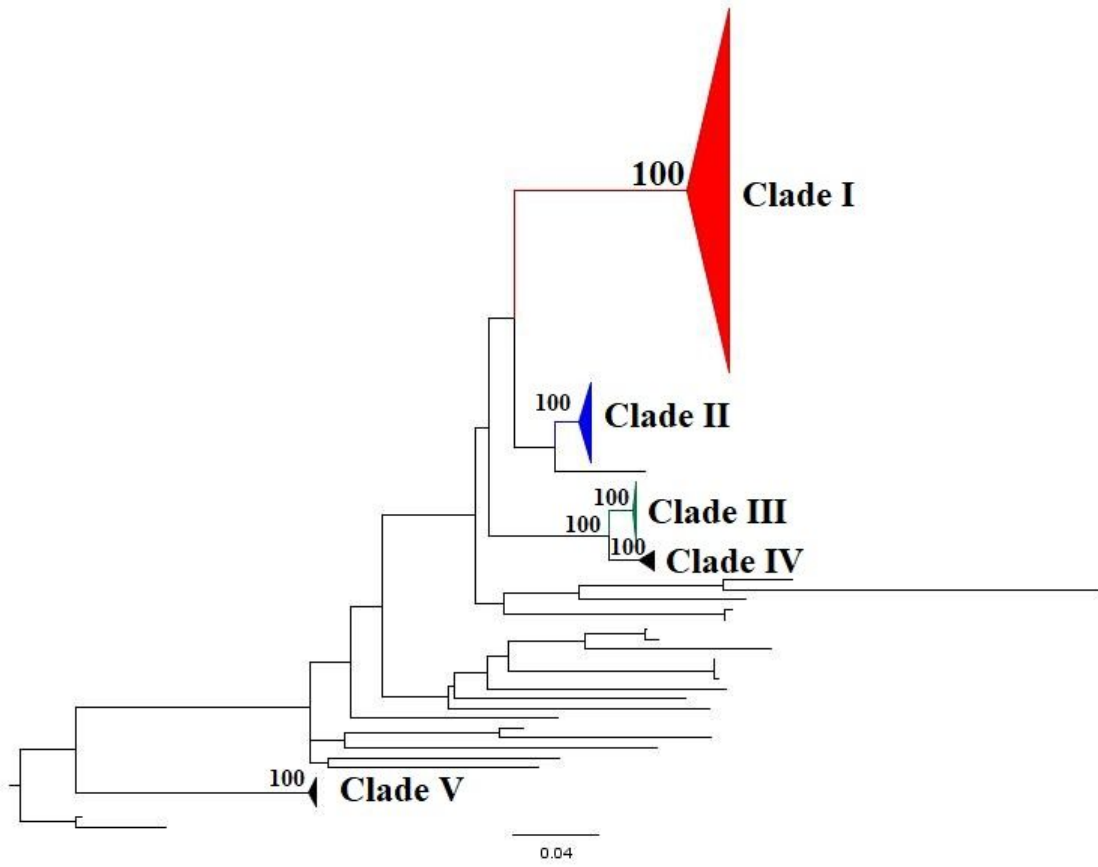


Figure 2

Phylogenetic tree showing major clades of species of *Mystus* identified as *M. tengara*, *M. vittatus* and *M. carcio* and their phylogenetic position within the genus

M. vittatus (KJ936696: Meghalaya)
M. vittatus (JX983385: Madhya Pradesh)
M. vittatus (JX260920: Maharashtra)
M. carcio (KX245092: Assam)
M. vittatus (KJ936695: Meghalaya)
M. vittatus (KJ936697: Meghalaya)
M. vittatus (KY769368: Telangana)
M. vittatus (MK359890: Korea)
M. vittatus (MK359926: Korea)
M. vittatus (MK359946: Korea)
M. vittatus (KJ959638: West Bengal)
M. vittatus (KF511563: West Bengal)
M. tengara (KJ909364: Bangladesh)
M. tengara (MK572352: Bangladesh)
M. tengara (MK599500: Arunachal Pradesh)
M. horai (FJ170791: Uttar Pradesh)
M. tengara (MT928147: Maharashtra Present study)
M. tengara (MT928145: Maharashtra Present study)
M. vittatus (JN228952: Tripura)
M. tengara (KJ909368: Bangladesh)
M. vittatus (MG641747: Andhra Pradesh)
M. tengara (MT928150: West Bengal Present study)
M. vittatus (JN628887: Assam)
M. tengara (MK572351: Bangladesh)
M. tengara (KT896742: Assam)
M. carcio (KT896740: Assam)
M. tengara (KJ909367: Bangladesh)
M. tengara (MT928148: West Bengal Present study)
M. vittatus (JN228950: Assam)
M. vittatus (JN228949: Assam)
M. vittatus (KT364780: Bangladesh)
M. vittatus (JO713855: Manipur)
M. vittatus (KY511143: Assam)
M. vittatus (MK359957)
M. tengara (MT928144: West Bengal Present study)
M. vittatus (JN228951: Assam)
M. tengara (MN083145: Bangladesh)
M. vittatus (JN228953: Assam)

Figure 3

Phylogenetic tree showing clade I (*M. tengara sensu stricto*)

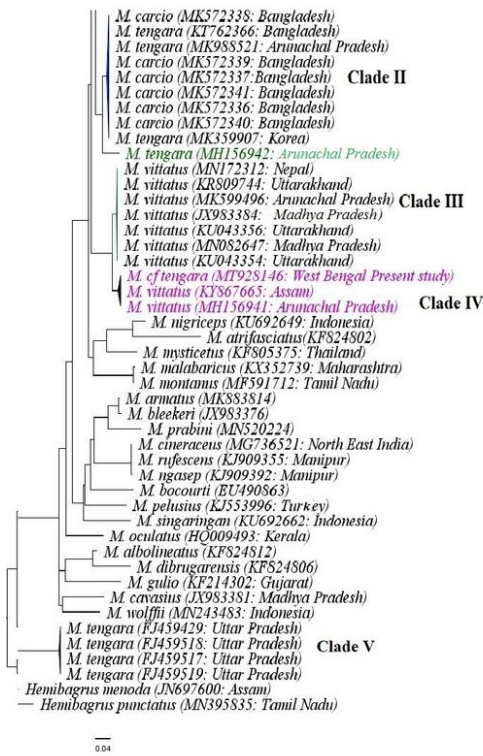


Figure 4

Phylogenetic tree of clade II, III, IV (Mystus identified as *M. tengara*, *M. vittatus* and *M. carcio*) and clade V (other species within the genus *Mystus*)

Supplementary Files

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