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ORIGINAL ARTICLE

The identity and distribution of striped bagrid catfish, *Mystus tengara* (Hamilton 1822) revealed through integrative taxonomy

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Abstract

Background The taxonomic status and geographical distribution of *M. tengara* are vague. No genetic diversity and phylogenetic study have been done till now to resolve its identity and distribution. In the present study, an integrated taxonomic approach has been applied to clarify the taxonomic status, identity, and distribution of bagrid catfish, *Mystus tengara*.

Methods and results Comparative morphometric evaluation of *M. tengara* identified in the present study from distant geographical locations 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 and Bayesian 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.

Conclusion The observation of paraphyletic subclades and evaluation of genetic distance between subclades reveals the presence of four cryptic species. Further confirmation on the identity of *M. vittatus* and *M. carcio*, by an integrated taxonomic approach based on fresh specimens collected from the type locality, is required.

Keywords Bagrids · Genetic distance · *Mystus tengara* · Phylogeny

Introduction

Genus *Mystus* Scopoli 1777 (Teleostei: Bagridae) comprises 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 additional six species, described from India, requires confirmation as they have been published in 'predatory journals' and are considered 'unavailable' [2].

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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, Bloch [9] described *M. vittatus* from Tranquebar (Tamil Nadu), India, and subsequently, *M. tengara* and *M. carcio* were described from the erstwhile Bengal Presidency by Hamilton [10]. As the original description of *M. tengara*

and *M. carcio* were based on the 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.* 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 the south-eastern part of 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.

Materials and methods

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°32′05.9″ N, 75°20′09.7″ E), Maharashtra, India. For molecular analysis, muscle tissue along the left side of the specimens were stored in 95% ethanol. All samples were preserved in 10% formalin for morphological studies.

Morphometrics and meristics

The morphometric characters were measured with an automated digital caliper (to the nearest 0.1 mm), and counts were recorded from the left side of the fish, following Chakraborty and Ng [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 [3, 10, 11, 17, 19, 26, 27]

DNA isolation and PCR amplification

Total genomic DNA was isolated from the muscle tissue (*n*=6) using the Phenol-Chloroform method [28]. The partial fragment of mitochondrial cytochrome c oxidase (*COI*) gene was amplified out using the method described by Ward et al. [29]. The PCR amplified products were purified using GelExtraction Kit (Qiagen, Germany) and both sense and antisense strands were sequenced by Xcelris Lab Limited (Gujarat, India). The generated sequences were deposited in GenBank with accession numbers MT928144- MT928148 and MT928150.

Molecular data analysis

A 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 NCBI GenBank (*M. tengara*-27, *M. vittatus*-41, *M. carcio*-8 and other species of the genus *Mystus*-22) (Online Resource 1). Sequences of *Hemibagrus menoda* and *H. punctatus* were used as outgroup. All the sequences were aligned using Clustal W program [30] (Online Resource 2). The phylogenetic tree was built using the maximum likelihood (ML) approach employing PhyML plugin and Bayesian (BI) approach using MrBayes 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), [32]. The 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].

Results

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 20.06–26.80% 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.

Body moderately compressed. Dorsal profile rising evenly from tip of snout to origin of the dorsal fin and sloping ventrally from the origin of the dorsal fin to end of caudal peduncle. Ventral profile more convex up to anal fin

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

	Present study				Darshan et al. [11]		Sudasinghe et al. [24]				Darshan et. al. [19]	
	M. tengara(n = 5) West Bengal		*M. tengara*(n = 9) Maharashtra		*M. tengara* (n = 36)		*M. tengara* (n = 6)		*M. vittatus* (n = 6)		*M. carcio* (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	–	–	–
In percentage (%) of SL												
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

Table 1 (continued)

	Present study				Darshan et al. [11]		Sudasinghe et al. [24]				Darshan et. al. [19]	
	M. tengara(n = 5) West Bengal		*M. tengara*(n = 9) Maharashtra		*M. tengara* (n = 36)		*M. tengara* (n = 6)		*M. vittatus* (n = 6)		*M. carcio* (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
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
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 depthat 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
In percentage (%) of HL												
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 diam-eter	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 mandibu-lar 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

Table 1 (continued)

	Present study				Darshan et al. [11]		Sudasinghe et al. [24]				Darshan et. al. [19]	
	M. tengara(n = 5) West Bengal		*M. tengara*(n = 9) Maharashtra		*M. tengara* (n = 36)		*M. tengara* (n = 6)		*M. vittatus* (n = 6)		*M. carcio* (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
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

base, then sloping slightly dorsally to end of caudal peduncle. Bony elements of the dorsal surface of head covered with thin skin. Anterior cranial fontanel extending from the level of posterior nasal opening to posterior orbital margin. Posterior cranial fontanel long, invading the region of supraoccipital bone and reaching the 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 the dorsal half of head. Gill membranes free from isthmus. Mouth subterminal, 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 the occipital process; inner mandibular barbel reaching pectoral fin base, and outer mandibular barbel reaching the 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 the 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 i,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.

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, the dorsal surface of the head and body pale brown; the 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.

Phylogenetic and genetic distance analysis

The maximum likelihood (Figs. 2 , 3, and 4) and Bayesian tree (Online Resource 3) revealed a similar topology. In the phylogenetic tree, sequences labeled as *M. tengara, M. vittatus*, and *M. carcio* formed four paraphyletic clades with significant bootstrap values However, these values were not high to signify the relationship between clades.

In the maximum likelihood tree, Clade I comprises *Mystus tengara* (samples collected from Maharashtra, Western India, and West Bengal, Eastern India, as a part of present

Fig. 1 *Mystus tengara* collected from West Bengal (67.95 mm SL)

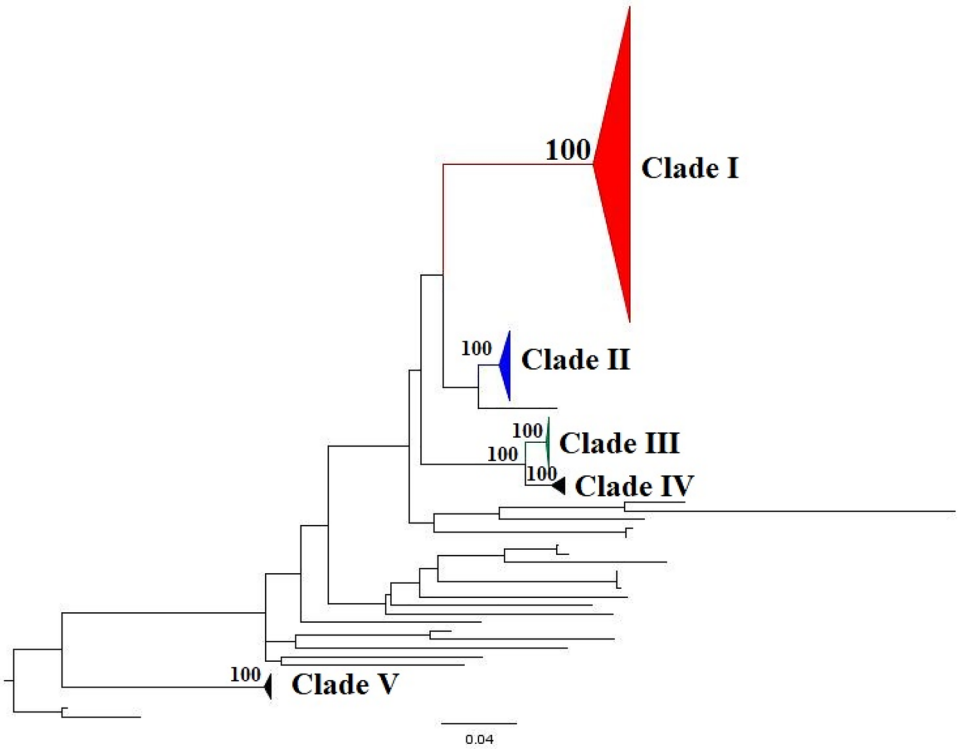

Fig. 2 Phylogenetic tree showing major clades ofspecies of *Mystus* identified as *M. tengara, M. vittatus,* and *M. carcio*and their phylogenetic position within the genus

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). *Mystus tengara*, 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 that occupied the basal position in the phylogenetic tree (Clade V).

The average genetic distance values within and between clades are provided in Table 2. Within *M. tengara* (the present study samples), the genetic distance values are ranged from 0.2 %(West Bengal- Maharashtra) 0.4% (West Bengal). Between *Mystus* cf. *tengara* and *M. tengara*, the average genetic distance value was 12.6 %. The genetic divergence value among clades ranged from 9.0 to 11.3 (Clade I-II),

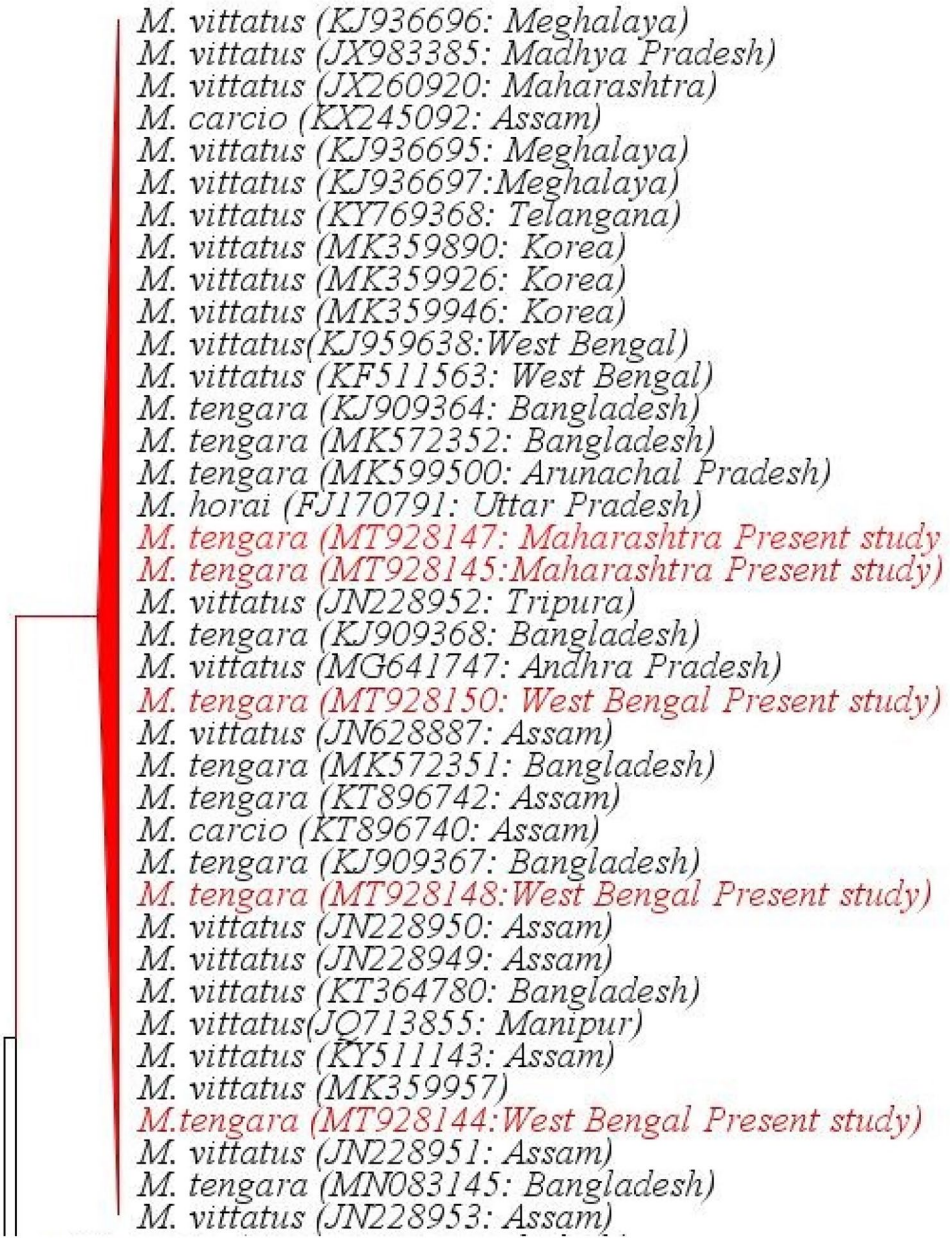

Fig. 3 Phylogenetic tree showing clade I (*M. tengara* sensustricto)

12.1–18.0 (Clade I–II), 12.6–13.8 (Clade I–IV), 17.8–19.0 (Clade I–V), 9.1–10.3 (Clade II-III), 8.2–9.3 (Clade II–Clade IV), 16.7–18.4 (Clade II–V),2.8-3.8 (Clade III–IV), 15.0-15.7 (clade III–V) and 15.9–17.4 (Clade IV–V) (Online Resource 2).

Discussion

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 the pectoral fin, length of four barbels longer than the 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 a small interspace between the occipital process and dorsal fin base.

M. tengara is differentiated from *M. vittatus* [10] by the absence of serrations in the dorsal spine (vs. presence), a character which is suggested to be an error [27], based on Gunther's description of "*Macrones tengara*". *M. tengara* further differentiated from *M. vittatus* [26] by median

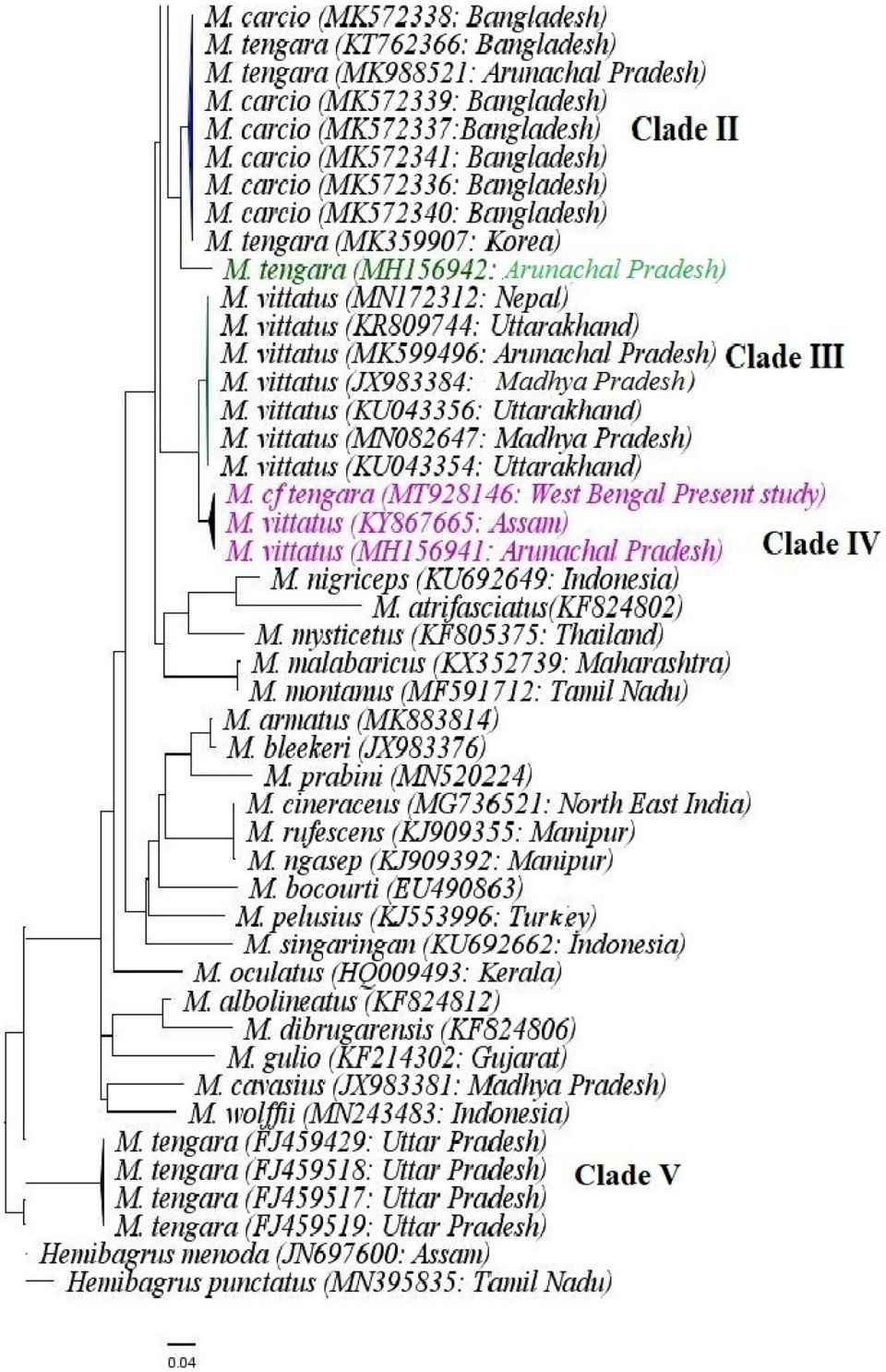

Fig. 4 Phylogenetic tree showing clade II, III, IV (*Mystus* identified as *M. tengara, M. vittatus*, and *M. carcio)* and clade V (*M. tengara*)

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

Darshan et al. [11] re-described *M. tengara* to establish and confirm its taxonomic identity and differentiated this species from *M. vittatus*. The specimens of *M. tengara* identified by Darshan et al. [11] varied from *M. vittatus* in having

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	0.4				
Clade II	10.6	0.4			
Clade III	12.5	9.8	0.2		
Clade IV	12.9	8.9	3.2	0.6	
Clade V	18.2	17.8	15.3	16.5	0.2

Values in the diagonal cell represents within clade genetic distance; values in below the diagonal represents between clade genetic distance values

a longer maxillary barbel (254.5–360.5 vs. 214.3–244.9), longer dorsal spine (12.3–17.2 vs. 10.7–12.2), and length of the 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 vs. 11.44–13.31), and extent of the occipital process (reaching dorsal fin base vs. not reaching) but, without any variations from the morphometric description of *M. tengara* and *M. vittatus* given by Darshan et al., and Sudasinghe et al., [11, 24], which shows that the effects of environmental variations in this trait cannot be ruled out.

DNA barcoding and phylogenetic study

DNA barcodes have been used for confirming the identity of species and their distribution [35]. Previous studies have shown that a genetic divergence value of 2–3% at DNA barcoding gene (COI) could be used as a threshold value to discriminate species [36, 37]. Accordingly, conspecific individuals show a 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%. The average genetic distance value among *M. tengara* specimens of the present study is 0.3%. These observations suggest that the sequences identified and labeled as *M. carico/M. vittatus/M. horai* in GenBank could be misidentifications of *M. tengara.*

Hamilton [10] described *M. carcio* and distinguished 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). Darshan et al. [19] revalidated *M. carcio* and distinguished it from *M. tengara* and *M. vittatus* based on shorter adipose-fin base length (8.5–11.9 vs. 24.0–31.7 and 21.5–26. 0 respectively) and posterior fontanel length (reaching the base of supraoccipital process vs. not reaching the middle of supra-occipital bone vs. terminating at the anterior tip of supraoccipital respectively). The description of the median longitudinal groove in *M. vittatus* terminating at the anterior border of supraoccipital bone, not invading the supraoccipital region by Darshan et al. [19], is not in agreement with Day [26]. Further, the description of the median longitudinal groove in adult specimens of *M. tengara* not reaching beyond the middle of supra-occipital bone by Day [26], is not in agreement with the present study. However, *M. carcio*, re-described by Darshan et al. [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 were grouped with *M. tengara* sensustricto, whereas, specimens identified as *M. carcio*, from Bangladesh, grouped with *M. tengara* clade II with significant genetic variation.

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 the 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 the middle of the pectoral fin vs. reaching to the base of pelvic fin). These finding indicates that these characters may be influenced by the size of the fish and the environment, in which they inhabit and cannot be considered as good diagnostic characters for these species [3].

Molecular evidence reveals cryptic species

In clade II, sequences identified and labeled 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 a higher genetic distance with sequences in clade I and could be a distinct species. Clade III is 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 3.2% suggesting the occurrence of distinct lineages or cryptic species in this group. Further based on morphometric and meristic data (Online Resource 4), we could not differentiate it from *M. tengara* and requires further confirmation based on more number of specimens. 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], which has been demonstrated recently in hill stream 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* sensustricto, 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 an integrated taxonomic approach based on freshly specimens collected from the type locality, is required. The observation of paraphyletic subclades 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.

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Data availability The sequence data generated in this study are deposited in NCBI GenBank. Details have been provided in the Material and Methods Sect. 2.3.

Code availability Not applicable.

Declarations

Conflict of interest The authors report no conflict of interest in authorship and publication.

Ethical approval 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.

Consent to participate Not applicable.

Consent for publication The authors give consent for the publication.

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