



Unveiling the Genetic Resemblances and Phylogenetic Relationship of Species Belonging to the Family *Mastacembelidae* (Teleostei: *Synbranchiiformes*) from Indian Waters

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study has been carried out to evaluate the genetic divergence among species of the family *Mastacembelidae* (common name: spiny eels) from India and to establish their phylogenetic relationship. Twenty reference sequences from NCBI GenBank database were obtained for five

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species viz. *Macrognathus aral*, *Macrognathus aculeatus*, *Macrognathus guentheri*, *Macrognathus pancalus* and *Mastacembelus armatus*, and used for the molecular analysis. The within species difference ranged between 0.32 to 1.12% except *Mastacembelus armatus* (9.00%). The lowest genetic distance was between *Macrognathus guentheri* and *Macrognathus pancalus* while the highest genetic distance was evident between *Macrognathus aculeatus* and *Mastacembelus armatus*. The findings of the present study may be used as baseline information on the molecular divergence of species belonging to the family *Mastacembelidae* from Indian water.

Keywords: Genetic divergence; *Mastacembelidae*; Phylogenetic; Indian water.

ABBREVIATIONS

NCBI : National Center for Biotechnology Information

COI : Cytochrome C oxidase subunit 1

NJ : Neighbour Joining

1. INTRODUCTION

India is one of the mega biodiversity hotspots, out of 36 global biodiversity hotspots, contributing to the World's biological resources from the Eastern Ghats on the East, the greater Himalaya range on the Northern Plains and the Western Ghats on the west [1]. In India, a total of 3439 species has been reported by Chandra et al. [2], out of which 1027 inhabits freshwater ecosystems [3,4,5]. Although, *Cypriniformes* is the most dominant order within the realm of freshwater habitats, other orders like *Siluriformes*, *Osteoglossiformes*, *Anabantiformes*, *Synbranchiformes* etc. also contribute significantly to the genetic diversity and hence, well-functioning of the ecosystems.

The order *Synbranchiformes*, commonly known as swamp eels comprise four families namely, *Synbranchidae*, *Chaudhuriidae*, *Indostomidae* and *Mastacembelidae*, with 18 genera and about 138 species [6], primarily found in fresh waters. Within the order *Synbranchiformes*, members of the family *Mastacembelidae* (Spiny eels) exhibits a wider distribution range in tropical Africa, the Middle East, Southeast Asia, China, and Korea, inhabiting diverse freshwater habitats such as lakes, streams and rivers [7]. *Mastacembelids* are anguilliform fishes [8], characterized by the presence of continuous dorsal and anal fins with a moderate caudal fin, tube-like lateral extension of the central rostral tentacle of the rostral appendage, and the absence of interarticular cartilage [9]. The tooth plates are merged to the hypobranchial, and the anterior nostrils are situated on the distal ends of the central rostral tentacle. The gill opening of *Mastacembelidae* species is reduced, owing to a connection of the

opercular membrane with the lateral wall of the body. The spiny eels have a long series of well-separated dorsal spines, a comparatively short series of anal spines, and are devoid of pelvic fins and the pelvic girdle. The long, eel-like body is covered by a large number of small cycloid scales in most species [8]. However, despite the available well described taxonomic characteristics, the identification and classification of the family *Mastacembelidae* is complicated.

Currently, the family *Mastacembelidae* is represented by three valid genera namely, *Macrognathus*, *Mastacembelus*, *Sinobdella* [9-11], consisting of a total of 97 valid species [6]. Some of these species can grow to a maximum length of about 1 m. However, even though they resemble the eel-like appearance of *Anguilliformes*, the family *Mastacembelidae* is not related to the afore-mentioned family [12]. In India, 10 species belonging to the family *Mastacembelidae* (Table 1) has been reported by Froese and Pauly [13]. Some of these species are used as food fishes, and some regularly appear in the aquarium trade owing to their brightly coloured body with distinctive patterns. Species of the family *Mastacembelidae* possess air-breathing ability and prefer to live in muddy water by burrowing in the mud [14].

DNA barcoding has proven to be an efficient tool to resolve the taxonomic ambiguities of morphologically similar species as well as to describe new species. It has also been successfully used in the freshwater fishes [15,16]. Moreover, phylogenetic relationships helps scientist to determine the phylogenetic value of species to assess priority for conservation activity [17]. Even though a few studies have been carried out to evaluate the phylogenetic relationship of species in the *Maastacembalidae* family around the globe, no such work has been reported from India. Hence, the present study has been undertaken to evaluate the existing genetic distance among the

Table 1. Checklist of the species belonging to the family *Mastacembelidae* from India (Froese and Pauly, 2024)

Sl. no	Species	Occurrence	Common name	IUCN status	Sequence obtained from NCBI
1.	<i>Macrognathus aculeatus</i> (Bloch, 1786)	Native	Lesser spiny eel	LC	MF122463, MF122462, MF122461, MF122460
2.	<i>Macrognathus albus</i> Plamoottil & Abraham, 2014	Native	Spotless spiny eel	NE	NA
3.	<i>Macrognathus aral</i> (Bloch & Schneider, 1801)	Native	One-stripe spiny eel	LC	MK572316, KY290064, MK572315, KY290063
4.	<i>Macrognathus fasciatus</i> Plamoottil & Abraham, 2014	Native	Yellow banded spiny eel	NE	NA
5.	<i>Macrognathus guentheri</i> (Day, 1865)	Endemic	Malabar spiny eel	LC	MZ312376, OR613465, MG923382, MG923381
6.	<i>Macrognathus lineatamaculatus</i> Britz, 2010	Native	NA	DD	NA
7.	<i>Macrognathus malabaricus</i> (Jerdon, 1849)	Native	NA	NE	NA
8.	<i>Macrognathus morehensis</i> Arunkumar & Tombi Singh, 2000	Native	NA	LC	NA
9.	<i>Macrognathus pancalus</i> Hamilton, 1822	Native	Barred spiny eel	LC	OR145341, OR145340, OR145339, OR145338
10.	<i>Mastacembelus armatus</i> (Lacepède, 1800)	Native	Zig-zag eel	LC	MW888796, MT805186, MT805185, MZ312377

* NA – Not Available, LC - Least Concern, NE – Not Evaluated, DD – Data Deficient

Mastacembelidae species through the use of the cytochrome c oxidase subunit I gene and to establish their phylogenetic relationships.

2. MATERIALS AND METHODS

2.1 Data Acquisition and Analysis

A checklist of all the species belonging to the family *Mastacembelidae* from Indian waters is provided in Table 1. For the present study, 20 random sequences of the mitochondrial cytochrome c oxidase subunit I gene of five species namely, *Macrogathus aral*, *Macrogathus aculeatus*, *Macrogathus guentheri*, *Macrogathus pancalus*, and *Mastacembelus armatus* were obtained from the NCBI GenBank database with confirmed species identification (Table 1). Sequences of the other five species were not available in the NCBI GenBank library and hence were excluded from the study. Subsequently, the sequences were aligned using the Clustal W [18], implemented in MEGA 11 (MEGA 11: Molecular Evolutionary Genetics Analysis). Genetic distances within and between the species were calculated using the Kimura-2 parameter (K2P) distance model implemented in the MEGA 11 programme. Hebert et al. [19] proposed the DNA barcoding method for species identification based on the K2P divergences, which are measured in terms of nucleotide substitutions per site *d*. A Neighbour-Joining (NJ) tree with a bootstrap value of 1000 [20] was constructed using MEGA 11 based on the pairwise genetic distances calculated using K2P model from all sequences of the COI gene. To assure the reliability of the NJ tree, the bootstrap value of 1000 was

preferred. Moreover, to account for the possibility of misidentification, nearly 80 more sequences were obtained from the NCBI database and used for constructing the phylogenetic tree.

3. RESULTS

In the present study, analysis of the mitochondrial COI gene sequences of *Mastacembelidae* species was conducted to assess the genetic divergence among the species. The pairwise genetic distance values among the five species are presented in Table 2. Within-species differences among the five species ranged between 0.32 to 1.12 %, except for *Mastacembelus armatus* (9.00%). *Macrogathus guentheri* and *M. pancalus* showed the lowest genetic distance value of 12.23%, whereas the highest distance was evident between *M. aculeatus* and *Mastacembelus armatus*. Among the genus *Macrogathus*, *M. guentheri* and *M. pancalus* were found to be the closest relatives with a distance value of 12.23%, followed by *M. aral* and *M. guentheri* (14.64%). The highest distance among the *Macrogathus* species was observed between *M. aculeatus* and *M. pancalus* (17.51%). *M. aral* showed distance values of 15.17% and 15.77% with *M. pancalus* and *M. aculeatus*, respectively. Additionally, the average nucleotide frequencies of the sequences were calculated. The nucleotide compositions of the *Mastacembelidae* species were, T: 29.08, C: 28.24, A: 26.42 and G: 16.26. The average GC content at 1st, 2nd and 3rd codon positions of the COI sequences were 42.66%, 36.02% and 54.96%, respectively (Fig. 1).

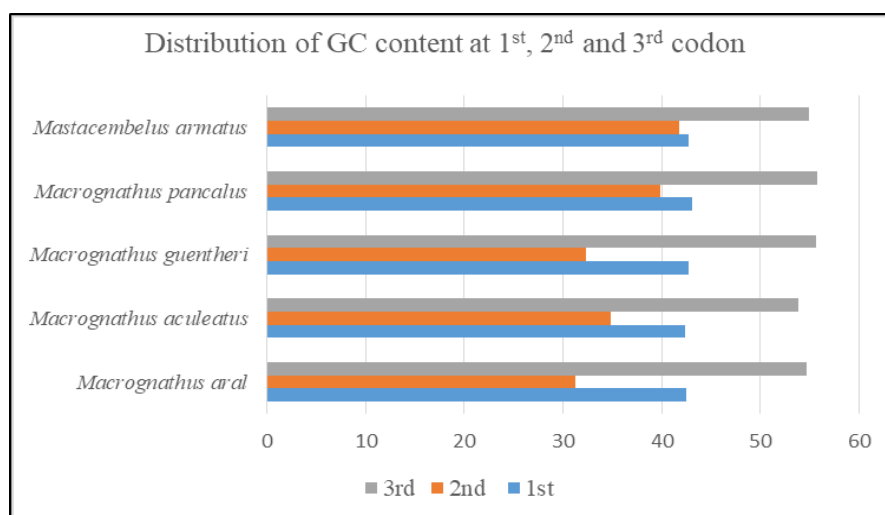


Fig. 1. Graphical representation of GC content at 1st, 2nd and 3rd codon of the sequences

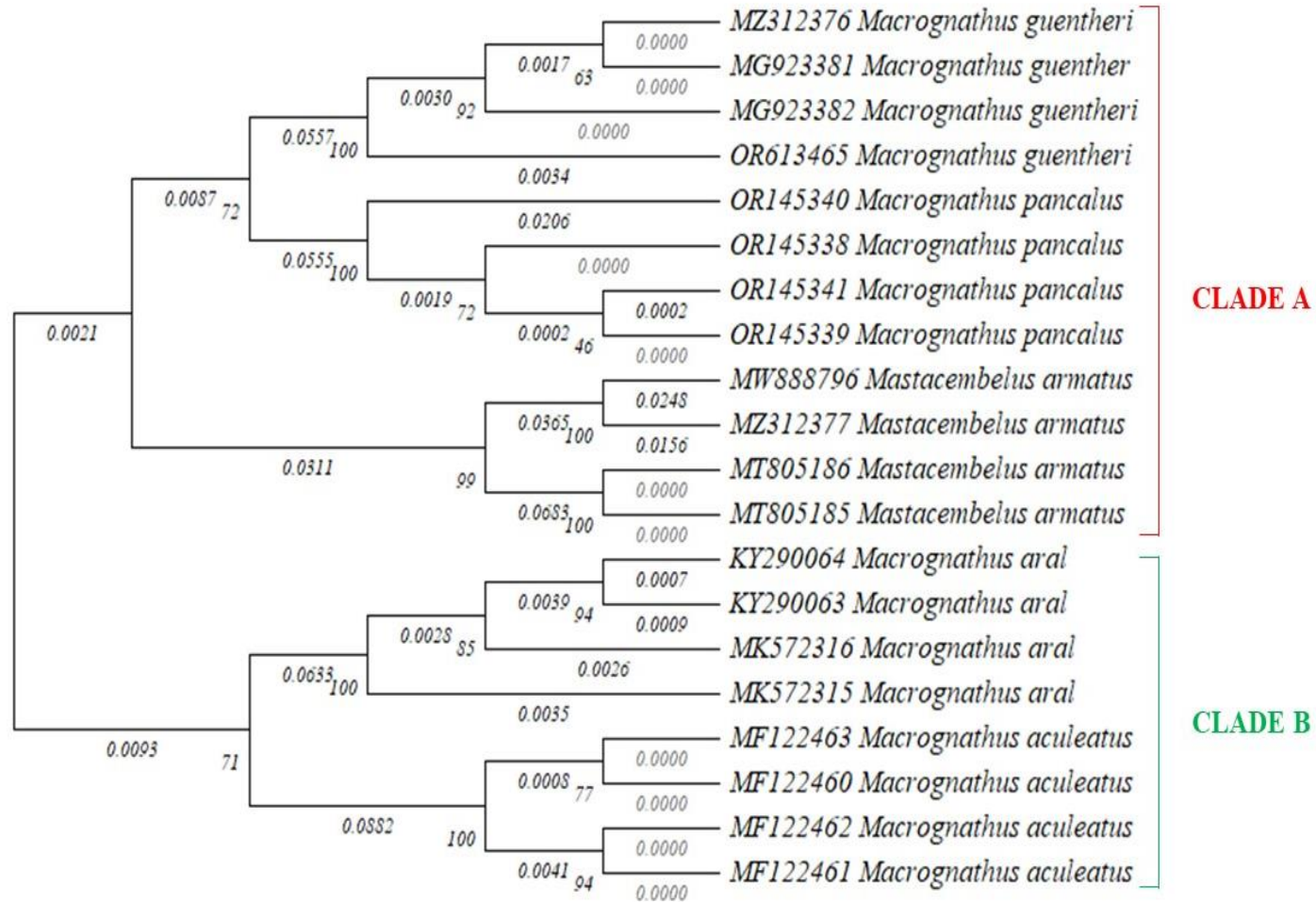


Fig. 2. A Neighbour Joining tree based on the Kimura 2 parameter model for the Mastacembelid species

Table 2. The pairwise genetic distance among the Mastacembelidae species

Species	1	2	3	4	5
<i>Macrognathus aral</i>	0.0077				
<i>Macrognathus aculeatus</i>	0.1577	0.0032			
<i>Macrognathus guentheri</i>	0.1464	0.1644	0.0041		
<i>Macrognathus pancalus</i>	0.1517	0.1751	0.1223	0.0112	
<i>Mastacembelus armatus</i>	0.1772	0.1923	0.1642	0.1635	0.0900

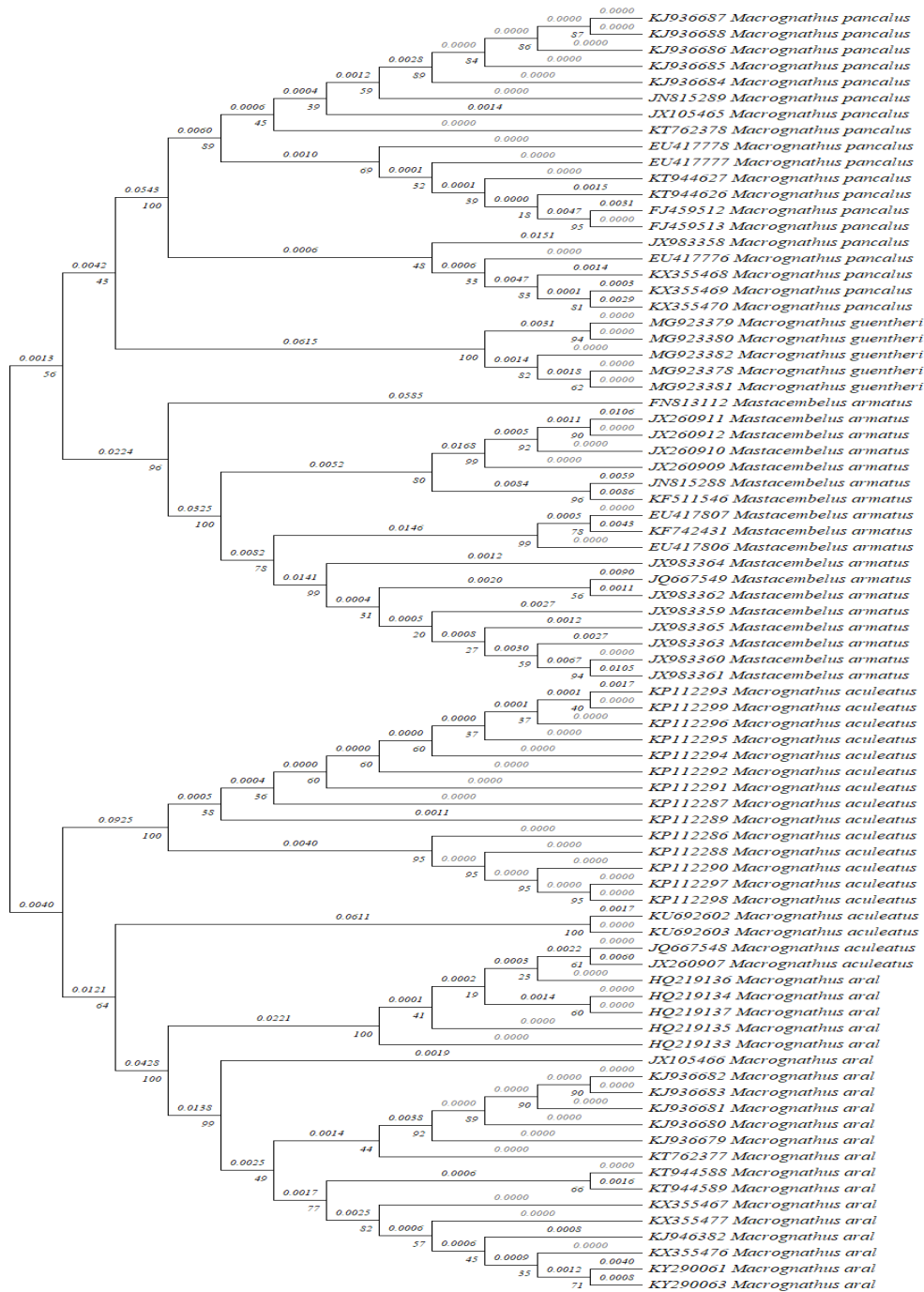


Fig. 3. A Neighbour Joining tree based on the Kimura 2 parameter model with the 80-reference sequence obtained from NCBI for the Mastacembelid species

A Neighbour Joining tree was constructed with the sequences obtained from the NCBI database to infer the existing phylogenetic relationship among the species (Fig. 2). The tree revealed five distinct clades, each corresponding to a species. Two major clusters, CLADE A and CLADE B, were observed from the tree. CLADE A comprised of three species: *Macrogathus guentheri*, *M. pancalus* and *Mastacembelus armatus*. The second clade showed a close relationship between *Macrogathus aral* and *M. aculeatus*. Furthermore, the constructed NJ tree with 80 reference sequences (Fig. 3) confirmed the same phylogenetic relationships among the aforementioned species.

4. DISCUSSION

Freshwater fishes exhibit extraordinary taxonomic breadth, endemism, and a wide range of geographic distribution [21]. A wide range of morphological, behavioural, and life history characteristics among these species aids in resolving their taxonomic diversity. DNA barcoding serves as a valuable tool to complement traditional taxonomy and reveal genetic divergence among the species.

Molecular phylogenetic analysis of the *Mastacembelid* species was explored by Brown et al. [22] for the first time and reported the Africa–Asia split of mastacembelid eels at ~19 Mya. In the present study, molecular analysis of the mt COI gene sequence has been performed for five species of the family *Maastacembalidae* from Indian waters. Genetic divergence within and between the species has been evaluated. Among the five species, the within genetic divergence ranged from 0.32% to 1.12 %, except for *Mastacembelus armatus*, which exhibited a divergence of 9.00 %. This finding aligns with previous studies by Kundu et al. [23] and Qayoom et al. [24], who reported divergence of 7.49% and 4.6%, respectively, for *M. armatus*. *Macrogathus pancalus* showed divergence value of 1.12%, which is lower than the 2.36% reported by Kundu et al. [23]. Lakra et al. [16] reported an average genetic distance of 0.35% within individuals of a species and 18.685% between species of the family *Mastacembelidae*, which is concurrent with the between-species distance value observed in the present study (16.06%).

The pairwise genetic distances between the species ranged from 12.23% between *Macrogathus guentheri* and *M. pancalus* to

19.23% between *M. aculeatus* and *Mastacembelus armatus* (19.23%). Kundu et al. [23] reported the distance of 17.7% between *Macrogathus aral* and *M. pancalus*, which closely matches the 15.17% observed in the present study. Lakra et al. [16] reported a distance of 15.00% between *M. pancalus* and *M. armatus*, whereas in this present study, it was found to be 16.35%. The average genetic distances within species, within genus and within family were 2.3%, 14.80 and 16.06%, respectively, which is similar to the observation made by Chakraborty and Ghosh [15] and Lakra et al. [16] for other Indian freshwater fish.

Sequence analysis revealed the average nucleotide frequencies of T: 29.08, C: 28.24, A: 26.42, and G: 16.26, similar to findings of Lakra et al. [16] for four species of the Synbranchids. Mitochondrial genomes exhibit wide variation in their GC content, and the average GC content of partial COI gene at 1st, 2nd and 3rd codon positions was 42.66%, 36.02% and 54.96%, respectively, with an average value of 44.55%. In this study, the GC content of the partial COI gene averaged 45.00%. Ward et al. [25] reported nearly similar observations for GC content, ranging from 38.4 to 43.2% and 42.2 to 47.1%, which can mostly be attributable to 3rd base variation.

The constructed NJ tree based on COI gene sequences of five *Mastacembelid* species revealed distinct phylogenetic relationships among them. The tree showed five distinct clusters corresponding to conspecific individuals without any misplacement. Two major clades were identified, supported by high bootstrap values, indicating a strong phylogenetic signal of the COI gene sequence, as also observed by Lakra et al. [26].

5. CONCLUSION

DNA barcoding is solely based on substantial taxonomic knowledge in the barcode reference library and aims to augment the morphological taxonomy. The present study extends the knowledge on the genetic distinctness among the *Mastacembelid* species of Indian waters along with their evolutionary relationships which will be useful in identification of the particular group and furthermore, their successful conservation. The study strongly supports the use of DNA barcoding technology based on the COI gene for correct identification and delineation of freshwater fishes. However, while

using NCBI reference sequences for analysis and comparison, the potential chance of misidentification by the authors of the respective sequences should be considered. Further research efforts on the biological and culture aspects of the species should be taken up for successful conservation of these germplasms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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