



First record of the one stick sting fish *Minous coccineus* Alcock, 1890 from the continental shelf of Bangladesh

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Abstract. A new record of the one stick sting fish *Minous coccineus* (Scorpaeniformes: Synanceiidae) was documented based on morphological traits and DNA barcoding. The species was acquired by the Department of Fisheries during the yearly survey of "RV Meen Sandhani" from the maritime area of Bangladesh's continental shelf in the Bay of Bengal from 2018 to 2019. The first record of *Minous coccineus* from Bangladeshi marine waters has been verified by DNA barcoding and morpho-meristic analysis.

Keywords: *Minous coccineus*, Synanceiidae, The Bay of Bengal, DNA barcoding

Introduction

The species of the family Synanceiidae commonly referred to as Stone fishes and also known as sting fishes and comprise 141 valid species of 52 genera (Fricke *et al.* 2022). They have relatively robust, slightly compressed bodies with spiky heads that can inject the most lethal fish neurotoxins through needle-like venom glands at the base of their dorsal fin spines (Nelson *et al.* 2016). The majority of the tiny, bottom-dwelling stone fishes can be found in shallow waters near the shore or at depths of up to 420 meters throughout the tropical marine waters of the Pacific and Indian Oceans, from Japan to Australia west to the western Indian Ocean and Red Sea (Eschmeyer *et al.* 1979, Amaoka and Kanayama 1981, Mandrytsa 1990 & 1993, Nelson *et al.* 2016, Naranji *et al.* 2018, Fricke *et al.* 2022). Stone fishes are most frequently seen in close proximity to coral reefs, where they dwell benthically and blend in with muddy or sandy substrates by disguising themselves as rocks and "walking" on the bottom (Nelson *et al.* 2016).

The genus *Minous* Cuvier, 1829 are small-size fish and poorly known stonefish with bodies devoid of scales and lowermost pectoral fin rays free from the rest of the pectoral fin. Most of them inhabit at depths between 10-420 meters on the muddy and sandy bottoms of coastal marine waters, east into the Western Pacific, north to Japan and south to Australia (Eschmeyer *et al.* 1979). The stonefish genus comprises 15 valid species distributed in the Indo-Pacific region (Matsunuma *et al.* 2017, Fricke *et al.* 2022), five of which have been reported as occurring in Indian waters, namely: *Minous pictus* Günther, 1880, *Minous coccineus* Alcock, 1890, *Minous inermis* Alcock, 1889, *Minous dempsterae* Eschmeyer, Hallacher & Rama-Rao, 1979 and *Minous monodactylus* (Bloch and Schneider 1801) (Russell 1803, Munro 1955, de Beaufort and Briggs 1962, Eschmeyer *et al.* 1979, Jones and Kumaran 1980, Krishnan and Mishra 1993, Kapoor *et al.* 2002, Smith and Heemstra 2012, Naranji *et al.* 2017, Matsunuma and Motomura 2018). However, only two species of stonefish, *Minous pictus* Günther, 1880 and *Minous monodactylus* (Bloch and Schneider 1801) have been recorded from the sea waters of Bangladesh (Habib and Islam 2021).

Minous inermis Alcock, 1889, *Minous coccineus* Alcock, 1890 and *Minous dempsterae* Eschmeyer, Hallacher & Rama-Rao, 1979 are the other three species that have not yet been found in the northern shore of the Bay of Bengal near Bangladesh's maritime region. In the current study, *Minous coccineus* Alcock, 1890 was first identified using morphological characteristics. Since DNA barcoding is one of the most reliable ways for verifying species identity, it was then utilized as a tool to further corroborate the morphological identification of the species (Hebert *et al.* 2003).

Material and Methods

Sample collection: Several stonefish individuals were taken in February 2019 during the yearly expedition of "RV Meen Sandhani" (2018-2019). The Department of Fisheries (DoF), Bangladesh conducted this trip in the Bay of Bengal's continental shelf off the coast of Bangladesh (Fig. 1). Through examination of morpho meristic traits, the researchers were able to identify some of them. However, they became perplexed by one individual who had some distinctive traits and were unable to identify it at the species level. They forwarded the sample to the Aquatic Bioresource Research Laboratory (ABR Lab) of Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, for species identification. In the lab, species identification was done using morpho-meristic analyses, and DNA barcoding was used to further validate the identification.

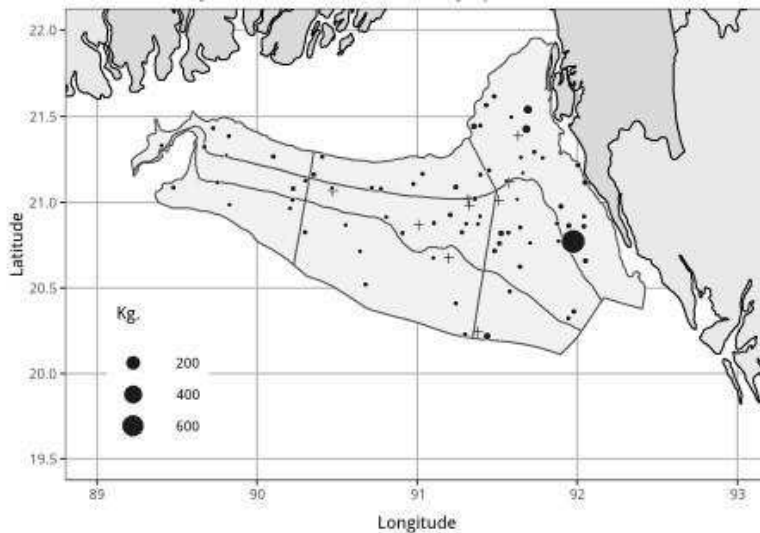


Fig. 1. Sampling locations of the expedition in continental shelf of Bangladesh maritime area of the Bay of Bengal. (Source: Fanning *et al.* 2019).

Morphological analysis: Taxonomic keys from Randall and Heemstra (1991), Heemstra and Randall (1993), Baldwin and Johnson (1994), and Elamin *et al.* (2011) were used for morphological identification. Abbreviations: D1 - First dorsal fin, P1 - Pectoral fin, P2 - Pelvic fin, A- Anal fin, C - Caudal fin.

Molecular study: A portion of muscle tissue was taken and kept in 95% ethanol for molecular research. A preserved tissue sample was used to extract genomic DNA using the TIANamp Marine Animals DNA Kit (TIANGEN). Mitochondrial COI gene's DNA barcode region was amplified using FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1: 5'-

TAGACTTCTGG GTGGCCAAAGAATCA-3' (Ward *et al.* 2005). In a thermal cycler, 50 reactions of a polymerase chain reaction (PCR) mixture were conducted (2720 Thermal Cycler, Applied Biosystems). Thermal cycling settings included an initial denaturation temperature of 95 °C for 2 minutes, followed by 35 cycles with denaturation at 94 °C for 40 seconds, annealing at 54 °C for 40 sec, and extension at 72°C for 1 min. Finally, for the final extension at 72 °C for 10 min was maintained. PCR results were analyzed using a 100 bp DNA ladder and 1% agarose gel electrophoresis. The Sanger standard technique of sequencing was used with the PCR primers in a typical automatic sequencer (Model 3730xI DNA analyzer). The National Center for Biotechnology Information (NCBI/BLAST)'s search engine and BOLD database were used to check the DNA sequence for molecular identification. We chose a total of nine COI sequences for phylogenetic analysis, including the sequence used in this study. These sequences included eight from the genus *Minous* (e.g., *M. coccineus*, *M. quincarinatus*, *M. Pictus* and *M. inermis*) and one con familiar sequence from *Trachicephalus uranoscopus* as an out group. These sequences were found in the GenBank BLAST search result (Altschul *et al.* 1990). The genetic distance between the sequences was calculated using the Kimura-2 parameter (K2P) distance model (Kimura 1980) in MEGA-11. Utilizing MEGA11, sequences were aligned, and a phylogenetic tree was created using the Neighbor Joining (NJ) method (Kumar *et al.* 2016). Bootstrap analysis with 10,000 replications was used to assess the robustness of the phylogenetic connections (Felsenstein 1985). The studied specimen was submitted to the Department of Fisheries Biology and Genetics, Aquatic Bioresource Research Lab, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Results

Material examined: Specimen collected from the Bangladesh maritime area of the Bay of Bengal; February 2019; one specimen; specimens voucher nos. F1902DoF-50 (Fig. 2). GenBank Accession No. OP942241.

Habitat: This fish was found in the deep sea in the continental shelf of Bangladesh.

Diagnostic Characters: D1 XI, 12; P1 11+1 (pectorals with 1 free rays); P2 I, 5; A II, 10; C 12. There is a gas bladder present in the dorsal area of the inner abdomen, dorsal spines are sharp and moderately strong, the first dorsal spine is extremely small and concealed under the skin, the head spines are moderately developed, and the maxillary bone has two sharp spines.

Color: The upper portion of the flanks have alternate white bars that are flanked by smaller dark brown lines, with the body's ground color being brown which becomes paler ventrally. The body has a few small, dispersed dark patches, with the exception of the lower flanks. Edges of both jaws have a mild brown pigmentation, with the upper part of the skull being brown and the area below the suborbital being gray to silvery. The dorsal fin is brown; anterior spinous dorsal membranes are blackish distally; posterior spinous dorsal membranes and soft dorsal fin have pale bands that reach obliquely from the fins to the upper surface of the body; soft dorsal border is blackish. Free pectoral-fin ray cap yellow; pectoral-fin base pale brown, darker at distal half; inner surface of pectoral fin with irregular, dark brown spots of various sizes on tan background; spots loosely organized near base and gradually closer to one another towards tip. Brown pigmentation at the pelvic base; proximally, the pelvic and anal fins are pale brown, and the tips are dark brown. Pale brown caudal fin with sporadic black coloring.

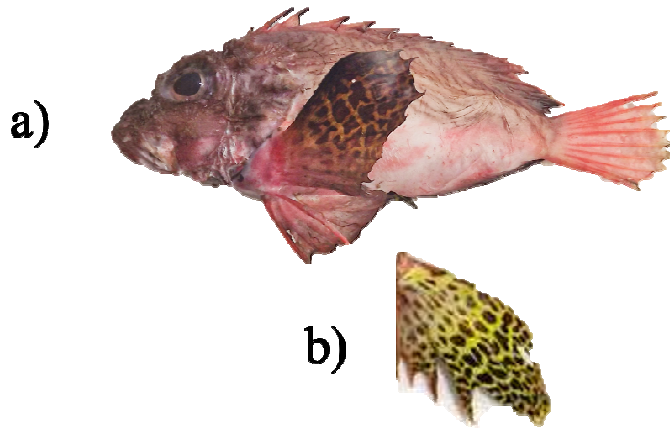


Fig. 2. Fresh specimen of *Minous coccineus* (F1803SM-43, 125 mm TL) (a); medial side of pectoral fin (b).

The morpho-meristic counts and measurements of the collected specimens of *Minous coccineus* and its comparison with the records of Ray *et al.* (2021) and Naranji *et al.* (2022) are given in Table I.

Molecular identification: After eliminating problematic sequences close to the primer ends, an unambiguous nucleotide sequence of 670 base pairs for the mtDNA COI gene was found. The sequence lacked any stop codons, insertions, or deletions. The amplified sequence is consistent with being a functional mitochondrial COI sequence because there are no stop codons. Our determined COI barcode area of the *M. coccineus* specimen was compared to other conspecific and congeneric species downloaded from the GenBank in order to confirm the outcome of morphological identification. The Bay of Bengal-based *M. coccineus* sequence from the current investigation, along with one sequence from an Indonesian individual, formed a single clade in the phylogeny (Fig. 3).

Table I. Comparison of morphological and meristic characters of collected specimen *Minous coccineus* of the present study and the records of Naranji *et al.* (2022) and Ray *et al.* (2021)

Morphometric parameters	<i>Minous coccineus</i> Present study F1902DoF-50	<i>Minous coccineus</i> (Naranji et al. 2022) n=62 DMLRAU-00201	<i>Minous coccineus</i> (Ray et al. 2021) n=4 MARC/ZSI/F3334
Total Length (TL)	125	133.4 (120.6-140.7)	
Standard Length (SL)	95	75.83 (73.87-78.76)	83.5 (73-94)
Percentage of standard length			
Head length (HL)	45.26	40.8 (34.2-46.4)	47.31
Body depth pectoral fin origin (BDPO)	26.32		
Body depth anal fin origin (BDAO)	30.53	32.80 (28.5-37.3)	43.71

Pre-dorsal length (PDL)	13.68	27.00 (24-32.8)	
Pre-pectoral length (PPL)	34.74	39.00 (34-45.2)	
Pre-pelvic length (PPvL)	33.68	34.80 (28.5-38.8)	
Pre-anal length (PAL)	62.11	63.6 (57.1-67.7)	
Dorsal-fin base length (DFBL)	69.47	75.6 (67.8-79.6)	
Dorsal Fin length (DFL)	22.11		
Pectoral-fin base length (PFBL)	11.58	13.5 (9.7-17.6)	
Pectoral-fin length (PFL)	36.84	36.9 (32.1-41.0)	
Pelvic-fin base length (PvFBL)	8.42		
Pelvic-fin length (PvFL)	26.32		
Anal-fin base length (AFBL)	33.68		
Anal-fin length (AFL)	17.89		
Caudal-fin base length (CFBL)	8.42		
Caudal-fin length (CFL)	32.63		
Percentage of head length			
Inter orbital wide (IOW)	17.68	23.6 (20-28.1)	18.92
Pre orbital length (POL)	41.98	22.7 (18.1-27.7)	
Post orbital length (PoOL)	37.56	42.2 (37.8-48.3)	39.11
Eye diameter (ED)	24.30	30.8 (26.6-37.0)	26.53
Snout length (SnL)	26.51	29.0 (25.6-35.7)	26.53
Caudal peduncle length (CPL)	8.84		
Caudal peduncle depth (CPD)	17.68	10.3 (5.5-14.5)	
Meristic parameters	<i>Minous coccineus</i> Present study F1902DoF-50	<i>Minous coccineus</i> (Naranji et al. 2022) n=62 DMLRAU-00201	<i>Minous coccineus</i> (Ray et al. 2021) n=4 MARC/ZSI/F3334
First dorsal-fin spines	XI	X-XII	XI
Second dorsal-fin soft rays	12	11-12	12
Pectoral-fin soft rays	11+1	11+1	
Pelvic-fin spines	I		

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Pelvic-fin soft rays	5		
Anal-fin spines	II	II-III	II
Anal-fin soft rays	10	9-11	10
Caudal-fin rays	12	12	

There was no genetic distance between the sequences of *M. coccineus* of the present study and sequences from Indonesia (HM422406). Between the current study's *M. coccineus* and the phylogenetically closest-related species, *M. pictus* reported from China, there was a genetic gap of 6.15% (SE 0.011). However, the genetic distance between the *M. coccineus* used in our investigation and the *M. inermis* that originated from China was calculated to be 14.6% (SE 0.020). The COI gene sequences included in the study had an overall mean distance of 12.9%.

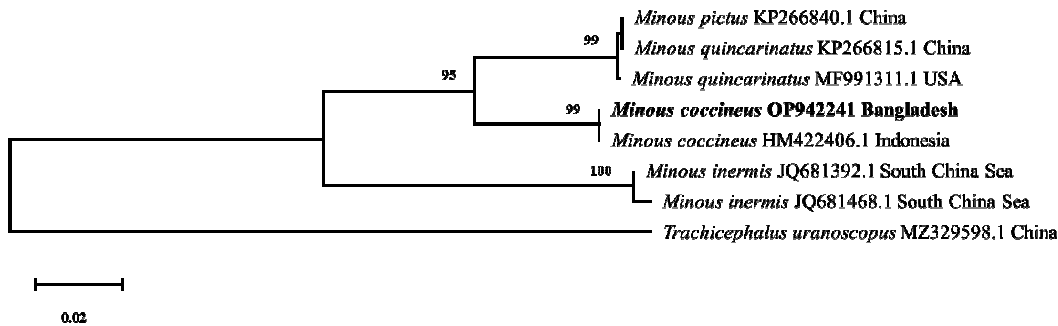


Fig. 3. Neighbor-joining tree of COI gene of *Minous coccineus*. The accession number and the country of each sequence are given beside the species name. Bootstrap support above 90% is shown above branches. The scale bar represents the substitution rate per site.

Discussion

Based on morphological characteristics and DNA barcoding, a record of *Minous coccineus* Alcock, 1890 (Scorpaeniformes: Synanceiidae) was reported for the first time in the maritime area of Bangladesh. Only one specimen was identified throughout the search, and it was then prepped for additional examination. Other studies, including those by Vella *et al.* (2016), Sen *et al.* (2019), and De La Cruz *et al.* (2020) similarly used the record of a single specimen to determine whether to include a species in a region's or nation's fish inventory. The morphometric traits were measured, enumerated, and photographed before being compared to the records of Heemstra and Randall (1993) and Elamin *et al.* (2011). All meristic characteristics are consistent with the species' earlier records (Table I). The majority of morphological traits either overlap or transcend the range, which is appropriate given the variations in growth rate, even though some morphological characters are not exactly similar to the range observed by Naranji *et al.* (2022) and Ray *et al.* (2021).

For identifying species, COI sequencing is regarded as an efficient and trustworthy method (Hebert *et al.* 2003). With one sequence of *Minous coccineus* of Indonesian provenance deposited in the GenBank database, the query sequence displayed a high identity (99%). (accession numbers: HM422406.1; Fig. 3). *M. coccineus* COI sequences formed a monophyletic group that was distinct from other species of the same genus. Strong support for *M. coccineus* species

validity was supplied by the results of the genomic analysis. The results of the phylogenetic analysis also demonstrated that *M. coccineus* sequences could be distinguished from those of other species belonging to the same genus by a genetic separation of between 6.2% and 15.1%.

The Indo-Pacific region is home to 15 recognized species of stonefish, five of which have been reported to inhabit Indian waters. These species are *Minous pictus* Günther 1880, *Minous inermis* Alcock, 1889, *Minous coccineus* Alcock, 1890, *Minous dempsterae* Eschmeyer, Hallacher & Rama-Rao 1979 and *Minous monodactylus* (Block and Schneider 1801) (Russell 1803, Munro 1955, de Beaufort and Briggs 1962, Eschmeyer *et al.* 1979, Jones and Kumaran 1980, Krishnan and Mishra 1993, Kapoor *et al.* 2002, Smith and Heemstra 2012, Naranji *et al.* 2017, Matsunuma and Motomura 2018). Only two of them, *Minous monodactylus* (Bloch and Schneider 1801) and *Minous pictus* (Günther 1880), have so far been identified in Bangladeshi marine waters (Habib and Islam 2021). However, our study verifies the existence of *M. coccineus* in the continental shelf of Bangladesh's maritime region in the Bay of Bengal and suggests that additional species of the genus *Minous* that have been missed in earlier studies may exist in Bangladeshi marine waters.

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