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Documentation of Vulnerable Bull Shark (*Carcharhinus leucas*) Occurrence in the Hooghly River, East Coast of India

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Abstract

The bull shark (*Carcharhinus leucas*) is a species of requiem shark distributed worldwide, which inhabits estuaries, nearshore areas, and the continental shelf waters and opportunistically in rivers within tropical and sub-tropical regions. In the present study, we report the first occurrence of bull shark from inland waters of the Hooghly River in the Ganga River Basin based on molecular and morphometric investigations. The identification of the specimen was confirmed through distinct morphological features, including a blunt snout, large dorsal fin, crescent-shaped mouth with sharp triangular teeth, and a muscular asymmetrical upturned tail. The percent identity of the specimen was 100% based on both the 16s rRNA and ND4 sequences. Phylogenetic analysis revealed that *Carcharhinus leucas* and *Glyphis glyphis* are closely related, forming a well-supported sister clade, while *Carcharhinus falciformis* was identified as a more distantly related sister taxon. This report documents the occurrence of the bull shark in the Ganga River system at the farthest inland distribution limit of the species in India. Our findings extend the known range of the bull shark within India's inland waters and contribute valuable baseline data on biodiversity, highlighting the ecological significance of this vulnerable species within the Ganga River system.

Keywords: Aquatic conservation, distribution, freshwater, molecular assessment, range extension

Introduction

River ecosystems face numerous challenges from water development projects, overfishing, habitat destruction, pollution, and climate-induced changes (Pittock *et al.*, 2008). These stressors collectively or acting independently have resulted in a decline in many freshwater and marine megafaunas (He *et al.*, 2024). Sharks serve as apex predators and play a significant role in local and regional trophic dynamics (Hammerschlag *et al.*, 2022). Despite, their crucial role in ecological processes they are exploited for meat, fins and liver oil for human consumption (Clarke *et al.*, 2005). Globally, the shark populations are under severe threat due to direct and indirect human activities and concerns are being voiced in response to reports of declining numbers due to destructive fishing, industrial activities, and habitat degradation (Airoldi *et al.*, 2008; Dulvy *et al.*, 2014). Despite their recognition of trophic dynamics, interventions focused on the conservation of sharks are impeded by a scarcity of distribution records of many species (Gausmann & Hasan, 2022; Haque *et al.*, 2021).

The bull shark (*Carcharhinus leucas* Müller & Henle, 1839) is a euryhaline migratory species belonging to the family Carcharhinidae. The global decline in the bull shark population, primarily due to overfishing, has led to its classification as Vulnerable on the International Union for Conservation of Nature (IUCN) Red List (Rigby *et al.*, 2021a; Postaire *et al.*, 2024). The species has a wide global distribution, inhabiting tropical to warm temperate waters (Compagno, 1990). Fossil records indicate the species has existed for 23 million years, with evidence spanning the former Tethys Sea, from present-day Peru to the Mekong River (Gausmann, 2021). The bull shark occupies a range of habitats, including rivers, estuaries, nearshore areas, and continental shelf waters in tropical and subtropical regions (Rigby *et al.*, 2021a). Their occurrences in inland rivers are not rare; historically, the species apart from the coast region has been reported inland worldwide (Gausmann, 2021). Recently, the species has been reported in new inland distributions in five different river basins

in Indonesia (Gausmann & Hasan, 2022). Bull shark can survive for extended periods in freshwater systems (Thorson *et al.*, 1973; Chen *et al.*, 2015) and rivers and their mouths are known to be essential refuges for neonates and juveniles, offering safer environments and abundant food resources (Pillans *et al.*, 2020; Simpfendorfer *et al.*, 2005). In India, the bull shark has only been reported from the western coast and eastern coast (Purushottama *et al.*, 2013), with no inland records. Accurate identification of the bull shark remains a persistent challenge due to their close resemblance to other shark species, particularly the Ganges shark (*Glyphis gangeticus*) and other members of the genus Carcharhinus (Compagno, 2007; Haque & Das, 2019; Rigby *et al.*, 2021b). The morphological similarity could lead to the misidentification of the shark species, where multiple species are known to occur (Compagno, 2007). Therefore, in the present study, we utilized a combination of morphometric and molecular assessments to report the first inland record of a bull shark from India.

Methodology

Study Area

The Hooghly River, a significant distributary of the Ganga River in eastern India's West Bengal state, originates from the confluence of the Bhagirathi and Jalangi rivers at Nabadwip (Ranjan & Ramanathan, 2018). The Hooghly River estuary is located in the south-western flank of the Ganga–Brahmaputra delta, which flows through Nadia, Hooghly, North 24 Parganas, South 24 Parganas, Howrah and East Medinipur districts before it drains into the Bay of Bengal at Ganga Sagar (Chugh, 1961) (Figure 1). The dynamic interaction between freshwater and estuarine environments along the Hooghly River supports diverse habitats that sustain a wide range of aquatic resources, including numerous fisheries of high ecological and economic importance (Rakshit *et al.*, 2017). The water from the Ganga River is diverted *via* the Farakka Barrage through a feeder canal, which supplies the Hooghly River with sufficient water. The physicochemical parameters such as dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity, pH, and NO3- recorded at the specimen collection site reflect the freshwater characteristics of the Hooghly River (Prakash *et al.*, 2023) (Supplementary Table S1).

Specimen collection

The specimen of a potential bull shark (*C. leucas*) was captured near Chandni Ghat, Hooghly (22° 54' N; 88° 23' E), situated 180 km upstream from the mouth of the Hooghly River, on April 26, 2021. The specimen was preserved in 70% ethanol for morphological and molecular assessment. The specimen was opportunistically captured in a fishing net during a moderate tidal phase from a meandering river channel with a width of 420 meters and a depth of 8.8 meters. At the time of collection, the DO concentration was measured at 7.66 mg/L, and salinity was recorded at 0.19 ppt. Additionally, 16 active fishing nets, 12 fishing boats, and 8 ferry boats were observed in the vicinity of the collection site (Supplementary Table S1).

Morphological assessment

We used the approach of Irschick & Hammerschlag (2014) to measure morphometric characteristics, and the specimen was identified following the field identification guide (McAuley *et al.*, 2002). We obtained the following morphometric and meristic traits using a standard metric flexible tape (accurate to 1 mm): total body length (TBL), fork length (FL), pre-caudal length (PCL), pre-orbital length (POB), pre-pectoral length

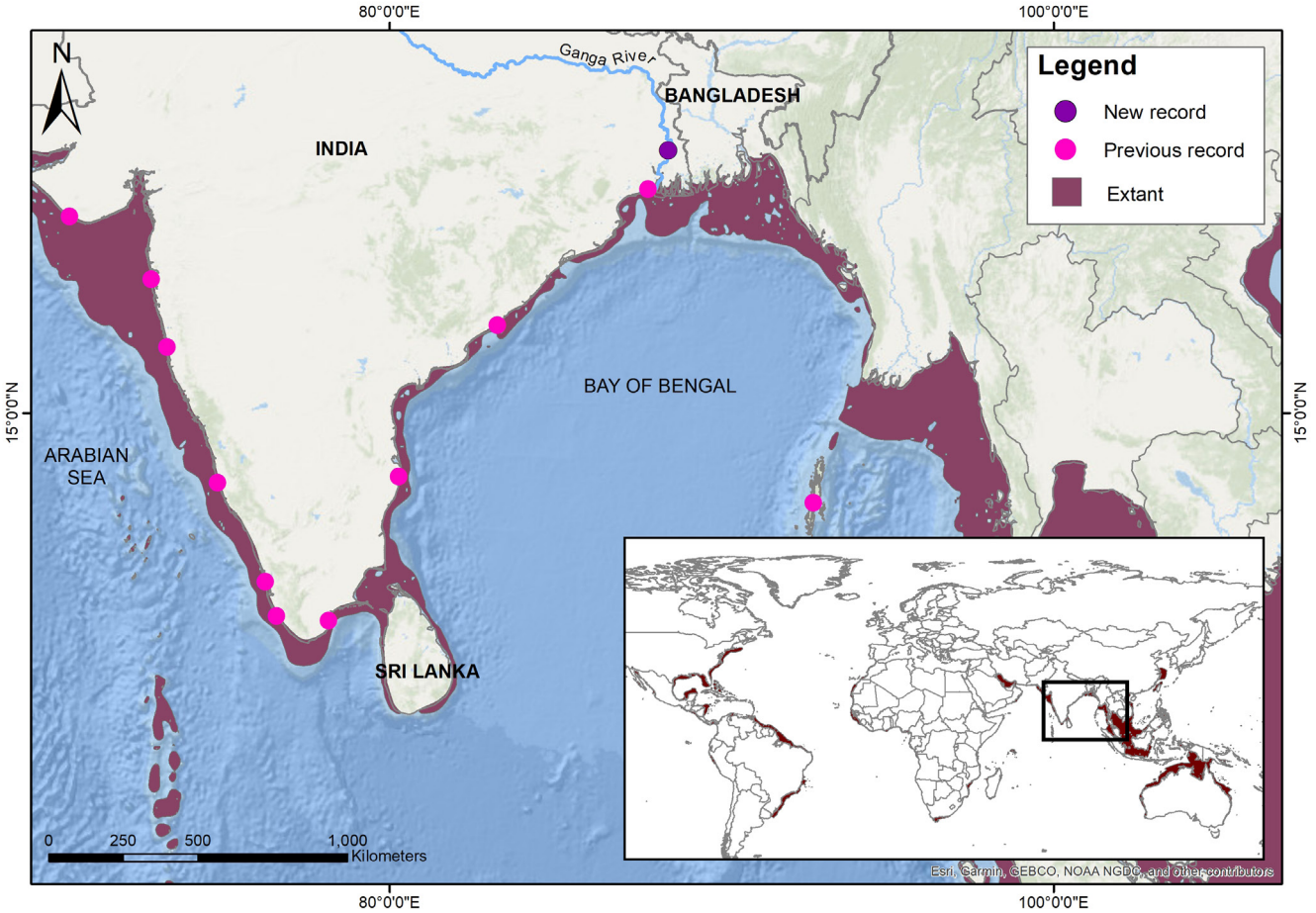

Figure 1. Occurrence records of bull shark (*Carcharhinus leucas*) from the eastern and western coast of India and the first inland record from Hooghly River, India.

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(PPL1), pre-pelvic length (PPL2), pre-anal length (PAL), girth at first dorsal fin (GDF), snout-vent length (SVL), mouth length (ML), mouth width (MW), head length (HL), tail height (TH), sex, weight. These morphometric measurements were used to confirm species identification as well as age class.

Molecular assessment

DNA extraction, PCR amplification, and DNA sequencing
A small section of tail tissue was collected from the specimen for molecular assessment. Total genomic DNA was extracted using a DNeasy blood and tissue kit following the manufacturer's protocol (QIAGEN Inc. USA) and quantified using a Quantus™ Fluorometer (Promega Corporation, Woods Hollow Road, USA). We used two partial mitochondrial DNA (mtDNA) fragments 16s rRNA and NADH dehydrogenase subunit 4 (ND4) for species identification and inferring phylogenetic relationship (Table 1).

Table 1. Details of primers used for amplification of mitochondrial DNA 16s rRNA and ND4 for molecular assessment.

Gene/ fragment	Primer sequence (5'—3')	Reference
16s rRNA	CGCCTGTTTATCAAAAACAT	Chapela *et al.* (2002); Palumbi (1991)
	CTCCGGTTTGAACTCAGATC	
ND4	TGACTACCAAAAGCTCATGTACAAGC	Engstrom *et al.* (2002)
	CCTATTTTAGAGCCACAGTCTAAT	

Polymerase chain reaction (PCR) was carried out in a 10 µL reaction volume containing 5 µL of QIAGEN multiplex PCR master mix, 0.25 µL (3 pmol) of each primer, and 1 µL of template DNA (20-40 ng/µL) and 3.5 µL of RNase-Free water. The PCR was conducted under the following conditions: initial denaturation at 95°C for 15 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at Ta=56°C for 40 seconds, and extension at 72°C for 90 seconds and final extension at 72°C for 30 min. The amplified PCR products were maintained at 4°C until further use. The amplified PCR products were visualized in 2% agarose gel electrophoresis. The amplified PCR products were purified using Exo-nuclease I and FastAP buffer and sequenced using BrilliantDye™ v3.1 Terminator Cycle Sequencing Kit (NimaGen Inc.) and sequenced in ABI Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following manufacturers protocol.

The sequences generated were first inspected manually for any error and the quality (Phred Score) of generated DNA sequences was checked in FinchTV Version 1.4.0 (Geospiza Inc. Seattle, WA, USA). The species identification of the generated sequences was confirmed by nucleotide BLAST (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The generated sequences were then aligned with sequences of *C. leucas* other closely related species downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/).

Data analysis

Phylogenetic analysis and haplotype network
We constructed a Bayesian phylogenetic tree based on the concatenated dataset of 1292 bp (16s rRNA - 571 bp and ND4 - 721 bp) of *Carcharhinus leucas* along with other shark species (Table S2). *Chimaera monstrosa* was taken as an out group species to construct the phylogenetic tree. The appropriate nucleotide substitution model was selected based on the Akaike information criterion (AIC) values using the program jModelTest Version. 2.1.10 (Darriba *et al.*, 2012). The tree was constructed in MrBayes Version 3.2. (Ronquist *et al.*, 2012) using the GTR+I+Gamma model. Two independent MCMC chains of 100 million simulations sampling at every 10,000 generations and 25% of the initial runs as burn-in was performed. The phylogenetic tree was visualized using FigTree version.1.4.4 (Rambaut *et al.*, 2018) and the haplotype network was constructed to assess genealogical relationships using a median-joining network in PopArt (Leigh *et al.*, 2015). Phylogenetic analysis is the most reliable method for reconstructing evolutionary connections and distances between nucleotide sequences. The pairwise nucleotides differences and evolutionary link between the DNA sequences were estimated in MEGA v11.0 (Tamura *et al.*, 2021).

Results

The total length (TL) of the specimen was 83 cm and the weight was 4.5 kg (Table 2). The morphological and meristic features indicate that the captured specimen was a juvenile bull shark. The species identification was confirmed by its distinct morphological features such as, blunt snout, large dorsal fin, crescent-shaped mouth with sharp triangular teeth, and a muscular asymmetrical upturned tail (Figure 3). The coloration was grey on the upper side and pale underneath. As a cartilaginous fish, it possesses two dorsal fins without a skin ridge between them. The characteristic of having five-gill slits, with the last one to three located over the pectoral fin. The eyes were round, and equipped with internal nictitating eyelids (Figure 2).

Figure 2. Lateral view of bull shark (*Carcharhinus leucas*) specimen captured from the Hooghly River, India.

The detailed morphometric measurements of the bull shark specimen recovered from the Hooghly River are provided in Table 2. The gill slits are moderately long. An inter-dorsal ridge is absent. The first dorsal fin is large and broadly triangular, featuring a pointed or sharply rounded. The origin of the first dorsal fin is typically over or just behind pectoral fin insertions, occasionally closer to their free rear tips. The inner margin of the first dorsal is short, measuring less than a third of the dorsal base or slightly less. The second dorsal fin is large and tall and located near the anal origin. Pectoral fins are generally large and broad, featuring narrow, pointed apices.

Molecular analysis
The 571 bp ND4 and 721 bp 16s rRNA fragments were sequenced for accurate identification of the specimen. The BLAST query of both fragments showed 100% similarity with the bull shark sequence (Accession No. OP007121.1). The sequences generated were submitted to GeneBank (Accession No. PP748259 and PP780003). The phylogenetic tree constructed using a concatenated dataset of 16s rRNA and ND4

Figure 3. Specimen of bull shark (*Carcharhinus leucas*) captured from the Hooghly River, India. (A) dorsolateral view, (B) ventral view, (C) dorsolateral view of head showing gill slits. (1) notch in the anal fin, (2) subterminal notch in the caudal fin, (3) minute eyes.

positioned the sequence with a sequence from the Arabian Sea with strong Bayesian posterior probability (Figure 4). The pairwise genetic distance among the sequences analyzed ranged between 0.0 (United Arab Emirates, Japan, Australia and Papua New Guinea) and 0.02 (Seychelles, Thailand & Sri Lanka) (Table S2).

We identified a total of 15 haplotypes in 364 bull shark sequences across 15 countries, including India. The haplotype CLH01 was the most dominant exhibited in 115 (31.7%) sequences, followed by CLH09 found in 61 (16.8%) sequences (Figure 5). The CLH01 has the widest distribution and it is found across ~60% of the countries including Australia, Indonesia, Japan, Taiwan Strait, Thailand, Sri Lanka, Papua New Guinea, United Arab Emirates and India. Our sequence also exhibited CLH01 haplotype, indicating a closer affinity and shared genetic lineage across regions.

Discussion

We report the first occurrence of bull shark in the Hooghly River, West Bengal, eastern India. Both morphological and molecular assessments strongly support the identification of the specimen, as a juvenile bull shark. Considering the ability of bull shark to thrive in freshwaters and recent records of the species in inland waters globally, its occurrences in the Hooghly River are no surprise (Compagno *et al.*, 2005; Hasan *et al.*, 2021) (Figure 1). It has also been observed that bull shark exhibits habitat preference based on size, with small individuals such as juveniles utilizing riverine habitats, while larger individuals prefer marine systems (Brunnschweiler & Barnett, 2013). The inland record of juvenile bull shark in the present study also supports the findings that young individuals utilize riverine habitats preferably guided by predator avoidance, and prey distribution (Glaus *et al.*, 2019). The bull shark species acts as a 'mobile link' species and plays an important role in the stability and functioning of the marine and freshwater ecosystems

Table 2. Morphometric measurements (cm) of the bull shark (*Carcharhinus leucas*) specimen collected from the Hooghly River, India.

Morphometric Measurements (cm)	CMFRI (2005)	Batcha & Reddy (2007)	Purushottama et al., (2013)	Sureandiran & Karuppasamy (2022)	Present study
Total length (TL)	356	330-350	82	180-248	83
Fork length (FL)	...	...	65	145-197	67
Pre-Caudal length (PCL)	...	...	...	...	60
Pre-Orbital length (POB)	...	...	...	...	3
Pre-Pectoral length (PPL1)	...	...	...	...	16
Pre-Pelvic length (PPL2)	...	...	...	...	39
Pre-Anal length (PAL)	...	...	...	...	51
Girth at first dorsal fin (GDF)	...	...	...	...	43
Snout-vent length (SVL)	...	...	43	122-143	43.2
Mouth length (ML)	...	...	7.9	21.5-25.7	7.2
Mouth width (MW)	...	...	9.2	25.5-29.6	8
Head length (HL)	...	...	18.3	55.3-57.9	17
Tail height (TH)	...	...	9.8	52-56	9.7
Sex	F	F	M	F	F
Weight (kg)	320	325-335	3.7	129-235	4.5

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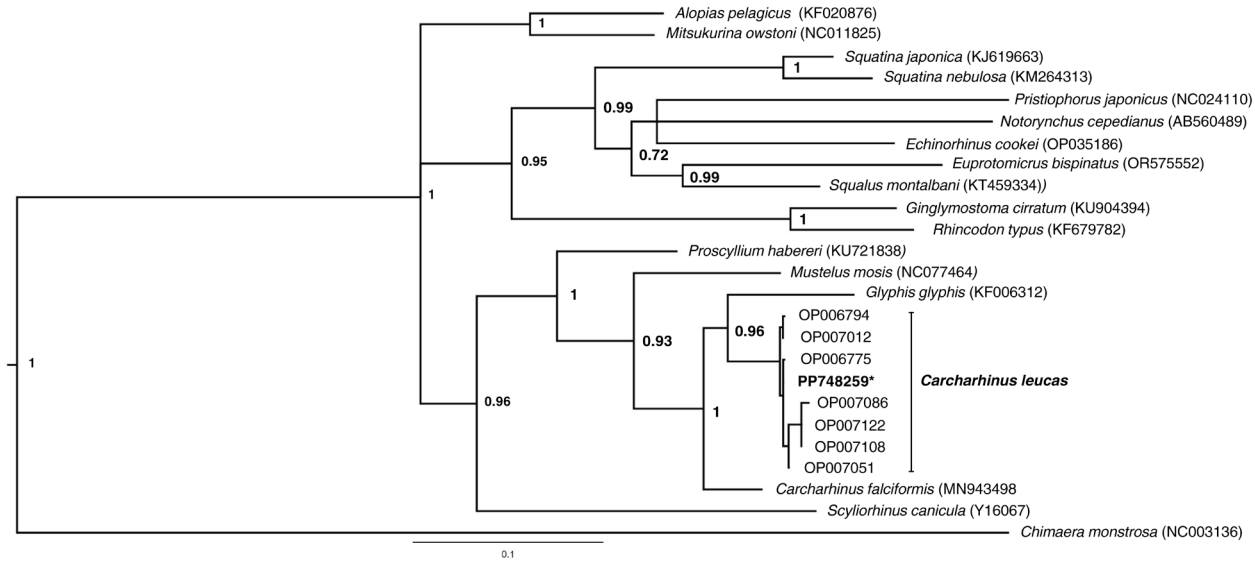

Figure 4. Phylogenetic tree of bull shark (*Carcharhinus leucas*) specimen using 1292 bp of concatenated mitochondrial 16s rRNA and ND4 sequence.

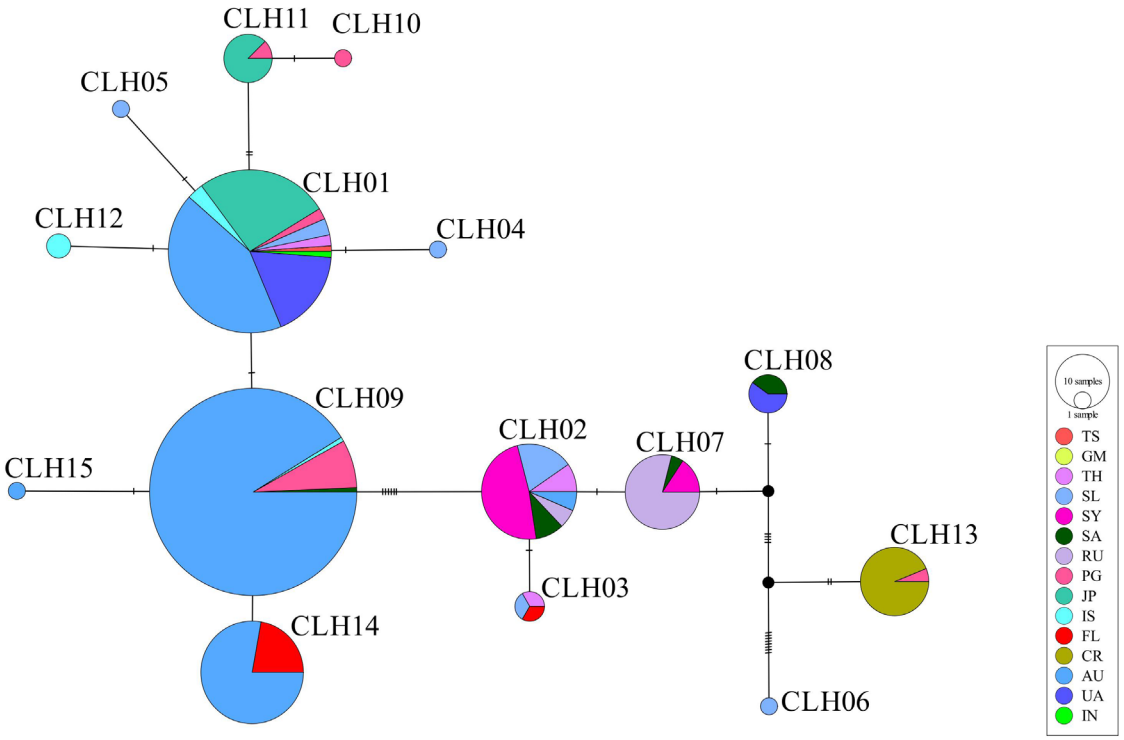

Figure 5. Haplotype network of bull shark (*Carcharhinus leucas*) specimen, constructed using 16s rRNA. Circle size reflects haplotype frequency. Different colors indicate haplotypes detected in different countries. TS-Taiwan Strait, GM- Gulf of Mexico, TH- Thailand, SL- Sri Lanka, SY-Seychelles, SA-South Africa, RU- Reunion, PG-Papua New Guinea, JP- Japan, IS-Indonesia, FL-Fiji, CR- Costa Rica, AU-Australia, UA- United Arab Emirates and IN- India.

(Lundberg & Moberg, 2003; McCann *et al.*, 2005). The increasing occurrences of bull shark in inland water systems worldwide have prompted the necessity for research on the factors that contribute to their presence in the inland waters (Werry *et al.*, 2012). Additionally, bull shark is known to exhibit a unique method of salinity regulation by actively moving between areas with differing salinity levels, rather than relying solely on physiological osmoregulation (Curtis *et al.*, 2013).

The identification of bull shark based on morphological traits and their phylogenetic placement remains somewhat ambiguous due to their notable resemblance with other species in the genus Carcharhinus and Ganges shark (da Cunha *et al.*, 2017; Haque & Das, 2019). Consequently, both the species - bull shark and Ganges shark - are often misidentified for each other (Martin, 2005; Compagno, 2007). Moreover, anecdotal records of these species, particularly Ganges sharks, might actually refer to bull shark and vice versa (Compagno, 1997), potentially explaining the scarcity of bull shark sightings in inland Indian waters. Therefore, identification through an integrated approach utilizing key morphological characteristics and molecular markers is crucial for the accurate identification of

these species (Haque & Das, 2019). Key morphological features such as minute eyes, a notch in the anal fin, and a subterminal notch in the caudal fin are helpful in distinguishing the bull shark from the Ganges shark (Compagno, 1997) (Figure 3). Our findings based on the 16s rRNA and ND4 genes, confirmed the identification of the specimen as bull shark, and phylogenetic analysis revealed that C. leucas forms a distinct lineage, exhibiting a sister clade relationship with Glyphis glyphis and C. falciformis. The widespread distribution of haplotype CLH01 indicates substantial gene flow across regions, including India. In contrast, the dominance of CLH09 in other regions such as Australia, Papua New Guinea and Indonesia highlights potential regional structuring (Devloo-Delva *et al.*, 2023). However, the limited number of sequences from India may have introduced bias in these interpretations, emphasizing the need for additional sampling to achieve a more comprehensive understanding of population connectivity within bull shark populations. Further studies have highlighted that genetic divergence in bull shark is primarily driven by significant biogeographic barriers and their philopatric behavior, which restricts gene flow and population isolation (Karl *et al.*, 2011; Devloo-Delva *et al.*, 2023; Postaire *et al.*, 2024).

Understanding the ecological drivers behind the inland distribution of bull sharks remains critical. Bull sharks are known for their remarkable tolerance to both freshwater and marine environments, attributed to their unique osmoregulatory capabilities. This adaptability allows them to migrate hundreds of kilometers upstream in large river systems like the Ganga and its tributaries, including the Hooghly River. One of the primary drivers of their inland movement is the fluctuation of salinity levels in riverine systems. Seasonal variations in salinity, particularly during monsoon and post-monsoon periods, create favorable conditions for bull sharks to move further upstream (Pillans *et al.*, 2020). Juveniles are often found in low-salinity or freshwater zones, which are thought to serve as nursery grounds, providing a refuge from larger predators and supporting early growth stages (Blanco-Parra *et al.*, 2022).

Overall, the first record of bull shark in the inland water system constitutes a noteworthy addition to our understanding of the aquatic biodiversity of the Ganga river basin. Effective conservation efforts, especially for critical nursery habitats, are crucial for the survival of these populations. This highlights the importance of the ongoing efforts made under the 'Namami Gange' program in protecting and revitalizing these crucial ecosystems, by enhanced river monitoring and interventions aimed at preserving the river and its biodiversity (Hussain & Badola, 2020).

Furthermore, understanding behavior, habitat preferences, and migratory patterns of bull sharks can provide insights into the ecological process and interconnectedness of marine and freshwater ecosystems (Heupel *et al.*, 2010; Curtis *et al.*, 2013). Such understanding is essential for the effective management of populations and, for mitigating negative interactions between sharks and humans (Pinel *et al.*, 2023).

Conclusion

Marine apex predators, particularly elasmobranch species like bull shark, play a critical role in maintaining the stability and functioning of marine and estuarine ecosystems. However, they are increasingly threatened by anthropogenic pressures and global climate change. Effective management and conservation strategies for these species require a thorough understanding of their movement patterns and spatial distribution. Due to the limited knowledge of the long-term migration patterns of bull sharks in riverine and estuarine habitats, the report of bull shark in Hooghly River provides valuable insights crucial for conservation and management.

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CONFLICT OF INTEREST

S. A. Hussain is an academic editor at Journal of Wildlife Science. However, he did not participate in the peer review process of this article except as an author. The authors declare no other conflict of interest.

DATA AVAILABILITY

Data will be made available upon reasonable request.

AUTHORS' CONTRIBUTION

G.C.D collected the biological samples and did the morphometric measurements, designed the methodology and wrote the original manuscript. S.P.S and S.T. designed the methodology for molecular analysis, performed the experiment, data curation, data analysis and wrote the original manuscript. S.A.H. acquired resources, and permission to collect biological samples, develop the concept and design the framework and reviewed the manuscript. All the authors approved the final version of the manuscript.

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