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Research Article

Extending the known northern range of *Carcharhinus amboinensis* (Müller & Henle, 1839) in the Bay of Bengal: First record from the Odisha coast, India

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A morphometric study corroborated by DNA barcoding confirms the presence of an under-reported carcharhinid shark species, *Carcharhinus amboinensis* (Müller & Henle, 1839) [Pigeye shark] from Odisha (northern Bay of Bengal), for the first time. This is the northernmost record of the species along the coastline of India and the Bay of Bengal. *Carcharhinus amboinensis* closely resembles its sister species, the euryhaline *Carcharhinus leucas* (Valenciennes, 1839) (Bull shark), in morphological features such as the absence of interdorsal ridge, body colour, and snout and dental morphology. The presence of shared similar morphological traits and their common co-occurrence (sympatric distribution) have been the reasons behind the paucity of records. However, it differs from its sister species by having a larger first dorsal fin than the second dorsal fin, a lesser number of teeth on the lower jaw, and fewer precaudal vertebrae. The present report raises the reported number of carcharhinids from Odisha to thirteen, thereby highlighting the evergrowing, diverse marine environment of Odisha's rich coastal belt.

[**Keywords**: Bay of Bengal, Bull shark, Carcharhinidae, Odisha, Pigeye shark]

Introduction

The shark order Carcharhiniformes (Compagno, 1977) represents a highly diverse and extensive group of sharks comprising 12 families, 61 genera and 350 species[1,2]. India is home to 50 species of the order, distributed across ten families[3,4]. Sharks of the family Carcharhinidae (Jordan & Evermann, 1896) (Requiem sharks) are top predators, consisting mostly of cosmopolitan species inhabiting warm-temperate to tropical waters. Carcharhinid sharks play a crucial role in maintaining the balance in marine ecosystems and have high commercial importance for artisanal and industrial fisheries, which makes it an important family[5]. This family comprises 59 valid species in 11 genera worldwide[2,5], while 28 species in nine genera are found in Indian waters[3,4,6,7].

There are 38 species in the genus *Carcharhinus* Blainville 1816 worldwide[2], of which 18 have been reported from the Indian coast[3,4]. This study reports and confirms the presence of *Carcharhinus amboinensis* (Müller & Henle, 1839) [Pigeye Shark] in the northeastern coast (Odisha) for the first time with genetic evidence. *Carcharhinus amboinensis* belongs to the large-bodied members of the genus *Carcharhinus*, which occur worldwide and are distributed across all three major ocean basins[8]. It was assessed as the closest relative of *C. leucas* as genetic studies revealed[9]. *Carcharhinus amboinensis* has often been misidentified with the similar-looking *C. leucas*, which complicates the reconstruction of its distribution[8]. Even though, *C. amboinensis* has been reported from the southern states of India and the Andaman Islands, it has yet to be reported from the Bay of Bengal. The present report extends its known northern range to the north Odisha coast.

Materials and Methods

A shark specimen was encountered on a routine survey to the Bahabalpur fish landing centre (21°30'47" N, 87°06'52" E) on 10th August, 2024. On enquiry, the fisherman informed that the shark specimen was caught about 22 kms southeast of the landing centre. For species identification, taxonomic keys and morphometric measurements proposed by Compagno[5] were followed. Owing to the large size of the shark specimen, collection or retention was not

feasible. Hence, the shark specimen was documented with photographs, and morphometric measurements were recorded in the field using a measuring tape (to the nearest millimetre). For confirmation of species identification and molecular characterisation, a biopsy was performed to collect (n = 02) white muscle samples from the base of the anal fin. Samples were subsequently preserved in absolute ethanol (EtOH) at room temperature for further analyses.

HiPurA® Insect DNA Purification kit was used to isolate 30 µl of genomic DNA according to the manufacturer's protocols. COI gene segment of the extracted DNA was amplified through PCR employing primers Fish F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and Fish R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') in 25 µL reactions. PCR amplification was achieved in a Thermo Fisher Scientific PCR machine in 25 µL reactions [12.5 µL of 2X Hi G9 Taq PCR Master Mix, 6.5 µL of nuclease-free water, 0.5 µL of forward primer Fish F1, 0.5 µL of reverse primer Fish R1 and 5 µL of DNA Template]. PCR protocol included an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec and extension at 72 °C for 35 sec, repeated for 35 cycles, followed by a final extension for 7 min at 72 °C. PCR products were visualised under UV illumination in 1.2 % Agarose gel. The PCR product was outsourced to HKP Scientific, and the targeted COI gene segment was generated using the Sanger dideoxy termination sequencing method. Phylogenetic analyses and GenBank data uploading were carried out according to Mohapatra *et al.*[10].

The consensus sequence was generated from the forward and reverse mtCOI gene sequences using the software BioEdit[11]. The gene sequence was uploaded to the public gene bank repository of NCBI, and the accession number PV164037 was obtained. For the construction of a phylogeny tree and genetic distance analysis, gene sequences of other congeners, along with those of the outgroup, were obtained (Table 1). The sequence alignment was performed using the MAFFT online platform[12]. The aligned sequence was converted to NEXUS format in Mesquite version 3.81[(ref. 13)]. The best nucleotide substitution model was tested in PAUP[14] using MrModeltest version 2[(ref. 15)] (GTR+G). The rates of substitution of different nucleotides, *viz*., rAC, rAG, rAT, rCG, rCT, and rGT were taken as 0.7801, 16.3501, 3.5598, 1.6967, 48.1558, and 1.0000, respectively. The Gamma distribution's shape parameter was taken as 0.1424. The chain length was taken as 20 million. The phylogeny was constructed in BEAST version 2.7.7[(ref. 16)]. The tree was visualised using FigTree version 1.4.4[(ref. 17)]. The pairwise genetic distance was checked using the Kimura-2-Parameter model[18] in MEGA version XII[19].

Results

***Carcharhinus amboinensis* (Müller & Henle, 1839)**

Common Name: Pigeye shark/Java shark

Material examined: One adult male (TL: 225 cm), observed at Bahabalpur fish landing centre (21°30′47″ N, 87°06′52″ E), Balasore, Odisha, India on 10.VII.2024.

IUCN Status: Vulnerable A2d[20]; Appendix-II of CITES[21].

Table 1 — Details of COI gene sequences of *Carcharhinus* sp. used for phylogenetic analysis

Sl. No.	Species name, author name	NCBI accession number	Geo-location
1		PV164037 (Present Study)	Bahabalpur, Balasore, Odisha, India
2	*Carcharhinus amboinensis* (Müller & Henle 1839)	KF899793.1	India
3		JF493048.1	KwaZulu-Natal, Zinkawazi, South Africa
4	*Carcharhinus amblyrhynchoides* (Whitley 1934)	JN082185.1	Kuwait
5		MF508664.1	Papua New Guinea
6	*Carcharhinus brevipinna* (Valenciennes 1839)	KF899797.1	India
7	*Carcharhinus leucas* (Valenciennes 1839)	KF899808.1	India
8		MF508679.1	Papua New Guinea
9	*Carcharhinus limbatus* (Valenciennes 1839)	KF899813.1	India
10		EU398624.1	Queensland, Australia
11	*Carcharhinus macloti* Müller & Henle 1839)	KF913239.1	India
12	*Carcharhinus melanopterus* (Quoy & Gaimard	KF899824.1	India
13	1824)	JN313260.1	Australia
14	*Sphyrna lewini* (Griffith & Smith 1834)	HQ171764.1	Madagascar
15	*Glyphis gangeticus* (Müller & Henle 1839)	MH244901.1	Bangladesh

Table 2 — Morphometric details of observed *Carcharhinus amboinensis* specimen (Present study) in percentage of total length

Morphometric and meristic data	*Carcharhinus amboinensis*, male (in cms) (This study)	
Total Length (TL) in cm	225	
		%TL
Eye diameter (vertical)	1.5	0.67
Pre-orbital Length (POL)	19	8.4
Head Length (HL)	54.5	24.2
Pre-first Dorsal length (PD1)	71	31.6
Inter-Dorsal Space (IDS)	46.4	20.62
Pre-second Dorsal length (PD2)	147	65.33
Fork Length (FOR)	179	79.55
First dorsal fin height	35.5	15.78
Second dorsal fin height	7.5	3.33
Pectoral fin anterior margin	48	21.33

Diagnostic characters: A large, stout male shark with a total length of 225 cm (adult specimen[22]), was observed. Eyes circular and small, pupil black and elliptical; snout evenly rounded and short, upper labial furrows very short, inter-dorsal ridge absent, pectoral fins large and broadly triangular. First dorsal fin large and broadly triangular, origin of the first dorsal fin well behind the pectoral fin insertion; first dorsal fin height 4.73 times of second dorsal fin height, second dorsal fin height 3.3 % of total length. Second dorsal fin origin is slightly anterior to anal fin origin. Internarial distance less than pre-oral length. Caudal fin heterocercal and asymmetric (Table 2).

Colour: Deep grey above and pale below. Body grey; pectoral fins dark grey in colour; black lining on edges of both dorsal fins. Dorsal and ventral margins of caudal fin dusky (Fig. 1a – c).

Distribution: Eastern Atlantic: Nigeria. Indo-West Pacific: Persian Gulf, Gulf of Aden, South Africa, Madagascar, Pakistan, Sri Lanka, Indonesia, Papua New Guinea, and Australia[22]. Known distribution in India: Karnataka[23], Kerala[24], Tamil Nadu[25,26], Andaman & Nicobar Islands[7], and Odisha (present study) (Fig. 2).

The present study reports the northernmost record of *C. amboinensis* in the Indian coastline, from the state of Odisha.

Carcharhinus amboinensis has an intra-specific genetic distance of 0.2 %. The phylogeny shows that all three *C. amboinensis* sequences form a clade, thus corroborating the morphological identification. The details of K2P distance are provided in Table 3.

Discussion

Odisha coast is known to harbour 12 species of the family Carcharhinidae (Jordan & Evermann, 1896),

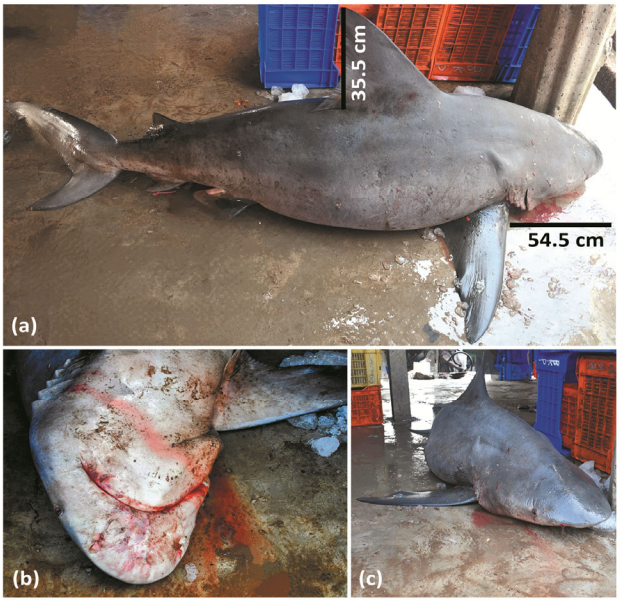

Fig. 1 — *Carcharhinus amboinensis* specimen at Bahabalpur fish landing centre, Balasore, Odisha: a) Lateral view; b) Ventral view of head; and c) Frontal view

with six species belonging to the genus *Carcharhinus* Blainville 1816[refs. 27,28], *viz*., *C. amblyrhynchoides* (Whitley, 1934), *C. dussumieri* (Valenciennes, 1839), *C. leucas* (Valenciennes, 1839), *C. limbatus* (Valenciennes, 1839), *C. melanopterus* (Quoy & Gaimard, 1824), and *C. sorrah* (Müller & Henle, 1839).

With a blunt snout, an internarial space slightly less than the preoral length, a dusky second dorsal fin tip, and the dorsal fin origin over or just behind the pectoral insertion, *C. amboinensis* closely resembles its congener *C. leucas*[3,5]. Notwithstanding, *C. amboinensis* differs from *C. leucas* in having a larger first dorsal fin relative to the second dorsal fin (> 3.1 times in *C. amboinensis vs*. < 3.1 times in *C. leucas*)[3,5]. *Carcharhinus amboinensis* exhibits 11 teeth on each side of the lower jaw (*vs*. 12 – 13 in *C. leucas*)[3,5]. Both species further differ in the number of precaudal vertebrae (89 – 95 in *C. amboinensis*; 101 – 123 in *C. leucas*)[3,5]. In addition, molecular characterisation conclusively rules out any scope of misidentification with *C. leucas*. The genetic distance between the two similar species is 4.9 – 5.6 % (Table 3), and both occur in different distant clades in the molecular phylogeny (Fig. 3).

Among Carcharhinids, *C. amboinensis* (Pigeye shark) has a sympatric distribution with *C. leucas* (Bull shark), which is found ubiquitously[5]. Homologous traits such as short, rounded snouts with

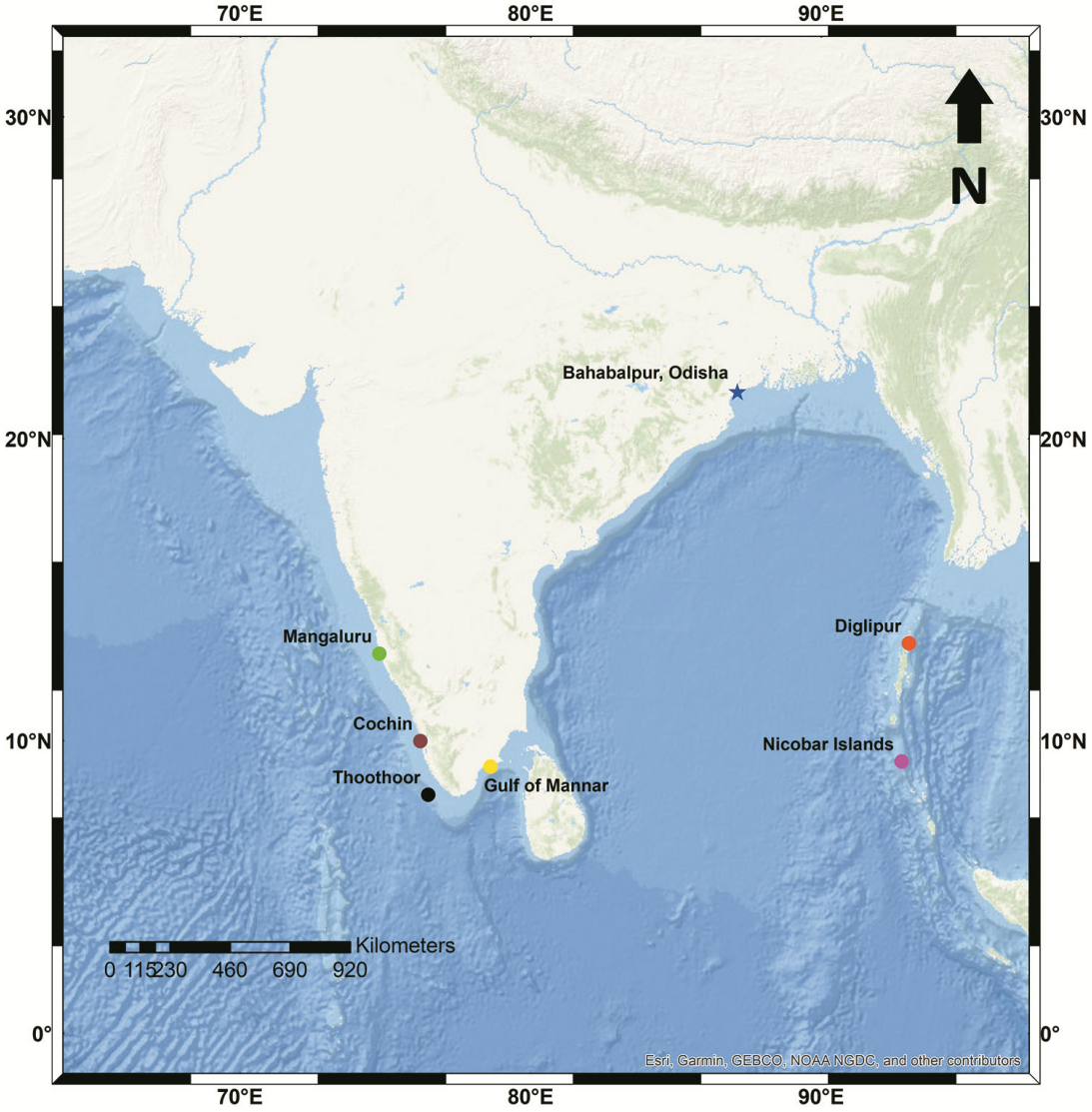

Fig. 2 — Distribution map of *C. amboinensis* along the Indian coastline. Bahabalpur: This study; Mangaluru: Thomas *et al.*[23]; Cochin: Gopalakrishnan *et al.*[24]; Thoothoor: Sahaya *et al.*[26]; Diglipur and Nicobar Islands: Tyabji *et al.*[7]; Gulf of Mannar: Joshi *et al.*[25]

triangular, serrated upper teeth and the absence of interdorsal ridges lead to common misidentification in the field[29]. This could be one of the major reasons for under-reportage and the absence of any previous record of this species from the Northern Bay of Bengal.

Even though the two species co-occur, they exhibit different physiological responses to salinity. *Carcharhinus leucas* is an euryhaline species commonly found in coastal marine habitats, with numerous recorded instances of freshwater penetration[30-32]. It can tolerate salinities from 0 (fresh water) to 53 psu[5,32]. *Carcharhinus amboinensis*, on the other hand, is an occasional marginal marine species which seldom ventures into freshwater[30,31]. Juveniles of Pigeye sharks actively demonstrate avoidant responses to freshwater inflow in estuaries[33]. Juvenile *C. amboinensis* behave totally opposite to juvenile *C. leucas*, which rely on low-salinity environments during the first years of their lifetime because they provide low-mortality habitats[34]. The present record of the shark of 225 cm seems to be a mature male[8] collected from a medium salinity zone along the close proximity of estuarine flow in the Northern Bay of Bengal. The Bay of Bengal receives a substantial freshwater influx after the summer monsoon, which inhibits vertical nutrient mixing[35,36]. A haline gradient is established, characterised by lower sea surface salinity, which leads to lower biological productivity[37]. This freshening effect of the northeastern Bay of Bengal is compounded by eddies,

Table 3 — Genetic distance of *Carcharhinus amboinensis* from its congeners

	C. amboinensis	*C. melanopterus*	*C. amblyrhynchoides*	*C. limbatus*	*C. leucas*	*G. gangeticus*	*S. lewini*	*C. brevipinna*	*C. macloti*
C. amboinensis	0.22								
C. melanopterus	6.47–6.65	0.30							
C. amblyrhynchoides	5.43–5.72	4.84–5.30	0.65						
C. limbatus	5.32–5.72	4.76–4.95	0.16–0.62	0.15					
C. leucas	4.90–5.60	6.60–6.80	4.60–5.30	4.52–4.96	0.5				
G. gangeticus	8.43–9.43	10.20–10.30	8.00–8.25	7.80–8.00	7.65–8.00				
S. lewini	10.89–11.72	12.00	11.80–12.70	12.00–12.20	10.93–11.25	13.45			
C. brevipinna	4.31–4.53	6.30	5.00	4.80–50	5.20–5.30	8.75	11.83		
C. macloti	4.81–5	7.00	5.00–5.30	4.80–50	5.07–5.30	8.00	11.64	4.64	

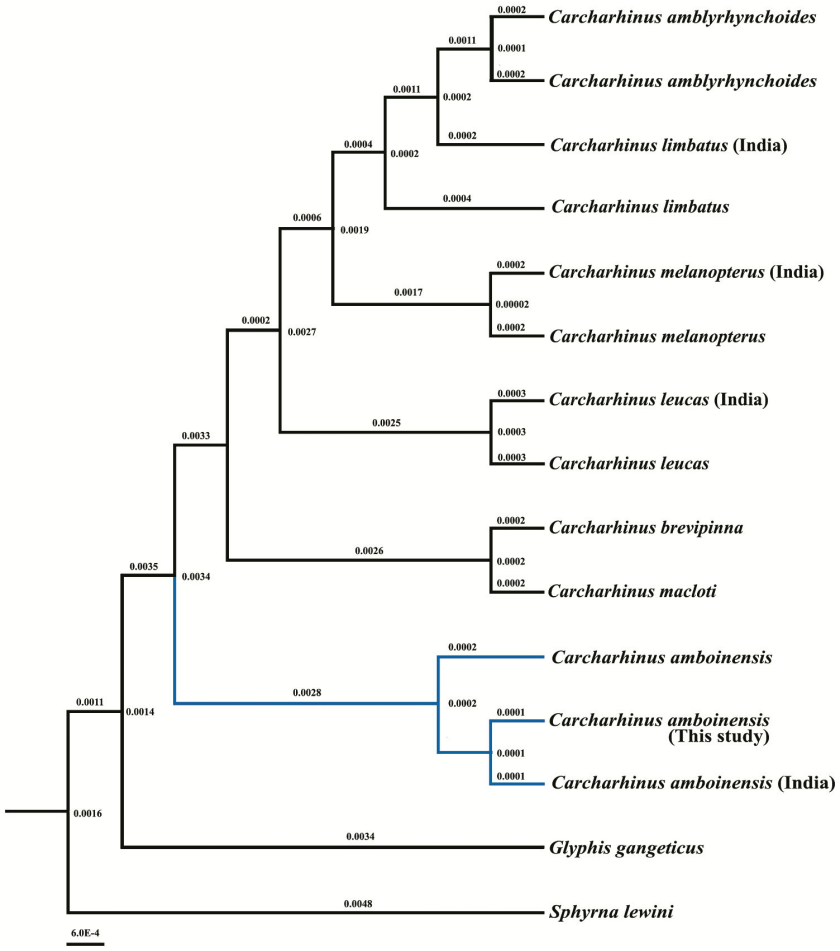

Fig. 3 — Bayesian inference phylogenetic tree of *C. amboinensis*

which push freshwater back into offshore areas[38]. The present study's recording of *C. amboinensis* catch during the peak rainfall season (August, 2024) in the northern Bay of Bengal could be pertinent for gathering information on the physiology of *C. amboinensis*, since the salinity of this region decreases due to the high influx of freshwater from rivers[39]. Strong water currents and tidal flow in the northern Bay of Bengal provide an

experimental ground to study variations in space use and home range of *C. amboinensis* in response to freshwater influx, preferably by undertaking a tagging study. Such future studies could focus on better understanding the behavioural consequences of physiological adaptations.

Conclusion

The present study conclusively demonstrates the presence of *C. amboinensis* (as corroborated by DNA barcoding and morphological identification) in the northeastern Bay of Bengal and extends its range northward along the eastern coastline of India. The present documentation contributes to the growing list of Odisha's marine megafauna biodiversity by increasing the reported number of sharks of the family Carcharhinidae (Jordan & Evermann, 1896) in the state to 13, and seven in the genus *Carcharhinus* Blainville 1816. The study may provide an opportunity to gather valuable information on the elasmobranch fauna of the Odisha coast.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

The studied shark species is not listed under any scheduled groups in the Wildlife Protection Act, 2022, but is included in Appendix II of CITES, and only a tissue sample was collected from the specimen, which was brought dead to the landing centre.

Author Contributions

TKST: Collection, preservation, identification, DNA isolation, and manuscript preparation; SKM: Collection and preparation of maps; SA: DNA isolation, and DNA sequence interpretation; BB, SSM & AM: Identification confirmation, manuscript preparation, and critical analysis.

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