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DNA Barcoding Confirms the Occurrence Rare Elasmobranchs in the Arabian Sea of Indian EEZ

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Abstract: The present paper reports and confirms the occurrence of *Rhynchobatus australiae* Whitley, 1939, *Dasyatis microps* Annandale (1908), *Himantura granulata* (Macleay, 1883), *Aetomylaeus vespertilio* (Bleeker, 1852) in the Arabian Sea coast of India and an extension of from their known distribution range by mitochondrial DNA (COI) analysis based on specimens/tissues collected from southwest coast of India. The obtained 640 bp COI sequence fragments perfectly matched with specific sequences available on the Barcode of Life Data (BOLD) system database.

Key words: Distribution extension · *Rhynchobatus australiae* · *Dasyatis microps* · *Himantura granulata* · *Aetomylaeus vespertilio* · Arabian Sea · India

INTRODUCTION

India has one of the largest chondrichthyan fishery in the world and a very diverse fauna of chondrichthyans. The chondrichthyan species have been catalogued by several researchers [1, 2], but taxonomic studies based on molecular tools are necessary to solve taxonomic issues in chondrichthyans especially when many large families have wider distribution, complex variations within and have complex taxonomic history. In the recent years several species have been added to chondrichthyan checklist of Indian waters [3-6] which clearly states that diversity of this group is far greater than expected in Indian waters.

The marine waters of India support a diverse chondrichthyan fauna consisting of more than 110 known species [1], the fishery of which is considered to be the one of the largest in the world [7]. However, the species composition in the chondrichthyan fishery along the Indian coast are poorly known and documented. During a survey of fish landing centers along the south west coast of India during 2008-2013, some rare species of elasmobranchs were observed. The present article provides the evidence of the occurrences of white spotted wedge fish, small eye stingray, ornate eagle ray and whitetail whipray from the southwest coast of India.

MATERIALS AND METHODS

Specimens were collected/observed from Cochin Fisheries Harbour (CFH), Kochi, Kerala and identified. Species identification based on [8], [9]. Since there is no issues in the field identification, except white spotted wedge fish which is deposited in the National Marine Biodiversity Museum (Designated National Repository) in the Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala, India (Accession No: GA.11.14.1.2).

Samples of white muscle tissues were collected from species were preserved in 95% ethanol used for DNA extraction and sequencing. The purified DNA samples were extracted from white muscle tissues of specimens using the Qiagen DNeasy Blood and Tissue kit (Kochi, India) and stored in AE buffer. The partial sequence of

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COI gene was amplified using primer 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') [10] in 25 µl reactions containing 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA polymerase and 20 ng of template DNA. The thermal condition consisted of initial preheat at 95°C for 3 min, denaturation 94°C for 30 s, annealing 50°C for 30 s, extension 72°C for 35 s, repeated for 29 cycles, followed by a final extension for 3 min at 72°C. The PCR products ranged from 620 to 655 bp and were visualized on 1.2% agarose gels. Samples with intense bands were selected for sequencing. Sequencing reactions used a BigDye terminator v3.1 cycle sequencing kit (applied Biosystems, Inc). All samples were sequenced bidirectionally using an ABI3730 capillary sequencer following the manufacture's protocol. The raw DNA sequences were edited and aligned using BioEdit sequence alignment editor version 7.0.5.2 [11]. Sequence divergences were calculated using Kimura 2 parameter (K2P) distance, clustered by neighbour joining and bootstrapped using MEGA 4.0 with 1000 replications. Twelve COI sequences of *Rhynchobatus* were obtained from GenBank with the following accession numbers: *Rhynchobatus australiae* Whitley, 1939 (EU 399009, EU 399007, DQ 108199 and EU 399008), *Rhynchobatus* cf. *laevis* (EU 399010, DQ 108198 and DQ 108192) and *Rhynchobatus djeddensis* (JF 4943386, JF 4943385, JF 4943384 and GU 805049). The COI sequences for *Glaucostegus typus* Bennett, 1830) used as an outgroup was obtained from GenBank (EU 398999). Twelve COI sequences generated in the present study were submitted to GenBank (www.ncbi.nlm.nih.gov/ GenBank) and Barcode of Life Database (www.barcodelife.org) with the following GenBank accession numbers: *Rhynchobatus australiae* ((JN022595, JN022596, JN108018 and JN108019), *Himantura granulata* (KF899471 and KF899472) and *Aetomylaeus vespertilio* (KF899584, KF899585 and KF899586).

RESULTS AND DISCUSSION

Order: Rajiformes

Family: Rhynchobatidae

***Rhynchobatus australiae* Whitley, 1939:** The genus *Rhynchobatus* comprises at least eight species of moderate size to giant shark like batoids in the family Rhynchobatidae [12, 8, 13], which are widespread and common in inshore tropical continental shelf waters of the eastern Atlantic, Indian and western Pacific Oceans [8, 13]. *Rhynchobatus* species are termed 'wedgefishes' because of their distinctive wedge shaped discs and snouts and are commonly caught as bycatch of demersal fisheries. The genus *Rhynchobatus* is represented by two species, *R. laevis* (Bloch and Schneider, 1801) and *R. djiddensis* (Forsskål, 1775) from Indian waters. *Rhynchobatus australiae* Whitley, 1939, originally described from New South Wales, Australia is a common species found in the Indo-West Pacific from the Gulf of Thailand in Thailand, Madagascar, East Indies, Java, Bali, Lombok and Papau in Indonesia, Philippines and Queensland (Australia) and has been given a status as endangered in IUCN red list [14].

The present study confirms the occurrence of *Rhynchobatus australiae* in the Arabian Sea (Figures 1 and 2). Several different colour morphs of *Rhynchobatus australiae* are present presumably representing different species (P. Last, pers. Comm). COI DNA sequences of 640 bp were generated for four specimens of *R. australiae* from India. The four specimens of *R. australiae* from southwest coast of India had an identical COI barcode sequence (0.0%) and this was very similar (0.9% divergent) to the specimens from Western Australia. (Fig. 3). The interspecific distance between *R. australiae* and *R. djiddensis* from was 3%. All four sequences were BLAST against BOLD and GenBank databases to confirm the identification. Our sequences showed 99% similarity with *R. australiae* in GenBank and 100% similarity in BOLD.

Order: Myliobatiformes

Family: Dasyatidae

***Dasyatis microps* Annandale (1908):** *Dasyatis microps* is a large stingray, described based on a single specimen collected from northern Bay of Bengal, off the Chittagong coast. Subsequently, [15]Annandale (1909) reported *D. microps* from Orissa coast and later [16] reported from the Gulf of Mannar. Since then, there are no records of the occurrence of this species from anywhere in India, except figured in [17]. Wide distribution range for *D. microps* in the western Indian Ocean and its distribution records were also provided [18]. The reports of *D. microps* from Arabian Sea are limited to Maldives [19] and off the coast of Oman. Due to its rarity in collections and scarce information on distribution, populations and fishing pressure, *D. microps* has been assessed as Data Deficient by the IUCN [20].

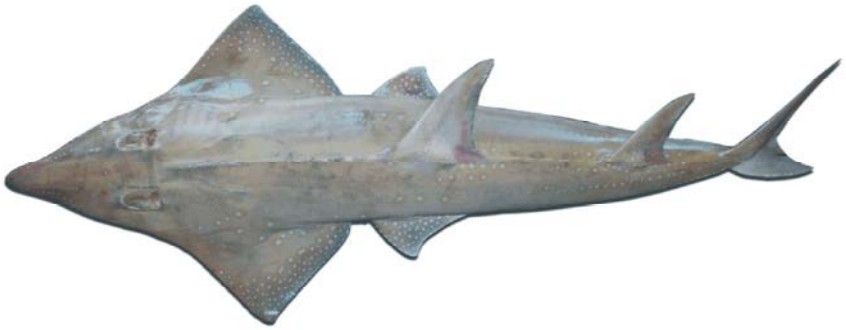

Fig. 1: *Rhynchobatus australiae* dorsal view (Adult) landed at Cochin Fisheries Harbour, Kerala

Fig. 2: *Rhynchobatus australiae* dorsal view (juvenile) landed at Cochin Fisheries Harbour, Kerala

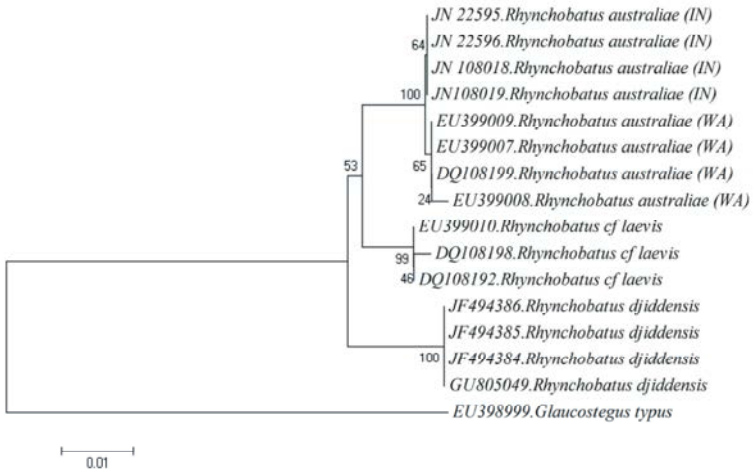

Fig. 3: K2P distance neighbour -joining tree of *Rhynchobatus australiae* from Western Australia (WA) and India (IN).

Five barcodes were generated for this species represents new sequences for both *BOLD* and GenBank databases. Sequences for *D. microps* have no matching sequence in both BOLD and GenBank databases.

Order: Myliobatiformes

Family: Dasyatidae

***Himantura granulata* (Macleay, 1883):l** Whitetail whipray *Himantura granulata* (Macleay, 1883) was described from a single female specimen from Port Moresby, Papua New Guinea. This species has a wide distribution range in the Indo-West Pacific extending from the Maldives to Australia. The Western Indian Ocean reports are from Seychelles [21] and Maldives [22]. A single specimen (Fig. 6) was observed during survey of fishery landings at Cochin Fisheries Harbour. Due to the fishing pressure in the coastal waters of occurrence, *H. granulata* has been assessed as Near Threatened by the IUCN [23].

Fig. 4: *Dasyatis microps* (142 cm DW) landed at Cochin Fisheries Harbour, Kerala.

Fig. 5: *Dasyatis microps* with *Taeniura meyeni* in the landings at Cochin Fisheries Harbour, Kerala

Two sequences were generated. Our sequences in Gen Bank had 98% similarity with *Himantura granulata* (JQ765514) from Australia and 98-99% similarity in BOLD with *H. granulata*. The Indian sequence is showing 2% divergence with Australian sequences warrants further morphological examination between geographically distant forms.

Order: Myliobatiformes
Family: Myliobatidae

***Aetomylaeus vespertilio* (Bleeker, 1852):** Ornate eagle ray or the reticulated eagle ray, *Aetomylaeus vespertilio* (Bleeker, 1852) is a rare species in the western Indian Ocean, where it has been previously reported from the Red Sea [24] Maldives [19] and Mozambique [25]. Due to wide distribution range [26] suggested its possible occurrence in Indian coast. Recently it has been observed in Cochin and has been reported [27, 28]. Specimens of *A. vespertilio* (Fig. 7) were observed in the fishery landings at Kochi. Due to the very high (and

Fig. 6: *Himantura granulata* (74 cm DW) with *Taeniura meyeni* in landings at Cochin Fisheries Harbour, Kerala, India

Fig. 7*: Aetomylaeus vespertilio* landed at Cochin Fisheries Harbour, Kerala, India

increasing) level of fishing pressure in inshore regions of occurrence, *A. vespertilio* has been assessed as Endangered by the IUCN [29].

Two sequences were generated. Our sequences in GenBank had 99% similarity with *Aetomylaeus vespertilio* (EU398512 and EU398511) from Australia and 99% similarity in BOLD with *A. vespertilio*.

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