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First Record of African Angel Shark, *Squatina africana* (Chondricthyes: Squatinidae) in Indian Waters, Confirmed by DNA Barcoding[1]

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Abstract—A single specimen of African angel shark, *Squatina africana* (Regan, 1908) was caught off Lakshadweep (11°5′47″ N; 72°2′21″ E), India in September 2016. The present study is a new report of the above species from Indian waters. In addition to classical methodologies, DNA barcoding was also adopted for species identification. The 650 bp-long region of mitochondrial Cytochrome Oxidase subunit I was sequenced to obtain the DNA barcode for the species under study. The sequence divergence value within species and between species was calculated using MEGA V.7.0, where Kimura 2 parameter (k2p) model was chosen as a distance model. The average k2p distance separating individuals within species was 1.76% and inter specific divergence was 8–10%. A neighbour joining network was constructed to provide a graphical representation of divergence between the species. Using the maximum identity with Gen Bank database, K2P divergence distance, NJ-network and traditional morphological approach, we could identify the given specimen as a mature male African angel shark.

Keywords: first record, Squatinidae, cytochrome c oxidase, *Squatina africana*, India

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INTRODUCTION

Angel sharks belonging to the family Squatinidae (Chondrycthyes, Elasmobranchii, Squatiniformes, Squatinidae) are dorso-ventrally flattened benthic sharks that are globally distributed in temperate and tropical marine waters (Colonello et al., 2007; Stelbrink et al., 2010). The family Squatinidae consists of a single genus, *Squatina* (Dumeril, 1806) with approximately 19 valid species (Walsh, 2011). They are common benthic and epibenthic sharks found on the continental shelf and upper slope, from the surf line close inshore to about 500 m depth (Compagno, 1984; Cliff, 2004). Among the various species of angel sharks, *Squatina africana* (Regan, 1908) is one of the smaller species. The species has been reported only from the South Western Indian Ocean between Tanzania and Eastern Cape of South Africa, where it is commonly found between 60 to 300 m depth (Compagno, 1984; Stelbrink et al., 2010). As the Squatina species are ambush predators with bottom dwelling habit, the majority of them are restricted to a small area (Stelbrink et al., 2010). Though one species of angel shark, *Squatina squatina* has been reported from Indian waters during 2000–2002 (Joshi, 2008), the present collection is the first report of the African angel shark, *Squatina africana* from Indian waters.

MATERIALS AND METHODS

The present sample was obtained on 28 September 2016 during our regular fishery observation survey at Cochin Fisheries Harbour (CFH), Kochi, Kerala, India. The specimen collected for this study was caught in a mechanised gillnet unit operated off Lakshadweep (11°5′47″ N; 72°2′21″ E) at depths of 100–500 m. The taxonomic identification of specimen was done according to FAO Species identification sheets for fishery purposes (Fischer and Bianchi, 1984). The morphometric measurements were made using a digital Vernier caliper (0.1 mm accuracy) following (Walsh and Elbert, 2007; Walsh et al., 2011). The specimen is deposited in the National marine biodiversity referral museum at CMFRI, Kochi (Deposition ID: GA. 15.2.5.4).

Apart from the traditional morphometric methods, DNA barcoding was also done for conclusive identification of the species. The tissue sample was collected and preserved in 95% ethanol at –20°C for analysis. Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue kit (QIAGEN, Germany)

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Table 1. Measurements of *Squatina africana* landed off Kochi

Morphometric characters	Measurements	Morphometric characters	Measurements
Total length, *TL*	691	Pectoral Fin posterior margin, P1P	13.59
Pre-Caudal Length, PRC	82.32	Pectoral-Pelvic Space, PPS	14.23
Pre-Orbital Length, POB	2.74	Pelvic Fin Length, P2L	21.34
Pre-Spiracle Length, PSP	7.21	Pelvic Fin Anterior Margin, P2A	12.07
Pre-Branchial Length, PG1	13.86	Pelvic Fin Base, P2B	10.73
Head Length, HDL	16.63	Pelvic Fin Height, P2H	10.04
Pre-Pectoral Length, PP1	17.03	Pelvic Fin Inner Margin, P2I	10.70
Pre-Pelvic Length, PP2	37.21	Pelvic Fin Posterior margin, P2P	16.68
Snout-Vent Length, SVL	43.04	Pelvic Caudal Space, PCS	32.53
Pre-1st Dorsal Length, PD1	61.32	1st Dorsal Fin Length, D1L	8.05
Pre-2nd Dorsal Length, PD2	72.46	1st Dorsal Fin Anterior Margin, D1A	8.59
Mouth Length, MOL	12.13	1st Dorsal Fin Base, D1B	3.59
Mouth Width, MOW	5.53	1st Dorsal Fin Height, D1H	6.34
Internarial Width, INW	6.09	1st Dorsal Fin Inner Margin, D1I	2.73
Nostril Width, NOW	1.52	1st Dorsal Fin Posterior Margin, D1P	3.90
Anterior Nasal Flap Length, ANF	1.59	2nd Dorsal Fin Length, D2L	8.00
Upper Lip Arch width, UAW	3.24	2nd Dorsal Fin Anterior Margin, D2A	8.18
Upper Lip Arch Height, UAH	0.90	2nd Dorsal Fin Base, D2B	3.43
Eye Length, EYL	2.03	2nd Dorsal Fin Height, D2H	6.83
Eye Height, EYH	1.37	2nd Dorsal Fin inner Margin, D2I	2.85
Inter-orbital Space, INO	7.42	2nd Dorsal Fin Posterior Margin, D2P	2.84
Spiracle Length, SPL	2.87	Inter-dorsal space, IDS	7.43
Eye-Spiracle Space, ESL	1.81	Dorsal caudal space, DCS	17.29
Inter Spiracle Space, ISP	7.10	Caudal peduncle Height, CPH	6.29
Head Height, HDH	3.54	Caudal peduncle Width, CPW	3.42
Head Width, HDW	20.06	Dorsal-Caudal Fin Margin, CDM	11.09
Trunk Height, TRH	3.94	Pre-ventral Caudal Fin Margin, CPV	13.39
Trunk Width, TRW	20.42	Lower Post ventral Caudal Margin, CPL	6.15
Pectoral Fin Length, P1L	30.38	Upper Post ventral Caudal Margin, CPU	5.33
Pectoral Fin Anterior Margin, P1A	26.61	Sub-terminal Caudal Fin Margin, CST	3.20
Pectoral Fin Base, P1B	8.91	Clasper inner length, CLI	17.39
Pectoral Fin Height, P1H	25.08	Clasper outer length, CLO	12.62

Total length (*TL*) is given in millimeters (mm), all other measurements are percent of *TL*.

following manufacturer's protocol. Polymerase Chain Reaction (PCR) was performed in order to amplify the Cytochrome Oxidase subunit I (COI) barcode fragments using two 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') (Ward et al., 2005). PCRs for COI were performed in a reaction volume of 25 μL consisting of 12.5 μL of Orion X Taq PCR smart mix (Origin, Kerala), 0.5 μL of each primer, 10.5 μL water and 1 μL of template DNA. PCR was carried out in thermal cycler (BIORAD T-100). The thermocyclic condition of PCR included the initial denaturation at 94°C for 3 min, followed by denaturation at 94°C for 30 s, annealing at 59.5°C for 30 s and extension at 72°C for 1 minute for 34 cycles followed by final extension at 72°C for 7 min. PCR products were visualized using Agarose gel electrophoresis (1.2%) and sequenced bi-directionally using AB 1 3730×1 capillary Sequencer following the manufacturer's instruction. The resulting sequences were assembled and edited using BioEdit sequence alignment editor (Hall, 1999). Sequences were compared using Basic Local Alignment Search Tool (BLAST) of NCBI (Altschul et al., 1990) (http://www.ncbi.nih.gov/BLAST) and also through BOLD species identification portal (http://www.barcodinglife.org). The edited sequences were submitted to GenBank. The sequence divergence value within and between species were calculated using the Kimura two parameter (k2p) distance model (Kimura, 1980) implemented in MEGA V.7.0 (Kumar et al., 2016). A neighbour joining network (French, 2014) was constructed with PopART V.1.7 (http://popart.otago.ac.nz), using the sequence from the present study and sequences available from NCBI (HQ945823, HQ945896, FN431674, FN431680, FN431681, FN431682, FN431684, and FN431688). JN641253, EU399039, FN431752, FN431717 were taken as out group.

RESULTS AND DISCUSSION

In the present study, we identified the specimen as *Squatina africana* based on the characters which fit the

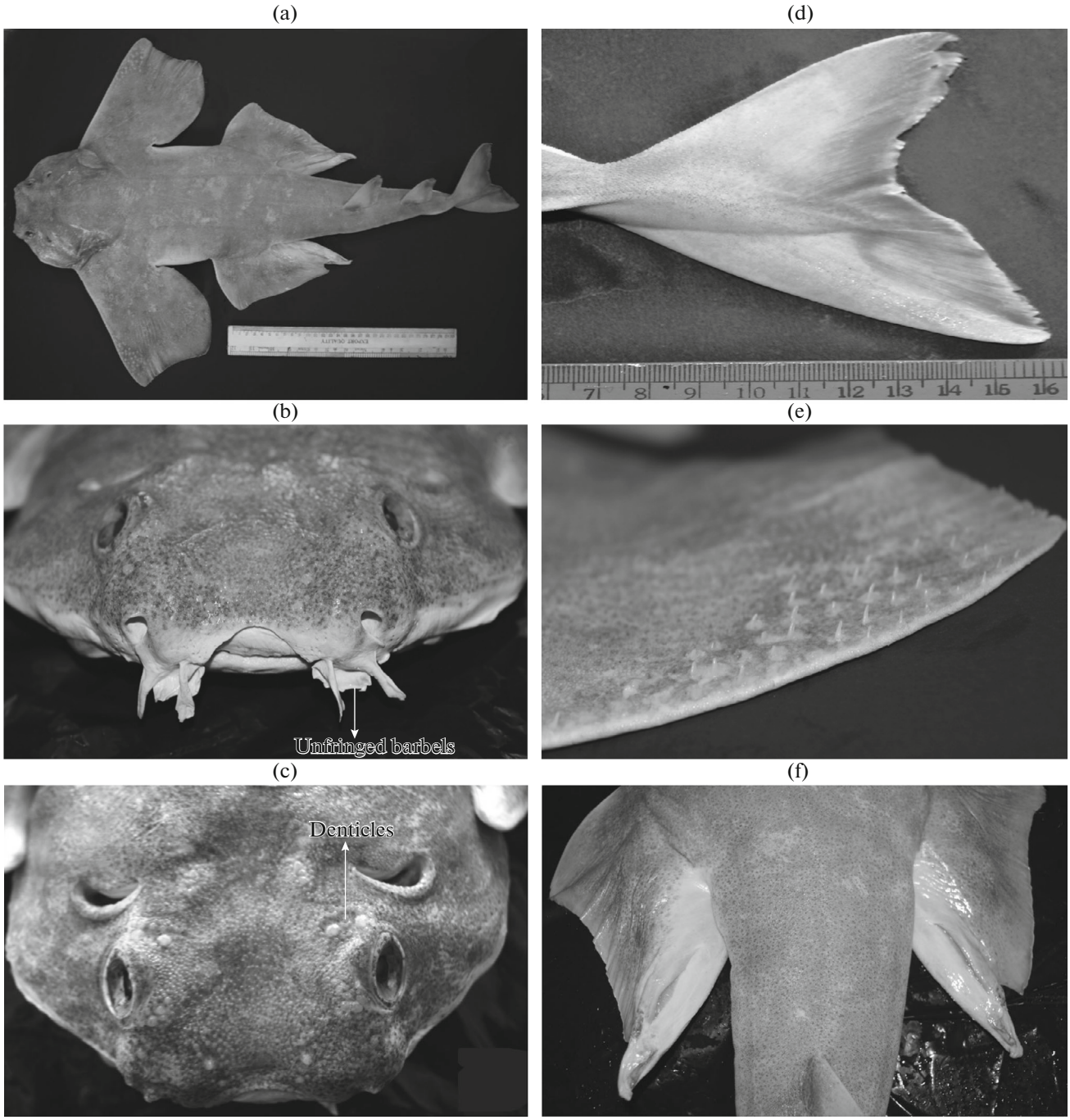

Fig. 1. African angel shark, *Squatina africana*: (a) mature male, 691 mm; (b) close-up view of snout, (c) close-up view of denticles on head, (d) caudal fin, (e) thorn shaped denticles on pectoral fin, (f) a pair of claspers.

description of the species by FAO (Compagno, 1984; Fischer and Bianchi, 1984). The morphometric measurements of the collected specimen are given in Table 1. The specimen was a mature male of 568.8 mm precaudal length (PCL) and weighed 2.665 g. Angel sharks belong to the family Squatinidae (Order: Squatiniformes) and are identified by their batoid shape, terminal mouth and nostrils, with nasal barbels on the anterior margin. Their eyes and large spiracles are found on dorsal surface of the head with gill slits on the sides of head. Presence of enlarged thorns or denticles on the head between eyes and spiracles distinguished the African angel sharks from other species. The distance from eye to spiracle is less than 1.5 times eye diameter. They have two small spineless dorsal fins, which are found behind the pelvic fins. Nostrils are at the tip of snout with unfringed barbels. Teeth present in both jaws with short cusps and no cusplets.

Table 2. Pair-wise genetic distances (Kimura 2 parameter) based on COI sequences from *Squatina* spp.

Species with Genbank Accession number		1	2	3	4	5	6	7	8	9	10	11	12	13
1.	*S. africana* INDIA	0.000												
2.	*S. africana* HQ945896	0.013	0.000											
3.	*S. africana* HQ945823	0.015	0.002	0.000										
4.	*S. africana* FN431680	0.025	0.021	0.023	0.000									
5.	*S. africana* FN431681	0.025	0.021	0.023	0.000	0.000								
6.	*S. africana* FN431682	0.025	0.021	0.023	0.000	0.000	0.000							
7.	*S. africana* FN431684	0.025	0.021	0.023	0.000	0.000	0.000	0.000						
8.	*S. africana* FN431674	0.025	0.021	0.023	0.000	0.000	0.000	0.000	0.000					
9.	*S. africana* FN431688	0.025	0.021	0.023	0.000	0.000	0.000	0.000	0.000	0.000				
10.	*S. occulta* FN431752	0.078	0.074	0.074	0.083	0.083	0.08	0.083	0.083	0.083	0.000			
11.	*S. californica* FN431717	0.083	0.081	0.083	0.091	0.091	0.09	0.091	0.091	0.091	0.020	0.000		
12.	*S. formosa* EU399039	0.091	0.083	0.081	0.096	0.096	0.10	0.096	0.096	0.096	0.089	0.094	0.000	
13.	*S. squatina* JN641253	0.100	0.101	0.103	0.110	0.110	0.110	0.110	0.110	0.110	0.108	0.106	0.082	0.000

They have large, angular pectoral fins with triangular anterior lobe and broad pelvic fin. First dorsal fin is seen posterior to free rear tip of pelvic fins and caudal fin is short with nearly symmetrical lobes. Caudal peduncle is with a pair of short keels and a weak upper precaudal pit. Ocelli are absent on the body (Compagno, 1984; Fischer and Bianchi, 1984; *FishBase*..., 2014; Miller, 2015) (Fig. 1).

In a study conducted on the KwaZulu-Natal coast of South Africa, Shelmerdine and Cliff (2006) reported that males mature between 640 and 700 mm and females at about 700 mm PCL. Thorn shaped denticles, each about 2 mm, were present on the anterior dorsal margin of both pectoral and pelvic fins of the identified specimen with a pair of calcified claspers (Figs. 1e, 1f). The thorns in mature males were directed backwards and were not arranged in distinct rows. According to Baremore (2010), the spines present in males are the general characteristics of angel sharks and as an indicative of maturity. The spines have been previously reported on the pectoral fins of mature males of *S. africana* (Shelmerdine and Cliff, 2006) and *S. guggenheim* (Colonello et al., 2007), though they have no known function. According to Luer and Gilbert (1985), the presence of spines was a secondary sexual characteristic and an indicative of maturity. As like the alar thorns in skates (Rajidae) they may be used during copulation for maintaining posture (Shelmerdine and Cliff, 2006).

DNA barcoding was employed in this study for confirmation of species identity. We constructed a comparative phylogeography of the squatinid sharks based on the mitochondrial marker, Cytochrome Oxidase subunit I (COI). The amplified sequences of the mitochondrial COI gene obtained from *S. africana* were about 635 bp in length after trimming. The sequence was submitted to GenBank under accession number (KY497255). To calculate the pairwise genetic distance, the COI sequences of *Squatina* (12 Nos) were retrieved from Genbank with the following accession numbers: *S. africana* (HQ945823, HQ945896, FN431674, FN431680, FN431681, FN431682, FN431684, FN431688), *S. squatina* (JN641253), *S. formosa* (EU399039), *S. occulta*

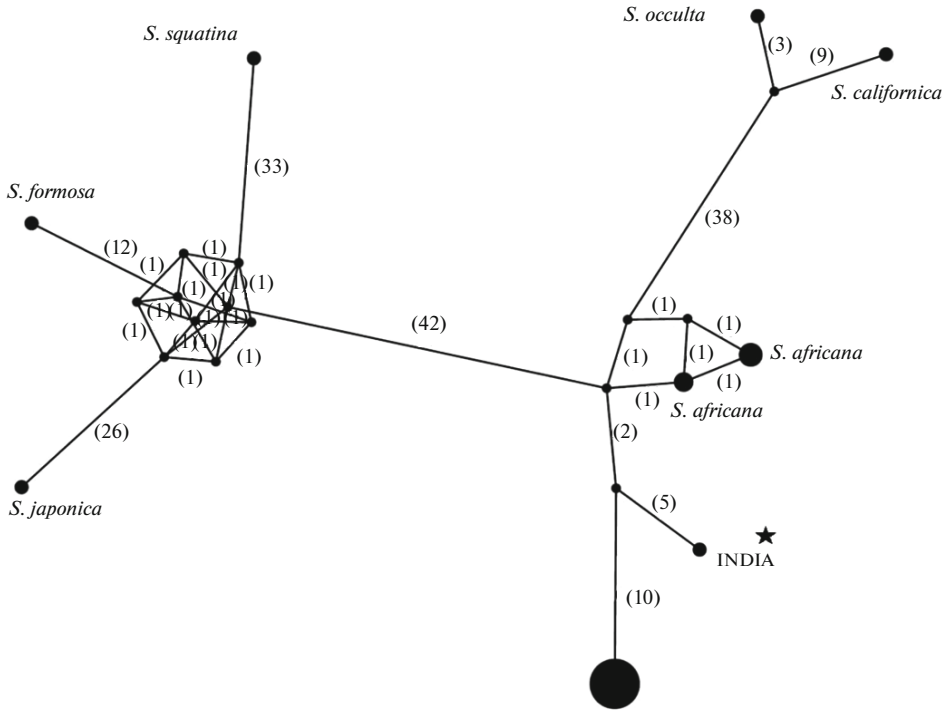

Fig. 2. Neighbour joining network for COI haplotypes in present and previously studied samples. Circle size is proportional to the number of samples. Number indicated in brackets—mutation steps between haplotypes.

(FN431752) and *S. californica* (FN431717) (Ward et al., 2008; Stelbrink et al., 2009; Moftah et al., 2011; Steinke et al., 2016). The pairwise genetic distance value (k2p) based on COI sequences were given in Table 2. From this study, the average k2p distance of individuals within species was 1.76%, and between species was 10% (*S. squatina*, JN641253), 9.1% (*S. formosa*, EU399039), 8% (*S. occulta*, FN431752) and 8.3% (*S. californica*, FN431717). Stelbrink et al. (2009) reported inter-specific genetic distance between the different species of *Squatina* ranged from 2.3 to 9.4%. But previously reported *S. africana* from South African east coast (FN431674, FN431680-82, FN431684, and FN431688) and from Tugela Deep, South Africa, showed 2.3% sequence divergence. From this study we observed that the inter-specific divergence varied from 7.4 to 10.8% in *Squatina* with a minimum intraspecific distance of 1.3% in *S. africana*. The neighbor joining network showed 8 and 9 mutation steps between the present sample (KY497255) and S. *africana* (HQ945896 and HQ945905) from Tugela Deep (Steinke et al., 2016) and 15 mutation steps between the present study and *S. africana* (FN431674, FN431680-82, FN431684, FN431688) from South African east coast (Stelbrink et al., 2010) (Fig. 2). The neighbor joining network also supported the similarity of our sample to the South African Tugela deep specimen of *S. africana* (HQ945896).

Squatina africana is known to be distributed only along the east coast of South Africa and has been poorly studied worldwide because of its limited potential in the fishery industry. So far, the species has been reported in the by catch of only two fisheries in Kwazulu-Natal of South Africa and Tugela Bank prawn trawl fishery. According to Cliff (2004), the current distribution of this species is widespread in the Western Indian Ocean. Shelmerdine and Cliff (2006) have found that angel sharks may undertake seasonal movements, either in response to environmental conditions or to relieve reproductive pressure.

CONCLUSION

From this study we have identified the specimen obtained as *Squatina africana,* a first report of this species in Indian waters. The taxonomy of angel shark was done based on the morphological characters combined with molecular methods. The DNA barcode of our sample showed 99% similarity and 1.3% genetic distance with the *S. africana* from Tugela Deep of South Africa. Since the species is a native of the Western Indian Ocean, we assume that the species is extending its distribution from the east coast of South Africa to the south west coast of India.

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