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RESEARCH ARTICLE

DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery

K. K. Bineesh[a], A. Gopalakrishnan[b], K. V. Akhilesh[b], K. A. Sajeela[a], E. M. Abdussamad[b], N. G. K. Pillai[b], V. S. Basheer[a], J. K. Jena[c] and Robert D. Ward[d]

[a]National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus Ernakulam North, Kochi, Kerala, India; [b]Central Marine Fisheries Research Institute, Ernakulam North, Kochi, Kerala, India; [c]National Bureau of Fish Genetic Resources, Dilkusha, Lucknow, India; [d]CSIRO National Research Collections Australia, Hobart, Tasmania, Australia

ABSTRACT

DNA barcoding was successfully used for the accurate identification of chondrichthyans in the Indian commercial marine fishery. About 528 specimens of 111 chondrichthyan species and 34 families, collected from the Indian EEZ, were barcoded for a 655 bp region of the mitochondrial gene cytochrome c oxidase subunit 1 (COI). Generally, five specimens per species were barcoded, but numbers ranged from 2 to 13. The average Kimura 2 parameter (K2P) distance separating individuals within species was 0.32%, and the average distance separating species within genera was 6.73%. Ten species were suggested as putative new species requiring formal descriptions. Based on the morphology and molecular support, 11 elasmobranch species were confirmed first records for Indian waters. The present study confirms the ability of DNA barcoding for the accurate identification of sharks, rays, and their products from Indian waters.

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Barcoding; chondrichthyans; identification; COI; Indian Ocean; taxonomy

Introduction

Chondrichthyans (chimaeras, sharks, rays, and skates) are widely distributed in all the oceans, but are most diverse in the tropical and subtropical Indo-Pacific (Bonfil, 2002). Chondrichthyans are exploited in commercial, artisanal, and recreational fishing activities but the major catch occurs as bycatch in the commercial fishery. They are highly vulnerable to over exploitation and habitat degradation due to their K-selected life history (Dulvy et al., 2014; Stevens et al., 2000). Overfishing and bycatch have significantly reduced many populations of these apex predators (Baum et al., 2005; Graham et al., 2010; Robbins et al., 2006). Concerns over the impact of fishing on elasmobranch population are being raised at international levels and many programs are being initiated to recover and protect this group through sustainable management plans (Dulvy et al., 2014; Ward-Paige et al., 2012). Accurate species identification is critical to the design of fishery conservation and management plans. (FAO, 1997; Last, 2007; White & Last, 2012). However, field identification of several closely related sharks (including carcharhinid, centrophorid, and triakid sharks) and batoids (Whiptail stingrays and skates) are often difficult (Tillett et al., 2012; Veríssimo et al., 2014), and can lead to erroneous species compositions and diversity in catch reports (Camhi et al., 2009).

India is one of the leading chondrichthyan fishing nations for past several years (FAO, 2013), in 2013, the estimated landing of 46,471 tonnes (sharks 45.5%, rays 49.5%, and guitarfishes 5%) accounting for 1.23% of its total marine fish landings (CMFRI, 2013). However, these catch data largely include the easily identifiable species and others will be often put in group names only (sharks, rays or *Carcharhinus* spp, *Himantura* spp., etc.). Despite the rich diversity and long history of the elasmobranch fishery, only a few detailed studies have been undertaken on the taxonomy and diversity of this group in India. For a long period, this important group was neglected by researchers due to impediments including taxonomic problems and large specimen sizes and costs. Nevertheless, elasmobranchs found in Indian waters have been catalogued by several researchers (Day, 1889; Misra, 1952, 1969; Raje et al., 2007; Talwar & Kacker, 1984). In recent years, several species have been added to the list (Akhilesh et al., 2010, 2013a,b; Bineesh et al., 2014, Babu et al., 2011; Soundararajan & Roy 2004; Sutaria et al., 2015). An updated and extended checklist of 227 chondrichthyan species reported/listed as occurring in Indian waters, put together by Akhilesh et al. (2014), suggested that 27 species (12%) had questionable status in India and another 41 species (18%) required additional confirmation with regard to their occurrence suggested there is an urgent need for a re-assessment of chondrichthyan diversity in Indian waters. However, it is possible that the actual species diversity occurring in the fishery or in Indian seas could have been underestimated, for many reasons: an extended long coastline, diverse habitat, a large number of widely distributed landing centers, varied operational depths and regions, limited chondrichthyan exploratory surveys, and low numbers of well-trained observers

CONTACT K.K. Bineesh, Scientist kkbineesh@gmail.com National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus, P.B. No. 1603, Ernakulam North, Kochi, Kerala 682 018, India

able to field identify morphologically similar species – data collection of elasmobranchs in all India level is a very difficult task. According to White & Last (2012), the Indian elasmobranch fauna is poorly known and requires more scientific exploration and investigation. Resolving taxonomic ambiguities is the first, and necessary, step towards developing a comprehensive conservation plan for chondrichthyans from Indian waters.

The Indian Wildlife (Protection) Act, 1972 (MoEF, Government of India) lists 10 elasmobranchs in Schedule I part 2(A) since 2001, which is the highest protected status for animals in India. Recently, the Ministry of Commerce and Industry, Government of India, prohibited the export of shark fins of all shark species (notification no. 110 (RE – 2013)/2009–2014 dated February 6), with immediate effect. Additionally, five shark species and two manta ray species found in Indian waters were included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), meaning that international trade of these species has to be monitored and regulated based on the sustainability and traceability. All these protected groups/species have to be identified accurately in the field or at the export/trade level to ensure their effective protection and prevention of illegal trade. However, identification of many of these protected and vulnerable species is very difficult in the field, especially in processed forms, due both to taxonomic ambiguities and to extensive morphological similarities. Accurate identification has become a major obstacle to basic cataloging, monitoring, and conservation of biodiversity (Vecchione & Collette, 1996).

DNA barcoding uses the nucleotide sequence of a region of the mitochondrial cytochrome oxidase I (COI) gene for rapid and accurate animal species identification (Hebert et al., 2003), including all life history stages. Hebert et al. (2004a,b) showed that the COI gene can discriminate between closely related species across diverse animal phyla. This approach has been very successful in discriminating marine and freshwater fish species (Hajibabaei et al., 2005; Hubert et al., 2008; Ward et al., 2005). Ward et al. (2005) validated the efficacy of COI barcodes for identifying chondrichthyans by sequencing 61 species of sharks and rays from Australian waters. Spies et al. (2006) confirmed the utility of DNA barcodes as a robust method by discriminating 15 skates species from North Pacific Ocean and Bering Sea. Ward et al. (2008) barcoded 210 species of sharks and rays from Australian waters, showing the utility of the approach for helping to resolve taxonomic issues and for new species discovery. Holmes et al. (2009) used the DNA barcode approach to identify shark and ray species from dried fins from northern Australian waters, showing that data can be used by enforcement authorities to manage the trade of chondrichthyan species. Santander-Neto et al. (2011) successfully identified a shark carcass by DNA barcoding. Pawan-Kumar et al. (2015) barcoded 18 elasmobranch species from Indian waters. Recent taxonomic studies coupled with molecular markers on chondrichthyan species around the world (Australia, Indonesia, Taiwan, and Argentina) have resulted in taxonomic resolution of complexes and also discovery of several new species (Ebert et al., 2010; Last et al., 2007, 2008a,b, 2010b; Naylor et al., 2012; Ruocco et al., 2012; White et al., 2013).

The present study substantially progresses our understanding of chondrichthyan diversity in the Indian commercial fishery, including bycatch landings, and develops DNA barcodes/reference sequences for >100 species. These barcodes will facilitate accurate specimen identification, and will lead to the improved implementation of chondrichthyan conservation and management programs in Indian waters. Our results, including the finding of several unidentified/cryptic species, also confirm the need for further systematic taxonomic studies of Indian chondrichthyans, from wide regional samplings. Such studies, incorporating DNA analysis, will no doubt reveal still greater diversity.

Materials and methods

Sample collections

Tissue samples from more than 500 specimens of 111 chondrichthyan species, landed at 11 locations (Figure 1) from the east and west coasts of India, were collected from 2009 to 2013. Approximately 100 mg of white muscle or gill tissue was collected from each specimen and preserved in 95% ethanol. Species identification was based on Alcock (1899), Misra (1969), Compagno (1984a,b), Talwar & Kacker (1984), Compagno et al. (2005), and other available publications related to respective taxa. All specimens were photographed and subsequently small- to medium-sized specimens were retained as whole animal vouchers. The vouchers are in the Marine Biodiversity Museum of Central Marine Fisheries Research Institute (CMFRI), Kochi, and National Bureau of Fish Genetic Resources (NBFGR), Kochi. Species, family and GenBank accession numbers are given in Table 1.

DNA isolation

Whole genomic DNA was isolated for most samples using the protocol of Miller et al. (1988), but for some degraded samples DNAeasy (Qiagen, Valencia, CA) was used following instructions of the manufacture, and eluted in 50–200 µl of AE buffer. Extracted DNA was checked by 0.8% agarose gel electrophoresis with ethidium bromide incorporated in 1 × TBE buffer. The concentration of isolated DNA was diluted to a final concentration of 100 ng/µl using a UV spectrophotometer.

Amplification and sequencing

The barcode sequence of the COI gene was PCR amplified using the 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) in 25 µl reactions containing 1 × 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. Thermal conditions 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.

PCR products were visualized in a 1.2% agarose gel. Samples with intense bands were selected for sequencing. Sequencing

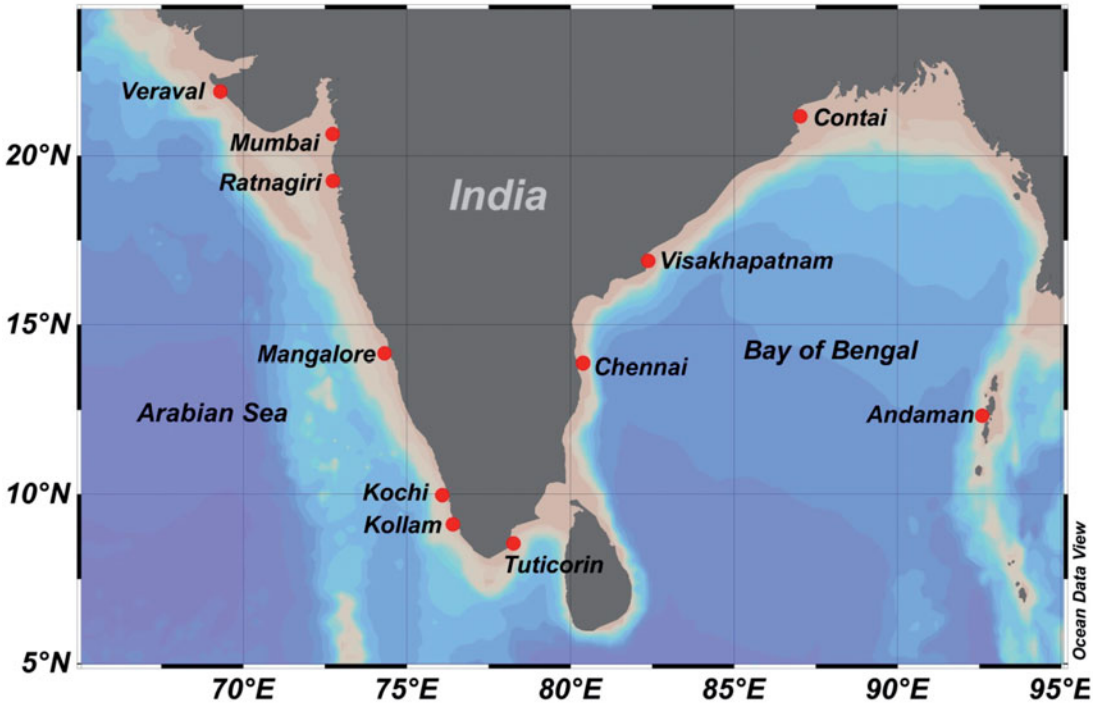

Figure 1. Map showing the sampling sites for Indian chondrichthyan species.

reactions used a BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc., Foster City, CA). All samples were sequenced bidirectionally using an ABI 3730 capillary sequencer following the protocol of the manufacture.

Sequence analysis

We assembled and edited the forward and reverse DNA sequences using the BioEdit sequence alignment editor, version 7.0.5.2 (Hall, 1999). Clean sequences were trimmed to the start and length of baseline shark barcode sequences downloaded from GenBank, and exported as FASTA files for molecular evolutionary analysis. Edited sequences were submitted to GenBank (Table 1). Sequences were aligned using ClustalW software as implemented in MEGA 5.05 (Tamura et al., 2011). Sequence divergence values within and between species were calculated using the Kimura 2 Parameter (K2P) distance model of nucleotide substitution. Neighbor-joining (NJ) trees of K2P distance were created to provide graphic representation of divergence, with 1000 bootstrap replications.

We used nucleotide searches of both public databases GenBank and BOLD to verify our initial morphological identifications at species level. We downloaded, from GenBank, representative sequences from widely different localities for taxa that have taxonomic uncertainties for character based identification (e.g. *Pastinachus* spp. and *Rhynchobatus* spp.).

Results

General findings

A total of 528 individuals of 111 species of Chondrichthyans (two chimaeroids, 60 sharks, and 49 batoids) were successfully barcoded for a minimum of 642 bp. However, several species generated sequences of insufficient quality (*Hexatrygon bickelli, Narke dipterygia, Torpedo* sp. B, and *Rhinobatos granulatus*) were excluded due to bad sequence quality and/or smaller sequence size. All sequences were compared with those in the NCBI GenBank and BOLD databases to verify initial identifications. An overall NJ tree based on K2P distance was produced from the 528 sequences. Sample size per species ranged from 2 (*Echinorhinus brucus* and *Rhinobatos thouin* – poor amplification) to 13 (*Himantura gerrardi* – morphotypes), with an average of 5.1. Representatives from 60 genera, 34 families, 10 orders, and two subclasses (Holocephali and Elasmobranchii) were barcoded. An average sequence length was 650.5 bp. Forty of the 111 species had not been previously barcoded; these species (comprising 184 sequences), and their GenBank accession numbers, are identified in Table 1. Overall nucleotide contents across all samples for chimaeras, rays, and sharks were estimated (Table 2). Similar base compositions were observed, with GC% around 44% for chimaeras and rays and 41% for sharks. Mean K2P distances within species, genera, family, and order increase with taxonomic rank, and average conspecific and congeneric distances are 0.325 and 6.73%, respectively (Table 3). Only one chondrichthyan species showed more than 1% intraspecies divergence, with the highest divergence in *Dipturus* sp. A (1.6%).

Comments on some individual taxa

Chimaeriformes: Two species of the subclass Holocephali were sequenced: one from the family Rhinochimaeridae (*Neoharriotta pinnata*) and other from the Chimaeridae

Table 1. List of species DNA barcoded with major collection locations and GenBank accessions.

Order	Family	Species	Location	GenBank accession no.
Chimaeriformes	Rhinochimaeridae	*Neoharriotta pinnata* (Schnakenbeck, 1931)*	KOC	KJ749670–KJ749676
	Chimaeridae	*Hydrolagus africanus* (Gilchrist, 1922)	KOM	KJ749662, KF899529–KF899531
Hexanchiformes	Hexanchidae	*Hexanchus griseus* (Bonnaterre, 1788)	KOC	KF899461–KF899464
		Heptranchias perlo (Bonnaterre, 1788)	KOM	KF899456–KF899460
Echinorhiniformes	Echinorhinidae	*Echinorhinus brucus* (Bonnaterre, 1788)*	KOC	KJ749661, HM467790
Orectolobiformes	Hemiscylliidae	*Chiloscyllium arabicum* (Gubanov, 1980)*	MUM	KF899620–KF899351
		Chiloscyllium griseum (Müller & Henle, 1838)	KOC	KF899625–KF899628
		Chiloscyllium hasselti (Bleeker, 1852)	MUM	KF899352, KF899629–KF899631
	Stegostomatidae	*Stegostoma fasciatum* (Hermann, 1783)	KOC	KF899640–KF899644
	Ginglymostomatidae	*Nebrius ferrugineus* (Lesson, 1831)	KOC	KF899635–KF899639
	Rhincodontidae	*Rhincodon typus* (Smith, 1828)	KOC	KF899632–KF899634
Lamniformes	Odontaspididae	*Odontaspis noronhai* (Maul, 1955)*	KOM	KF899559–KF899560
	Alopiidae	*Alopias pelagicus* (Nakamura, 1935)	KOC	KF899545–KF899548, HM239672
		Alopias superciliosus (Lowe, 1841)	KOC	KF899549–KF899556, HM239673
		Alopias vulpinus (Bonnaterre, 1788)	KOC	KF899557–KF899558
	Lamnidae	*Isurus oxyrinchus* (Rafinesque, 1810)	KOC	KF899536–KF899541
		Isurus paucus (Guitart, 1966)	KOC	KF899542–KF899544
	Pseudocarchariidae	*Pseudocarcharias kamoharai* (Matsubara, 1936)	KOC	KF899532–KF899535
Carcharhiniformes	Scyliorhinidae	*Apristurus* sp. A*	KOC	KF899717–KF899721
		Apristurus sp. B*	KOC	KT766183–KT766185
		Apristurus sp. C*	KOC	KT766186–KT766187
		Apristurus sp. D*	KOC	KT766188–KT766190
		Cephaloscyllium silasi (Talwar, 1974)*	KOM	KF899707–KF899711, HM467791
		Halaelurus quagga (Alcock, 1899)*	KOM	KF899712–KF899716, JF260984
		Bythaelurus hispidus (Alcock, 1891)*	KOC	KF899701–KF899706
	Proscylliidae	*Eridacnis radcliffei* (Smith, 1913)*	KOC	KF899421–KF899425
	Triakidae	*Iago* sp. A*	KOC	KF899737–KF899742
		Iago sp. B*	KOC	KF899730KF899736, KF899365
	Hemigaleidae	*Paragaleus randalli* (Compagno et al., 1996)	CHE	KF913243, KF913245
		Hemipristis elongata (Klunzinger, 1871)	KOC	KF899451–KF899455, HM239669
		Hemigaleus microstoma (Bleeker, 1852)	AND	KF899786
	Carcharhinidae	*Carcharhinus altimus* (Springer, 1950)	KOC	KF899783–KF899788
		Carcharhinus amblyrhynchos (Bleeker, 1865)	KOC	KF899789–KF899792
		Carcharhinus albimarginatus (Ruppel, 1837)	KOC	KF899778–KF899782
		Carcharhinus amboinensis (Müller & Henle, 1839)	KOC	KF899793–KF899796
		Carcharhinus brevipinna (Müller & Henle, 1839)	KOC	KF899797–KF899802
		Carcharhinus falciformis (Müller & Henle, 1839)	KOC	KF899803–KF899807
		Carcharhinus macloti (Müller & Henle, 1839)	KOC	KF913239–KF913242
		Carcharhinus leucas (Müller & Henle, 1839)	KOC	KF899803–KF899807
		Carcharhinus limbatus (Müller & Henle, 1839)	KOC	KF899813–KF899816
		Carcharhinus longimanus (Poey, 1861)	KOC	KF899773–KF899777
		Carcharhinus melanopterus (Quoy & Gaimard, 1824)	KOC	KF899817–KF899818
		Carcharhinus sorrah (Müller & Henle, 1839)	KOC	KF899817–KF899822
		Galeocerdo cuvier (Péron & Lesueur, 1822)	MAN	KF89942–KF89949, HM23967
		Loxodon macrorhinus Muller & Henle, 1839	AND	KF899752–KF899757
		Lamiopsis temminckii (Müller & Henle, 1839)*	MUM	KF899562–KF899563
		Prionace glauca (Linnaeus, 1758)	KOC	KF899650–KF899653
		Rhizoprionodon acutus (Rüppell, 1837)	KOC	KF899684–KF899688
		Rhizoprionodon oligolinx Springer, 1964	MUM	KF899752–KF899757
		Scoliodon laticaudus (Müller & Henle, 1838)	KOC	KF899694–KF899700
		Triaenodon obesus (Rüppell, 1837)	KOC	KF899764–KF899768
	Sphyrnidae	*Sphyrna lewini* (Griffith & Smith, 1834)	KOC	KF899746–KF899751
		Sphyrna zygaena (Linnaeus, 1758)	KOC	KF899752–KF899757
Squaliformes	Squalidae	*Squalus* sp. A*	KOC	KF899758–KF899763
	Centrophoridae	*Centrophorus granulosus* (Bloch & Schneider, 1801)	KOM	KF899391–KF899393
		Centrophorus squamosus (Bonnaterre, 1788)	KOM	KF899383–KF899386
		Centrophorus atromarginatus (Garman, 1913)	KOM	KF899388–KF899390
		Centrophorus zeehaani (White et al., 2008)	KOM	KF899394–KF899399
		Deania profundroum (Smith & Radcliffe, 1912)	KOC	KF899378–KF899382
	Etmopteridae	*Etmopterus pusillus* (Lowe, 1839)	KOC	KF899426–KF899428
	Somniosidae	*Centroselachus crepidater* (Bocage & Capello, 1864)	KOM	KF899400–KF899401
		Zameus squamulosus (Günther, 1877)	KOC	KF899769–KF899772
Torpediniformes	Torpedinidae	*Torpedo* sp. A*	KOC	KF899725–KF899729
		Torpedo sinuspersici (Olfers, 1831)	KOM	KF899723–KF899724
	Narcinidae	*Benthobatis moresbyi* (Alcock, 1898)*	KOM	KJ768659–KJ768663
		Narcine sp. A*	TUT	KF899601–KF899605
		Narcine sp	KOM	KF899606–KF899608
		Narcine maculata (Shaw, 1804)*	VIS	KF899598–KF899600
Rajiformes	Rhinidae	*Rhina ancylostoma* (Bloch & Schneider, 1801)	KOC	KF899659–KF899663
	Rhynchobatidae	*Rhynchobatus australiae* (Whitley, 1939)	KOC	JN022595–JN022598
		Rhynchobatus cf. *laevis* (Bloch & Schneider, 1801)	MUM	KF899376–KF899693

(continued)

Table 1. Continued

Order	Family	Species	Location	GenBank accession no.
	Rhinobatidae	*Glaucostegus obtusus* (Müller & Henle, 1841)*	KOC	KF899437–KF899441
		Rhinobatos punctifer (Compagno & Randall, 1987)*	KOC	KF899664–KF899668
		Rhinobatos lionotus (Norman, 1926)*	CHE	KF899669–KF899672
		Rhinobatos variegatus (Nair & Lal Mohan, 1973)*	KOC	KF899673–KF899678, HM467794
	Rajidae	*Dipturus johannisdavesi**	KOC	KF899412–KF899415
		Dipturus sp. B*	KOC	KF899416–KF899420
		Dipturus sp. A*	KOC	KF899402–KF899411
		Okamejei powelli (Alcock, 1898)*	TUT	KF899614–KF899619
		Okamejei sp. A*	AND	KT766191–KT766192
Myliobatiformes	Plesiobatidae	*Plesiobatis daviesi* (Wallace, 1967)	KOC	KF899645–KF899649, HM467801
	Dasyatidae	*Dasyatis microps* (Annandale, 1908)*	KOC	KJ749656–KJ749660
		Himantura undulata (Bleeker, 1852)	MUM	KF899503–KF899506
		Himantura sp. C*	KOC	KF899488–KF899491
		Himantura fai (Jordan & Seale, 1906)	KOC	KF899473–KF899475
		Himantura gerrardi (Gray, 1851)	KOC	KF899364, KF899476–KF899487
		Himantura granulata (Macleay, 1883)	KOC	KF899471–KF899472
		Himantura imbricata (Bloch & Schneider, 1801)	KOC	KF899354–KF899356, KF899512–KF899521
		Himantura jenkinsii (Annandale, 1909)	TUT	KF913237–KF913238
		Himantura leoparda (Manjaji-Matsumoto & Last, 2008)	MUM	KF899353, KF899500–KF899502
		Himantura sp. A*	KOC	KF899465–KF899470
		Himantura sp. B*	KOC	KF899360–KF899363, KF899522–KF899528
		Himantura uarnacoides (Bleeker, 1852)	KOC	KF899357–KF899359, KF899492–KF899496
		Himantura uarnak (Forsskål, 1775)	KOC	KF899507–KF899511
		Neotrygon kuhlii (Müller & Henle, 1841)	TUT	KF899609–KF899613, HM467799
		Pastinachus atrus (Macleay, 1883)	KOC	KF899370–KF899375
		Pastinachus sephen (Forsskål, 1775)	KOC	KF899366–KF899369
		Pteroplatytrygon violacea (Bonaparte, 1832)	KOC	KF899654–KF899658
		Taeniura meyeni (Müller & Henle, 1841)*	KOC	KF899825–KF899829, HM467797
		Urogymnus asperrimus (Bloch & Schneider, 1801)	MUM	KT766193–KT766194
	Gymnuridae	*Gymnura poecilura* (Shaw, 1804)	KOM	KF899442–KF899446
		Gymnura sp. A*	MUM	KF899447–KF899450
	Myliobatidae	*Aetobatus ocellatus* (Kuhl, 1823)	KOC	KF899589–KF899595
		Aetobatus sp. A*	MUM	KF899596–KF899597
		Aetomylaeus vespertilio (Bleeker 1851)	KOC	KF899584–KF899586
		Aetomylaeus maculatus(Gray, 1832)	VIS	KF899587–KF899588
	Mobulidae	*Manta birostris* (Walbaum, 1792)	KOC	KF899564–KF899569
		Mobula japanica (Muller & Henle, 1841)	KOC	KF899570–KF899575
		Mobula kuhlii (Müller & Henle 1841)	KOC	KF899581–KF899583
		Mobula tarapacana (Philippi, 1892)	KOC	KF899576–KF899580
	Rhinopteridae	*Rhinoptera jayakari* (Boulenger, 1895)*	KOC	KF899679–KF899683

VER, Veraval; KOC, Kochi; TUT, Tuticorin; RAT, Ratnagiri; MAN, Mangalore; CHE, Chennai; VIS, Visakhapatnam; CON, Contai; MUM, Mumbai; KOM, Kollam; AND, Andaman; Asterisk shows sequences for the first time on the GenBank.

Table 2. Percent nucleotide composition, with s.e. GC% given overall, and for codon positions 1, 2 and 3 chimaeroids, rays and sharks.

%	All species (111)	chimaeroids (2)	Rays (49)	Sharks (60)
G	16.9	16.5	17.5	16.5
C	25.3	23.6	26.7	24.1
A	25.7	26	24.9	26.3
T	32.1	33.9	30.9	33.1
GC	42.2	40.1	44.2	40.6
GC1	47.7	47.8	47.6	46.2
GC2	30.5	34.6	27	28.1
GC3	48.5	50.3	47.8	46.1

Numbers in parenthesis are numbers of species – only one representative per species used for those species with multiple specimens.

Table 3. Means and ranges of K2P distance values, *D* (percent), by taxonomic rank among 528 sequences and 111 species of sharks and rays.

	No. of taxa	Mean *D*	Minimum	Maximum
Within species	102 (111)	0.32	0	0.54
Within genus	13 (60)	6.73	1.01	13.76
Within family	18 (34)	8.21	1.47	16.22
Within order	9 (10)	13.3	4.19	18.83

Number of taxa is given both as number of contributing taxa and (in parenthesis) total number.

(*Hydrolagus africanus*). These two chimaeroids had a high interspecies distance of 21.7%.

Myliobatidae & Mobulidae: *Aetomylaeus vespertilio* and *A. maculatus* show a very high congeneric divergence of about 24.8%. The Chilean devil ray *Mobula tarapacana* diverged considerably from the Shortfin devil Ray *M. kuhlii* (7.3%) and *M. japonica* (8.3%). Another species of *Aetobatus* sp. A from northern Arabian Sea (similar to *A. ocellatus*) showed a separate clade warranting further studies.

Carcharhinidae

Eighteen species of carcharhinid sharks from seven genera of the order Carcharhiniformes were analyzed, 12 of the genus *Carcharhinus* and one species from each of six different genera (*Galeocerdo, Lamiopsis, Prionace, Rhizoprionodon, Scoliodon*, and *Triaenodon*). Average genetic distance within species was 0.23% and the average genetic distance between species was 8.25%. The NJ tree showed that sequences from each species of *Carcharhinus* fell into very distinct clusters (Figure 2).

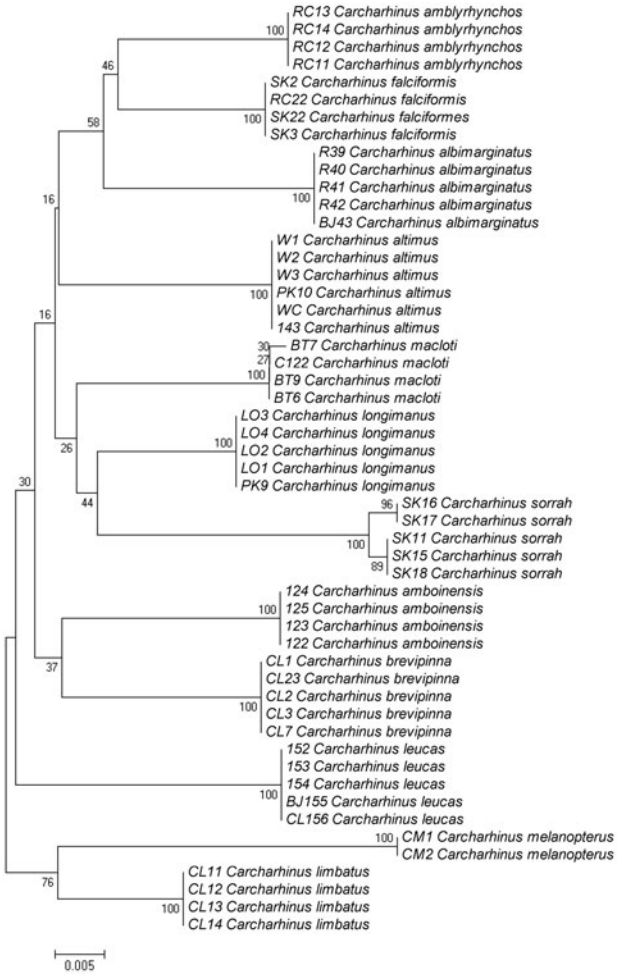

Figure 2. K2P distance neighbor-joining tree of COI sequence from 12 species of *Carcharhinus*.

Scyliorhinidae

Seven species were used for study (Figure 3), including four *Apristurus* species as yet formally identified and named here as *Apristurus* sp. A, *Apristurus* sp. B, *Apristurus* sp. C, and *Apristurus* sp. D. The average distance between species within family was 15.2%, while the average interspecies distance within the *Apristurus* was very high 8.3%. The high genetic distance was observed 11.7% between *Apristurus* sp. A and *Apristurus* sp. B and the minimum distance was 4.9% observed between *Apristurus* sp. C and *Apristurus* sp. D. *Halaelurus quagga,* described from off the Malabar coast, India, and *Cephaloscyllium silasi,* described from off Kollam (Arabian Sea), both show 0.3% intraspecies variation. The specimens of *Bythaelurus hispidus* (type locality, Andaman Sea) were collected from a wide geographic area (off Chennai, Bay of Bengal and off Kollam, Arabian Sea) but showed no intra-species variation.

Rajidae

Five species belonging to two genera (*Dipturus* and *Okamejei*) were examined (Figure 4), with an average interspecies distance of 7.9% in the two genera. Two are putative new species, yet to be formally described, named here *Dipturus* sp. A (KKB2014) and *Okamejei* sp. A (KKB2014). Intraspecific variation in *Dipturus* sp. A was relatively high, at 1.6%. Specimens from southern Arabian Sea and off Chennai showed a maximum divergence of 1.3%, perhaps warranting further investigation. Its barcodes best matched, at c.96–98% (BOLD ID engine), with other skates currently named as *Dipturus* sp. 1 and *Dipturus* sp. 2, from Indonesia. The taxonomic status of two other rajids in our collection could not be confirmed: namely *Dipturus johannisdavisi* and *Dipturus* sp. B. *Dipturus johannisdavisi* (Alcock, 1899) is the only valid deepwater skate reported from India, but the record is based on a single juvenile specimen from off the Travancore coast; this holotype is in bad condition and could not be reliably compared with our specimens. The best BOLD match of our field identified *Dipturus johannisdavisi* to other rajid is 96.5%, to our *Dipturus* sp. A. *Dipturus* sp. B needs more morphological and genetic analysis to determine its species status. Taxonomic clarification and description of these potentially new rajids are ongoing. The Indian ringed skate, *Okamejei powelli*, clustered distantly with the three *Dipturus* species. Two samples of *Okamejei* sp. A from Andaman waters not yet confirmed to species level. Sequences of *Okamejei powelli* from off Kollam, south-eastern Arabian Sea, and *Okamejei* sp. A samples separate into two clusters with high interspecific distance (3.3%).

Dasyatidae

Twelve species were examined, three of which appear to be unrecognized and are designated here as *Himantura* sp. A, *Himantura* sp. B, and *Himantura* sp. C. BOLD searches reveal that *Himantura* sp. A best matched, at c. 96%, *H. randalli* from Kuwait, *H.* sp. B. at 100% with some other *Himantura* specimens from Sri Lanka and India (some without a species epithet, some apparently wrongly designated as, for example, *H. imbricata*), and *Himantura* sp. C best matched, at 98%, *H. pastinacoides* from Malaysia. Planned in-depth investigations are required to determine if these three species are truly undescribed or whether they are synonymized species that require resurrecting as valid species. The average genetic distance within species was 0.84% and among species was 7.45%. Eleven specimens of *Himantura* sp. B clustered with, but were well separated from ($D = 6.7\%$), the 13 specimens of *H. imbricata*. The NJ tree revealed very distinct species clusters (Figure 5). The average interspecies distance (19 species) in the family Dasyatidae was 10.32%. *Himantura gerrardi* represents a complex of species which are widely distributed in the Indo-Pacific region and requires taxonomic revisions (Ward et al., 2008). This study included 17 specimens with the basic character and color pattern of *H. gerrardi*. Even though there were different morphotypes displaying slight morphometric variations and higher spot variations within forms, they all clustered into one single *Himantura gerrardi* lineage. Four specimens designated as *Himantura* sp. C that shows unique haplotypes well distantly separated from *Himantura gerrardi*.

Centrophorus and Deania (Centrophoridae)

Five species of Centrophoridae were sequenced. Intraspecific distances ranged from 0.0% (*Centrophorus squamosus*) to 0.3%

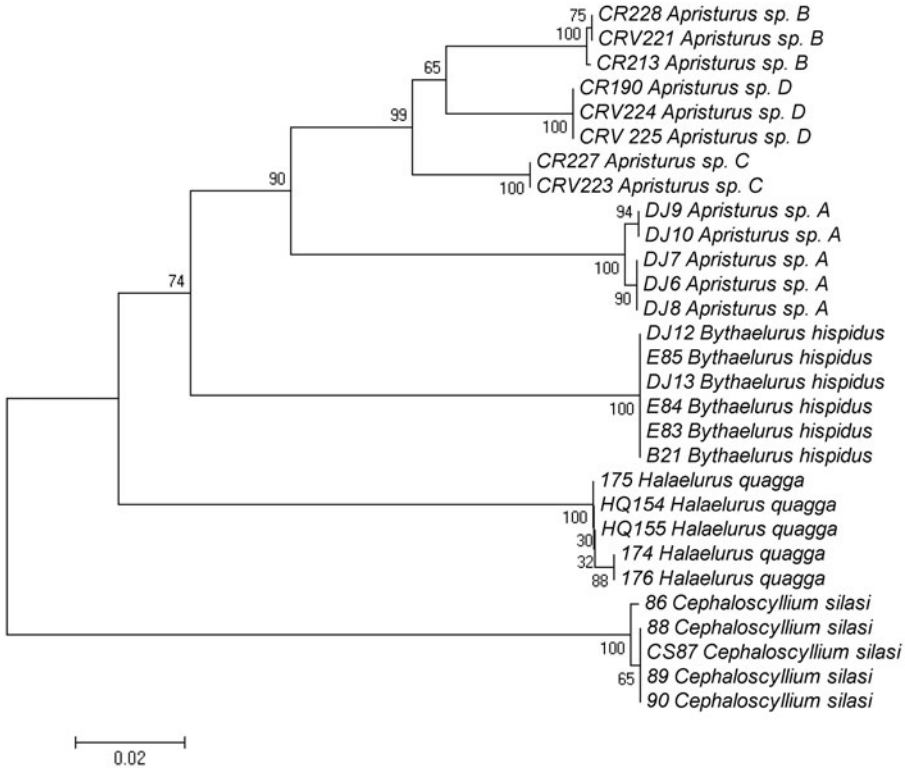

Figure 3. K2P distance neighbor joining tree of COI sequences from Scyliorhinidae.

(*Centrophorus zeehaani*). The average interspecies distance in the family was 6.1% and, in the genus, *Centrophorus* was 4.4%. Three specimens of *Centrophorus granulosus* clustered closely with, but separated from ($D = 2.7\%$), the four specimens of *Centrophorus squamosus* (Figure 6).

Rhinobatidae (Rajiformes)

Five species belonging to two genera (*Glaucostegus* and *Rhinobatos*) were examined (Figure 7), with an average interspecies distance of 15.5%. The Stripenose guitarfish, *Rhinobatos variegatus,* described from the Gulf of Mannar, was distantly placed with other two *Rhinobatos* species. The intraspecific genetic distance was relatively high in *Rhinobatos variegatus*, at 0.6%.

Lamniformes

Seven species from four genera (*Alopias*, *Odontaspis*, *Pseudocarcharias,* and *Isurus*) belonging to the families Odontaspididae, Alopiidae, Lamnidae, and Pseudocarchariidae were characterized. The mean interspecies distance within the order was 15.1%. The species *Odontaspis noronhai*, the sole representative collected in the family Odontaspididae, fell within the *Alopias* cluster of three species (family Alopiidae).

Orectolobiformes

Six species from four genera (one species each from *Nebrius, Stegostoma,* and *Rhincodon,* three species from *Chiloscyllium*) and four families (Ginglymostomatidae, Stegostomatidae, Rhincodontidae, and Hemiscylliidae, respectively) were barcoded. The mean interspecies distance within the order was 14.5%.

Torpedeniformes

The electric rays of three genera, namely *Torpedo* (two species, family Torpedinidae*)*, *Narcine* (three species, family Narcinidae), and *Benthobatis* (one species, family Narcinidae), were characterized. The mean interspecies distance within the order was 15.8%. Mean interspecies distances within the families Torpedinidae and Narcinidae were 18.3% and 20.1%, respectively.

Comparisons with GenBank and BOLD

Of the 111 species sequences we tested, 88 species showed GenBank and/or BOLD matches of 98% similarity or greater (Table 4). Table 4 shows the results of comparing a representative sequence from each species with GenBank and BOLD records. Eighteen taxa could not be fully identified to species level in our study (*Apristurus* sp. A, *Apristurus* sp. B, *Apristurus* sp. C, *Apristurus* sp. D, *Iago* sp. A, *Iago* sp. B, *Squalus* sp. A, *Aetobatus* sp. A, *Gymnura* sp. A, *Himantura* sp. A, *Himantura* sp. B, *Himantura* sp. C, *Dipturus* sp. A, *Dipturus* sp. B, *Narcine* sp, *Narcine* sp. A, *Okamejei* sp. A, and *Torpedo* sp. A). Among these species, 10 taxa are confirmed as new taxa by morphological examination of the closely related species. Most of the relatively few apparent misidentifications using the existing databases arise in taxonomically difficult or as yet not fully-resolved genera. For example, the specimens we identified as *H. leoparda* best matched (99.68%) with three sequences of *H. uarnak* on BOLD, but also matched (99.53%) two sequences of *H. leoparda* and two sequences of *H. uarnak*. The resulting BOLD identification tree revealed two clades separated by

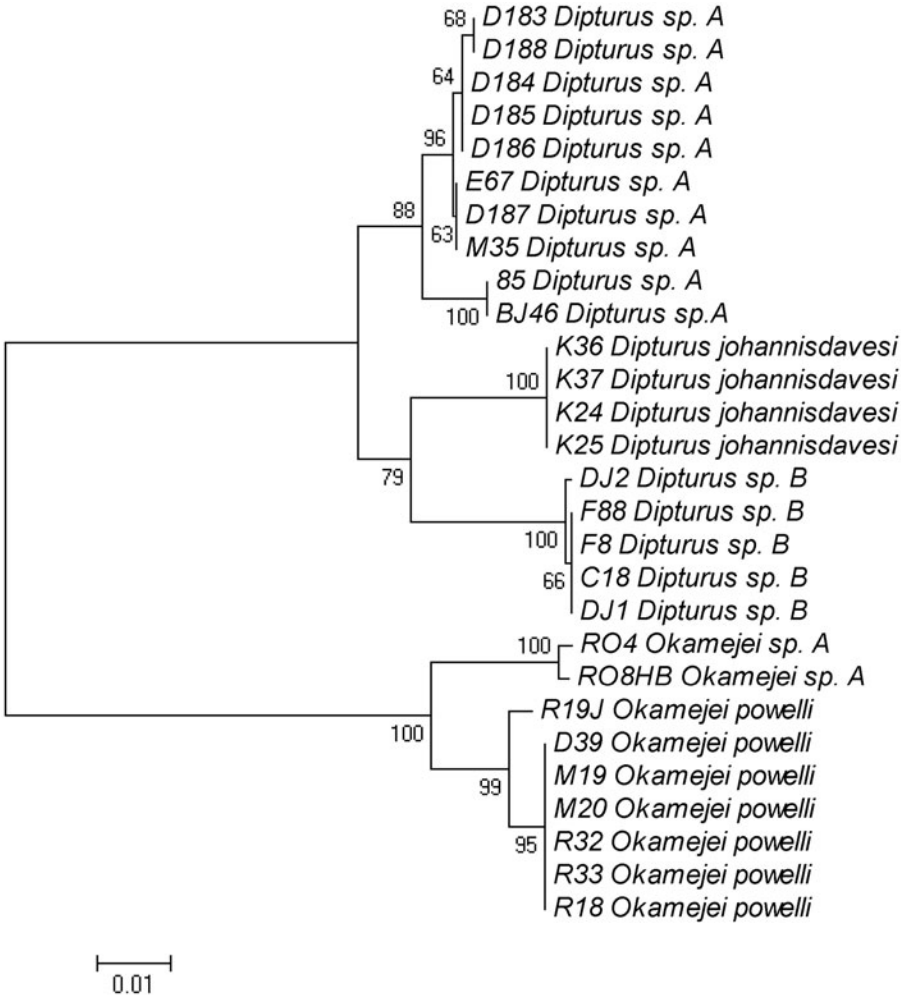

Figure 4. K2P distance neighbor joining tree of COI sequences from Rajidae.

about 8% distance, but with both clades consisting of a mix of *H. leoparda* and *H. uarnak*. The taxon we identified as *Aetobatus ocellatus* matched on BOLD 100% with a single specimen of *A. narinari*, 99.84% with a specimen of *A.* cf. *narinari* and another specimen of *A. narinari*, but matched specimens of *A. ocellatus* at values of 99.2% and 98.9%. Twenty-one species showed GenBank and BOLD matches of 97% or less. Unsurprisingly, given the low similarity values, none of these taxa could be accurately identified to species level using either GenBank or BOLD databases (Table 4b), although most (GenBank) or all (BOLD) taxa could be identified to genus. Ten of the 21 species had been morphologically identified to species level; none of these species were hitherto represented in existing databases and, therefore, these 10 represent new barcode species records. These species barcode records include *Benthobatis moresbyi, Chiloscyllium arabicum, Dasyatis microps, Echinorhinus brucus, Gymnura poecilura, Himantura imbricata, Odontaspis noronhai, Pastinachus sephen*, and *Scoliodon laticaudus*.

Discussion

In this study, 111 species representing 10 orders and 34 families of chondrichthyans from Indian waters were characterized for DNA barcode variability, making this the largest such study in the region. No insertions, deletions, or stop codons were observed in any of the barcodes, indicating that all were derived from functional mitochondrial COI sequences. Sequencing *c.*650 bp region of mtDNA COI permitted the discrimination of every one of these 111 species. The average degree of intraspecies divergence, 0.35%, is very similar to the values of 0.39% previously estimated for 143 species of teleosts and 64 species of chondrichthyans (Ward et al., 2005) and 0.37% for 210 species of chondrichthyans (Ward et al., 2008).

The average number of specimens per species was only 5.1. Small sample sizes can lead to under estimates of both intraspecific variability and the extent of spatial genetic differentiation, and potentially can reduce the precision of DNA barcoding (Moritz & Cicero, 2004). The Shortfin devil ray *Mobula kuhlii* and Chilean devil ray *Mobula tarapacana* are reported to be rare in Indian seas. Our COI barcodes of *Mobula kuhlii* and *Mobula tarapacana* match 100% with GenBank and BOLD database records of these two species, confirming their distribution in Indian waters and verifying morphological identifications from the southwest coast of India.

In the Hemiscyllidae, *Chiloscyllium griseum* and *Chiloscyllium hasseltii* are very similar with many overlapping morphometric characters, and the main differences relate to juvenile color. After morphological examination, we assigned specimens to *Chiloscyllium griseum* and to *Chiloscyllium* sp. Later barcode

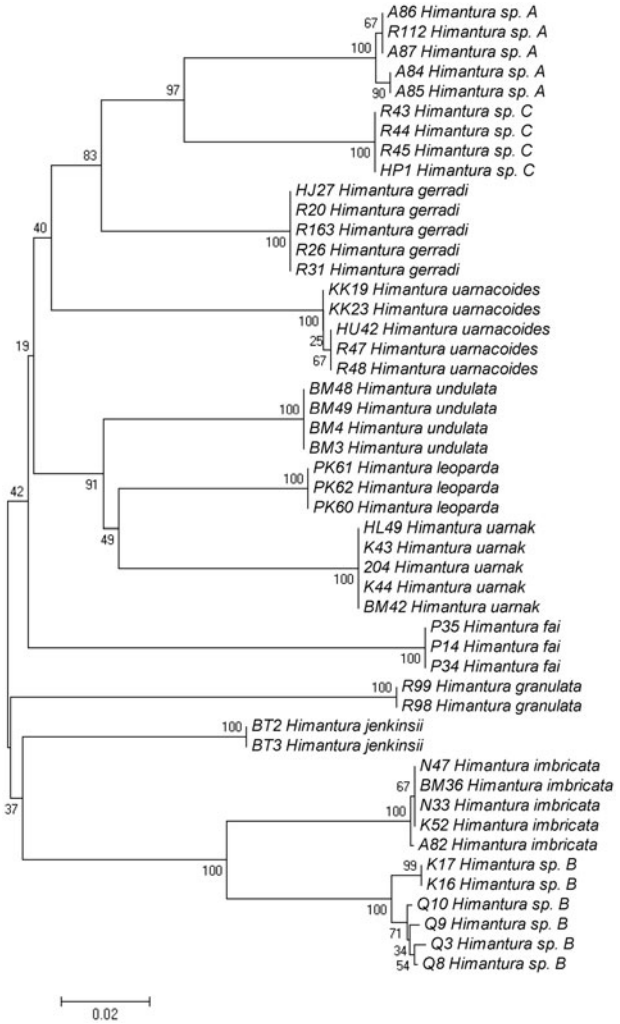

Figure 5. K2P distance neighbor joining tree of COI sequences from the genus *Himantura*.

analysis using BOLD and GenBank comparisons confirmed the identity of the former group and allocated specimens of the second group to *Chiloscyllium hasseltii*. Genetic divergence and analysis of NJ tree showed that *Chiloscyllium griseum* is closely related to *C. hasseltii* ($D = 1.8\%$). The third species in this genus that we collected is *Chiloscyllium arabicum*, and is relatively distant from both these species ($D = 8.8\%$ and 7.8%, respectively).

The catshark family Scyliorhinidae comprises sharks of small size that are exclusively found in deeper areas at depth ranges of 200–1000 m and have no commercial value. Most specimens are discarded at sea and knowledge on exact species diversity in Indian waters is very poor, most species are described from few specimens. Eight of the 17 species from seven genera reported from Indian waters have questionable status (Akhilesh et al., 2014). Seven species, *Cephaloscyllium silasi, Halaelurus quagga, Bythaelurus hispidus,* and *Apristurus* spp. were barcoded. *Apristurus investigatoris* (Misra, 1952), described from the Andaman Sea from one specimen, is the only valid Indian species of *Apristurus,* and our present *Apristurus* is morphologically very different from *A. investigatoris*. The position of the dorsal fins, interdorsal distance, and teeth structures varies greatly between the two species. As mentioned earlier, our *Apristurus* sp. A barcode best matched *Apristurus nakayai* (94% similarity), but is clearly a different species. Taxonomic clarification of this potentially new *Apristurus* is currently in progress.

Skates (order Rajiformes, family Rajidae) are an extremely diverse group of fishes, characterized by high morphological conservatism (Ebert & Compagno, 2007; McEachran & Dunn, 1998). *Dipturus* Rafinesque, 1810, is the second most speciose genus of the family Rajidae. The high level of species diversity coupled with morphological and ecological conservatism makes specimen identification very difficult. Eleven species are reported from Indian waters, three of which have questionable status (Akhilesh et al., 2014). The species referred to in this paper as *Dipturus* sp. A is a putative new species to science, while *Dipturus* sp. B and *Dipturus johannisdavisi* both need more research to determine their true species designations. Taxonomic clarifications of these deep-sea skates are in progress. Much effort has been made to generate validated reference barcode sequences for skates globally (Coulson et al., 2011; Moura et al., 2008; Serra-Pereira et al., 2011; Ward et al., 2008). These reference sequences facilitate molecular studies of species identification, market mislabeling, and forensic identification (Marko et al., 2004; Wong & Hanner, 2008). Reference sequences for most Indian species are not available in public databases. In the present study, the four species collected from this family were found to be genetically distinct from each other with no haplotype sharing. Much further analysis, perhaps involving nuclear genes, of large data sets from all described species of the family Rajidae will be needed to resolve the taxonomic make-up of this family.

The family Centrophoridae consists of medium-sized demersal sharks from two genera, *Centrophorus* and *Deania*. The genus *Centrophorus* is the one of the most taxonomically complex and confusing elasmobranch groups. White et al. (2013) published a revision of part of this group, finding that *Centrophorus acus* and *C. niaukang* are junior synonyms of *C. granulosus*. Eight species of Centrophoridae are listed in Indian waters, three of which need confirmation (Akhilesh et al., 2014). An interesting finding from our studies was that specimens of *Centrophorus zeehani* caught locally showed 100% similarity with Australian specimens. Similarly, Naylor et al. (2012) using NADH2 sequences found very high similarity among specimens collected from widely different regions (Australia and Atlantic Ocean). But *Centrophorus zeehani* is considered to be a southern Australian endemic species (White et al., 2008). The existence of deep water marine superhighways (Broecker, 1991) may facilitate long distance movement and exchange of genetic material across regions. However, more studies on this group are needed to validate the species identity of *Centrophorus zeehani* in Indian waters.

The family Hexanchidae comprises three genera with four species. All four species have been listed from India, but the occurrence of *Hexanchus nakamurai* and *Notorynchus cepedianus* needs additional confirmation (Akhilesh et al., 2014). We barcoded specimens of *Hexanchus griseus* ($n = 4$, from off Kollam) and *Heptranchias perlo* ($n = 5$, from off Kochi). The latter barcodes matched 100% with a Portugese specimen (EU869819) of *Heptranchias perlo,* but are 2% divergent from Western Australia specimens (EU869817-18). NADH2 data of

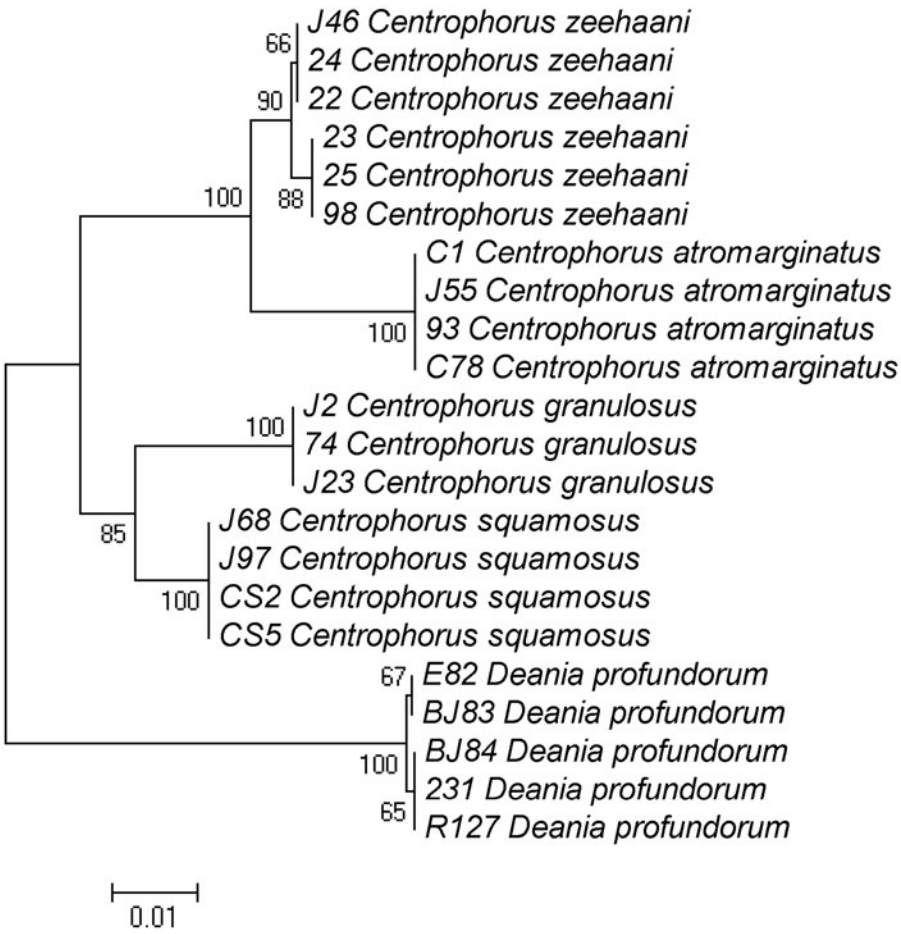

Figure 6. K2P distance neighbor joining tree of COI sequences from the family Centrophoridae.

this species from around the oceans are very variable, but again the Western Australia population is very distinct from other populations (Naylor, personal communication). There is a possibility that *Heptranchias dakini* Whitley, 1931, described from Australia, currently a synonym of *Heptranchias perlo,* may be a valid species. Further morphological and genetic comparisons between widely separated geographic samples of this species are required.

During 1801–1909, nine *Himantura* species were described from the east coast of India, but now only six are considered valid (Eschmeyer & Fong, 2013). We found 12 species of *Himantura*, including three undescribed or unrecognized species (we found a further five other undescribed/unrecognized batoids). The analysis included specimens from Kochi, Mumbai, Tuticorin, and Kolkata. The unrecognized species have been designated *Himantura* sp. A., *Himantura* sp. B, and *Himantura* sp. C, but in depth, taxonomic investigations are required to determine whether they represent undescribed species or synonymized/unidentified species which need to be resurrected as valid species. Many new species of rays have been uncovered recently by elasmobranch surveys off Australia, Indonesia, and Malaysia, with many unrecognized species in commercial trawl landings (Last et al., 2005, 2008a, 2010a,b); our findings are similar. Last et al. (2012) commented that *Himantura uarnacoides* (Bleeker, 1852) has not been recorded from the western Indian Ocean, here in, we clarify that *Himantura alcockii* known in the literatures from northern Arabian Sea (mostly Maharashtra, India) are misidentifications of *Himantura uarnacoides*.

Eleven elasmobranch species, confirmed by morphological data and COI sequences, were recorded for the first time from Indian waters, namely *Isurus paucus, Paragaleus randalli, Chiloscyllium hasseltii, Deania profundorum, Centrophorus zeehaani, Hexanchus griseus, Odontaspis noronhai, Zameus squamulosus, Rhynchobatus australiae, Aetomylaeus vespertilio,* and *Himantura granulata*.

Recent chondrichthyan taxonomic research integrated with genetic studies have resulted in the description of many new species and better taxonomic resolution of species complexes (e.g. Last et al., 2008a, 2010a; Naylor et al., 2012; White et al., 2010a–c). Our DNA barcoding study from India supported with morphology-based taxonomy also reveals under-estimated species diversity. We also note that while more than 150 species of chondrichthyans have been reported in Indian waters, there are very few representative specimens in ichthylogical collections and these are insufficient for detailed taxonomic studies. Some reported species are questionable and whether or not they are true records needs to be resolved. Better documentation of this fauna is essential. Without this, there is little scope for more effective conservation and management of these vulnerable and exploited species.

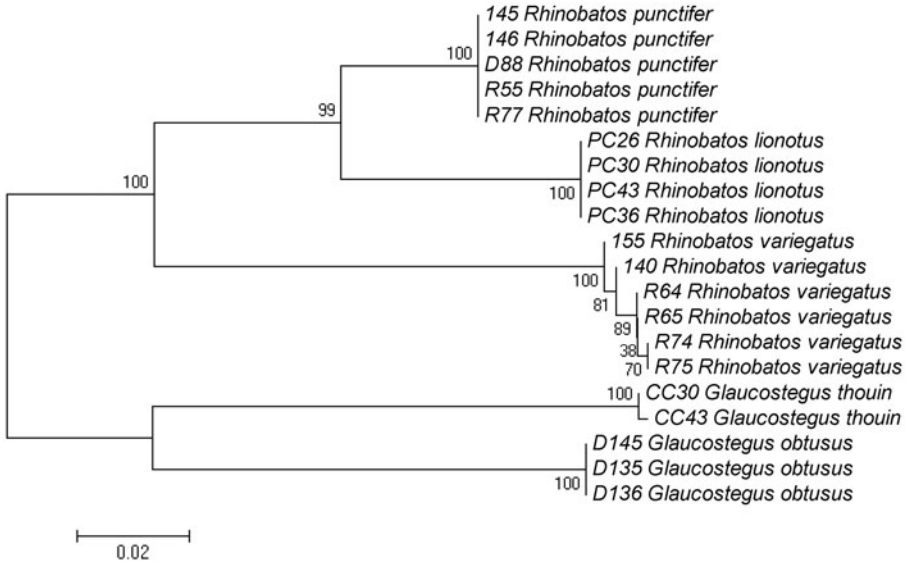

Figure 7. K2P distance neighbor joining tree of COI sequences from the family Rhinobatidae.

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Declaration of interest

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Table 4. Identification of sampled elasmobranchs using GenBank and BOLD databases.

(a) GenBank and/or BOLD identification percent 98% or greater. Identifications with less than 98% similarity given in parentheses.

Morphological identification	GenBank identification	%	BOLD identification	%	Species compatibility	
					GB	BOLD
Aetobatus sp. A	(*Aetobatus narinari*)	−97	*Aetobatus cf. ocellatus*	99	–	?
Aetobatus ocellatus (Kuhl, 1823)	*Aetobatus ocellatus*	99	*Aetobatus narinari*	100	√	X
Aetomylaeus maculatus (Gray, 1832)	*Aetomylaeus maculatus*	99	*Aetomylaeus maculatus*	99	√	√
Aetomylaeus vespertilio (Bleeker 1851)	*Aetomylaeus vespertilio*	99	*Aetomylaeus vespertilio*	99	√	√
Alopias pelagicus (Nakamura, 1935)	*Alopias pelagicus*	100	*Alopias pelagicus*	100	√	√
Alopias superciliosus (Lowe, 1841)	*Alopias superciliosus*	100	*Alopias superciliosus*	100	√	√
Alopias vulpinus (Bonnaterre, 1788)	*Alopias vulpinus*	98	*Alopias vulpinus*	98	√	√
Benthobatis moresbyi (Alcock, 1898)	(*Benthobatis kreffti*)	−94	*Benthobatis moresbyi*	99	–	√
Bythaelurus hispidus (Alcock, 1891)	*Bythaelurus sp*	99	(*Bythaelurus dawsoni*)	90	√	–
Carcharhinus albimarginatus (Ruppel,1837)	*Carcharhinus albimarginatus*	100	*Carcharhinus albimarginatus*	100	√	√
Carcharhinus altimus (Springer, 1950)	*Carcharhinus altimus*	100	*Carcharhinus altimus*	100	√	√
Carcharhinus amblyrhynchos (Bleeker, 1865)	*Carcharhinus amblyrhynchos*	99	*Carcharhinus amblyrhynchos*	99	√	√
Carcharhinus amboinensis (Müller & Henle, 1839)	*Carcharhinus amboinensis*	100	*Carcharhinus amboinensis*	100	√	√
Carcharhinus brevipinna (Müller & Henle, 1839)	*Carcharhinus brevipinna*	100	*Carcharhinus brevipinna*	100	√	√
Carcharhinus falciformis (Müller & Henle, 1839)	*Carcharhinus falciformis/Isurus oxyrinchus*	100	*Carcharhinus falciformis*	100	√	√
Carcharhinus leucas (Müller & Henle, 1839)	*Carcharhinus leucas*	99	*Carcharhinus leucas*	99	√	√
Carcharhinus limbatus (Müller & Henle, 1839)	*Carcharhinus limbatus*	100	*Carcharhinus limbatus*	100	√	√
Carcharhinus longimanus (Poey, 1861)	*Carcharhinus longimanus*	100	*Carcharhinus longimanus*	100	√	√
Carcharhinus macloti (Müller & Henle, 1839)	*Carcharhinus macloti*	100	*Carcharhinus macloti*	100	√	√
Carcharhinus melanopterus (Quoy & Gaimard, 1824)	*Carcharhinus melanopterus*	99	*Carcharhinus melanopterus*	99	√	√
Carcharhinus sorrah (Müller & Henle, 1839)	*Carcharhinus sorrah*	99	*Carcharhinus sorrah*	100	√	√
Centrophorus atromarginatus (Garman, 1913)	*Centrophorus atromarginatus*	99	*Centrophorus atromarginatus*	98	√	√
Centrophorus granulosus (Bloch & Schneider, 1801)	*Centrophorus granulosus*	100	*Centrophorus niaukang*	100	√	X
Centrophorus squamosus (Bonnaterre, 1788)	*Centrophorus squamosus*	100	*Centrophorus squamosus*	100	√	√
Centrophorus zeehaani (White et al., 2008)	*Centrophorus zeehaani*	99	*Centrophorus zeehaani*	99	√	√
Centroselachus crepidater (Barbosa du Bocage & de Brito Capello 1864)	*Centroselachus crepidater*	100	*Centroscymnus crepidater*	100	√	√
Chiloscyllium arabicum (Gubanov, 1980)	(*Chiloscyllium hasseltii*)	−93	*Chiloscyllium* sp	99	–	√
Chiloscyllium griseum (Müller & Henle, 1838)	*Chiloscyllium griseum*	100	*Chiloscyllium griseum*	100	√	√
Chiloscyllium hasseltii (Bleeker, 1852)	*Chiloscyllium hasseltii*	100	*Chiloscyllium hasseltii*	100	√	√
Dasyatis microps (Annandale, 1908)	(*Myliobatis* sp)	−88	*Himantura* sp	100	–	X
Deania profundroum (Smith & Radcliffe, 1912)	*Deania profundroum*	99	*Deania profundroum*	99	√	√
Echinorhinus brucus (Bonnaterre, 1788)	No match	–	No match	–	–	–
Etmopterus pusillus (Lowe, 1839)	*Etmopterus pusillus*	99	*Etmopterus pusillus*	99	√	√
Galeocerdo cuvier (Péron & Lesueur, 1822)	*Galeocerdo cuvier*	100	*Galeocerdo cuvier*	100	√	√
Gymnura poecilura (Shaw, 1804)	(*Aetoplatea zonura*)	−91	*Gymnura poecilura*	98	–	√
Gymnura sp. A	*Gymnura poecilura*	100	*Gymnura poecilura*	100	?	?
Halaelurus quagga (Alcock, 1899)	*Halaelurus natalensis*	100	(*Halaelurus sellus*)	−93	X	–
Hemipristis elongata (Klunzinger, 1871)	*Hemipristis elongata*	100	*Hemipristis elongata*	100	√	√
Heptranchias perlo (Bonnaterre, 1788)	*Heptranchias perlo*	100	*Heptranchias perlo*	100	√	√
Hexanchus griseus (Bonnaterre, 1788)	*Hexanchus griseus*	99	*Hexanchus griseus*	100	√	√
Himantura fai (Jordan & Seale, 1906)	*Himantura fai*	99	*Himantura fai*	99	√	√
Himantura fava (Müller & Henle, 1837)	*Himantura fava*	99	*Himantura fava*	98	√	√
Himantura gerrardi (Gray, 1851)	*Dasyatis microps*	99	*Himantura gerrardi*	100	X	√
Himantura granulata (Macleay, 1883)	*Himantura granulata*	98	*Himantura granulata*	99	√	√

(continued)

Table 4. Continued

(a) GenBank and/or BOLD identification percent 98% or greater. Identifications with less than 98% similarity given in parentheses.

Morphological identification	GenBank identification	%	BOLD identification	%	Species compatibility	
					GB	BOLD
Himantura imbricata (Bloch & Schneider, 1801)	*(Himantura walga)*	−92	*Himantura imbricata*	98	–	√
Himantura jenkinsii (Annandale, 1909)	*Himantura jenkinsii*	100	*Himantura jenkinsii*	100	√	√
Himantura leoparda (Manjaji-Matsumoto & Last, 2008)	*Himantura leoparda*	100	*Himantura uarnak*	100	√	X
Himantura sp. B	*Dasyatis lata*	99	*Himantura* sp	100	X	√
Himantura sp. C	*Himantura pastinacoides*	98	*Himantura pastinacoides*	99	?	?
Himantura uarnacoides (Bleeker, 1852)	*Himantura uarnacoides*	99	*Himantura uarnacoides*	99	√	√
Himantura uarnak (Forsskål, 1775)	*Himantura uarnak*	99	*Himantura uarnak*	99	√	√
Hydrolagus africanus (Gilchrist, 1922)	*Hydrolagus africanus*	99	*Hydrolagus africanus*/sp	99	√	√
Iago sp. B	*Mustelus mosis*	99	*Iago omanensis*	100	X	?
Isurus oxyrinchus (Rafinesque, 1810)	*Isurus oxyrinchus*	99	*Isurus oxyrinchus*	99	√	√
Isurus paucus (Guitart Manday, 1966)	*Isurus paucus*	100	*Isurus paucus*	100	√	√
Lamiopsis temminckii (Müller & Henle, 1839)	*Lamiopsis temminckii*	98	*Lamiopsis temminckii*	99	√	√
Manta birostris (Walbaum, 1792)	*Manta birostris*	100	*Manta birostris*	100	√	√
Mobula japanica (Muller & Henle, 1841)	*Mobula japanica*	100	*Mobula japanica*	100	√	√
Mobula kuhlii (Müller & Henle 1841)	*Mobula kuhlii*	100	*Mobula kuhlii*	100	√	√
Mobula tarapacana (Philippi 1892)	*Mobula tarapacana*	100	*Mobula tarapacana*	100	√	√
Nebrius ferrugineus (Lesson, 1831)	*Nebrius ferrugineus*	99	*Nebrius ferrugineus*	99	√	√
Neoharriotta pinnata (Schnakenbeck, 1931)	*Neoharriotta pinnata*	99	No match	–	√	–
Neotrygon kuhlii (Müller & Henle, 1841)	*Neotrygon kuhlii*	99	*Neotrygon kuhlii*	98	√	√
Odontaspis noronhai (Maul, 1955)	(*Odontaspis ferox*)	−93	*Odontaspis noronhai*	99	–	√
Paragaleus randalli (Compagno et al., 1996)	*Paragaleus randalli*	100	*Paragaleus randalli*	100	√	√
Pastinachus atrus (Macleay, 1883)	*Pastinachus atrus*	99	*Pastinachus atrus*	99.84	√	√
Pastinachus sephen (Forsskål, 1775)	(*Pastinachus solocirostris*)	−94	*Pastinachus sephen*	100	–	√
Plesiobatis daviesi (Wallace, 1967)	*Plesiobatis daviesi*	99	*Plesiobatis daviesi*	99	√	√
Prionace glauca (Linnaeus, 1758)	*Prionace glauca*	100	*Prionace glauca*	100	√	√
Pseudocarcharias kamoharai (Matsubara, 1936)	*Pseudocarcharias kamoharai*	99	*Pseudocarcharias kamoharai*	99	√	√
Pteroplatytrygon violacea (Bonaparte, 1832)	*Pteroplatytrygon violacea*	99	*Pteroplatytrygon violacea*	99	√	√
Rhina ancylostoma (Bloch & Schneider, 1801)	*Rhina ancylostoma*	99	*Rhina ancylostoma*	98	√	√
Rhincodon typus (Smith, 1828)	*Rhincodon typus*	100	*Rhincodon typus*	100	√	√
Rhinobatos punctifer (Compagno & Randall, 1987)	*Rhinobatos penggali*	99	*Rhinobatos penggali*	98	X	X
Rhinobatos variegatus (Nair & Lal Mohan, 1973)	*Rhinobatos annandalei*	99	*Rhinobatos variegatus*	99	X	√
Rhinoptera jayakari (Boulenger, 1895)	*Rhinoptera javanica*	99	*Rhinoptera javanica*	100	√	√
Rhizoprionodon acutus (Rüppell, 1837)	*Rhizoprionodon acutus*	99	*Rhizoprionodon acutus*	99	√	√
Rhynchobatus australiae (Whitley, 1939)	*Rhynchobatus australiae*	100	*Rhynchobatus australiae*	100	√	√
Rhynchobatus cf. *laevis* (Bloch & Schneider, 1801)	*Rhynchobatus* cf. *laevis*	99	*Rhynchobatus* sp	99	√	√
Scoliodon laticaudus (Müller & Henle, 1838)	(*Scoliodon macrorhynchos*)	−97	*Scoliodon laticaudus*	99	–	√
Sphyrna lewini (Griffith & Smith, 1834)	*Sphyrna lewini*	100	*Sphyrna lewini*	100	√	√
Sphyrna zygaena (Linnaeus, 1758)	*Sphyrna zygaena*	100	*Sphyrna zygaena*	100	√	√
Squalus sp. A	*Squalus mitsukurii*	98	*Squalus mitsukurii*	98	?	?
Stegostoma fasciatum (Hermann, 1783)	*Stegostoma fasciatum*	100	*Stegostoma fasciatum*	100	√	√
Taeniura meyeni (Müller & Henle, 1841)	*Taeniura meyeni*	99	*Taeniura meyeni*	99	√	√
Triaenodon obesus (Rüppell, 1837)	*Triaenodon obesus*	99	*Triaenodon obesus*	99	√	√
Urogymnus asperrimus (Bloch & Schneider,1801)	*Urogymnus asperrimus*	99	*Urogymnus asperrimus*	99	√	√
Zameus squamulosus (Günther, 1877)	*Zameus squamulosus*	99	*Zameus squamulosus*	99	√	√

Table 4. Continued.

(b) GenBank and BOLD identification percent 97% or less.

Morphological identification	Sequence #	GenBank identification	%	BOLD identification	%	Genus compatibility	
						GB	BOLD
Apristurus sp. A		*Apristurus nakayai*	94	*Apristurus sp.*	94	√	√
Cephaloscyllium silasi (Talwar, 1974)		*Cephaloscyllium ventriosum*	92	*Cephaloscyllium ventriosum*	92	√	√
Dipturus sp. B		*Dipturus springeri*	96	*Dipturus* sp. 1	97	√	√
Dipturus sp. C		*Dipturus argentinensis*	95	*Dipturus* sp. 2	96	√	√
Dipturus sp. A		*Dipturus confusus*	95	*Dipturus* sp. 2	97	√	√
Eridacnis radcliffei (Smith, 1913)		*Proscyllium habereri*	86	*Eridacnis sp*	95	x	√
Glaucostegus obtusus (Müller & Henle, 1841)		*Glaucostegus typus*	87	*Glaucostegus typus*	87	√	√
Glaucostegus thouin (Anonymous [Lacepède] 1798)		*Glaucostegus typus*	90	*Glaucostegus typus*	90	√	√
Himantura sp. A		*Himantura pastinacoides*	91	unable to match	n.a.	√	-
Iago sp. A		*Mustelus mosis*	94	*Iago omanensis*	94	x	√
Narcine maculata (Shaw, 1804)		*Narcine brunnea*	87	*Narcine brunnea*	86	√	√
Narcine sp		*Narcine brunnea*	95	*Narcine brunnea*	95	√	√
Narcine sp. A		*Narcine brevilabiata*	94	*Narcine brevilabiata*	94	√	√
Okamejei powelli (Alcock, 1898)		*Raja rhina*	88	*Okamejei* sp.	94	x	√
Rhinobatos lionatus Norman, 1962		*Rhinobatos penggali*	94	*Rhinobatos penggali*	94	√	√
Torpedo sinuspersici (Olfers, 1831)		*Torpedo fuscomaculata*	96	*Torpedo fuscomaculata*	95	√	√
Torpedo sp. A		*Torpedo californica*	95	*Torpedo tokionis*	97	√	√

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