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Couverture / *Cover* :
Underside of the head *Rhinoptera jayakari* Boulenger, 1895.

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First record and DNA Barcoding of Oman cownose ray, *Rhinoptera jayakari* Boulenger, 1895 from Andaman Sea, India

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ABSTRACT
Strategically located to the southeast of the Bay of Bengal and northwest of the Malay Peninsula, the Andaman Sea houses rich and varied biological diversity both in its flora and fauna which is under explored. The present study reports the occurrence of Oman cownose ray, *Rhinoptera jayakari* Boulenger, 1895 as a new record from this region. A male specimen with a disc width of 494 mm was landed by a motorized longline boat in Junglighat fishing harbour. Port Blair, Andaman and Nicobar Islands fills the wide gap in the known distribution range of the species. A detailed morphological description of freshly collected specimen in comparison with those from the previous published reports elsewhere was carried out. Molecular marker based taxonomical annotation using Mitochondrial COI gene sequencing confirmed the identity of the specimen.

KEY WORDS
Rays,
myliobatiformes,
Andaman,
Nicobar waters,
first report.

RÉSUMÉ

Première signalisation et premier barcode pour la raie mourine à queue courte Rhinoptera jayakari *Boulenger, 1895 en mer d'Andaman, Inde.*

Stratégiquement située au sud-est de la baie du Bengale et au nord-ouest de la péninsule malaise, la mer d'Andaman abrite une diversité biologique riche et variée tant pour sa flore que pour sa faune qui est sous explorée. La présente étude mentionne la présence de la raie mourine à queue courte, *Rhinoptera jayakari* Boulenger, 1895 comme une nouvelle signalisation dans cette région. Un spécimen mâle avec une largeur de disque de 494 mm a été débarqué par un palangrier motorisé dans le port de pêche de Junglighat. Port Blair, Andaman et Nicobar Islands comblent le large fossé dans la distribution connue de l'espèce. Une description morphologique détaillée des spécimens fraîchement collectés a été réalisée en comparaison avec les publications précédentes. L'identité de l'échantillon a été confirmée par annotation taxonomique moléculaire en séquençant le gène mitochondrial COI.

MOTS CLÉS
Raies,
myliobatiformes,
mers d'Andaman et de Nicobar,
signalisation nouvelle.

INTRODUCTION

Rays of the family Rhinopteridae Bonaparte, 1835, are distributed in warm temperate and tropical seas except around oriental Pacific Island (McEachran & Fechhelm 1998; Last & Stevens 2009; Domingues *et al.* 2009; Froese & Pauly 2017). They are semi pelagic and gregarious, often forming large schools (Bogorodsky *et al.* 2014). Weigmann (2016) listed eight species under genus *Rhinoptera* Cuvier, 1829 and mentioned that *R. jayakari* Boulenger, 1895 is represented by three unresolved subgroups in the western Indian Ocean, based on the NADH2 gene (Naylor *et al.* 2012). *Rhinoptera sewelli* Misra, 1946 from the Arabian Sea, questionably valid following Compagno & Last (1999), was considered to be possibly conspecific with *R. jayakari* (Last *et al.* 2010). These species are strongly allopatric with widespread geographic ranges believed to reflect their high mobility (Schwartz 1990). Species of cownose rays are very similar to one another and most of the classification work is based on number of tooth plate rows or series. However, dentition is highly variable in some species and the family is in urgent need of revision (Bigelow & Schroeder 1953; James 1970; Sandoval & Olivares 2011). Presently eight species under the genus *Rhinoptera* are considered valid (Bailly 2014; Last *et al.* 2016).

Previous records of the Oman Cownose Ray (*Rhinoptera jayakari* Boulenger, 1895) in the north-western Indian Ocean include those from the Gulf of Oman (type locality), southeastern Arabian Sea coast of Oman (Henderson & Reeve 2011), Gulf of Aden (Bonfil & Abdallah 2004) and Red Sea (Bogorodsky *et al.* 2014). From Indian waters, it has been listed by Akhilesh *et al.* (2014) and Joshi *et al.* (2016), but without morphological details. DNA barcoding has previously been performed for the specimens collected from Kochi, Southwest coast of India (Bineesh *et al.* 2016) and deposited in the Genbank, NCBI. The aim of the current study is to utilize the mitochondrial COI molecular marker to confirm the taxonomic identity of a specimen identified as *R. jayakari* collected from the Andaman Sea and report the species occurrence in that area for the first time, thus filling the gap in the known distribution range of this species.

MATERIAL AND METHODS

A single specimen of *Rhinoptera jayakari* was collected from Junglighat fishing landing centre, Port Blair, South Andaman (Fig. 1). It was caught by a motorised fishing boat with longline gear at a depth of 30-50 m in the waters of the Andaman and Nicobar islands of the Andaman Sea. Morphometric measurements were recorded and expressed as proportion of disc width (DW), according to Last & Stevens (2009) and White *et al.* (2010). These measurements were compared with the published data by Bogorodsky *et al.* 2014 (Table 1).

The specimen was preserved in the museum of the zonal base of Fishery Survey of India, Port Blair. The tissue sample of *R. jayakari* was collected and preserved in 99.5% ethanol at ambient temperature and DNA was extracted by standard protocols (Miller *et al.* 1988). The extracted DNA was checked by agarose (0.8%) gel electrophoresis.

The partial sequence of mitochondrial COI gene was amplified using specific universal 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 reaction volume containing 1× assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM MgCl2, 0.5 µl of each primer, 200 µM of each dNTPs (Genei, Bangalore, India), 1.5 U Taq DNA polymerase, and 20 ng of template DNA. PCR reactions were carried out in Veriti TM 96-Well Thermal Cycler (Applied Biosystems, USA) following initial preheating at 95° C for 5 min, denaturation 94° C for 30 s, annealing 53° C for 45 s, extension 72° C for 1 min, repeated for 30 cycles, followed by a final extension for 10 min at 72° C. The PCR products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide (10 µg/ml) to ensure that a fragment of the correct size had been amplified. The gel was visualized on a Gel documentation system (Bio-Rad, USA). The purified PCR Products were labeled using Big Dye Terminator V.3.1 cycle sequencing kit (Applied Biosystems Inc.) and sequenced bidirectionally using ABI 3730 capillary sequencer using the primers Fish F1 and Fish R1.

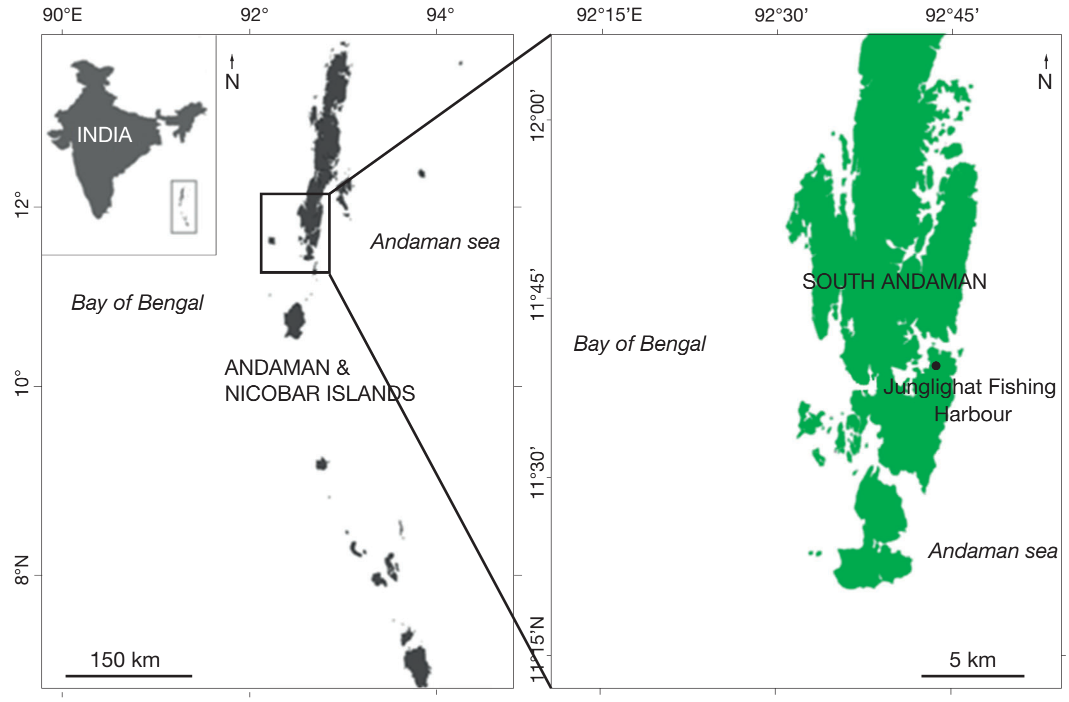

FIG. 1. — Junglighat Fishing Harbour (Sampling station), South Andaman.

Sequences were analyzed and the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul *et al.* 1990) was used to search the GenBank database for homologous sequences (http://www.ncbi.nlm.nih.gov/). The sequences were multiple aligned and were used to construct a phylogenetic tree of K2P distance were created to provide graphic representation of divergence by the neighbor-joining (NJ) method (Saitou & Nei 1987), using the MEGA V. 6.0 package (Tamura *et al.* 2013). Bootstrap analysis was based on 1000 replicates.

SYSTEMATICS

Family RHINOPTERIDAE Bonaparte, 1835
Genus *Rhinoptera* Cuvier, 1829

Rhinoptera jayakari Boulenger, 1895

Rhinoptera jayakari Boulenger, 1895: 1520-1523.

MATERIAL EXAMINED. — MUS/FSI/PB/EB/09/2016, 1 ♂ *Rhinoptera jayakari* of Disc Width (DW) 494 mm and weight 2.9 kg (Fig 2 A), collected from Junglighat fishing landing centre, Port Blair, South Andaman. The specimen was caught by a motorized fishing boat with longline gear at a depth of 30-50 m in the Andaman and Nicobar waters of the Andaman Sea.

DESCRIPTION OF SPECIMEN FROM THE ANDAMAN SEA

Disc length 1.5 in disc width; near straight anterior margin, posterior margin concave, apices acutely pointed (Fig. 2); disc mostly naked, except for small and stellate-based prickles dorsally on snout and inter orbital space (Fig. 3B); trunk thick and broad; head relatively broad and its width between posterior margin of spiracles 5.3 in disc width; upper part of the snout bilobed, separated almost at level of mouth from a lower, forward-projecting and also the bilobed part by a continuous deep groove around front of head; eyes moderately large, lateral on head and just anteromedial to spiracles (Fig. 2B), eyes 1.8 in spiracle length; mouth transverse, straight, and narrow (Fig. 3A), preoral distance 8.4 in disc width; anterior nasal flaps long medially expanded and fused into a broad, elongated fringed nasal curtain overlapping mouth; nostrils slightly anterior to mouth, connected by broad nasoral grooves with mouth (Fig. 3A); tail short, 1.4 in disc length; base of tail with relatively high, rounded dorsal fin originating over pelvic-fin insertions; posterior margin of the dorsal-fin almost upright; a serrate spine on tail behind dorsal fin (Fig. 3D), its base beneath dorsal-fin inner margin; teeth flat and pavement-like, in 9 series in upper jaw (Fig. 3C), 3 medial rows of broad teeth and 3-5 rows of narrower diamond-shaped teeth on each side.

Colour

Dorsal surface of disc dark olivaceous, ventral surface of disc white, tail dark olivaceous gradually becoming black distally.

DNA barcoding

The obtained partial sequence of COI gene of was submitted to the NCBI database with an accession number KY574494.

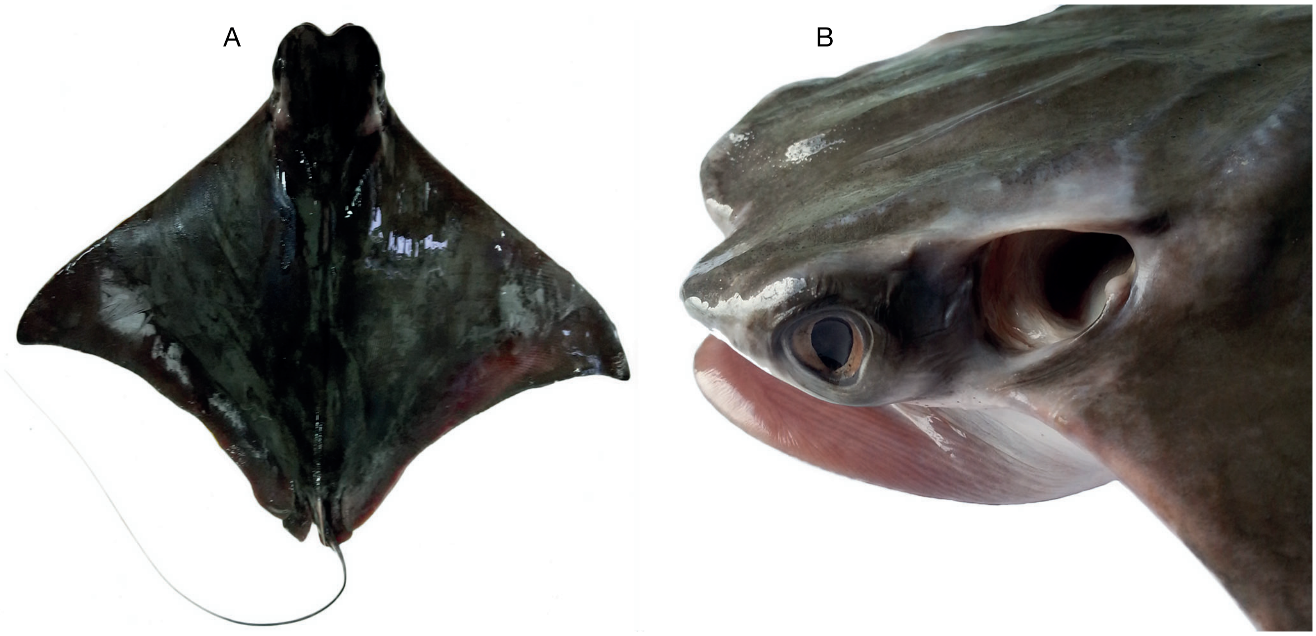

FIG. 2. — **A**, Dorsal view of *Rhinoptera jayakari* Boulenger, 1895; **B**, antero-lateral view of the head with upper and lower lobes. Size of specimen: 494 mm DW.

TABLE 1. — Morphometric details of *Rhinoptera jayakari* Boulenger, 1895 (in mm and % Disc Width [DW]) in comparison with previous records.

	SMF 34988 Yemen	**SMF 34989 Jizan**	**KAUMM 36 Jizan**	**Present specimen**	
	%DW	**%DW**	**%DW**	**%DW**	**mm**
Disc width	727	684	771		494
Total length, from medial point of snout	100.8	108.2	91.4	111.13	549
Tail length	44	45.3	36.5	47.77	236
Disc length (direct) (snout to rear edge of pectoral fin along axis)	61.5	66.7	62.1	64.57	319
Disc thickness	11.7	11.7	11.9	11.34	56
Disc, anterior margin of spiracle to pectoral insertion	49.1	53	51	55.87	276
Disc, snout to maximum width	36.9	37.4	36.4	38.87	192
Head length	26.7	28.2	26.1	26.52	131
Snout-preobital	11.2	10.9	11.5	10.73	53
Snout preoral	11	11.5	10.8	11.94	59
Snout-prenasal	11.9	11.4	12	11.34	56
Lower-flap length	12.8	12.4	12.8	12.35	61
Eye Diameter	2.3	2.3	2.2	2.23	11
Inter-eye width	17.5	17.7	17	17.61	87
Orbit and spiracle length	8.5	9.4	8.5	9.31	46
Spiracle length	4.2	4.2	4	4.05	20
Interspiracular width, anteriorly	15.5	16.2	15.6	15.99	79
Interspiracular width, posteriorly	18.2	19.1	18	19.03	94
Mouth width, between edges of teeth	8.4	8.8	8.5	8.70	43
Mouth width, between folders	13.1	13.1	13.2	13.16	65
Nostril length	1.5	1.3	1.4	1.21	6
Nasal curtain width	14.7	15.3	14.6	15.38	76
Nasal curtain length	4.7	4.85	4.4	4.86	24
Internasal width, anteriorly (between bases of nasal curtain)	9.4	9.9	9.3	9.72	48
Internasal width, posteriorly (between posterior out ends of nostrils)	11.3	11.7	11.2	11.74	58
Width 1st gill slit	2.8	2.8	2.6	2.83	14
Width 5st gill slit	1.9	1.9	1.7	1.82	9
Distance between 1st gill slits	18.2	18.5	18.8	18.62	92
Distance between 5st gill slits	12.3	13	12.6	12.75	63
Pelvic fin, length	13.8	13.5	11.5	13.66	67.5
Pelvic fin. Base width	6.3	6.7	5.8	6.56	32.4
Tail width, axil of pelvic fin	3.5	3.5	3.2	3.36	16.6
Tail width, base of sting	2	1.9	1.8	1.82	9
Tail height, base of sting	2.3	2.1	2.2	2.15	10.3
Spine length	broken	7.3	7.1	0.00	broken
Snout to origin of cloaca	52.4	58.8	53.6	58.50	289
Cloaca origin to sting	11.5	13.1	11.4	12.45	61.5
Cloaca origin to tail tip	48.8	49.1	40	48.79	241
Cloaca length	3.6	5.1	2.7	4.86	24
Clasper, post cloacal length (from posterior edge of cloaca to tip of clasper)	14.6	13.5	13.4	13.36	66
Clasper, length from pelvic axil (from insertion of pelvic fin to tip of clasper)	12.9	13.1	13	12.75	63
Dorsal-fin base length	5.8	7	6.6	6.48	32
Dorsal-fin height	2.3	5.3	5.3	5.16	25.5
Teeth rows on upper jaw	7	9	9		9

Fig. 3. — *Rhinoptera jayakari* Boulenger, 1895: **A**, underside of the head; **B**, location of prickles on dorsal surface of the head; **C**, nine series of teeth, upper jaw; **D**, dorsal fin along with spine. Size of specimen: 494 mm DW.

The partial sequence of mitochondria COI gene produced a mean value of 623 nucleotide base pairs. BLAST result shows a 99 % match with the existing COI sequences of *R. jayakari* from India (GenBank: KF 899679.1, KF 899683.1, and KM 0703015.1) in the NCBI database. It also forms a distinctive clade in Neighbour Joining (NJ) phylogenetic tree inferred from mitochondrial COI gene sequences (Fig. 4). The pair wise genetic distances values (K2P) based on COI sequences are given in Table 2. The interspecies distance ranged from 0.002 to 0.008 and the genetic distance within the genus *Rhinoptera* ranged from 0.002 to 0.093.

DISCUSSION

In present scenario, the importance of biological collections with clear and descriptive morphological details in conjunction with the state of art molecular marker based studies in taxonomy has become the need of the hour. The limited basic taxonomical studies in the batoid members have paved hurdles in front of researchers round the globe. The Andaman Sea had remained prestine in this context and had come to limelight with upcoming development of basic science research. Hence, the morphological and molecular identification of organisms,

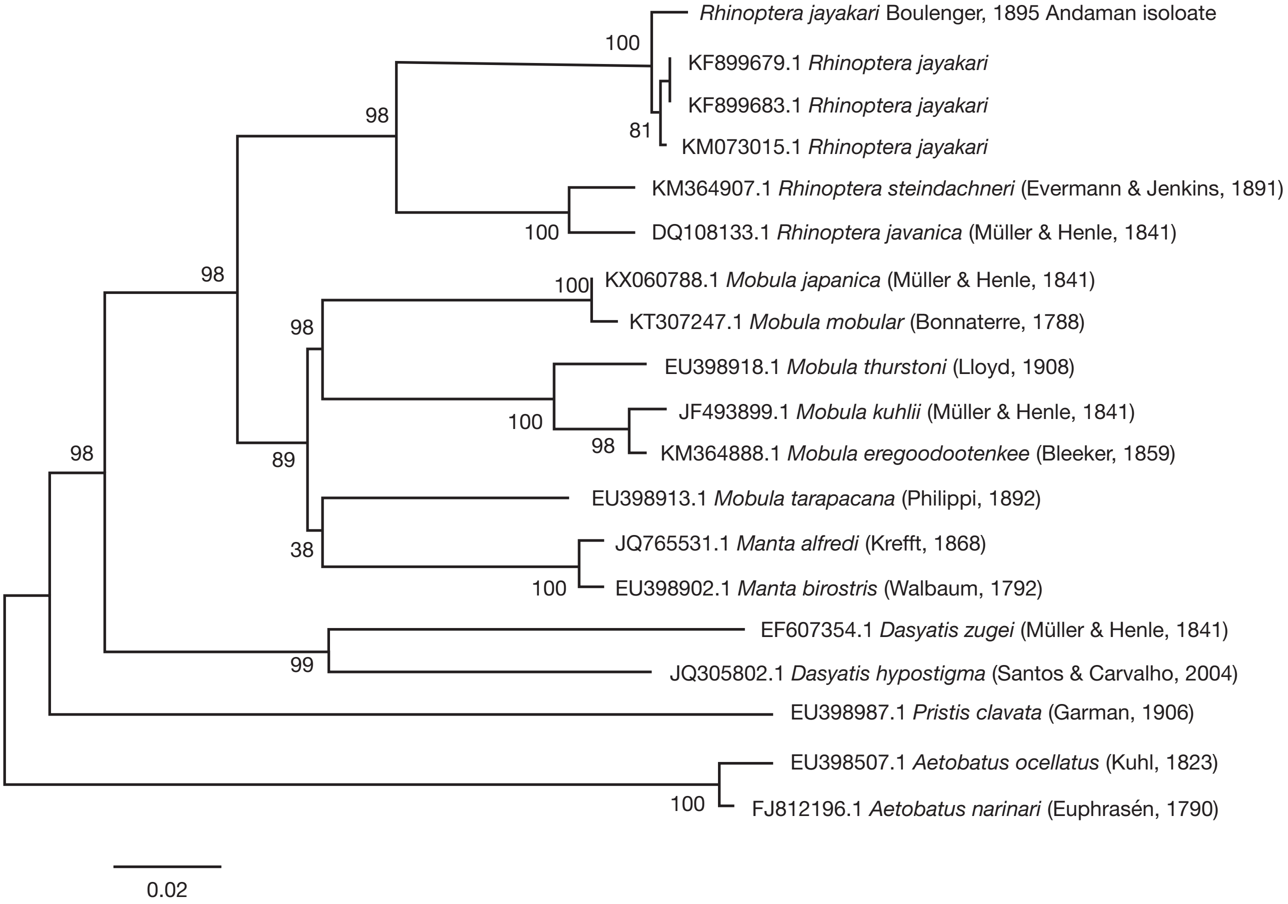

Fig. 4. — Neighbour Joining (**NJ**) phylogenetic tree of *Rhinoptera jayakari* Boulenger, 1895 and related species inferred from mitochondrial COI gene sequences.

especially batoids, had been difficult due to the limited available descriptions in literature globally.

Rhinoptera jayakari had been previously reported from gulf of Oman to Red Sea along northwestern Indian Ocean (Bonfil & Abdallah 2004; Henderson & Reeve 2011; Bogorodsky *et al.* 2014). Ahmad *et al.* (2013) has mentioned a number of identification characters of *R. Jayakari* in a field guide on look-alike Sharks and Rays species of the Southeast Asian Region but they are not enough to confirm the identity of the specimen. From, Indian waters *R. jayakari* has been listed by Akhilesh *et al.* (2014), reported from Gulf of Mannar (Joshi *et al.* 2016) and DNA barcoding of *R. Jayakari* based on partial COI gene sequences has been performed from Kerala coast (Bineesh *et al.* 2016). Only a single species of *Rhinoptera* (*R. javanica* Müller & Henle, 1841) has been reported from Andaman and Nicobar waters of the Andaman Sea (Rajan *et al.* 2013).

Differentiation of closely related members of *Rhinoptera* clade is quite difficult solely based on morphological characteristics. Compagno & Last (1999) separated *R. adspersa* from *R. javanica* in having fewer rows of teeth in upper jaw (7 vs 9). However, the number of rows appears to be a variable character, so *R. adspersa* has been placed in the synonymy of *R. javanica.* The Australian species *R. neglecta* can be distinguished from both *R. javanica* and *R. jayakari* in having a smooth disc without prickles, tail as long as the disc length and origin of the dorsal fin in front of the pelvic-fin insertions. Recent works by Bogorodsky *et al.* in 2014 on the morphological studies of three specimens from Red Sea mentioned the difference in the number of teeth rows in the upper jaw: one specimen has nine rows; two specimens have 12 rows of teeth (with the outermost rows variable present). Thus, posing another hurdle on morphological identification keys. Hence, the molecular marker based taxonomic annotation becomes the perfect tool for species level confirmation. *Rhinoptera jayakari* has been confused with *R. javanica* but it differs in having a broader head (17.6% disc width vs 14.5-16% disc width in *R. javanica*), shorter tail (1.4 in disc length vs 0.5-0.8 in disc length in *R. javanica*), and dorsal-fin origin over pelvic-fin insertions (dorsal-fin origin behind pelvic-fin insertions in *R. javanica*). Morphometrical characters of the present specimen like, disc length (1.5), head (5.3), preoral distance (8.4), tail (1.5) in disc width and eyes 1.8 in spiracle length, fall well within the range given by Bogorodsky *et al.* (2014) for *R. jayakari* collected from Red Sea. This is the first study of mitochondrial COI gene sequence of *R. jayakari* from the Andaman Sea. The DNA barcode sequences based on partial sequence information of COI gene has been widely

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

Species (with GenBank Acc. No.)	**1**	**2**	**3**	**4**	**5**
Rhinoptera jayakari KY574494					
Rhinoptera jayakari KF899679.1	0.008				
Rhinoptera jayakari KF899683.1	0.008	0.000			
Rhinoptera jayakari KM073015.1	0.006	0.002	0.002		
Rhinoptera steindachneri KM364907.1	0.088	0.093	0.093	0.090	
Rhinoptera javanica DQ108133.1	0.093	0.093	0.093	0.095	0.020

used in identification and validation of species (Ward *et al.* 2005). The phylogenetic tree derived from mitochondrial COI gene strongly confirms the present specimen was *R. jayakari* which helps to avoid the ambiguity in identification based on external characteristics. Hence, the present study not only adds a new species to fish diversity of Andaman and Nicobar waters but also fills the wide gap in the known distribution range of this species.

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