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Morphology and Molecular Biology of Benthic Java Sea Shark Ray *Rhina ancylostoma* Bloch and Scheider 1801 (Elasmobranchia: Rhinidae)

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Authors' contributions

The work had been carried out in collaboration among all authors responsibility. Author AH as the first author is research coordinator, main concept and manuscript finalization. Author DP as second author contribute for DNA analysis and discussion. Author ACA contribute for laboratory analysis. All authors read and approve the final manuscript.

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ABSTRACT

Aims: Morphology and molecular biology of a rare shark-rays *Rhina ancylostoma* caught from Java sea.Indonesia.

Study Design: Morphology, morphometry and DNA analysis of the fish specimen.

Place and Duration of Study: Sample wascollected from fish auction hall at north Java, specimen now stored in Lab of Ichthyology. Department of Fisheries. Faculty of Fisheries and Marine Science. Diponegoro University, between March to December 2019.

Methodology: DNA extraction, amplification and molecular identification of fish sample.Yield of DNA supernatant transferred in an appendeorf tube and stored in 4°C for further process. PCR

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amplification. Part of Mitochondrial DNA COI (Cytochrome Oxidase subunit-I) gen was amplified using Polymerase Chain Reaction (PCR) method. Then matched up with GenBank database at NCBI using Basic Local Alignment Search Tool (BLAST) analysis. Phylogenetic tree was set using Neighbor-Joining method, Kimura-2 parameter model and 1,000 bootstrap replication. Some sequence from NCBI GenBank were inputted into phylogenetic tree as comparison.

Results: *Rhina ancylostoma* as one of demersal shark rays catch from tropical shallow Java sea. Range of Total Length 73 – 200 cm and dominant Total Length catch 125 cm. Morphology is characterised with three lines of spine thorn on the head, morphometry characterwise of head to TL ratio 0.77. DNA analysis had confirmed the specimen of AH2 as *Rhina ancylostoma* based on homological match up of sequence of Gen Bank database with reference accession number KU721837.1 with length sequence of 665 bp and identical similarity of 99.84% for specimen Accession number LC 505461.

Conclusion: *Rhina ancylostoma* as one of demersal shark rays catch from Java sea. Range Total Length (TL) of catch 73 – 200 cm and dominant TL catch range 101 – 125 cm. Morphologically character of wide head ratio with three lines of spine thorn on the head. Nearest genetic distance of 0.02 to *Rhincobatus horkelii* and 0.017 to *R. australiae*. Longest genetic distance of 0.243 to *Potamotrygon motoro*.

Keywords: Morphology; molecular biology; shark-ray; Java-sea; Rhinidae.

1. INTRODUCTION

High biodiversity of tropical seawater teleost and elasmobranch of Indonesia is very well known. High biodiversity and high consumption of the fish is need to be carefully managed for sustainable fish resource use [1]. Consumption need in nowadays demand for the elasmobranch fish was even higher than last decades demands. Specifically for elasmobranch sharks market demand as high price commodity is mainly for the shark fins for Asean demand market [2]. These high demand had driven vastly for fisherman to catch more sharks and mainly for its fin inconsiderably. In other sector had increasing capability of sharks fish processing for food and vitamin extraction [3]. Indonesia had been known as one of the biggest sharks catch country in the world with 103,245 tons in 2011 and 105,230 tons in 2018. Sharks can be as the main target as well as by catch in fishing operations [4]. As mention by [5] high demand for shark fins and rays for soup, celebratory dish in Asian cuisines and more specifically of the pre-branchial appendages or gill rakers of mobulid rays (Family Mobulidae) is reality. Also the demand for medicinal purposes had drives to uncontrolled exploitation of the vulnerable elasmobranch fishes [6-9]. One of rare demersal shark catch from Java sea is *Rhina ancylostoma*. The International Union for Conservation of Nature (IUCN) had classified *R. ancylostoma* as in category of vulnerable since it was widely caught by artisanal and commercial fishery especially for its valuable fins. As by-catch of bottom trawlers and tuna long liners it was

regarded will damage the net unit by its thorny skin, but still collect the catch for high price of its fins only. Habitat degradation and the fishing operations had more challenge to these sharks survival and populations especially at Java Sea. Sharks known as demersal fish and mostly inhabit on the bottom of the sea and thus assumed to have correlation with the depth of the sea as the habitat [10]. Aim of study is to explore morphology and molecular biology of demersal *R. ancylostoma* catch from Java Sea landed at Cirebon, Tegal and Rembang fishing port.

2. METHODOLOGY

2.1 Samples and Study sites

The data of demersal shark-rays *R. ancylostoma* were collected from fisherman catch from Java sea landed at Cirebon, Tegal and Rembang fishing port (Fig. 1). Quantitative yearly data of sharks catch was collected from three fishing port at north Java for period of 2011-2018. Shark catch operation by fishermen at Java sea were mostly using bottom gillnet, locally called as 'liong bun' net. Morphology and morphometric measurement involving of the main taxonomical measurements such as Total Length, Standard Length, fin length, distance between dorsal fin-I and II, caudal fin and ventral fin and ratios [11].

DNA extraction, amplification and molecular identification of fish specimen. Tissue sampling and collection of fish specimen using scalpel and pinset and sterilized using ethanol 96% washing with sterilized seawater. Genomic DNA

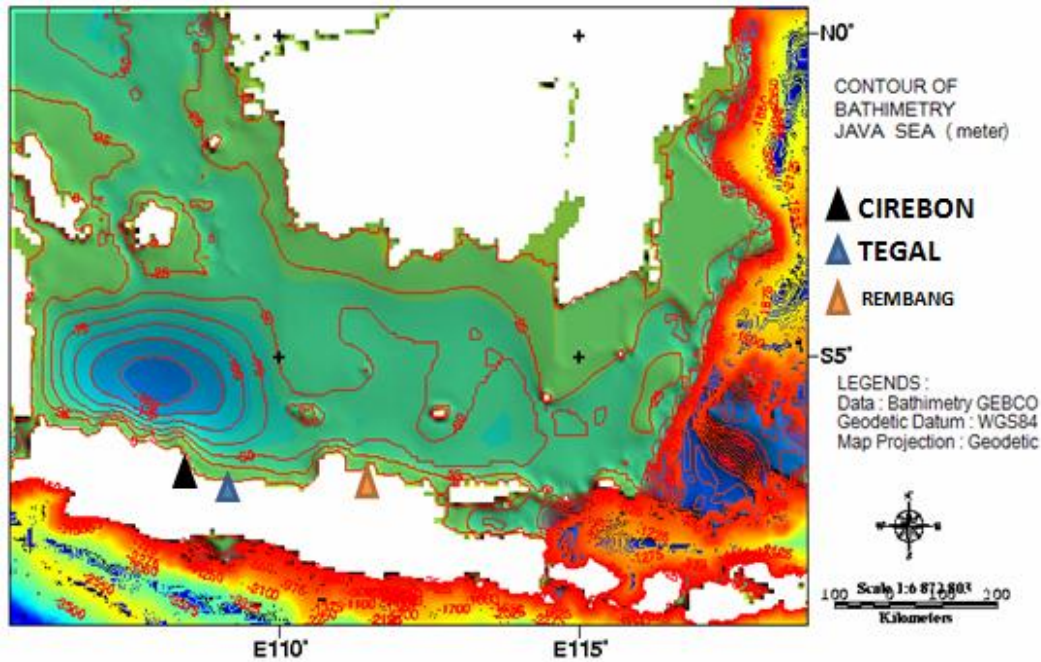


Fig. 1. Bathymetry of Java Sea and fish landing site

extraction using modified Chelex 100 method [12,13]. One gram of sample of muscle tissue put in the Eppendorf tubes filled with 500 µl of Chelex 10% solution combined with 7 µl proteinase K (10 mg/ml). Specimen sample then incubated in heating block at 55°C for 90 minute for DNA release, followed with 100°C for 20 minute for proteinase-K deactivation and centrifuge at 9,000 RPM for 15 minute. Yield of DNA supernatant transferred into eppendorf tube and stored in 4°C for further process. PCR amplification. Part of Mitochondrial DNA COI (Cytochrome Oxidase subunit -I) gen was amplified using Polymerase Chain Reaction (PCR) method.

An amount of 26 µl mix solution of 1µl DNA template, 12.5 µl Bioline My Taq™ HS Red Mix, 1.25 µl forward primer 10 mM, 1.25 µl reverse primer 10 mM and 10 µl aquabidest. Primer used was Fish BCL: 5'-TCA ACY AAT CAY AAA GAT ATY GGC AC-3' forward and Fish BCH: 5'-ACT TCY GGC TGR CCR AAR AAT CA-3' reverse [13,14]. The mix then put into Thermal Cycle for PCR process, with pre-denaturation at 95 °C for 5 minute, followed with 38 cycles of denaturation at 94°C for 30 second. Annealing process at 48 °C for 30 second then extension at 72°C for 45 second and final extension at 72°C for 5 minutes. Sample of PCR then visualized to determine the presence DNA band by

electrophoresis method using 1% agarose and gel-rad colouring. PCR specimen with DNA visualization then purified and sequenced at Genetica Science Indonesia company. Examination on result of forward and reverse sequence, sorted and edited using Mega 7.0 software [13,15]. Then matched up with GenBank database at NCBI using Basic Local Alignment Search Tool (BLAST) analysis. Phylogenetic tree was set using Neighbor-Joining method, Kimura-2 parameter model and 1,000 bootstrap replication. Some sequence from NCBI Gen Bank were inputted into phylogenetic tree as comparison.

3. RESULTS AND DISCUSSION

3.1 Morphology

As widely known that teleost group of Gobiidae as the most rich species group with about 2,228 species found in the tropical seas of Iran, Malaysia, Indonesia and Fiji [16]. The species of *Rhina ancylostoma* group of elasmobranchia Rhinidae inhabit the bottom of up to 200 m depth, was regarded as one rare and not well documented species and references. Unlike the case of Mediteransea receiving many migratory and climate cycle changes species [17]. The demersal elasmobranch of *R. ancylostoma* in the study was regarded as a semi enclosed

ecosystem distributed in a shallow Java sea (Fig. 2). Systematic taxonomy Kingdom:Animalia, Phylum: Chordata, Class: Chondrichthyes, Order: Rajiformes, Family: Rajidae - J. P. Muller and Henle, 1941, Genus: Rhina- Bloch and J.G Schneider, 1801, Species: *R. ancylostoma*.

Shark ray *R. ancylostoma* catch from Java sea is locally known as 'Mimi shark', or other local name at TegalsariTegal fishing port locally called as 'kupu-kupu' orbutterfly. Specimen of *R. ancylostoma* in the study was caught from the depth range of 40 – 60 m at Java sea by fisherman fishing operation. Described by [1], with morphological characters are as follows. The first base dorsal fin positioned in front of ventral fin base, caudal fin as sickle like form. Snout thick and rounded, no skin denticulation on back edge spiracel. Eye circle at middle of head body with three lines of sharp spine thorns at dorsal. Most of the species distributed along west Indo–Pacific to Papua New Nugini and north Australia and south Africa south Africa. Commonly live at bottom of the sea. Viviparous with dependency of embryo to the egg yolk biologically not much understood. As predator group with main feeding were on crustacean and mollusc [1]. Snout wedge-shape of Rhynchobatus. Snout broadly rounded of Rhina, three cluster lines of sharp thorns and Rhinagrouped in Rhinidae. Rhinobatidae, body less thick and less shark-like; pectoral fins with origins in front of nostrils and with free rear tips posterior to pelvic-fin origins. Dorsal fins smaller, first dorsal fin with origin behind rear tips of pelvic fins. Key to the species of Rhinidae, snout and anterolateral edges of pre-pectoral head broadly rounded, with a deep indentation separating it from pectoral-fin origin on each side, orbits, mid-dorsal line and shoulders with prominent ridges bearing clusters of small, medium-sized and large triangular thorns or spines. The large spines on the midback triangular and erect like saw teeth. The shoulder spines on 2 prominent ridges, spiracular folds absent, pectoral and pelvic fins high and angular. Caudal fin almost lunate with pre-ventral margin nearly as long as dorsal margin. Maximum total length at 270 cm, males adolescent between 157 - 178 cm and reaching at range 206 - 220 cm. Wide ranging of batoid in the tropical Indo-West Pacific. Mostly bottom dweller in coastal areas and inshore coral reefs. Feeding on bottom crustaceans including crab and mollusc. Caught by line gear or nets including shrimp trawls and marketed fresh for consumption. Very sharp spines and spiky, become problem for net gears, difficult to handle

and cause damage the whole fish catch. Distributed along the Indo-West Pacific, from Natal coast South Africa, Mozambique, East Africa, Seychelles, Red Sea, Arabia, Oman, the Persian Gulf, India, Sri Lanka, Malaysia, Indonesia (Borneo), Philippines, New Guinea, Thailand, Viet Nam, China, Taiwan Province of China, Korea, Japan, Australia from Western Australia to Northern Territory, Queensland and New South Wales [18,19]. Order RHINOBATIFORMES. Family: RHINIDAE or Rhynchobatidae. Taxonomic characters of large shark-like batoids, adults from 71 cm to over 3 m total length, with long stout tails and small pectoral discs. Tiny dermal denticles or placoid scales covering the body. Enlarged denticles or thorns present on dorsal surface on snout, orbits, dorsal and midline of trunk and tail. Body slightly flattened and shark-like and thick trunk. Pre-caudal tail moderately depressed, with lateral ridges on sides, tail not abruptly narrower than trunk, no barbed sting or stinging spine on dorsal surface of tail behind dorsal fins. Head narrow to broad and moderately depressed and snout short to moderately elongated, bluntly rounded or angular. Snout supported by a stout rostral cartilage and not formed into a rostral saw and without lateral saw teeth. Five small gill openings on underside of front half of pectoral-fin bases, not visible in lateral view, no gill sieves or rakers on internal gill slits. Eyes dorsolateral on head and just anterior to spiracles. Mouth more or less arched and with prominent knobs and depressions. Nostrils anterior to mouth and separated from it by less than their own widths. Always separate from each other and mouth; anterior nasal flaps short, not connected with each other and not reaching mouth. Oral teeth small to moderately large, rounded-oval in shape and without cusps on their crowns, not laterally expanded and plate-like, similar in shape and in 32 to 76 rows in either jaw. Pectoral fins small, originating in front of mouth but behind nostrils, attached to post-rostral head and ending anterior to pelvic-fin origins. No electric organs at bases of pectoral fins. Pelvic fins angular, not divided into anterior and posterior lobes. Two large subequal and widely separated dorsal fins present, these of similar falcate shark-like shape with distinct apices, anterior, posterior and inner margins and free rear tips. First dorsal fin originates over anterior half of total length, origin anterior to rear tips of pelvic fins and junction between trunk and tail. Caudal fin large, shark-like and asymmetrical, with vertebral axis raised above body axis, lower caudal-fin lobe present and large. Color of dorsal surface yellowish,

brownish, grey-brown, or greenish, white below. Dorsal surface with small to large white spots and sometimes dark blotches and dark ocelli on pectoral fins [18-20].

3.2 Morphometry

Morphometric measurement on the specimen collected from the fish auction hall of north Java coast have Total Length (TL): 162 cm, Standard Length (SL): 128 cm, wide of ventral fin: 66 cm and weight: 65 kg (Fig. 2). As comparison this shark TL can reach upto 270 cm at Cilacap fishing port south Java fishing port from the Indian ocean fishing operation. Complete morphometric measurements as presented in Table 1. Based on ratio of Body Width to Standard Length (SL): 0.77 was almost near to 1.0 meaning that Body depth to SL ratio has describe the specimen as a wide body and a depressed head type of fish. Based on shark catch data collected from Cirebon, Tegal and Rembang fishing port revealed that Total Length (TL) of *R. ancylostoma* in range of 76 – 200 cm, with most dominant TL was in the class category of 101 – 125 cm (Fig. 3). Mature male was found in the TL of 157 – 178 cm. Dominant TL was found in the range of 120 cm and least dominant TL was in the range of 76-100 cm and 151-175 cm [3,21].

Fundamentally, two pattern of sharks spatial distribution at Java sea, that is around west Java sea during west season but spread along west to east of Java sea during east season. Based on assumption that these sharks are bottom living species, then bathymetric or depth related distribution analysis on shark catch of *Rhina ancylostoma*, *Rhynchobatus djiddensis* (Junjuran shark) and *Rhinobatosschegellii* (Depo shark) was spatially distributed due to their ecological suitable depth. Sharks catch along Java sea was exclusively dominated by *R. schlegelii* and *R. djiddensis*. Highest monthly catch of *R. djiddensis* during west season (rainy season) was 7,440 kg and highest monthly catch 7,383 kg of *R. schlegelii* during east season (dry season). Catch of the three main sharks *R. ancylostoma*, *R. djiddensis* and *R. schegellii* (Ordo Rajiformes) at Java sea was distributed in the depth range of 11 – 167 m during east season. Specific highest catch was found at 167 m depth for *R. schegellii* and the east season catch was higher than west season. Depth distribution during west season at the depth range of 14 – 134 m and specifically around 47 m depth for *R. djiddensis*. Much lower catch

during west season because of heavy rain and high waves in the sea. Also noted that *R. djiddensis* catch was always found in every depth range [22]. The study also revealed that depth and sea bottom substrate which are mostly silt-clay had related to the sharks spatial distribution. Which is further to form as ecological-cluster for most of demersal fish such as sharks spatial distribution [23,24]. Monthly averages of sharks catch year 2018 at north Java sea highest catch in May was 20,617 kg and lowest catch in January with 2,122 kg and average yearly total catch of 72,480 Kg the catch of north Java sea is 26.08 percent compared to shark catch of south Java (Indian ocean) average yearly catch 2018 Cilacap fishing port south Java sea 1,877,844 kg with highest catch in September with 331,928 kg and lowest catch in November with 69,630 kg. About 16 family and 38 species of sharks catch had been recorded (Table 2). The most dominant of shark catch from Java sea was *Alopiassupercilliosus* with 33.43%, the smallest catch was *Sphyrnazygaena* with 0.01% while *R. ancylostoma* with 0.54% of the total yearly catch data [21].

DNA Amplification. Result on DNA amplification of isolate sample AH2 had exhibit of a single band with size of 700 base pair (bp) according to the comparison of DNA marker of the DNA electrophoresis and DNA sequencing as in Fig. 4. Band size was corresponding with fish DNA gene that commonly 700 length bases pair (Pringgenis and Susilowati, 2016). Analysis of Molecular Phylogenetic. Based on DNA sequencing as in Figs. 4 and 5, then result was followed with molecular phylogenetic analysis of sample AH2 based on neighbor joining method with 1000x bootstrap as presented in Fig. 6.

Result on sequencing of isolate AH2 sample, followed with further search in the DNA database GenBank using BLAST system on the [25] National Center for Biotechnology Information, National Institute for Health, USA (<http://www.ncbi.nlm.nih.gov>). Result search of AH2 isolate had confirmed homology on 100% gene 700 bp as mitochondria DNA Co1 and identified as *Rhina ancylostoma* with reference Access Number KU721838.1 (Table 1). Result of sequencing on sample AH2 as species of *Rhina ancylostoma* with Accession No LC505461 as in Tabel 2. The nearest genetic distance of sample AH2 to *Rhina ancylostoma* 0.02 and thus include with family of *Rhynchobatus horkellii* of 0.017 and *R. australiae*, while the longest distance to *Potamotrygon motoro* is 0.243 (Fig. 6 and Tables 3,4 and 5).

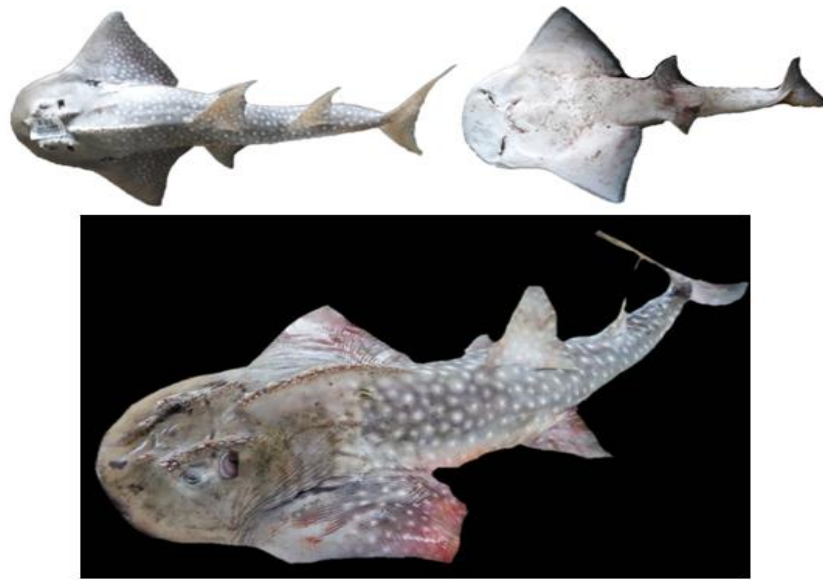


Fig. 2. Morphology (Dorsal and ventral) of specimen AH2 - *Rhina ancylostoma*

Table 1. Morphometric measurement of *R. ancylostoma*

Characters	%	Characters	%
Standard length (cm)	128	Head length (cm)	22
Body depth/SL	32/128 (0.25)	Body depth/HL	32/22 (1.45)
Body width/SL	98/128 (0.77)	Body width/HL	98/22 (4.45)
Head length/SL	22/128 (0.172)	Head length/HL	22/22 (1.0)
Snout length/SL	16/128 (0.125)	Snout length/HL	16/22 (0.73)
Eye diameter/SL	4.5/128 (0.035)	Eye diameter/HL	4.5/22 (0.205)
Predorsal length/SL	56.5/128 (0.441)	Predorsal length/HL	56.5/22 (2.57)
Prepelvic length/SL	76.5/128 (0.597)	Prepelvic length/HL	76.5/22 (3.48)
First dorsal base length/SL	12/128 (0.094)	First dorsal base length /HL	12/22 (0.55)
Second dorsal fin base length/SL	9.5/128 (0.074)	Second dorsal fin base length/HL	9.5/22 (0.43)
Anal base length /SL	52/128 (0.406)	Anal fin base length /HL	52/22 (2.36)
Caudal fin length /SL	8/128 (0.063)	Caudal fin length /HL	8/22 (0.36)
Pectoral fin length /SL	12.5/128 (0.098)	Pectoral fin length /HL	12.5/22 (0.57)

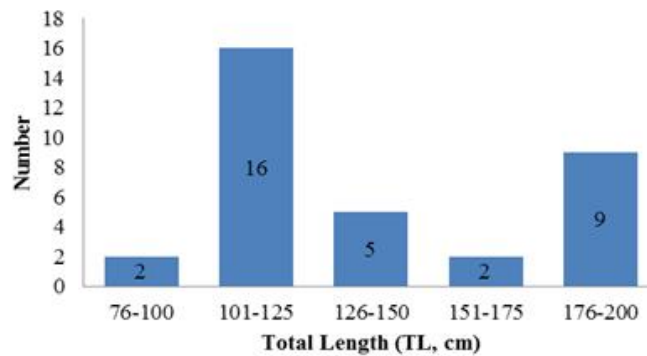


Fig. 3. Total length (TL, cm) variation *R. ancylostoma* catch from Java Sea

Table 2. Species of sharks catch from Java Sea 2011-2018

Ordo	Family	No.	Species	Common name	
Squaliformes	Squalidae	1.	<i>Squalus</i> sp.	Indonesian GreeneyeSpurdog	
Squatiformes	Squatinaidae	2.	<i>Squatina</i> sp.	Indonesian Angelshark	
Orectolobiformes	Hemiscyllidae	3.	<i>Chiloscyllium indicum</i>	Slender Bambooshark	
		4.	<i>Chiloscyllium plagiosum</i>	WhitespottedBambooshark	
		5.	<i>Chiloscyllium punctatum</i>	BrownbandedBambooshark	
	Rhincodontidae	6.	<i>Rhincodon typus</i>	Whale Shark	
		Stegostomatidae	7.	<i>Stegostoma fasciatum</i>	Zebra Shark
	Lamniformes	Alopiidae	8.	<i>Alopias pelagicus</i>	Pelagic Thresher
			9.	<i>Alopias superciliosus</i>	Bigeye Thresher
Lamnidae		10.	<i>Isurus oxyrinchus</i>	ShortfinMako	
		11.	<i>Isurus paucus</i>	LongfinMako	
Odontaspidae		12.	<i>Odontaspis ferox</i>	Sandtiger Shark	
Pseudocarchariidae		13.	<i>Pseudocarcharias kamoharai</i>	Crocodile Shark	
Carcharhiniformes	Carcharhinidae	14.	<i>Carcharhinus brevipinna</i>	Spinner Shark	
		15.	<i>Carcharhinus dussumieri</i>	Whitecheek Shark	
		16.	<i>Carcharhinus falciformis</i>	Silky Shark	
		17.	<i>Cacharhinus limbatus</i>	Common Blacktip Shark	
		18.	<i>Carcharhinus macloti</i>	Hardnose Shark	
		19.	<i>Carcharhinus plumbeus</i>	Sandbar Shark	
		20.	<i>Carcharhinus sealei</i>	Blackspot Shark	
		21.	<i>Carcharhinus sorrah</i>	Spot-tail Shark	
		22.	<i>Galeocerdo cuvier</i>	Tiger shark	
		23.	<i>Lamiopsis temmincki</i>	Broadfin Shark	
		24.	<i>Loxodon macrorhinus</i>	Sliteye Shark	
		25.	<i>Prionace glauca</i>	Blue Shark	
		26.	<i>Rhizoprionodon oligolinx</i>	Grey Sharpnose Shark	
		Hemigaleidae	27.	<i>Chaenogaleus macrostoma</i>	Hooktooth Shark
			28.	<i>Hemigaleus microstoma</i>	Sicklefin Weasel Shark
			29.	<i>Hemipristis elongata</i>	Fossil Shark
		Scyliorhinidae	30.	<i>Atelomycterus marmoratus</i>	Coral Catshark
			Sphyrnidae	31.	<i>Sphyrna lewini</i>
		32.		<i>Sphyrna mokarran</i>	Great Hammerhead

Ordo	Family	No.	Species	Common name
		33.	<i>Sphyrna zygaena</i>	Smooth Hammerhead
	Triakidae	34.	<i>Mustelus cf manazo</i>	Sparse-spotted Smoothhound
Rhinobatiformes	Rhinidae	35.	<i>Rhina ancylostoma</i>	Shark Ray
	Rhinobatidae	36.	<i>Rhinobatos thouin</i>	Clubnose Guitarfish
		37.	<i>Rhinobatos typus</i>	Giant Shovelnose Ray
	Rhynchobatidae	38.	<i>Rhynchobatus australie</i>	Whitespotted Guitarfish

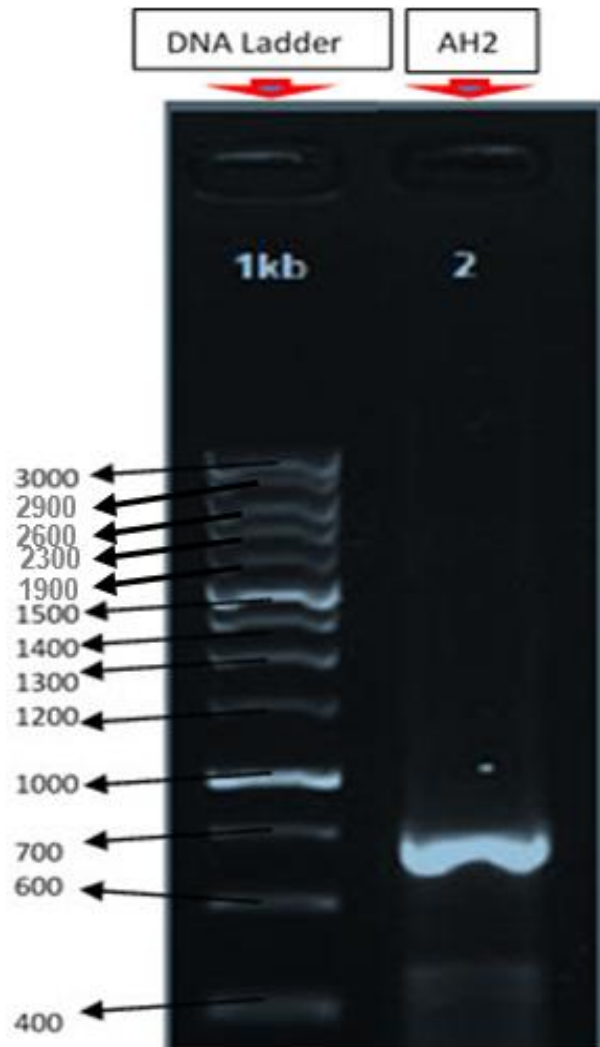


Fig. 4. DNA electrophoresis of AH2 sample

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GGCACCTTATATTTGATCTTTGGTGCTTGAGCAGGAATAGTTGGTACTGGTCTAAGTT
TACTTATTCGAACAGAGCTTAGCCAACCCGGAACACTTCTTGGAGATGACCAAATCTA
TAATGTAGTTGTAACAGCCCATGCCTTCGTAATGATTTTCTTCATAGTCATGCCAATC
ATAATTGGAGGGTTTGGTAATTGATTAATTCCTTTAATAATTGGCGCCCAGACATAG
CATTCCCACGAATAAATAATAAGCTTCTGATTGTTACCTCCATCATTCTTCTTTTA
TTAGCCTCTGCTGGAGTTGAAGCTGGAGTCGGAACAGGTTGAACTGTATACCCCCCA
CTTGCTGGTAACCTCGCCCATGCTGGAGCCTCCGTAGACTTAGCCATTTTTTCATTAC
ATTTAGCCGGGGTATCCTCTATCCTAGCATCCATTAATTTTATTACAACAATCATTAC
ATAAAACCCCAGCAATCTCTCAGTATCAGACACCTTTATTTGTGTGGTCAATTC TTG
TAACAACCGTTCTTCTACTACTCTCATTACCTGTACTAGCAGCGGGTATTACCATACT
CCTCACAGATCGAAATCTTAATACTACTTTCTTTGACCCAGCAGGAGGCGGGAGATCC
AATCCTTTATCAACATTTATTCTGATT
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Fig. 5. Result on DNA sequencing of sample AH2

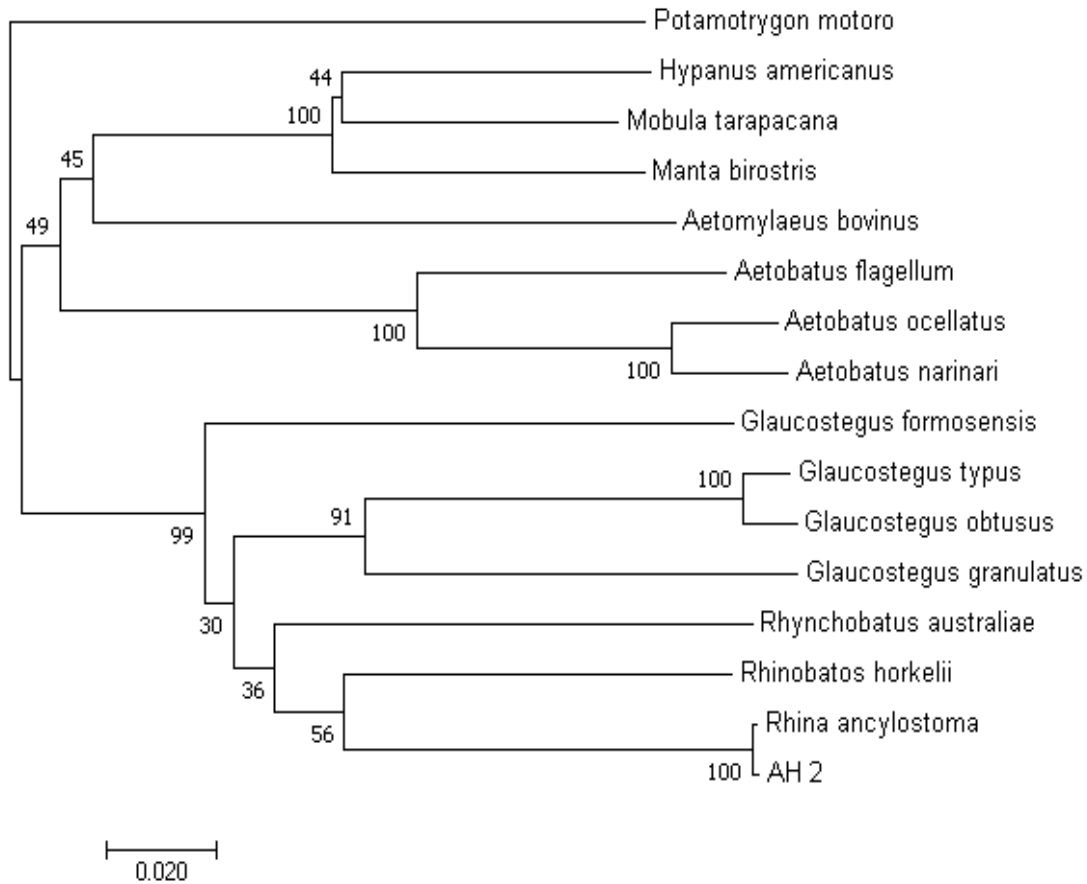


Fig. 6. Phylogenetic tree of sample DNA (AH2) confirmed species *Rhina ancylostoma*

Table 3. Homological match up result of sequence with Gen Bank database

No.	Original code	Species Identification (BLAST)	Access number	Length sequence (bp)	Ident similarity (%)	Query cover (%)
1.	AH2	<i>Rhina ancylostoma</i>	KU721837.1	665	99,84%	100%

Table 4. Sample accession number

No.	Original code	Species	Accession number
1.	AH2	<i>Rhina ancylostoma</i>	LC505461

The series of combination in line to species characterisation has been developed. Started with morphological character with its very distinctive three lines of spine thorn on the head. Morphometry was characterised with ratio of width of head to Standard Length (SL) is 0.77 as wide depressed head fish. The use of the right DNA solvent and fish primer lead to the category of the right for fish molecular weight of the DNA marker [26]. The

result of DNA based on phylogenetic tree had confirmed the nearest genetic distance of 0.02 to *Rhincobatus horkelii* and 0.017 to *R. australiae*. Longest genetic distance of 0.243 to *Potamotrygon motoro*. In comparison to reality on diversity of shark catch from Java sea are *Rhinobatos thouin*, *Rhinobatos typus* related group in the family, where *Rhincobatus horkelii* was not found in the catch from Java sea.

Table 5. Genetic distance of sample AH2 to other species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.Potamotrygon motoro		0.214	0.221	0.258	0.252	0.245	0.237	0.267	0.267	0.261	0.243	0.234	0.249	0.255	0.261	0.243
2.Hypanus americanus	0.021		0.107	0.235	0.243	0.259	0.117	0.272	0.277	0.245	0.264	0.209	0.251	0.246	0.222	0.262
3.Mobula tarapacana	0.022	0.013		0.227	0.251	0.230	0.107	0.261	0.263	0.240	0.239	0.217	0.250	0.248	0.239	0.233
4.Rhyncobatus australiae	0.023	0.022	0.021		0.174	0.171	0.244	0.203	0.193	0.182	0.200	0.233	0.272	0.292	0.284	0.173
5.Rhinobatos horkelii	0.023	0.023	0.023	0.018		0.147	0.230	0.195	0.197	0.211	0.164	0.253	0.283	0.277	0.267	0.145
6.Rhina ancylostoma	0.023	0.024	0.021	0.018	0.017		0.249	0.201	0.203	0.199	0.218	0.227	0.280	0.293	0.274	0.002
7.Manta birostris	0.023	0.015	0.014	0.022	0.022	0.023		0.259	0.271	0.264	0.252	0.199	0.240	0.235	0.226	0.251
8.Glaucostegus typus	0.024	0.024	0.023	0.020	0.018	0.019	0.022		0.018	0.151	0.197	0.275	0.261	0.257	0.244	0.199
9.Glaucostegus obtusus	0.024	0.025	0.023	0.020	0.018	0.020	0.023	0.005		0.164	0.187	0.278	0.251	0.252	0.244	0.201
10.Glaucostegus granulatus	0.023	0.021	0.021	0.018	0.020	0.019	0.023	0.018	0.018		0.220	0.266	0.271	0.267	0.252	0.201
11.Glaucostegus formosensis	0.022	0.024	0.022	0.021	0.018	0.021	0.024	0.020	0.019	0.021		0.279	0.259	0.259	0.254	0.216
12.Aetomylaeus bovinus	0.023	0.021	0.021	0.022	0.024	0.023	0.021	0.025	0.025	0.024	0.025		0.251	0.239	0.228	0.230
13.Aetobatus ocellatus	0.023	0.023	0.023	0.023	0.025	0.024	0.023	0.023	0.023	0.023	0.024	0.023		0.041	0.116	0.283
14.Aetobatus narinari	0.023	0.023	0.023	0.025	0.024	0.026	0.023	0.022	0.022	0.023	0.024	0.023	0.008		0.130	0.296
15.Aetobatus flagellum	0.024	0.021	0.022	0.025	0.023	0.025	0.023	0.021	0.021	0.023	0.023	0.023	0.014	0.015		0.272
16.AH-2	0.022	0.024	0.021	0.018	0.016	0.002	0.023	0.019	0.020	0.020	0.021	0.023	0.024	0.026	0.025	

4. CONCLUSION

Rhina ancylostoma is one of demersal shark rays catch from Java Sea. Range of Total Length (TL) of catch 73 – 200 cm with dominant TL fish catch 125 cm. Morphological character with three lines of spine thorn on the head, morphometry with wide head ratio to SL 0.77 as wide depressed head. DNA analysis had confirmed the specimen of AH2 as *Rhina ancylostoma* based on homological match up of sequence of GenBank database with reference accession number KU721837.1 with length sequence of 665 bp identical similarity of 99.84 % for specimen Accession number LC 505461. Nearest genetic distance of 0.02 to *Rhincobatushorkelii* and 0.017 to *R. australiae*. Longest genetic distance of 0.243 to *Potamotrygon motoro*.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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