



## Close genetic relatedness of whale sharks, *Rhincodon typus* in the Indo-Pacific region

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**Abstract.** The goal of our study was to evaluate genetic diversity of whale sharks (*Rhincodon typus*) observed in Cenderawasih Bay, Indonesia, compared with that of whale sharks elsewhere in the Indo-Pacific region using mitochondrial DNA. We collected tissue samples from 31 whale sharks, virtually all adolescent males, in Cenderawasih Bay and then extracted, amplified, and sequenced mitochondrial DNA from the cytochrome oxidase I (COI) gene of the mtDNA COI gene. Genetic diversity of whale sharks in Cenderawasih Bay was low. We detected seven haplotypes and estimated haplotype diversity at 0.187 and nucleotide diversity at 0.002. The average number of pairwise differences was 0.254. These data suggest that the whale sharks that we encountered in Cenderawasih Bay were closely related and less related to whale sharks elsewhere in the Indian Ocean and western Pacific Ocean.

**Key Words:** *Rhincodon typus*, Cenderawasih Bay, haplotype diversity, nucleotide diversity, Coral Triangle.

**Introduction.** Cenderawasih Bay (also known as Teluk Cenderawasih) is located on the north side of Papua and West Papua, Indonesia. It is the largest bay in Indonesia and a major priority for conservation of marine resources owing to its rich and diverse marine life, including at least 500 species of corals, more than 1,000 species of fish, and a variety of invertebrates, turtles, and marine mammals (Allen & Erdmann 2009). Commercial fishing, including illegal and unauthorized activities, and harvesting of coral for various purposes has degraded many habitats and reduced some species from overharvesting (Mangubhai et al 2012).

The whale shark (*Rhincodon typus*) is a prominent species in Cenderawasih Bay (Stewart 2011; Hoegh-Guldberg et al 2009). Its recent association with moored lift-net fisheries there has led to an increase in tourism that facilitates encounters for human snorkelers and SCUBA divers with the sharks (Stewart 2011). This association has also supported opportunities for research to discover various elements of their biology, ecology, movements and genetics.

An understanding of the genetic composition of the sharks in Cenderawasih Bay and their relationships to whale sharks elsewhere in the Indian Ocean and Western Pacific Ocean is important for their conservation and to help design marine protected areas. Castro et al (2007) reported on patterns of global genetic diversity in whale sharks using mitochondrial DNA loci but that study did not include samples from Indonesian waters. Our goal was to specifically assess the molecular genetics of whale sharks in Cenderawasih Bay relative to the results of studies conducted elsewhere in the Indian Ocean and the Western Pacific Ocean using the cytochrome oxidase I (COI) gene of mtDNA.

## Material and Method

**Sample collection.** We collected skin samples from whale sharks in Cenderawasih Bay (Figure 1) in November 2012 (14 samples), April 2013 (2 samples), and June 2013 (15 samples) with modified hog ear notch pliers and small biopsy tips. The samples were preserved in 96% ethanol and stored at 4°C in the laboratory until DNA extraction.



Figure 1. The location of Cenderawasih Bay (black triangle) in West Papua Province, Indonesia.

**DNA extraction, PCR, sequencing.** We extracted mitochondrial DNA (mtDNA) from the samples using Chelex 5-10% (Bioradm Hercules, CA; Walsh et al 1991) and then amplified the cytochrome oxidase I (COI) gene using the Hot-start and Gold program (Saiki et al 1988) with the modified protocol of Barber & Erdmann (2000) and the primer FISH-BCH (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and primer FISH-BCL (5'-TCA ACY AAT CAY AAA GAT ATY GGC AC-3; Baldwin et al 2009). We visualized the amplified products by electrophoresis with agarose gel 1% (b/v) following Davis (1964), purified using *Shrimp Alkaline Phosphatase* (Amersham Biosciences Corporation, Arlington Heights, Illinois, USA) and *Exonuclease* (Amersham; SAP/EXO). We sequenced COI mtDNA using Big Dye® terminator chemistry (Perkin Elmer). Sequenced products were purified using isopropanol precipitation and visualized using the automatic sequencer ABI377 (Applied Biosystems).

**Data analysis.** We edited the COI gene sequences with automatic sequencer ABI 377 (Applied Biosystems) and Mega5 (Tamura et al 2011). The nucleotide composition was analysed using Mega5 (Tamura et al 2011). We used the program DnaSP 5.1001 (Librado & Rozas 2009) to assess genetic diversity (polymorphisms, haplotypes, and nucleotides). We compared the results from whale sharks in Cenderawasih Bay with those available in GenBank for nearby areas of the Indian and Western Pacific oceans with the neighbor-joining method (Saitou & Nei 1987) and bootstrap test 1000 times (Felsenstein 1985) using Mega5 (Tamura et al 2011). The analysis was done with representatives of Indo-Pacific region of whale shark sequences available in GenBank (Table 1). Evolutionary distances were calculated using the LogDet method (Tamura & Kumar 2002) and haplotype network with the Network 4.6.

Table 1

The whale shark (*Rhincodon typus*) sequences available in GenBank from representatives of Indo-Pacific region, with the accession number

<i>Location</i>	<i>Accession number</i>	<i>Reference</i>
Seychelles	FJ519244	Wong et al (2009)
India	FJ456922	Sajeela et al (2010)
India	FJ375725	Benson et al (2009)
Taiwan	NC_023455	Alam et al (2013)
Taiwan	FJ519250, FJ519251	Wong et al (2009)
Taiwan	KF679782	Alam et al (2013)
Taiwan	EU398993	Ward et al (2008)
Philippines	GU440502	Benson et al (2009)
South Africa	FJ519247	Wong et al (2009)
South Africa	HQ945887, HQ945888, HQ945889	Benson et al (2009)
Western Australia	FJ519248, FJ519249	Wong et al (2009)
Mozambique	FJ519245, FJ519246	Wong et al (2009)

## Results and Discussion

**Genetic characteristics.** The fragment length of the COI gene in 32 whale sharks from Cenderawasih Bay ranged from 382 to 731 base pairs (bp). All samples identified in Genbank as, *Rhincodon typus* (Smith 1828) had a similarity percentage of 99-100%. The mean nucleotide composition was C: 22.27%; T: 32.28%; A: 29.61%; G: 15.83%. The changes of mtDNA COI gene nucleotide in Cenderawasih Bay were relatively small and slow, shown by pairing and segregation differences (Figures 2 and 3). Under constant conditions, a mismatch distribution is not significantly different between observation and expectation. A genetic lineage estimation indicates sequence data to be 0.00004 for DeltaSt, 0.01659 for GammaSt, and 14.82 for Nm.

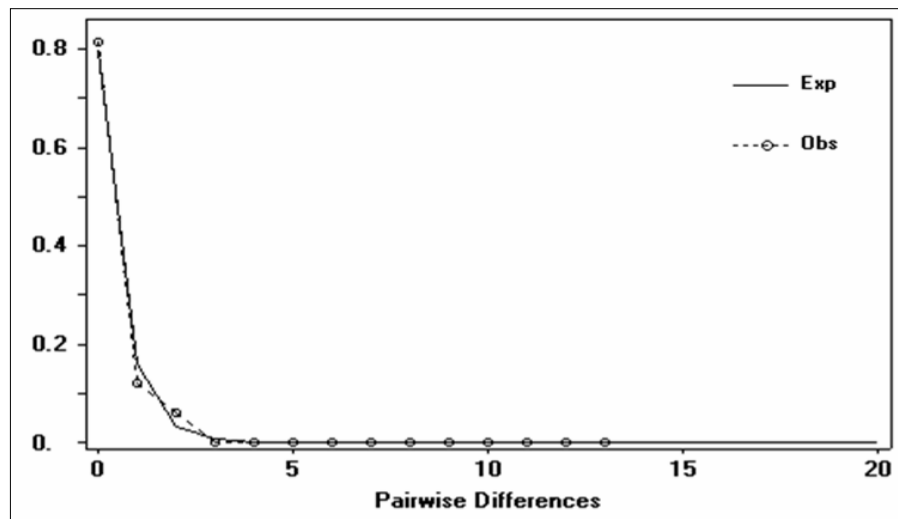


Figure 2. Nucleotide change with pairwise differences variation in constant population.

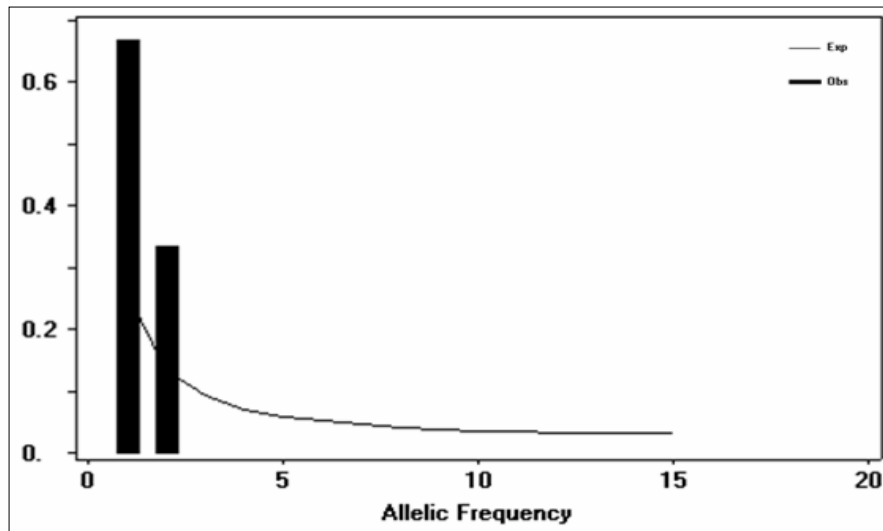


Figure 3. Nucleotide changes with allelic frequency variation in constant population.

**Genetic diversity and relatedness.** We detected seven haplotypes among the 31 whale sharks sampled in Cenderawasih Bay for a haplotype diversity ( $H_d$ ) of 0.1871 (variance = 0.00859, s.d. = 0.093). The nucleotide diversity ( $\pi$ ) was 0.00244 ( $\pm 0.0013$ ) and the values for theta were 0.75094 per shark and 0.00722 per site.

The neighbour-joining tree for individual whale sharks from Cenderewasih Bay and other regions are shown in Figure 4. All 31 whale sharks that we sampled in Cenderawasih Bay appeared to be closely related to each other. They also appeared to be closely related to whale sharks sampled elsewhere in the Indian and Western Pacific oceans (Figure 5; Chi-square,  $\chi^2$ : 3.620, df: 5, p: 0.605). Haplotype one has highest frequencies (37 individuals), one haplotype has two individuals, and six other haplotypes has each one individual. The three last haplotypes has just one individual, respectively.

The whale sharks that we sampled in Cenderawasih Bay had low genetic diversity (haplotype, nucleotide and polymorphic), similar to that reported for whale sharks in other areas (Castro et al 2007; Schmidt et al 2009). Sharks sampled from other nearby areas similarly had low genetic diversity indicating some degree of connection within this regional group though still distinct from whale sharks sampled in the Atlantic ocean (Castro et al 2007). Sakai et al (2001) argued that genetic diversity reflects the capacity of a population to adapt to changes in environmental conditions. It might also have a role in the invasive potential of a population or species (Drake & Lodge 2006; Lavergne & Molofsky 2007). Some have also suggested that populations or species with lower genetic diversity might be more susceptible to disease, pollution, or changes in habitat or climate (Spielman et al 2004; Hauser et al 2002) though there are a number of examples that challenge that hypothesis.

The distribution of haplotypes among whale sharks in Cenderawasih Bay and nearby areas of the Indian Ocean and Western Pacific Ocean indicates that they are all relatively closely related and that none of the sampled areas are genetically isolated from the others. Other ecological study (Himawan et al 2015) has suggested that individuals in Cenderawasih Bay National Park were dominated by males categorized as juveniles (3-6 m), suggests the area serves as important habitat for young whale sharks, which is in turn important to preserve their genetic diversity.

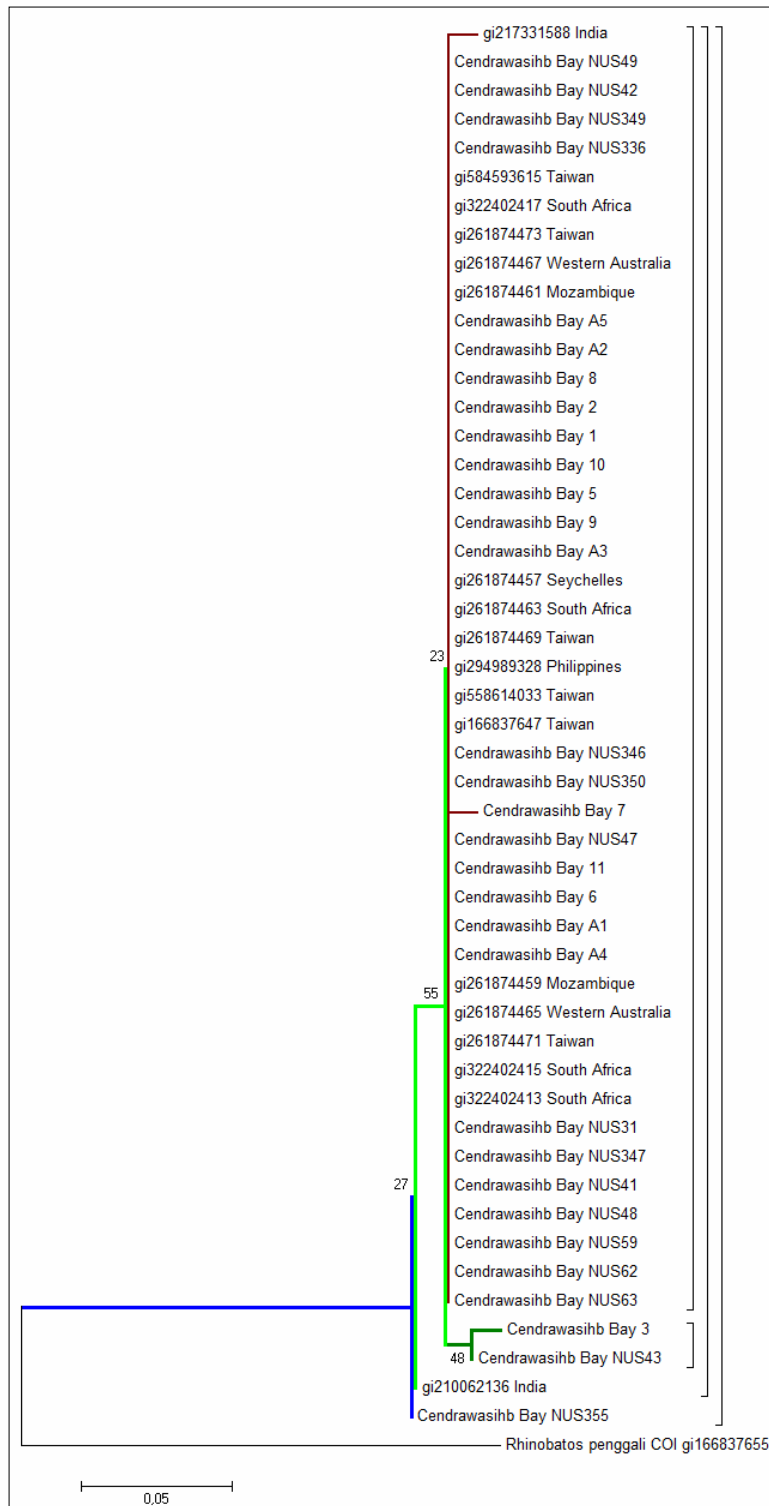


Figure 4. The Neighbour-joining tree on whale shark individual from Cenderwasih Bay and other region, based on COI sequence data using Kimura-two-parameter substitution model with 1000 bootstrap.

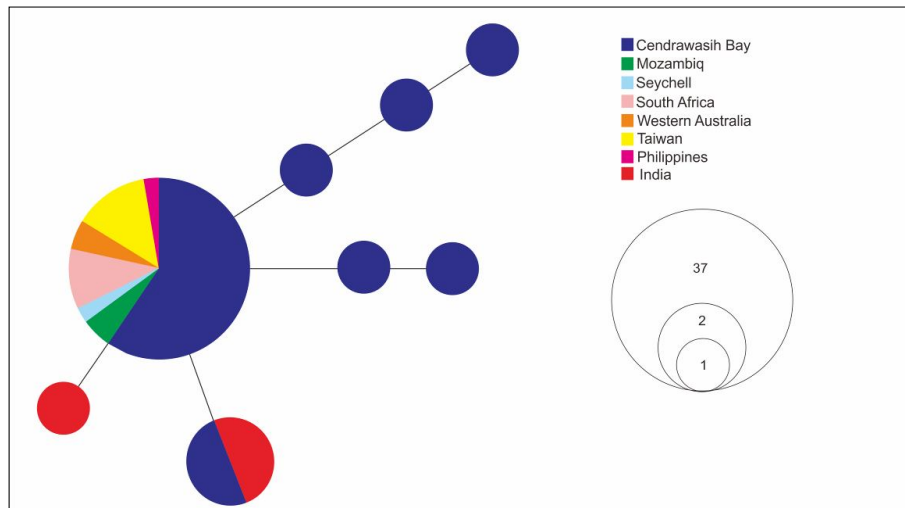


Figure 5. The haplotype network of whale shark individuals within Indo-Pacific region. Circle represents kinds of haplotype with their different frequencies. Cenderawasih Bay consists of seven haplotypes. The color of the fill for each haplotype is indicative of their specific site.

**Conclusions.** We found that the 31 whale sharks that we sampled in Cenderawasih Bay were very closely related and that these sharks were closely related to whale sharks elsewhere in the Indian and Western Pacific oceans. The extent of movements among reproductive sharks in this large region is unknown as is the frequency and magnitude of mating among sharks from these different areas. Further research is needed to monitor the long-term movements of sharks within and among regions, particularly reproductively mature males and females to help design conservation plans for whale sharks and the habitats that they might depend on for feeding and reproducing.

**Acknowledgements.** The research was a collaborative study with MB-RAI-Universitas Negeri Papua, Laboratory of Marine Biodiversity and Biosystematics Bogor Agricultural University, WWF-Indonesia, and Hubbs-SeaWorld Research Institute. We thank Balai Taman Nasional Teluk Cenderawasih for its help in issuing a research permit for collection of skin samples, Marine Biodiversity of Raja Ampat Islands (sponsor USAID-NSF program PEER), IBRC-Bali for sending PCR products to the Cornell University sequencing facility, and Andri Kuncoro, Andika Ariwibowo and Laboratorium Genetika UNIPA for their generous help in the laboratory.

## References

- Alam M. T., Petit R. A. III, Read T. D., Dove A. D. M., 2013 The complete mitochondrial genome sequence of the world's largest fish, the whale shark (*Rhincodon typus*), and its comparison with those of related shark species. *Gene* 539(1):44-49.
- Allen G. R., Erdmann M. V., 2009 Reef fishes of the Bird's Head Peninsula, West Papua, Indonesia. *Check List* 5:587-628.
- Baldwin C. C., Weigt L. A., Smith D. G., Mounts J. H., 2009 Reconciling genetic lineages with species in Western Atlantic *Coryphopterus* (Teleostei: Gobiidae). *Smithsonian Contributions to the Marine Sciences* 38:111-138.
- Barber P. H., Erdmann M. V., 2000 Molecular systematics of the Gonodactylidae (Stomatopoda) using mitochondrial cytochrome oxidase C (subunit 1) DNA sequence data. *Journal of Crustacean Biology* 20:20-36.
- Benson D. A., Karsch-Mizrachi I., Lipman D. J., Ostell J., Sayers E. W., 2009 GenBank. *Nucleic Acids Research* 37:26-31.
- Castro A. L., Stewart B. S., Wilson S. G., Hueter R. E., Meekan M. G., Motta P. J., Bowen B. W., Karl S. A., 2007 Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular Ecology* 16:5183-5192.

- Davis B. J., 1964 Disc electrophoresis-II. Method and application to human serum proteins. *Annals of the New York Academy of Sciences* 121:404-427.
- Drake J. M., Lodge D. M., 2006 Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. *Biological Invasions* 8:365–375.
- Felsenstein J., 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Hauser L., Adcock G. J., Smith P. J., Bernal Ramírez J. H., Carvalho G. R., 2002 Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the USA* 99:11742-11747.
- Himawan M. R., Tania C., Noor B. A., Wijonarno A., Subhan B., Madduppa H., 2015 Sex and size range composition of whale shark (*Rhincodon typus*) and their sighting behaviour in relation with fishermen lift-net within Cenderawasih Bay National Park, Indonesia. *AACL Bioflux* 8(2):123-133.
- Hoegh-Guldberg O., Hoegh-Guldberg H., Veron J. E. N., Green A., Gomez E. D., Lough J., King M., Ambariyanto H., Hansen L., Cinner J., Dews G., Russ G., Schuttenberg H. Z., Penaflor E. L., Eakin C. M., Christensen T. R. L., Abbey M., Areki F., Kosaka R. A., Tewfik A., Oliver J., 2009 The Coral Triangle and climate change: ecosystems, people and societies at risk. WWF Australia, Brisbane, 276 pp.
- Lavergne S., Molofsky J., 2007 Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the USA* 104:3883–3888.
- Librado P., Rozas J., 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451-1452.
- Mangubhai S., Erdmann M. V., Wilson J. R., Huffard C. L., Ballamu F., Hidayat N. I., Hitipeuw C., Lazuardi M. E., Muhajir, Pada D., Purba G., Rotinsulu C., Rumetna L., Sumolang K., Wen W., 2012 Papuan Bird's Head SEASCAPE: emerging threats and challenges in the global center of marine biodiversity. *Marine Pollution Bulletin* 64(11):2279-2295.
- Saiki R. K., Gelfand D. H., Stoffel S., Scharf S. J., Higuchi R., Horn G. T., Mullis K. B., Erlich H. A., 1988 Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.
- Saitou N., Nei M., 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.
- Sajeela K. A., Rakhee C., Rekha J. N., Gopalakrishnan A., Basheer V. S., Shoba J. K., Joe K., Lakra W. S., 2010 Mitochondrial DNA sequences for forensic identification of the endangered whale shark, *Rhincodon typus* (Smith, 1828): a case study. *Tools for Identifying Biodiversity: Progress and Problems*, pp. 353-354.
- Sakai A. K., Allendorf F. W., Holt J. S., Lodge D. M., Molofsky J., With K. A., Baughman S., Cabin R. J., Cohen J. E., Ellstrand N. C., McCauley D. E., O'Neil P., Parker I. M., Thompson J. N., Weller S. G., 2001 The population biology of invasive species. *Annual Review of Ecology and Systematics* 32:305–332.
- Schmidt J. V., Schmidt C. L., Ozer F., Ernst R. E., Feldheim K. A., Ashley M. V., Levine M., 2009 Low genetic differentiation across three major ocean populations of the whale shark, *Rhincodon typus*. *PLoS ONE* 4:e4988.
- Spielman D., Brook B. W., Frankham R., 2004 Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the USA* 101:15261–15264.
- Stewart B. S., 2011 Workshop and monitoring training for whale sharks in Cendrawasih Bay National Park, West Papua. 2–7 May 2011, Nabire, Papua. Hubbs-SeaWorld Research Institute Technical Report 2011-375:1-27.
- Tamura K., Kumar S., 2002 Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Molecular Biology and Evolution* 19:1727-1736.

- Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10):2731–2739.
- Walsh P. S., Metzger D. A., Higuchi R., 1991 Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques* 10:506–513.
- Ward R. D., Holmes B. H., White W. T., Last P. R., 2008 DNA Barcoding Australian Chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research* 59:57-71.
- Wong E. H. K., Shivji M. S., Hanner R. H., 2009 Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach. *Molecular Ecology Resources* 9:243-256.

Received: 22 December 2015. Accepted: 13 February 2016. Published online: 17 May 2016.

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How to cite this article:

Toha A. H., Widodo N., Subhan B., Himawan M. R., Tania C., Noor B. A., Stewart B. S., Madduppa H. H., 2016 Close genetic relatedness of whale sharks, *Rhincodon typus* in the Indo-Pacific region. *AAFL Bioflux* 9(3): 458-465.