Sequence variation among populations of sawfishes (Pristiformes: Pristidae) from Indonesia and Australia

SUTARNO1*, A. BUDIHARJO1, A.D. SETYAWAN1, A.J. LYMBERY2

1Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia. Tel./Fax. +62-271-663375, *email: nsutarno@yahoo.com, volatileoils@gmail.com
2Fish Health Unit, School of Veterinary and Biomedical Sciences, Murdoch University. Murdoch 6150, West Australia, Australia

Manuscript received: 5 January 2016. Revision accepted: 30 April 2017.

Abstract. Satarno, Budiharjo A, Setyawan AD, Lymbery AJ. 2017. Sequence variation among populations of sawfishes (Pristiformes: Pristidae) from Indonesia and Australia. Biodiversitas 18: 850-856. The sawfishes (Pristiformes: Pristidae) are very rare and critically endangered species globally, calling for conservation efforts around the world. This species is taxonomically interesting because molecular research in recent years has led to re-groupings in some species. The aim of this research was to sequence the control region (CR) of mitochondrial DNA of sawfishes from Indonesia and Australia, compare the sequences between samples, and construct phylogenetic trees to know the relationship of those samples. To achieve these aims, dried rostra of samples from both countries are collected, total DNA were purified and amplified using PCR, followed by sequencing reaction. The sequence data were then lined followed by constructing phylogenetic trees. The results of the BLASTN and phylogenetic tree analysis found two species, the all Australian samples belong to Pristis pristis (formerly P. microdon), one Indonesian sample also belongs to P. pristis, while the other Indonesian samples belong to Anoxypristis cuspidata.

Keywords: Anoxypristis cuspidata, mitochondrial DNA, Pristis pristis (P. microdon), sawfishes

INTRODUCTION

Sawfishes (Pristiformes: Pristidae) are a small group of elasmobranch large shark-like batoid of the order Pristiformes, and the unique family of Pristidae (Compagno and Last 1999; Sutarno et al. 2012). The taxonomy of sawfishes is confusing (c.f. Sutarno et al. 2012), but refer to current molecular and morphological studies there are five species of sawfishes from two genera, Pristis and Anoxypristis (Last et al. 2016). Faria et al. (2013) suggest that based on a combination of mitochondrial DNA and morphological characters, the three previously known species (Pristis pristis, P. microdon and P. perotteti) appear to be one species, Pristis pristis.

The all five species of sawfishes are found in the Indo-West Pacific, where Anoxypristis cuspidata (Latham, 1794), Pristis pristis (Linnaeus, 1758) (formerly known as Pristis microdon Latham, 1794), and Pristis zijsron (Bleeker, 1851) has widespread distribution; and Pristis clavata (Garman 1906) appears to be limited to northern Australia (Compagno and Last 1999; Last and Stevens 2009; Phillips et al 2011); whereas, Pristis pectinata (Latham, 1794) reportedly originated from the Red Sea and East Africa to the Philippines (Compagno and Last 1999; Last and Stevens 2009; Phillips et al. 2011). In the East-Pacific Atlantic are found two species, including P. pristis and P. pectinata (Compagno and Last 1999; Last and Stevens 2009; Faria et al. 2013). In Southeast Asian waters, these five species of sawfishes can be recorded, including Pristis pristis (P. microdon), P. clavata, P. zijsron, P. pectinata and Anoxypristis cuspidata (Sutarno et al. 2012).

Sawfishes are characterized by a distinctive flattened, greatly elongated rostrum armed on each side with a row of the large transverse teeth, presumably used for hunting and defense (Bigelow and Schroeder 1953; Wueringer et al. 2009; Sutarno et al. 2012). The rostral teeth grow continuously from the base and attach to the rostrum through the alveoli (Slaughter and Springer 1968; Compagno and Last 1999). The peduncle is not expanded and the dentine cap is easily removed (Slaughter and Springer 1968). The maximum total length is around 7 m (Last and Stevens 1994).

Sawfishes usually inhabit shallow inner shelf and coastal habitat, in tropical and subtropical waters including estuarine and freshwater habitats (Nelson 2006; Wueringer et al. 2009; Waters et al. 2014; Hollenese et al. 2015), as well as marine environments to a maximum depth of 122 m (McEachran and de Carvalho 2002; Simpfendorfer 2006). While presenting a wide spectrum of salinity, sawfish prefer coastal marine and estuarine habitats (Peverell 2005; Whitty et al. 2009; Norton et al. 2012). They can migrate from euryhaline, brackish and freshwaters habitats, as a possible behavioral mechanism to cleanse the body of undesirable parasites (Morgan et al. 2010).

Sawfishes have been important as a source of food and medicines as well as religious and cultural symbols (Gonzalez MMB. 2005; Robillard and Sere 2006; Clarke et al. 2007). The pressure from commercial and recreational fishing, the loss of near-shore habitat due to development, and low reproductive potential lead to a decrease in population (Thorson 1982; Simpfendorfer 2000; Seitz and Pouikis 2006; Carlson and Simpfendorfer...
Nowadays, sawfishes are among the most threatened marine fishes, with declining numbers and reduced distributions worldwide (Wuringer et al. 2009; Simpfendorfer 2005; Faria et al. 2013). All five species face a very high risk of global extinction (Dulvy et al. 2014). The decline has been observed in all parts of their distribution range (Leeney and Poncelet 2013; Moore 2014; Fernandez-Carvalho et al. 2014). Sawfishes are highly sensitive to exploitation and habitat destruction due to their large size and their coastal and riverine habitats (Robillard and Séret 2006).

All five sawfishes species have now been listed as endangered or critically endangered species by the International Union for Conservation of Nature (Carlson et al. 2013, D’Anastasi et al. 2013; Kyne et al. 2013a, 2013b; Simpfendorfer 2013; IUCN 2017). They have also been listed in Appendix I of CITES, which prohibits their international trade (CITES 2007). In Indonesia, populations of sawfishes have declined severely, with recent surveys of several sites where the species has historically been recorded, including Lake Sentani and the Mahakam River, finding no individuals. Legislation has recently been enacted to protect all member of Pistis genus (as well as Anoxypristis) in Indonesia, where overfishing and habitat destruction has severely reduced population numbers (PP 7/1999). Population declines in northern Australia have not been as severe, although the species is still listed as vulnerable under the Australian Environmental Protection and Biodiversity Conservation Act 1999 (EPBC 1999).

Genetic studies are essential for the effective conservation of sawfishes, because (i) there is confusion over the taxonomic status of sawfishes (Faria et al. 2013; Sutarno et al. 2012), and (ii) population genetic data can be used provide information on current and historical population sizes and migration rates, which determine how the species should be managed. Molecular genetic studies using mitochondrial DNA sequencing analysis was used in the research. Skin tissue from dried rosta of sawfishes was obtained from collections and purchased from individuals and markets in Indonesia. This means that genetic data were obtained using previously collected samples, without the need to obtain further specimens of this highly endangered species to determine the relationship and taxonomic status of Australian and Indonesian populations of sawfishes.

The aim of this research was to sequence the control region (CR) of mitochondrial DNA of sawfishes from Indonesia and Australia, compare the sequences between samples, and construct phylogenetic trees to know the relationship of those samples.

**MATERIALS AND METHODS**

**Sampling**

Tissue samples were obtained from dried rostra sourced from the museum, university and private collections in Indonesia and Australia, and purchased from markets throughout Indonesia (from a five-year survey, 2011-2015). Fifteen samples from Australian collection (DNA material stored in the Fish Health Unit, Murdoch University, Australia) and seven samples from Indonesian collection (rostra stored at the Department of Biology, Universitas Sebelas Maret, Surakarta, Indonesia; this is part of 24 samples collected over five years of field research) were used in this study. A total of 22 samples were assessed. Sampling from dried rostra obviates the need to catch and obtain tissue samples of these rare and threatened fishes.

**DNA extraction**

Total genomic DNA will be extracted from tissue obtained from dried rostra using a Masterpure™ (Epicentre Technologies, Sydney) DNA extraction kit, and following the procedures of Phillips (2006) and Phillips et al. (2009). To minimize the risk of contamination with non-target DNA, all genetic work involving dry rostra samples will be carried out referring to the protocols for working with ancient DNA set out by Mulligan (2005). The quantity and quality of the extracted DNA will be assessed through the appearance of a 2 µL aliquot of the extract on a 2% agarose gel subject to electrophoresis for 25 min at 46 mAmps, stained with ethidium bromide, and illuminated with UV light.

**mtDNA sequencing**

Polymerase chain reaction (PCR) will be used to amplify a 353-351-bp portion of the control region of the mtDNA of sawfishes using the forward primer (CRF: 5‘-ACGTATCCGTAATACCTAT and reverse primer (CRR: 5‘-ATGCAATAATTATATGTCGAGG), as described by Phillips et al. (2008). The PCR amplification will be carried out in a reaction mixture containing about 10 ng of DNA template, 10 mM Taq buffer with 1.5 mM MgCl2 (Roche), 0.1 mM of each of the dNTPs (Promega), 0.5 U of Taq polymerase (Roche), 20 µmol of each primer, and adjusted to the final volume of 50 µL with PCR-grade water. The amplification conditions will consist of an initial 5 min denaturation phase at 94°C, followed by 35 cycles, each cycle consisting of 30 sec of denaturation at 94°C, 30 sec of annealing at 59°C, and 30 sec of extension at 72°C; followed by a final 7 min extension phase at 72°C.

Prior to sequencing, the PCR products will be cleaned by using Qiaquick columns (Qiagen), referring to the manufacturer’s protocol. The sequencing will be carried out by using the dye terminator cycle sequencing method. Each sequencing reaction will be prepared using approximately 30 ng of clean PCR product, 3.2 pmol of the forward or reverse primer and a Big Dye 3.1 terminator cycle sequencing ready reaction kit following the manufacturer’s protocol (Applied Biosystems Inc. 2001). The sequencing products will be electrophoresed, and the raw data chromatograms generated using an Applied Biosystems 3230 DNA Analyzer automated sequencer.

**mtDNA data analysis**

The forward and reverse sequences of the mitochondrial control region will be aligned using GeneTool™ Lite v1.0, the primer sequences removed from both ends and a forward reading consensus sequence generated. Tajima’s
The results of the sequencing reaction to 22 samples from Indonesia and Australia have then checked the similarity with BLASTN data, and the results are presented in Table 1.

The similarity percentage between CR region of mtDNA of Indonesian and Australian sawfishes after comparing it to the central data of GeneBank using BLASTN indicated that there are two species of the sawfishes found in Indonesia, P. pristis and A. cuspidata. The P. pristis was found from Samarinda, a region in Kalimantan (Borneo) island, while A. cuspidata was found from Merauke in Papua island. Unfortunately, the sample of Samarinda was only found 1 sample from this island due to the species was facing extinction since couple years ago. The sawfishes samples from Australia all belong to P. pristis. The sequences resulted were analyzed using the program MEGA 5.10 to construct the phylogenetic tree, and the result is shown in Figure 1.

The phylogenetic trees presented in Figure 1 indicated the relationship between the population of P. pristis from Samarinda and Australia. The Pristis of Samarinda population is at the same taxonomic group of most populations from Australia, indicating that the Samarinda populations have high sequence similarity compared to most Australian populations. While samples of sawfishes from Merauke in Papua island are all indicating taxonomic different, and it belongs to species of A. cuspidata.

Similarity analysis using BLASTN between the sequence of CR region of Sawfishes from Indonesia and Australia to the central data of GeneBank indicated that there are variations found in those samples both from Indonesia and Australia. Analysis of phylogenetic trees to find out the relationship between those samples indicated that one of Indonesian Pristis (from Samarinda) is closely related to PM1023, PM10220, PM3196, PM1027 and Flyma03; the other samples from Merauke belongs to different species of origin compared to any other samples studied, A. cuspidata.

**Conservation effort**

The decline and extinction of sawfishes have been reported or found from across the Indonesian archipelago. This is evident from five years of field and market surveys (2011-2015) in various regions of Indonesia, where part of the sample is used in this study. The field research and market surveys cover the western coast of Sumatra and the Mentawai Islands, the northern coast of Sumatra (Aceh), the eastern coast of Sumatra (Jambi), the western coast of Kalimantan and the mouth of Kapuas and Peniti rivers, the southern coast of Kalimantan and the mouth of the Barito River; the Mahakam River estuary in East Kalimantan, the coastal areas of Manado (Sulawesi), the coastal areas of Sorong and Manokwari (west of Papua), the northern coast of Papua and Lake Sentani, and the river estuaries and coastal areas of Merauke, southern Papua, and several Fish Auction Places (TPI) in Java's northern coastal fishing port, especially in Juwana and Pekalongan.

In the field research and market surveys (2011-2015), there are only 24 individual sawfishes were recorded, represented by the rostrum. A total of two samples of P. pristis were recorded, i.e. from Lake Sentani, Papua (1 sample) and Mahakam river estuary, East Kalimantan (1 sample was used in this study and the identity was confirmed as P. pristis, under the code of Samarinda); besides, 21 A. cuspidata was recorded from south coast of Merauke (6 samples were used in this study and the identity was confirmed as A. cuspidata, under the code of Merauke, HGM1, HGM2, PMB141, PMB148, PMC143).
Table 1. Percentage of similarity between CR region of mtDNA of Indonesian and Australian sawfishes comparing to the central data of GeneBank using BLASTN

| Sequence | Query length | The most sequence having similarity | Identity | Access no. |
|----------|--------------|-------------------------------------|----------|------------|
| **Australia** |              |                                     |          |            |
| 1121 (Australia) | 397 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7879 (Australia) | 401 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7881 (Australia) | 402 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7883 (Australia) | 401 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7885 (Australia) | 399 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7891 (Australia) | 401 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 98% | GQ980007.1 |
| 7899 (Australia) | 401 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| 7901 (Australia) | 329 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 93% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 93% | GQ980007.1 |
| Flyma02 (Australia) | 404 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| Flyma03 (Australia) | 410 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| PM10220 (Australia) | 370 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 89% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 89% | GQ980007.1 |
| PM10231 (Australia) | 395 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| PM1027 (Australia) | 407 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| PM1028 (Australia) | 425 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |
| PM3196 (Australia) | 411 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |

| **Indonesia** |              |                                     |          |            |
| Merauke       | 364 | Anoxypristis cuspidata haplotype 2 D-loop, partial sequence; mitochondrial | 93% | JQ026199.1 |
| HGM1 (Merauke) | 405 | Anoxypristis cuspidata haplotype 2 D-loop, partial sequence; mitochondrial | 99% | JQ026199.1 |
| HGM2 (Merauke) | 404 | Anoxypristiscuspidata haplotype 2 D-loop, partial sequence; mitochondrial | 99% | JQ026199.1 |
| PMB141 (Merauke) | 402 | Anoxypristiscuspidata haplotype 6 D-loop, partial sequence; mitochondrial | 98% | JQ026203.1 |
| PMB148 (Merauke) | 342 | Anoxypristiscuspidata haplotype 5 D-loop, partial sequence; mitochondrial | 92% | JQ026202.1 |
| PM3196 (Merauke) | 368 | Anoxypristiscuspidata haplotype 5 D-loop, partial sequence; mitochondrial | 99% | JQ026202.1 |
| Samarinda     | 395 | Pristis microdon haplotype 1 control region, partial sequence; mitochondrial | 99% | GQ980006.1 |
|              |            | Pristis microdon haplotype 2 control region, partial sequence; mitochondrial | 99% | GQ980007.1 |

Note: Refer to Faria et al. (2013), Pristis microdon (Latham, 1794) should be included in Pristis pristis (Linnaeus, 1758)
Figure 1. Phylogenetic trees of Indonesian and Australian sawfishes based on the CR gene sequences analyzed using MEGA 5.10.

In the previous 20-25 years, fishermen in the surveyed places still catch and sell sawfishes, but at the time of the study (2011-2015), fishermen could no longer catch sawfishes, except on the coast of Merauke. The intensive research at Merauke in 2014 shows that sawfishes rostrum are sold freely as souvenirs in stores around the harbor, but to bring out the island special permits are required. Some fishermen said still to catch and sell sawfishes and offer to provide fresh (or alive) sawfishes caught on the demersal coast or river mouths of the southern coast of Merauke, between Yos Sudarso Island and Wasur National Park. The lack of public awareness to keep the animals protected by law (PP 7/1999) and inadequate law enforcement causing hunting, trade, and consumption of protected animals is still common in this region, not only for sawfishes but also rays, sharks, deer, kangaroos, birds, etc. The pressure of development and conversion of natural habitats is likely to immediately reduce the protected animals if the environmental law enforcement and public awareness to participate does not arise. In PP 7/1999 it is said that the protected sawfishes are *Pristis* spp., but the *Anoxypristis cuspidata* should also be treated equally because this is only a taxonomic case.

In Australia, all sawfishes species have decreased significantly, although most are unquantified. In places, viable populations exist, representing some of the last surviving populations in the Indo-West Pacific, with Australia being one of a number of global strongholds for sawfishes (Stevens et al. 2005), they can find in the Gulf of Carpentaria, Western Australia, Northern Territory and Queensland coastlines, with the distribution of each species and gender is not always equitable (Phillips et al. 2011).

The rate of decline in the Genus *Pristis* is generally higher than that of *A. cuspidata*, because the latter species have smaller body sizes that require less food and space, and have a high reproductive capacity. *P. pristis* and *A. cuspidata* are the most distributed species, but in places where some sawfishes are found, *A. cuspidata* is the easiest to be found (Thorburn et al. 2007; Peverell 2009). Although *A. cuspidata* is relatively common throughout their global distribution, it is relatively declined throughout its range. The accurate information about the decline of sawfishes population is difficult because baseline data is not species-specific (Peverell 2009; D’Anastasi 2010; Harry et al. 2011), misidentification, weak reporting and not appropriate for the observation program. In general, the decline of sawfishes population occurred since the 1960s and in the last 20 years, the remaining population tends to be 20% from the 1960s (D’Anastasi et al. 2013). The appropriate regulation, law enforcement, accurate databases and the growth of environmental awareness are expected to prevent the extinction of sawfishes populations, although unavoidable global climate change can affect the population.
ACKNOWLEDGEMENTS

The authors thank “The Foreign Collaborative Research and International Publication”, Directorate General of Higher Education, Indonesian Ministry of Education that has supported the funding for the research. Thanks also Dr. Iwan Suryatna (Universitas Mulawarman, Samarinda) and Dr. Abdul Hamid Toha (Universitas Papua, Manokwari) for Indonesian sample of sawfishes, as well as M. Jaftron, Ida and Nunung for laboratory works.

REFERENCES

Bigelow HB, Schroeder WC. 1953. Fishes of the western North Atlantic, Part 2. Sawfishes, guitarfishes, skates, rays, and chimaeroids. In: Tee Van J, Reder CM, Hildebrand SF, Parr AE, Schroeder WC (eds). Fishes of the western North Atlantic. Yale University, New Haven.

Carlson J, Wiley T, Smith K. 2013. Pristis pectinata. The IUCN Red List of Threatened Species 2013: e.T18157A43398238. DOI: 10.2305/IUCN.UK.2013-3.RLTS.T18157A43398238.en.

Carson JK, Simpfendorfer CA. 2015. Recovery potential of smalltooth sawfish, Pristis pectinata, in the United States determined using population viability models. Aquat Conserv Mar Freshw Ecosyst 25: 187-200.

CITES. 2007. CITES Appendix I FAMILY listing Pristidae spp. Effective for Indonesian sample of sawfishes, as well as M. Jafron, Dr. Abdul Hamid Toha (Universitas Papua, Manokwari) and Iwan Suryatna (Universitas Mulawarman, Samarinda) has supported the funding for the research. Thanks also Dr. Higher Education, Indonesian Ministry of Education that has supported the funding for the research. Aquat Conserv Mar Freshw Ecosyst. DOI: 10.2305/aqc.2441.

Dulvy NK, Davidson LK, Kyne PM, Simpfendorfer CA, Harrison LR, Compagno LJV, Last PR. 1999. Order Pristiformes. In: Carpenter KE, Clarke S, Milner-Gulland EJ, Cemare TB. 2007. Social, economic and regulatory drivers of the shark fin trade. Mar Res Econ 22: 305-327.

Garza JC, Thompson WD. 2000. Analysis of substitution rate variation among sites. Mol Biol Evol 14: 1106-1113.

Gonzalez MMB. 2005. Use of Pristis spp. (Elasmobranchii: Pristidae) as a host for the Indian parasitic copepod, Caligus furciisetid Redkar, Rangnekar et Murti, 1949 (Sphyonophoridae). New records from northern Australia. Acta Parasiitologica 55 (4): 419-423.

IUCN. 2017. The IUCN Red List of Threatened Species Version 2016-3. [www.iucnredlist.org]. [6 March 2017]

IUCN. 2017. The IUCN Red List of Threatened Species. Version 2016-3. [www.iucnredlist.org]. [6 March 2017]

Johnson D (eds.). Interrelationships of the fishes of the western North Atlantic, Part 2. Sawfishes, guitarfishes, skates, rays, and chimaeroids. In: Tee Van J, Reder CM, Hildebrand SF, Parr AE, Schroeder WC (eds). Fishes of the western North Atlantic. Yale University, New Haven.

Last PR, Stevens JD. 1994. Family Pristidae. Sawfishes. In: Last PR, Stevens JD (eds.). Sharks and Rays of Australia. CSIRO, Melbourne, VIC.

Leeney RH, Ponecet P. 2013. Using fishes’ ecological knowledge to assess the status and cultural importance of sawfish in Guinea-Bissau. Aquat Conserv Mar Freshw Ecosyst. DOI: 10.2305/aqc.2441.

McEachran JD, Dunn KA, Miyake T. 1996. Interrelationships of the batoid fishes (Chondrichthyes: Batoidea). In: Sisastnny, Parenti L, Johnson D (eds.). Interrelationships of fishes. New York Academic Publishers, New York.

Moore ABM. 2014. A review of sawfishes (Pristidae) in the Arabian region: diversity, distribution, and functional extinction of large and historically abundant marine vertebrates. Aquat Conserv Mar Freshw Ecosyst. DOI: 10.2305/aqc.2441.

Morgan DL, Tang D, Peverell SC. 2010. Critically endangered Pristis microdon (Elasmobranchii, as a host for the Indian parasitic copepod, Caligus furciisetid Redkar, Rangnekar et Murti, 1949 (Sphyonophoridae): New records from northern Australia. Acta Parasiitologica 55 (4): 419-423.

Mulligan CJ. 2005. Isolation and analysis of DNA from archaeological, clinical, and natural history specimens. Meth Enzymol 395: 87-102.

Norton SL, Wiley TR, Carlson JK, Frick AL, Poulakis GR, Simpfendorfer CA. 2012. Designating critical habitat for juvenile endangered smalltooth sawfish in the United States. Mar Coast Fish: Dynamic Manage Ecosyst Sci 4: 473-486.

Peverell SC. 2005. Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfishes ecology. Environ Biol Fish 73: 391-402.

Peverell SC. 2009. Sawfish (Pristidae) of the Gulf of Carpentaria, Queensland, Australia. [Thesis]. James Cook University, Townsville, QLD.

Phillips N, Chaplin J, Morgan D, Peverell S. 2009. Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics. Pac Conserv Biol 15: 128-134.

Phillips N. 2006. Development of Genetic and Morphological Methods for the Study of Sawfishes (Pristidae) Populations via Analysis of Old Rostra. [Hon. Thesis]. MurdochUniversity, Perth, WA.

Phillips NM, Chaplin JA, Morgan DL, Peverell SC, Thorburn DC. 2008. Genetic diversity and population structure of the Freshwater Sawfishes (Pristis microdon) in Australian waters. In: Whitty JM, Phillips NM, Whitly JM, Morgan DL, Chaplin JA, Thorburn DC, Peverell SC (eds). Habitat associations of freshwater sawfishes (Pristis microdon) and northern river shark (Glyphis sp. C): including genetic analysis of P. microdon across northern Australia. Centre for Fish, Fisheries Research, Murdoch University, Perth, WA.

Phillips NM, Chaplin JA, Morgan DL, Peverell SC. 2009. Microsatellite and mitochondrial DNA assessment of the genetic diversity and population structure of the freshwater sawfishes (Pristis microdon) and the green sawfishes, Pristiszijsrion, respectively, in Australian waters: preliminary results. In: Phillips NM, Whitly JM, Morgan DL, Chaplin JA, Thorburn DC, Peverell SC (eds). Freshwater sawfishes (Pristis microdon) movements and demographics in the Fitzroy River, Western Australia and genetic analysis of P. microdon and Pristis zijsrion. Centre for Fish & Fisheries Research, Murdoch University, Perth, WA.

Phillips NM, Chaplin JA, Morgan DL, Peverell SC. 2011. Population genetic structure and genetic diversity of three critically endangered Pristis sawfishes in northern Australian waters. Mar Biol 158: 903-915.

PP 71999. Indonesian Government Regulation No. 7 of 1999 concerning Preservation of Plants and Animals. [Indonesian]

Robillard M, Sëret B. 2006. Cultural importance and decline of sawfish (Pristidae) populations in west Africa. Cybium 30 (4) suppl.: 23-30.

Seitz JC, Poulakis GR. 2006. Anthropogenic effects on the smalltooth sawfish (Pristis pectinata) in the United States. Mar Poll Bull 52: 1533-1540.

Simpfendorfer C. 2013. Pristiszijsrion. The IUCN Red List of Threatened Species 2013: e.T39393A18620401. DOI: 10.2305/IUCN.UK.2013-1.RLTS.T39393A18620401.en.

Simpfendorfer CA. 2000. Predicting population recovery rates for endangered western Atlantic sawfishes using demographic analysis. Environ Biol Fish 58: 371-377.
Simpfendorfer CA. 2006. Threatened fishes of the world: Pristis pectinata Latham, 1794 (Pristidae). Environ Biol Fishes 73: 20. DOI:10.1007/s10641-004-4174-9.

Slaughter BH, Springer S. 1968. Replacement of rostral teeth in sawfishes and sawsharks. Copeia 3:499-506.

Sutarno, Setyawan AD, Suyatna I. 2012. Species diversity of critically endangered pristid sawfishes (Elasmobranchii: Pristidae) of Nusantara waters (Maly Archipelago). Biodiversitas 13 (4): 161-171.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619-633.

Thorburn DC, Morgan DL, Rowland AJ, Gill HS. 2007. Freshwater Sawfishes Pristis microdon Latham, 1794 (Chondrichthyes: Pristidae) in the Kimberley region of Western Australia. Zootaxa 1471: 27-41.

Thorson TB. 1982. The impact of commercial exploitation on sawfish and shark populations in Lake Nicaragua. Fisheries 7: 2-10.

Waters JD, Coelho R, Fernandez-Carvalho J, Timmers AA, Wiley T, Seitz JC, McDevitt MT, Burgess GH, Poulakis GR. 2014. Use of encounter data to model spatio-temporal distribution patterns of endangered smalltooth sawfish, Pristis pectinata, in the Western Atlantic. Aquat Conserv Mar Freshw Ecosyst 24: 760-776.

Whitty JM, Morgan DL, Thorburn DC, Fazeldean T, Peverell SC. 2008. Tracking the movements of Freshwater Sawfishes (Pristis microdon) and Northern River Sharks (Glyphis sp. C) in the Fitzroy River. In: Whitty JM, Phillips NM, Morgan DL, Chaplin JA, Thorburn DC, Peverell SC (eds.). Habitat associations of freshwater sawfishes (Pristis microdon) and northern river shark (Glyphis sp. C): including genetic analysis of P. microdon across northern Australia. Centre for Fish, Fisheries Research, Murdoch University, Perth, WA.

Wueringer BE, Squire L, Collin SP. 2009. The biology of extinct and extant sawfish (Batoidea: Sclerorhynchidae and Pristidae). Rev Fish Biol Fish 19: 445-464.