

Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics

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DNA was obtained from the skin tissue of a high proportion (198/236) of dry rostra collected during the past ~30 years from the sawfishes *Pristis microdon*, *P. clavata*, *P. zijsron* and *Anoxypristis cuspidata*. DNA obtained from the dry rostra was usually either non-degraded, or only partly degraded, and was of a quality whereby a 465–468-bp portion of the mtDNA 12S rRNA gene could be PCR-amplified. These findings demonstrate that it is possible to use the dry rostra, which are generally readily available, as a source of DNA in conservation genetic studies of sawfishes. Such studies are needed for the development of effective conservation strategies for these critically endangered species, but would otherwise be greatly constrained by the difficulties of obtaining samples from sufficient numbers of living sawfishes.

Key words: *Pristis*, *Anoxypristis*, sawfish, conservation genetics, rostrum

INTRODUCTION

SAWFISHES (Family Pristidae) are large, iconic elasmobranchs characterized by a saw-like projection of the upper jaw, termed a rostrum (Bigelow and Schroeder 1953) that is used to hunt and stun prey (Compagno 1977; Last and Stevens 1994). Extant sawfishes are divided into two genera, *Pristis* and *Anoxypristis* (Last and Stevens 1994). At least three of the six described species of *Pristis* (*P. microdon*, *P. zijsron* and *P. clavata*), as well as the sole described species of *Anoxypristis* (*A. cuspidata*), occur in the Indo-west Pacific region. Sawfishes occur in marine waters and estuaries and sometimes also rivers and lakes (Compagno and Cook 1995). It is generally believed that the abundance of sawfishes has declined in recent times due to the combined effects of by-catch mortality, habitat deterioration and exploitation (Simpfendorfer 2000; Pogonoski *et al.* 2002; Stobutzki *et al.* 2002; Peverell 2005). However, while all sawfish species are currently listed as critically endangered worldwide on the International Union of the Conservation of Nature (IUCN) Red List (IUCN 2006), they are poorly studied and the concomitant lack of information on their population biology hinders the development of effective strategies for their conservation (Peverell 2005). Population genetic information can be used to estimate a variety of demographic parameters, such as population structure, size and history, which are central to the development of such strategies and typically unattainable by other means (DeSalle and Amato 2004).

Research into the conservation genetics of sawfishes is challenging because sampling is difficult as the sawfishes typically occur in low

numbers in remote regions with restricted access and seasonal flooding (Thorburn *et al.* 2003). If DNA could be obtained from the large numbers of dry sawfish rostra already held in museum and private collections, then the difficulty of finding adequate samples could partially be overcome. However, these dry rostra have not typically been stored for genetic analyses and the DNA therein may be degraded, limiting their utility in this regard. In general, tissues that have not been specifically stored for genetic analyses yield much lower quantities of DNA compared to preserved tissue (O'Rourke *et al.* 1996; Wandeler *et al.* 2007). A loss of yield is potentially a major problem for conservation genetic studies, which typically require relatively large sample sizes and therefore cannot invest large amounts of time and money obtaining DNA from single samples.

The purpose of this study was to ascertain whether it is possible to obtain DNA from a relatively high proportion of dry rostra of sawfishes and to assess whether the quality of the rostral DNA is adequate for the PCR-amplification of genetic markers. If so, this should remove a major impediment for much-needed conservation genetic studies of these species as it would allow for adequate sample sizes without the difficulties associated with sampling living sawfishes.

METHODS

Experimental design

The first phase of this research was to identify a tissue type on the dry rostra of sawfishes that might consistently yield DNA. Four soft tissue types, namely skin (L1), tissue under the skin

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around the teeth (L2), tissue on the side of the rostrum base (L3), and tissue from the cavity of the rostrum (L4) (see Fig. 1), as well as rostral teeth were assayed. Six samples of each soft tissue type were taken from each of six rostra for a total of 36 extraction attempts per tissue type. Three of these rostra were from *P. microdon* and three were from *P. clavata* and were randomly selected from a larger pool of rostra for each species. The number of attempts to extract DNA from rostral teeth was limited to two teeth from each of four rostra (two *P. microdon* and two *P. clavata*) for a total of eight extraction attempts.

The results of the first phase of this research indicated that, of the tissue types tested, skin was likely to be the most reliable source of DNA from rostra. However, since these results were based on a limited number of rostra and species, the second phase of this research investigated the reliability (success rate) of obtaining DNA from skin tissue on an additional 230 dry rostra from *P. microdon*, *P. clavata*, *P. zijsron* and *A. cuspidata*. Up to three attempts, using independent skin samples, were made to extract DNA from a particular rostrum. If all three attempts failed, it was concluded that it was not possible to obtain DNA from that rostrum for the purposes of this experiment.

Since the results of the second phase of this research indicated that it was possible to obtain DNA from the skin tissue of a relatively high proportion of dry rostra, the next phase was to determine whether a genetic marker could be retrieved from the extracted DNA. Specifically attempts were made to PCR-amplify a portion of the 12S rRNA gene in the mitochondrial

DNA (mtDNA) from seven dry rostra from each of *P. microdon* and *P. clavata*, two from *P. zijsron* and one from *A. cuspidata*. The 12S rRNA gene was targeted because it is relatively conserved, e.g., in comparison to the control region (see Kocher *et al.* 1989). This meant that it was possible to use the same set of primers to amplify this marker from all four sawfish species. It should also be possible to amplify the marker from some other taxa using these primers, which was important in the test for contaminants (see below).

Since dry rostra have not been specifically preserved for genetic analysis, contamination of a sample with DNA from either other sawfish samples or external sources is potentially a problem (see Mulligan 2005; Wandeler *et al.* 2007). Thus, this study followed the recommendations of Mulligan (2005) regarding the genetic assays. This included undertaking the rostral work in a laboratory that had not previously held contemporary tissue of any elasmobranch. In addition, negative controls were included in all PCR runs, but not positive controls because of the risk of contamination from the positive control itself (see Mulligan 2005). Regardless, the fourth phase of the research investigated the possibility that the putative rostral DNA samples were contaminated with non-target DNA, as follows. (1) The nucleotide sequence of the amplified portion of the 12S rRNA gene from a particular dry rostrum was compared to those from ethanol-preserved muscle tissue from individuals of the same species. This was to test whether the DNA in the putative rostra sample was, in fact, DNA of the target species of sawfish. (2) The

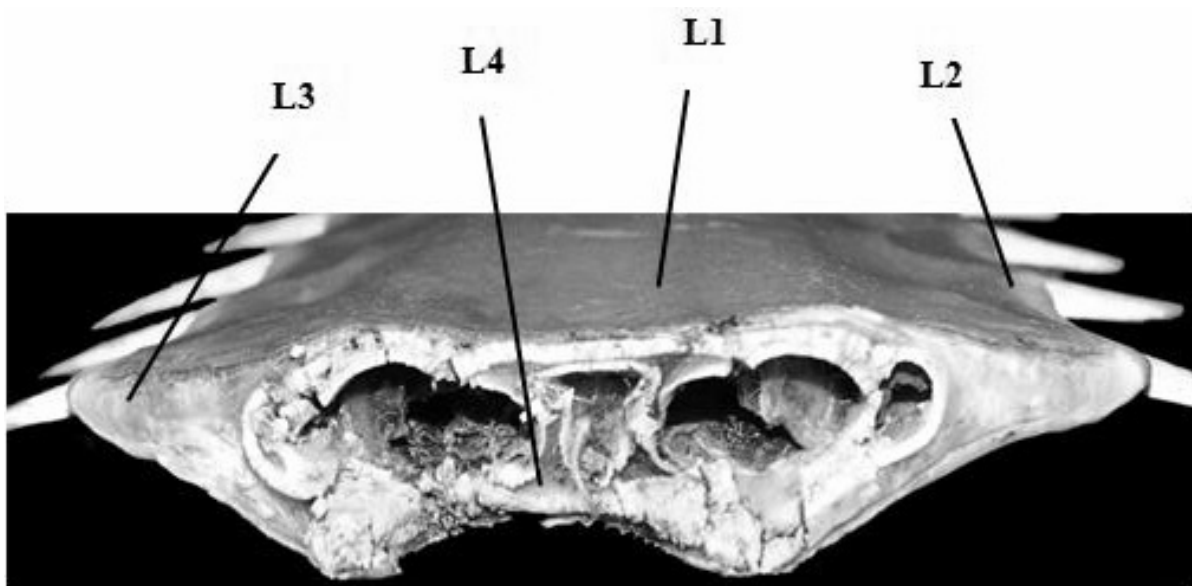


Fig. 1. A dry rostrum showing the four different types of soft tissue from which attempts to extract DNA were made. These tissue types were: skin from the dorsal surface (L1), tissue from around the tooth base under the skin (L2), tissue from the side of the rostrum base (L3), and tissue from within the rostrum cavity (L4).

nucleotide sequence of the amplified portion of the 12S rRNA gene from a dry rostrum from each of *P. microdon* and *P. clavata* was compared to a second such sequence from the same rostrum but obtained from an independent tissue sample and extraction assay. This was done to test whether the 12S rRNA sequence obtained from a putative rostral DNA sample was reproducible, which is unlikely to be the case if DNA from multiple sources was present on the rostrum (see Pääbo *et al.* 2004; Mulligan 2005). In this analysis, the 12S rRNA sequence data were generated using DNA from five dry rostra from each of *P. microdon* and *P. clavata*, and one from each of *P. zijsron* and *A. cuspidata* and from ethanol-preserved tissue from five individuals of each of *P. microdon* and *P. clavata* and four individuals of each of *P. zijsron* and *A. cuspidata*.

Sample collection and identification

The dry rostra used in this study were obtained from fisheries departments, museums, private collections, and research surveys by Morgan *et al.* (2002; 2004) and Thorburn *et al.* (2003). These rostra came from individuals of *P. microdon*, *P. clavata*, *P. zijsron* and *A. cuspidata* collected between the 1970's and 2006 in north-western Australia, with the exception of two which were from the Gulf of Carpentaria. The ethanol-preserved muscle tissues used in this study came from sawfishes collected from the Gulf of Carpentaria and associated rivers in north-eastern Australia by Peverell (2005).

The dry rostra of the four species of sawfish included in this study were identified based on either observations of the entire animals from which the rostra were taken or on the following morphological characteristics of the rostra — (1) teeth start at the base of the rostrum in *Pristis* but not in *Anoxypristis* (Last and Stevens 1994); (2) the number of left or right rostral teeth in *P. zijsron* is usually more (typically 24 – 31) than that of either *P. microdon* (17 – 24) or *P. clavata* (18 – 24) (see Last and Stevens 1994; but note that the range in the teeth number for each of the three *Pristis* species has been expanded in the present study); and (3) the ratio of teeth spacing at the base of the rostrum versus the tip is larger in *P. clavata* than in *P. microdon* (unpublished data).

Genetic methods

Attempts to extract total genomic DNA from the soft tissues of the sawfish rostra were made using 5 mg pieces of tissue and a Masterpure™ (Epicentre Technologies, Sydney) DNA extraction kit, according to the manufacturer's recommendations. This method was also used for attempts to extract DNA from the rostral

teeth, except that prior to extraction, the teeth were ground into a powder and de-calcified with 1.5 ml of 5 M EDTA at 37°C (Yang *et al.* 1997), which was changed daily until it was colourless (three days). The de-calcified tissue was then rinsed several times with deionized water and incubated in a Proteinase K solution at 67°C until the tissue was completely digested (18 h). The outcome of a DNA extraction was determined via agarose gel electrophoresis. The quality of the DNA in successful extracts was broadly categorized as: (1) non-degraded when the DNA was present as a compact, high molecular weight band; (2) partly degraded when present as a relatively high molecular weight smear; or (3) severely degraded when present as a relatively low molecular weight smear (Fig. 2).

A 465–468-bp portion of the 12S rRNA gene in the mtDNA was amplified from selected DNA extracts via PCR using the forward primer 12SF: 5'-CAAACCTAGGATTAGATACCC-3' and the reverse primer 12SR:5'CACTTACCATGTTACGACTT-3'. These primers were designed in the present study using the 12S rRNA sequences of other elasmobranchs obtained from GenBank. PCR amplification was performed in a reaction mixture containing variable amounts of DNA template (usually about 10 ng), 10 mM of TAQ buffer with 1.5 mM of 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 a final volume of 50 μl with PCR-grade water. The amplification conditions consisted of an initial denaturation phase at 94°C for 5 minutes, followed by 30 cycles, with each cycle consisting of 30 seconds of denaturation at 94°C, 30 seconds of annealing at either 64°C (*P. microdon* and *P. clavata*) or 62°C (*P. zijsron* and *A. cuspidata*), and 30 seconds of extension at 72°C, followed by a final extension at 72°C for 7 minutes. PCR products were cleaned with a Qiagen PCR purification kit and sequenced 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 at half reactions. Raw sequence data chromatograms were generated using an Applied Biosystems 3230 DNA Analyzer automated sequencer and associated software.

RESULTS

The results of the first phase of this research, which was based on tissue samples from three rostra from each of *P. microdon* and *P. clavata*, indicated that skin tissue was a reliable source of DNA from dry rostra. In particular, DNA was extracted from all 36 of the skin tissue samples, but with less success from other soft tissue types

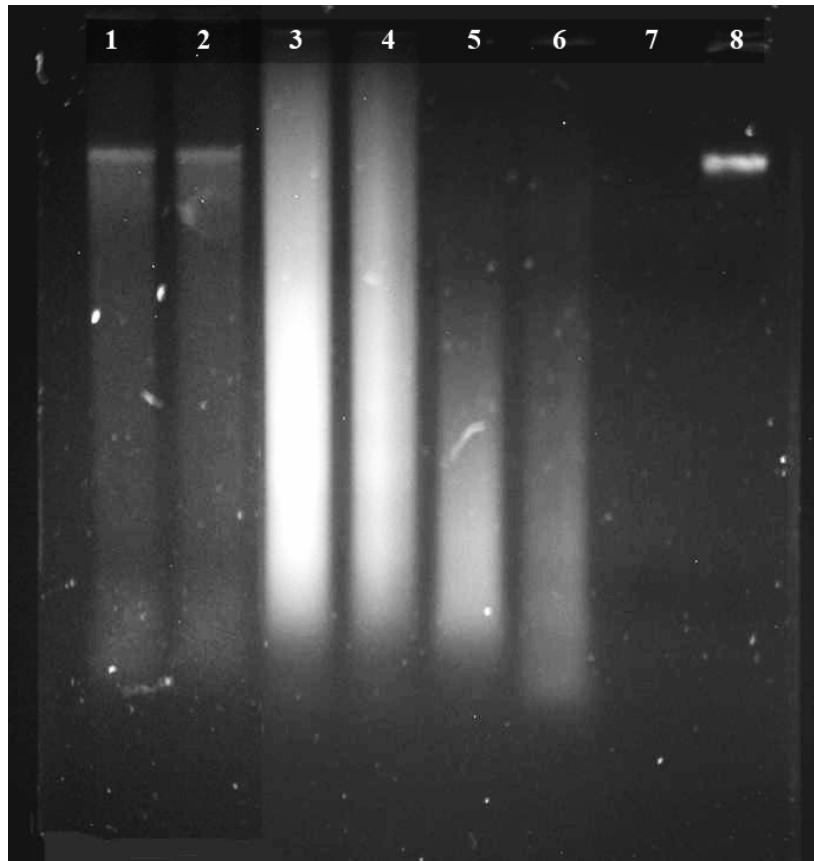


Fig. 2. Agarose gel showing DNA extracted from dried sawfish rostra and demonstrating the three categories of DNA quality. Lanes 1 and 2 are extracts containing non-degraded DNA; Lanes 3 and 4 are extracts with degraded DNA of relatively high molecular weight; Lanes 5 and 6 are extracts with severely degraded, low molecular weight DNA; Lane 7 shows a failed extraction (yielded no DNA); and Lane 8 shows a 48.5 kbp λ marker (5 μ l/ml).

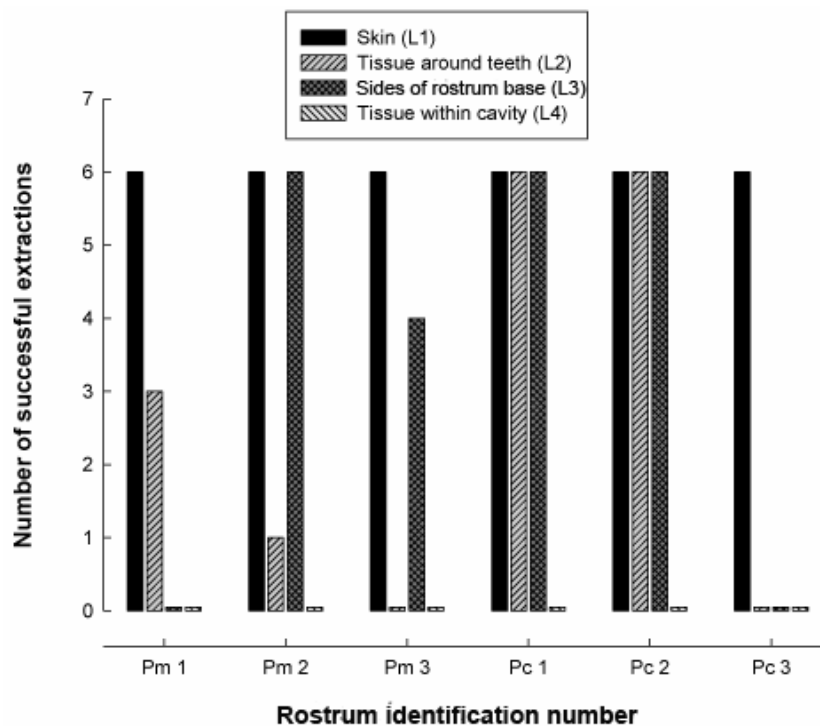


Fig. 3. The effects of tissue type on the reliability of DNA extraction from the rostra of *Pristis microdon* (Pm) and *P. clavata* (Pc). Data are the number of successful DNA extractions from six replicate samples of each tissue type.

Table 1. The number of successful DNA extractions in the investigation into the reliability of obtaining DNA from skin tissue on 230 dry rostra from each of *Pristis microdon*, *P. clavata*, *P. zijsron* and *Anoxypristis cuspidata*. The total number of rostra tested per species is in parentheses and the number of successful DNA extractions per species is broken down into the number of non-degraded, partly degraded and severely degraded samples (see Fig. 2).

Species	<i>A. cuspidata</i>	<i>P. microdon</i>	<i>P. clavata</i>	<i>P. zijsron</i>
No. of successful extracts	2 (4)	47 (61)	117 (127)	26 (38)
Quality of extracts				
Non-degraded	2	25	81	18
Partly degraded	0	16	33	7
Severely degraded	0	6	3	1

(Fig. 3) and from only two teeth on the same *P. microdon* rostrum.

When the reliability of extracting DNA from skin tissue was further assessed using the additional 230 dry rostra, DNA was obtained from 192 of these rostra and at least 68% of those tested for each of the three *Pristis* species ($n \geq 38$) and from 50% of the rostra tested for *A. cuspidata* ($n = 4$) (Table 1). If the six rostra from the tissue comparison experiment are included, DNA was obtained from a total of 84% (198/236) of the dry rostra tested.

The DNA obtained from the dry rostra was usually non-degraded, although sometimes it was partly degraded and occasionally severely degraded (Table 1). A 465–468-bp portion of the 12S rRNA gene was PCR-amplified and sequenced from all of the non-degraded and partly degraded DNA extracts from the small number of dry rostra that were so tested for each species. However, attempts to PCR-amplify this marker from severely degraded DNA from the dry rostra of five individuals of the three *Pristis* species were not successful.

Comparisons with the nucleotide sequence of the entire 12S rRNA gene in an individual of *A. cuspidata* on GenBank (AF 447988) showed that the amplified PCR products correspond to the right domain (about positions 454–919) of this gene. A representative sequence for each *Pristis* species has been deposited in GenBank under the accession numbers EU 784160, EU 784161, and EU 784162, while the sequences obtained for *A. cuspidata* in this study were virtually the same to that of the individual already in GenBank.

The total number of different 12S rRNA sequences (haplotypes) from the dry rostra and ethanol-preserved sawfish samples was eleven; the number per species ranged from two to four. The minimum amount of raw sequence divergence between any two haplotypes of any two species was 33 bp or 6.5% (for a haplotype of *P. clavata* and one of *P. zijsron*). In contrast, the maximum amount of raw sequence

divergence between any two haplotypes of the same species, including rostra and ethanol-preserved samples, was 8 bp or 2% (for two haplotypes of *P. clavata*). Thus, each of the four sawfish species had a characteristic 12S rRNA sequence profile and the profiles obtained from the rostral DNA extracts were consistent with the expectations for that species on the basis of ethanol-preserved samples. This proves that the 12S rRNA fragment amplified from the rostral samples of each species was always that of the target species. Similarly, the 12S rRNA sequence data generated from independent skin samples from a rostrum from *P. microdon* were identical, as was also the case for a rostrum from *P. clavata*.

DISCUSSION

Specimens in natural history collections potentially provide a valuable source of DNA for studies into the conservation genetics of elasmobranchs, whose populations are generally in decline (e.g., Ahonen and Stow 2008). The results of the present study indicate that it will be feasible to use dry rostra from *Pristis* and *Anoxypristis* sawfishes, which are typically readily available from private and public collections, as a source of DNA for conservation genetic studies of these species. However, since some of the genetic variation represented in some of the older rostra in particular may have since been lost from populations, it is important to supplement the rostral samples with samples from extant sawfish populations (see Austin and Melville 2006).

Studies into the conservation genetics of sawfishes will require large sample sizes. In order for dry rostra to be useful as a source of DNA for such studies, it must be possible to obtain DNA, relatively easily and inexpensively, from a high proportion of rostra. In this study, DNA was obtained, using a routine extraction method, from the skin tissue of at least 50% of the dry rostra assayed for each of *P. clavata* ($n = 127$), *P. microdon* ($n = 61$), *P. zijsron* ($n = 38$) and *A. cuspidata* ($n = 4$), with an overall success rate of 84%. This high rate of success was

probably DNA from dry rostra was probably due to the desiccated nature of the tissue, as DNA stored in a dry state is generally relatively stable for decades or even longer (Anchordoquy and Molina 2007). Age and the environmental conditions of storage are probably the main determinants of the quality and quantity of DNA in a particular rostrum (see Watts *et al.* 2007); although a variety of other factors could also play a role (see Wandeler *et al.* 2007).

The DNA in specimens in natural history collections is often degraded, thus limiting the size and types of genetic markers that can be amplified. Specifically, large fragments are unlikely to remain intact in degraded DNA (Pääbo *et al.* 2004) and some regions of the genome tend to degrade relatively rapidly (e.g., Gilbert *et al.* 2003). Nevertheless, the DNA in the vast majority of rostral extracts was either non-degraded or only partly degraded. Accordingly, it was possible to PCR-amplify a 465–468-bp mtDNA marker, which is at the upper end of the size range for DNA fragments that can typically be amplified from natural history specimens (Pääbo *et al.* 2004; Wandeler *et al.* 2007). Although attempts to PCR-amplify this particular marker from severely degraded rostral DNA extracts were unsuccessful, it might be possible to amplify smaller fragments as they are more likely to remain intact in degraded DNA (Pääbo 1989).

Although contamination of samples is a significant risk in studies involving natural history specimens (see Cooper and Poinar 2000; Bandelt 2005; Wandeler *et al.* 2007), it does not appear to have been a factor in this study. This is because the 12S rRNA fragment amplified from the rostral samples of each species was always that of the target species. Furthermore, sequence data produced for a rostrum from each of a *P. microdon* and *P. clavata* were reproducible, which is unlikely to be the case if DNA from multiple sources is present, at least on these particular rostra (see Mulligan 2005). Nevertheless, since contamination is an ongoing risk, future studies using dry rostra as a source of DNA should follow the recommendations of Mulligan (2005) for working with natural history specimens.

Management Implications

The conduct of studies into the conservation genetics of sawfishes is currently hindered by the difficulties of obtaining samples from sufficient numbers of living individuals. By showing that it is possible to obtain useable DNA from a high proportion of dry rostra collected during the past ~30 years, which are readily available from museum and private collections, the results of this study have opened the way for conservation

genetic studies of sawfishes. The results of genetic studies can be used to provide information about the population structure, size and history of sawfish populations, which is urgently required for input into the development of strategies for the conservation of these critically endangered species.

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REFERENCES

- Ahonen, H. and Stow, A. J., 2008. Shark jaws and teeth: an unexploited resource for population genetic studies. *J. Fish Biol.* **73**: 450–455.
- Anchordoquy, T. J. and Molina, M. C., 2007. Preservation of DNA. *Cell Preserv. Technol.* **5**(4): 180–188.
- Austin, J. J. and Melville, J., 2006. Incorporating historical museum specimens into molecular systematic and conservation genetics research. *Mol. Ecol. Notes* **6**: 1089–1092.
- Bandelt, H. J., 2005. Mosaics of ancient mitochondrial DNA: positive indicators of nonauthenticity. *Eur. J. Hum. Genet.* **13**: 1106–1112.
- Bigelow, H. B. and Schroeder, W. C., 1953. Sawfishes, guitarfishes, skates and rays Fisheries of the Western North Atlantic. *Memoir. Sears. Mem. Found. Mar. Res.* **1**: 1–514.
- Compagno, L. J. V., 1977. Phyletic relationships of living sharks and rays. *Am. Zool.* **17**: 303–322.
- Compagno, L. J. V. and Cook, S. F., 1995. The exploitation and conservation of freshwater elasmobranchs: status of taxa and prospects for the future. *J. Aquacult. Aquat. Sci.* **7**: 62–90.
- Cooper, A. and Poinar, H. N., 2000. Ancient DNA: do it right or not at all. *Science* **289**: 1139–1141.
- DeSalle, R. and Amato, G., 2004. Expansion of conservation genetics. *Nat. Rev. Genet.* **5**: 702–711.
- Gilbert, M. T. P., Willerslev, E., Hansen, A. J., Barnes, I., Rudbeck, L., Lynnerup, N. and Cooper, A., 2003. Distribution patterns of postmortem damage in human mitochondrial DNA. *Am. J. Hum. Genet.* **72**: 32–47.

- IUCN 2006. *2006 IUCN Red List of Threatened Species*. [Internet]: www.iucnredlist.org. Accessed August 5, 2006.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. and Wilson, A. C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci.* **86**: 6196–6200.
- Last, P. R. and Stevens, J. D., 1994. *Sharks and Rays of Australia*. CSIRO Division of Fisheries, Melbourne, Australia. 600 pages.
- Morgan, D., Allen, M., Bedford, P. and Horstman, M., 2002. "Inland Fish Fauna of the Fitzroy River Western Australia, including the Bunuba, Gooniyandi, Ngarinyin, Nyikina and Walmajarri Aboriginal names." Technical report to the Natural Heritage Trust, Australia. 56 pages.
- Morgan, D., Allen, M., Bedford, P. and Horstman, M., 2004. Fish fauna of the Fitzroy River in the Kimberley region of Western Australia-including the Bunuba, Gooniyandi, Ngarinyin, Nyikina and Walmajarri Aboriginal names. *Rec. West. Aust. Mus.* **22**: 147–161.
- Mulligan, C. J., 2005. Isolation and analysis of DNA from archaeological, clinical, and natural history specimens. *Methods Enzymol.* **395**: 87–102.
- O'Rourke, D. H., Carlyle, S. W. and Parr, R. L., 1996. Ancient DNA: methods, progress, and perspectives. *Am. J. Hum. Biol.* **8**: 557–571.
- Pääbo, S., 1989. Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Nat. Acad. Sci. USA* **86**: 1939–1943.
- Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2004. Genetic analysis from ancient DNA. *Annu. Rev. Genet.* **38**: 645–679.
- Peverell, S. C., 2005. Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfish ecology. *Environ. Biol. Fishes* **73**: 391–402.
- Pogonoski, J. J., Pollard, D. A. and Paxon, J. R., 2002. Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes. Environment Australia, Canberra, Australia. 375 pages.
- Simpfendorfer, C. A., 2000. Predicting population recovery rates for endangered western Atlantic sawfishes using demographic analysis. *Environ. Biol. Fishes* **58**: 371–377.
- Stobutzki, I. C., Stevens, J., Gribble, N., Buchworth, R., Lenanton, R., McAuley, R. and McLoughlin, K., 2002. Sustainability of elasmobranchs caught as bycatch in a tropical prawn (shrimp) trawl fishery. *Fish. Bull.* **100**: 800–821.
- Thorburn, D. C., Peverell, S., Stevens, J. D., Last, P. R. and Rowland, A. J., 2003. "Status of freshwater and estuarine elasmobranchs in northern Australia." Technical report to the Natural Heritage Trust, Australia. 76 pages.
- Wandeler, P., Hoeck, P. E. A., and Keller, L. F., 2007. Back to the future: museum specimens in population genetics. *TRENDS Ecol. Evol.* **22**(12): 634–642.
- Watts, P. C., Thompson, D. J., Allen, K. A. and Kemp, S. J., 2007. How useful is DNA extracted from the legs of archived insects for microsatellite-based population genetic analyses? *J. Insect Conserv.* **11**: 195–198.
- Yang, H., Golenberg, E. M. and Shoshani, J., 1997. Proboscidean DNA from museum and fossil specimens: an assessment of ancient DNA extraction and amplification techniques. *Biochem. Genet.* **35**(5/6): 165–179.