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TECHNICAL NOTE

Genetic tools to support the conservation of the endangered smalltooth sawfish, *Pristis pectinata*

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Abstract The smalltooth sawfish, *Pristis pectinata*, is protected under the US Endangered Species Act (ESA) and all forms of international trade of this species are prohibited under Appendix I of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES). Although it is illegal to land or trade *P. pectinata* within the US or across its borders, it is difficult to enforce these regulations for some sawfish body parts because they resemble legally-traded shark body parts (e.g. dried fins). There is also a growing need for conservation genetics research on this species and its relatives, including assessments of population structure and genetic diversity. Given these pressing trade monitoring and research needs, we developed: (1) a rapid PCR-based test to identify *P. pectinata* body parts in trade in the US and western Atlantic, (2) a DNA-barcode based on 520 bp of cytochrome b that resolves *P. pectinata* and five other extant sawfish species and (3) a suite of 11 polymorphic *P. pectinata* microsatellite markers that can be used in a variety of conservation genetics applications for this and other sawfish species. We anticipate that this suite of genetic tools will contribute to the conservation of this critically endangered species and its relatives by reinforcing landings and trade restrictions and by enabling future conservation genetics research.

Keywords Sawfish · Microsatellites · DNA barcoding · Wildlife trade

Introduction

Genetic profiling often paints the most accurate picture of the wildlife trade, a global industry that annually generates billions in revenue but is very difficult to monitor (Baker 2008). Often, the first step to elucidating how trade affects a particular species is to develop molecular markers that can resolve species, populations, and individuals from samples of its commercial products (e.g. Baker and Palumbi 1996;

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Chapman et al. 2003; Michelini et al. 2007). In some cases it is not until these molecular tools are developed and applied that any evidence of trade in these species is found (e.g. Chapman et al. 2003; Shivji et al. 2005).

The smalltooth sawfish, *Pristis pectinata*, is a large-bodied (maximum length 540 cm) elasmobranch (i.e. sharks, batoids) that was once abundant along the United States (U.S.) Atlantic and Gulf of Mexico coasts, ranging from New York to Texas (Bigelow and Schroeder 1953). Verifiable records of this species only exist from the Atlantic basin, while Indo-Pacific records are the result of confusion with other species (Simpfendorfer 2005; Faria 2007). The US *P. pectinata* population suffered significant bycatch mortality in coastal net fisheries that arose in the early 1900s and by the 1950s had declined to probably less than 5% of its size at the turn of the century (Simpfendorfer 2000; NMFS 2009). As sawfish were extirpated from several major estuarine systems (e.g. Indian River Lagoon, FL, Snelson and Williams1981; Schmid et al. 1988) a dramatic range contraction also took place and the only breeding areas still known to exist are located in southwest Florida (Simpfendorfer 2000; Seitz and Poulakis 2002; Poulakis and Seitz 2004; NMFS 2009). In 2003, the smalltooth sawfish became the first fully marine fish species to be listed under the US Endangered Species Act (NMFS 2009). The species is now prohibited from fisheries landings, although they are occasionally landed as bycatch in some recreational and commercial fisheries (Seitz and Poulakis 2002, 2006; Carlson et al. 2007). All sawfish species were listed by the Convention on International Trade in Endangered Species (CITES) in 2007, with *P. pectinata* and others being of sufficiently critical status to warrant placement on Appendix I, which prohibits all international trade in these species and their body parts (NMFS 2009). Part of the rationale for this listing is that the rostra and dried fins of sawfish are highly valued in the curio and Asian dried seafood trades, which could encourage illicit landing and international trade despite the existence of domestic protective legislation in the United States (McDavitt unpublished).

The US has implemented a recovery plan for *P. pectinata* (NMFS 2009), and it is probable that interactions between this species and fisheries will gradually increase as the sawfish recover (Carlson et al. 2007). Although sawfish can survive if handled carefully when captured by a variety of gear (e.g. nets, hook and line), the reputed high value of sawfish rostra and fins in Asian markets may encourage unlawful retention and trade rather than release (McDavitt unpublished). Trade in sawfish fins may be especially easy to conceal from law enforcement officials because some sawfish fins resemble shark fins (Fig. 1), which still legally enter the international fin trade from the US Monitoring. This high volume trade for rare species is potentially costly and time-consuming, which will necessitate the development of high-throughput, cost-effective genetic tests.

Fig. 1 First dorsal fin of *Pristis pectinata*

We report on the development of genetic markers to improve conservation genetics research and trade monitoring for *P. pectinata*. We first developed a PCR-based DNA assay that can be used to rapidly and inexpensively screen "shark" body parts (e.g. fins, meat) that enter the international market for the presence of illicit *P. pectinata* in the US and western Atlantic region. We also identified a region of cytochrome b that can serve as a DNA barcode for *P. pectinata* and five other extant sawfish species, providing robust sequence-based confirmation of species identity once sawfish body parts are initially detected in trade. Lastly, we developed a suite of 11 polymorphic microsatellite loci that can be used to resolve whether *P. pectinata* body parts detected in trade are from one or multiple individuals. These microsatellite markers can also be used for conservation genetics applications in *P. pectinata* (e.g. assessments of genetic diversity, population structure, parentage, breeding frequency), with some loci also proving useful for its similarly endangered congeners.

Materials and methods

Sample collection and DNA extraction

Pristis pectinata samples ($N = 70$) were obtained from wild-captured specimens in Florida under Endangered Species Act (ESA) permits issued to Mote Marine Laboratory (MML), National Marine Fisheries Southeast Fisheries Science Center (NMFS-SEFSC) and the Florida Fish and Wildlife Conservation Commission (FWC). Two additional samples were collected in Bimini, Bahamas by scientists at the Bimini Biological Field Station (BBFS). Samples consisted of small (~ 1 cm^2) clips of fin tissue

taken non-destructively from live animals that were captured during research activities. Samples were stored in 95% reagent grade ethanol at room temperature. Three extraction methods were used to obtain genomic DNA from ~10 to 25 mg of fin tissue: (1) a salting-out procedure (Sunnucks and Hales 1996), (2) the DNeasy (Qiagen, Valencia, California) commercial kit and (3) The DirectAmp kit (Denville Scientific, Denville, New Jersey). The latter was trialed for three shark and three *P. pectinata* samples to determine whether this rapid (~15 min) extraction method could yield sufficient quality and quantity of genomic DNA for PCR-based identification of species from elasmobranch fin tissue, as a means to further streamline the testing process.

Cytochrome b sequencing

The most recent taxonomic work, utilizing extensive molecular and morphological data, has concluded that there are seven species of sawfish in the Family Pristidae (Faria 2007). The nominal species *Pristis pristis* has been associated with features from several species and apparently does not exist in nature. In addition, the putative species *Pristis zephyreus* has only been genetically identified by a single museum specimen, which was unavailable for study. Thus, we sequenced the mitochondrial cytochrome b (cyt b) from *P. pectinata* and five other sawfish species for which samples were available (1–6 individuals per species) using custom amplification and sequencing primers (PrisCBF1: 5′-TGAGGACAAATATCCTTCTGAGGTGC-3′, PrisCBR1: 5′-AAGTAGGTGATTGAGGCGATTTGGCC-3′). These primers were designed from a *P. perotteti* sequence that was available on GenBank (Accession number D50024). PCR reactions were performed in a 10 μL volume consisting of 1 μL of genomic DNA, 0.50 μM of each primer, 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3, 1.5 mM $MgCl_2$), 0.1 μM dNTPs, 5× BSA, and 1 unit of Roche *Taq* polymerase (Madison, WI, USA). Thermal cycling consisted of an initial denaturing step of 95°C for 5 min followed by 35 cycles of 94°C for 15 s, 52°C for 15 s, and 72°C for 60 s, and a final elongation step of 72°C for 5 min. PCR products were cleaned using Exonuclease I and Shrimp Alkaline Phosphatase according to the manufacturer's protocol (USB Corporation, Cleveland, Ohio). DNA sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Sequencing reactions were precipitated with ethanol and 125 mM EDTA and run on an ABI 3730 DNA Analyzer. Sequences were aligned and edited in the program GeneDoc 2.6.002 (Nicholas et al. 1997) and pairwise genetic distances (Kimura 2 parameter) within and between species were calculated in MEGA 4.1 (Kumar et al. 2004).

Rapid PCR test

Cytochrome b sequences were obtained for *Pristis pectinata* (n = 8), *Pristis clavata* (n = 6), *P. microdon* (n = 6), *P. perotteti* (n = 1), *P. zijsron* (n = 6), and *Anoxypristis cuspidata* (n = 5). These were aligned with the original *P. perotteti* sequence and three other elasmobranch cytochrome b sequences obtained from GenBank (*Negaprion brevirostris* [Accession: L08039], *Carcharodon carcharias* [Accession: L08031], *Aetobatus narinari* [Accession: AB021502]). We developed species-specific forward (PpeF3 5′-GCTTCTTTATCCTAATCTCATTACTCACCC-3′) and reverse primers (PpeR4 5′-AGGATTTGTGT GATGG GTCGTAATGTTAA-3′) that were designed to amplify *P. pectinata* DNA templates. We anticipated that there was sufficient interspecific variation in this region of the cyt b to prevent amplification from other sawfish and shark species. For example, the 30 bp annealing site of the primer PpeF3 differed between the target *P. pectinata* and the non-target elasmobranch species in the alignment by 6–14 substitutions. Correspondingly, this primer set was anticipated to generate a 281 bp amplicon from *P. pectinata* genomic DNA templates, but not from non-target species (Figure 2a). To provide an internal positive control for the PCR reaction (i.e. to avoid interpreting non-amplification as a "negative" result for *P. pectinata* DNA) we multiplexed this primer pair with universal primers that amplify the entire internal transcribed spacer 2 (ITS2) locus in sharks and sawfish (Shivji et al. 2002; this study; FISH5.8SF 5′-TTAGCGGTGGAT CACTCGGCTCGT-3′, FISH28SR 5′-TCCTCCGCTTAGTAATATGCTTAAAT TCAGC-3′). Thus, the multiplex assay was expected to produce two amplicons from *P. pectinata* genomic DNA templates (i.e. a "positive control" ITS2 and a 281 bp species-specific cyt b), but only one amplicon (i.e. a "positive control" ITS2) from non-target shark and sawfish species (Figure 2a).

To assess the efficacy of this multiplex PCR assay as a diagnostic tool for *P. pectinata* we attempted to amplify DNA from 33 other shark or sawfish species (N = 129 individuals, Table 1). For sharks, we selected species that were common or potential in landings of the US commercial and recreational shark fisheries (Morgan et al. 2009), including all of the major species in global shark fin landings (Clarke et al. 2006). PCR reactions were performed in a volume of 50 μL consisting of 1 μL of the extracted DNA, 10 pmol of each ITS2 primer, 13 pmol of each cytochrome b primer, 1× PCR buffer, 40 μM dNTPs, and 1 unit of HotStar© Taq polymerase (Qiagen, Valencia, California). All reactions were run with a positive (i.e. *P. pectinata* DNA) and negative control (i.e. no DNA). Thermal cycling conditions consisted of a 5 min activation of the polymerase at 95°C, followed by 35 cycles of 1 min

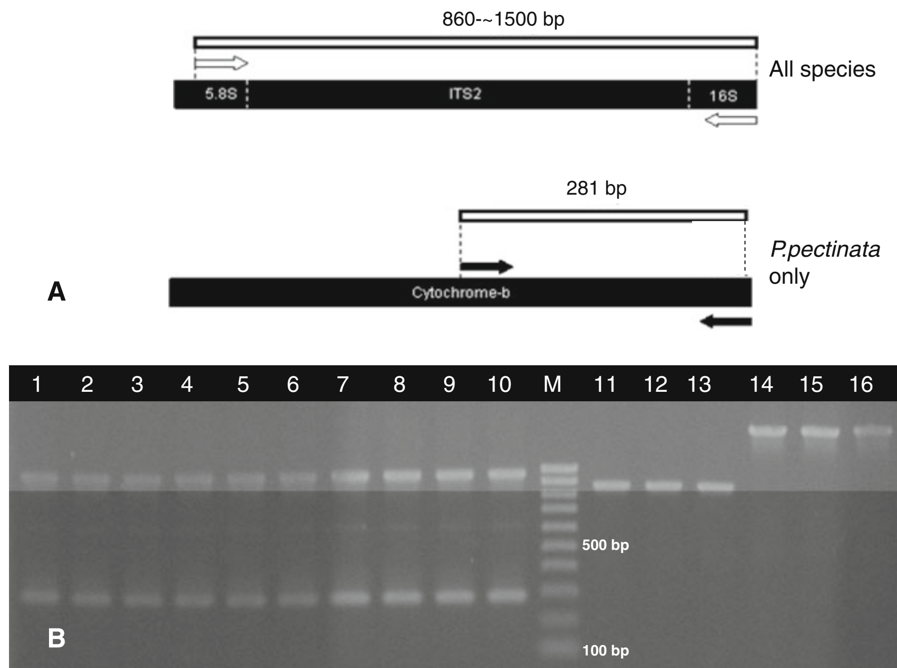

Fig. 2 **a** Above-Schematic of the internal transcribed spacer 2 (ITS2) locus and approximate annealing locations of the universal shark and sawfish primers (FISHF, FISHR; *white arrows*). The size range of the amplicon expected after PCR using any shark or sawfish genomic DNA template is shown above. Below-Schematic of the mitochondrial cytochrome b locus showing the approximate annealing sites of the species-specific *Pristis pectinata* primers (PpeF3, PpeR4; *black arrows*). The size of the expected amplicon after PCR using a *P. pectinata* genomic DNA template is shown above. **b** Agarose gel (1.2%) showing amplification results after multiplex PCR using the four primers. Lanes 1–10 are all *Pristis pectinata*, M is a marker (1 KB ladder, Denville Scientific, Denville, NJ), 11–13 are *Sphyrna lewini*, *S. mokarran* and *S. zygaena* (scalloped, great and smooth hammerhead sharks, respectively) and 14–16 are *Carcharhinus plumbeus* (sandbar shark), *C. limbatus* (blacktip shark) and *C. obscurus* (dusky shark). The 500 and 100 bp fragments on the 1 KB ladder are indicated. The smaller fragment in lanes 1–10 corresponds to the 281 bp fragment produced by PpeF3 and PpeR4, the larger fragment is the internal positive control (i.e. the entire ITS2 amplified by FISHF and FISHR). All other sample lanes only exhibit an entire ITS2 fragment

at 95°C, 1 min at 65°C and 2 min at 72°C, followed by a final extension step of 72°C for 5 min. Amplicons were resolved on a 1.2% agarose gel that had been run at 80 volts for ~40 min and were visualized using ethidium bromide. Four different DNA analysts in two different laboratories used reagents and thermal cyclers originating from multiple manufacturing companies to confirm that the assay was robust and transferable between labs.

Microsatellites

A *P. pectinata* microsatellite library was developed using an enrichment protocol (Glenn and Schable 2005). This protocol, which employs streptavidin-coated magnetic beads and biotin-labeled repetitive probes, has been described elsewhere for developing microsatellite markers for elasmobranchs (Feldheim et al. 2007). We optimized 11 microsatellite loci isolated from the *P. pectinata* genomic library. Primer sequences, repeat motif and the size of the cloned allele for each locus are shown in Table 2. We amplified these loci in a sample of 30 individual *P. pectinata* from Southwest Florida. To incorporate a fluorescent label into PCR products, a universal M13 sequence was added to the 5′ end of all forward primers following Schuelke (2000). In addition, to facilitate the addition of adenine to all PCR products, reverse primers were tailed on the 5′-end with GTGTCTT as suggested by Applied Biosystems. PCR amplifications were carried out in 10 µL reactions containing 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 0.12 mM of each dNTP, 10× BSA, 0.16 µM of the fluorescently labeled universal M13 primer and the species-specific reverse primer, 0.04 µM of the species-specific forward primer with a 5′-M13 tail (Schuelke 2000), 1U Taq DNA polymerase, and approximately 25–50 ng of template DNA. Thermal cycling consisted of the two-step cycling conditions following Schuelke (2000) with an initial denaturation of 94°C for 4 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, followed by 8 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 10 min. Amplification products were run with an internal size standard (LIZ-500, Applied Biosystems) on

Table 1 Species, geographic origin, and sample size (N) used as test subjects for the multiplex *Pristis pectinata* PCR assay

Species	Origin	*N*
Order Pristiformes		
Pristis pectinata	Florida, USA	70
	Bimini, Bahamas	2
P. perotteti	Texas, USA	1
P. clavata	Australia	4
P. microdon	Australia	4
P. zijsron	Australia	4
Anoxypristis cuspidata	Australia	4
Order Carcharhiniformes		
Carcharhinus acronotus	USA	4
C. altimus	USA	4
C. brevipinna	USA	4
C. falciformis	USA	4
C. leucas	USA	4
C. limbatus	USA	4
C. longimanus	USA	4
C. obscurus	USA	4
C. perezi	USA	4
C. plumbeus	USA	4
C. signatus	USA	4
Galeocerdo cuvier	USA	4
Negaprion brevirostris	USA	4
Prionace glauca	USA	4
Rhizoprionodon terranovae	USA	4
R. porosus	USA	4
Sphyrna lewini	USA	4
S. mokarran	USA	4
S. tiburo	USA	4
S. zygaena	USA	4
Order Lamniformes		
Alopias superciliosus	USA	4
A. vulpinus	USA	4
Carcharias taurus	USA	4
Carcharodon carcharias	USA	4
Cetorhinus maximus	USA	4
Isurus oxyrhyncus	USA	4
Lamna nasus	USA	4
Order Orectolobiformes		
Ginglymostoma cirratum	USA	4

an ABI 3730 Genetic Analyzer and scored by two experienced analysts (DDC, KAF) using ABI PRISM GeneMapper® Software v3.7 (Applied Biosystems). We used the program Fstat (Goudet 2002) to describe the genetic diversity of the sample, and the program Genepop v3.4 (Raymond and Rousset 1995) to test for linkage disequilibrium and deviations from Hardy–Weinberg expectations. We calculated parental exclusion probabilities using GERUD 2.0 (Jones 2005) and probability of identity ($P_{(ID)}$, Paetkau and Strobeck 1994) and probability of identity among siblings ($P_{(ID)sibs}$, Evett and Weir 1998) using DROPOUT v2.3 (McKelvey and Schwartz 2005). Finally, we attempted to amplify these loci in the other sawfish species, using identical PCR conditions, except that the annealing temperature for the first 30 cycles was dropped to 50°C.

Results

Cytochrome b DNA barcodes

Partial mitochondrial cyt b sequences (~500 bp) were obtained from *P. pectinata* and five other valid sawfish species (Accession numbers GU139174–GU139187). Average genetic distances between individuals within species (0–0.006, Table 3) were all more than an order of magnitude lower than average genetic distances between any of the species pairs (0.092–0.152, Table 4).

Rapid PCR test

The putative cyt b species-specific primers amplified the expected 281 bp fragment in all *P. pectinata* specimens tested ($N = 72$, Table 1, Figure 2b). The universal ITS2 primers amplified in all specimens as well, producing a fragment ~950 bp (Figure 2b). Thus, the assay produced the expected two-amplicon "fingerprint" for all *P. pectinata*. In contrast, although all other non-target species tested (Table 1) exhibited the ITS2 positive control amplicon (from 860 to ~1,500 bp, depending on species; Figure 2b), none exhibited the 281 bp cyt b amplicon. The assay was consistent regardless of DNA extraction method, PCR reagent, or thermal cycler manufacturer, and was successfully replicated in two laboratories by four different DNA analysts.

Microsatellites

We developed 11 microsatellite loci for *P. pectinata* (Table 2), which exhibited 7–29 alleles per locus with high levels of observed (0.357–0.966) and expected (0.406–0.968) heterozygosity. All loci conformed to Hardy–Weinberg expectations and no pair exhibited linkage disequilibrium. The $P_{(ID)}$ at these 11 loci was 2.72×10^{-18} while the $P_{(ID)sibs}$ was 6.76×10^{-6}. The probability of parental exclusion (neither parent known) of this suite of markers was 0.9999. From one to five of the eleven loci successfully amplified and were polymorphic in the other sawfish species (Table 5).

Table 2 Primer sequences, number of alleles (k), expected (H_{exp}) and observed (H_{obs}) heterozygostites, and Genbank accession numbers for 11 *Pristis pectinata* microsatellite loci

Locus	Primer sequences (5′–3′)	k	H_{exp}	H_{obs}	Genbank Accession #
Ppe5	F: M-CGGATATAGATGAATGTTTGGTG	24	0.959	0.964	GU066301
	R: GCGTGAAGAGAGTTAGTACAGCA				
Ppe77	F: M-TGTGGCAGCAGTACAAGACG	16	0.910	0.931	GU066302
	R: TCTTCTCCTCCGCCTCATTA				
Ppe88	F: M-CCTGCTGCTCATCGAGTGTA	29	0.968	0.931	GU066292
	R: ACTCGTCCATTCCATCCAAG				
Ppe107	F: M-CGTACAAACATACACGCCTAGC	17	0.929	0.965	GU066293
	R: CCTTCGGAAAGGATGAATCAC				
Ppe114	F: M-CCATGGCTCATATTCTCTCG	21	0.945	0.965	GU066294
	R: AGATAGAGATTGACATGATAGGTTGA				
Ppe122	F: M-GCTGCAAGCAAATTTTTCAC	9	0.749	0.7	GU066295
	R: GTGCCAGAGTGATGGAGACC				
Ppe157	F: M-GTGCCCAAACTTTTGCATGT	14	0.877	0.966	GU066296
	R: CTGTACATCCATCAGACACCAA				
Ppe160	F: M-TTCACCATCAGTACACAATTTACA	7	0.406	0.357	GU066297
	R: GCAACAAGCTTGAGTCTTTTCA				
Ppe165	F: M-CCTTCTTCCATATTTATGACTCCA	14	0.895	0.896	GU066298
	R: CAGTGCTGGACCTTCAGTGT				
Ppe168	F: M-GGGTTTCCCCAGTACCAGTC	12	0.840	0.833	GU066299
	R: TCCCATTTTGTAAGTCGCTGT				
Ppe186	F: M-CAAAGTTGAAAGGTGGTTGGA	19	0.919	0.966	GU066300
	R: AATGATGCCCAAATCCAAAA				

"M" indicates the M13 primer was added to the 5′ end of the primer. All reverse primers were tailed with GTGTCTT on the 5′ end (see text)

Table 3 Average pairwise genetic distance (Kimura-2 parameter distance) between conspecifics for each of the sawfish species using 520 bp of cytochrome b sequence

Species	D
Pristis pectinata	0.003
P. perotteti	0
P. clavata	0
P. microdon	0.001
P. zijsron	0.006
Anoxypristis cuspidata	0.002

Table 4 Average interspecific pairwise genetic distances (Kimura-2 parameter distance) between sawfish using 520 bp of cytochrome b (Ppec = *Pristis pectinata*, Pper = *P. perotteti*, Acus = *Anoxypristis cuspidata*, Pcla = *P. clavata*, Pmic = *P. microdon*, Pzij = *P. zijsron*)

	Ppec	Pper	Pcla	Pmic	Pzij
Pper	0.136				
Pcla	0.115	0.160			
Pmic	0.136	0.014	0.160		
Pzij	0.107	0.143	0.127	0.143	
Acus	0.137	0.176	0.160	0.181	0.162

Discussion

Wildlife products originating from rare species, like *P. pectinata*, are extremely difficult to detect in trade when their presence is potentially obscured by morphologically similar products from other more common species. For example, even though there was virtually no evidence at the time that fins from protected white sharks (*Carcharodon carcharias*) occurred in the US multi-species shark fin trade, the development of a PCR-based diagnostic assay for this species (Chapman et al. 2003) soon revealed their presence (e.g. Shivji et al. 2005, Shivji unpublished data). This illustrates the importance of developing DNA-based identification assays for protected species even when the threat posed by trade is uncertain.

We developed a primer pair that can provide a partial cytochrome b sequence that is useful as a DNA barcode to distinguish individual sawfish species. This sequence can be used by law enforcement to identify species-of-origin from any illegally traded sawfish body part, which is important because all of these species are listed on the appendices of CITES (i.e. all international trade is prohibited or restricted). However, although DNA sequencing costs have dramatically decreased in the past few years,

Table 5 Cross species amplification of microsatellite loci originally isolated from a *Pristis pectinata* genomic library in other sawfish species. Loci that did not amplify are indicated by a "–"

Species	Ind #	Ppe5	Ppe88	Ppe114	Ppe122	Ppe165	Ppe168	Ppe186
A. cuspidata	1	–	–	–	256/264	–	–	–
A. cuspidata	2	–	–	–	268/284	–	–	–
A. cuspidata	3	–	–	–	240/248	–	–	–
A. cuspidata	4	–	–	–	248/260	–	–	–
A. cuspidata	5	–	–	–	236/268	–	–	–
P. clavata	1	321/351	–	280/296	223/227	296/328	–	266/272
P. clavata	2	333/337	–	268/276	231/235	316/316	–	254/286
P. clavata	3	313/321	–	264/292	231/235	312/320	–	254/270
P. clavata	4	313/329	–	–	223/227	304/312	–	252/278
P. clavata	5	317/337	–	264/264	223/227	296/300	–	250/254
P. microdon	1	313/353	–	P	231/237	–	275/275	245/253
P. microdon	2	299/337	–	P	235/239	–	275/275	245/285
P. microdon	3	313/321	–	P	235/251	–	249/275	253/285
P. microdon	5	283/337	–	P	235/251	–	251/267	269/285
P. zijsron	2	–	339/351	–	–	286/286	253/253	310/310
P. zijsron	3	–	285/351	–	–	278/294	253/253	302/314
P. zijsron	4	–	327/347	–	–	284/314	253/253	310/314
P. zijsron	5	–	289/343	–	–	260/286	253/253	314/340

Ind ID# denotes different test individuals for a given species. "P" indicates that the locus amplified but was difficult to score. Numbers indicate allele size in base pairs. Four loci (Ppe77, Ppe107, Ppe157, and Ppe160) did not amplify in any other species

barcoding is likely to be prohibitively expensive for screening a large enough volume of shark fins or meat in trade to detect what is likely to be a comparatively rare occurrence of sawfish. At present, the adoption of routine genetic testing of the shark fin and meat trades for the illicit presence of sawfish requires a more cost-effective approach than DNA-barcoding, at least for the initial detection phase. A PCR assay for *P. pectinata* similar to the *C. carcharias* PCR assay described previously could provide a cost-effective means to monitor the shark fin trade and objectively assess the threat this trade poses to the recovery of *P. pectinata* in the US Atlantic.

To address this issue, we developed a simple-to-perform, economical PCR assay that can rapidly and reliably identify tissues of *P. pectinata* from the US Atlantic. All *P. pectinata* samples that we tested exhibited the expected 281 bp species-specific cyt b amplicon and the non species-specific ~950 bp ITS2 amplicon. Notably, our samples originated from a wide geographic range in Florida (the Dry Tortugas to the Florida Panhandle) that encompassed the known US breeding grounds (Simpfendorfer 2000; Seitz and Poulakis 2002; Poulakis and Seitz 2004). In addition, the assay successfully amplified two specimens from the western Bahamas. None of the other non-target elasmobranchs tested, which constitute most of the species harvested in US commercial and recreational shark fisheries (Hale and Carlson 2007; Morgan et al. 2009), exhibited the 281 bp species-specific amplicon, despite the presence of the general ITS2 amplicon in all samples. Notably, we also found that a rapid (~15 min) DNA isolation protocol using the DirectAmp kit provided sufficient quantity and quality of DNA for this assay from sawfish and shark tissues, including dried fins.

The polymorphic microsatellite loci we isolated provide genetic markers that will be useful for a variety of conservation and trade-monitoring applications for *P. pectinata*. These markers are sufficiently polymorphic to permit robust identification of individuals, which could be useful for determining how many individual fish contribute to products such as fins and processed meat intercepted in trade. The high parental exclusionary power of these markers also demonstrates that if captive breeding is ever pursued for this species (i.e. in order to restock wild populations), then they would be useful for verifying studbooks and guiding breeding programs aimed at maximizing genetic diversity. Moreover, these markers enable assessment of genetic diversity and population structure in wild *P. pectinata*, which will be useful for informing future management activities. In addition, we show that from four to five loci can be amplified in each of the Indo-Pacific *Pristis* species, all of which could help to enable similar research and trade monitoring for these endangered sawfish as well.

Although there is evidence that many elasmobranchs have declined in response to fishing pressure in the US Atlantic (e.g. NMFS 2006; Cortés et al. 2006; Hayes et al. 2009), *P. pectinata* are arguably among the closest to actual biological extinction. The prevention of illegal landings and trade will be an integral component of the successful management of this species, while insights into population structure and genetic diversity are also necessary. The suite of genetic tools provided here will enable this type of research and management of *P. pectinata* in the US and will also contribute directly or as a model for the management of endangered sawfish elsewhere.

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