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ARTICLE

Molecular Identification of Hammerhead Shark Trunks from the Southern Gulf of California using Multiplex PCR

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ABSTRACT

The demand for shark fins in Asiatic markets has resulted in excessive increases in shark catches, even for species that may be under protection or subject to management. As such, it has been necessary to develop and promote monitoring efforts for exploited species and taxonomic groups in order to improve fishing management strategies for elasmobranchs. Identifying species from landings is one of many fishing management problems because landed organisms have usually already been processed and are therefore incomplete, which makes identification problematic, impedes the generation of proper species records, and leads to poor fishery assessments. Tools that can correctly identify species, such as various molecular techniques, have become essential for accurate fishery assessments. In this study, 30 hammerhead trunks from artisanal fisheries from the southern portion of the Gulf of California were identified using multiplex PCR (17 *Sphyrna lewini* and 13 *Sphyrna zygaena*). The total fee to identify each trunk with this technique was ~ $3.80 and the procedure required 2 to 5 days. When compared with other widely-used methods, such as PCR-RFLP or barcoding, multiplex PCR is fast, efficient, low-cost, and easy to implement in a laboratory.

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1. Introduction

Shark landings have increased in recent decades due to the demand for shark fins in Asiatic markets and shark meat in locations, such as Hong Kong, China, and Korea [1-6]. Shark meat is a relatively inexpensive protein source and is consumed by both humans and domesticated animals [7]. Sharks fins are a traditional ingredient in Chinese cuisine and are considered to possess beneficial properties[8]. Moreover, shark fins continue to be viewed as status symbol due to the associated exclusiveness and exoticism of the product and shark fins are judged based on the length, thickness, and texture of their fin needles, or ceratotrichia [8]. Not all the larger shark species possess the most attractive fin needles, but a particular high demand exists for the cartilaginous ceratotrichia found on of the Sphyrnidae family, specially in the dorsal fin and lower lobe of the caudal fin[8-10].

In Mexico, shark fisheries represent an important economic activity. Along the Mexican Pacific, hammerhead sharks comprise up to 30% of elasmobranch catches [11]. The scalloped hammerhead, *Sphyrna lewini*, and the smooth hammerhead, *Sphyrna zygaena*, make up the majority of catches for this group. The distributions of these species primarily overlap along the coast of Sinaloa [12]. Although these two species are morphologically distinct as adults (*S. lewini* has an extra notch in the center of the head), juveniles and shark trunks are difficult to identify in the field and in landings [3,13]. Since 2014, both *S. lewini* and *S. zygaena* have been listed in CITES Appendix II with the specification that their commercial exploitation must not be detrimental to the survival of their populations [11]. Therefore, accurate fishery assessments are necessary for the effective management and conservation of these species and depend on the correct identification of landed specimens [14].

In developing countries few records of species-specific shark catches are available as official statistics only report landings based on common or group names (i.e., a species complex). Moreover, the lack of biological data and the misidentification of organisms and body parts has hindered the proper identification of species in landings [15]. For example, hammerhead fisheries often land sharks without heads, trunks, or fins or eviscerated sharks, making species identification problematic [1,16,17]. These factors have led to questionable fishery assessments and the biological characteristics of a species are generally not considered in management plans [1,16,18]. As such, the implementation of new tools to identify species in landings, such as the use of molecular markers, is essential for reliable fishery assessments and effective management plans. Molecular markers are species-specific and may be used by any molecular lab with the necessary equipment to identify organisms within hours or days given that their use requires following established protocols [3,19,20,21,22]. Furthermore, the use of low-cost molecular tools in developing countries where resources are often limited may facilitate the proper management and conservation of fishery resources [3,23].

Molecular methods of organism identification are diverse, yet the most common method is DNA barcoding, which is comprised of a PCR and the subsequent sequencing of a DNA fragment (COI and/or rDNA[15,22,24]). Techniques like PCR-RFLPs that involve a PCR, enzyme digest, and agarose electrophoresis [25] reduce costs and sample processing times. Furthermore, multiplex-PCR allows for the identification of multiple species in a single PCR reaction using a primer set that amplifies target sequences from multiple species at an affordable cost [3,23,26]. Although some multiplex PCR primers have been validated, multiplex-PCR is not commonly used for the identification of shark trunks or fins in shark fishery assessments [26,27].

Multiplex PCR is an accurate, rapid, and simple technique that may be implemented in any molecular biology lab to identify pelagic shark species [2]. The technique consists in a single PCR reaction with a primer set to simultaneously discriminate between the DNA of different species. The primer design is derived from DNA sequences that have been previously identified for each species. Each primer only hybridizes target DNA and additional primer design involves amplifying fragments of different sizes for each species. Therefore, a subsequent agarose or polyacrylamide electrophoresis is enough to identify the species present in the sample without a further manipulation of amplicons by restriction endonuclease digestion or sequencing [2].

Species of Sphyrnidae family are of the most caught sharks worldwide, and a large percentage of the fins sold in Asian markets come from this family [3,21,28,29]. Given their importance, identification protocols using molecular techniques have been implemented for sphyrnid species and have primarily consisted of DNA barcoding and multiplex PCR[3,19,21,27,29-37]. In fact, species-specific primers and a five-primer multiplex PCR have been developed and extensively tested worldwide for *S. lewini*, *S. mokarran*, and *S. zygaena* [3].

This work aimed to identify the trunks of hammerhead sharks from artisanal fisheries in the southern portion of

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the Gulf of California in Mexico using multiplex-PCR. This technique has been shown to identify sphyrnid species correctly, efficiently, and reliably from a variety of tissue types [3] and is thus amply suited for the identification of hammerhead sharks from Mexican artisanal fisheries. The results of this study provide an efficient solution to the identification problems associated with landed sphyrnid organisms as well as samples from ichthyological collections. A proper record of sphyrnid catches in Mexico will improve landing records and consequently improve fishery assessments and management in the country.

2. Material and Methods

Thirty hammerhead trunk samples from the Mexican artisanal fisheries of Mazatlán (23°12'18.66''N, 106°24'36.35''W) and Teacapán (22° 31'45.44''N, 105°44'09.53''W) in Sinaloa, and from Santa Rosalia (27°20'13.72''N, 112°15'45.79''W) in Baja California Sur (Figure 1) were collected. Some of the sample trunks were identified by fishers using local species names (hammerhead, *Sphyrna lewini*, and the black hammerhead, *S. zygaena*).

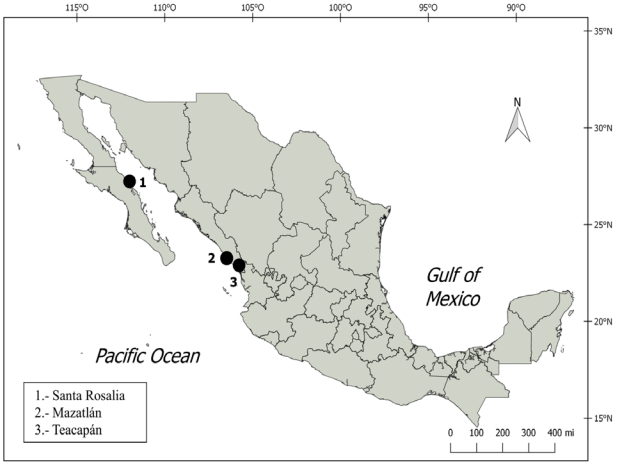

Figure 1. Location of sample sites in the Gulf of California

Tissues from Sinaloa were collected in 2017 and kept in salt-saturated DMSO (SSD: saturated NaCl, ethylenediaminetetra-acetic acid, and dimethylsulfoxide 20%) and preserved at room temperature before being placed in long-term storage at 4 °C. Tissues from Baja California Sur were collected in 2011 and stored in ethanol at room temperature. A Student´s t-test was used to compare DNA quality and quantity between the different preservation methods (salt-saturated DMSO or ethanol; Table 1 [38]).

Table 1. Concentration and quality of hammerhead shark DNA. DNA quality is measured by the absorbance rate A260/A280. Sample IDs are composed of species abbreviations (SZY= S. *zyganea*; SLE= S. *lewini*), sample location (IP= Isla de la Piedra; TE= Teacapán; SR= Santa Rosalía), and consecutive number. Bold sample IDs correspond to the samples used in Figure 2. Results of the t-test to detect differences among preservation methods: DNA quality (t= 1.57; p= 0.13) and concentration (t= -0.46; p= 0.64)

ID sample	Sample year	Preservation Solution	Quality	Concentration (ng/µl)
SLETE-25	2017	SSD	1.95	774.2
SLETE-26	2017	SSD	1.98	1292.6
SLETE-27	2017	SSD	2.01	844.1
SLETE-28	2017	SSD	**1.98**	**222.8**
SLETE-29	2017	SSD	1.98	565.5
SLETE-30	2017	SSD	1.94	1560.1
SLETE-31	2017	SSD	1.99	392.9
SLETE-32	2017	SSD	**1.59**	**2055.7**
SLETE-33	2017	SSD	2.05	532.8
SLETE-34	2017	SSD	**1.97**	**742.9**
SLETE-35	2017	SSD	2.06	433.8
SLEIP-29	2011	SSD	2.03	173.0
SLEIP-30	2011	SSD	2.00	53.8
SLEIP-31	2011	SSD	2.06	206.8
SLEIP-32	2011	SSD	**2.1**	**184.8**
SLEIP-33	2011	SSD	2.08	201.1
SLEIP-34	2011	SSD	2.00	156.5
SZYIP-23	2011	SSD	1.98	185.6
SZYIP-24	2011	SSD	2.07	120.7
SZYSR-1	2011	ethanol	2.08	335.9
SZYSR-2	2011	ethanol	2.04	228.4
SZYSR-3	2011	ethanol	1.99	360.7
SZYSR-4	2011	ethanol	**2.05**	**151.0**
SZYSR-6	2011	ethanol	**1.99**	**239.1**
SZYSR-7	2011	ethanol	**1.98**	**417.2**
SZYSR-8	2011	ethanol	2.01	478.6
SZYSR-9	2011	ethanol	**1.91**	**655.8**
SZYSR-10	2011	ethanol	2.00	161.5
SZYSR-11	2011	ethanol	2.04	134.0
SZYSR-12	2011	ethanol	1.98	85.8
Average			2.00	464.9

Genomic DNA was extracted from 50 mg of tissue using standard proteinase K digestion and purified with a lithium chloride salting-out protocol, followed by organic extraction using chloroform-isoamyl alcohol and subsequent ethanol precipitation [39]. Nuclear ribosomal DNA

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(rDNA) was amplified. We used a PCR assay with three optimal species-specific primers (*S. lewini*, *S. zygaena*, and *S. mokarran*) combined in a 5-primer (pentaplex) reaction [3]. The pentaplex PCR included: shark universal primers FISH5.8S-F (5´-TTAGCGGTGGATCACTCG-GCTCGT-3´) and FISH28S-R (5´-TCCTCCGCTTAG-TAATATGCTTAAATTCAGC-3´ [23]) to amplify an rDNA fragment containing the entire nuclear ribosomal DNA internally transcribed spacer (*ITS*2) region plus short portions of the flanking *5.8S* and *28S* ribosomal RNA genes, and the species-specific primers for *S. lewini* (with a *ITS*2 fragment of 445bp; ScHH401F 5´-GGTAAAG-GATCCGCTTTGCTGGA-3), *S. mokarran* (with a *ITS*2 fragment of 782bp; GtHH123F 5´-AGCAAAGAGCGT-GGCTGGGGTTTCGA-3´), and *S. zygaena* (with a frag-ment of 249bp; SmHH630 5´-TGAGTGCTGTGAGGG-CACGTGGCCT-3´ [3]).

Nuclear rDNA has been extensively tested as a shark species diagnostic tool and has proven to be 100% accu-rate for identification [2,3,23]. The advantage of using inter-genic regions, such as *ITS*2, is the presence of multiple insertion and deletions (indels), which are rare and reduce erroneous identification between DNA samples of differ-ent species because each species is tagged with a numeric profile of fragment lengths.

All PCR reactions contained 0.2 mM dNTPs, 1x PCR buffer (10 mM Tris HCL, 50 mM KCl and 1.5 mM $MgCl_2$), 0.4 µM of each primer, 0.5 U of Taq DNAPol (NEB, Ipswich, MA), and 10 ng of template DNA in a to-tal volume of 10 µl. Amplifications were performed with the following thermal profiles: 94 ºC for 2 min, 35 cycles of 95 ºC for 1 min, 65 ºC for 1 min, and 72 ºC for 2 min, followed by a final incubation at 72 ºC for 10 min.

Positive DNA controls of previously identified sphyr-nids (*S. lewini* and *S. zygaena*) and negative controls with no target shark species were included in all PCRs. The sphyrnid specimens that were used as positive controls were collected from an artisanal fishery in Mazatlán. These specimens were complete specimens and were positively identified morphologically by a specialist. The *ITS*2 region of the DNA samples collected from these specimens was sequenced to confirm their morphological identification. These samples were used as positive con-trol in each PCR multiplex to identify thirty hammerhead trunk samples.

3. Results and Discussion

A total of 30 samples were successfully identified to the species level using the pentaplex method proposed by Ab-ercrombie [3]. The amplified samples presented 2-3 rDNA fragments (Fig. 2). Bands of ~900 bp were complete fragments of the *ITS*2 region for both species, while the smaller bands were species-specific. The rDNA fragments of 249 bp pertained to *S. zygaena*, while fragments of 445 bp pertained to *S. lewini*. In some samples, we observed bands of ~2000 bp, which represented the unspecific amplification of a region of rDNA. Of the 30 samples analyzed, 17 pertained to *S. lewini* (6 from Mazatlán and 11 from Teacapán) and 13 to *S. zygaena* (2 from Mazat-lán and 11 from Santa Rosalia). The DNA samples of the adult *S. lewini* and *S. zygaena* organisms that had been correctly identified and used as the positive controls in the multiplex PCR were used to confirm the corresponding *ITS*2 fragments and multiplex PCR results, as has been done in previous studies [3,35].

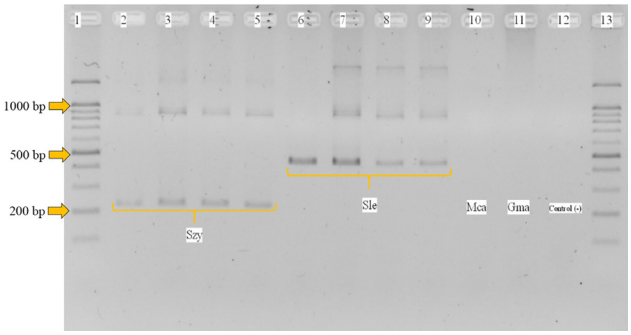

Figure 2. Agarose electrophoresis (2%) showing multi lex PCR results. Lane (1) DNA ladder, lanes (2-5) rDNA of *S. zygaena* (Szy), lanes (6-9) rDNA of *S. lewini* (Sle), lanes (10-11) samples no target (Mca=*Mustelus californicus* and Gma= *Gymnura marmorata*), lane (12) negative control, lane (13) DNA ladder. Amplicons of universal primers were~900 bp.

Multiplex PCR and electrophoresis allowed us to identify hammerhead shark species quickly, accurately, and at low cost. Furthermore, the advantages associated with multiplex PCR allowed us to make proper use of the samples collected along the Mexican Pacific, which may not have been possible with other methods. Our results, and those of previous studies, indicate that multiplex PCR can serve as a useful monitoring tool to identify ham-merhead shark meat and fins in landings, markets, and in mislabeled products worldwide [3,19,40]. This application is particularly important because *S. lewini*, *S. zygaena*, and *S. mokarran* are listed in CITES Appendix II and their commercialization is regulated. In Mexico, the molecular identification of hammerhead sharks using multiplex PCR during the seasonal elasmobranch fishing ban (from May 1st to July 31st; DOF 2007) will support law enforcement and resource management efforts. Given the results of our study, we suggest that multiplex PCR should be used to identify hammerhead species in future evaluations of

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hammerhead landings in the Mexican Pacific and elsewhere.

Although a higher concentration of DNA was observed when samples were preserved with SSD than with ethanol, a statistical comparison between the preservation methods found no significant differences in either DNA concentration or quality (Table 1). In the absence of an experimental test for the preservation efficiency of target tissues, the use of either ethanol or the SSD buffer is appropriate as neither method compromises the effectiveness of amplification [41]. This is also true for samples that have been stored for several years.

The multiplex PCR results agree with the distributions of both species near the mouth of the Gulf of California. Artisanal fisheries land both *S. lewini* and *S. zygaena* in this region and particularly in Sinaloa [42,43]. The organisms collected in Tecapán were *S. lewini* juveniles, which also agree with previous studies that have designated the Tecapan lagoon system as a possible nursery area for this species [42,44]. The organisms collected in Santa Rosalia were *S. zygaena* individuals, which coincides with the distribution of this species in temperate zones [45]. As expected, *S. mokarran* was not present among the sampled tissues. This is likely because there have been few records of *S. mokarran* in the Mexican Pacific over the last two decades [46] and there have only been a few catch records in the southern Mexican Pacific [12,47]. While *S. media* and *S. tiburo* showed high catch rates in 1960s in the Gulf of California, no catch records for these species are available for the last decade. As such, these species have likely been extirpated from the Gulf of California [12,46]. According with this historic data, we did not expect to find these species among landed specimens.

Multiplex PCR produces accurate and efficient results after the designation of species-specific molecular markers, which requires a preliminary study involving the comprehensive sampling of organisms and loci. The disadvantages to using multiplex PCR include the initial investment costs required to develop the primers and that it can only be currently used to identify a small group of hammerhead species [2,3,19]. By taking into account species distributions and regional fishing records, it is possible to generate protocols for a given region. This implies that shark species identification protocols using multiplex PCR can be generated to identify species among samples, and that only those samples that produce questionable results may need to be sequenced[27]. A potential disadvantage to multiplex PCR is the possible presence of inhibitory substances in the DNA template for PCR. Similarly, errors in setting up the reaction may result in false negatives and the erroneous interpretation of the absence of target species. Even though preliminary studies involve an additional investment, multiplex PCR remains the best approach to rapidly identify species of importance to conservation efforts or commercial interests at an overall low cost.

We evaluated the time and costs associated with multiplex PCR and found that the generation of results requires 2-5 days, depending on the DNA extraction method, at an expense of ~ $3.80 (reagent cost) per sample. When comparing multiplex PCR with other methods, such as PCR-RFLPs or DNA barcoding, the associated costs are lower for multiplex PCR. For example, PCR-RFLPs require DNA extraction, a PCR ($3.80 per sample), and an enzyme digest, which carries an additional charge of 0.60 cents per enzyme and adds an additional day to the time required for the analysis. The DNA barcoding method requires DNA extraction and a PCR ($3.80 per sample), but it also requires Sanger sequencing, which brings an additional fee of $8.00 (http://www.macrogen.com) and 2-3 additional days are required to send sample by mail and have it processed in an external laboratory. Furthermore, PCR-RFLPs are generally inefficient because some enzymes can produce similar banding, making it necessary to use more than one enzyme [48], which is reflected in increased laboratory costs. Likewise, barcoding requires the use of external sequencing services to obtain the final result, which implies not only extra cost but also additional time.

Identifying species from landings is one of many common fishing management problems. Landed organisms have usually already been processed and are therefore incomplete, which makes identifying species highly problematic. Hammerhead sharks in landings are particularly difficult to identify morphologically as they are often landed without heads. Therefore, molecular tools that can correctly identify species, such as multiplex PCR, have become essential for better catch records. Given that the species-specific primers used in our study have been extensively tested and have been proven to be reliable[3,19,27], we were able to identify 30 hammerhead trunks from artisanal fisheries from the southern portion of the Gulf of California using multiplex PCR (17 *Sphyrna lewini* and 13 *Sphyrna zygaena*).

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Declaration of Interest: none.

Authors Contribution

TAAR, JJRH and VOG manuscript preparation and data analysis, ECOG and ODD data collection and manuscript preparation, and NCSS manuscript preparation and funding.

References

[1] Bonfil R. Overview of world elasmobranch fisheries[R]. FAO Fisheries Technical Paper No. 341. Roma, 1994.

[2] Shivji M, Clarke S, Pank M, Natanson L, Kohler N, Stanhope M. Genetic identification of pelagic shark body parts for conservation and trade monitoring[J]. Conservation Biology, 2002, 16: 1036-1047. DOI: 10.1046/j.1523-1739.2002.01188.x

[3] Abercrombie D, Clarke SC, Shivji MS. Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement[J]. Conservation Genetics, 2005, 6: 775-788. https://doi.org/10.1007/s10592-005-9036-2

[4] Lack M, Sant G. Trends in global shark catch and recent developments in management[M]. TRAFFIC International, 2009: 30.

[5] Musick JA, Musick S. Sharks[R]. FAO Fisheries and Aquaculture Reviews and Studies. FAO. Rome, 2011.

[6] Dent F, Clarke S. State of the global market for shark products[R]. FAO Fisheries and Aquaculture Technical Paper No. 590. FAO. Rome, 2015: 187.

[7] Dulvy NK, Simpfendorfer CA, Davidson LNK, Fordham SV, Bräutigam A, Sant G, Welch DJ. Challenges and Priorities in Shark and Ray Conservation[J]. Current Biology, 2017, 27, 11: R565-R572. https://doi.org/10.1016/j.cub.2017.04.038

[8] Clarke SC, Milner-Gulland EJ, BjØrndal T. Social, Economic, and Regulatory Drivers of the Shark Fin Trade[J]. Marine Resource Economics, 2007, 22, 3: 305-327.

[9] Musick JA. Shark utilization. In Musick J and Bonfil R (eds) Elasmobranch fisheries management techniques[R]. FAO Fisheries Technical Paper. No. 474. Rome, 2005.

[10] Steinke D, Berdard AM, Horn RL, Hilton P, Hanner R, Shivij MS. DNA analysis of traded shark fins and mobulid gill plates reveals a high proportion of species of conservation concern[J]. Scientific Reports, 2017, 7(9505). https://doi.org/10.1038/s41598-017-10123-5

[11] Castillo-Géniz JL, Tovar-Ávila J. Tiburones mexicanos de importancia en la CITES. [R]. Instituto Nacional de la Pesca. México, 2016: 11-15.

[12] Pérez-Jiménez JC. Historical records reveal potential extirpation of four hammerhead sharks (Sphyrna spp.) in Mexico Pacific waters[J]. Reviews in Fish Biology and Fisheries, 2014, 24: 671–683. https://doi.org/10.1007/s11160-014-9353-y

[13] Castro JI. The Sharks of North American Waters[M]. Texas: Texas A&M University Press, College Station, 1983: 180.

[14] Dudgeon CL, Blower DC, Broderick D, Giles JL, Holmes BJ, Kashiwagi T, Krück NC, Morgan JAT, Tillett BJ, Ovenden JR. A review of the application of molecular genetics for fisheries management and conservation of sharks and rays[J]. Journal of Fish Biology, 2012, 80: 1789-1843. DOI: 10.1111/j.1095-8649.2012.03265.x

[15] Holmes HH, Steinke D, Ward RD. Identification of shark and ray fins using DNA barcoding[J]. Fisheries Research, 2009, 95, 2–3: 280-288. https://doi.org/10.1016/j.fishres.2008.09.036

[16] Castro JI, Woodley C, Brudek R. A preliminary evaluation of the status of shark species[R]. FAO Fisheries Technical, Roma, 1999, 380: 72.

[17] INP. Plan de Manejo de la Pesquería de Tiburones y Rayas del Pacífico Mexicano[R]. Dirección General Adjunta de Investigación Pesquera en el Pacífico. México: Instituto Nacional de Pesca, 2012.

[18] Smith E, Au D, Show C. Intrinsic rebound potentials of 26 species of Pacific sharks[J]. Marine and Freshwater Research, 1998, 49, 7: 663 - 678. https://doi.org/10.1071/MF97135

[19] Clarke SC, Magnussen JE, Abercrombie DA, McAllister MK, Shivji MS. Identification of shark species composition and proportion in the Hong Kong shark fin market base on molecular genetics and trade records[J]. Conservation Biology, 2006, 20: 201-211. DOI: 10.1111/j.1523-1739.2005.00247.x

[20] Liu SYV, Chan CLC, Lin O, Hu CS, Chen CA. DNA barcoding of shark meats identify species composition and CITES-listed species from the markets in Taiwan[J]. PLOS ONE, 2013, 8, 11: e79373. https://doi.org/10.1371/journal.pone.0079373

[21] Chuang PS, Hung TC, Chang HA, Huang CK, Shiao

JC. The species and origin of shark fins in Taiwan´s fishing ports, markets, and customs detention: a DNA barcoding analysis[J]. PLOS ONE, 2016, 11, 1: e0147290.
https://doi.org/10.1371/journal.pone.0147290

[22] Feitosa LM, Barbosa-Martins AP, Giarrizzo T, Macedo W, Monteiro IL, Gemaque R, Silva-Nuñes JL, Gomes F, Schneider H, Sampaio I, Souza R, Sales JB, Rodrigues-Filho LF, Tchaicka L, Carvalho-Costa LF. DNA-based identification reveals illegal trade of threatened shark species in a global elasmobranch conservation hotspot[J]. Scientific Reports, 2018, 8: 3347.
https://doi.org/10.1038/s41598-018-21683-5

[23] Pank M, Stanhope M, Natanson L, Kohler N, Shivji M. Rapid and simultaneous identification of body parts from the morphologically similar sharks Carcharhinus obscurus and Carcharhinus plumbeus (Carcharhinidae) using multiplex PCR[J]. Marine Biotechnology, 2001, 3: 231–240.
https://doi.org/10.1007/s101260000071

[24] Chapple DG, Ritchie PA. A retrospective approach to testing the DNA barcoding method[J]. PLOS ONE, 2013, 8, 11: e77882.
https://doi.org/10.1371/journal.pone.0077882

[25] Mendonça FF, Hashimoto DT, Porto-Foresti F, Oliveira C, Gadig OB, Foresti F. Identification of the shark species Rhizoprionodon lalandii and R. porosus (Elasmobranchii, Carcharhinidae) by multiplex PCR and PCR-RFLP techniques[J]. Molecular Ecology Resources, 2009, 9: 771-773.
DOI: 10.1111/j.1755-0998.2009.02524.x

[26] Caballero S, Cardeñosa D, Soler G, Hyde J. Application of multiplex PCR approaches for shark molecular identification: feasibility and applications for fisheries management and conservation in the Eastern Tropical Pacific[J]. Molecular Ecology Resources, 2012, 12: 233-237.
DOI: 10.1111/j.1755-0998.2011.03089.x

[27] Sebastian H, Haye PA, Shivhi MS. Characterization of the pelagic shark-fin trade in north-central Chile by genetic identification and trader surveys[J]. Journal of Fish Biology, 2008, 73: 2293–2304.
DOI: 10.1111/j.1095-8649.2008.02016.x

[28] Clarke SC, McAllister MK, Milner-Gulland EJ, Kirkwood GP, Michielsens CGJ, Agnew DJ, Pikitch EK, Nakano H, Shivji MS. Global estimates of shark catches using trade records from commercial markets[J] Ecology Letters, 2006, 9: 1115-1126.
DOI: 10.1111/j.1461-0248.2006.00968.x

[29] Sembiring A, Pertiwi NPD, Mahardini A, Wulandari R, Kurniasih EM, Kuncoro AW, Cahyani NKD, Anggoro AW, Ulfa M, Madduppa H, Carpenter KE, Barber PH, Mahardika GN. DNA barcoding reveals target fisheries for endangered sharks in Indonesia[J]. Fisheries Research, 2015, 164: 130-134.
https://doi.org/10.1016/j.fishres.2014.11.003

[30] da Silva Rodrigues-Filho LF, da Rocha TC, Sena do Rêgo P, Schneider H, Sampaio I, Vallinoto M. Identification and phylogenetic inferences on stocks of sharks affected by the fishing industry off the Northern coast of Brazil[J]. Genetics and Molecular Biology, 2009, 32, 2: 405-413.
DOI: 10.1590/S1415-47572009005000039

[31] Jabado RW, Al Ghais SM, Hamza W, Herderson AC, Spaet JLY, Shivji MS, Hanner RH. The trade in sharks and their products in the United Arab Emirates[J]. Biological Conservation, 2015, 181: 190-198.
https://doi.org/10.1016/j.biocon.2014.10.032

[32] Vélez-Zuazo X, Agnarsson I. Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes)[J]. Molecular Phylogenetics and Evolution, 2011, 58, 2: 207-217.
https://doi.org/10.1016/j.ympev.2010.11.018

[33] Bineesh KK, Gopalakrishnan A, Akhilesh KV, Sajeela KA, Abdussamad EM, Pillai NGK, Basheer VS, Jena JK, Ward RD. DNA barcoding reveals species composition of shark and rays in the Indian commercial fishery[J]. Mitochondrial DNA Part A, 2016, 28(4): 458-472.
DOI: 10.3109/19401736.2015.1137900

[34] O´Bryhim JR, Parsons ECM, Lance ST. Forensic species identification of elasmobranch products sold in Costa Rica markets[J]. Fisheries Research, 2017, 186, 1: 144-150.
https://doi.org/10.1016/j.fishres.2016.08.020

[35] Almerón-Souza F, Sperb C, Castillo CL, Figueiredo PICC, Gonçalves LT, Machado R, Oliveira LR, Valiati VH, Fagundes JR. Molecular identification of shark meat from local markets in Southern Brazil base on DNA Barcoding: evidence for mislabeling and trade of endangered species[J]. Frontiers in Genetics, 2018, 9: 138.
https://doi.org/10.3389/fgene.2018.00138

[36] Appleyard SA, White WT, Vieira S, Sabub B. Artisanal shark fishing in Milne Bay Province, Papua New Guinea: biomass estimation from genetically identified shark and ray fins[J]. Scientific Reports, 2018, 8, 1: 6693.
DOI: 10.1038/s41598-018-25101-8

[37] Kuguru G, Maduna S, da Silva C, Gennari E, Rhode C, Bester-van der Merwe A. DNA barcoding of chondrichthyans in South African fisheries[J]. Fisheries Research, 2018, 206: 292-295.

DOI: https://doi.org/10.30564/jfsr.v2i1.1685

https://doi.org/10.1016/j.fishres.2018.05.023

[38] Zar JH. Biostatistical analysis[M]. 3rd ed. Prentice Hall Internationl. London, 1996: 944.

[39] Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques[J]. Nucleic Acids Research, 1997, 25(2): 4692–4693.
https://doi.org/10.1093/nar/25.22.4692

[40] Chapman D, Abercrombie D, Douady C, Pikitch E, Stanhope M, Shivji M. A streamlined, bi-organelle, multiplex PCR approach to species identification: application to global conservation and trade monitoring of the great white shark, Carcharodon carcharias[J]. Conservation Genetics, 2003, 4: 415–425.
https://doi.org/10.1023/A:1024771215616

[41] Gaither MR, Szabó Z, Crepeau MW, Brid CE, Toonen RJ. Preservation of corals in salt-saturated DMSO buffer is superior ethanol for PCR experiments[J]. Coral Reefs, 2011, 30: 329–333.
https://doi.org/10.1007/s00338-010-0687-1

[42] Bizzarro JJ, Smith WD, Castillo–Géniz JL, Ocampo-Torres A, Márquez–Farías JF, Hueter RE. The seasonal importance of small coastal sharks and rays in the artisanal elasmobranch fishery of Sinaloa, Mexico[J]. Pan-American Journal of Aquatic Sciences, 2009, 4(4): 513-531.

[43] Corro-Espinosa D. La importancia pesquera de los tiburones incluidos en el apéndice II de la CITES en aguas de México. Sinaloa[M]. In: Castillo-Géniz JL and Tovar-Ávila J (eds) Tiburones mexicanos de importancia en la CITES. Instituto Nacional de Pesca, Mexico, 2016: 29 – 93.

[44] Coiraton C, Amezcua F, Salgado-Ugarte IH. Estructura de longitudes de las capturas del tiburón martillo común (*Sphyrna lewini*) en el Pacífico mexicano[J]. Ciencia Pesquera, 2017, 25: 27-40.

[45] Castro JI. Sharks of North America[M]. Oxford University Press USA, 2011: 613.

[46] Saldaña-Ruíz LE. The artisanal shark fishery in the Gulf of California: Historical catch reconstruction and vulnerability of shark species to the fishery[D]. Doctor of Science thesis. Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California, México, 2017: 73.

[47] Tovar-Ávila J, Gallegos-Camacho R. Oldest estimated age for *Sphyrna mokarran* (Carcharhiniformes: Sphyrnidae) in the Mexican Pacific[J]. Hidrobiológica, 2014, 24(2): 163-165.

[48] Cocolin L, D'Agaro ED, Manzano M, Lanari D, Comi G. Rapid PCR-RFLP method for the identification of marine fish fillets (Seabass, Seabream, Umbrine, and Dentex)[J]. Journal of Food Science, 2000, 65: 1315-1317.
DOI: 10.1111/j.1365-2621.2000.tb10604.x

 DOI: https://doi.org/10.30564/jfsr.v2i1.1685