Food Control

DNA Barcoding for the Identification of shark lips (鱼唇): a nationwide survey for analyzing a never investigated product in the Chinese market

--Manuscript Draft--

Manuscript Number:	FOODCONT-D-21-00182R1
Article Type:	Research Paper
Keywords:	Shark food products; DNA Barcoding; Prionace glauca; Carcharhinus spp.; Sphyrna spp.; labelling system; fraudulent substitution
Corresponding Author:	Jing Wen Lingnan Normal University Zhanjiang, CHINA
First Author:	Xia Zhang
Order of Authors:	Xia Zhang
	Andrea Armani
	Jing Wen
	Alice Giusti
	Siyun Xie
	Huiru Kang
	Juan Zhao
	Xuyan Li
	Yuqi Yan
Abstract:	Shark food products are extremely popular in China. Fins are especially considered a delicacy, but also other part of the carcass, such as the skin sold as “shark lips”, are consumed. The high fishing pressure has contributed to shark population decreasing, and many species are currently endangered and/or with a strictly regulated commerce. A nationwide survey aimed at authenticating species in shark lips products (鱼唇) (n=252) by full DNA COI barcoding (FDB; 652 bp) is presented. In addition, the efficiency of the FDB and of the mini DNA barcode (MDB; 127 bp) proposed by Fields et al. (2015) (PloS one, 10, e0114844.) in identifying the shark species detected in this study was compared. Despite the manufacturing process, the total DNA of the samples presented a medium low fragmentation degree, and the FDB was obtained from all the samples, which were allocated to species level in 96.4% of the cases. This confirms the importance to perform a preventive evaluation of the level of DNA degradation before selecting cost and time-consuming procedures. Of the 7 identified species, Prionace glauca was the most recovered (65.5%). The other six detected species were Carcharhinus falciformis (11.5%), Sphyrna lewini (6.7%), S. zygaena (3.6%), Isurus oxyrinchus (3.6%), C. longimanus (3.2%) and C. sorrah (2.4%), 5 of which are threatened and 4 are subject to global commerce regulation. Overall, issues in discriminating among some Carcharhinus spp. were highlighted both using the FDB and the MDB. Outcomes of this study confirms the need to improve the Chinese traceability system. In fact, even though a legislation for seafood labelling supported by an official system for name attribution not always ensure the sector safeguarding from frauds, absent or weak traceability system certainly facilitate illegal practices.

Dear Editor,

please find enclosed the manuscript entitled "*DNA Barcoding for the Identification of shark lips (鱼唇): a nationwide survey for analyzing a never investigated product in the Chinese market*" to be considered for publication in Food Control.

Shark food products are extremely popular in China and the growing demand has contributed to shark population decreasing. Shark fins are especially considered a delicacy in Asia, but also other part of the carcass, such as the skin sold as shark lips, are consumed.

DNA barcoding technique facilitates accurate species identification in seafood, and the standard target for DNA barcoding of animal species is generally a ~650 bp region (full DNA barcode – FDB) of the mitochondrial gene coding for cytochrome c oxidase subunit I (COI). However, mini DNA barcodes (MDB) can be used in processed products with highly degraded DNA.

We present a nationwide survey aimed at authenticating species in 252 shark lips products labelled as *yu chun* (鱼唇) marketed in 31 cities across China by DNA barcoding approach targeting the FDB. In addition, the discriminatory ability of the FDB and MDB proposed by Fields et al. (2015) in identifying the detected shark species, was compared.

To the best of our knowledge, this is the first surveys investigating shark products in mainland China and the first analysing shark lips at global level.

The total DNA of the samples presented a medium low fragmentation degree, and the FDB was obtained from all the samples, which were allocated to species level in 96.4% of the cases. *Prionace glauca* was the most recovered (65.5%). The other six detected species were *Carcharhinus falciformis* (11.5%), *Sphyrna lewini* (6.7%), *S. zygaena* (3.6%), *Isurus oxyrinchus* (3.6%), *C. longimanus* (3.2%) and *C. sorrah* (2.4%), 5 of which are threatened and 4 are subject to global commerce regulation. Both FDB and MDB were proved efficient in discriminating the detected shark species, with some limits related to some *Carcharhinus* spp.

Other than shed a light on a never investigated shark food product, outcomes from this study can contribute to improve the selection of a suitable analytical method for monitoring the illegal traffic of sharks. Moreover, the need to improve the Chinese traceability system was highlighted.

Best Regards,

Jing Wen

Dear Editor, we are sending you back the revised version of the manuscript entitled "***DNA Barcoding for the Identification of shark lips (鱼唇): a nationwide survey for analyzing a never investigated product in the Chinese market*".** The manuscript has been improved according to the reviewers' suggestions.

We thank the reviewers for they useful comments.

Reviewers' comments:

Reviewer #1: The manuscript deals with a nationwide survey for authenticating species in shark lips products by DNA barcoding targeting COI gene. The objective and design of this study is clear and was satisfactorily carried out. The introduction and methods are written nicely with precise language and format.

Minor comments:

The abstract is too long. Please shorten it.

The abstract was shortened (250 words)

Methods:

Add the cat number of all used reagents.

Done

It is unnecessary to mention some references in the methods part such as "Barbuto et al., 2010" in line 159.

In our opinion these references are necessary to support the identity thresholds used in the study.

Line 130, 168 & 335: Subtitles are so long. Shorten them without upsetting the meaning.

Line 130: the subtitle has been modified. A dedicated subsection to the COI amplification and sequencing has been produced.

Line 168: the subtitle has been shortened as requested.

Line 335: the subtitle has been shortened as requested.

Results and discussion: Some ideas are not clear. Please separate results from the discussion part.

Dear Editor, we have gone throughout the entire manuscript trying to solve eventual issues due to lack of clarity. However, also considering the comments of the other 2 reviewers, we prefer to maintain the original version in which Results and Discussion are presented together. We hope you understand our choice.

Also shorten the Caption of figure and tables. You can add symbols for each province instead of its full name (i.e. the first 1 or 2 letters of its name). You can also write these symbols (with

mentioning in the text) on the figure itself instead of numbers. OR remove the name of the province from the caption. Adding it in the text only.

We think that the figure 2 caption should be maintained in the current form as all the reported information are informative. We also think that changing the full province name with symbols may result less intuitive for the reader. The Tables caption were shortened as suggested by the reviewer.

Legend of table 1: Change it to: Shark species included in the Appendix II of the CITES with their IUCN relative conservation status. "Critically endangered; endangered & vulnerable": These can be added in the specified place in the table itself.

Done

Table 4: The same.

Table 4 was not amended as requested because it was impossible according to the lay-out and organization of the Table.

Reviewer #2: This manuscript describes DNA barcoding used to identify shark lips sold on the Chinese market. The manuscript is novel, well-organized and clear. The material and method section is clear and detailed, a large number of samples from 31 cities were collected. The authors did a good job of nationwide survey and comparison on efficiency of FDB and MDB. I am recommending only minor revisions prior to publication.

Line 60: "The trend of globally trading of shark meat reached 42% from 2000 to 2011", increased from?% to 42% from 2000 to 2011?

As highlighted by the reviewer, the term “reached” was not appropriate. The sentence was modified.

Line 231: "For the remain MDB (sample JN16), the nearest match was with Sphyrna lewini, .but the homology score was too low (97.62%) to allow species identification." Table 4, JN16 identified as S. lewini or S. zygaena by BLAST and identified as S. lewini by BOLD, add the Identity (%).

Done

Reviewer #3: In the MS, the authors performed molecular identification of the species used in 252 shark lips products sampled from 31 cities across China. Since shark lips are heavily processed products, the authors, first, evaluated quality and fragmentation of the DNA extracted. Then, standard DNA barcoding region of the mitochondrial COI gene (~650 bp region full DNA barcode - FDB) was generated and, in case of amplification failure, a shorter length COI barcode (~127 bp mini-DNA barcode - MDB) was generated using the internal reverse primer Shark COI-MINIR described by Fields et al. (2015). The discriminatory ability at species level of the FDB and MDB was compared. Results showed that, even if shark lips are heavily processed products, the full barcode was successfully generated from almost all the samples (99.2%) and only in two cases the minibarcode was instead needed. Moreover, the identification at species level was possible in the 96.4% of the cases. Problems of

discrimination at species level were reported for Carcharhinus spp. and Prionace glauca was the most recovered species. Moreover, most of the detected species were threatened.

The manuscript is generally clearly written. The experimental design and data analyses are adequate and the results are well discussed in comparison to the literature available. Overall, the MS deserves to be published after some minor revisions.

Minibarcode was generated only from two samples (failing the sequencing of the full barcode FDB) (lines 190-192). However, the abstract (lines 35-36) reported different information FDB was obtained from all the samples. Please verify.

The abstract has been modified accordingly.

The percentages of success of discrimination at species or genus level need to be checked. Based on the abstract lines 35-36, the FBD were generated for all samples (252) with 96.4% assigned at species level. However, in lines 202-203 the percentage 96.4 (same of the abstract) was calculated including all the molecular data generated (250 FBD + 2MDB). The percentage of FDB was instead reported in line 204 '97.2% (243 out of 250 sequenced FDB) species identification rate'. Moreover, seems that the 2 sequences generated by MDB aren't counted in the percentage of success reported in line 246 (94%). However, in terms of power of discrimination of MDB you should include all the sequences (250 FDB trimmed + 2 MDB generated). So, please check the percentage and organize the results based on three different points of view: i) the samples (252), ii) the FDB (250) iii) the MDB (250 FDB trimmed + 2 MDB generated).

The percentage of identification success now reported in line 262 has been modified accordingly.

Lines 153-157: The sentence isn't clear. What do you mean? Please try to be more precise.

The sentence was modified.

The MDB in-silico analysis it is very interesting but it wasn't described in the M&M.

A specific section as regard the in-silico analysis have been now added in the M&M.

Please, include this part. Results from MDB in-silico analysis could be easily compared to FDB including a new column in Table 4.

We reported in the manuscript that 94% of the samples were identified at species level and in these cases the ID percentage was similar or identical to that of the FDB. We therefore think that this additional info may be omitted.

One useful information for the readers is absent in MDB in-silico analysis: Are the internal reverse primer Shark COI-MINIR fully conserved in your data? Are mismatches present in this region in the multialignment?

Done. The primer matching was assessed and discussed (lines 255-260).

Lines 234-235 and 244-246: Move the sentences in M&M. In results & discussions clarify that this is an in-silico analysis.

Done

Lines 247-248: What is the meaning of 'similar homologies'? 'in particular, P. glauca, C. falciformis, S. lewini, S. zygaena, I. oxyrinchus and C. sorrah were detected with similar homologies observed for FDB'

This is linked with the fact that the authors used in MS the term homology as synonymous of similarity/identity. This is absolutely wrong. Homology is qualitative concept and you cannot report the results from a blast search in terms of '% of homology'. Similarity and identity are synonymous and quantitative measures. The homology is another concept. In other fields of research, value of similarity is used to infer homology among genes but homologous genes can also have low level of similarity. I saw that some papers you cited made the same mistake using homology instead of similarity. However, even if this erroneous use of terms doesn't affect the experiment, it isn't a good idea to continue to widespread errors. Please, change the term homology with similarity/identity in the MS.

Thank you for this useful comment. The term homology has been replaced with identity all over the manuscript.

Lines 180-181: Please explain better the concept of complementary of FDB and MDB.

The term means that both methods must be used together to fully resolve the identification at species level. However, the sentence has been modified.

Lines 220-222: Can you better explain the meaning of the sentence?

The sentence has been modified.

Line 286: please check the number reported in %

The sentence has been verified according to Fields et al., 2018.

DNA Barcoding for the Identification of shark lips (鱼唇): a nationwide survey for analyzing a never investigated product in the Chinese market.

Xia Zhang[a1], Andrea Armani[b1], Jing Wen[c*], Alice Giusti[b*], Xiaoguo Ying[d], Sigang Fan[e], Juan Zhao[c], Xuyan Li[c]

[a] *Henry Fok College of Food Science, Shaoguan University, Shaoguan, 512005, China*

[b]*FishLab, Department of Veterinary Sciences, University of Pisa, 56124, Pisa, Italy*

[c]*Department of Biology, Lingnan Normal University, Zhanjiang, 524048, China*

[d]*Key Laboratory of Health Risk Factors for Seafood of Zhejiang Province, College of Food Science and Pharmacy, Zhejiang Ocean University, Zhoushan 316022, China*

[e]*Key Laboratory of South China Sea Fishery Resources Utilization, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, 510300, China*

[1] These authors equally contributed to this work.

*Corresponding authors:

Jing Wen, Lingnan Normal University, Zhanjiang, 524048, China

Email: jw82123@126.com

Alice Giusti, Department of Veterinary Sciences, University of Pisa, 56124, Pisa, Italy

Email: alice.giusti183@outlook.it

Abstract

~~Shark food products are extremely popular in China. Fins are especially considered a delicacy, but also other part of the carcass, such as the skin sold as "shark lips", are consumed. The high fishing pressure has contributed to shark population decreasing, and many species are currently endangered and/or with a strictly regulated commerce.~~ A nationwide survey conducted in China aimed at authenticating species in shark lips products (鱼唇) (n=252) by full DNA *COI* barcoding (FDB; 652 bp) is presented. In addition, the efficiency of the FDB and of the mini DNA barcode (MDB; 127 bp) proposed by Fields et al. (2015) (PloS one, 10, e0114844.) in identifying the shark species detected in this study was compared. Despite the manufacturing process, the total DNA of the samples presented a medium low fragmentation degree, and the FDB was obtained from almost all the samples except for two (99.2%) from which the MDB was instead successfully obtained.

~~from all the s~~ Samples~~, which~~ were allocated to species level in 96.4% of the cases. This confirms the importance to perform a preventive evaluation of the level of DNA degradation before selecting cost and time-consuming procedures. Of the 7 identified species, *Prionace glauca* was the most recovered (65.5%). The other six detected species were *Carcharhinus falciformis* (11.5%), *Sphyrna lewini* (6.7%), *S. zygaena* (3.6%), *Isurus oxyrinchus* (3.6%), *C. longimanus* (3.2%) and *C. sorrah* (2.4%), 5 of which are threatened and 4 are subject to global commerce regulation. Overall, issues in discriminating among some *Carcharhinus* spp. were highlighted both using the FDB and the MDB. Outcomes of this study confirms the need to improve the Chinese traceability system. In fact, even though a legislation for seafood labelling supported by an official system for name attribution not always ensure the sector safeguarding from frauds, absent or weak traceability system certainly facilitate illegal practices.

Keywords: Shark food products, DNA barcoding, *Prionace glauca*, *Carcharhinus* spp., *Sphyrna* spp., labelling system, fraudulent substitution

1. Introduction

In the last decades, mainly due to the large-scale growth in Asian economies and with increased standards of living in China, the shark fisheries expanded considerably (Erikson & Clarke, 2015) and China accounts for over 80% of the world's shark trade (Chuang, Hung, Chang, Huang, & Shiao, 2016). The Chinese growing demand has been a driving force for the global fishing pressure increasing, which has contributed to shark population decreasing and collapsing world-wide (Erikson & Clarke, 2015). The trend of global~~ly~~ trading of shark meat ~~reached~~ increased 42% from 2000 to 2011 (FAO, 2015; Almerón -Souza et al., 2018). Characterized by a life history of slow growth, late maturity, low fecundity, and low population resilience to overfishing, sharks are in fact extremely vulnerable to overexploitation, that is probably the dominant driver of population declines (Graham, Spalding, & Sheppard, 2010; Dulvy et al., 2014). Consequently, many populations of sharks are considered threatened or endangered by both targeted and incidental catches (Erikson & Clarke, 2015). According to the most recent systematic analysis performed by the International Union for the Conservation of Nature (IUCN) Shark Specialist Group (SSG), 74 of the 465 (15.9%) shark species included in the IUCN Red List are threatened (Dulvy et al., 2014).

For partially facing this issue, parties to the Convention on International Trade in Endangered Species (CITES) included in the Appendix II of the Convention several shark species (Table 1) that, although not necessarily endangered, factually could be threatened without a strictly regulated commerce. Basically, the international trade may be authorized by the granting of an export permit or re-export certificate and they should only be granted if the relevant authorities are satisfied that certain conditions are met, above all that trade will not be detrimental to the survival of the species in the wild (https://www.cites.org/). Moreover, a network of Regional Fishery Management Organizations (RFMOs) handles with varying degrees of legal competence for setting limits on fishing for sharks (https://www.iucnssg.org/rfmos.html). Nevertheless, these actions' regulatory

effects have been minimal, partly due to the difficulty in identifying a species and its origin (Chuang et al., 2016).

To overcome these challenges, several DNA-based analyses have been developed for the identification of shark species (Dudgeon et al., 2012; But, Wu, Shao, & Shaw, 2020). Among the other, the DNA barcoding technique facilitates a rapid and accurate species identification, which is essential for enforcing regulations (Chuang et al., 2016). The standard target for DNA barcoding of animal species is generally a ~650 bp region (full DNA barcode – FDB) of the mitochondrial gene coding for cytochrome c oxidase subunit I (*COI*) (Hebert, Ratnasingham, & De Waard, 2003). Even though this method can be successfully used for the identification of fresh products, it has shown some weaknesses in the case of processed seafood products; the DNA degradation that occurs during processing can in fact hamper the amplification of FDB from processed shark products (Fields, Abercrombie, Eng, Feldheim, & Chapman, 2015). Therefore, also shorter length *COI* barcodes (mini-DNA barcode – MDB) of variable lengths were used (Table 2). Scientific studies dealing with identification of shark species in food products by DNA-barcoding (Table 2) often highlighted the illegal presence of species listed in the CITES appendices or considered near-threatened, vulnerable or endangered according to the IUCN Red List. Moreover, the presence of mislabelled or potentially mislabelled shark products is reported, and especially refers to illegal practices of using cheaper species to replace more popular ones, or even selling threatened species (Marchetti et al., 2020).

The most valuable part of the shark carcass are fins, that are one of the most expensive seafood items in the world (https://theaseanpost.com/article/malaysias-appetite-shark-fin), especially considered a delicacy in different parts of Asia (Lehr, 2015; Iloulian, 2016; Thomas, 2019). However, other part as meat, cartilage, liver oil, and skin are used for human consumption. Even though the most part of the shark skin is used as leather, those from frozen or chilled shark carcasses intended for human consumption is usually so damaged that it is unsuitable for leather factoring. Therefore, skin is used as food product after a process that involves the removing of the denticles

from the dried skin followed by bleaching with hydrogen peroxide and re-drying before marketing. Cooked skin (rehydrated before cooking) is soft, smooth, and juicy and it is sold in Singapore and Malaysia under the name fish lips (Vannuccini, 1999). In East and South-East Asia, they are traditionally known as "shark lips" (Vannuccini, 1999; Lehr, 2015). Shark skin, as other fish parts rich in gelatine, is also processed into gelatinous food products. In a small mountainous part of Northern Japan there seems be a tradition to eat shark aspic ("Nikogori") for new year celebration (Lehr, 2015). In China, shark lips are labelled as *yu chun* (鱼唇) and they are among the top sea treasures used to prepare valuable dishes at superior banquets (Vannuccini, 1999). Surprisingly, despite the popularity that shark food products have in China, only one study was conducted to investigate species composition in gill plates in the Chinese mainland (Steinke et al., 2017 in Table 2). Moreover, to the best of our knowledge, no surveys aimed at identifying species in shark lips have been performed yet (Table 2). We therefore present a nationwide survey aimed at authenticating species in shark lips products labelled as *yu chun* (鱼唇) marketed in 31 cities across China by DNA barcoding approach targeting the FDB. In addition, the discriminatory ability of the FDB and MDB proposed by Fields et al. (2015) in identifying the detected shark species, was compared. Other than shed a light on a never investigated shark food product, outcomes from this study can contribute to improve the selection of a suitable analytical method for monitoring the illegal traffic of sharks.

2. Materials and Methods

2.1. Sampling

A total of 252 dried shark lip products (Table 3; Figure 1) were purchased from seafood shops in 31 cities belonging to 22 provinces, 5 autonomous regions and 4 municipalities (Figure 2). At least 6 products per city were collected. The samples were collected when reporting the Chinese terms 鱼唇-*yuchun* (shark's lip in English) on the label or on an information sign next to the product. The

sampling was conducted to include a proportional number of products per price, according to the market availability.

2.2. Total DNA extraction, evaluation of DNA quality and fragmentation, ~~*COI* gene amplification and sequencing~~

Total DNA extraction was performed from 30 mg of tissue using the TIANamp Marine Animals DNA Kit (TIANGEN, China, Cat. no. DP324) according to the manufacturer's instructions. DNA concentration and quality were evaluated using a U-1800 spectrophotometer (Hitachi, Japan). One thousand ng of the total DNA extracted from each sample were run on 1% agarose gel previously and visualized under UV light. The degree of DNA fragmentation was assessed by comparison the DL2000 DNA marker (TaKaRa, Japan, Cat no. 3452).

2.3. *COI* gene amplification and sequencing

The primer pairs FishF1 and FishR1, FishF2 and FishR2 (Ward et al., 2005) were used alternatively for the amplification of the FDB (652 bp without primers). PCR amplification was performed using 100 ng of template DNA and 50 µL master mix containing 2 µL each primer (10 µmol/L), 5 µL of 10×Ex Taq buffer (20 mmol/L Mg2+ plus), 4 µL dNTP mixture (2.5 mmol/L each, TaKaRa, Japan, Cat no. 4030), and 0.25 µL Ex Taq DNA polymerase (2 U/µL) (TaKaRa, Japan, Cat no. RR001). PCR was carried out in a C1000 touch thermal cycler (Bio-Rad, USA). Amplification conditions included a denaturing step at 94 °C for 2 min, 35 cycles of 30 s at 94 °C for denaturation, 30 s at 52 °C for annealing, 1 min at 72 °C for extension, and a final extension at 72 °C for 10 min. In case of amplification failure, the forward primer FishF2 by Ward et al. (2005) was used with the reverse primer Shark COI-MINIR (5'-AAGATTACAAAAGCGTGGGC-3') projected by Fields et al. (2015) for the amplification of the MDB region (127 bp). Amplification conditions included a denaturing step at 95 °C for 15 min, 35 cycles of 1 min at 94 °C for denaturation, 30 s at 52 °C for annealing, 2 min at 72 °C for extension, and a final extension at 72 °C for 5 min. The presence of the expected amplicon and the final concentration were verified by

comparison with the DL2000 DNA marker (TaKaRa, Japan, Cat no. 3452). PCR products purification, sequencing and sequence editing were performed according to Zeng et al. (2019).

2.43. Sequences comparison with genetic databases and species identification

The sequences were queried against the reference sequences available on GenBank (http://www.ncbi.nlm.nih.gov) and Barcode of Life Data system (BOLD) using the algorithms ~~by~~ Basic Local Analysis Search Tool (BLAST) and ~~by the~~ BOLD Identification System (ID's) (Ratnasingham & Hebert, 2007) ~~against the reference sequences available on GenBank (http://www.ncbi.nlm.nih.gov) and Barcode of Life Data system (BOLD)~~ (http://www.boldsystems.org/), respectively. In both the databases, the similarity scores of the top 100 matches were considered. For the FDB, a match with a sequence similarity of at least 98 was used to designate potential species identification (Barbuto et al., 2010). The sample identification was considered achieved only when both databases produced specie-specific identity value higher than the proposed threshold value. Given the short length of the MDB (127 bp), a species level identification was only achieved when the highest match on BLAST and BOLD ID's was the same, exclusive to a single species and with >99% ~~homology~~ identity score (query coverage 99-100%), according to Hobbs et al. (2019). The shark products identified through databases comparison were analysed based on their average price and sampling site, and the IUCN and CITES status of the recovered species was evaluated.

2.5. *In-silico* analysis for assessing the MDB species discrimination ability

An *in-silico* analysis aimed at evaluating the MDB species discrimination ability for all the samples analysed in this study was performed. The analysis was performed on the MDB proposed by Fields et al. (2015), All the obtained FDB (n=250) were trimmed to obtain the 127 bp MDB (Fields et al., 2015), which was then compared with both GenBank and BOLD databases as reported in section 2.4.

3. Results and discussion

3.1 Total DNA extraction, ~~evaluation of DNA quality and fragmentation,~~assessment and *COI* gene amplification and sequencing

Chemical-physical treatments used to produce shark food products such as dried fins, soups, cartilage pills, often results in a high DNA fragmentation (fragments <500 bp) (Fields et al., 2015; Hellberg et al., 2019; Hobbs et al., 2019; Muttaqin et al., 2019). Therefore, alternative primers for the amplification of shorter barcodes were used (Wainwright et al., 2018; Muttaqin et al., 2019; Abdullah et al., 2020). Fields et al. (2015) developed a mini-barcode *COI* assay that yielded a truly short (~110–130 bp) sequence specifically from degraded shark products. To date, this MDB approach was applied by Hellberg et al. (2019) and Hobbs et al. (2019) for species identification in processed shark products sold in USA and UK, while Zahn, Silva, & Hellberg (2020) recently used the MDB region to develop a MDB protocol for the identification of elasmobranch species in shark cartilage pills, which was tested and validated on samples from another previous study (Hellberg et al., 2019). Hellberg et al. (2019) proved that FDB and MDB ~~are complementary~~ can be used in association ~~… in their ability~~ to identify ~~of~~ processed shark products so that a combination of these approaches was suggested. Considering that shark lips undergo a process involving a chemical "bleaching" (Vannuccini, 1999), we decided to assess the level of degradation of the total DNA before selecting the best molecular marker (FDB or MDB). In fact, MDB was already reported as not able to discriminate some shark species in previous work (see section 3.2). This simple preliminary evaluation step could allow to speed up and reduce the cost of the analysis by optimizing the amplification procedure (Armani et al., 2015). The total DNA electrophoresis showed that all the DNA samples presented a medium-low fragmentation degree (500-700 bp). In addition, the spectrophotometric analysis, showed medium-high DNA yield and quality (A260/A280 and A260/A230 ratio >2.0) (data not shown). In fact, the target FDB that was selected as elective genetic marker, was successfully amplified, and sequenced from almost all the samples except for two (99.2%) from which the MDB was instead successfully amplified and sequenced. In this respect,

literature reports contrasting findings. Hellberg et al. (2019), which analysed highly processed shark products, reported that the amplification rate of FDB (8.6%) was considerably lower respect to MDB (54.3%). Issues in FDB amplification from highly processed shark products were also highlighted by Muttaqin et al. (2019) and the amplification of this molecular marker even totally failed in the study by Abdullah et al. (2020), where the analysed products were cooked, smoked and salted. Contrariwise, other authors highlighted a high FDB amplification rate even in processed shark products (Holmes et al., 2009; Sembiring et al., 2015). This confirms the importance to perform a preventive evaluation of the level of DNA degradation before selecting cost and time-consuming procedures.

3.2 Sequence comparison with genetic databases and species identification

The sequence comparison with databases (GenBank and BOLD) allowed to solidly allocate 243 out of the 252 analysed samples (96.4%) to species level. All of them belonged to FDB region (Table 4), that showed a 97.2% (243 out of 250 sequenced FDB) species identification rate. Seven species were detected: *Prionace glauca* (165 samples, 65.5%), *Carcharhinus falciformis* (29 samples, 11.5%), *Sphyrna lewini* (17 samples, 6.7%), *S. zygaena* (9 samples, 3.6%), *Isurus oxyrinchus* (9 samples, 3.6%), *Carcharhinus longimanus* (8 samples; 3.2%) and *Carcharhinus sorrah* (6 samples, 2.4%) (Table 4). For the remaining 9 samples (3.6%), 7 FDB and 2 MDB, a species level match could not be achieved by sequences comparison with databases. The 7 FDB samples were allocated to *Carcharhinus* sp. (Table 4). In both the databases, these samples showed 99-100% ~~homology~~ identity with different species of this high populated genus (http://www.marinespecies.org/aphia.php?p=taxdetails&id=105719), especially with *C. limbatus, C. brevipinna, C. leiodon, C. amblyrhynchoides* and *C. tilstoni*. *Carcharhinus sp.* was also identified in 1 MDB (sample HH5), that simultaneously matched with *C. altimus, C. plumbeus* and *C. amblyrhynchos* with solid ~~homologies~~ identities in both the databases. The difficulty in discriminating within this genus was also highlighted in other studies (Holmes et al., 2009; Liu et

al., 2013) and it might be because, while the mean sequence divergence between congeneric species of sharks is 7.48%, some congeners are known to have very low sequence divergence (Ward, Holmes, White, & Last, 2008). For example, the interspecies sequence divergence between *C. limbatus*, *C. amblyrhynchoides* and *C. tilstoni* averages only 0.45% (Ward et al., 2008). ~~In situations~~ W~~w~~here ~~taxa share sequences with~~species ~~-~~divergence is less than 1% ~~divergence~~, the databases show all possible species assignments (Holmes et al., 2009). It should be also underlined that several closely related species of the genus *Carcharhinus* are morphologically similar to each other and difficult to identify (Ward et al., 2008; Liu et al., 2013), such in the case of *C. limbatus*/*C. tilstoni* that are indistinguishable except for precaudal vertebral counts that separate them (Last & Stevens, 2009), and *C. amblyrhynchoides* is also very similar to them (Ward et al., 2008). *C. limbatus, C. tilstoni, C. leiodon,* and *C. amblyrhynchoides* were also defined as the "blacktip species complex" (Cardeñosa et al., 2018). This high species similarity could sometimes lead to the presence of wrongly deposited sequences on official databases due to specimen misidentification. Several errors among the shark sequences in GenBank were for instance detected (Fields et al., 2015; Fields et al., 2018). For the remain MDB (sample JN16), the nearest match was with *Sphyrna lewini*, but the ~~homology~~ identity score was too low (97.62%) to allow species identification in both the databases (Table 4). Since Hellberg et al. (2019) proved that the MDB was more effective than FDB for detecting species within highly processed samples containing degraded DNA, we therefore performed an *in-silico* analysis aimed at evaluating the MDB species discrimination ability for all the samples analysed in this study. In fact, the possibility that highly degraded DNA in shark food products occur should not be excluded, considering their manufacturing. We decided to focus this analysis on the MDB proposed by Fields et al. (2015), which is the shorter among those reported in literature (Table 2). In fact, considering the abovementioned difficulties in discriminating among some *Carcharhinus* spp. even with FDB, we deemed unnecessary evaluating the species discrimination power of other MDBs and we considered more appropriate to deepen the

identification performance of a MDB whose use is essential in presence of highly degraded DNA samples. Moreover, MDB by Fields et al. (2015) is the only amplified by a primer (Shark COI-MINIR) especially projected for shark species, while "generic" universal primers were used for the amplification of the other MDBs (Table 2). Therefore, all the obtained FDB (n=250) were trimmed to obtain the 127 bp MDB (Fields et al., 2015), which was then compared with both GenBank and BOLD databases. The Shark COI-MINIR primer matching with the sequences of the species identified in this study by using the FDB was assessed. Given the fact that mismatches found (from 2 to 4) –did not prevent the amplification of the samples HH5 (*Carcharhinus* spp.) and JN16 (*Sphyrna* spp.), and considering thehigh number of species successfully amplified by Fields et al., (2015) (included those considered in this study), we think it is highly possible that all the species can be amplified using this primer.

~~Overall,~~Therefore, considering the 250 obtained FDB and the 2 MDB amplified in this study, 235 (93.2~~4~~%) were identified at species level; in particular, *P. glauca*, *C. falciformis*, *S. lewini*, *S. zygaena*, *I. oxyrinchus* and *C. sorrah* were detected with similar ~~homologies~~ identities observed for FDB. MDB region was instead proved as not enough informative to go beyond the genus level in the 8 samples identified as *C. longimanus* with FDB. Likewise, Hobbs et al. (2019) reported that, although most of the analysed samples were successfully assigned to a single species, cases of samples assigned back to a range of closely related sharks of *Carcharhinus* sp., where the identification could not be made beyond genus level, occurred. Factually, Fields et al. (2015) partially anticipated these outcomes, by observing that the MDB sequences of *C. logimanus*, *C. obscurus* and *C. galapagensis* were identical or nearly identical. Therefore, in presence of highly degraded DNA samples, alternative methods for amplifying the FDB should be considered. For instance, Cardeñosa et al. (2017) developed a multiplex PCR mini-barcode assay to identify processed shark products using the MDB described in Fields et al. (2015) as starting point and projecting a second mini-barcoding primer that could be additionally used. They predicted that a

multiplex of these primers would potentially yield up to three amplicons allowing the sequencing of the entire FDB (Cardeñosa et al., 2017).

Overall, the species detected in this study reflected in many cases the findings of other studies (Table 1SM). *P. glauca* accounted alone for more than 30% of the samples collected by Chuang et al. (2016) and Fields et al. (2018) in Taiwan and Hong Kong, followed by *C. falciformis*; *C. falciformis, I. oxyrinchus,* and *P. glauca,* together with *A. pelagicus* represented 80% of shark meats also collected in Taiwan a few years before (Liu et al., 2013); *P. glauca, C. falciformis* and *S. lewini* were also among the most common species recovered in other studies investigating fins and/or other shark products sampled in Indonesia (Sembiring et al., 2015), Hong Kong (Cardeñosa et al., 2018) and Singapore (Wainwright et al., 2018). *P. glauca*, *I. oxyrinchus*, *S. lewini*, *S. zygaena*, *C. falciformis* and *C. logimanus,* together with some other species belong to *Carcharhinus* and *Alopias* genera, are in fact the dominant species of the Western Pacific Ocean and they are especially caught in Taiwanese waters (Liu et al., 2013), so that their presence in products marketed in China and South East Asia is easy to understand. Although most of these shark species have extensive ranges, the shark products in Taiwanese market seem in fact more dominated by domestic supplies than by international sources (Chuang et al., 2016), with exception of some species that are most likely a result of international trade (Table 1SM). Moreover, *S. lewini*, *S. zygaena* and *C. longimanus* are by far the species whose fins were traded in Hong Kong, that was reportedly the world's top legal importer of fins from CITES listed sharks (Cardeñosa et al., 2018).

Because of its cosmopolitan distribution (Chuang et al., 2016; Almerón-Souza et al., 2018), *P. glauca* was often recovered also in extra-Asian markets (Table 1SM) such as in products sold in Canada (Steinke et al., 2017), Brazil (Almerón-Souza et al., 2018; da Silva Ferrette et al., 2019) and Italy (Marchetti et al., 2020) (Table 1SM). However, when found in frozen filets, it was suggested that these individuals were captured in Asia, especially in Taiwan, and subsequently imported (Almerón-Souza et al., 2018). Overall, the observation of shark species trade (Table 1SM), jointly

with the results from this study, confirm the scenario described by Fields et al. (2018), in which, despite high species diversity, the contemporary shark trade is dominated by only 8 species or complexes, likely comprising more than 1%: *P. glauca*, *C. falciformis*, *S. lewini* and *S. zygaena*, *Carcharhinus* spp., *C. brevipina*, *C. leucas*, and *Isurus* spp. Other Mediterranean species were also found in Greece (Pazartzi et al., 2019) and Italy (Marchetti et al., 2020) (Table 1SM).

No differences were observed among the recovered species based on the product type, except for the study of Hobbs et al. (2019), where most of the species detected in meat samples from UK were different from those found in fins analysed in the same study and overall, poorly represented in literature (Table 1SM).

3.3. *Species composition vs average price, collection site and IUCN and CITES status.*

The samples showed market prices ranging from 25 to 86 US $/kg (60.3±15.2 US $/kg) (Table 3). Since most of the global market is addressed to meat and fins, while some other shark products, such as shark lips, are separately recorded in trade statistics (Hellberg et al., 2019), we cannot perform a price comparison with global market trends. Overall, shark lips price appeared lower than shark fins, reported as 168.8 US $/kg, when excluding the highest and lowest observation (Lehr, 2015). Among our samples, highest prices (≥80 US $/kg) were observed in samples collected in Beijing (municipality) (site 13 in Figure 2), Chongqing (municipality) (site 10), Tianjin (municipality) (site 24), Yinchuan (Ningxia Hui autonomous region) (site 4) and Zhengzhou (Henan province) (site 16). Overall, 7 out of the 8 samples generally identified as *Carcharhinus* sp. showed the higher average price (74.7 US $/kg), together with all the 29 samples identified with *C. falciformis* (73.8 US $/kg) and the 8 samples identified as *C. longimanus* (72.5 US $/kg) (Table 4). These products were collected in Chengdu (Sichuan province) (site 6 in Figure 2) Chongqing (municipality) (site 10), Harbin (Heilongjiang province) (site 21), Hangzhou (Zhejiang province) (site 29), Ji'nan (Shandong province) (site 25), Nanning (Guangxi Zhuang autonomous region) (site 12), Tianjin (municipality) (site 24) and Yinchuan (Ningxia Hui autonomous region) (site 4). Since

the collection sites do not correspond to the sites with the highest average prices in 4 out of the 7 cases, the price is presumably related to the species, since overall shark prices are greatly related on this aspect (Vannuccini, 1999).

Five out of the 7 detected species are included in the IUCN Red List as vulnerable, endangered, or critically endangered and 4 of them (66.7%) were included in the CITES Appendix II (Table 4). Similar findings were reported in all the studies on species identification in shark food products, as most of the detected species currently cover a threatened IUCN conservation status and they are proved as commonly exploited regardless or they are included in the CITES Appendix II (Table 1SM). Most cases of threatened or CITES-listed species recovering involved processed products (such as fins), where the morphological features of the species lack. The high frequency of these species strongly suggests that they are not the result of by-catch or small-scale artisanal fisheries, but instead result from large-scale targeted shark fisheries (Sembiring et al., 2015). Despite the public awareness for the shark conservation, are currently poorly applied by governments; in Taiwan, for instance, *Rhincodon typus* it is the only species with a restricted ban on fishing and trading (Liu et al., 2013). However, it should be noted that sustainable fisheries do exist for some of these species in specific geographic regions. For example, the National Oceanic and Atmospheric Administration (NOAA) Fish Watch considers U.S. wild-caught *I. oxyrinchus* to be sustainably managed and responsibly harvested (Hellberg et al., 2019). *P. glauca*, the dominant species in this study and in many other (section 3.2; Table 1SM) is categorized as a near-threatened species in the IUCN Red List. Since 1980s, a progressive population declines in this species might have resulted from the rapid expansion of directed fisheries (Chuang et al., 2016). Therefore, efforts in reducing fishing pressure in this species should be implemented. In Brazil, for instance, species assessed as non-threatened should be prioritized for research and conservation measures according to a specific ordinance (da Silva Ferrette et al., 2019). It should be therefore highlighted that shark species in the

non-threatened categories face fishing pressure, putting at risk of extinction data-deficient species that could already be threatened (da Silva Ferrette et al., 2019).

3.4 ~~Shortcomings in seafood labelling enforcement in Asian countries hamper m~~Mislabelling assessment in shark products~~.~~ in Asian countries

As it can be observed, the mislabelling evaluation of shark products was not performed in this study, as well as in all the studies which analysed Asian products (Table 1SM), because a legislation framework to regulate seafood naming, labelling and traceability do not exist. All the collected samples were in fact sold without reference to any species on the label. Most of seafood products sold in Asian commercial markets do not display label information regarding species authentication such as scientific name and origin (Abdullah et al., 2020). The lack of a mandatory legislation on seafood traceability and official naming system is especially alarming in China, as often highlighted in literature (Xiong et al., 2016a; Xiong et al., 2016b; Zeng et al., 2019; Zhang et al., 2021). In this respect, the comparison between the species identified by DNA barcoding and the declared name of the shark was therefore not feasible.

Potential mislabelling or fraudulent substitution cases were evaluated in 6 studies (Table 1SM) Barbuto et al. (2010) found 77.8% species substitutions cases in shark slices sold in Italy under the vernacular name of ‘‘*palombo*” (that is referred to *Mustelus mustelus* and *M. asterias* for the Italian regulation) with low-value species. Still in Italy, the results of a more recent investigations revealed a high occurrence of incorrect species declaration in 45.4% shark meat products, also in this case especially involving “*palombo*” (Marchetti et al., 2020). In Greece, UK and USA, 55.81%, 34.1% and 19%, non-compliances, respectively, were detected between the name reported on the label and the shark species identified (Pazartzi et al., 2019; Hobbs et al., 2019; Hellberg et al., 2019). In the study of Pazartzi et al. (2019), which analysed shark meat products collected in Greek retailers, over half of products originated from species that are listed as threatened by the IUCN Red List, and of the mislabelled products, 23% originated from species with prohibitions on landings or CITES

listings. Equally, threatened and/or CITES listed species were found in products sold in countries having specific legislation on seafood labelling (Table 1SM). Hellberg et al. (2019) also found one sample of shark fin soup to be potentially mislabelled due to the detection of teleost fish instead of shark. One explanation for this finding is that the restaurant intentionally did not include shark in the product because it is illegal to sell shark fin in California. The assessing of the mislabelling rate in these studies was possible due to the existence of a specific legislation in the countries where the samples were collected: the EU has a legislation on seafood labelling requiring indication of commercial designation, scientific name, method of production, geographical origin and fishing-gear category (Regulation EU No 1379/2013). In the USA, the U.S. Food and Drug Administration has produced and maintains a list of Acceptable Market Names which are allowed for seafood species (Food Integrity Project, 2018). Likewise, the Ministry of Agriculture, Livestock, and Food Supply (MAPA – Ministério da Agricultura, Pecuária e Abastecimento), responsible for ensuring accurate labelling of foodstuff at federal level in Brazil, produced an official list of legal commercial names and Latin scientific names to facilitate market regulation (Carvalho et al., 2017). Therefore, also in the study of Calegari et al. (2019) conducted in Brazil the mislabelling rate was calculated: 100% of products sold as shark filets were instead Striped catfish (*Pangasianodon hypophthalmus*).

4. Conclusion

Sharks include many species of conservation concern. If the fins, the most valuable product obtained from the shark carcass, have been rather highly investigated, literature dealing with species identification in other shark products is scarce and no surveys have been especially provided for the Chinese market. In this study, performed in China, where shark lips were investigated for the first time, DNA barcoding targeting the standard *COI* 650 bp region (FDB) was proved as an effective tool for detecting species in this kind of products, with some limitation in presence of low intra-species sequence divergences among some *Carcharhinus* spp. Alternatively, short length barcodes MDBs) can be successfully used in cases of highly degraded DNA, despite the even lower

discrimination power among *Carcharhinus* spp. Most of the detected species are included in the IUCN Red List as threatened and/or whose commerce is strictly regulated. The lack of information on the products label, related to a well-known seafood weak labelling system in China, did not allow to evaluate the overall mislabelling rate. In this respect, even though the presence of a specific legislation for seafood labelling supported by an official system for name attribution not always ensure the sector safeguarding from illegal practices, absent or weak traceability system and consumer information policies inevitably facilitate the implementation of fraudulent market channels (e. g. commercial frauds and IUU fishing).

Acknowledgments and Funding

This work was supported by the National Natural Science Foundation of China (Award Number 31872571).

References

1. Abdullah, A., Nurilmala, M., Muttaqin, E., & Yulianto, I. (2020). DNA-based analysis of shark products sold on the Indonesian market towards seafood labelling accuracy program. *Biodiversitas Journal of Biological Diversity, 21(4),* 1385-1390. https://doi.org/10.13057/biodiv/d210416
2. Almerón-Souza, F., Sperb, C., Castilho, C. L., Figueiredo, P. I., Gonçalves, L. T., Machado, R., ... & Fagundes, N. J. (2018). Molecular identification of shark meat from local markets in Southern Brazil based on DNA barcoding: evidence for mislabeling and trade of endangered species. *Frontiers in Genetics, 9,* 138. https://doi.org/10.3389/fgene.2018.00138
3. Armani, A., Guardone, L., Castigliego, L., D'Amico, P., Messina, A., Malandra, R., ... & Guidi, A. (2015). DNA and Mini-DNA barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market. *Food Control, 50*, 589-596. https://doi.org/10.1016/j.foodcont.2014.09.025
4. Baldwin, C. C., Mounts, J. H., Smith, D. G., & Weigt, L. A. (2009). Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx andAstrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx. *Zootaxa*, 1-22.
5. Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P., & Casiraghi, M. (2010). DNA barcoding reveals fraudulent substitutions in shark seafood products: the Italian case of "palombo" (*Mustelus* spp.). *Food Research International, 43(1),* 376-381. https://doi.org/10.1016/j.foodres.2009.10.009
6. But, G. W. C., Wu, H. Y., Shao, K. T., & Shaw, P. C. (2020). Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use. *Scientific Reports, 10(1),* 1-14. https://doi.org/10.1038/s41598-020-61150-8
7. Calegari, B. B., Reis, R. E., & Alho, C. S. (2019). DNA barcode identification of shark fillet reveals fraudulent commerce in Brazil. *Canadian Society of Forensic Science Journal, 52(2),* 95-100. https://doi.org/10.1080/00085030.2019.1581692
8. Cardeñosa, D., Fields, A., Abercrombie, D., Feldheim, K., Shea, S. K., & Chapman, D. D. (2017). A multiplex PCR mini-barcode assay to identify processed shark products in the global trade. *PloS one, 12(10),* e0185368. https://doi.org/10.1371/journal.pone.0185368
9. Cardeñosa, D., Fields, A. T., Babcock, E. A., Zhang, H., Feldheim, K., Shea, S. K., ... & Chapman, D. D. (2018). CITES- listed sharks remain among the top species in the contemporary fin trade. *Conservation Letters, 11(4),* e12457. https://doi.org/10.1111/conl.12457

10. Carvalho, D. C., Guedes, D., da Gloria Trindade, M., Coelho, R. M. S., & de Lima Araujo, P. H. (2017). Nationwide Brazilian governmental forensic programme reveals seafood mislabelling trends and rates using DNA barcoding. *Fisheries Research, 191*, 30-35. https://doi.org/10.1016/j.fishres.2017.02.021
11. Chuang, P. S., Hung, T. C., Chang, H. A., Huang, C. K., & Shiao, J. C. (2016). The species and origin of shark fins in Taiwan's fishing ports, markets, and customs detention: A DNA barcoding analysis. *PloS one, 11(1)*, e0147290. https://doi.org/10.1371/journal.pone.0147290
12. da Silva Ferrette, B. L., Domingues, R. R., Ussami, L. H. F., Moraes, L., de Oliveira Magalhães, C., de Amorim, A. F., ... & Mendonça, F. F. (2019). DNA-based species identification of shark finning seizures in Southwest Atlantic: implications for wildlife trade surveillance and law enforcement. *Biodiversity and Conservation, 28(14)*, 4007-4025. https://doi.org/10.1007/s10531-019-01862-0
13. Dudgeon, C. L., Blower, D. C., Broderick, D., Giles, J. L., Holmes, B. J., Kashiwagi, T., ... & Ovenden, J. R. (2012). A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology, 80(5)*, 1789-1843. https://doi.org/10.1111/j.1095-8649.2012.03265.x
14. Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., ... & Pollock, C. M. (2014). Extinction risk and conservation of the world's sharks and rays. *elife, 3*, e00590.
15. Eriksson, H., & Clarke, S. (2015). Chinese market responses to overexploitation of sharks and sea cucumbers. *Biological Conservation, 184*, 163-173. https://doi.org/10.1016/j.biocon.2015.01.018
16. FAO (2015). State of the Global Market for Shark Products. FAO, Rome.
17. Fields, A. T., Abercrombie, D. L., Eng, R., Feldheim, K., & Chapman, D. D. (2015). A novel mini-DNA barcoding assay to identify processed fins from internationally protected shark species. *PloS one, 10(2)*, e0114844. https://doi.org/10.1371/journal.pone.0114844
18. Fields, A. T., Fischer, G. A., Shea, S. K., Zhang, H., Abercrombie, D. L., Feldheim, K. A., ... & Chapman, D. D. (2018). Species composition of the international shark fin trade assessed through a retail- market survey in Hong Kong. *Conservation Biology, 32(2)*, 376-389. https://doi.org/10.1111/cobi.13043
19. Folmer, O., Black, M., Hoeh,W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial Cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol. 3*, 294–299.
20. Food Integrity Project (2018). A guide to food authenticity issues and analytical solutions. Edited by Jean-François Morin and Michèle Lees, Published by Eurofins Analytics France.
21. Graham, N. A., Spalding, M. D., & Sheppard, C. R. (2010). Reef shark declines in remote atolls highlight the need for multi-faceted conservation action. *Aquatic Conservation: Marine and Freshwater ecosystems, 20(5)*, 543-548.
22. Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1)*, S96-S99. https://doi.org/10.1098/rsbl.2003.0025
23. Hellberg, R. S., Isaacs, R. B., & Hernandez, E. L. (2019). Identification of shark species in commercial products using DNA barcoding. *Fisheries Research, 210*, 81-88. https://doi.org/10.1016/j.fishres.2018.10.010
24. Hobbs, C. A., Potts, R. W., Walsh, M. B., Usher, J., & Griffiths, A. M. (2019). Using DNA Barcoding to Investigate patterns of species Utilisation in UK shark products Reveals threatened species on sale. *Scientific Reports, 9(1)*, 1-10. https://doi.org/10.1038/s41598-018-38270-3
25. Holmes, B. H., Steinke, D., & Ward, R. D. (2009). Identification of shark and ray fins using DNA barcoding. *Fisheries Research, 95(2-3)*, 280-288. https://doi.org/10.1016/j.fishres.2008.09.036
26. Iloulian, J. (2016). From shark finning to shark fishing: A strategy for the US & EU to combat shark finning in China & Hong Kong. *Duke Environmental Law & Policy Forum, 27*, 345.
27. Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D.N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes, 7*, 544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x
28. Last P. R., & Stevens, J. D. (2009) Sharks and Rays of Australia. CSIRO Australia, Collingwood, Vic.
29. Lehr, H. (2015). Traceability study in shark products. Report prepared for the CITES Secretariat.
30. Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., ... & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in zoology, 10(1)*, 34. https://doi.org/10.1186/1742-9994-10-34
31. Liu, S. Y. V., Chan, C. L. C., Lin, O., Hu, C. S., & Chen, C. A. (2013). DNA barcoding of shark meats identify species composition and CITES-listed species from the markets in Taiwan. *PloS one, 8(11)*, e79373. https://doi.org/10.1371/journal.pone.0079373

32. Lobo, J., Costa, P. M., Teixeira, M. A., Ferreira, M. S., Costa, M. H., & Costa, F. O. (2013). Enhanced primers for amplification of DNA barcodes from a broad range of marine metazoans. *BMC Ecology, 13(1)*, 34. https://doi.org/10.1186/1472-6785-13-34
33. Marchetti, P., Mottola, A., Piredda, R., Ciccarese, G., & Di Pinto, A. (2020). Determining the Authenticity of Shark Meat Products by DNA Sequencing. *Foods, 9(9)*, 1194. https://doi.org/10.3390/foods9091194
34. Md-Zain, B. M., Abid-Kamal, S. N. A., Aifat, N. R., Abdul-Latiff, M. A. B., Mohd-Hashim, A. B. U., Ampeng, A., ... & Samat, A. (2018). Molecular identification of shark fins in Malaysian Borneo's local markets. *Biodiversitas Journal of Biological Diversity, 19(3)*, 1035-1043. https://doi.org/10.13057/biodiv/d190336
35. Muttaqin, E., Abdullah, A., Nurilmala, M., Ichsan, M., Simeone, B. M., Yulianto, I., & Booth, H. (2019). DNA-barcoding as molecular marker for seafood forensics: Species identification of locally consumed shark fish products in the world's largest shark fishery. In *IOP Conference Series: Earth and Environmental Science* (Vol. 278, No. 1, p. 012049). IOP Publishing.
36. Naylor, G. J., Caira, J. N., Jensen, K., Rosana, K. A. M., White, W. T., & Last, P. R. (2012). A DNA sequence–based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History, 2012(367)*, 1-262. https://doi.org/10.1206/754.1
37. Palumbi, S. R., Martin, A. P., Romano, S., Mcmillian, W. O., Stice, L., & Grabowski, G. (1991). The simple fool's guide to PCR. Special publication. Honolulu: University of Hawaii, Dept. of Zoology
38. Pazartzi, T., Siaperopoulou, S., Gubili, C., Maradidou, S., Loukovitis, D., Chatzispyrou, A., ... & Imsiridou, A. (2019). High levels of mislabeling in shark meat–Investigating patterns of species utilization with DNA barcoding in Greek retailers. *Food Control*, 98, 179-186. https://doi.org/10.1016/j.foodcont.2018.11.019
39. Ratnasingham, S., & Hebert, P. D. N. (2007). Bold: The barcode of life data system. *Molecular Ecology Notes, 7(3)*, 355e364. http://www.barcodinglife.org.
40. Regulation (EU) No 1379/2013 of the European parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products, amending Council regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council regulation (EC) No 104/2000. Official journal of the European union, L 354.
41. Sembiring, A., Pertiwi, N. P. D., Mahardini, A., Wulandari, R., Kurniasih, E. M., Kuncoro, A. W., ... & Mahardika, G. N. (2015). DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. *Fisheries Research*, *164*, 130-134. https://doi.org/10.1016/j.fishres.2014.11.003
42. Shokralla, S., Hellberg, R. S., Handy, S. M., King, I., & Hajibabaei, M. (2015). A DNA mini-barcoding system for authentication of processed fish products. *Scientific Reports*, 5, 15894. https://doi.org/10.1038/srep15894
43. Steinke, D., Bernard, A. M., Horn, R. L., Hilton, P., Hanner, R., & Shivji, M. S. (2017). DNA analysis of traded shark fins and mobulid gill plates reveals a high proportion of species of conservation concern. *Scientific Reports, 7(1)*, 1-6. https://doi.org/10.1038/s41598-017-10123-5
44. Sultana, S., Ali, M. E., Hossain, M. M., Naquiah, N., & Zaidul, I. S. M. (2018). Universal mini COI barcode for the identification of fish species in processed products. *Food Research International, 105*, 19-28. https://doi.org/10.1016/j.foodres.2017.10.065
45. Thomas, J. (2019). Malaysia's appetite for shark fin. The Asean post, 20 september 2019. Available at https://theaseanpost.com/article/malaysias-appetite-shark-fin
46. Vannuccini, S. (1999). Shark utilization, marketing and trade. FAO Fisheries Technical Paper. No. 389. Rome, FAO. 1999. 470p.
47. Wainwright, B. J., Ip, Y. C. A., Neo, M. L., Chang, J. J. M., Gan, C. Z., Clark-Shen, N., ... & Rao, M. (2018). DNA barcoding of traded shark fins, meat and mobulid gill plates in Singapore uncovers numerous threatened species. *Conservation Genetics, 19(6)*, 1393-1399. https://doi.org/10.1007/s10592-018-1108-1
48. Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462)*, 1847-1857. https://doi.org/10.1098/rstb.2005.1716
49. Ward, R. D., Holmes, B. H., White, W. T., & Last, P. R. (2008). DNA barcoding Australasian chondrichthyans: results and possible uses in conservation. *Marine Freshwater Research, 59*, 57–71. https://doi.org/10.1071/MF07148
50. Xiong, X., Guardone, L., Giusti, A., Castigliego, L., Gianfaldoni, D., Guidi, A., & Andrea, A. (2016a). DNA barcoding reveals chaotic labeling and misrepresentation of cod (鳕, Xue) products sold on the Chinese market. *Food Control, 60*, 519-532. https://doi.org/10.1016/j.foodcont.2015.08.028

51. Xiong, X., D'Amico, P., Guardone, L., Castigliego, L., Guidi, A., Gianfaldoni, D., & Armani, A. (2016b). The uncertainty of seafood labeling in China: A case study on Cod, Salmon and Tuna. *Marine Policy, 68*, 123-135. https://doi.org/10.1016/j.marpol.2016.02.024
52. Zahn, R. J., Silva, A. J., & Hellberg, R. S. (2020). Development of a DNA mini-barcoding protocol targeting COI for the identification of elasmobranch species in shark cartilage pills. *Food Control, 109*, 106918. https://doi.org/10.1016/j.foodcont.2019.106918
53. Zeng, L., Armani, A., Wen, J., Lin, H., Xu, Y., Fan, S., ... & Li, X. (2019). Molecular identification of seahorse and pipefish species sold as dried seafood in China: A market-based survey to highlight the actual needs for a proper trade. *Food Control, 103*, 175-181. https://doi.org/10.1016/j.foodcont.2019.04.007
54. Zhang, X., Armani, A., Giusti, A., Wen, J., Fand, S., Yinge, X. (2021). Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat. *Food Control*, In Press.

Figure 1. Some dried shark's lip products (鱼唇-*Yu chun*) collected in this study. Bar= 5 cm.

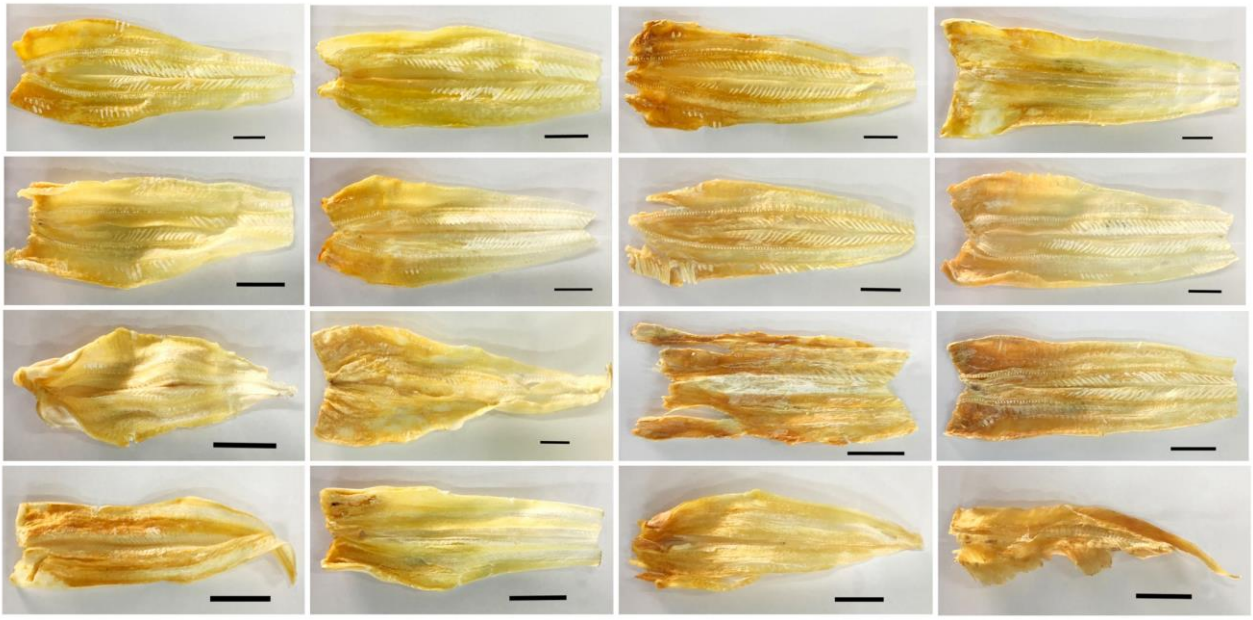
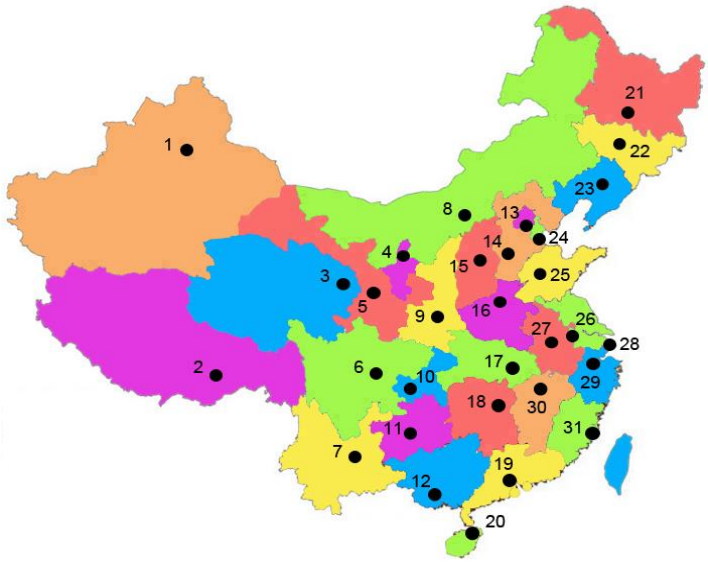


Figure 2. Origin (cities and provinces) of collected samples. 1: Urumqi (Xinjiang Uygur autonomous region); 2: Lhasa (Tibet autonomous region); 3: Xining (Qinghai province); 4: Yinchuan (Ningxia Hui autonomous region); 5: Lanzhou (Gansu province); 6: Chengdu (Sichuan province); 7: Kunming (Yunnan province); 8: Hohhot (Inner Mongolia autonomous region); 9: Xi'an (Shaanxi province); 10: Chongqing (municipality); 11: Guiyang (Guizhou province); 12: Nanning (Guangxi Zhuang autonomous region); 13: Beijing (municipality); 14: Shijiazhuang (Hebei province); 15: Taiyuan (Shanxi province); 16: Zhengzhou (Henan province); 17: Wuhan (Hubei province); 18:Changsha (Hunan province); 19: Guangzhou (Guangdong province); 20: Haikou (Hainan province); 21: Harbin (Heilongjiang province); 22: Changchun (Jilin province); 23: Shenyang (Liaoning province); 24: Tianjin (municipality); 25: Ji'nan (Shandong province); 26: Nanjing (Jiangsu province); 27: Hefei (Anhui province); 28: Shanghai (municipality); 29: Hangzhou (Zhejiang province); 30: Nanchang (Jiangxi province); 31: Fuzhou (Fujian province).


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

581

Highlights

1. A survey for authenticating species in shark lips products sold in China is presented.
2. Full and mini DNA barcoding were applied and their discrimination power was compared.
3. The molecular targets were able in discriminating species, except for *Carcharhinus* spp.
4. 7 species were detected, of which 5 threatened and 4 subject to commerce regulation
5. *Prionace glauca* was the most recovered species (65.5%)

Declarations of interest: none

CRediT author statement

Xia Zhang: **Investigation, Data curation, writing Original Draft**

Andrea Armani: **Writing - Review & Editing, Supervison**

Jing Wen: **Conceptualization; Writing - Review & Editing, Funding aquisiton**

Alice Giusti: **Writing - Review & Editing**

Siyun Xie: **Conceptualization; Writing - Review & Editing**

Huiru Kang: **Conceptualization; Data curation**

Juan Zhao: **Conceptualization; Writing - Review & Editing**

Xuyan Li: **Conceptualization; Writing - Review & Editing, Funding aquisiton**

Yuqi Yan: **Conceptualization; Data curation**

Table 1SM. Species detected in studies on species identification in shark food products by DNA-barcoding with current IUCN status, presence in the CITES Appendix II and products mislabelling rate. Given the high number of detected species, only shark species found in percentages ≥ 1% were reported, while the other species can be checked in the original papers. Species are listed in descending order based on their occurrence, except for references with (*) where the percentage occurrence is not indicated in the original paper. [a]only percentages of the most found species were reported (when available). CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened; LC: Least Concern; DD: Data Deficient; nr: not reported in IUCN list

Reference	Country	products	Detected species (%)[a]	IUCN	CITES	mislabelling rate
Holmes et al. (2009)	Australia	fins	*Carcharhinus dussumieri* (21.8%)	EN	-	Not evaluated
			Carcharhinus tilstoni (14.0%)	LC	-	
			Carcharhinus sorrah (9.3%)	NT	-	
			Sphyrna lewini (6.7%)	CR	✓	
			Carcharhinus amboinensis (6.2%)	DD	-	
			Carcharhinus macloti (5.7%)	NT	-	
			Carcharhinus brevipinna	VU	-	
			Carcharhinus limbatus	NT	-	
			Eusphyra blochii	EN	-	
			Sphyrna mokarran	CR	✓	
			Triaenodon obesus	VU	-	
			Carcharhinus leucas	NT	-	
			Carcharhinus obscurus	EN	-	
			Carcharhinus amblyrhynchos	EN	-	
			Rhizoprionodon acutus	VU	-	
			Rhizoprionodon taylori	LC	-	
Barbuto et al. (2010)	Italy	Slices or filet	*Squalus acanthias* (67.6%)	VU	-	77.8%
			Isurus oxyrinchus (17.6%)	EN	✓	
			Mustelus mustelus (8.3%)	VU	-	
			Prionace glauca (8.3%)	NT	-	
			Alopias superciliosus (2.9%)	VU	✓	
			Galeorhinus galeus (2.9%)	CR	-	
Liu et al. (2013)	Taiwan	Filets and fins	*Alopias pelagicus* (22.8%)	EN	✓	Not evaluated
			Carcharhinus falciformis (22.8%)	VU	✓	
			Prionace glauca (17.9%)	NT	-	
			Isurus oxyrinchus (16.8%)	EN	✓	

			Alopias superciliosus	VU	✓	
			Sphyrna lewini	CR	✓	
			Carcharhinus albimarginatus	VU	-	
			Carcharhinus longimanus	CR	✓	
			Galeocerdo cuvier	NT	-	
Sembiring et al. (2015)	Indonesia	fins	*Carcharhinus falciformis* (19.1%)	VU	✓	Not evaluated
			Sphyrna lewini (10.5%)	CR	✓	
			Prionace glauca (8.2%)	NT	-	
			Alopias superciliosus (7.6%)	VU	✓	
			Alopias pelagicus (7.2%)	EN	✓	
			Carcharhinus sorrah	NT	-	
			Carcharhinus limbatus	NT	-	
			Isurus oxyrinchus	EN	✓	
			Rhizoprionodon acutus	VU	-	
			Squalus hemipinnis	VU	-	
			Galeocerdo cuvier	NT	-	
			Isurus paucus	EN	✓	
			Carcharhinus longimanus	CR	✓	
			Centrophorus granulosus	EN	-	
			Carcharhinus melanopterus	VU	-	
			Carcharhinus sealei	NT	-	
			Carcharhinus brevipinna	VU	-	
			Hemipristis elongata	VU	-	
			Nebrius ferrugineus	VU	-	
			Carcharhinus amblyrhynchos	EN	-	
			Hemitriakis falcata	LC	-	
			Mustelus lenticulatus	LC	-	
			Hemigaleus microstoma	VU	-	
			Loxodon macrorhinus	LC	-	
			Sphyrna zygaena	VU	✓	
Chuang et al. (2016)	Taiwan	Fresh tissue	*Prionace glauca* (47.2%)	NT	-	Not evaluated
			Carcharhinus falciformis (7.8%)	VU	✓	
			Alopias superciliosus (7.4%)	VU	✓	
			Alopias pelagicus (6.9%)	EN	✓	
			Etmopterus pusillus (6.9%)	LC	-	
			Isurus oxyrinchus (5.6%)	EN	✓	

			Centrophorus granulosus	EN	-	
			Sphyrna zygaena	VU	✓	
			Galeus sauteri	LC	-	
			Sphyrna lewini	CR	✓	
		fins	*Prionace glauca* (34.3%)	NT	-	
			Carcharhinus falciformis (12.1%)	VU	✓	
			Carcharhinus coatesi (8.6%)	LC	-	
			Carcharhinus macloti (7.5%)	NT	-	
			Sphyrna lewini (4.7%)	CR	✓	
			Hemigaleus australiensis	LC	-	
			Carcharhinus longimanus	CR	✓	
			Callorhinchus callorynchus	VU	-	
			Carcharhinus sorrah	NT	-	
			Alopias pelagicus	EN	✓	
			Isurus oxyrinchus	EN	✓	
Steinke et al. (2017)	Canada	fins	*Alopias pelagicus*	EN	✓	Not evaluated
			Alopias superciliosus	VU	✓	
			Isurus oxyrinchus	EN	✓	
			Prionace glauca	NT	-	
			Sphyrna lewini	CR	✓	
			Lamna nasus	VU	✓	
			Rhincodon typus	EN	✓	
			Isurus paucus	EN	✓	
			Lamna ditropis	LC	-	
			Rhizoprionodon acutus	VU	-	
			Sphyrna mokarran	CR	✓	
			Carcharhinus leiodon	EN	-	
	Hong Kong, mainland China and Sri Lanka	gill plates	All ray (Mobulidae) species	-	-	
Almerón-Souza et al. (2018)	Brazil	filets	*Prionace glauca* (23.8%)	NT	-	Not evaluated
			Sphyrna lewini (22.2%)	CR	✓	
			Rhizoprionodon lalandii	VU	-	
			Carcharhinus brachyurus	VU	-	
			Carcharhinus falciformis	VU	✓	

			Sphyrna zygaena	VU	✓	
			Squalus mitsukurii	EN	-	
			Galeorhinus galeus	CR	-	
			Rhizoprionodon porosus	LC	-	
			Squalus cubensis	LC	-	
			Squatina occulta	CR	-	
			Squatina guggenheim	EN	-	
Cardeñosa et al. (2018)	Hong Kong	fin trimmings	*Prionace glauca*	NT	-	Not evaluated
			Carcharhinus falciformis	VU	✓	
			C. limbatus, *C. tilstoni*, *C. leiodon*, *C. amblyrhynchoides*	-	-	
			Sphyrna lewini	CR	✓	
			Sphyrna zygaena	VU	✓	
Fields et al. (2018)	Hong Kong	fin trimmings	*Prionace glauca* (34.0%)	NT	-	Not evaluated
			Carcharhinus falciformis (10.1%)	VU	✓	
			C. limbatus, *C. amblyrhynchoides*, *C. leiodon*, *C. tilstoni*.	-	-	
			Sphyrna lewini	CR	✓	
			Sphyrna zygaena	VU	✓	
			Isurus oxyrinchus	EN	✓	
			Carcharhinus sp.	-	-	
			Carcharhinus leucas	NT	-	
			Rhizoprionodon acutus	VU	-	
			Carcharhinus brevipinna	VU	-	
			Carcharhinus amboinensis	DD	-	
			Dalatias licha	VU	-	
			Carcharhinus sorrah	NT	-	
			Carcharhinus longimanus	CR	✓	
Md-Zain et al. (2018)*	Malaysia	fins	*Alopias pelagicus*	EN	✓	Not evaluated
			Carcharhinus brevipinna	VU	-	
			Carcharhinus limbatus	NT	-	
			Carcharhinus sorrah	NT	-	
			Lamiopsis tephrodes	nr	-	
			Loxodon macrorhinus	LC	-	
			Sphyrna mokarran	CR	-	
			Sphyrna lewini	CR	✓	

			Chiloscyllium griseum	VU	-	
Wainwright et al. (2018)	Singapore	fins, meat	*Prionace glauca* (6.7%)	NT	-	Not evaluated
			Carcharhinus falciformis	VU	✓	
			Sphyrna lewini	CR	✓	
			Scoliodon laticaudus	NT	-	
			Rhizoprionodon oligolinx	LC	-	
			Galeocerdo cuvier	NT	-	
			Hemipristis elongata	VU	-	
			Carcharhinus leucas	NT	-	
Calegari et al. (2019)	Brazil	filets	*Pangasianodon hypophthalmus* (non-shark species) (100%)	-	-	100%
da Silva Ferrette et al. (2019)	Brazil	fins	*Prionace glauca* (33.7%)	NT	-	Not evaluated
			Isurus oxyrinchus (27.7%)	EN	✓	
			Carcharhinus porosus (13.1%)	CR	-	
			Carcharhinus acronotus	NT	-	
			Carcharhinus falciformis	VU	✓	
			Rhizoprionodon porosus	LC	-	
			Sphyrna tudes	CR	-	
			Isurus paucus	EN	✓	
			Sphyrna tiburo	EN	-	
			Carcharhinus perezi	NT	-	
Hellberg et al. (2019)	USA	shark jerky, fin soup, cartilage pills, filets	*Carcharhinus sorrah* (32%)	NT	-	19%
			Galeorhinus galeus (16%)	CR	-	
			Carcharhinus falciformis (12%)	VU	✓	
			Alopias pelagicus	EN	✓	
			Isurus oxyrinchus	VU	✓	
			Alopias vulpinus	VU	✓	
			Carcharhinus melanopterus	VU	-	
			Prionace glauca	NT	-	
			Carcharhinus sealei	NT	-	
Hobbs et al. (2019)	UK	Meat	*Squalus acanthias*	VU	-	31.4%
			Mustelus asterias	LC	-	
			Scyliorhinus stellaris	NT	-	
			Squalus suckleyi	LC	-	
			Prionace glauca	NT	-	
		fins	*Carcharhinus leucas*	NT	-	

			Sphyrna lewini	CR	✓	
			Isurus oxyrinchus	VU	✓	
			Sphyrna tudes	CR	-	
			Carcharhinus sp.	-	-	
Mattaqin et al. (2019)	Indonesia	meat (fresh and smoked), skin, fins, cartilage	*Isurus oxyrinchus*	VU	✓	Not evaluated
			Alopias pelagicus	EN	✓	
			Carcharhinus falciformis	VU	✓	
			Sphyrna lewini,	CR	✓	
			Carcharhinus sorrah	NT	-	
			Galeocerdo cuvier	NT	-	
Pazartzi et al. (2019)	Greece	filets	*Mustelus mustelus* (36%)	VU	-	55.81%
			Scyliorhinus canicular (23.2%)	nr	-	
			Squalus blainville (13.9%)	DD	-	
			Mustelus asterias	LC	-	
			Prionace glauca	NT	-	
			Mustelus punctulatus	DD	-	
			Squatina squatina	CR	-	
			Alopias vulpinus	VU	✓	
			Heptranchias perlo	NT	-	
			Galeorhinus galeus	CR	-	
			Hexanchus griseus	NT	-	
Abdullah et al. (2020)	Indonesia	various fresh and processed shark products	*Carcharhinus falciformis*	VU	✓	Not evaluated
			Carcharhinus sorrah,	NT	-	
			Alopias pelagicus	EN	✓	
			Galeocerdo cuvier	NT	-	
			Sphyrna lewini	CR	✓	
			Carcharhinus leucas	NT	-	
			Carcharhinus brevipinna	VU	-	
Marchetti et al. (2020)	Italy	filets	*Prionace glauca*	NT	-	45.4%
			Scyliorhinus canicula	LC	-	
			Mustelus asterias	LC	-	
			Mustelus punctulatus	DD	-	
			Isurus oxyrinchus	EN	✓	

Table 2. Studies on species identification in shark food products by DNA-barcoding. *Amplification of various length MDBs to obtain FDB sequences.

Reference	Product	Sampling (n)	Country	Molecular target	Size (bp)	Primers
Holmes, Steinke, & Ward (2009)	dried fins	211	Australia	*COI*	652–655	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005); HCO2198 (rev) (Folmer, Black, Hoeh, Lutz, &Vrijenhoek (1994)
Barbuto et al. (2010)	Slices or fillets	45	Italy	*COI*	550	Shark int (fwd) (Barbuto et al., 2010); FishR2 (rev) (Ward et al., 2005)
Liu, Chan, Lin, Hu, & Chen (2013)	fillets and fins	548	Taiwan	*COI*	~655	LCO1490 (fwd); HCO2198 (rev) (Folmer et al., 1994)
					391	*In silico* analysis
Sembiring et al. (2015)	fins	582	Indonesia	*COI*	600-654	Fish-BCL (fwd); Fish-BCH (rev) (Baldwin, Mounts, Smith, & Weigt, 2009)
Chuang et al. (2016)	fresh tissue	231	Taiwan	*COI*	~655	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005)
	fins	429				
Steinke et al. (2017)	fins	71	Canada	*COI*	652	C_FishF1t1, C_VF1LFt1 (fwd); C_FishR1t1 or and C_VR1LRt1 (rev) (Ivanova, Zemlak, Hanner, & Hebert., 2007)
				16SrRNA	~500	16sarl-L (fwd) 16sbr-H49 (rev) (Palumbi et al., 1991)
	gill plates	58	Hong Kong, mainland China and Sri Lanka	*COI*	652	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005)
				16SrRNA	~500	16sarl-L (fwd) 16sbr-H (rev) (Palumbi et al., 1991)
Almerón-Souza et al. (2018)	fillets	63	Brazil	*COI*	~650	FishF2 (fwd); FishR2 (rev) (Ward et al., 2005)
Cardeñosa et al. (2018)	fin trimmings	9200	Hong Kong	*COI*	650*	Shark474F (fwd) (Cardeñosa et al., 2017); FishF2 (fwd) FishR1, FishR2 (rev) (tailed with M13) (Ward et al., 2005); Shark COI-MINIR (rev) (Fields et al., 2015)
Fields et al. (2018)	fin trimmings	4800	Hong Kong	*COI*	130	FishF2_t1 (fwd), VF2_t1 (fwd) (Ward et al., 2005; Ivanova et al., 2007); Shark COI-

						MINIR (rev) (Fields et al., 2015)
Md-Zain et al. (2018)	fins	24	Malaysia	*COI*	~750	VF2_tl (fwd), FR1d_tl (rev) Ward et al., 2005; Ivanova et al., 2007)
Wainwright et al. (2018)	fins, meat	207	Singapore	*COI*	313	mlCOIintF (fwd) (Leray et al. 2013); LoboR1 (Lobo et al., 2013)
Calegari, Reis, & Alho (2019)	filets	7	Brazil	*COI*	610	LCO1490 (fwd); HCO2198 (rev) (Folmer et al., 1994)
da Silva Ferrette et al. (2019)	fins	800	Brazil	*COI*	~650	FishF1 (fwd); FishR1 (rev) (Ward et al., 2005)
Hellberg Isaacs, & Hernandez (2019)	shark jerky, fin soup, cartilage pills, fillets	35	USA	*COI*	652-658	FishF1t1, C_VF1LFt1 (fwd), C_FishR1t1, C_VR1LRt1 (rev) (Ivanova et al., 2007)
					127	C_FishF1t1 (fwd) (Ivanova et al., 2007), Shark COI-MINIR (rev) (Fields et al., 2015)
Hobbs, Potts, Walsh, Usher, & Griffiths (2019)	meat	117	UK	*COI*	~650	FishF2_t1 (fwd), VF2_t1 (fwd) (Ward et al., 2005; Ivanova et al., 2007)
	fins	40			~130	FishF2_t1 (fwd), VF2_t1 (fwd) (Ward et al., 2005; Ivanova et al., 2007); Shark COI-MINIR (rev) (Fields et al., 2015)
Muttaqin et al. (2019)	meat (fresh and smoked), skin, fins, cartilage	40	Indonesia	*COI*	655	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005)
					295	Primer by Sultana, Ali, Hossain, Naquiah, & Zaidul (2018)
Pazartzi et al. (2019)	filets	87	Greece	*COI*	670	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005)
				16SrRNA	600	16sarl-L (fwd) 16sbr-H (rev) (Palumbi et al., 1991)
Abdullah, Nurilmala, Muttaqin, & Yulianto (2020)	various fresh and processed shark products	36	Indonesia	*COI*	226	SHE-F (fwd), SHE-R (rev) (Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015)
Marchetti, Mottola, Piredda, Ciccarese, & Di Pinto (2020)	filets	130	Italy	*COI*	~655	FishF1, FishF2 (fwd); FishR1, FishR2 (rev) (Ward et al., 2005)
				NADH2	~1050	Primers by Naylor et al. (2012)

Table 3. Samples collected and analysed in this study with relative market price. *numbers refer to the origin (cities and provinces) in Figure 2.

Code	Number of samples	Origin*	Price (US $/kg)
BJ1-BJ6	6	13	80
CC1-CC6	6	22	55
CD1-CD6	12	6	49
CD7-CD12			71
CQ1-CQ6	6	10	86
CS1-CS6	6	18	65
FZ1-FZ6	6	31	58
GY1-GY6	6	11	62
GZ1-GZ6	24	19	26
GZ7-GZ12			32
GZ13-GZ18			52
GZ19-GZ24			68
HB1-HB6	6	21	74
HF1-HF6	6	27	72
HH1-HH6	6	8	68
HK1-HK6	6	20	68
HZ1-HZ6	6	29	68
JN1-JN6	18	25	46
JN7-JN12			49
JN13-JN18			25
KM1-KM6	6	7	49
LS1-LS6	6	2	78
LZ1-LZ6	6	5	43
NC1-NC6	6	30	49
NJ1-NJ6	6	26	74
NN1-NN6	12	12	71
NN7-NN12			65
SH1-SH6	12	28	74
SH7-SH12			56
SJ1-SJ6	12	14	74
SJ7-SJ12			46
SY1-SY6	6	23	58
TJ1-TJ6	12	24	80
TJ7-TJ12			68
TY1-TY6	6	15	55
UQ1-UQ6	6	1	54
WH1-WH6	6	17	55
XA1-XA6	6	9	55
XN1-XN6	6	3	46
YC1-YC6	12	4	43
YC7-YC12			80
ZZ1-ZZ6	6	16	86
Total	**252**		

Table 4. Shark species identified in collected samples ~~by means of sequence comparison with databases~~ with relative average market price, IUCN status and presence in the CITES Appendix II. *only one matching sequence; ~~**Identity values <98%;~~ CR: Critically Endangered; EN: Endangered; NT: Near Threatened; VU: Vulnerable.

Sample code	Number of samples (%)	Molecular target	BLAST	Identity (%)	BOLD ID's	Identity (%)	Sample ID	Average price	IUCN	CITES
BJ1-BJ6, CC1-CC6, CD1-CD6, CS1-CS6, FZ1-FZ6, GY1-GY6, GZ1-GZ12, GZ19-GZ24, HH1-HH4, HH6, HK1, HK2, HK5, HK6, JN7-JN12, KM1-KM6, LS1-LS6, LZ1-LZ6, NC1-NC6, NJ1-NJ6, SH7-SH12, SJ7-SJ12, SY1-SY6, TJ7-TJ12, TY1-TY6, UQ1-UQ6, WH1-WH6, XA1-XA6, XN1-XN6, YC1-YC6, ZZ1-ZZ6	165 (65.5%)	FDB	*Prionace glauca*	100	*P. glauca*	100	*P. glauca*	56.8 ± 13.7	NT	-
CD8, CD11, CQ2, HB2, HB4, HK3, HK4, HZ1-HZ6, NN7-NN10, TJ1-TJ6, YC7-YC12	29 (11.5%)	FDB	*Carcharhinus falciformis*	100	*C. falciformis*	100	*C. falciformis*	73.8 ± 6.6	VU	✓

GZ15-GZ17, NN11, NN12, SH1-SH6, SJ1-SJ6	17 (6.7%)	FDB	*Sphyrna lewini*	100	*S. lewini*	100	*S. lewini*	60.1 ± 8.7	CR	✓
HF1-HF6, JN14, JN17, JN18	9 (3.6%)	FDB	*Sphyrna zygaena*	100	*S. zygaena*	100	*S. zygaena*	56.3 ± 23.5	VU	✓
GZ13, GZ14, GZ18, NN1-NN6	9 (3.6%)	FDB	*Isurus oxyrinchus*	100	*I. oxyrinchus*	100	*I. oxyrinchus*	64.7 ± 9.5	EN	✓
CD7, CD9, CD10, CD12, HB1, HB3, HB5, HB6	8 (3.2%)	FDB	*Carcharhinus longimanus*	100	*C. longimanus*	100	*C. longimanus*	72.5 ± 1.6	CR	✓
CQ1, CQ3-CQ6, JN1, JN2	7 (2.8%)	FDB	*Carcharhinus limbatus* *C. brevipinna* *C. leiodon* *C. amblyrhynchoides* *C. tilstoni*	100 99.85 99.69	*C. limbatus* *C. brevipinna* *C. leiodon* *C. amblyrhynchoides* *C. tilstoni*	100	*Carcharhinus* sp.	74.7 ± 19.5	-	-
JN3-JN6, JN13, JN15	6 (2.4%)	FDB	*Carcharhinus sorrah*	100	*C. sorrah*	100	*C. sorrah*	39 ± 10.8	NT	-
HH5	1 (0.4%)	MDB	*Carcharhinus altimus* *C. plumbeus* *C. amblyrhynchos*	100 99.21	*Carcharhinus* sp. *C. altimus* *C. plumbeus*	100	*Carcharhinus* sp.	68	-	-
JN16	1 (0.4%)	MDB	*Sphyrna lewini* *S. zygaena*	**97.62 96.06	*S. lewini* -	**97.62 -	-	25	-	-

Table 1. Shark species included in the Appendix II of the ~~Convention on International Trade in Endangered Species (~~CITES) with their IUCN relative conservation status ~~according to International Union for the Conservation of Nature (IUCN) Red List of Threatened Species~~. ~~CR: Critically Endangered; EN: Endangered VU: vulnerable.~~

			IUCN status
Carcharhiniformes	Carcharhinidae	*Carcharhinus falciformis*	Vulnerable~~VU~~
		Carcharhinus longimanus	Critically Endangered~~CR~~
	Sphyrnidae	*Sphyrna lewini*	Critically Endangered~~CR~~
		Sphyrna mokarran	Critically Endangered~~CR~~
		Sphyrna zygaena	Vulnerable~~VU~~
	Alopiidae	*Alopias* spp.	Endangered~~EN~~ (*A. pelagicus*) Vulnerable~~VU~~ (*A. superciliosus; A. vulpinus)*
	Cetorhinidae	*Cetorhinus maximus*	Endangered~~EN~~
Lamniformes	Lamnidae	*Carcharodon carcharias*	Vulnerable~~VU~~
		Isurus oxyrinchus	Endangered~~EN~~
		Isurus paucus	Endangered~~EN~~
		Lamna nasus	Vulnerable~~VU~~
Orectolobiformes	Rhincodontidae	*Rhincodon typus*	Endangered~~EN~~