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Identification of Shark Species in Commercial Products using DNA Barcoding

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25 **Abstract**

26   Sharks are harvested globally and sold in a variety of commercial products. However,

27 they are particularly vulnerable to overfishing and many species are considered protected or

28 endangered. The objective of this study was to identify species in various commercial shark

29 products and to assess the effectiveness of three different DNA barcoding primer sets. Thirty-

30 five products were collected for this study, including fillets, jerky, soup, and cartilage pills.

31 DNA barcoding of these products was undertaken using two full-length primer sets and one

32 mini-barcode primer set within the cytochrome c oxidase subunit (COI) gene. Successfully

33 sequenced samples were then analyzed and identified to the species level using sequence

34 databases and character-based analysis. When the results of all three primer sets were combined,

35 74.3% of the products were identified to the species level. Mini-barcoding showed the highest

36 success rate for species identification (54.3%) and allowed for a wide range of identification

37 capability. Six of the 26 identified products were found to be mislabeled or potentially

38 mislabeled, including samples of shark cartilage pills, shark jerky, and shark fin soup. Six

39 products contained species listed in the Convention on International Trade in Endangered

40 Species of Wild Fauna and Flora (CITES) Appendices and 23 products contained near-

41 threatened, vulnerable or endangered species according to the International Union for the

42 Conservation of Nature (IUCN) Red List. Overall, this study revealed that a combination of

43 DNA barcoding primers can be utilized to identify species in a variety of processed shark

44 products and thereby assist with conservation and monitoring efforts.

45 **Keywords:** DNA barcoding; elasmobranchs; shark cartilage; shark meat; species identification

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48   **Introduction**

49   Sharks are harvested worldwide both in targeted fisheries and as bycatch in other fishing

50   operations (Bräutigam, et al., 2015). There is a wide diversity of shark products on the global

51   marketplace, including meat, fins, skin, oil, and cartilage (S. Clarke, 2004; Dent & Clarke,

52   2015). The greatest consumer demand is for shark meat and fins; however, other shark products

53   are not recorded separately in trade statistics, making them difficult to track. Sharks are

54   particularly vulnerable to overfishing due to their late maturity, relatively long gestation periods,

55   and low fecundity (Bräutigam, et al., 2015). Many populations of sharks and rays are considered

56   threatened or endangered: close to 20% of the 1,038 species of sharks and rays assessed by the

57   International Union for the Conservation of Nature (IUCN) Red List of Threatened Species have

58   been categorized as Critically Endangered, Endangered, or Vulnerable, and another 12% have

59   been categorized as Near Threatened (Bräutigam, et al., 2015). Furthermore, the Convention on

60   International Trade in Endangered Species of Wild Fauna and Flora (CITES) has 13 Appendix II

61   listings for sharks and rays, meaning that international trade of these organisms must be

62   controlled through the use of export permits (CITES, 2018). For proper enforcement of CITES,

63   it is essential that customs agents are able to identify these species in globally traded shark

64   products.

65   Intact, unprocessed shark specimens can often be identified to the species level by expert

66   taxonomists using morphological indicators (Hanner, Naaum, & Shivji, 2016; Marshall &

67   Barone, 2016). Some shark fins can be identified in this way as well; however, extensive

68   training is required and identification can be problematic due to species that are similar in

69   appearance and the focus on at-risk species. In order to overcome these challenges, a number of

70   DNA-based analyses have been developed for the identification of shark species (reviewed in

71   Dudgeon, et al., 2012; Hanner, et al., 2016; Rodrigues-Filho, Pinhal, Sondre, & Vallinoto, 2012).

72   These methods are largely based on the use of polymerase chain reaction (PCR) for amplification

73   of universal or species-specific DNA regions. Several multiplex species-specific PCR assays

74   have been developed to assist with shark conservation efforts and monitoring of international

75   trade (Abercrombie, Clarke, & Shivji, 2005; Chapman, et al., 2003; S. C. Clarke, Magnussen,

76   Abercrombie, McAllister, & Shivji, 2006; M. Shivji, et al., 2002; M. S. Shivji, Chapman,

77   Pikitch, & Raymond, 2005). These studies have revealed trade of shark fins from protected

78   species such as white shark (*Carcharodon carcharias*) and hammerhead sharks (*Sphyrna* spp.).

79   While species-specific PCR assays are favored for the rapid identification of known target

80   species, a universal approach, such as DNA barcoding, is advantageous in applications where a

81   wide range of species is possible.

82   DNA barcoding is a sequencing-based technique that utilizes universal primers targeting

83   a short, standardized genetic region for the identification of species (Hebert, Cywinska, Ball, &

84   DeWaard, 2003). The standard target for DNA barcoding of animal species is a ~650 bp region

85   of the mitochondrial gene coding for cytochrome *c* oxidase subunit I (COI). Because of

86   campaigns such as the Fish Barcode of Life Initiative (http://www.fishbol.org/), DNA barcoding

87   is supported by a large database of sequence information to assist with species identification.

88   DNA barcoding of elasmobranchs has been investigated in numerous studies and has proven to

89   be effective in identifying a wide range of species (Bineesh, et al., 2017; Doukakis, et al., 2011;

90   Ward, Holmes, White, & Last, 2008; Wong, Shivji, & Hanner, 2009). This method has also

91   been utilized to reveal mislabeling of shark products, as well as trade of threatened and

92   endangered shark species (Asis, Lacsamana, & Santos, 2016; Barbuto, et al., 2010; Cardeñosa, et

93   al., 2017; Holmes, Steinke, & Ward, 2009; Liu, Chan, Lin, Hu, & Chen, 2013; Moore, Almojil,

94 Harris, Jabado, & White, 2014; Naaum Amanda & Hanner, 2015; Sembiring, et al., 2015;

95 Steinke, et al., 2017). However, it can be challenging to recover the full-length DNA barcode

96 from products that have undergone extensive processing as the DNA is often degraded and

97 highly fragmented (Fields, Abercrombie, Eng, Feldheim, & Chapman, 2015; Shokralla,

98 Hellberg, Handy, King, & Hajibabaei, 2015). To address this, Fields et al. (2015) developed a

99 mini-barcoding assay for shark species identification that targets a shorter 110-130 bp region

100 within the full-length COI barcode. This assay was shown to be effective in identifying sharks to

101 the species or genus level in 100% of processed fins tested and 62% of shark fin soup samples.

102 These results indicate potential use of the shark mini-barcoding assay for species identification in

103 other highly processed shark products, such as shark cartilage supplements.

104   The objective of this study was to use DNA barcoding to identify shark species in

105 commercial products and to compare the effectiveness of three different barcoding methods:

106 shark mini-barcoding, fish full barcoding, and mammalian full barcoding.

107 **2.   Materials and Methods**

108 *2.1 Sample collection*

109   A total of 35 commercial shark products were collected for this study. The products were

110 purchased online and from restaurants or retail outlets in Orange and Los Angeles Counties, CA,

111 USA. A variety of products were collected, including shark jerky (n = 3), shark fin soup (n = 1),

112 shark cartilage pills (n = 29), and fresh or grilled shark fillets (n = 2). Following collection, each

113 product was assigned a sample number and catalogued. Products were then held at their

114 recommended storage temperatures until DNA extraction. DNA was extracted from perishable

115 items within two days of collection.

116 *2.2 DNA extraction*

5

117   Sterile forceps were used to sample tissue from the jerky, soup (ceratotrichia), and fillet

118   samples. Cartilage pills in capsule form were twisted open and the powder was poured directly

119   into a sterile microcentrifuge tube for weighing, while tablets (solid form) were broken up with

120   sterile forceps and then placed into a sterile microcentrifuge tube. DNA was extracted from ~25

121   mg of each sample using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), Spin-

122   Column protocol, according to the manufacturer's instructions, with modifications made to the

123   final elution step. DNA was eluted using pre-heated (37°C) AE buffer at a volume of 60 µl for

124   cartilage pill samples and 100 µl for all other samples. A reagent blank negative control with no

125   sample added was included with each set of DNA extractions.

126   *2.3 PCR*

127   DNA extracts from each sample underwent PCR using three different primer sets (Table

128   1): a shark mini-barcode primer set (Fields, et al., 2015) and two full-barcode primer sets ('fish

129   full barcode' and 'mammalian full barcode') used in a previous study on shark species

130   identification (Wong, et al., 2009). With the exception of Shark COI-MINIR, all primers

131   included M13 tails to facilitate DNA sequencing (Table 1). Amplification of shark mini-

132   barcodes was carried out with the following reaction mixture: 25 µl HotStar Taq Master Mix

133   (2X) (Qiagen), 22 µl of molecular-grade sterile water, 1 µl of 10 µM C_FishF1t1 (Table 1), 1 µl

134   of 10 µM Shark COI-MINIR (Table 1), and 1 µl of template DNA. Fish and mammalian full

135   barcodes were amplified using the following reaction mixture: 25 µl HotStar Taq Master Mix

136   (2X) (Qiagen), 23 µl of molecular-grade sterile water, 0.5 µl of 10 µM forward primer cocktail

137   (Table 1), 0.5 µl of 10 µM reverse primer cocktail (Table 1), and 1 µl of template DNA. A no-

138   template control (NTC) with molecular-grade sterile water instead of DNA was included

139   alongside each set of reactions. PCR was carried out using a Mastercycler nexus Gradient

140 Thermal Cycler (Eppendorf). The cycling conditions for shark mini-barcoding were: 95°C for

141 15 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min; and a final extension

142 step at 72°C for 5 min. The cycling conditions for fish full barcoding were: 95°C for 15 min; 35

143 cycles of 94°C for 30 s, 52°C for 40 s, and 72°C for 1 min; and a final extension step at 72°C for

144 10 min. The cycling conditions for mammalian full barcoding were: 95°C for 15 min; 5 cycles

145 of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min; 35 cycles of 94°C for 30 s, 55°C for 40 s,

146 and 72°C for 1 min; and a final extension step at 72°C for 10 min.

147 *2.4 PCR product confirmation and DNA sequencing*

148   Confirmation of PCR products was achieved using 2.0% agarose E-Gels (Life

149 Technologies, Carlsbad, CA) run on an E-Gel iBase (Life Technologies). A total of 16 µl of

150 sterile water and 4 µl of PCR product were loaded into each well (Hellberg, Kawalek, Van,

151 Shen, & Williams-Hill, 2014). Each sample with a visible PCR product on the agarose gel was

152 purified with the QIAquick PCR Purification Kit using a Microcentrifuge (Qiagen), according to

153 the manufacturer's instructions. Purified PCR products were sequenced at the GenScript facility

154 (Piscataway, NJ) with M13 primers. Mini-barcode products were only sequenced in one

155 direction using the forward M13 primers, as described in Fields et al. (2015), while all full-

156 barcoding products were sequenced bi-directionally (Ivanova, Zemlak, Hanner, & Hebert, 2007).

157 DNA sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life

158 Technologies) and a 3730xl Genetic Analyzer (Life Technologies).

159 *2.5 Sequencing results and analysis*

160   Raw sequence data was assembled and edited using Geneious R7 [(Biomatters, Ltd.,

161 Auckland, New Zealand) (Kearse, et al., 2012)]. The resulting sequences were trimmed to the

162 appropriate full-barcode (652-658 bp) or mini-barcode (127 bp) regions. Trimmed sequences

163 with < 2% ambiguities were queried through the Barcode of Life Database (BOLD) Animal

164 Identification Request Engine (http://www.boldsystems.org/), Species Level Barcodes. Any

165 sequences that could not be identified to the species level in BOLD were next queried in

166 GenBank with the Nucleotide Basic Local Alignment Search Tool (BLASTn;

167 http://blast.ncbi.nlm.nih.gov/Blast.cgi). The top species matches were recorded. Sequences with

168 multiple top species matches and/or secondary matches with ≥ 98% genetic similarity were next

169 examined using character-based analysis, as described in Wong et al. (2009). The conservation

170 status of each identified species was determined using the IUCN Red List of Threatened Species

171 (http://www.iucnredlist.org/).

172 **3. Results**

173 *3.1 Species identification using DNA barcoding*

174   DNA barcodes were obtained from at least one primer set for 26 of the 35 commercial

175 shark products tested in this study (Fig. 1). DNA barcodes were recovered from 100% of the

176 jerky, fillet, and soup products, but only 69% of the 29 shark cartilage pill samples. The one

177 shark cartilage tablet collected for this study failed PCR with all three primer sets, while 20 of

178 the 28 capsules collected were sequenced by at least one method (Table 2). The shark mini-

179 barcoding primer set was the most successful at identifying shark or other fish species in the

180 products tested, with identification success in 19 of the 35 products (Fig. 1). The mammalian

181 full-barcoding primer set allowed for species identifications in 16 of the 35 products; however,

182 only 10 of the products were identified as shark or other fish species. The remaining six

183 products were identified as wild rice (*Oryza rufipogon*). The fish full-barcoding primer set was

184 the least successful and was only able to identify species in 3 of the commercial shark products.

185   In cases where one sequence matched multiple species with a genetic similarity of ≥

186   98%, character analysis was applied (Wong et al. 2009). The use of character analysis allowed

187   for five of the shark cartilage products (S19, S22, S26, S31, and S35) sequenced across the mini-

188   barcode region to be identified to species level. Character analysis also reduced the number of

189   secondary species matches obtained for three other samples (S21, S27, and S33) sequenced

190   across the mini-barcode region. For example, the mini-barcode sequence for S33 showed a top

191   species match with 99.12% genetic similarity to spot-tail shark (*Carcharhinus sorrah*) and a

192   secondary species match to night shark (*Carcharhinus signatus*) with 98.92% genetic similarity.

193   However, character analysis revealed that the sequence did not contain one of the nucleotides

194   determined to be diagnostic for night shark.

195   Despite the use of character analysis, eight of the samples sequenced with mini-barcoding

196   continued to have at least one secondary species match with genetic similarity ≥ 98% (Table 2).

197   This occurred with seven samples containing spot-tail shark and one sample containing blacktip

198   reef shark (*Carcharhinus melanopterus*). In most cases, the secondary matches were to other

199   *Carcharhinus* spp. These results are consistent with previous DNA barcoding research that has

200   reported less than 1% genetic divergence among some members of the *Carcharhinus* genus

201   (Ward, et al., 2008). Five products sequenced with the shark mini-barcode (S21, S22, S33, S35,

202   and S36) showed equivocal BOLD matches (99.1-100%) to both spot-tail shark and blacktip

203   shark (*Carcharhinus limbatus*). Upon further examination, it was found that each sample

204   matched numerous published entries for spot-tail shark and only one entry for blacktip shark,

205   which was an Early-Release sequence and not publicly accessible. When the sequences were

206   queried in GenBank, they all matched spot-tail shark with no equivalent match to blacktip shark.

207   Therefore, these samples were determined to be spot-tail shark.

208   None of the shark species detected with mammalian full barcoding showed multiple

209 species matches with ≥ 98% genetic similarity. All of the samples identified as wild rice showed

210 secondary matches in BOLD to other plant species, such as meadow grass (*Poa annua*) and

211 ryegrass (*Lolium rigidum*). The two samples identified as winter skate (*Leucoraja ocellata*) with

212 full fish barcoding (S05 and S16) each showed a secondary match to one sequence labeled as

213 little skate (*Leucoraja erinacea*). However, upon further investigation, it was found that this

214 sequence (BOLD Sample ID JF894896) was misidentified and is actually derived from winter

215 skate (Coulson et al. 2011).

216   Mammalian full barcoding generated barcodes for two samples (S09 and S22) that did

217 not show a species match with ≥ 98% genetic similarity in BOLD. Therefore, these samples

218 were instead identified with GenBank. Sample S09, labeled as "Shark's Fin Soup," was

219 identified as delagoa threadfin bream (*Nemipterus bipunctatus*) with 94% genetic similarity, and

220 sample S22, a bottle of shark cartilage capsules, was identified as blackspot shark (*Carcharhinus*

221 *sealei*) with 96% genetic similarity. In both cases, the sequence quality was relatively low, with

222 < 23% high quality (HQ) bases. Similarly, the mini-barcode primer set generated a barcode for

223 the shark fin soup sample (S09) with a low HQ score (9.9%) that did not show a species match

224 with ≥ 98% genetic similarity in BOLD. The top species match for this sample in GenBank was

225 red bigeye (*Priacanthus macracanthus*) with 90% genetic similarity.

226 *3.2 Mislabeled products*

227   Among the 26 samples for which sequences were obtained, 5 samples (19%) were

228 determined to be mislabeled and one was considered to be potentially mislabeled. The five

229 mislabeled samples claimed to be manufactured in the United States and consisted of one "mako

230 shark" jerky product (S12) identified as thresher shark (*Alopias vulpinus*); two shark cartilage

231   pill products (S05, S16) containing undeclared winter skate and no shark species; and two shark

232   cartilage pill products (S19 and S26) containing undeclared rice ingredients in addition to shark

233   species. Another shark cartilage product (S27) that tested positive for rice in addition to shark

234   contained cellulose as an ingredient, which may have been the source of the rice. Therefore, this

235   product was not considered to be mislabeled. The one sample of shark fin soup (S09) tested was

236   determined to be potentially mislabeled due to the detection of teleost fish in the product instead

237   of shark. Of note, the mislabeled jerky product (S12) was obtained from a different brand and

238   online distributor as compared to the correctly labeled sample of mako shark jerky (S02). The

239   two samples containing winter skate were sold under different commercial brand names but were

240   purchased from the same online distributor and originated from the same manufacturer. In

241   contrast, the two shark cartilage pill products identified as containing undeclared rice were

242   purchased from different sellers and originated from different manufacturers.

243   *3.3 Conservation status of identified species*

244   Six of the commercial shark products tested in this study were found to contain CITES-

245   listed shark species: silky shark (*Carcharhinus falciformis*) and thresher sharks [(*Alopias* spp.)

246   (Table 3)]. However, it should be noted that the CITES listings for these species were not

247   effective until after this study was completed (effective date: 4 October 2017). The three

248   products containing thresher sharks consisted of two jerky samples and one fillet, while silky

249   shark was detected in three shark cartilage pill samples. All 10 species of sharks and skate

250   detected in this study appear on the IUCN Red List of Threatened Species (IUCN, 2017). These

251   species were detected in 23 different commercial products, with some products found to contain

252   multiple species (Table 2). Five of these species are considered to be near threatened, four are

253   considered vulnerable, and one is considered endangered.

254 **4. Discussion**

255 *4.1 Comparison of DNA barcoding methods*

256   Using a combination of three DNA barcoding primer sets, species identification

257 (including rice, teleost, and elasmobranch species) was possible in the majority (74.3%) of

258 commercial shark products tested (Fig. 1). On an individual basis, shark mini-barcoding had the

259 highest identification rate (54.3%), followed by mammalian full-barcoding (45.7%), and fish

260 full-barcoding (8.6%). The three DNA barcoding primer sets proved to be complementary in

261 that they allowed for a wide range of species to be identified. Despite the low success rate of the

262 fish full-barcode primer set, it was the only method that enabled the identification of winter skate

263 in shark cartilage pills (Table 2). Along these lines, the other two primer sets also showed

264 advantages for identification of certain shark species, such as spot-tail shark with mini-barcoding

265 and pelagic thresher (*Alopias pelagicus*) with mammalian full barcoding. Mammalian full

266 barcoding not only amplified shark species but also resulted in the detection of wild rice in

267 products, indicating the universal nature of this primer set. However, it is important to note that

268 any plant species identifications based on COI DNA barcoding must be verified using a plant-

269 specific DNA barcoding assay, such as that used by Newmaster et al. (2013).

270   The mini-barcode was most effective for detecting species within the shark cartilage pills,

271 demonstrating the benefits of using shorter barcodes on highly processed samples containing

272 degraded DNA. The mammalian full barcode was more effective with lightly processed products

273 likely due to the better DNA quality within these samples. Interestingly, there was only one

274 instance in which all three primer sets were successful with the same product (S32), which was

275 identified as tope shark (*Galeorhinus galeus*). In three cases (S21, S22, and S33), the use of

276 multiple primer sets allowed for the identification of more than one shark species in shark

277 cartilage pills. For example, mammalian full barcoding enabled the identification of tope shark

278 in two cartilage pill samples (S21 and S33), while mini-barcoding enabled the identification of

279 spot-tail shark in these products. With regards to CITES-listed species, shark mini-barcoding

280 allowed for the identification of silky shark and thresher shark in products, but not pelagic

281 thresher. On the other hand, mammalian full barcoding allowed for the identification of thresher

282 and pelagic thresher but not silky shark. These results indicate potential complementary uses of

283 these primer sets in identifying CITES-listed species, which require strict monitoring of trade by

284 all member parties.

285   While all jerky, fillet, and soup products were identified to the species level, only 69% of

286 the shark cartilage pill samples were successfully sequenced and identified. In comparison,

287 Wallace et al. (2012) reported a success rate of only 20% for DNA barcoding of five animal

288 product capsules. The one capsule (velvet antler) that was successfully sequenced by Wallace et

289 al. (2012) failed with full-length DNA barcoding, but was recovered using a universal mini-

290 barcode primer set. The reduced success with shark cartilage pills in the current study may have

291 been due to several factors, including DNA degradation during processing, the presence of

292 species that could not be amplified with the primer sets used, and/or the use of species mixtures.

293 Because DNA barcoding primers are able to amplify a wide range of species, the presence of

294 multiple species in a single product can lead to an unreadable electropherogram and sequencing

295 failure. The presence of species mixtures may also explain the relatively low genetic similarity

296 (94-96%) obtained for the top species matches for two samples: a sample of shark fin soup (S09)

297 and a shark cartilage product (S22). Both samples had sequences with relatively low quality

298 scores, which may have been a result of simultaneous amplification of multiple species in a

299 single product.

300   *4.2 Mislabeling of commercial products*

301   Potential mislabeling was detected in a variety of product types, including jerky, soup,

302   and shark cartilage supplements (Table 2). Species substitution was the most common type of

303   mislabeling detected, followed by the use of undeclared fillers. As previously mentioned, the

304   one sample of shark fin soup tested was found to be potentially mislabeled due to the detection

305   of teleost fish instead of shark. One explanation for this finding is that the restaurant

306   intentionally did not include shark in the product because it is illegal to sell shark fin in

307   California under A.B. 376, Shark fins (2011). In contrast to these results, a large-scale survey on

308   shark fin soup from U.S. restaurants detected a number of shark species, including tope shark,

309   blue shark (*Prionace glauca*), and other *Carcharhinus* spp., with no reports of teleost fish

310   species (Fields, et al., 2015).

311   Among the product types tested, mislabeling was detected most frequently in the shark

312   cartilage supplements. Out of the 20 supplements with a recoverable barcode, 20% were found

313   to be mislabeled. Similarly, Wallace et al. (2012) reported 2 of 10 shark natural health products

314   collected in North America to be mislabeled, including one sample of shark bones and one dried,

315   shredded shark fin. Undeclared rice was detected in two of the shark cartilage products tested in

316   the current study (S19 and S26). Rice is a common filler used in dietary supplements; however,

317   additional testing of the shark cartilage products using plant-specific barcodes would be needed

318   to confirm this detection. The presence of undeclared fillers has previously been reported in

319   herbal products sold in North America (Newmaster, et al., 2013). In comparison to the current

320   study, which found undeclared fillers in 7% of shark cartilage supplements tested, Newmaster et

321   al. (2013) reported the presence of undeclared fillers (rice or wheat) in 21% of herbal products

322   tested. The presence of undeclared fillers such as these in a product can be a health risk for

323   individuals with allergies.

324   Three bottles of shark cartilage pills were found to contain rice, with no shark species

325   detected in the products (S08, S18, and S30). However, all of these samples included rice flour

326   or rice powder in the ingredient list. Due to the possibility that these products contained shark

327   DNA that could not be amplified by the methods used in this study, they were not considered to

328   be mislabeled. One of the samples (S30) specifically stated that it contained dogfish shark,

329   which is considered an acceptable market name for a number of species, including *Squalus* spp.

330   (FDA, 2016). Dogfish from the *Squalus* genus was detected previously with the shark mini-

331   barcoding method in a sample of shark fin soup (Fields, et al., 2015) and the authors predicted

332   that the shark mini-barcoding assay described in their study would be capable of amplifying all

333   or most shark species. However, the use of fillers, such as rice, can be problematic for DNA

334   sequencing, as this can result in an unreadable mixed signal due to the simultaneous

335   amplification of multiple species.

336   *4.3 Conservation issues*

337   This study revealed the presence of near threatened, vulnerable, and endangered

338   elasmobranch species on the U.S. commercial marketplace. Many of these species are

339   considered to be of concern because they are under heavy fishing pressure, targeted by

340   unmanaged and unreported fisheries, and known to be exploited for their fins and meat (IUCN,

341   2017). However, it should be noted that sustainable fisheries do exist for some of these species

342   in specific geographic regions. For example, the National Oceanic and Atmospheric

343   Administration (NOAA) FishWatch considers U.S. wild-caught shortfin mako (*Isurus*

344   *oxyrinchus*) to be sustainably managed and responsibly harvested (NOAA, 2017).

345   Winter skate, which was found in two products, was the only species detected in this

346   study that is considered to be endangered by IUCN. This species inhabits shelf waters of the

347   northwest Atlantic Ocean and it is primarily harvested for use in skate wings (Kulka, Sulikowski,

348   & Gedamke, 2009). The IUCN considers this species to be endangered globally due to the

349   observance of substantial declines in major areas of the species' range. However, according to

350   NOAA FishWatch, winter skate that is wild-caught in the United States is considered to be

351   sustainably managed and responsibly harvested (NOAA, 2017).

352   The most common species detected varied depending on the type of commercial product.

353   For example, all of the jerky, steak, and fillet samples were found to contain shortfin mako,

354   pelagic thresher or thresher. All three species are considered vulnerable according to the IUCN

355   Red List and the latter two are CITES-listed. On the other hand, the majority of shark cartilage

356   pills contained spot-tail shark, a near threatened species, with other commonly detected species

357   being tope shark (vulnerable) and silky shark (near threatened and CITES listed). Less

358   frequently detected species include winter skate and two near threatened species (blue shark and

359   blackspot shark). Previous studies reported the presence of blue shark in a sample of dried shark

360   cartilage (Wallace, et al., 2012) and basking shark (*Cetorhinus maximus*) in a cartilage pill

361   product (Hoelzel, 2001). Similar to the results of the current study, Fields et al. (2015) primarily

362   detected requiem sharks (*Carcharhinus* spp.) followed by tope (school) sharks, blue sharks, and

363   spot-tail shark in dried processed fin samples from Hong Kong. These results support earlier

364   reports that shark cartilage is utilized as a by-product of existing shark fisheries (Rose, 1996).

365   Currently, shark cartilage is not separately recorded as part of global trade statistics and there is a

366   lack of information on the quantities being traded and the exact species that are used.

367   **5. Conclusions**

368   This study revealed the effectiveness of DNA barcoding for the identification of species

369   in commercial shark products. The three primer sets examined in this study proved to be

370   complementary in their ability to identify a range of elasmobranch species. Shark mini-

371   barcoding was found to be the most successful assay for identification of shark species in highly

372   processed shark cartilage pills, while mammalian full barcoding was the most effective at

373   identifying species in lightly processed products, such as fillets and jerky. This study also

374   revealed the ability of these assays to detect trade of threatened and endangered species in

375   commercial shark products, including several CITES-listed species, thereby facilitating

376   conservation efforts and monitoring of international trade. While many of the shark species

377   detected in this study have been reported in the global shark fin trade, this is the most extensive

378   report to-date of shark species in commercial shark cartilage supplements. Many of the species

379   identified in these supplements are known for being targeted in the commercial shark fin trade

380   and the results indicate that they are also being used for shark cartilage production. Furthermore,

381   this is the first report of the use of winter skate as a substitute for shark species in cartilage pill

382   supplements. Although DNA barcoding was successful with lightly processed products,

383   detection of species in shark cartilage pills was relatively challenging and may benefit from

384   further optimization.

385   **Acknowledgements**

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506

Table 1. Details for the PCR primer sets and M13 tails used in this study.

Primer set	Primer cocktail	Primer name	Primer sequence (5'-3')[a]	Ratio in Cocktail	Barcode length	Reference
Shark mini-barcode	C_FishF1t1	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAA AGACATTGGCAC	1	127 bp	Ivanova, et al. (2007)
		FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAA AGATATCGGCAC	1		
	N/A	Shark COI-MINIR	AAGATTACAAAAGCGTGGGC	N/A		Fields, et al. (2015)
Fish full barcode	C_FishF1t1	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAA AGACATTGGCAC	1	652 bp	Ivanova, et al. (2007)
		FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAA AGATATCGGCAC	1		
	C_FishR1t1	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACC GAAGAATCAGAA	1		
		FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCC GAARAAYCARAA	1		
Mammalian full barcode	C_VF1LFt1	LepF1_t1	TGTAAAACGACGGCCAGTATTCAACCAATCA TAAAGATATTGG	1	658 bp	Ivanova, et al. (2007)
		VF1_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCA CAAAGACATTGG	1		

		VF1d_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCA CAARGAYATYGG	1			
		VF1i_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCA IAAIGAIATIGG	3			
	C_VR1LRt1	LepRI_t1	CAGGAAACAGCTATGACTAAACTTCTGGATG TCCAAAAAATCA	1			
		VR1d_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTG GCCRAARAAYCA	1			
		VR1_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTG GCCAAAGAATCA	1			
		VR1i_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTG ICCIAAIAAICA	3			
M13	N/A	M13F (−21)	TGTAAAACGACGGCCAGT		N/A	N/A	Messing (1983)
	N/A	M13R (−27)	CAGGAAACAGCTATGAC		N/A	N/A	

[a]Shaded portions indicate M13 tails

Table 2. Species identified in the 26 commercial shark products successfully sequenced by at least one of the primer sets tested in this study. Products found to be mislabeled or potentially mislabeled are shown in boldface.

Sample ID	Sample description	Identified species		
		Fish full barcode	Mammalian full barcode	Shark mini-barcode
S01	Mako shark steak, grilled	Failed PCR	Shortfin mako (*Isurus oxyrinchus*)	Shortfin mako (*Isurus oxyrinchus*)
S02	Mako shark jerky	Failed PCR	Shortfin mako (*Isurus oxyrinchus*)	Shortfin mako (*Isurus oxyrinchus*)
S05	**Shark cartilage capsules**	**Winter skate (*Leucoraja ocellata*)[a]**	**Failed PCR**	**Failed sequencing**
S08	Shark cartilage capsules	Failed PCR	Wild rice (*Oryza rufipogon*)[a]	Failed PCR
S09	**Shark's fin soup**	**Failed sequencing**	**Delagoa threadfin bream (*Nemipterus bipunctatus*)[b]**	**Red bigeye (*Priacanthus macracanthus*)[b]**
S10	Thresher shark fillet, fresh/frozen	Failed PCR	Pelagic thresher (*Alopias pelagicus*)	Failed PCR
S11	Shark jerky	Failed PCR	Pelagic thresher (*Alopias pelagicus*)	Failed PCR
S12	**Mako shark jerky**	**Failed PCR**	**Thresher (*Alopias vulpinus*)**	**Thresher (*Alopias vulpinus*)**
S13	Shark cartilage capsules	Failed PCR	Failed PCR	Spot-tail shark (*Carcharhinus sorrah*)[a]
S14	Shark cartilage capsules	Failed PCR	Failed sequencing	Spot-tail shark (*Carcharhinus sorrah*)[a]
S16	**Shark cartilage capsules**	**Winter skate (*Leucoraja ocellata*)[a]**	**Failed sequencing**	**Failed PCR**
S17	Shark cartilage capsules	Failed PCR	Failed PCR	Tope shark (*Galeorhinus galeus*)
S18	Shark cartilage capsules	Failed PCR	Wild rice (*Oryza rufipogon*)[a]	Failed PCR
S19	**Shark cartilage capsules**	**Failed PCR**	**Wild rice (*Oryza rufipogon*)[a]**	**Silky shark (*Carcharhinus falciformis*)[c]**
S21	Shark cartilage capsules	Failed sequencing	Tope shark (*Galeorhinus galeus*)	Spot-tail shark (*Carcharhinus sorrah*)[a]
S22	Shark cartilage capsules	Failed sequencing	Blackspot shark (*Carcharhinus sealei*)[b]	Spot-tail shark (*Carcharhinus sorrah*)[ac]
S23	Shark cartilage capsules	Failed PCR	Failed sequencing	Spot-tail shark (*Carcharhinus sorrah*)[a]

S26	**Shark cartilage capsules**	**Failed PCR**	**Wild rice (*Oryza rufipogon*)**[a]	**Silky shark (*Carcharhinus falciformis*)**[c]
S27	Shark cartilage capsules	Failed sequencing	Wild rice (*Oryza rufipogon*)[a]	Blacktip reef shark (*Carcharhinus melanopterus*)[a]
S28	Shark cartilage capsules	Failed sequencing	Failed sequencing	Blue shark (*Prionace glauca*)
S30	Shark cartilage capsules with dogfish shark	Failed PCR	Wild rice (*Oryza rufipogon*)[a]	Failed PCR
S31	Shark cartilage capsules	Failed PCR	Failed sequencing	Silky shark (*Carcharhinus falciformis*)[c]
S32	Shark cartilage capsules	Tope shark (*Galeorhinus galeus*)	Tope shark (*Galeorhinus galeus*)	Tope shark (*Galeorhinus galeus*)
S33	Pacific Ocean shark cartilage capsules	Failed sequencing	Tope shark (*Galeorhinus galeus*)	Spot-tail shark (*Carcharhinus sorrah*)[a]
S35	Shark cartilage capsules	Failed PCR	Failed PCR	Spot-tail shark (*Carcharhinus sorrah*)[ac]
S36	Shark cartilage capsules	Failed PCR	Failed PCR	Spot-tail shark (*Carcharhinus sorrah*)[a]

[a]Sequence had secondary species matches with ≥ 98% genetic similarity that could not be ruled out with character analysis
[b]Top species match was < 98% genetic similarity
[c]Species identification included the use of character analysis

Table 3. Conservation status of the elasmobranch species detected in commercial products tested in this study.

Elasmobranch species	Common name	CITES Listing	IUCN Red List status	Number of products containing species
Leucoraja ocellata	Winter skate	Not listed	Endangered	2
Alopias pelagicus	Pelagic thresher	Appendix II (October 2017)	Vulnerable	2
Alopias vulpinus	Thresher	Appendix II (October 2017)	Vulnerable	1
Galeorhinus galeus	Tope shark	Not listed	Vulnerable	4
Isurus oxyrinchus	Shortfin mako	Not listed	Vulnerable	2
Carcharhinus sorrah	Spot-tail shark	Not listed	Near Threatened	8
Carcharhinus falciformis	Silky shark	Appendix II (October 2017)	Near Threatened	3
Carcharhinus melanopterus	Blacktip reef shark	Not listed	Near Threatened	1
Prionace glauca	Blue shark	Not listed	Near Threatened	1
Carcharhinus sealei	Blackspot shark	Not listed	Near Threatened	1

Figure caption

Figure 1. Percentage of commercial shark products (n = 35) identified through DNA barcoding with three different primer sets.