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Identification of Shark Species in Commercial Products using DNA Barcoding

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1 **Title: Identification of shark species in commercial products using DNA barcoding**

2

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Abstract

Sharks are harvested globally and sold in a variety of commercial products. However, they are particularly vulnerable to overfishing and many species are considered protected or endangered. The objective of this study was to identify species in various commercial shark products and to assess the effectiveness of three different DNA barcoding primer sets. Thirty-five products were collected for this study, including fillets, jerky, soup, and cartilage pills. DNA barcoding of these products was undertaken using two full-length primer sets and one mini-barcode primer set within the cytochrome *c* oxidase subunit (COI) gene. Successfully sequenced samples were then analyzed and identified to the species level using sequence databases and character-based analysis. When the results of all three primer sets were combined, 74.3% of the products were identified to the species level. Mini-barcoding showed the highest success rate for species identification (54.3%) and allowed for a wide range of identification capability. Six of the 26 identified products were found to be mislabeled or potentially mislabeled, including samples of shark cartilage pills, shark jerky, and shark fin soup. Six products contained species listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendices and 23 products contained near-threatened, vulnerable or endangered species according to the International Union for the Conservation of Nature (IUCN) Red List. Overall, this study revealed that a combination of DNA barcoding primers can be utilized to identify species in a variety of processed shark products and thereby assist with conservation and monitoring efforts.

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

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*

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

pill products (S05, S16) containing undeclared winter skate and no shark species; and two shark cartilage pill products (S19 and S26) containing undeclared rice ingredients in addition to shark species. Another shark cartilage product (S27) that tested positive for rice in addition to shark contained cellulose as an ingredient, which may have been the source of the rice. Therefore, this product was not considered to be mislabeled. The one sample of shark fin soup (S09) tested was determined to be potentially mislabeled due to the detection of teleost fish in the product instead of shark. Of note, the mislabeled jerky product (S12) was obtained from a different brand and online distributor as compared to the correctly labeled sample of mako shark jerky (S02). The two samples containing winter skate were sold under different commercial brand names but were purchased from the same online distributor and originated from the same manufacturer. In contrast, the two shark cartilage pill products identified as containing undeclared rice were purchased from different sellers and originated from different manufacturers.

3.3 Conservation status of identified species

Six of the commercial shark products tested in this study were found to contain CITES-listed shark species: silky shark (*Carcharhinus falciformis*) and thresher sharks [(*Alopias* spp.) (Table 3)]. However, it should be noted that the CITES listings for these species were not effective until after this study was completed (effective date: 4 October 2017). The three products containing thresher sharks consisted of two jerky samples and one fillet, while silky shark was detected in three shark cartilage pill samples. All 10 species of sharks and skate detected in this study appear on the IUCN Red List of Threatened Species (IUCN, 2017). These species were detected in 23 different commercial products, with some products found to contain multiple species (Table 2). Five of these species are considered to be near threatened, four are 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.

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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	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	1	127 bp	Ivanova, et al. (2007)
		FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	1		
	N/A	Shark COI-MINIR	AAGATTACAAAAGCGTGGGC	N/A		Fields, et al. (2015)
Fish full barcode	C_FishF1t1	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	1	652 bp	Ivanova, et al. (2007)
		FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC	1		
	C_FishR1t1	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA	1		
		FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	1		
Mammalian full barcode	C_VF1LFt1	LepF1_t1	TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG	1	658 bp	Ivanova, et al. (2007)
		VF1_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG	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 $\geq 98\%$ 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.