

Species authentication of canned mackerel: challenges in molecular identification and potential drivers of mislabelling

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Abstract

The market for canned mackerel is growing, due to their competitive price and to the excellent properties of the meat. However, the weak rules of current legislation, coupled with the loss of discrimination power due to being obliged to use short barcode regions, open up new challenges for traceability in processed products. In this study, for the first time, we applied a two-step mini-barcoding approach to identify canned mackerel sold in Italian markets and make assumptions on the potential drivers of mislabelling. Preliminary identification was performed using mini-barcode universal primers targeting a COI-fragment; then, in order to discriminate within the complex *Scomber colias*/*S. japonicus*/*S. australasicus*, we applied a second step, using new specific primers designed in the mitochondrial control region (D-loop). Comparison between labels and molecular identifications highlighted that the chance of mislabeling could be either 45% or 0%, depending on the interpretation of the generic terms used on the labels. Therefore, the study revealed that the ambiguous use, in the ingredient list, of ‘umbrella’ terms for naming fish species, such as ‘sgombro’ could be related to geographic origin of products and creates opportunities for fraudulent activities, but also misleads consumers. In light of this pattern, an urgent update of European legislation is needed to face current and future challenges for full chain traceability.

Keywords: canned fish; mini-barcoding; seafood mislabelling; COI; D-loop; *Scomber*; mackerel

1. INTRODUCTION

During recent decades, the consumption of fish and fish products has increased globally due to a rising awareness among consumers of their nutritional value, sensorial characteristics and an overall increase in demand for healthy food products (FAO, 2020a). At the same time, important changes in lifestyles, socio-economic status and cultural behaviour have reduced the time set aside for preparing dishes and pushed consumer preferences towards convenience foods, which are easy to prepare and have a long shelf-life (Infante & Manchado, 2006; Mottola et al., 2020). Canned seafood products, such as tuna, mackerel, salmon, anchovies, clams, mussels are the most widely-consumed across the world as they represent the best compromise between nutritional value, practicality of use (Akhbarizadeh et al., 2020; Bell et al., 2019), and are assumed to be associated with fairly low levels of food loss and waste (FAO, 2020b). In particular, the market for canned mackerel is growing also due to an evolution in the product itself that, in the past, was traditionally sold as fillets in oil, but which is now available in a wide range of products (*i.e.* steaks, in brine, grilled, as sausages), often in combination with several other ingredients such as tomato, olive, spices, etc. Such a new appeal, combined with their competitive price and the excellent properties of the meat, increased European average annual per capita consumption of canned mackerel from 0.58 kg in 2016 to 0.65 kg in 2017 (<https://www.cbi.eu/market-information/fish-seafood/canned-fish/market-potential>).

The European legislation for the labelling of processed food is Regulation (EU) 1169/2011, which provides general rules about the mandatory information required on labels to guarantee safety and consumer information. Based on such legislation, the mandatory information does not include the commercial denomination or scientific name of the fish species sold in processed products, since they are simply one of the ingredients. Although not mandatory, in Resolution No. 2016/2532, the European Parliament encourages the producers of each European Member State to voluntarily provide more details on the labels so as to enhance transparency and conscious food choices on the part of consumers. However, in the event of voluntary declaration in processed

78 seafood, the correspondence between the commercial and the scientific name follows the same rules
79 defined for fresh products. Such correspondence is based on a list defined and deposited by each
80 European country ([https://ec.europa.eu/oceans-and-fisheries/fisheries/markets-and-trade/seafood-](https://ec.europa.eu/oceans-and-fisheries/fisheries/markets-and-trade/seafood-markets/commercial-and-scientific-name-species_it)
81 [markets/commercial-and-scientific-name-species_it](https://ec.europa.eu/oceans-and-fisheries/fisheries/markets-and-trade/seafood-markets/commercial-and-scientific-name-species_it)), as established by Article 37 of EU Regulation
82 1379/2013. Based on Annex 1 of Italian MiPAAF Decree dated 22 September 2017, the term
83 ‘Mackerel’ (without the ‘s’) labelled as ‘Sgombro’ refers only to the species *Scomber scombrus*
84 (Linnaeus, 1758) commonly known as ‘Atlantic mackerel’ and widely appreciated by the European
85 fishing industry for its productivity, the excellent properties of its meat and its economic importance
86 (EUMOFA, 2020; Infante et al., 2007). Other species, such as *S. colias* (Gmelin, 1789) (Atlantic
87 chub mackerel) and *S. japonicus* (Houttuyn, 1782) (Pacific chub mackerel/Japanese mackerel)
88 should be labelled as ‘Lanzardo o sgombro occhione’; *S. australasicus* (Cuvier, 1832) (Pacific
89 mackerel) as ‘Sgombro australe’, and *Rastrelliger kanagurta* (Cuvier, 1816) (Indian mackerel) as
90 ‘Sgombro indopacifico’ (Supplementary Table 1 and Figure 2).

91 Seafood authentication involves DNA methodologies in most studies (Luque & Donlan,
92 2019). In particular, DNA barcoding is the most important policy instrument for species
93 identification (Clark, 2015) and is applied by the U.S. Food and Drug Administration (FDA) to
94 identify fish species in food for regulatory purposes (Handy et al., 2011). The DNA barcoding
95 approach proposes the use of a short, standardized region (650 bp of the mitochondrial cytochrome
96 c oxidase subunit I (COI) for species discrimination (Hebert et al., 2003). This region has been
97 widely applied, both in biodiversity assessments and food identification, but other mitochondrial
98 and nuclear regions have also been used to support or reach identification at species level
99 (Fernandes et al. 2020). In highly processed foods such as canned fish, the technological processes
100 which the food undergoes, such as thermal treatment for sterilization, causes considerable
101 degradation of DNA molecules. This requires the use of a shorter barcode region (Fernandes et al.
102 2020) and, to this purpose, fragments of smaller size (<300 bp) known as mini-barcodes, were
103 developed from COI or other markers (Meusnier et al., 2008; Shokralla et al., 2015; Xing et al.,

2020). However, such a reduction in molecular information makes it impossible to discriminate rigorously between species in numerous fish products using a single-locus approach, especially in the cases of species hybridization, introgression, incomplete lineage sorting or sister species (Bucklin et al., 2011).

This study will explore the mislabelling rate in canned mackerel by the application of DNA mini-barcoding. To this end, molecular identifications will be performed on a short region of the mitochondrial COI gene using universal primers previously described by Shokralla et al. (2015). Then, in order to distinguish down to species level a complex of taxa sharing the same COI mini-barcode (*S. colias*, *S. japonicus* and *S. australasicus*) a new primer pair from the mitochondrial control region (D-loop) will be designed. In the light of the results achieved for canned mackerel, we discuss future improvements to current legislation and potential drivers of fraud.

2. MATERIALS AND METHODS

2.1 Sample collection

In spring 2020, a total of 40 canned mackerel products of different brands and type of preparation (in olive oil, sunflower oil, natural, grilled), were purchased from supermarkets located in the Apulia region (south-eastern Italy) (Table 1).

2.2 Analysis of labels and Mislabelling assessment

For each sample, mandatory information required by Regulation (EU) No 1169/2011 (*i.e.* commercial name, ingredient list, net quantity, conservation instructions, best before date, company name or code and nutrition declaration, voluntary claims) were checked. Non-compliance was established when any of the following criteria were met: a) labels did not report the mandatory information reported above; b) the voluntarily declared scientific name did not correspond to that detected by the molecular analysis; c) the voluntarily declared commercial designation did not correspond to that detected by the molecular analysis.

2.3 DNA Extraction and Purification

130 Prior to DNA extraction, samples were pre-processed by removing oil and lipids by washing
131 the fish sample in Nuclease-Free Water (Thermo Fisher Scientific, Waltham, USA) (1:100v/vol).
132 Then, the extraction and purification of genomic DNA were performed starting from 25 mg aliquots
133 of sample, using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) as reported by
134 other authors (Marchetti et al., 2020). To verify the purity of the extraction reagents, no added tissue
135 was included as negative extraction control. The DNA concentration and purity were established by
136 evaluating the A260 nm/A280 nm ratio using a BioPhotometer D30 filter (Eppendorf, Milan, Italy).

137 2.4 Oligonucleotide Primers and Reference Genes

138 Preliminary identification (COI-fragment) was performed using the SH-E mini-barcode
139 primer set (Table 2), as previously described by Shokralla et al. (2015). Their protocol included the
140 use of M13 universal non-homologous oligonucleotide forward and reverse tails to improve
141 amplification and DNA sequencing performance (Table 2). Then, a new primer pair targeting the
142 mitochondrial control region (D-loop), was designed to differentiate a group of species (*S. colias*, *S.*
143 *japonicus* and *S. australasicus*) for which the COI mini-barcode fragment was not able to provide
144 an unambiguous identification at species level. To this aim, 120 reference sequences of the
145 mitochondrial Control Region, available in Genbank/NCBI for *Scomber* species (*S. colias*, from
146 AB361495 to AB361549; *S. japonicus*, from AB361550 to AB361562; *S. australasicus*, from
147 AB458834 to AB458848; *S. scombrus*, from AB466271 to AB466275) were downloaded and
148 aligned. Then, we identified a new primer pair targeting a fragment of 207 bp comprised between
149 the nucleotide position 325 and 531 of the Control Region, including several polymorphic sites
150 among the three target *Scomber* species. Primers were designed using Primer3 software
151 (Untergasser et al., 2012) and were synthesized by EUROFINS GENOMICS s.r.l. (Ebersberg,
152 Germany).

153 2.5 COI and D-loop PCR Assay

154 PCR reactions were carried out in a 25 μ L reaction volume using 12.5 μ L of HotStarTaq
155 Master Mix 2X (QIAGEN, Hilden, Germany), containing 2.5 units of HotStarTaq DNA

156 Polymerase, 1.5 mM of MgCl₂ and 200 µL of each dNTP. Then, 0.5 µM of each primer and 1 µL of
157 DNA template were added. Both mini-COI and mini-D-loop PCR amplification profiles are
158 reported in Table 3. Negative controls were run. The PCR reactions were processed in an Applied
159 Biosystems™ SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Milan, Italy). All reactions
160 were performed in duplicate. For analysis, PCR products were electrophoresed on a 1.5% (wt/vol)
161 agarose NA gel (Pharmacia, Uppsala, Sweden) in 1X Tris-acetate-EDTA (TAE) buffer containing
162 0.089 M Tris, 0.089 M acetic acid, and 0.002 M EDTA, pH 8.0 (USB Corp., Cleveland, OH), and
163 stained with Green Gel Safe 10,000× Nucleic Acid Stain (5 µL/100 mL; Thermo Fisher Scientific,
164 Waltham, MA). The GeneRuler 50 bp DNA Ladder (Thermo Fisher Scientific, Waltham, USA) was
165 used as the molecular weight standard to estimate the amplicon sizes. Image acquisition was
166 performed using Gel Doc EZ System-Image Lab Version 6.0.1 (Bio-Rad). PCR product purification
167 and sequencing analysis of mini-COI and mini-D-loop products were performed by EUROFINIS
168 GENOMICS S.r.l. (Milan, Italy).

169 *2.6 Analysis of Sequences and Identification*

170 The electropherograms were checked using Bioedit version 7.0.9 (Hall et al., 2011) and
171 SeaView v.4.0 (Gouy et al., 2010). The sequences generated were dereplicated to identify and
172 remove identical sequences using mothur v.1.44.1 (Schloss et al., 2009). Identification was
173 performed by analysis of similarity, in the case of COI fragments, with a blast search against the
174 GenBank nucleotide database (<https://www.ncbi.nlm.nih.gov>) and Species Level Barcode Records
175 database within BOLD SYSTEMS (http://www.boldsystems.org/index.php/IDS_OpenIdEngine),
176 whereas the D-loop fragments were blasted against the GenBank nucleotide database. A
177 contingency table was generated for the two variables, *i.e.* Country of production reported on labels
178 (European vs non-European origin) and correct or incorrect Labelling. The Chi-Squared test of
179 independence was applied (Null hypothesis: Country and Labelling are independent; Alternative
180 hypothesis: Country and Labelling are correlated in some way). Moreover, we computed the
181 measure of association between the two variables (Country and Labelling) using Crammer's V

182 (value between 0 and +1; 0 corresponding to no association between the variables and 1 to complete
183 association). The analyses were conducted within the R environment (R Core Team, 2020). Map of
184 the five species of mackerels, included in the Italian legislation (Supplementary Table 1) , was
185 generated with ggplot2 (Wickham, 2016) and maps 3.4.0 R packages using coordinates of native
186 distributions provided by AquaMaps (Kashner et al., 2019).

187

188 **3 RESULTS**

189 *3.1 Analysis of labels*

190 Analysis of the labels revealed that all 40 samples were correctly labelled according to the
191 mandatory provision required by Regulation (EU) No. 1169/2011. Exploration of the labels showed
192 that 38/40 (95%) samples were sold as ‘Filetti di sgombro’ (Atlantic mackerel fillets) and 2/40 (5%)
193 samples were sold as ‘Filetti di sgombro occhione’ (Atlantic/Pacific chub mackerel fillets under the
194 current Italian legislation). Within ‘Sgombro’ fillet’ (Atlantic mackerel) samples, we found
195 incongruences between the name of the food (printed in boldface type on the package in Italian) and
196 the commercial designation of species reported in the ingredient list. In particular, 4/38 (10.53%)
197 product reported in the list of ingredients the generic term ‘Sgombri’ (mackerels), and 8/38 (21.1%)
198 the term ‘Sgombro occhione’ (corresponding to Atlantic or Pacific chub mackerel). All the other
199 samples reported congruent names of food and commercial designation (Table 1 and Supplementary
200 Table 2). Only 2/40 (5%) samples reported details regarding the catch area (FAO 27) and 12/40
201 (30%) samples included the scientific name within the ingredient list (8/40 *S. scombrus*; 2/40 *S.*
202 *colias*; 2/40 *S. colias-japonicus*) (Table 1).

203 *3.2 Molecular analysis*

204 *3.2.1 Molecular species identification*

205 DNA of sufficient yield (about 20 ng/μL) and quality (A260 nm/A280 nm ratio ranging
206 from 1.5 to 1.8) was successfully extracted from all 40 the samples. PCR products were clearly
207 visible as single bands and of expected size on agarose gel (*i.e.*, ~ 226bp for mini-COI and ~207bp

for mini-D-loop, respectively). Sanger sequences were generated for all analysed samples (40 for COI and 32 for D-loop). From the 40 COI fragments sequenced, the generation of unique sequences identified only two different haplotypes with 16 polymorphic sites: Haplotype COI 1 including 8 samples (S19-S26), and Haplotype COI 2 grouping 32 samples (S1-S18, S27-S40). Identification by similarity against GenBank and Bold systems for COI-haplotype 1 showed unambiguous identification as *S. scombrus* (MT455184.1), whereas for COI-haplotype 2 identification at species level was not possible because the same haplotype was shared among *S. australasicus*, *S. colias*, and *S. japonicus* (100% similarity) (Supplementary Figure 1). The sequencing of D-loop region for those 32 samples generated two haplotypes with 8 polymorphic sites: Haplotype D-loop 1 including 4 samples (S1, S7, S10, S16), and Haplotype D-loop 2 grouping 28 samples (S2-S6, S8-S9, S11-S15, S17-S18, S27-S40). Identification by similarity against GenBank for Haplotype D-loop 1 showed unambiguous identification as *S. australasicus* (100% similarity AB458843.1), while Haplotype D-loop 2 was identified as *S. colias* (100% similarity AB488406.1) (Figure 1 and Supplementary Figure 1).

3.2.2 Mislabelling assessment

On the basis of the mislabelling criterion defined, molecular analysis showed 18/40 (45%) cases of mislabelling, always involving samples sold as ‘Sgombro’ (Atlantic Mackerel) (Table 1). The majority of these, *i.e.* 14/18 (77.8%) were identified as *S. colias* (Atlantic Chub mackerel), while 4/18 (22.2%) were identified as *S. australasicus* (Pacific Mackerel). The remaining 22 samples highlighted compliance between the commercial designation and/or the scientific name voluntarily reported on the ingredient list and the name of the food (Table 1, and Supplementary Table 2). The two samples sold as *S. japonicus-colias* were identified as *S. colias*.

Result of the Chi-Squared test rejected the null hypothesis of independence between Country of production and mislabelling (p -value= 0.0011), and the value of Cramér's V (0.51) confirmed a moderate degree of association between the two variables.

234 4 DISCUSSIONS

235 Few data are available on the mislabelling of canned seafood products using molecular
236 approaches and these are principally focused on tuna products (Boughattas et al., 2019; Chang et
237 al., 2021; Nagalakshmi et al., 2016; Sotelo et al., 2018). Our results highlight cases of mislabeling
238 in 45% of canned mackerel, higher than the mislabeling rate previously reported by other studies,
239 with the exception of Boughattas et al. (2019) who detected a similar mislabeling rate (40%) in
240 canned tuna, and Parrondo et al. (2021) reporting 100% mislabelling in five canned Zamburiñas
241 (*Mimachlamys varia*) analyzed. All the other cases, including herrings, anchovies, tuna and
242 octopus, showed a lower mislabeling rate, ranging from 0% to 16.9% (Chang et al., 2021; Chin
243 Chin et al., 2016; Giusti et al., 2019; Nagalakshmi et al., 2016; Sotelo et al., 2018; Tinacci et al.,
244 2019; Velasco et al., 2021). This contrasting scenario could be generated by the limited number of
245 studies focused on canned seafood, but also to the weak and often ambiguous European legislation,
246 which allows the use of generic information of species of fish used. Indeed, our mislabelling cases
247 are due to labels using the term ‘sgombro’ which can be ambiguously interpreted. Indeed, if
248 ‘sgombro’ is regarded as the commercial name, it corresponds exclusively to ‘*Scomber scombrus*’
249 (Annex I, Italian Mipaaf Decree, 2017) and this leads to 45% of the mislabelling. Otherwise, if
250 ‘sgombro’ is regarded as the common name, it could include any species within the *Scomber* genus
251 (*Scomber* spp.), as well as ‘*Rastrelliger kanagurta*’ (Annex I, Italian Mipaaf Decree, 2017), a
252 situation which would engender complete compliance between labels and molecular identification.
253 The freedom to shift between common or commercial designation without any declaration on the
254 label reduces the possibility to identify *aliud pro alio* frauds and, doubtless, promotes economic
255 gain for producers who would have less constraints in the choice of raw materials for their
256 processed products. Not good news, however, for consumers who are very often unfamiliar with the
257 outward appearance of commercial fish species (Cusa et al., 2021) and what is more are also
258 unaware of any precise correspondence between commercial denomination and scientific name
259 (Annex I, Italian Mipaaf Decree 2017) or of the potential ambiguity inherent in using a common

260 name. Consumers are supposed to be the main beneficiaries of the transparent food labelling
261 systems implemented both by the European General Food Law and by the specific food labelling
262 laws (Reg. CE 178/02; Reg. UE 1169/2011). However, an update to the current legislation,
263 forbidding the use of common names to define species, or by extending Reg. (UE) No 1379/2013
264 to processed seafood is needed. Furthermore campaigns to improve consumer knowledge on food
265 labelling system , would be required if we are to achieve conscious choices (Di Pinto et al., 2016;
266 Piredda et al., 2022). Indeed, the fact that ‘umbrella’ terms for naming fish species (*e.g.* tuna, shark,
267 mackerel, etc.) in the list of ingredients, or worse still the generic term ‘fish’, creates opportunities
268 for substitution and fraud, but also misleads consumers. Moreover, it could promote the inadvertent
269 consumption of species potentially causing hazards and constituting various risks (Di Pinto et al.,
270 2015, 2016; Marchetti et al., 2020; Piredda et al., 2022; Tantillo et al., 2015), by infringing the
271 requirements of Article 8 of Regulation (EC) No 178/2002. On the other hand, the evolution of
272 canned products is moving from a single canned species towards a full meal product, since fish are
273 often canned alongside other ingredients, thus shifting consumer attention away from the fish
274 species (the main ingredient) and more towards the ‘associated’ ingredients or flavours (*e.g.*
275 tomatoes, olives, spices, lemon, cereals, legumes, etc.).

276 Although the molecular approach is a powerful tool for species identification in the
277 absence of morphological traits, as in canned products, the high degradation of DNA molecules
278 does not allow the use of a full barcode region, forcing the use of a shorter region. This difficulty
279 within the *Scombridae* family has previously been reported not only in *Scomber* but also in the
280 *Thunnus* and *Sardinella* genera (Catanese et al., 2010; Chan et al., 2019; Krčmář et al., 2019; Viñas
281 & Tudela, 2009) and between *Engraulis japonicus* and *E. encrasicolus* (Giusti et al., 2019). Our
282 study reveals that the COI mini-barcode applied is able to unambiguously identify only *S.*
283 *scombrus*, the most distant species within the *Scomber* genus (Catanese et al., 2010; Scoles et al.,
284 1998). By contrast, the other species, *i.e.* *S. colias*, *S. japonicus* and *S. australasicus*, share the same
285 COI haplotype, probably due to episodes of gene flow and introgressive hybridization among them

286 (Catanese et al., 2010). Thus, for processed food, the approach based on a single locus could require
287 a second step with a complementary marker for the identification in the case of sister species or
288 complex evolutionary patterns. In our study, the new primer pair is designed on D-loop, a
289 regulatory non coding region with high potential power for species identification (Karaïskou et al.,
290 2004; Lalitha & Chandavar, 2018). Such region has proven to be helpful for discriminating, to
291 species level, the complex of *S. colias*-*S. japonicus*-*S. australasicus*, as already found for *Thunnus*
292 genus and *S. japonicus* haplotypes (Mariani et al., 2015; Viñas & Tudela, 2009; Yan et al.,
293 2015). The two-step barcoding approach, already suggested for other taxa (Jin et al., 2018;
294 Pawlowski et al., 2012), consists in a first step, in which universal primers are used to reach at least
295 the genus level, followed by specific primers targeting problematic taxa. However, besides the
296 complex taxa, such a strategy could be the winning approach for degraded food where, the
297 fragmentation of molecular information reduces the power of a single locus, whatever it is, and
298 there is need for support from a second fragment to achieve resolution to species level.

299 Revision of taxonomy can also affect our ability to detect fraud. Within the *Scomber* genus,
300 recent studies using mitochondrial and nuclear markers recognised the taxa *S. japonicus*-*colias* as
301 two allopatric species, of which *S. japonicus* inhabits the Pacific Ocean and *S. colias* the
302 Atlantic/Mediterranean Sea (Figure 2) (Catanese et al., 2010; Collette, 2003; Espiñeira et al., 2009;
303 Infante et al., 2007; Scoles et al., 1998). For this reason, signals of contrasting or old nomenclature
304 can be reflected in commercial designations, as found in two sample labels, where the old scientific
305 name *S. japonicus*-*colias* is still used. Moreover, molecular entries associated with outdated
306 taxonomic nomenclature still available in public repositories, could generate doubtful identification.
307 For instance, in our study, the identification Haplotype D-loop 2 shows the best hits with 100% of
308 similarity with *S. colias*, but also includes a hit using the old taxonomic nomenclature as *S.*
309 *japonicus* (KJ578977.1) (Allaya et al., 2015). However, such a molecular sequence was generated
310 from a specimen collected in Tunisia (Mediterranean Sea), an area recognised as being inhabited
311 only by *S. colias*. For this sequence, the correct denomination (*S. colias*) was later included by the

312 authors in another publication (Allaya et al., 2016), even though the accession number KJ578977.1
313 is still associated with the previous taxonomic nomenclature (Allaya personal communication).

314 The relationship between frauds and geography is far from simple to explore, since the
315 indication of the geographical origin of processed products is not mandatory under current
316 legislation. However, tracing the geographic origin of fishery products is important and can help
317 consumers move towards sustainable fishery, also promoting more local and traditional fish
318 products. On the other hand, geographical information is useful for stakeholders (competent
319 authorities of official controls, producers, consumers) to understand and track the routes of
320 processed seafood and then to implement the best strategies for the monitoring and prevention of
321 fraudulent activities. For example, sympatric substitutions of morphologically similar species could
322 be due to an accidental mistake in sorting onboard, if the species are caught in mixed
323 catches/harvests, whereas substitutions by an allopatric would most probably be deliberate (Blanco-
324 Fernandez et al., 2021). Yet, our results show dependence between country of production and
325 mislabelling, thus confirming the relationship between expansion of the supply chain and major risk
326 of fraudulent behaviour (D'Amico et al., 2016; Fox et al., 2018). In particular, most of the
327 mislabelling cases in which the ambiguous term 'sgombro' was used, showed Morocco as the
328 country of production. However, in the absence of other information about the full supply chain of
329 those products and based on the small number of data used for inference, drivers of this pattern
330 cannot be clearly identified, even though it could be related to problems with phonetic translations
331 from Arabic, as previously reported for sushi labels phonetically translated from Japanese (But et
332 al., 2019).

333

334 5 Conclusions

335 This study implements a DNA barcoding pipeline including, for the first time, a two-step
336 approach for the identification of *Scomber* species while the use of a D-loop fragment enables each
337 species in the complex *S. colias*-*S. japonicus*-*S. australasicus* to be distinguished, thus allowing

338 traceability of processed products. In terms of mislabelling, our study highlights the difficulties
339 inherent in quantifying frauds when the ambiguous term ‘sgombro’ is used on the labels, generating
340 45% or 0% of non-compliance based on the various different interpretations. The extension of the
341 fishery supply chain is confirmed to be a potential driver of fraudulent activities, although more
342 details on the entire supply chain, including catch, post-harvest treatment, handling and storage,
343 processing, distribution, and retail, should be mandatory for labelling processed seafood products,
344 both for implementing controls and for consumer protection. Food is changing, in line with our
345 changing habits, technological innovations, reductions in transportation costs and improved
346 logistics. The strategies used to commit fraudulent activities are changing too, and an urgent update
347 to European legislation is required to respond to current and future challenges for full chain
348 traceability.

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357

358 **Figure captions**

359 **Figure 1. Summary of two-steps mini-barcoding approach applied for species identification**
360 **for canned products.** In the step 1 the generation of COI mini-barcode region was not able to
361 discriminate at species level 32 samples. In these cases, the application of Step 2 with the
362 amplification of D-loop mini-barcode region allowed the full identification of canned samples.
363

364 **Figure 2. Native distribution of the five species included in the Italian legislation.** Identification
365 of allopatric and sympatric species is traced based on the information available on AquaMaps.

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