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Abstract: Fishery products imported from Third countries in the European Union is constantly rising. The aim of this study was to conduct a survey on labeling non-compliances on fishery products imported from Third countries. Conduced in collaboration with the veterinary staff of the Italian Ministry of Health Border Inspection Post of Livorno-Pisa (BIP), this study is the first survey on mislabeling in products sampled at BIPs in Italy. In particular, the correspondence between the products' identity and the scientific denominations reported on the accompanying certificates was checked using the DNA barcoding method. Overall, 277 products belonging to different categories (fish, cephalopods, crustaceans, bivalves, amphibian) were submitted to analysis for species identification. The comparison of the molecular results and the scientific names declared on accompanying documents highlighted that mislabeling interested the 20.6% of the analyzed products. In particular, the highest percentage was observed on cephalopods based products (43.8%), followed by crustaceans (17%) and fish (14%). A higher rate of mislabeling was found in products imported from China, Vietnam and Thailand. Altogether, this study provided data that highlight the need of implementing analytical checks, based on DNA barcoding, on incoming fishery products.

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Dear Editor,

Please find enclosed the manuscript entitled "**DNA barcoding as a tool for detecting mislabeling on incoming fishery products from Third countries: an official survey conducted at the Border Inspection Post of Livorno-Pisa (Italy)"** to be considered for publication in Food Control.

Fishery products are the most commercialized products of animal origin in the world. In the EU seafood is largely imported from Asian countries, in particular Thailand, India, China and Vietnam, followed by African countries such as Tunisia and Morocco and by North America.

At a global level, seafood is among the foodstuffs most prone to illegal practices since it represents the second food product (after oil) and the first among foods of animal origin, most affected by frauds. According to Council Directive 97/78/EC and Commission Regulation (EC) n. 136/2004 all food of animal originated from an extra-EU country have to pass through a BIP for veterinary border controls. These consist of a series of documentary, identity and physical checks carried out on each imported consignment or on sample, depending on several factors, such as type and characteristics of consignment, exporting country, exporter reputation, history of non-compliance and latest advice from the European Commission.

While documentary and identity checks are performed on all consignments, physical checks are conducted only on a percentage of them. However, this could be particularly important in the light of the data provided by the EU Food Fraud Network (FFN) that among the most common fraudulent activity (on all food products), there were those related to labelling (36%) and illegal exports (18%). In particular, the highest number of alleged violations concerned fish and fish products.

The aim of this study was to conduct a survey on labeling non-compliances on fishery products imported from Third countries. In particular, the analysis was conducted to verify the scientific denominations declared on the accompanying documents. Conduced in collaboration with the veterinary staff of the Italian Ministry of Health Border Inspection Post of Livorno-Pisa (BIP), this study is the first survey on mislabeling in products sampled at BIPs in Italy. The correlation of the products found most at risk of fraud for species substitution with their countries of origin will allow to better address future checks.

The manuscript has not been published elsewhere nor is it being considered for publication elsewhere. All authors have approved this manuscript, agree to the order in which their names are listed, declare that no conflict of interests exists and disclose any commercial affiliation.

Yours sincerely,

Andrea Armani

DNA barcoding as a tool for detecting mislabeling on incoming fishery products from Third countries: an official survey conducted at the Border Inspection Post of Livorno-Pisa (Italy)

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Abstract

Fishery products imported from Third countries in the European Union is constantly rising. The aim of this study was to conduct a survey on labeling non-compliances on fishery products imported from Third countries. Conduced in collaboration with the veterinary staff of the Italian Ministry of Health Border Inspection Post of Livorno-Pisa (BIP), this study is the first survey on mislabeling in products sampled at BIPs in Italy. In particular, the correspondence between the products' identity and the scientific denominations reported on the accompanying certificates was checked using the DNA barcoding method. Overall, 277 products belonging to different categories (fish, cephalopods, crustaceans, bivalves, amphibian) were submitted to analysis for species identification. The comparison of the molecular results and the scientific names declared on accompanying documents highlighted that mislabeling interested the 20.6% of the analyzed products. In particular, the highest percentage was observed on cephalopods based products (43.8%), followed by crustaceans (17%) and fish (14%). A higher rate of mislabeling was found in products imported from China, Vietnam and Thailand. Altogether, this study provided data that highlight the need of implementing analytical checks, based on DNA barcoding, on incoming fishery products.

Keywords: Seafood products, Border Inspection Post, fraud, mislabeling, DNA barcoding, official controls.

1. Introduction

Global fish production has grown steadily in the last five decades and currently seafood is the most traded food commodity in the world (Asche *et al*., 2015). In 2022, according to FAO, the world seafood production is expected to rise to 181 million tons, of which at least 42% will come from aquaculture (FAO, 2014). World *per capita* fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2014) and to meet the domestic demand many Countries worldwide must necessarily import a growing share of seafood from abroad. Currently, the major fish exporting countries are in Asia, where fish production (both from catch and aquaculture) has grown dramatically in the last twenty years, accounting now for about 70% of the global production (FAO, 2014). In the European Union (EU) seafood is largely imported from Eastern countries, especially China and Vietnam. These countries annually export to the Community market 5.3 million tons (9% of the total volume of EU seafood imports from Third countries) and 2.9 million tons (5%) of fish, respectively (European Market Observatory for Fisheries and Aquaculture Products, 2015). Asian countries, in particular Thailand, India, China and Vietnam, are also responsible for most of the Italian imported seafood, followed by African countries such as Tunisia and Morocco and by North America (Italian Ministry of Health, 2015).

The complexity of trade flows that characterize the fishery sector makes it difficult to trace back seafood origin (Sterling and Chiasson, 2014). Seafood often covers very long distances, changing hands several times among various intermediaries (brokers, wholesalers, processors and retailers) and this can favor the loss of traceability information along the chain as well as encourage frauds and commercialization of Illegal, Unreported, and Unregulated (IUU) fishing products (Miller and Sumalia, 2014; Sterling and Chiasson, 2014; Pramod *et al*., 2014). At a global level, seafood is among the foodstuffs most prone to illegal practices since it represents the second food product (after oil) and the first among foods of animal origin, most affected by frauds (Spink and Moyer, 2011; Johnson, 2014). Therefore, it is imperative that accurate and stringent checks are carried out by official authorities at border posts on incoming foodstuffs.

Veterinary border checks are key pillars for preventing the introduction of possible health risks and non-compliant goods into a country and ensuring incoming foodstuffs meet the specific import and transit conditions (Hinrich *et al*., 2010). In the early 1990's, the EU provided for the establishment in all major Community ports, airports and land borders of veterinary offices called Border Inspection Posts (BIPs) (Hinrich *et al*., 2010; Department for Environment, Food & Rural Affairs of UK, 2013). According to Council Directive 97/78/EC and Commission Regulation (EC) n. 136/2004 all food of animal originated from an extra-EU country have to pass through a BIP (which must be authorized to receive that specific category of animal foodstuffs). Currently, in the EU there are 222 veterinary BIPs. However, the list of the approved BIPs, which is laid down in the Commission Decision 2009/821/EC and its amendments, is frequently updated. (Directive 1997/78/CE; Commission Decision 2009/821/EC; Italian Ministry of Health, 2015). Animal foodstuffs covered by the border checks regime are reported in Commission Decision 2007/275/EC.

Veterinary border controls are a series of documentary, identity and physical checks carried out on each imported consignment or on sample, depending on several factors, such as type and characteristics of consignment, exporting country, exporter reputation, history of non-compliance and latest advice from the European Commission (Hinrich *et al*., 2010; Department for Environment, Food & Rural Affairs of UK, 2013; European Commission, 2013). All products of animal origin consignments must be pre-notified to the BIP and presented with the correct documentation, including the health certificate issued by the competent authority in the Third Country (as required by Commission Regulation (EC) No 2074/2005 and Commission Implementing Regulation (EU) No 1012/2012). Moreover, for fishery products, covered by the Fish Labelling Regulations (Art. 35 of the Regulation (EU) n. 1379/2013), accompanying documents must also report the commercial designation and the scientific name of the fish species, the production method, the catch area and the fishing gears used must be notified (D'Amico *et al*., 2016). Moreover, starting from 31st December 2009, also a validated catch certificate, as required

by the IUU Regulation (Council Regulation (EC) n. 1224/2009), has to be presented to the receiving BIP.

While documentary and identity checks are performed on all consignments, a physical check is conducted only on a percentage of them. The frequency of physical checks is established by the Commission Decision 94/360/EC (recently amended by Commission Decision 2006/590/EC). According to FAO, there is a general rule of 1-5 percent random sampling at EU BIPs (Ababouch *et al*., 2005) but such percentage can increase where serious infringement, such as presence of unauthorized substance or exceeding of a maximum residue limit, are revealed (European Commission, 2013). Physical checks may include sampling the product to detect pathogens or illegal contaminants (veterinary drugs residues or heavy metals) or even physical tests, such as cutting and cooking, sensory testing, control of temperature, weight and wrapping materials (Hinrich *et al*., 2010; Department for Environment, Food & Rural Affairs of UK, 2013).

The BIP of Livorno-Pisa (port), along with that of Genoa (port), Fiumicino (airport) and Malpensa (airport), is one of the Italian BIPs with the highest volume of traffic (Italian Ministry of Health, 2015). In 2015, according to the most recent data of the Italian Ministry of Health, 7383 consignments passed through at the BIP of Leghorn-Pisa (port) and 78% (5767) of these were fishery products (Fig. 1) (Italian Ministry of Health, 2015).

Analytical methods based on DNA may be a useful tool to support physical checks, overall in case of processed fishery products, in order to deter operators from falsely labelling catches and prevent frauds for species substitution. Despite the widespread use of these techniques for research purposes (Cawthorn et al., 2015; Pardo *et al*., 2016; Vandamme *et al*., 2016) in assessing the identity of products along the fishery supply chain, the use of this analysis for regulatory forensic programs is still limited (Carvalho *et al*., 2015; Chang *et al*., 2016). However, this could be particularly important in the light of the data provided by the EU Food Fraud Network (FFN) (European Commission, 2015a). The FFN Activity Report 2015 has showed that the highest number of alleged violations concerned fish and fish products and among the most common

fraudulent activity (on all food products), there were those related to labelling (36%) and illegal exports (18%) (European Commission, 2015a).

The aim of this work was to conduct an analysis based DNA barcoding to investigate labeling non conformities on fishery products imported from Third Countries and entering the European Union through the BIP of Livorno-Pisa. In particular, the analysis was conducted to verify the scientific denominations declared on the accompanying documents. The correlation of the products found most at risk of fraud for species substitution with their countries of origin will allow to better address future checks.

2. Materials and methods

2.1 Sample collection and tissue sampling

A total of 277 fishery products unprocessed (simply frozen) or processed (salted, canned and smoked), whole or prepared in various forms (filleted, pieces, threads), variously packaged (pre-packaged, canned, under vacuum) or in bulk, were collected at the port of Livorno-Pisa BIP between April 2015 and June 2016 (Table 1SM-7SM).

The collected products were brought to the FishLab, Department of Veterinary Science, University of Pisa, where they were visually inspected, registered by an internal code and photographed. Tissue samples were collected and stored at -20 °C until further analysis. For those products which, on the basis of the available information and of the visual inspection, appeared to be composed only of a single seafood species, a variable number of tissue samples were taken, in relation to the number of specimens in the product. In particular, in the case of products made of a maximum of 4 specimens, a tissue sample was taken from each of them; in case of 5-10 specimens 3 samples were taken and in case of more than 10 specimens 5 samples were taken. In both cases the samples were randomly chosen. In the case of products made of a mix of different species, at least one sample per species was taken. From these 277 products, 1010 tissue samples were obtained.

2.2 Molecular analysis

148 *2.2.1 DNA extraction and evaluation of DNA quality and concentration.* Total DNA extraction
149 was performed from all samples starting from 100 mg of tissue as described by Armani *et al*.,
150 (2014). The DNA quality and quantity was determined with a NanoDrop ND-1000
151 spectrophotometer (NanoDrop Technologies, Wilmington, DE, US). In the case of samples which
152 showed low amplification performances (see section 3.2), a total DNA run was performed: one
153 thousand nanograms of the total DNA extracted from the samples was electrophoresed on 1%
154 agarose gel GellyPhorLE (Euroclone,Wetherby, UK), stained with GelRed™ Nucleic Acid Gel
155 Stain (Biotium, Hayward, CA, USA), and visualized on a ultraviolet transilluminator (UVP,
156 Benchtop Variable Transilluminator, Cambridge, UK). DNA fragments' size was estimated by
157 comparison with the standard marker SharpMass™50-DNA ladder and Sharp- Mass™1-DNA
158 ladder (Euroclone S.p.A-Life Sciences Division, Pavia, Italy).
159 *2.2.2. DNA amplification and sequencing.* Different primer pairs for the amplification of
160 mitochondrial and nuclear genes were chosen according to the product category (fish, molluscs,
161 crustaceans, amphibian) and, in the above mentioned cases, the level of DNA degradation.
162 Briefly, three primer pairs were used for the amplification of a long fragment of the
163 mitochondrial *COI* gene (Handy *et al*., 2011; Mikkelsen *et al*., 2007; Folmer *et al*., 1994) and one
164 for a short fragment of the same gene (Armani *et al*., 2015a); two primer pairs were used for the
165 amplification of the mitochondrial gene *16S rRNA*, targeting a long (Palumbi, 1996) or a short
166 (Armani *et al*., 2015b) fragment and one pair for the amplification of the nuclear gene PEPCK
167 encoding the enzime phosphoenolpyruvate carboxykinase (Tsang *et al*., 2008). Details of the
168 primers' sequences, references and PCR conditions are reported in Table 1. Five µL of each PCR
169 product were checked by electrophoresis on a 1.8% agarose gel and the presence of expected
170 amplicons was assessed by a comparison with the standard marker SharpMass™50-DNA ladder.
171 Amplicons were purified and sequenced by High-Throughput Genomics Centre (Washington,
172 USA). The results of the amplification and sequencing of the samples belonging to different product
173 categories have been evaluated and discussed separately.

174 *2.2.3. Sequences analysis and comparison with the databases.* The obtained sequences were
175 analyzed with Clustal W in Bio Edit version 7.0.9. (Hall, 1999). Fine adjustments were manually
176 made after visual checking. All the sequences were submitted to a BLAST analysis on GenBank
177 and analyzed using the Identification System (IDs) on BOLD (Species Level Barcode Records). A
178 match with a sequence similarity of at least 98% was used to designate potential species
179 identification for the *COI* gene (Barbuto *et al*., 2010). For what concerns the *16S rRNA*, a specific
180 identification was attributed only for identity values of 99-100% (Armani *et al*., 2015b), due to the
181 lower interspecific variability of this *locus*. The same cut-off was used for the PEPCK gene.
182 Since the sequences obtained in this study were not derived from voucher samples or expert-
183 identified fish specimens, these sequences were not submitted to any international online database.
184 *2.2.3. Statistical analysis.* The χ^2 test was performed for proportion comparison between the
185 product categories. In particular, fish (frozen, salted, smoked and canned), cephalopods, crustaceans
186 and bivalves categories were compared. Results were considered significant when if $p<0,05$. After
187 the overall significance was assessed k-1 χ^2 were performed in order to better assess the difference
188 **2.3 Comparison between the molecular results and the scientific name reported on the**
189 **health certificate**
190 The results of the molecular identification obtained after submitting the obtained sequences to
191 the databases were compared with the scientific name of the species declared on the health
192 certificate accompanying the products, in order to highlight cases of species substitution.
193 **3. Results and discussion**
194 ***3.1 Sample collection and tissue sampling***
195 The 277 products collected consisted of frozen fish (107), salted or smoked fish (3), canned fish
196 (19), cephalopods (64), crustaceans (53), a mix of cephalopods and crustaceans (6, mainly ready to
197 cook skewers) and bivalves (20) (Table 2). The remaining 5 were diverse products: 1 packet of frog
198 legs, 1 packet of ready to eat sushi, 1 loaf of fish skin and 2 products made of fish eggs. The
199 products showed a wide range of presentations. As regards fish, all the frozen products were

unprocessed. Only 11 products were composed of whole specimens, the remaining were filleted (74), beheaded fish (13) and fish slices (12). Among the processed products the 2 smoked fish were whole herring specimens and the only salted product was a fillet of cod. On the contrary, cephalopod products consisted in unprocessed whole specimens (31), mixed rings and arms (14), rings (7), mantle slices (9) and arms (3). Crustacean products consisted of peeled tails (30), whole specimens (9), not peeled tails (5), crustacean meat (3) and claws, legs or half body (6). In addition, as mentioned, 6 products were a mix of cephalopods and crustaceans: these were skewers of shelled shrimp tails and mantle slices (4) or shelled shrimp tails mixed with cephalopods arms and rings. For what concerns bivalves, 12 products were not shelled while 8 were shelled (see Section 3.2.5). Of the 1010 tissue samples obtained, 387 derived from fish tissue, 310 from cephalopods, 214 from crustaceans, 94 from bivalves and 5 from amphibian. In this case the tissue samples deriving from mixed products have been divided and counted in the corresponding *taxa* (mollusks and crustaceans).

3.2 Molecular analysis: DNA extraction, evaluation, amplification and sequencing

All the samples were extracted obtaining DNA of good quality and yield. Of the total 277 products collected, at least one readable sequence was obtained for all unprocessed, salted or smoked fish products (100%), for 15 canned fish (79%), for 59 cephalopods (92.2%), for 41 crustaceans (77.4%), for 12 bivalves (60%) and for 5 products composed of a mix of cephalopods and crustaceans (83.3%). The remaining 5 diverse products were all successfully sequenced.

3.2.1 Fish. Out of the total extracted DNA samples from frozen unprocessed fish products (311), 304 were successfully amplified (97.7%) and 288 gave a readable sequence (92.6%) targeting the *COI* gene. This category showed the highest sequencing rate. All the DNA samples were firstly amplified with the primers targeting the full *COI* barcode by using the primers of Handy *et al*., (2011). For the samples which showed none or poor amplification a short *COI* fragment was targeted (Table 1). Following this approach, 247 long *COI* fragments (average length 600 bp,

corresponding to 92% of the expected length, range 400-655 bp) and 41 short *COI* fragments (full length 139 bp) were obtained. The results are reported in Table 2 and in Table 1SM.

Out of the total DNA samples extracted from smoked and salted products (5), all were successfully amplified and sequenced targeting the *COI* gene. In particular, 2 full *COI* barcodes and 3 mini *COI* barcodes were obtained (Table 2 and Table 1SM).

For what concerns canned products, the *COI* gene (nor the full length nor the short length fragment) was not amplifiable. Thus, a short fragment of the *16S rRNA* was targeted using primers FOR16S-2 and REV16S-2 (Table 1). Out of the total extracted DNA samples (62), 48 were successfully amplified (77.4%) and 45 gave a readable sequence (72.5%) (the maximum length was obtained for each species, see Table 2SM). Further details are given in Table 2.

3.2.2 Cephalopods. Of the total extracted DNA samples (278), 232 were successfully amplified (83.4%) and 223 gave a readable sequence (80.2%). Firstly, the *COI* gene was targeted: initial amplifications were performed by using the primers designed by Mikkelsen *et al*., (2006). Since the amplification rate was very low a second pair of primers designed by Folmer *et al*., (1994) was introduced. For those samples that failed amplification also with this latter primer pair, the alternative target *16S rRNA* was amplified by using the primer pair proposed by Palumbi, (1996) (Table 1). With this approach, 180 long fragments of the *COI* gene (average length 633.5 bp, corresponding to 96.3% of the expected length, range 476-658 bp) and 43 long fragments of the *16S rRNA* gene (average length 493 bp, range 404-591 bp) were obtained. The results are reported in Table 2 and Table 3SM.

3.2.3 Crustaceans. Regarding crustaceans, 181 DNA samples were extracted, 152 were successfully amplified (83.9%) and 142 gave a readable sequence (78.4%). For crustaceans, the gene encoding for PEPCK was chosen as the first target, obtaining 129 sequences (average length 518 bp, range 412-603 bp). In the case of products that were not amplifiable with this approach the *COI* was targeted using the primers of Folmer *et al*., (1994) and 13 additional sequences were obtained (Table 2 and Table 4SM).

3.2.4 Mixed products made of cephalopods and crustaceans. As for the mixed products, 30 DNA samples were extracted from cephalopod tissue and 30 from crustacean tissue. Twenty-six samples of DNA samples extracted from cephalopods were successfully amplified (86,7%) and 22 sequences were obtained (73.3%). For these samples the primers of Folmer *et al*., (1994) were used for the amplification of the *COI* gene, obtaining 18 sequences (average length 621.1 pb, range 505-658 pb). Only for one product which failed amplification of the *COI* gene the *16S rRNA* gene was targeted and 4 long sequences were obtained (478 bp). For what concerns crustacean tissues, 19 amplicons (63,3%) and 14 readable sequences (46.7%, average length 525.9 pb, range 493-556 pb) were obtained targeting the PEPCK gene. For further details see Table 2 and Table 5SM.

3.2.5 Bivalves. Out of the total extracted DNA samples (94), only 53 (56.4%) were successfully amplified, of which 50 gave a readable sequence (53.2%). The *COI* gene was targeted as the first choice: amplifications were performed by using the primers designed by Mikkelsen *et al.*, (2006) and Folmer *et al*., (1994) obtaining *32* COI sequences (average length 594.5 bp, range 459-658 bp). For those samples that failed amplification the *16S rRNA* fragment of Palumbi, (1996) was targeted and 18 additional sequences were obtained (average length 449.4 bp, range 325-552 bp) (Table 2 and Table 6SM).

3.2.6 Diverse products. Both the 2 DNA samples extracted from fish eggs failed amplification of the long fragment of the *COI* gene, but the short fragment was successfully amplified. A long fragment of the *COI* gene was successfully amplified from all the 3 samples of fish skin and from 4 out of 5 frog tissue samples. Finally, for what concerns the ready to eat sushi product 4 long fragments of the *COI* gene were obtained from the fish and the cephalopod samples, while the 3 crustacean samples were successfully amplified targeting the PEPCK gene.

3.3 Comparison of molecular results with the scientific name reported on the health certificates: assessing the mislabelling rate

On the basis of the comparison between the molecular results and the scientific denomination reported on the accompanying documents, results were classified in different categories. A first

distinction was made between molecular results that allowed an identification to species level or not. When the result allowed specific identification, two possibilities occurred: i) the identified species matched the species declared on the label or ii) the identified species did not match the label declaration. On the other side, when the result did not allow specific identification, other possibilities occurred: i) the molecular result matched the declared genus, ii) the molecular result match the declared family, iii) the molecular result, although not specific, allowed to highlight the presence of mislabelling, iv) the molecular results and the declared information were not comparable. This latter case is generally due to the absence of reference sequences in the databases. The results are described according to the different categories in the following sections and shown in details in the corresponding SM tables. They are also summarized in Table 2.

3.3.1 Fish. Of the 288 sequences obtained from frozen fish samples, 142 (49.3%) allowed identification to species level. Of these, 125 samples (43.4%) matched with label declaration, while 17 (5.9%) did not match it, showing mislabelling. Of the other 146 samples (50.7%) for which identification to species level was not possible, 104 (36.1%) and 21 (7.3%) matched the declared genus or family, respectively, while in 7.3% of the cases (21 sequences) a mislabelling was observed (Table 2 and Table 1SM). In general, a difference was observed in the discriminatory performance of the full and the mini *COI* barcode: while the full *COI* barcode allowed specific identification in 136 samples (55.1%), the mini *COI* barcode was specifically discriminant in only 6 (14.6%) of the cases. However, in almost 50% of the cases the mini barcode still allowed identification to the genus level.

Overall, in unprocessed frozen fish mislabelling was identified in 38 samples (13.2%), belonging to 15 different products (14%) (Fig. 2). Details on the mislabelling are reported in Table 3 and described in section 3.4.

For what concerns smoked products, out of the 4 *COI* sequences obtained, 2 full DNA barcodes and 2 mini DNA barcodes were identified to species level and matched with label declaration (*Clupea harengus*), while 1 mini DNA barcode obtained from the salted cod only allowed

assignment to the family Gadidae (Table 2). Regarding canned products, 18 (40%) samples were identified to species level and matched the label information, while the remaining 60% could not be specifically identified, but matched the genus (15, 33.3%) or the family (12, 26.7%) (Table 2). Therefore, no cases of mislabelling were identified for salted, smoked or canned fish products.

3.3.2 Cephalopods. Of the 223 sequences obtained from cephalopods, 201 (90.1%) allowed identification to species level. Of these, 122 samples (54.7%) matched with label declaration, while 79 (35.4%) did not match it, showing mislabelling. Of the remaining 22 samples (9.9%) for which identification to species level was not possible, 7 (3.1%) and 3 (1.3%) matched the declared genus or family, respectively, while in in 5.4% of the cases (12 sequences) a mislabelling was observed (Table 2 and Table 3SM). Thus, in this category mislabelling was identified totally in 91 samples (40.8%), belonging to 28 products (43.8%) (Fig. 2).

3.3.3 Crustaceans. Of the 142 sequences obtained from crustaceans, 82 (57.7%) allowed identification to species level. Of these, 73 samples (51.4%) matched with label declaration, while 9 (6.3%) did not. The other 60 samples (42.3%) for which identification to species level was not possible gave the following results: 19 (13.4%) and 18 (12.7%) matched the declared genus or family, respectively; 12 (8.5%) showed a mislabel and in 11 cases (7.7%) the match was not verifiable (Table 2 and Table 4SM). Overall, in this category mislabelling was identified totally in 21 samples (14.8%), belonging to 9 products (17%) (Fig. 2).

3.3.4 Mixed products made of cephalopods and crustaceans. From the mixed products 36 sequences were obtained, of which 29 (80.5%) allowed specific identification. Of these, 18 (50%) corresponded with the certificates' declarations, while 11 (30.6%) showed a substitution. Of the remaining 7 samples (19.4%), for which the identification at the species level was not possible, 4 (11.1%) agreed with the documents regarding the family, while 3 (8.3%) showed a mislabelling (Table 2 and Table 5SM). Thus, a mislabelling was found in a total of 14 sequences (38.9%) belonging to 4 different products (66% of the products) (Fig. 2). In particular, in two products the

328 species of the class Cephalopoda were substituted, in one the crustacean species and in another one
329 both.

330 *3.3.5 Bivalves.* A total of 50 sequences were obtained from bivalves. Of these, 41 (82%%)
331 retrieved a specific identification: 36 (72%) matched with label declaration, while 5 (10%) were
332 mislabelled. Of the other 9 samples (18%) for which specific identification was not achieved, 6
333 (12%) and 3 (6%) matched the declared genus or family, respectively (Table 2 and Table 6SM).
334 The 5 mislabelled sequences belonged to one single product (5% of the total number of products)
335 that was declared *Meretrix lyrata* and was identified as *Gafrarium divaricatum* on the 5 mislabelled
336 samples.

337 *3.3.6 Diverse products.* The 2 samples of fish eggs were identified to species level and the
338 retrieved species corresponded to the declared ones (*Zeus faber* and *Thunnus albacares*). The same
339 occurred for the product consisting of frozen frog legs that were identified as *Hoplobatrachus*
340 *rugolosus*, matching the label declaration. For what concerns the fish skin loaf, declared to be
341 *Oreochromis niloticus*, the obtained sequences only allowed identification to the genus level
342 (*Oreochromis* sp.), due to similarity of the *COI* gene in congeneric species. Finally, for what
343 concerns the ready-to-eat sushi product, the sequences derived from the fish and crustacean samples
344 matched the species declared in the label (*Salmo salar* and *Litopenaeus vannamei*), while those
345 retrieved from cephalopod samples showed the substitution of *Uroteuthis chinensis* with *U.*
346 *duvaucelii*.

347 **3.4 Analysis of the cases of mislabelling**

348 The mislabelling rates found in the different categories were: 14% of the frozen fish products,
349 43.8% for cephalopod based products, 17% for products made of crustaceans, 66% for products
350 composed of a mix of cephalopods and crustaceans, and 5% for bivalves. No cases of mislabelling
351 were observed in salted, smoked and canned fish and in the diverse products (frog legs, fish skin
352 and eggs), except for the cephalopod sample in the ready-to-eat sushi (Table 2 and Fig. 2). Results
353 shows a significant difference between the positive proportion in the cephalopod category (χ^2=

354 31,42 p<0,01) and all the others, the differences among which were not statistically significant (χ
355 ^{2}2,11 p=0,35)χ.

356 The average value of mislabelling calculated on the total number of the analysed products was
357 20.7%. Interestingly, this value confirms the results of a recent report published by Oceana, in
358 which the results of more than 200 studies on mislabelling conducted in 55 globally distributed
359 countries were analysed (http://usa.oceana.org/sites/default/files/global_fraud_report_final_low-
360 res.pdf). In fact, from the results of the report, issued from the analysis of more than 25,000 samples
361 of fishery products, it was found that problems related to the replacement of species affected one in
362 five samples.

363 Mislabelling cases have been categorized and separately discussed in the following sections. The
364 results are also collected in Table 4.

365 *3.4.1 Substitutions between species belonging to the same genus.* In the cases of congeneric
366 species presenting high morphological similarities, overlapping distribution areas and shared
367 habitats, their erroneous identification can be the direct result of an unexperienced or not properly
368 formed operator. In fact, the increase of the variety of fish species fished and traded globally, makes
369 morphological identification by operators even more difficult (Rehbein, 2008; Armani *et al*.,
370 2015a). This might have happened when the substitutive and the declared species presented a
371 similar commercial value. Rather than to intentional frauds, these cases may be related to an
372 insufficient preparation of the personnel.

373 Concerning fishes, the likely unintentional mislabelling cases highlighted in this study involve 5
374 different genera. In fact, considering also the *post mortem* partial or total loss of livery colour, the
375 morphological characters distinguishing *Psettodes belcheri* and *Psettodes bennetti*, *Epinephelus*
376 *areolatus* and *Epinephelus bleekeri*, *Merluccius paradoxus* and *Merluccius capensis*, *Mustelus*
377 *mustelus* and *Mustelus punctulatus*, *Synaptura cadenati* and *Synaptura lusitanica*, may not be easily
378 appreciable (Govindaraju and Jayasankar, 2004; see also the specific pages on
379 http://www.fishbase.org/). The poor training of operators in discriminating between related species

may be confirmed by the fact that many products for which mislabelling was found originated from developing countries, such as Mauritania (see the cases of *Psettodes* spp. and *Mustelus* spp.) and Senegal (see the case of *Synaptura* spp.).

As regards the 2 cases of intra-genus substitutions involving *Epinephelus* spp., only 1 out of the 3 examined samples did not correspond to the declared species. Considering the partial substitution and the small size of the fillets and thus of the fished specimens (often young specimens are very similar among related species, Govindaraju and Jayasankar, 2004), it is possible to speculate that the presence of a different species may be due to the casual presence of a small number of specimens in the lot due to the by-catch. In relation to the small size of the fillets, we need to emphasize that fishing juvenile stages, other than constituting a further element of difficulty in the identification of species, it can be considered one of the causes of depletion of fish stocks globally (Froese, 2004).

For what concerns *Seriola dumerilii* and *Seriola quinqueradiata*, although they are morphologically similar, *S. dumerilii* is worldwide distributed and generally wild caught, *S. quinqueradiata* is only found in the Asiatic region, where it is also intensively farmed. Due to this fact its presence on Asiatic markets is constant and this has led to a decrease in its commercial value (http://www.fao.org/fishery/culturedspecies/Seriola_quinqueradiata/en). Thus, in this case it is plausible to hypothesize an intentional economically motivated adulteration.

Twenty-one cases of substitution among species of the same genus were found for cephalopods, involving 4 genera: *Loliolus* spp., *Sepia* spp., *Sepiella* spp. and *Uroteuthis* spp.. Almost all these non-conformities were found in products imported from Asian countries. As for fish species, it is plausible to hypothesize that most of these mislabelling may be due to the presence of similar species in the same fishing grounds. This can be the case of the substitution of *Loliolus japonica* with *L. beka*, of *Sepiella japonica* with *Sepiella inermis*, of *Sepia officinalis* with *Sepia hierreda*, or of the several substitutions (of part or all the samples of the product) found between *Uroteuthis chinensis, Uroteuthis edulis* and *Uroteuthis duvaucelii*. For what concerns the genus *Sepia* sp., on

406 the contrary, considering that *Sepia aculeata* and *Sepia pharaonis* present different morphological
407 characteristics that may be appreciated even by non-experts
408 (http://www.sealifebase.org/Photos/ThumbnailsSummary.php?ID=57882;
409 http://www.sealifebase.org/Photos/ThumbnailsSummary.php?ID=57301), it is possible to
410 hypothesize that the substitution is unintentional and it is likely due to limits in labelling rules
411 awareness, since both species have a high commercial value. This is confirmed by the fact that both
412 species were found alternatively substituted.
413 As regards crustceans, the only substitution among species of the same genus was found for
414 *Metapenaeus* spp., where the declared species *Metapenaeus monoceros* was substituted with the
415 species *Metapenaeus affinis*. These species share similar anatomical characters and geographical
416 distribution, so also in this case the mislabelling may be considered unintentional.
417 *3.4.2 Substitutions between species belonging to the same family*. Also in this case some species
418 substitutions may be caused by the inexperience of operators in distinguishing related species.
419 As regards fishes, this could be the case of the substitution of *Lepidotrigla microptera* with
420 *Chelidonichthys* sp.. Notwithstanding the morphological similarities between the species of these
421 genera, the high frequency and the recurrence of this substitution (total substitution in 6 products
422 from China), highlights again the existence of traceability and label issues in fishery products in
423 China (Xiong *et al*., 2016a, b, c).
424 On the countrary, the substitution of *Limanda aspera* with *Hippoglossoides* spp., may be
425 considered intentional due to their morphological differences and to the fact that while *L. aspera*
426 has a high commercial value, the two species belonging to the genera *Hippoglossoides* are of scarce
427 economic interest. Similarly, another possible example of intentional fraud may be represented by
428 the substitution of *Theragra chalcogramma* (pollack d'Alaska) with *Boreogadus saida*, considered
429 of low commercial value (http://www.fao.org/fishery/species/2233/en).
430 As regards cephalopods, probable accidental substitutions due to limits in labelling awareness
431 may have occurred in the case of *Cistopus indicus* substituted with *Amphioctopus* spp., or of

Uroteuthis duvaucelii with *Heterololigo bleekeri*. A different story may be hypothesized for the products for which the declared species was *Octopus membranaceus*. In fact, its substitution with *Amphioctopus fangsiao* may be explained considering that the stocks of the former species are depleted and it is very rarely fished (FAO, 2016), while *A. fangsiao* is not included in the Italian official list of seafood denominations. Therefore, selling a little known species under the name of a highly commercial and depleted species may consent economic advantages. The same hypothesis applies to the products in which *O. membranaceus* was substituted with *Cistopus* sp.

Also for crustaceans, in addition to unintentional or accidental mislabelling, some of them may have been perpetrated with the aim of commercializing little known species with more common ones. This might be the case of *Metanephrops thompsoni* substituted with species of the genus *Nephropsis* spp., of *Metapenaeus affinis* substituted with *Metapenaeopsis* sp., or of *Litopenaeus vannamei* substituted with *Parapenaeopsis* sp..

As for bivalves, the only mislabelling encountered was between *Meretrix lyrata* and *Gafrarium divaricatum*. Considering the morphological differences of the two species, this substitution is likely to be voluntary.

Particular attention must be given to the 6 products containing a mixture of cephalopods and crustaceans, since mislabelling was found in 4 of them (66%). Although the majority of the substitutions may be unintentional, the case of product PIF265 is particularly interesting, since in this product all the 3 species of cephalopods declared (*U. duvaucelii*, *S. pharaonis*, *O. membranaceus*) were found to be substituted (*U. edulis, S. aculeata, Cistopus* spp.). In addition, the only species of crustacean declared was also mislabelled (*Metapenaeus dobsoni* substituted with *Parapenaeopsis cornuta*). Although, except for the substitution involving *O. membranaceus*, these intra-genus and intra-family replacements can be considered accidental due to morphological similarities, they highlight strong limits in species identification and product traceability.

3.4.3 Substitutions between species belonging to the same order

457 The only substitution occurring between species belonging to the same order, but to different
458 families, regarded *Arnoglossus kessleri* which was replaced with *Citharus linguatula.* This
459 substitution is likely due to limits in labelling procedures.

460 **3.5 Relationship between countries of origin and mislabelling**

461 The majority of the products analysed in this study came from Asia (75.39%), followed by
462 Africa (18.46%), reflecting the high number of exports to the EU by developing countries in those
463 geographic areas (Smith *et al*., 2010) and confirming the data of the Italian Ministry of Health
464 (2015). In Fig. 3 the declared countries of origin for each category of product are shown. In
465 particular, 31.5% of collected samples had a Chinese origin. This high rate is not surprising
466 considering that China is one of the main producers of fishery products (FAO, 2014). As regards
467 mislabelling cases, the Third countries most frequently involved were China, Vietnam and
468 Thailand, which were also among the main importers by number of products (Table 4). A recent
469 survey aimed at analyzing the Chinese legislative framework in the seafood compart highlighted the
470 lack of a mandatory legislation on seafood traceability and of an official naming system (Xiong *et*
471 *al*., 2016a). Moreover, molecular analysis conducted on Chinese products highlighted an impressive
472 rate of mislabeling and substitution with toxic or endangered species (Xiong *et al*., 2016b; Xiong *et*
473 *al*., 2016c).

474 In 2016, of the total number of notifications transmitted through the Rapid Alert System for
475 Food and Feed (RASFF), about 18% of these were related to seafood and in particular fishery
476 products (63.5%), bivalve mollusks (16.5%), crustaceans (12.8%) and cephalopods (7.2%) (RASFF
477 Portal, 2016). Among the third countries with the highest number of notifications there are those of
478 Asian (mainly Thailand, Vietnam and China) and African (Ghana and Senegal) origin (RASFF
479 Portal, 2016). The data concerning Asian countries in particular are perfectly in line with the issues
480 observed in the present study. However, of the total RASFF notifications concerning seafood, only
481 a very low percentage (0.15%) was due to labeling problems (absent/incomplete/incorrect) in 2016.
482 The percentage varied from 0.01% to 0.18% between 2010 and 2015 (Table 5).

These percentages do not represent an accurate estimate of mislabeling cases involving fisheries products at EU border level. This is because RASFF data include mislabeling cases detected not only at BIPs but also in intra-Community trade and at local level (within each Member State) (RASFF, 2015). Moreover, it must not be underestimated that at the BIPs, physical and laboratory checks are not carried out on each consignments and therefore the mislabeling cases detected at BIPs do not rely on molecular analysis but just on documentary checks (Hinrich *et al*., 2010; Department for Environment, Food & Rural Affairs of UK). Considering that documents checks mainly focus on the verification of the approval number of the establishment of origin, product description, batch numbers and production dates, only the cases of broken labels, discrepancies between label and accompanying documents and fraudulent trademarks, descriptions or stamps can be revealed during border controls (European Commission, 2013). Therefore, other types of fraud that need specific analysis, such as fish substitution, are not usually detected. Border controls on fishery products are limited not only at European level, but also in the United States were is estimated that less than 2% of incoming seafood is inspected specifically for fraud (Warner *et al*., 2013). Therefore, it is likely that the data on mislabeling given by the RASFF are underestimated.

In this regard, data emerging from the coordinated testing program on fish species substitution, organized by the European Commission (after horsemeat scandal) and based on analysis of molecular identification, are more indicative (European Commission, 2015b). In 2015, during official controls, 27 Member States and 2 European Free Trade Association (EFTA) Member States collected 3906 samples of fish (predominantly white fish species) at different stages of the food chain, including BIPs (European Commission, 2015b). The results showed that 6% of unprocessed fish samples and 5% of processed ones were mislabeled. As it regards specifically the samples taken at the BIPs, 7% of the total (135) resulted mislabeled with regard to the species declared on the label (European Commission, 2015b). A higher mislabeling rate (14% for unprocessed fish products, 11.6% for all fish products) was found in the present study. Many similar labelling issues were found concerning species belonging to cod, haddock, grouper and flat fish

(https://ec.europa.eu/food/sites/food/files/safety/docs/official-controls_food-fraud_fish_test_substitution_table3.pdf). However, in our study cephalopods, which were not included in the EU study, were found to be the product most at risk for mislabeling.

4. Conclusions

While confirming the third countries characterized by the highest number of notification (RASFF Portal, 2016) as those at highest risk of frauds for species substitution, discrepancies between the available data (RASFF Portal, 2016; European Commission, 2015b) and the results of the present study were highlight for labelling. Moreover, our data show that, in addition to white fish, other categories of products, such as those made of cephalopods or of a mix of cephalopods and crustaceans, are at high risk of mislabelling. Therefore, the implementation of appropriate sampling plan (on the basis of the product category and of the Third country) together with the application of analytical methods (DNA barcoding) for the official control of incoming fishery products is needed.

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Figure captions

Fig. 1. Consignments passed through the BIP of Livorno-Pisa in 2015

Product category 1: Mollusks; 2: Fishery products, aquaculture products and mollusks; 3: Crustaceans; 4: Mixed consignments of fish and crustaceans, molluscs and other aquatic invertebrates and their preparations; 5: Mixed consignments of meat preparations, fish or crustaceans, molluscs or other aquatic invertebrates; 6 Products of fish or crustaceans, and molluscs and other aquatic invertebrates; dead animals of Chapter 3.

Fig. 2. Mislabeling rates in the different categories of analyzed products

Fig. 3. Geographical origin of the products in relation to the different categories

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***Highlights (for review)**

- Food of animal origin from an extra-EU country must undergo veterinary border controls
- A survey on labeling non-compliances on 277 imported fishery products was conducted
- Sampling was performed at the Border Inspection Post of Livorno-Pisa
- The overall mislabeling rate was 20.6%. The highest percentage was found for cephalopods.
- Analytical checks, based on DNA barcoding, on incoming fishery products are needed.

Figure

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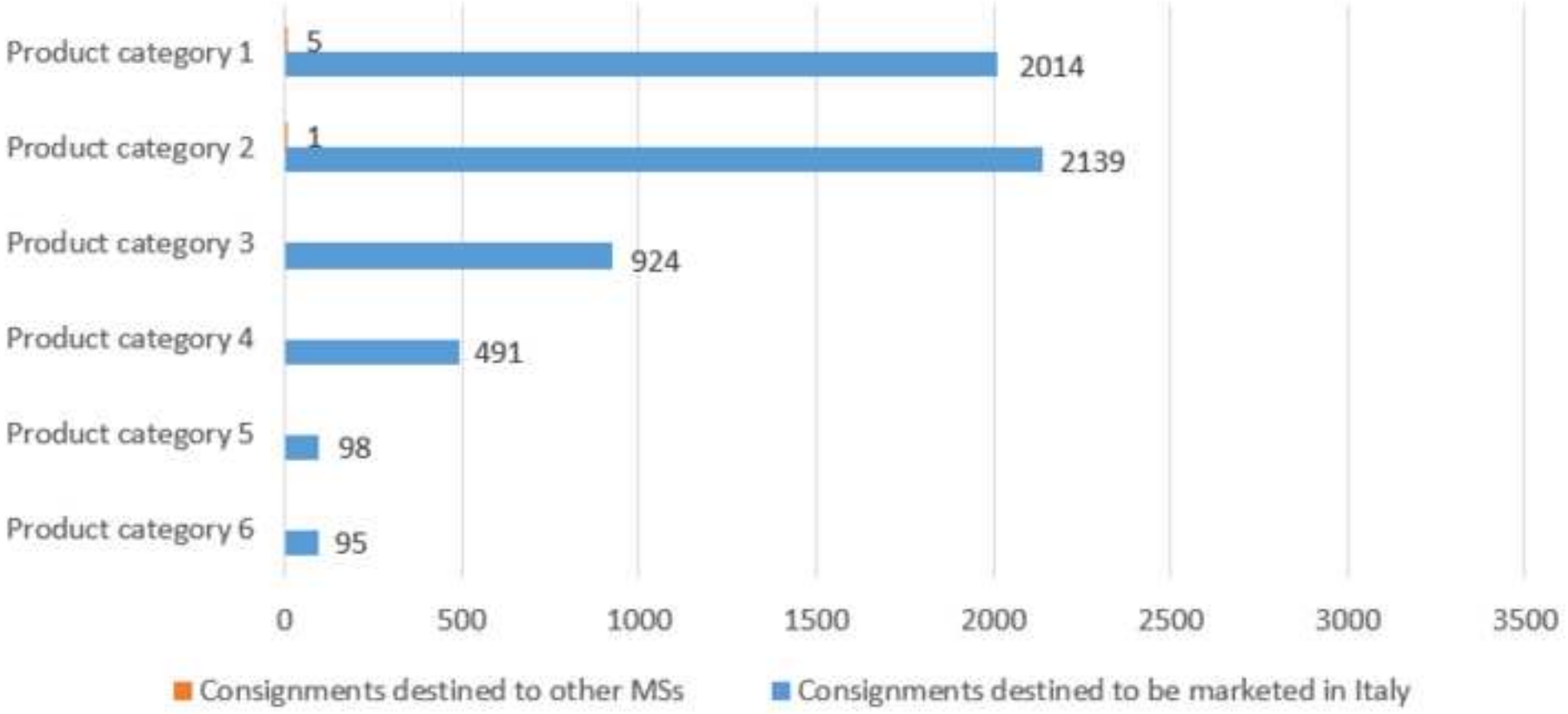

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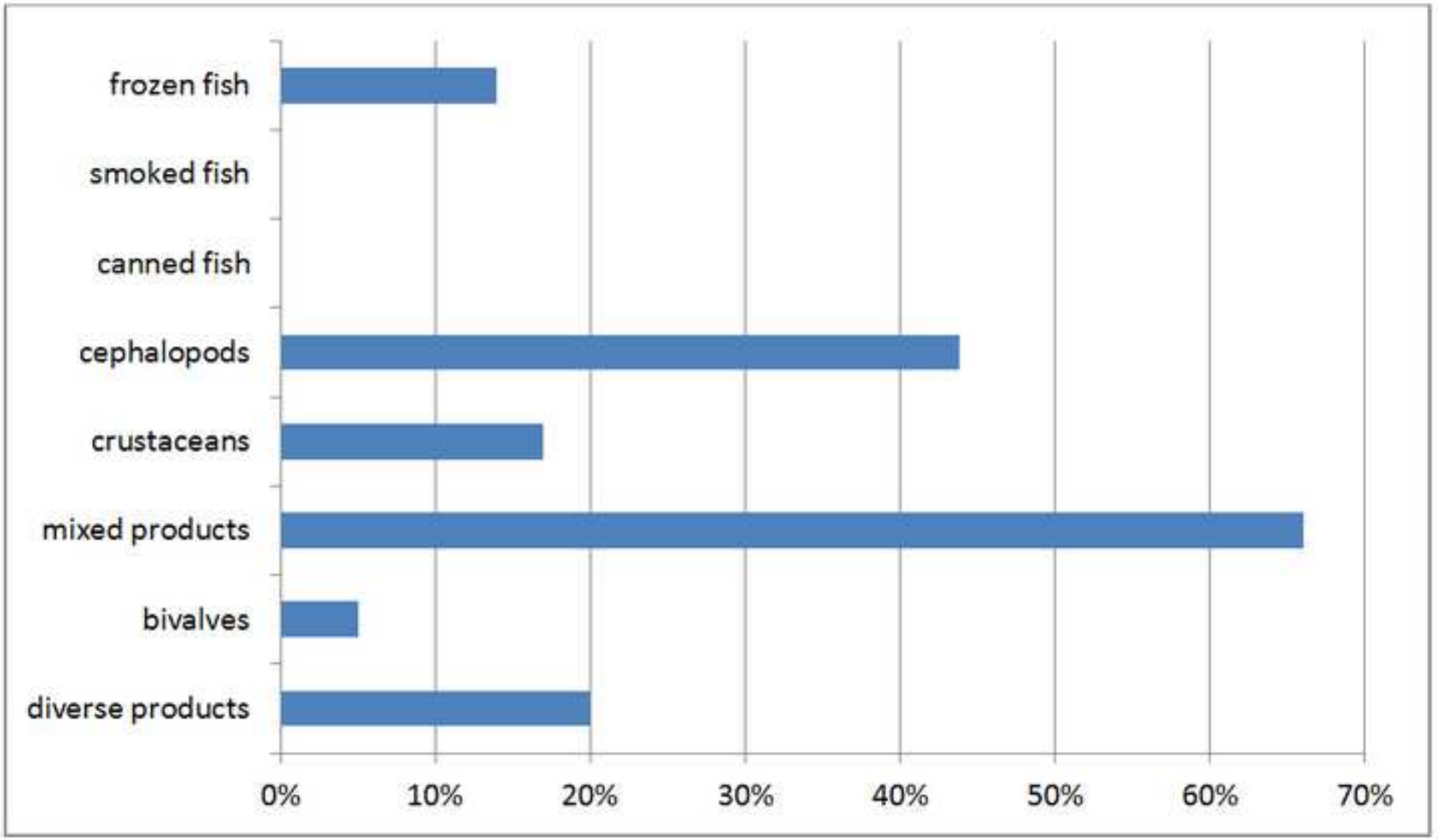

Figure
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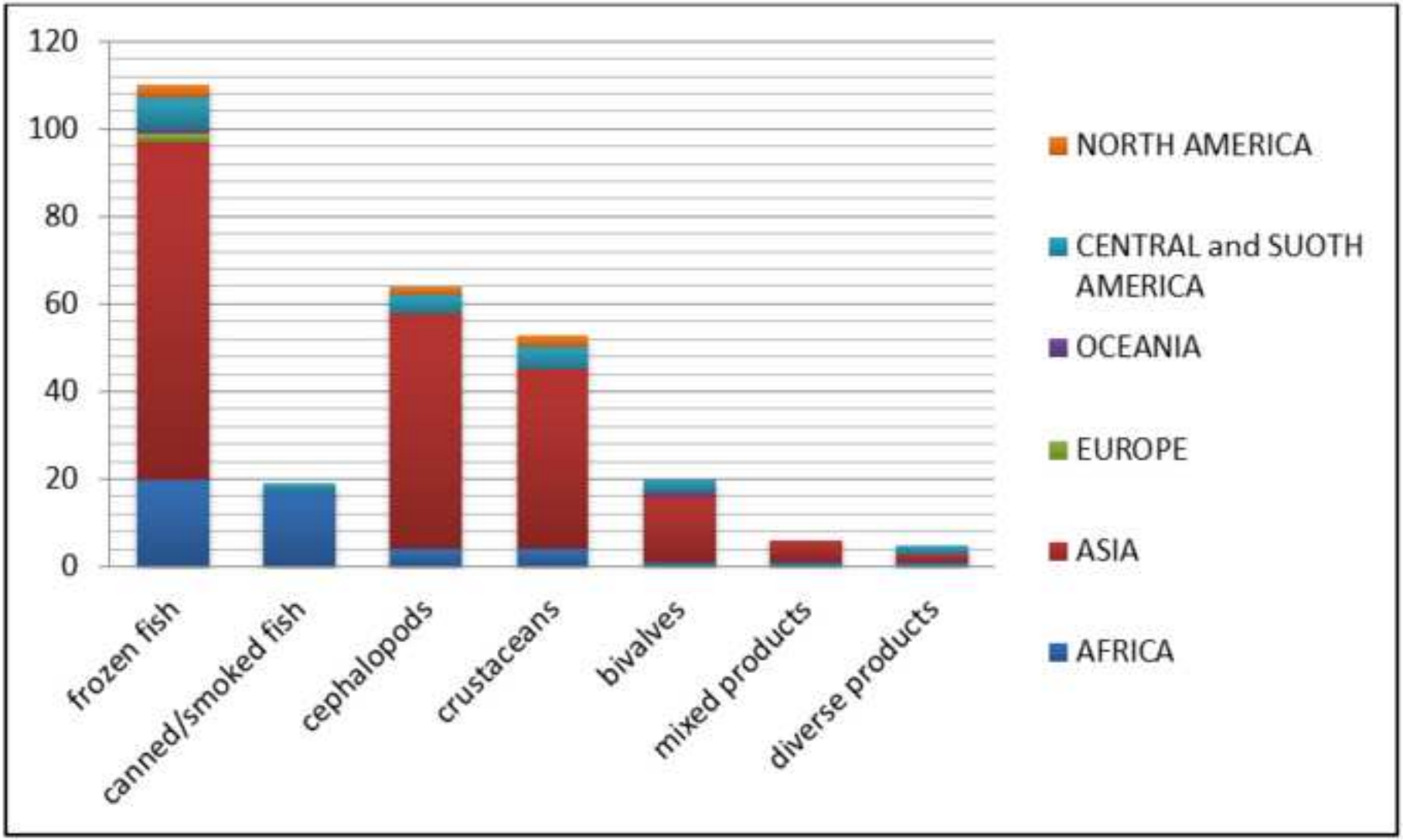

Table

Table 1. Universal primers used for the amplification of DNA samples extracted from the samples. PL: primer length; AL: amplicon length.
[a]Initial and final step (enzyme activation and final elongation) were set according to the manufacturer's instruction (5 Prime, Gaithersburg,USA) and were always performed at 94°C for 3 min and 72°C for 10 min. [b]Primer tailed with the oligonucleotides proposed by Steffens (1993) that are highlighted in grey. In bracket in the PL colon the primer length without the tail; [c]The length refers to the amplicon generated using the forward FISHCOILBC_ts; [d]The amplicon length varies according to the classes and to the species, due to the presence of insertions and deletions, typical of the 16S rRNA gene.

Primer Code	Target gene	Reference	Cycling conditions[a] (40 cycles) Temperature/time (sec)	Primer Sequence (5'-3')	PL (bp)	AL with and without primers
FISHCOILBC_ts[b]	COI	Handy *et al.*, 2011	94°C / 30 53°C / 30 72°C / 35	**CACGACGTTGTAAAACGA**CTCAACYAATCAYAAAGATATYGGCAC	45(27)	705/655
FISHCOIHBC_ts[b]				**GGATAACAATTTCACACAGG**ACTTCYGGGTGRCCRAARAATCA	43(23)	
COIFALT		Mikkelsen *et al.*, 2006	94°C / 30 47°C / 30 72°C / 30	ACAAATCAYAARGAYATYGG	20	698/650
COIRALT				TTCAGGRTGNCCRAARAAYCA	21	
LCO1490		Folmer *et al.*, 1994	94°C / 30 46°C / 30 72°C / 40	GGTCAACAAATCATAAAGATATTGG	25	710/659
HCO2198				TAAACTTCAGGGTGACCAAAAAATCA	26	
REVshort1[b]		Armani *et al.*, 2015a	94°C / 25 51°C / 30 72°C / 10	**GGATAACAATTTCACACAGG**GGYATNACTATRAAGAAAATTATTAC	46(26)	192/139[c]
16sar-L	16S rRNA	Palumbi 1996	94 °C / 25 57.5 °C / 15 72 °C / 2	CGCCTGTTTATCAAAAACAT	20	≈630/588[d]
16sbr-H				CCGGTCTGAACTCAGATCACGT	22	
FOR16S-2		Armani *et al.*, 2015b	94°C / 30 53°C / 20 72°C / 20	CTTMGGTTGGGGCGACC	17	≈152 /117[d]
REV16S-2				CTGTTATCCCTAGGGTAACT	20	
PEPCK for2	PEPCK	Tsang *et al.*, 2008	94°C / 30 59°C / 30 72°C / 35	GCAAGACCAACCTGGCCATGATGAC	25	644/598
PEPCK rev3				CGGGYCTCCATGCTSAGCCARTG	23	

Table

Table 2 Summary of the results obtained from the molecular analysis and after the comparison of the retrieved sequences with the databases.

COI LF: long fragment of the *COI* gene (obtained using the primers proposed by Handy *et al.*, 2011, Mikkelsen *et al*., 2006, Folmer *et al*., 1994); *COI* short: short fragment of the *COI* gene (obtained using the forward FISHCOILBC_ts proposed by Handy *et al.*, 2011 and REVshort_1 proposed by Armani *et al.*, 2015a); 16S: long fragment of the *16SrRNA* gene (obtained using the primers proposed by Palumbi, 1996); 16S short: short fragment of the *16SrRNA* gene (obtained using the primers proposed by Armani et al., 2015b).

Product category (n)	N. of DNA samples	N. of suitable PCR products	n. of sequences	Samples identified to species level			Samples not identified to species level					Total mislabelled samples	Total mislabelled products
				Matching with label declaration	Not matching with label declaration (mislabelled)	Total	Molecular matching		Mislabelled	Not verifiable	Total		
							Genre	Family					
Frozen fish (107)	311	304	Tot: 288	125 (43.4%)	**17 (5.9%)**	142 (49.3%)	104 (36.1%)	21 (7.3%)	**21 (7.3%)**	0	146 (50.7%)	**38 (13.2%)**	**15 (14%)**
			COI LF: 247	123 (49.8%)	**13 (5.2%)**		84 (34.0%)	12 (4.8%)	**15 (6.1%)**	0			
			COI short: 41	2 (4.9%)	**4 (9.7%)**		20 (48.8%)	9 (22.0%)	**6 (14.6%)**	0			
Salted or smoked fish (3)	5	5	Tot: 5	4	**0**	4	0	1	**0**	0	1	**0**	**0**
			COI LF: 2	2	**0**		0	0	**0**	0			
			COI short: 3	2	**0**		0	1	**0**	0			
Canned fish (19)	62	48	Tot (16S short): 45	18 (40%)	**0**	18 (40%)	15 (33.3%)	12 (26.7%)	**0**	0	27 (60%)	**0**	**0**
Cephalopods (64)	278	232	Tot: 223	122 (54.7%)	**79 (35.4%)**	201 (90.1%)	7 (3.1%)	3 (1.3%)	**12 (5.4%)**	0	22 (9.9%)	**91 (40.8%)**	**28 (43.8%)**
			COI LF: 180	96 (53.3%)	**70 (38.9%)**		6 (3.3%)	3 (1.7%)	**5 (2.8%)**	0			
			16S: 43	26 (60.5%)	**9 (20.9%)**		1 (2.3%)	0	**7 (16.3%)**	0			
Crustaceans (53)	181	152	Tot: 142	73 (51.4%)	**9 (6.3%)**	82 (57.7%)	19 (13.4%)	18 (12.7%)	**12 (8.5%)**	11 (7.7%)	60 (42.3%)	**21 (14.8%)**	**9 (17%)**
			PEPCK: 129	66 (51.2%)	**9 (7.0%)**		19 (14.7%)	12 (9.3%)	**12 (9.3%)**	11 (8.5%)			
			COI LF: 13	7 (53.8%)	**0**		0	6 (46.2%)	**0**	0			
Mix of cephalopods and crustaceans (6)	60	45	Tot*: 36	18 (50.0%)	**11 (30.6%)**	29 (80.5%)	0	4 (11.1%)	**3 (8.3%)**	0	7 (19.5)	**14 (38.9%)**	**4 (66%)**
			COI LF: 18	7 (38.9%)	**9 (50.0%)**		0	0	**2 (11.1%)**	0			
			PEPCK: 14	11 (78.6%)	**2 (14.3%)**		0	0	**1 (7.1%)**	0			
			16S: 4	0	**0**		0	4 (100%)	**0**	0			
Bivalves (20)	94	53	Tot: 50	36 (72%)	**5 (10%)**	41 (82%)	6 (12%)	3 (6%)	**0**	0	9 (18%)	**5 (10%)**	**1 (5%)**
			COI LF: 32	21 (65.6%)	**5 (15.6%)**		3 (9.4%)	3 (9.4%)	**0**	0			

	16S: 18	15 (83.3%)	**0**	3 (16.7%)	0	**0**	0

Table

PRODUCT CATEGORY	PIF CODE	TYPE OF PRODUCT AND N. OF SEQUENCES	ORIGIN	DECLARED SPECIES	FAO AREA AND IUCN STATUS	MOLECULAR IDENTIFICATION	FAO AREA AND IUCN STATUS	HYPOTHESIS ON MISLABELING

Table 3 Summary of the cases of mislabelling encountered in the study subdivided by categories.

FISH	PIF 11	fillets 1/3	Vietnam	*Epinephelus areolatus*	51 - 57 - 61 - 71 – 77 *Least concerned*	*Epinephelus bleekerii*	51 - 57 - 61 - 71 *Near threatened*	Likely unintentional due to morphological similarities. Mix of different species may be due to by-catch.
	PIF 78	fillets 1/3	Vietnam					
	PIF 71	fillets 2/2	Mauritania	*Psettodes belcheri*	34 *Not evaluated*	*Psettodes bennetti*	34 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 172	Pressed slices 4/4	China	*Theragra chalcogramma*	18 - 61 - 67 – 77 *Not evaluated*	*Boreogadus saida*	18 - 21 - 27 - 61 - 67 *Not evaluated*	Likely intentional. Lower values of the substituting species
	PIF 234	beheaded 2/2	South Africa	*Merluccius capensis*	47 - 51 *Not evaluated*	*Merluccius paradoxus*	47 - 51 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 252	whole 3/3	Morocco	*Arnoglossus kessleri*	37 *Data deficient*	*Citharus linguatula*	37 – 27 – 34 *Not Evaluated*	Likely unintentional due to labelling awareness limits
	PIF 264	beheaded 2/2	Mauritania	*Mustelus mustelus*	37 – 27 – 34 - 47 – 51 *Vulnerable*	*Mustelus punctatus*	37 – 27 – 34 *Data deficient*	Likely unintentional due to morphological similarities
	PIF 295	fillet with skin 1/1	Vietnam	*Seriola dumerili*	37 – 34 – 47 – 51 – 57- 71 – 61 – 77 – 31 - 41 *Not evaluated*	*Seriola quinqueradiata*	61 - 77 *Not evaluated*	Likely intentional. Lower values of the substituting species (farmed)
	PIF 312	whole 1/1	Senegal	*Synaptura cadenati*	34 *Not evaluated*	*Synaptura lusitanica*	27 - 34 - 37 – 47 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 32	fillets with skin 3/3	China	*Lepidotrigla microptera*	61 – 71 *Not evaluated*	*Chelidonichthys* sp.		Likely unintentional due to morphological similarities
	PIF 129	fillets with skin 3/3	China					
	PIF 162	fillets with skin 3/3	China					
	PIF 240	fillets with skin 3/3	China					
	PIF 63	fillets 3/3	China	*Limanda aspera*	61 – 67 *Not evaluated*	*Hippoglossoides* spp.		Likely intentional. Lower values of the substituting species
	PIF 300	whole 3/3	Senegal	*Pegusa lascaris*	27 – 37 – 34 – 47 - 41	Non id. ma non è *Pegusa lascaris*		
CEPHALOPODS	PIF 37	Arms and rings ¼	China	*Uroteuthis chinensis*	57 – 71 *Not evaluated*	*Uroteuthis edulis*	57 - 71 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 209	whole 5/5	China					
	PIF 53	whole 3/3	Thailand	*Uroteuthis chinensis*	57 – 71 *Not evaluated*	*Uroteuthis duvaucelii*	51 – 57 – 71 – 61 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 54	mantle slices 5/5	Thailand					
	PIF 56*	Rings 3/5	Thailand					

PIF 66	Mantello 5/5	Vietnam					
PIF 94	Mantello 3/3	China					
PIF 139	arms and rings 5/5	Thailand					
PIF 140	ciuffi 4/4	Thailand					
PIF 213	whole 5/5	Thailand					
PIF 67	arms and rings 3/3	Vietnam	*Uroteuthis edulis*	57 - 71 *Not evaluated*	*Uroteuthis chinensis*	57 – 71 *Not evaluated*	Likely unintentional due to morphological similarities
PIF 159	whole 5/5	Vietnam					
PIF 169	arms and rings 4/4	Vietnam					
PIF 280	arms and rings 4/4	Vietnam					
PIF 177	arms and rings 5/5	Vietnam	*Uroteuthis edulis*	57 - 71 *Not evaluated*	*Uroteuthis chinensis/ Uroteuthis duvaucelii*	57 – 71 *Not evaluated*	Likely unintentional due to morphological similarities
PIF 191	whole 5/5	Vietnam	*Octopus membranaceus*	51 – 57- 61- 71- 77 *Not evaluated*	*Amphioctopus fangsiao*	61 - 71 *Not evaluated*	Likely intentional. Lower values of the substituting species (less known). The substituted species is depleted
PIF 218	whole 5/5	China	*Loliolus japonica*	61 – 71 *Not evaluated*	*Loliolus beka*	61 *Not evaluated*	Likely unintentional due to morphological similarities
PIF 219	whole 3/3	Vietnam	*Sepiella japonica*	61 – 71 *Data deficient*	*Sepiella inermis*	51 – 57 – 61 – 71 *Data deficient*	Likely unintentional due to morphological similarities
PIF 224	whole 2/2	Thailand	*Sepia aculeata*	51 – 57 – 61 – 71 *Data deficient*	*Sepia pharaonis*	51 – 57 – 61 – 71 *Data deficient*	Likely unintentional due to labelling awareness limits
PIF 246	whole 2/2	Senegal	*Sepia offiChinalis*	27- 37 - 34 – 47 *Least concern*	*Sepia hierreda*	34 – 47 *Data deficient*	Likely unintentional due to morphological similarities
PIF 258*	arms and rings 1/5	Tunisia	*Doryteuthis gahi*	41 – 87 *Not evaluated*	*Sepioteuthis lessoniana*	51 – 57 – 71 – 61 *Not evaluated*	Likely unintentional due to morphological similarities (of the juvenile forms)
			Illex argentinus	41 *Least concern*			
PIF 265*	arms and rings 5/5	India	*Uroteuthis duvaucelii*	51 – 57 – 71 – 61 *Not evaluated*	*Uroteuthis edulis*	57 - 71 *Not evaluated*	Likely unintentional due to morphological similarities
			Sepia pharaonis	51 – 57 – 61 – 71 *Data deficient*	*Sepia aculeata*	51 – 57 – 61 – 71 *Data deficient*	Likely unintentional due to labelling awareness limits
			Octopus membranaceus	51 – 57- 61- 71- 77 *Not evaluated*	*Cistopus* sp.		Likely intentional. Lower values of the substituting species (less known). The substituted species is depleted
PIF 192	Arms 3/3	Thailand	*Octopus membranaceus*	51 – 57- 61- 71- 77 *Not evaluated*	*Cistopus* sp.		Likely intentional. Lower values of the substituting species (less known). The

								substituted species is depleted
	PIF 267	arms and rings 3/5	Malaysia	*Uroteuthis duvaucelii*	51 – 57 – 71 – 61 *Not evaluated*	*Uroteuthis chinensis*	57 – 71 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 270	Mantle 4/5	Vietnam	*Uroteuthis edulis*	57 - 71 *Not evaluated*	*Heterololigo blekerii*	61 *Not evaluated*	Likely unintentional due to labelling awareness limits
						Uroteuthis sp.		Likely unintentional due to morphological similarities
	PIF 136	Whole 1/1	Thailand	*Cistopus indicus*	51 – 57 – 71 – 61 *Not evaluated*	*Amphioctopus sp.*		Likely unintentional due to labelling awareness limits
	PIF 223	Whole 5/5	Vietnam	*Sepiella japonica*	61 – 71 *Data deficient*	*Sepia sp.*		Likely unintentional due to morphological similarities (of the juvenile forms)
	PIF 226	Whole 2/4	Thailand	*Sepia aculeata*	51 – 57 – 61 – 71 *Data deficient*	*Sepiella sp.*		Likely unintentional due to morphological similarities (of the juvenile forms)
CRUSTACEANS	PIF 106	Peeled tails 5/5	Thailand	*Litopenaeus vannamei*	31 – 41 – 61 – 71 – 77 – 87 *Not evaluated*	*Penaeus monodon*	51 – 57 – 61 71 – 67 – 77 - 87 *Not evaluated*	Likely intentional. Lower values of the substituting species (less known).
	PIF 187	Not peeled tails 4/4	India	*Metapenaeus monoceros*	37- 47 – 51- 57 *Not evaluated*	*Metapenaeus affinis*	51 – 57 – 71 *Not evaluated*	Likely unintentional due to morphological similarities
	PIF 265	Peeled tails 2/2	India	*Metapenaeus dobsoni*	51 – 57- 71 *Not evaluated*	*Parapenaeopsis cornuta*	51 – 57 – 71 – 61 *Not evaluated*	Likely unintentional due to labelling awareness limits
	PIF 84	Peeled tails 5/5	China	*Metanephrops thompsoni*	61 *Not evaluated*	*Nephropsis sp.*		Likely intentional. Lower values of the substituting species (less known).
	PIF 186	Peeled tails 3/3	China	*Solenocera melantho*	61 – 71 *Not evaluated*	Fam. *Penaeidae*		
	PIF 189	Peeled tails 3/5	China	*Solenocera melantho*	61 – 71 *Not evaluated*	*Parapenaeus* sp.		
	PIF 249	Peeled tails 1/1	Thailand	*Metapenaeus affinis*	51 – 57 – 71 *Not evaluated*	*Metapenaeopsis* sp.		Likely intentional. Lower values of the substituting species (less known).
	PIF 257*	Not peeled tails 1/1	Thailand	*Litopenaeus vannamei*	31 – 41 – 61 – 71 – 77 – 87 *Not evaluated*	*Parapenaeopsis* sp.		Likely unintentional due to morphological similarities
BIVALVES	PIF 38	Without shell 5/5	Vietnam	*Meretrix lyrata*	57 – 71 *Not evaluated*	*Gafrarium divaricatum*	51 – 57 – 61 – 71 *Not evaluated*	Likely intentional. Lower values of the substituting species (less known).

Table 4 Mislabeling cases divided according to the country of origin and the product category. In grey are highlighted the countries most frequently involved in mislabeling cases.

Country of origin	Total number of products by category	Number of mislabeling detected
	FISH	
China	60	6
Morocco	2	1
Mauritania	3	2
Vietnam	12	3
Senegal	5	1
South Africa	6	1
	CEPHALOPODS	
China	12	4
Malaysia	1	1
Senegal	2	1
Thailand	12	10
Vietnam	14	9
	CRUSTACEANS	
China	9	3
India	15	1
Thailand	9	2
	MIXED PRODUCTS	
India	1	1
Thailand	2	1
Tunisia	1	1
Vietnam	2	1
	BIVALVES	
Vietnam	9	1

Table

Table 5 - Percentage of labeling non-compliance for fishery products, reported by RASFF in the period 2010-2016
a: all notifications
b: notifications due to labeling problems (absent/incomplete/incorrect).

	2010		**2011**		**2012**		**2013**		**2014**		**2015**		**2016**	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Bivalve mollusks	78	0	69	0	54	1	123	0	125	0	60	0	83	0
Cephalopods	44	0	78	0	48	2	22	0	21	0	19	1	37	0
Crustaceans	78	0	75	0	60	0	53	0	71	0	59	0	65	1
Fishery products	452	2	481	4	369	4	311	1	321	3	294	7	321	7
TOTAL	652	2	703	4	531	7	509	1	538	3	432	8	506	8
Percentage	**0,03%**		**0,05%**		**0,13%**		**0,01%**		**0,05%**		**0,18%**		**0,15%**	

e-component

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