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Title: DNA and Mini-DNA Barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market.

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Keywords: DNA Barcoding, Mini-DNA Barcoding, Sparidae, COI gene, mislabeling, seafood identification.

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Abstract: The morphological similarity among Sparidae species, which are characterized by a different market price, represents a serious problem for their trade and for stock management, since it encourages fraud for substitution. The most accredited morphological method for their identification is based on the dental-plate, but this approach is not simple and cannot be used for prepared products. When molecular methods are used the DNA degradation induced by cooking is the main drawback. In this work, we collected 314 reference tissues belonging to 75 Sparidae species and we produced a dataset of full (FDB) and mini-barcode (MDB) reference sequences starting from DNA extracted from fresh and ethanol-preserved tissues using universal primes. Moreover, some fresh samples were cooked. The FDB was successfully amplified in 91% (fresh), 50% (cooked) and 81% (ethanol-preserved) samples, while the amplification rates of the MDB were considerably higher in case of cooked (100%) and ethanol-preserved (94%) samples. The same primers were used for the amplification of the DNA obtained from 58 market samples (MS). All the DNA barcodes were compared with BOLD and GenBank using IDs and BLAST analysis. FDB was able to provide unambiguous species-level identifications for 53 (78%) and 44 (64.7%) reference samples analyzed on BOLD and GenBank, respectively. Mini-DNA barcode (MDB) showed a lower discriminating power with 32 (45.7%) and 29 (41.4%) sequences unambiguously matched to a species on BOLD and GenBank. However, the MDB allowed to identify all the reference sequences as belonging to the Sparidae family. FDB and MDB showed a similar performance in analyzing the MS, allowing to highlight 21 (38%) mislabeled MS. Our study, while confirming the FDB as a reliable tool for fish authentication, proposes the MDB as a promising tool to recover molecular information in case of cooked products.

Dear Editor,

We would like to submit the following manuscript (19 pages) for possible publication:

"***DNA and Mini-DNA Barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market***"

Among the globally marketed fish, the species belonging to the family Sparidae are excellent food-fishes of high economic value. This family includes about 115 species divided in 33 genera and nowadays 85 species of Sparidae are commercialized worldwide.

The morphological similarity among Sparidae species, which are characterized by a different market price, represents a serious problem for their trade and for stock management. The specialized dentition is the most used criterion for their identification but, the marked similarities, which represent a problem even in the presence of whole specimens, make it almost impossible to distinguish the prepared or processed products during the inspection.

The DNA-based techniques are a useful tool to overcome the problems related to morphological identification and DNA barcoding has been successfully used to enforce traceability regulations in the seafood chain. Despite excellent performances when applied to fresh products, DNA barcoding has shown some weaknesses in case of processed products. For this reason, and considering that targeting a shorter region would increase the likelihood of successful amplification from degraded DNA, in this study, together with the full-barcode, the ability of a mini-DNA barcode was also assessed to produce a correct identification of Sparidae species.

In this work, we collected 314 reference tissues belonging to 75 Sparidae species and we produced a dataset of full and mini-barcode reference sequences using universal primes. The same primers were used for the amplification of the DNA obtained from 58 market samples (MS). All the DNA barcodes were compared with BOLD and GenBank using IDs and BLAST analysis. Full-DNA barcode was able to provide unambiguous species-level identifications for an higher percentage of samples than the mini-barcode on both databases. However, the mini-barcode allowed to identify all the reference sequences as belonging to the Sparidae family. Both barcodes showed a similar performance in analyzing the MS highlighting 21 mislabeled MS.

Our study, while confirming the full-DNA barcoding as a reliable tool for fish authentication, shows that the mini-barcode is a valid approach to recover molecular information from processed samples, allowing to assess the authenticity of imported products preventing commercial fraud, but also to enforce fishery control.

Best regards,

Andrea Armani

*Detailed Response to Reviewers

Dear Editor,
we revised the manuscript as suggested by the Reviewer and here below you can find our answers, comments and rebuttals. To facilitate the revision process all the manuscript revised sections have been written in green font.

Best Regards

Reviewers' comments:

The manuscript presents important and interesting results about the use of molecular markers in the identification of fishes trade as a monitoring tool. It also presents the development of a dataset of reference sequences to species of the Sapridae family. I saw these two subjects as the main objectives of the paper.

Although the modifications made in the text according to suggestions of previous reviewer, the text still confuse with the presentation of many information. I suggest that the text should be presented with two main sections. One: the data of the development of dataset of reference sequences presented as the traditional barcode papers and succinctly and; Two: the tests of market samples that could include the section of amplification problems in different types of preservation methods. This second section must receive greater attention and emphasis as it is the main objective of the article. At this time, this matter is diluted in the text.

The addition of section 3.4.3 (Mislabeled products: what and why?) was very interesting because it calls attention to main objective of paper.

Other specific comments:

Introduction

- Please add a reference of the information about the increase in the number of marketed species;

The sentence has been changed and a reference has been added (line 56-58)

Regarding Italy, we did not report any reference because this statement comes from the comparison of many Ministerial Decrees that have been issued from 2002 to 2010 by the Ministry of Agriculture Food and Forestry Policies. Considering that we made this analysis personally, we think this sentence could be considered as an authors' note (line 59).

- Lines 109-116 - The market samples were compared with dataset of reference samples after their deposit in Bold and Genbank databases or only with the sequences previously found in theses databases? It was not clear.

The market samples were compared to Bold and Genbank databases enriched by the reference sequences produced in this study, since they have been released immediately after submission. The sentence has been modified (line 111-114).

Material and Methods

- section 2.2 - What is the purpose to cooking some samples to the analysis? I believe that it was made to test the extraction method of DNA and the DNA integrity of processed samples, but it is not clear in the text. Link it with market samples that could be obtained in several forms.

A new sentence has been added in the section to better explain the purpose (line 128-129)

- lines 150-162 - the amplification protocol could be summarized for all sample type.

In our opinion the PCR protocol cannot be summarized. We have maintained the original organization of the text but we clarified the reason of the separation between fresh and other kind of samples (line 161).

- section 2.9 - What is the aim in use phylogenetic analysis? Link it with the sample identification and explain the differences in the success rate in the correct species and sequences identification of ID-BOLD, Blast and phylogenetic analysis.

We prefer to limit the Material and Methods section to a synthetic description of the analytical approach and methodologies. A short explanation was provided in Results and Discussions section (line 320-321).

Results and discussion

Overall the text presents many interesting information, but that making the text difficult to read. I suggest, if possible a reduction of the text in this section, highlighting only the main results.

Maybe some secondary finds could be presented as a supplementary material or even be removed. For example the analysis of identification problem in BOLD and Genbank are so long and diverts the reader's attention from the main purpose of the manuscript, the test of market samples.

I suggest highlight in this point only the possible causes of the misidentification and put the explanation for each case as a supplementary material.

- Section 3.1 - I suggest that the section will not splitted in subsections. It possible presenting and discussing these results of amplification in a single and more flow text.

- the results about % of amplification to different samples (lines 213-215 and 237-238) could be transfer to section 3.3 where these results are discussed. Here you could concentrate in the results and discussion about the rate of success of the sequences in discriminate the species.

The section 3.1 has been revised as suggested and the results about % of amplification of different samples have been moved to section 3.3. However, we prefer to keep two subsections (section 3.1.1 and 3.1.2) because, in our opinion, this can make easier the manuscript understanding.

Section 3.2 - the subsections 3.2.1, 3.2.2 and 3.2.3 could be reduced a only one subsection such as was made with MDB analysis (subsection 3.2.5). Beside this the text is very long and confused. I suggest the reduction of the entirely text highlighting only main results making a text more concise and clear to readers.

Subsections 3.2.2 and 3.2.3 have been incorporated in section 3.2.1. Secondary findings and specific explanations regarding identification issues found during the comparison to BOLD and

Genbank have been removed from the text and reported in the Table 6SM (new Table). The section has been shortened and focused on DNA barcoding results according to the suggestion.

Section 3.4 - It is not clear if to checking the MS sequences the authors used the dataset produced by them or only the sequences previously deposited in the BOLD and GenBank databases.

A sentence has been added at the beginning of the section (line 417).

***Highlights (for review)**

- Similarities among Sparidae species complicate morphological identification
- DNA barcoding has proven to be a useful tool for seafood products inspection
- Full and mini-DNA barcodes have been compared for the identification of Sparidae
- Full-barcode shows higher discriminatory ability but a lower amplification rate
- Analysis of marketed samples confirmed widespread mislabeling in the seafood chain

DNA and Mini-DNA Barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market.

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Abstract

The morphological similarity among Sparidae species, which are characterized by a different market price, represents a serious problem for their trade and for stock management, since it encourages fraud for substitution. The most accredited morphological method for their identification is based on the dental-plate, but this approach is not simple and cannot be used for prepared products. When molecular methods are used the DNA degradation induced by cooking is the main drawback. In this work, we collected 314 reference tissues belonging to 75 Sparidae species and we produced a dataset of full (FDB) and mini-barcode (MDB) reference sequences starting from DNA extracted from fresh and ethanol-preserved tissues using universal primes. Moreover, some fresh samples were cooked. The FDB was successfully amplified in 91% (fresh), 50% (cooked) and 81% (ethanol-preserved) samples, while the amplification rates of the MDB were considerably higher in case of cooked (100%) and ethanol-preserved (94%) samples. The same primers were used for the amplification of the DNA obtained from 58 market samples (MS). All the DNA barcodes were compared with BOLD and GenBank using IDs and BLAST analysis. FDB was able to provide unambiguous species-level identifications for 53 (78%) and 44 (64.7%) reference samples analyzed on BOLD and GenBank, respectively. Mini-DNA barcode (MDB) showed a lower discriminating power with 32 (45.7%) and 29 (41.4%) sequences unambiguously matched to a species on BOLD and GenBank. However, the MDB allowed to identify all the reference sequences as belonging to the Sparidae family. FDB and MDB showed a similar performance in analyzing the MS, allowing to highlight 21 (38%) mislabeled MS. Our study, while confirming the FDB as a reliable tool for fish authentication, proposes the MDB as a promising tool to recover molecular information in case of cooked products.

Keywords: DNA Barcoding, Mini-DNA Barcoding, Sparidae, *COI* gene, mislabeling, seafood identification.

1. Introduction

Trade globalization is one of the main challenges for the identification of fishery products. In fact, due to the depletion of the stocks of the most requested fish on the market, alternative and underutilized species are now exploited. As a consequence, a huge variety of species is nowadays commercialized over the world. For instance, 1700 different species of seafood are now available in the U.S. (FDA, 2014). In Italy, the number of official denominations for seafood species has increased from about two hundreds to more than nine hundreds in about ten years (authors' note).

The international authorities, due to an increased attention on nutritional, ecological and safety concerns related to seafood, have issued a traceability legislation in the fishery sector. The European Union has adopted a very stringent approach: seafood must be labeled with the commercial and the scientific name, the production method, the catch area (EU Reg. No. 104/2001 and 404/201) and, from the 1st January 2015, the category of fishing gear (EU Reg. No. 1379/2013).

A global seafood traceability network requires the harmonization of regulatory and commercial practices across the whole fishing sector. However, some developing Countries still have difficulties to conform to the rules of the international trade chain (Environmental Justice Foundation 2012; Armani, D'Amico, Castigliego, Sheng & Gianfaldoni, 2012a; Cawthorn, Steinman & Witthuhn, 2012; Clarke, 2009). Moreover, considering that a single commercial name can be used at the international level for different species, unscrupulous traders could take profit from this confusion by selling illegal products. Recent surveys showed that frauds are becoming widespread and seafood mislabelling has reached alarming levels (Armani, Tinacci, Giusti, Castigliego & Gianfaldoni, 2013; Carvalho, Neto, Brasil & Oliveira, 2011; Wong & Hanner, 2008).

Among the globally marketed fish, the species belonging to the family Sparidae (Porgies) are excellent food-fishes of high economic value (Antonucci, Costa, Aguzzi & Cataudella, 2009).

This family includes about 115 species divided in 33 genera (Nelson, 2006) although, according to Fishbase, the species are 133 and the genera 35 (http://www.fishbase.org/Nomenclature/FamilySearchList.php?). On the basis of the official lists

consulted (Table 1SM), 85 species of Sparidae are commercialized worldwide with different trade designations, and other unexploited species could attract the interest of the market in the future.

Porgies are very similar to each other and their morphological identification can only be performed by skilled operators. The specialized dentition, on the basis of which the Sparidae family has been grouped in six subfamilies, is the most used criterion for their identification (Smith & Smith 1986; Akazaki, 1962). These marked similarities, which represent a problem even in the presence of whole specimens, make it almost impossible to distinguish the prepared or processed products during the inspection.

The DNA-based techniques are a useful tool to overcome the problems related to the morphological identification (Armani, Castigliego & Guidi, 2012c) and the DNA barcoding, based on the analysis of the first part of the cytochrome *c*-oxidase I (*COI*) gene sequence, is the most promising approach (Hebert, Ratnasingham, & de Waard, 2003). In fact, this DNA region usually shows a greater interspecific than intraspecific variation (Hajiababei, Singer, Hebert & Hickey, 2007; Hebert *et al.,* 2003) allowing discrimination among species. Consequently, many researchers have investigated the use of DNA barcoding to enforce traceability regulations and to fight illegal fishing and frauds (Handy, Deeds, Ivanova, Hebert & Hanner, 2011; Ward, Hanner, & Hebert, 2009; Yancy, Zemlak, Mason, Washington & Tenge, 2008). Even though this method has been successfully used for the identification of fresh seafood products (Di Pinto, Di Pinto, Terio, Bozzo & Bonerba, 2013; Cawthorn *et al*., 2012; Barbuto, Galimberti, Ferri, Labra & Malandra, 2010; Wong & Hanner, 2008), it has shown some weaknesses in the case of processed products, due to the DNA fragmentation induced by heating (Cawthorn *et al.* 2012; Wong & Hanner, 2008). At the same time, the DNA degradation induced by prolonged storage in ethanol, which can occur in museum reference samples (Hajibabaei, de Waard, Ivanova, Ratnasingham & Dooh, 2005), could affect the amplification of the full *COI* barcode region, limiting the construction of sequence datasets, necessary for seafood "molecular inspection". These considerations and the possibility that fish substitutions could occur not only at the market level but also during catering activities, has

prompted us to assess, together with the full-DNA barcode (FDB) fragment, also the capability of a mini-DNA barcode (MDB) in identifying the Sparidae species of commercial interest for the international market.

In this work, we collected 75 species of Sparidae, from fresh and ethanol-preserved reference tissues, and we produced a dataset of full-length *COI* barcode reference sequences by using universal primers. Then, by aligning these sequences and those retrieved from databases, we developed a new reverse primer to amplify a mini-DNA *COI* barcoding region of ~ 190bp. The FDB and MDB obtained from the reference samples were compared to BOLD and GenBank databases and immediately released. The barcodes obtained from the 58 market samples were then compared to both databases enriched with the sequences produced in this study. Lastly, a phylogenetic analysis using the Neighbor-Joining (NJ) method was performed. The information on the label of the market samples were evaluated in the light of the molecular results.

2. Materials and Methods

2.1 Sample collection: reference and market samples

Eighty whole fresh fish were collected and morphologically identified by the Official veterinarian of the wholesale market of Milan. Two hundred thirty four ethanol-preserved reference tissues were kindly provided by Research Institutes. Overall, we collected 75 species, distributed across 26 genera, out of the 133 included in the Sparidae family (Table 2SM), and 72 out of the 85 species of commercial interest included in the consulted official lists (Table 1SM). The mean number of the collected specimens per species was 4.2 (range 1-11). Fifty-eight market samples (MS) were collected from retail markets, large-scale distribution and restaurants (Table 3SM). Each fish/tissue was labeled with an internal code and stored at -20°C.

2.2 Preparation of processed samples

Due to the fact that fish substitutions may occur not only at the market level but also during catering activities, where seafood could undergo different cooking treatments, 34 whole fresh fish were used for the preparation of processed samples according to standard recipes. Part of them was

baked as whole in an oven, preheated at 180°C, for a variable time (25-40 min) depending on the size. The rest were filleted and cooked in a frying pan for 10-15 min.

Fresh muscle tissue samples were collected before and after cooking and used for DNA extraction.

2.3 DNA extraction and evaluation of DNA fragmentation by gel electrophoresis

The ethanol-preserved reference samples were re-hydrated in 100 mM TRIS-base (pH 7.8) for 30 min at Room Temperature (RT) on a thermoshaker. Total DNA extraction was performed starting from at least 20 mg of tissue as described by Armani, Castigliego, Tinacci, Gandini & Gianfaldoni, (2012b). DNA from fresh and cooked samples was extracted as described by Armani, Tinacci, Xiong, Titarenko & Guidi (2014). The DNA quality and quantity was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, US).

One thousand nanograms of total DNA were electrophoresed on 1% agarose gel GellyPhorLE (Euroclone, Wetherby, UK), stained with GelRed™ Nucleid Acid Gel Stain (Biotium, Hayward, CA, USA) and visualized via UV transillumination. DNA fragment size was estimated by comparison with the marker SharpMass™50-DNA ladder (Euroclone, Wetherby, UK).

2.4 Amplification and sequencing of the full-COI barcode (FDB)

Several universal primers for the FDB region (Table 4SM) were aligned with the *COI* complete sequences of the Sparidae species available in GenBank. Those proposed by Handy *et al.* (2011) were selected. The reverse primer (SPACOIREV) was slightly modified and tailed as proposed by Steffens, Sutter, & Roemer (1993) (Table 4SM).

A 655bp fragment of the *COI* gene was firstly amplified from the DNA extracted from fresh reference specimens with the following PCR protocol: 20 µl reaction volume containing 2 µl of a 10x buffer (5Prime, Gaithersburg, USA), 100 µM of each dNTP (Euroclone, Pavia, Italy), 300 nM of forward primers, 400 nM of reverse primer, 25 ng/µL of BSA (New England BIOLABS® Inc. Ipswich, MA, USA), 1.25 U PerfectTaq DNA Polymerase (5Prime, USA), 100 ng of DNA and DNase free water (5Prime, USA) with the following cycling program: denaturation at 94 °C for 3

min; 45 cycles at 94°C for 30s, 53°C for 30s, 72°C for 35s; final extension at 72°C for 10 min. Five µL of PCR products were checked by electrophoresis on a 1.8% agarose gel and the presence of expected amplicons was assessed by a comparison with the standard marker SharpMass™50-DNA ladder. Amplicons were purified and sequenced by High-Throughput Genomics Center (Washington, USA). Once validated on fresh samples, the same PCR protocol was used for the amplification of cooked, ethanol-preserved and market DNA samples. The ethanol-preserved and the market DNA samples that gave the expected amplicon were sequenced.

2.5 Full-DNA barcode (FDB) sequence analysis and comparison with databases

The obtained sequences were analyzed using Clustal W in MEGA version 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Fine adjustments were manually made after visual inspection. Before the upload on the database, all the sequences were used to run a BLAST analysis on GenBank and analyzed using the Identification System (IDs) on BOLD (Species Level Barcode Records) (Ratnasingham & Hebert, 2007) to assess the concordance between the morphological and the molecular analysis (Ratnasingham & Hebert, 2013). A top match with a sequence similarity of at least 98% was used to designate potential species identification (Barbuto *et al*., 2010). Then, all the reference sequences were deposited on BOLD and GenBank (Table 5SM). Moreover, the sequences deposited on BOLD were used to produce a Barcode Index Number discordance report (BINdr). The mean genetic distances were calculated within species, genus and family using the Kimura 2-parameter model (Kimura, 1980) using the Distance Summary tool on BOLD.

The 55 *COI* sequences from MS, not originating from expert-identified specimens, were not submitted to the databases and were only used to assess the discriminatory ability of the barcoding region (Table 3SM).

2.6 Reverse primer design for the amplification of a mini barcoding region of the COI gene

Five hundred and sixty two reference sequences belonging to 73 Sparidae species available on GenBank and BOLD were downloaded and aligned with those produced in this study using Clustal W in MEGA. Once a potential region was found spanning from the 140th and the 190th bp, all the

sequences were examined for the presence of polymorphisms. The projected reverse primer (REVshort1) (Table 4SM) was tailed (Steffens *et al*., 1993).

2.7 Amplification and sequencing of the mini-barcode (MDB)

The DNA of the reference samples was used to test the performance of the primer pair FISHCOILBC_ts/REVshort1 for the amplification of a ~190bp DNA region (139bp without primers). The PCR was made in 20 µl reaction volume, containing 2 µl of a 10x buffer (5Prime, USA), 100 µM of each dNTP, 300 nM of primers, 25 ng/µL of BSA, 1.25 U PerfectTaq DNA Polymerase, 100 ng of DNA and DNase free water. The cycling program was the following: denaturation at 94 °C for 3 min; 45 cycles at 94°C for 25s, 51°C for 30s, 72°C for 10s; final extension at 72°C for 5 min. This protocol was also applied to samples for which the amplification of the 655bp *COI* barcoding region failed. All the PCR products were sequenced as reported in section 2.4.

2.8 Mini-DNA barcode (MDB) sequence analysis and comparison with databases

The obtained MDB were checked as reported in section 2.5 and those obtained from the reference samples were deposited in the European Bioinformatics Institute (EBI) (Table 5SM) due to the fact that BOLD and GenBank do not allow the submission of sequences shorter than 200bp. All the sequences were compared to the databases as reported in section 2.5. The mean genetic distances were calculated using the Kimura 2-p model in MEGA.

The sequences obtained from the MS were only used to assess labeling non conformities.

2.9 Phylogenetic analysis.

Two datasets were used to produce NJ dendrograms in MEGA computing the distance using the Kimura 2-parameter model with 2000 bootstrap re-samplings (Saitou & Nei, 1987).

In case of the FDB 460 reference sequences of 546bp (219 from this study and 241 from databases) and 52 sequences from MS were used while for the MDB 478 reference sequences of 138bp (254 from this study and 224 from databases) and 55 sequences from MS were used.

3. Results and Discussion

3.1 Development of a COI Barcode dataset for Sparidae

3.1.1 Full DNA barcode (FDB): Sequencing yielded 225 *COI* FDB with an average length of 650bp (520-655), without stop codons, insertions or deletions. We obtained at least one FDB for 68 species (91%), with an average of 3.3 (range 1-8) per species.

The sequences belonging to the species *Acanthopagrus palmaris*, *A. sivicolus*, *Calamus arctifrons*, *C. proridens*, *Dentex angolensis*, *D. canariensis*, *D. gibbosus*, *D. maroccanus*, *Diplodus noct*, and *Pagrus africanus* were obtained in this study for the first time.

As expected, the congeneric divergence was found to be higher than the conspecific divergence, with mean pairwise genetic distances of 0.43%, 9.16%, and 16.18% for conspecific, congeneric and confamilial, respectively. These values were very similar to those obtained by Keskin & Atar, (2013) and Ward *et al*., (2009).

3.1.2 Mini DNA barcode (MDB): When the FDB region was not obtained, a MDB region of ~ 190bp was amplified using the primer REVshort1. Thirty-four MDB with an average length of 135bp (60-139bp) were produced and registered and we obtained molecular data also for *D. cervinus* and *P. africanus*. No insertions, deletions or stop codons were found within the sequences, indicating that nuclear DNA sequences (NUMTs), described by Zhang & Hewitt (1996), were not amplified.

3.2 Testing the full (FDB) and mini-barcodes (MDB)

3.2.1 Full DNA barcodes (FDB) sequence analysis and comparison with databases. The BOLD System includes a tool for the characterization of unknown specimens, the Identification System (IDs) resource, that delivers a species identification if the query sequence shows a divergence less than 1% to a reference sequence. When less than 1% divergence is found with two or more taxa all possible species assignments are shown (Ratnasingham & Hebert, 2007). On the other hand, the BIN module assigns new *COI* sequences longer than 500bp to an existing or a new BIN, clustering them into OTUs independently from their previous taxonomic assignment. This analysis allows to confirm the concordance between barcode sequence clusters and species designations.

The IDs results and the BINdr are summarized in Table 5SM and 2, respectively. A maximum species identity in the range of 98–100% was obtained for 220 sequences (98%). For *C. arctifrons*, *D. canariensis* and *D. gibbosus*, the absence of reference sequences in the database resulted in "no match". The identification approach based on IDs results was coherent with the morphological approach for 39 species out of 68 (57.4%), according to an identity value ≥ 98%. Usually, when a sequence matches with more than one species, the highest value is obtained for the species inferred from the morphological identification (Table 5SM). A previous work suggested that a threshold value of 2% was effective in distinguish different species (Hebert *et al*., 2003). In this work this threshold did not allow to identify the remaining 29 species (42.6%). However, among these "non-identifiable" species, 9 (13.2%) were not identified due to the lack of reference sequences (Table 5SM).

We found that inconsistencies, such as indecision among species, were confirmed in most of the cases by the BINdr (Table 2). Among the 259 sequences that obtained a BIN, 37 were discordant at the genus level and 56 at the species level.

Considering the high number of "ambiguous" results we further investigate the issues highlighted by the IDs analysis and the BINdr, with the aim to interpret and possibly solve them.

In most of the cases, only a few sequences were responsible for the discordance at the genus level. These findings could be due to the fact that the barcodes are not filtered as they enter BOLD, even when show deep sequence divergence from existing records (Ratnasingham & Hebert, 2007) For this reason, when two or more species of the same genus cluster together, misidentification among them could have occurred (Costa et al., 2012). All these discrepancies are reported in Table 5SM, Table 2 and explained in Table 6SM.

Regarding the discrepancies at the species level, different issues were found (Table 2 and Table 6SM). Among these, to be highlighted are the many misidentifications among the species belonging to the genus *Acanthopagrus* that are very similar from both a genetic and a morphological point of view (Hsu, Guillén Madrid, Burridge, Cheng & Gwo, 2011).

Moreover, the occurrence of hybrid-like specimens among the *Acanthopagrus* species makes the study of this group even more difficult (Hsu *et al*., 2011). In fact, by using a mitochondrial gene, only the matrilineal lineage is examined (Carvalho *et al*., 2011; Costa, Landi, Martins, Costa & Costa, 2012). In this case, supplemental analyses on nuclear genes would be advisable. These considerations could explain the failure, in this work, to distinguish between *A. pacificus* and *A. berda and* among *A. schlegelii*, *A. schlegelii schlegelii*, and *A. sivicolus*,

Finally, DNA Barcode was not able to distinguish among *Pagrus* and *Diplodus* species due to their close phylogenetic relationship at the sub-species level (Table 2 and Table 6SM).

However, the DNA barcoding approach was always capable to distinguish the genus *Diplodus* from the others belonging to the family Sparidae.

On the basis of this elaboration process, 53 additional sequences (belonging to 14 species) were considered resolvable and therefore the IDs could discriminate 53 species out of 68 (78%), strongly increasing the ability of the FDB in discriminating among Porgies species. Summarizing, the system was not able to identify 15 species due to the lack of reference sequences (n=9) or due to close phylogenetic relationship among species (n=6) (Table 1).

When analyzing the sequences in GenBank, a maximum species identity in the range of 98–100% were obtained for 208 sequences (92.4%), belonging to 37 species out of 68 (54.4%).

The non-identification of the remaining 31 species was related to the absence of reference sequences or to the presence of problematic sequences (Table 5SM). In particular, identity values lower than 98% were obtained for *A. pacificus*, *C. arctifrons*, *C. leucosteus*, *C. proridens*, *D. canariensis*, *D. gibbosus*, *D. spariformis*, *V. acromegalus*, *O. melanura* and *A. spinifer* (Table 5SM).

As for BOLD, when a sequence matched with more than one species, the highest identity value was attained for the species inferred from the morphological identification (Table 5SM).

In the case of *D. puntazzo* and *P. aeneum*, the ambiguous identification was due to sequences of *D. labrax* and *P. sordida* (Moronidae and Lutjanidae family), while in the case of *D. holbrookii*, *D.*

287 *vulgaris*, *E. cardinalis*, *P. bellottii*, *P. auratus*, *P. major*, *P. pagrus*, and *S. cantharus* the
288 identification problems were the same observed on BOLD (Table 2, Table 5SM, Table 6SM).
289 However, for all of them, with the exception of *E. cardinalis*, the system was able to correctly
290 identify the sequences at the genus level.

291 Summarizing, the BLAST analysis could clearly discriminate 44 species out of 68 (65%),
292 increasing the ability of the FDB in discriminating among Porgies species (Table 1), while it was
293 not able to identify 24 species (35.3%), due to absence of reference sequences (n=17) or due to
294 close phylogenetic relationships (n=7).

295 We observed that the discriminatory ability of the FDB was strictly related to the availability of
296 correctly identified reference sequences. In fact, after the correction of the ambiguous results,
297 BOLD was able to identify 53 species (78%) while GenBank only 44 (64%). The higher resolution
298 of BOLD compared to GenBank agrees with the results obtained by Wong *et al*. (2008) and
299 Cawthorn *et al*. (2012), who analyzed different groups of fish. In our study, this could be due to the
300 fact that on BOLD only 9 reference sequences were missing, while on GenBank the lacking
301 sequences were almost twice.

302 Our results are similar to those obtained by Barbuto *et al*. 2010, who, using the DNA barcoding
303 approach for the identification of *Palombo,* recognized at the species level 34 out of 45 (75.6%)
304 samples. In fact, in the case of *Mustelus* spp., the high genetic correlations and morphological
305 similarities made their recognition by the IDs system difficult, as in the case of the species
306 belonging to the genus *Acanthopagrus* and *Diplodus.* On the contrary, in other studies the FDB
307 allowed to unequivocally identify a higher percentage of samples (Cawthorn *et al*., 2012; Keskin &
308 Atar, 2013). On the basis of this data, it seems that the DNA barcoding approach is more precise
309 when applied to species belonging to different genus and families.

310 Interesting to note that on BOLD the number of problematic sequences that could lead to
311 misinterpretation and need thorough analysis were higher (n= 73) than on GenBank (n=59). A
312 systematic revision (elaboration process) of the "raw data" obtained by the IDs system should be

313 performed to resolve "ambiguity" produced by unreliable sequences. Therefore, considering that
314 published sequences are susceptible to occasional inaccuracies, a more stringent process of
315 confirmation and validation is desirable. In fact, many cases of ambiguous results due to species
316 misidentification, wrong labeling or mistakes during sequences submission have been reported
317 (Barbuto *et al*., 2010; Carvalho *et al*., 2010). These types of mistakes that are readily detected when
318 specimens from different orders or families cluster together, must be carefully considered and
319 analyzed when species belonging to the same genus are involved.

320 *3.2.2 Phylogenetic analysis of the full-barcode (FDB).* A phylogenetic analysis was performed in
321 order to solve most of the issues highlighted with the DNA barcoding analysis. In particular, the
322 most part of the species and subspecies formed discrete clusters (Fig. 1SM), with bootstrap values >
323 70%, showing the presence of unique and diagnostic polymorphism. However, a few species still
324 could not be distinguished, such as: *D. maroccanus* from *D. angolensis*, *P. auratus* from *P. major*,
325 *A. sivicolus* from *A. schlegelliii*, *D. cervinus* from *D. cervinus hottentotus*, *S. chrysops* from *S.*
326 *caprinus*.

327 *3.2.3 139bp mini DNA barcodes (MDB) sequence analysis and comparison with databases.*
328 Hajibabaei *et al*., (2005) have tested "*in silico*" the possibility to use MDB of 218bp and 109bp for
329 the identification of fishes, observing that they generally provided sequence variability comparable
330 to that of FDB at both intraspecific and intrageneric levels.

331 Meusnier, Singer, Landry, Hickey & Hebert, (2008) found that, even though the FDB performed
332 slightly better (97% species resolution), 250bp MDB gave only slightly lower rates (95%), while
333 with 100bp MDB resolution decreased to 90%.

334 The MDB sequences were compared with BOLD and GenBank databases. The BINdr could not
335 be performed due to the limit of the system in processing sequences shorter than 500bp.

336 Only 251 MDB were used on BOLD because sequences shorter than 80bp cannot be processed
337 by the IDs. All the analyzed sequences retrieved a max identity value from 98 to 100% allowing to
338 unequivocally identify 28 species (40%). Of the remaining species, 10 (14.3%) were not identified

due to the absence of reference sequences, and 32 (45.7%) where not identifiable or showed ambiguous results. After an interpretation process, the number of correctly identified species rose to 32 (45.7%) (Table 1). Furthermore, the MDB allowed identifying at the genus level 50% of the remaining not identifiable 28 species.

Two hundred fifty five sequences were analyzed by BLAST analysis on GenBank and a max identity value ranging from 98 to 100% was obtained for 243 sequences (95.2%). Sequences from *C. arctifrons*, *D. macrophthalmus*, *D. spariformis*, *O. melanura*, *R. haffara*, and *V. acromegalus* gave lower identity values (95-97%). MDB allowed to unequivocally identify 26 species (37.1%). For the remaining species, 18 (25.7%) were not identified due to the absence of reference sequences and 26 (37.1%) showed ambiguous results or were not identifiable to the species level. Once these issues had been resolved the number of correctly identified species rose to 29 (41.4%). However, the 139 mini-barcode allowed to identify at the genus level 13 (56%) of the unidentifiable 23 species (Table 1).

The analysis of the MDB highlighted a similar discriminatory power on both databases, with a comparable number of species correctly identified (32 and 29, respectively) (Table 1). Even though the discriminatory power was lower than the FDB the MDB allowed to identify 60% and 65% of the species correctly identified analyzing the FDB on BOLD and GenBank, respectively. The higher discriminatory power associated to GenBank could be explained considering that, in this database, also shorter sequences are used by the identification engine.

Finally, the MDB allowed to unambiguously identify all the reference sequences as belonging to the Sparidae family. This is a further advantage when Porgies species are replaced with species belonging to different group of fish.

3.2.4 Phylogenetic analysis of the mini-barcode (MDB). The NJ phylogenetic analysis obtained with the MDB (Fig. 2SM), despite the average lower bootstrap values at species and subspecies level, were able to correctly cluster most of the reference sequences with the exception of: *D. maroccanus*, *D. angolensis*, *D. canariensis*, *P. auratus*, *P. major*, *E. cardinalis*, *P. edita*, *S.*

emarginatum, *S. cantharus*, C. *nodosus*, *C. calamus*, *D. sargus*, *D. noct*, *D. holbrookii*, *D. argenteus*, *A. sivicolus*, *A. schlegelliii*, *D. cervinus*, *D. cervinus hottentotus*, *S. chrysops* and *S. caprinus*.

3.3 Factors affecting PCR amplification when using full (FDB) and mini barcodes (MDB)

The DNA electrophoresis clearly showed that the cooked samples had a more degraded DNA with respect to the fresh ones (data not shown). The DNA degradation was extremely variable among the samples and in some cases, the degradation patterns revealed a scarce presence of fragments longer than 300bp. In particular, the level of degradation was higher in fish of smaller dimensions. No marked differences were observed between cooking processes. In case of ethanol preserved specimens the degradation patterns were variable, with a smear in the range of 100 to 1000bp, not always comparable between samples belonging to the same batch (Institution).

Since the different origin and preservation of tissue samples may affect the primers amplification performances, we calculated the specificity and the rate of successful amplifications on the number of the species collected rather than on the totality of the samples analyzed.

The primers selected in this study had a specificity of 100% for the target region corresponding to the FDB. Overall, the rate of successful amplifications was 95% and rose to 100% for fresh samples. The overall DNA amplificability was 85%. The DNA of the fresh specimens was successfully amplified in 91% of the cases. The rate drastically decreased to 50% after cooking. Considering that other DNA samples of the same species were amplified with the same primers, the amplification failure of the DNA extracted from fresh samples cannot be explained with an improper primers annealing, but it might be more likely caused by DNA degradation. In fact, in some cases, the DNA obtained from fresh tissues after 5 days of storing at 4°C can be fully degraded (Rodriguez-Ezpeleta, Mendibil, Álvarez & Cotano, 2013).

The reduced amplificability of the DNA extracted from the cooked products agrees with the observed degradation patterns. Thermal treatments, ingredients and storage conditions are among the most important factors that can induce DNA degradation (Armani et al., 2013; Armani,

391 Castigliego, Tinacci, Gianfaldoni & Guidi, 2012d; Rodriguez-Ezpeleta et al., 2013). In fact, even
392 though the cooking procedure used in this study was not comparable to that caused by canning
393 processes, the amplificability was strongly affected. Similar problems were reported by Wong &
394 Hanner, (2008) and Cawthorn et al., (2012), who were not able to produce the full-barcode from
395 smoked, pickled and canned products, confirming that DNA degradation is the main obstacle to the
396 application of the "classical DNA barcoding" approach.

397 The DNA amplificability of ethanol-preserved tissue was 81%. This lower rate could be due to
398 the preservation of samples in formalin or in ethanol for a long time. Many evidences suggest that
399 formaldehyde induces DNA degradation (Diaz-Cano & Brady, 1997), whereas alcoholic reagents
400 yield superior results in terms of DNA amplificability (Srinivasan, Sedmak & Jewell, 2002).
401 Therefore it is generally difficult to recover the full-barcode from museum specimens (Hajibabaei et
402 al., 2005). Nevertheless, even short-term conservation can affect DNA integrity. Rodriguez-
403 Ezpeleta et al., (2013) found that fish muscle stored in ethanol for 120 days showed a lower DNA
404 integrity than those stored for only 30 days. In accordance, we found that samples that were soaked
405 in ethanol just before the shipping showed a higher rate of DNA amplificability than those
406 preserved for a longer time.

407 In the light of the aforesaid issues, it would be advisable to collect many samples per species in
408 order to obtain at least 3 reference barcodes.

409 In the case of MDB, while the specificity was 100% as in the case of FDB, the overall rate of
410 successful amplification was 93% (70 species out of 75). In fact, the DNA of the 3 species that were
411 not amplified had been preserved in formalin or in ethanol for a long time. The DNA amplificability
412 was 95%, 100% and 94% for fresh, cooked and ethanol-preserved tissues. In the case of cooked and
413 ethanol-preserved samples the rates were considerably higher than with the FDB, demonstrating the
414 great utility of MDB in case of samples containing degraded DNA.

415 ***3.4 Mislabeling of commercial samples***

Fifty eight samples (43 from market and 15 from restaurant) have been collected throughout Italy. After submission and releasing of the reference sequences produced in this study, the 55 DNA FDB (average length 653bp) and the 58 MDB (average length 139bp) (55 extrapolated from the FDB) obtained were compared to the databases and used for the phylogenetic analysis.

3.4.1 Full-DNA barcodes (FDB) comparison with BOLD and GenBank. A maximum species identity in the range of 98–100% was obtained in BOLD for 54 sequences (98%) and in GenBank for 47 sequences (85%). On the basis of the identity value obtained and considering the correction factors already discussed (section 3.2) for the reference sequences, 45 samples (83%) and 38 samples (81%) were unambiguously identified at the species level on BOLD and GenBank, respectively. Only considering a top match of 100% the number of MS identify at the species level rises to 50 (91%) on BOLD and to 42 (89%) on GenBank (Table 3SM). Even though, on both databases 100% of the remaining MS not identified at the species level were identified at the genus level, this did not allow to verify the traceability information on the remaining samples.

Overall, the analysis performed on both databases matched and allowed to highlight 21 mislabeled samples (38%). In particular, we found 7 (33%) mislabeled restaurants products and 14 (67%) mislabeled samples from retail food and large-scale markets distribution.

3.4.2 Mini DNA barcodes (MDB) comparison with BOLD and GenBank. A maximum species identity in the range of 98–100% was obtained in BOLD for 58 sequences (100%) and in GenBank for 57 sequences (98.2%). On the basis of the identity value obtained, and considering the correction factors already discussed (section 3.2), 37 samples (64%) and 42 samples (74%) were unambiguously identified at the species level on BOLD and GenBank, respectively. Only considering a top match of 100% the number of MS identified to species level rises to 47 (81%) on BOLD and to 51 (89%) on GenBank (Table 3SM). The MDB confirmed the mislabeling already detected by the barcode. No additional mislabeling was found for the three MS for which only the short fragment was amplified.

441 In summary, we found that FDB and MDB applied to MS were characterized by a similar
442 discriminatory power on GenBank (89% vs 89%) while on BOLD a discrepancy was observed
443 (91% vs 81%). Interestingly, all the MS were correctly identified with the NJ analysis using the
444 FDB (Fig. 1SM), while using the MDB 5 MS could not be unequivocally assigned to a species (Fig.
445 2SM).

446 *3.4.3 Mislabeled products: what and why?*

447 This study confirmed that more than one third of the commercialized fish is mislabeled,
448 accordingly with what reported by Cawthorn *et al*., (2012) and Stiles, Lahr, Lahey, Shaftel &
449 Bethel, (2011).

450 On the contrary, our data are quite different from most of the studies reporting that the
451 mislabeling rate is usually higher in processed products (Carvalho *et al*., 2011; Cawthorn *et al*.,
452 2012). In this work, 71% of the mislabeled samples were sold as whole fish while the rest were
453 fillets. This could be explained taking into consideration the high morphological similarity among
454 Porgies.

455 Some of the mislabeling, such as *S. salpa* sold as S. *auratus*, *Diplodus* spp. sold as *O. melanura*,
456 and *Spicara maena* sold as *S. salpa*, could be voluntary and aimed at charging higher prices on low
457 commercial value species.

458 Other cases were due to the improper use of commercial denomination, such as the utilization of
459 a generic name for the whole genus rather than the specific commercial name stated in the Italian
460 list: Seabream (Pagello) instead of Red Pandora (Pagello fragolino) for *P. erythrinus*, Seabream
461 (Sarago) instead of Sharp snout seabream (Sarago pizzuto) for *D. puntazzo*, Dentex (Dentice)
462 instead of Canary dentex (Dentice atlantico) for *D. canariensis*.

463 In some European countries, such as Italy, many different commercial names have been issued
464 for the different species of Sparidae, while in the UK, all the species of the family Sparidae except
465 *Boops boops* (Bogue), *Diplodus sargus* (White sea bream) and *Pagrus auratus* (Golden seabream)
466 can be referred to as Porgy. The ratio among the total number of commercial denominations and the

467 total number of Porgies species considered in the official lists of seafood products analyzed in this
468 study reflects the different national approaches for the management of seafood products. In
469 particular, the percentage of family coverage varies from more than 79% (Australia, Canada and
470 Italy) to 2% for UK (Table 3). This discrepancy is probably due to different culinary traditions and
471 to a different attention paid to the preservation of the local products (D'Amico, Armani,
472 Castigliego, Sheng & Gianfaldoni, 2014). In this light, trade names associated to single species,
473 which often include geographical adjectives, can clearly differentiate national products from the
474 imported ones.

475 Unfortunately, the different approaches adopted from different countries can enormously
476 complicate the fair commerce of seafood species.

477 **Conclusion**

478 In this study, the DNA barcoding was confirmed as a reliable approach for supporting the
479 traceability in the seafood chain and ensure the correct information of consumers, in agreement with
480 what reported by the EU Reg. No. 1379/2013.

481 The analysis of MS sequences and their comparison with our dataset of reference sequences,
482 supported by the comparison performed on BOLD and GenBank, allowed to highlight commercial
483 frauds in the trade of Porgies' species.

484 Moreover, considering that targeting a shorter region would increase the likelihood of successful
485 amplification from degraded DNA, for the first time a mini DNA barcoding approach was proposed
486 for the identification of seafood species. In fact, considering that it is not possible to establish *a*
487 *priori* the degradation level of a DNA sample, the utilization of a MDB represents a valid, and
488 sometimes the only, approach to recover molecular information from an unknown sample.

489 Finally, our work highlighted that both BOLD and Genbank still lack of reference sequences and
490 host different kind of problematic sequences. For these reasons, it would be beneficial to use both
491 the databases, supported by a NJ analysis, and to perform a careful and aware analysis and
492 elaboration of the raw data in order to solve ambiguous results that could create misidentification.

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References

1. Akazaki, M. (1962). *Studies on the spariform fishes. Anatomy, phylogeny, ecology, and taxonomy*. Misaki Marine Biology Institute, Kyoto University, Spec. Rep. 1. Osaka: Kosugi Ltd.
2. Antonucci, F., Costa, C., Aguzzi, J., & Cataudella, S. (2009). Ecomorphology of Morpho-Functional Relationships in the Family of Sparidae: A Quantitative Statistic Approach. *Journal of Morphology*, 270, 843–855.
3. Armani, A., D'Amico, P., Castigliego, L., Sheng, G., Gianfaldoni, D., & Guidi, A. (2012)a. Mislabeling of an "unlabelable" seafood sold on the European market: the jellyfish. *Food Control*, 26(2), 247-251.

4. Armani, A., Castigliego, L., Tinacci, L., Gandini, G., Gianfaldoni, D., & Guidi, A. (2012)b. A rapid PCR–RFLP method for the identification of Lophius species. *European Food Research and Technology*, 235(2), 253-263.
5. Armani, A., Castigliego, L., & Guidi, A. (2012)c. Fish fraud: The DNA challenge. *CAB Animal Science Reviews*, 7, 227-239.
6. Armani, A., Castigliego, L., Tinacci, L., Gianfaldoni, D., & Guidi, A. (2012)d. Multiplex conventional and real-time PCR for fish species identification of Bianchetto (juvenile form of *Sardina pilchardus*), Rossetto (*Aphia minuta*), and Icefish in fresh, marinated and cooked products. *Food Chemistry*, 133(1), 184-192.
7. Armani, A., Tinacci, L., Giusti, A., Castigliego, L., Gianfaldoni, D., & Guidi, A. (2013). What is inside the jar? Forensically informative nucleotide sequencing (FINS) of a short mitochondrial COI gene fragment reveals a high percentage of mislabeling in jellyfish food products. *Food Research International*, 54(2), 1383-1393.
8. Armani, A., Tinacci, L., Xiong, X., Titarenko, E., Guidi, A., & Castigliego, L. (2014). Development of a simple and cost-effective bead-milling method for DNA extraction from fish muscles. *Food Analytical Methods*, 7, 946–955.
9. Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P., & Casiraghi, M. (2010). DNA barcoding reveals fraudulent substitutions in shark seafood products: the Italian case of ‘‘palombo” (Mustelus spp.). *Food Research International*, 43, 376–381.
10. Carvalho, D. C., Neto, D. A., Brasil, B. S., & Oliveira, D. A. (2011). DNA barcoding unveils a high rate of mislabeling in a commercial freshwater catfish from Brazil. *Mitochondrial DNA*, 22(S1), 97-105.
11. Cawthorn, D. M., Steinman, H. A., & Witthuhn, R. C. (2012). DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. *Food Research International*, 46, 30–40.
12. Clarke, S., (2009). Understanding China's fish trade and traceability. TRAFFIC, East Asia, Available at www.traffic.org/fisheries-reports/traffic_pub_fisheries9.pdf Accessed 22.03.13
13. Costa, F. O., Landi, M., Martins, R., Costa, M. H., Costa, M. E., Carneiro, M., Alves, M. J., Steinke, D., & Carvalho, G. R. (2012). A ranking system for reference libraries of DNA barcodes: application to marine fish species from Portugal. *PloSone*, 7(4), e35858.
14. D’Amico, P., Armani, A., Castigliego, L., Sheng, G., Gianfaldoni, D., & Guidi, A. (2014). Seafood traceability issues in Chinese food business activities in the light of the European provisions. *Food Control*, 35, 7-13.

15. Díaz-Cano, S. J., & Brady, S. P. (1997). DNA extraction from formalin-fixed, paraffin-embedded tissues: Protein digestion as a limiting step for retrieval of high-quality DNA. *Diagnostic Molecular Pathology*, 6(6), 342-346.
16. Di Pinto, A., Di Pinto, P., Terio, V., Bozzo, G., Bonerba, E., Ceci, E., & Tantillo, G. (2013). DNA barcoding for detecting market substitution in salted cod fillets and battered cod chunks. Food Chemistry, 141, 1757–1762.
17. Enviromental Justice Foundation (2012). Pirate fishing exposed: the fight against illegal fishing in West Africa and the EU. Avaiable at: http://ejfoundation.org/sites/default/files/public/Pirate%20Fishing%20Exposed.pdf
18. European Commission (EC) Council Regulation No. 104/2000 of 17 December 1999. (21st January 2000). On the common organization of the markets in fishery and aquaculture products. Official Journal of the European Union, L 17/22.
19. European Union (EU) Commission Implementing Regulation No. 404/2011 of 8 April 2011. (30th April2011). Laying down detailed rules for the implementation of Council Regulation (EC) No 1224/2009 establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy. Official Journal of the European Union, L 112/1.
20. European Union (EU) Regulation No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organization of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000.
21. FDA (2014). The seafood list. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=seafoodlist. Accessed 02 May 2014.
22. Hajibabaei, M., de Waard, J. R., Ivanova, N. V., Ratnasingham, S., Dooh, R. T., Kirk, S. L., Mackie, P. M., & Hebert, P. D. N. (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1959-1967.
23. Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N., & Hickey, D. A. (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, 23, 167–172.
24. Handy, S. M., Deeds, J. R., Ivanova, N. V., Hebert, P. D. N., Hanner, R. H., Ormos, A., Weigt, L. A., Moore, M. M., &Yancy, H. F. (2011). A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *Journal of Association of Official Analytical Chemists International*, 94, 201-210.

612 25. Hebert, P. D. N., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life:
613 cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the*
614 *Royal Society of London. Series B*, 270, 596–599.
615 26. Hsu, T. H., Guillén Madrid, A. G., Burridge, C. P., Cheng, H. Y., & Gwo, J. C. (2011).
616 Resolution of the *Acanthopagrus* black seabream complex based on mitochondrial and amplified
617 fragment-length polymorphism analyses. *Journal of fish biology*, 79(5), 1182-1192.
618 27. Keskin, E., & Atar, H. H. (2013). DNA barcoding commercially important fish species of
619 Turkey. *Molecular Ecology Resources*, 13(5), 788-797.
620 28. Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions
621 through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–
622 120.
623 29. Meusnier, I., Singer, G. A., Landry, J. F., Hickey, D. A., Hebert, P. D., & Hajibabaei, M. (2008).
624 A universal DNA mini-barcode for biodiversity analysis. *BMC genomics*, 9(1), 214.
625 30. Nelson, J. S., (2006). *Fishes of the world*. (6th ed.). Hoboken, New Jersey: John Wiley & Sons,
626 Inc.
627 31. Ratnasingham, S., & Hebert, P. D. (2007) BOLD: The Barcode of Life Data System
628 (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.
629 32. Ratnasingham, S., & Hebert, P. D. (2013). A DNA-based registry for all animal species: The
630 Barcode Index Number (BIN) System. *PLoSOne*, 8(7), e66213.
631 33. Rodriguez-Ezpeleta, N., Mendibil, I., Álvarez, P., &Cotano, U. (2013). Effect of fish sampling
632 and tissue storage conditions in DNA quality: considerations for genomic studies. *Revista de*
633 *Investigación Marina, AZTI-Tecnalia*, 20(6), 77-87
634 34. Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing
635 phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.
636 35. Smith, J. L.B. & Smith M. M. (1986). *Sparidae*. In Smith, M. M., & Heemstra P. C. (Eds.),
637 Smiths' sea fishes. Berlin: Springer-Verlag.
638 36. Srinivasan, M., Sedmak, D., & Jewell, S. (2002). Effect of fixatives and tissue processing on the
639 content and integrity of nucleic acids. *The American Journal of Pathology*, 161(6), 1961-1971.
640 37. Steffens, D. L., Sutter, S. L., & Roemer, S. C. (1993). An alternate universal forward primer for
641 improved automated DNA sequencing of M13. *Biotechniques*, 15, 580-582.
642 38. Stiles, M. L., Lahr, H., Lahey, W., Shaftel, E., Bethel, D., Falls, J., & Hirshfield, M. S., (2011).
643 Oceana, Bait and switch: how seafood fraud hurts our Oceans, our wallets and our health.
644 http://oceana.org/sites/default/files/reports/Bait_and_Switch_report_2011.pdf

645 39. Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular
646 Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.
647 40. Ward, R. D., Holmes, B. H., & Yearsley, G. K. (2008). DNA barcoding reveals a likely second
648 species of Asian sea bass (barramundi) (*Lates calcarifer*). *Journal of Fish Biology*, 72, 458–463.
649 41. Ward, R. D., Hanner, R., & Hebert, P. D. N. (2009). The campaign to DNA barcode all fishes,
650 FISH-BOL. *Journal of Fish Biology*, 74, 329-356.
651 42. Wong, E.H.K., & Hanner, R.H. (2008). DNA barcoding detects market substitution in North
652 American seafood. *Food Research International*, 41, 828–837.
653 43. Yancy, H. F., Zemlak, T. S., Mason, J. A., Washington, J. D., Tenge, B. J., Nguyen, N. L.,
654 Barnett, J. D., Savary, W. E., Hill, W. E., Moore, M. M., Fry, F. S., Randolph, S. C., Rogers, P.
655 L., & Hebert, P. D. (2008). Potential use of DNA barcodes in regulatory science: applications of
656 the Regulatory Fish Encyclopedia. *Journal of Food Protection*, 71, 210-217.
657 44. Zhang, D.X., Hewitt G.M. (1996). Nuclear integrations: challenges for mitochondrial DNA
658 markers. *Trends in Ecology & Evolution*, 6, 247-251.

Table

		Full-DNA barcodes (655bp)		Mini-DNA barcodes (139bp)	
		IDs BOLD	**BLAST NCBI**	**IDs BOLD**	**BLAST NCBI**
		Raw data			
Correctly identified	Sequences	134 – 59.6%	127 – 56.4%	97 – 38.6%	97 – 38%
	Species	39 – 57.4%	37 – 54.4%	28 – 40%	26 – 37.1%
Problematic*	Sequences	73 –32.4%	59 – 26.2%	132 – 52.6%	112 – 44%
	Species	20 –29.4%	14 – 20.6%	32 – 45.7%	26 – 37.1%
No reference sequences	Sequences	18 – 8%	39 – 17.3%	22 – 8.8%	46 – 18%
	Species	9 – 13.2%	17 – 25%	10 – 14.3%	18 – 25.7%
		After result elaboration			
Correctly identified	Sequences	187 – 83%	161 – 71.5%	110 – 43.8%	114 – 44.7%
	Species	53 – 78%	44 – 64.7%	32 – 45.7%	29 – 41.4%
No reference sequences	Sequences	18 – 8%	39 – 17.3%	22 – 8.8%	46 – 18%
	Species	9 – 13.2%	17 – 25%	10 – 14.3%	18 – 25.7%
Non identifiable	Sequences	20 – 9%	25 – 11.2%	119 – 47.4%	95 – 37.2%
	Species	6 – 8.8%	7 –10.3 %	28 – 40%	23 – 32.8%

Table 1. Summary of the results of the IDs analysis on BOLD and of the BLAST analysis on GenBank using the full and the mini DNA barcodes (655bp and 139bp, respectively), before and after the elaboration of the results. * Include the sequences that were not identified due to the presence of sequences belonging to misidentified specimens in the databases or to close relationship between species.

Table

Identification	Conflicting Taxon in BIN	Rank of Conflict	BIN	BIN Total Members	BIN Tax Variation
Boops boops	*Boops*	Genus	BOLD:AAB7806	59	***Boops* [78],** *Oblada* [2]
Cheimerius nufar	*Cheimerius*	Genus	BOLD:AAE2592	25	***Cheimerius* [24],** *Pagrus* [1]
Evynnis cardinalis	*Evynnis*	Genus	BOLD:AAC2906	22	***Evynnis* [19],** *Parargyrops* [3]
Evynnis tumifrons	*Evynnis*	Genus	BOLD:AAD0508	11	***Evynnis* [11],** *Dentex* [2]
Pagellus acarne	*Pagellus*	Genus	BOLD:AAC3611	35	***Pagellus* [45],** *Oblada* [2]
Pagellus bellottii	*Pagellus*	Genus	BOLD:AAF8829	8	***Pagellus* [5],** *Pagrus* [3]
Pagellus erythrinus	*Pagellus*	Genus	BOLD:AAC8525	39	***Pagellus* [52]**, *Oblada* [2]
Pagrus pagrus	*Pagrus*	Genus	BOLD:AAC8526	58	***Pagrus* [54],** *Oblada* [4], *Pagellus* [2]
Rhabdosargus haffara	*Rhabdosargus*	Genus	BOLD:ACG7708	3	***Rhabdosargus* [2],** *Sparus* [1]
Sarpa salpa	*Sarpa*	Genus	BOLD:AAE4266	41	***Sarpa* [41],** *Boops* [1]
Viridiidentex acromegalus	*Viridiidentex*	Genus	BOLD:ABX7583	8	***Pagellus* [5], *Viridiidentex* [3]**
Acanthopagrus pacificus	*Acanthopagrus pacificus*	Species	BOLD:ACF5415	7	***Acanthopagrus pacificus* [5],** *A. berda* [2]
Acanthopagrus schlegelii	*Acanthopagrus schlegelii*	Species	BOLD:AAF8876	29	***Acanthopagrus schlegelii* [13],** A. *schlegelii schlegelii* [11], ***A. sivicolus* [3]**
Acanthopagrus sivicolus	*Acanthopagrus sivicolus*				
Argyrops bleekeri	*Argyrops bleekeri*	Species	BOLD:AAB3719	13	***Argyrops bleekeri* [12],** *A. spinifer* [1]
Calamus proridens	*Calamus proridens*	Species	BOLD:AAU3000	3	***Calamus leucosteus* [2],** *C. proridens* [1]
Dentex angolensis	*Dentex angolensis*	Species	BOLD:AAE3470	10	*Dentex macrophthalmus* [5], ***D. angolensis* [3], *D. maroccanus* [2]**
Dentex maroccanus	*Dentex maroccanus*				
Diplodus cervinus hottentotus	*Diplodus cervinus hottentotus*	Species	BOLD:AAD3631	34	*Diplodus cervinus* [26], *D. fasciatus*[5], ***D. cervinus hottentotus* [3]**
Diplodus noct	*Diplodus noct*	Species	BOLD:ACE3794	62	***Diplodus sargus* [42],** *D. capensis* [11], ***D. noct* [3]**, *D. sargus helenae* [2], *D. sargus ascensionensis* [2], *D. sargus sargus* [1], *D. kotschyi* [1]
Diplodus sargus	*Diplodus sargus*				
Diplodus vulgaris	*Diplodus vulgaris*	Species	BOLD:AAC2260	47	***Diplodus vulgaris* [60],** *D. prayensis* [6], *D. sargus* [2], *D. fasciatus* [1]
Pagrus major	*Pagrus major*	Species	BOLD:AAC0553	43	***Pagrus major* [21], *Pagrus auratus* [19]**
Pagrus auratus	*Pagrus auratus*				
Stenotomus caprinus	*Stenotomus caprinus*	Species	BOLD:AAC4538	29	***Stenotomus chrysops* [24], *S. caprinus* [4]**
Stenotomus chrysops	*Stenotomus chrysops*				

Table 2: BIN discordance report.

Table

Country	N° of commercial denominations	N° of species	Percentage of coverage
Italy	28	35	80%
Spain	27	41	65%
UK	3	113	2%
France	36	47	76%
Germany	21	49	43%
USA	6	57	10%
Canada	23	29	79%
Australia	10	10	100%

Table 3. Percentage of coverage of the commercial denominations for the Sparidae family in different Countries.

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Scientific Name	*Sparidae* Official Trade Denominations								FAO English name
	Europe					Extra EU			
	Italy	Spain	France	Germany	United Kingdom[a]	USA	Canada	Australia	
Acanthopagrus australis								Yellowfin Bream	Surf bream
Acanthopagrus berda						Seabream, Porgie		Pikey Bream	Goldsilk seabream
Acanthopagrus bifasciatus	Pagro bifasciato								Twobar seabream
Acanthopagrus butcheri								Black Bream	N.R.
Acanthopagrus latus								Western Yellowfin Bream	Yellowfin seabream
Acanthopagrus palmaris								Northwest Black Bream	N.R.
Archosargus probatocephalus			Rondeau mouton			Sheepshead	Sheepshead Porgy, Seabream, Porgy		Sheepshead
Archosargus rhomboidalis						Sea Bream			Western Atlantic seabream
Argyrops bleekeri						Bream	Taiwan Thai, Bream	FrypanBream	Taiwan tai
Argyrops filamentosus	Pagro indiano		Spare de l'Océan indien						Soldier bream
Argyrops spinifer	Pagro reale		Spare royal			Bream	Long-spinedRedBream		King soldier bream
Boops boops	Boga	Boga	Bogue	Gelbstriemen	Bogue	Bream or Bogue	Bream		Bogue
Calamus arctifrons			Daubenet (*Calamus* spp.)			Porgy	Porgy (*Calamus* spp.)		Grass porgy
Calamus bajonado			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		Jolthead porgy
Calamus brachysomus			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		Pacific porgy
Calamus calamus		Pezpluma	Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		Saucereye porgy

Calamus campechanus			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		N.R.
Calamus cervigoni			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		N.R.
Calamus leucosteus			Daubenet (*Calamus* spp.)			Porgy	Porgy (*Calamus* spp.)		N.R.
Calamus mu			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		N.R.
Calamus nodosus			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy		N.R.
Calamus penna			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		Sheepshead porgy
Calamus pennatula			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		N.R.
Calamus proridens			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		Littlehead porgy
Calamus taurinus			Daubenet (*Calamus* spp.)			Porgy (*Calamus* spp.)	Porgy (*Calamus* spp.)		N.R.
Cheimerius nufar (Dentex nufar)	Dentale indiano (Dentice rosa)	Dentón nufar							Santer seabream
Chrysoblephus gibbiceps				Stumpfnase, Rote					Red stumpnose seabream
Pagrus auratus (Chrysophrys auratus)	Pagro rosa indo pacifico					Porgy		Snapper	N.R.
Dentex abei		Dentones (*Dentex* spp.)		Brasse, Meer, Dorade (*Dentex* spp.)					N.R.
Dentex angolensis	Dentice atlantico	Dentones (*Dentex* spp.)	Denté angolais	Brasse, Meer, Dorade (*Dentex* spp.)					Angolan dentex
Dentex barnardi	Dentice atlantico	Dentones (*Dentex* spp.)		Brasse, Meer, Dorade (*Dentex* spp.)					Barnard dentex
Dentex	Dentice	Denton Canario	Denté des	Brasse, Meer,					Canary

canariensis	atlantico		Canaries, Denté à tâche rouge	Dorade (*Dentex* spp.)					dentex
Dentex congoensis		Dentones (*Dentex* spp.)	Denté congolais	Kongo-Zahn-Brasse					Congo dentex
Dentex dentex	Dentice	Denton, Denton europeo	Denté commun, denté	Zahn-Brasse		Porgy	Dentex , Common Dentex		Common dentex
Dentex fourmanoiri		Dentones (*Dentex* spp.)		Brasse, Meer, Dorade (*Dentex* spp.)					N.R.
Dentex gibbosus	Dentice gibboso	Sama de pluma	Denté rose	Brasse, Dickkopfzahn		Porgy			Pink dentex
Dentex macrophthalmus	Dentice occhione	Cachucho	Denté à gros yeux	Brasse, Großaugenzahn					Large-eye dentex
Dentex maroccanus	Dentice marocchino	Sama	Denté du Maroc	Brasse, MarokkanischeZahn					Morocco dentex
Dentex spariformis		Dentones (*Dentex* spp.)		Brasse, Meer, Dorade (*Dentex* spp.)					N.R.
Diplodus annularis	Sarago sparaglione	Raspallon	Sparaillon commun, sparaillon	Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			Annular seabream
Diplodus argenteus argenteus	Sarago atlantico[b]	Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodusargenteus*)			South American silver porgy
Diplodus argenteus caudimacula	Sarago atlantico[b]	Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodusargenteus*)			N.R.
Diplodus bellottii		Sargos (*Diplodus* spp.)	Sparaillon africain, sparaillon	Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			Senegal seabream
Diplodus bermudensis		Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			N.R.
Diplodus capensis		Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			N.R.
Diplodus cervinus cervinus	Sarago[b]	Sargo breado	Sar à grosses lèvres, Sar	Bänder-Brasse		Porgy (*Diplodus* spp.)			Zebra seabream
Diplodus cervinus hottentotus	Sarago[b]	Sargos		Brasse, Meer, Dorade		Porgy (*Diplodus* spp.)			N.R.

		(*Diplodus* spp.)		(*Diplodus* spp.)					
Diplodus cervinus omanensis	Sarago[b]	Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			N.R.
Diplodus fasciatus		Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			Banded seabream
Diplodus holbrookii		Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy	Salema		Spottail seabream
Diplodus noct		Sargos (*Diplodus* spp.)		Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			Red Sea seabream
Diplodus prayensis		Sargos (*Diplodus* spp.)	Sar à tête noire du Cap Vert	Brasse, Meer, Dorade (*Diplodus* spp.)		Porgy (*Diplodus* spp.)			Two-banded seabream
Diplodus puntazzo	Sarago pizzuto	Sargo picudo	Sar à museau pointu, sar	Spitz-Brasse		Porgy			Sharpsnout seabream
Diplodus sargus ascensionis	Sarago[b]	Sargo[b]		Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			N.R.
Diplodus sargus cadenati	Sarago[b]	Sargo[b]		Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			N.R.
Diplodus sargus helenae	Sarago[b]	Sargo[b]		Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			N.R.
Diplodus sargus kotschyi	Sarago[b]	Sargo[b]		Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			N.R.
Diplodus sargus lineatus	Sarago[b]	Sargo[b]		Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			N.R.
Diplodus sargus sargus	Sarago[b]	Sargo[b]	Sarcommun, sar	Weiß-Brasse, GroßeGeiß-Brasse[b]		Porgy (*Diplodus* spp.)			White seabream
Diplodus vulgaris	Sarago	Mojarra	Sar à tête noire, sar	Zweibinden-Brasse		Porgy (*Diplodus* spp.)			Common two-banded seabream
Evynnis tumifrons						Sea Bream			N.R.

						(*Dentex tumifrons*)			
Lagodon rhomboides						Porgy	Pinfish		Pinfish
Lithognathus lithognathus			Marbré d'Afrique, dorade-marbré						White steenbras
Lithognathus mormyrus	Mormora	Herrera	Marbré commun, dorade-marbré	Marmor-Brasse, Meer-Brasse, Dorade					Sand steenbras
Oblada melanura	Occhiata	Oblada	Oblade	Brand-Brasse					Saddled seabream
Pagellus acarne	Pagello	Aligote	Pageot acarné	Achselfleck-Brasse		Sea Bream (*Pagellus* spp.)	Sea Bream, Axillary Seabream, Axillary bream		Axillary seabream
Pagellus affinis	Pagello indiano	Besugo arabe	Pageot d'Arabie, Pageot de la mer d'Oman	Brasse, Meer, Dorade (*Pagellus* spp.)		Sea Bream (*Pagellus* spp.)			Arabian pandora
Pagellus bellottii	Pagello atlantico	Brecachata	Pageot à tache rouge, Dorade rouge	Brasse, Meer, Dorade (*Pagellus* spp.)		Sea Bream (*Pagellus* spp.)	Red Pandora, Pandora		Red Pandora
Pagellus bogaraveo	Pagello	Besugo	Pageot rose, Dorade rose	Grau-Barsch, See-Karpfen		Sea Bream (*Pagellus* spp.)	Seabream , Porgy		Blackspot (=red) seabream
Pagellus erythrinus	Pagello fragolino	Breca	Pageot mommun, Pageot	Rot-Brasse		Bream			Common pandora
Pagellus natalensis		Besugos (*Pagellus* spp.)		Brasse, Meer, Dorade (*Pagellus* spp.)		Sea Bream (*Pagellus* spp.)			Natal pandora
Pagrus africanus	Pagro africano			Brasse, Meer, Dorade (*Pagrus* spp.)					Southern common seabream
Pagrus auratus									Silver seabream
Pagrus auriga	Pagro	Urta	Pagre rayé	Brasse, Meer, Dorade (*Pagrus* spp.)					Redbanded seabream
Pagrus caeruleostictus	Pagro	Zapata	Pagre à points bleu, Dorade	Brasse, Meer, Dorade			Seabream, Porgy, Bluespotted		Bluespotted seabream

				(*Pagrus* spp.)			Seabream		
Pagrus major	Pagro del Giappone			Brasse, Meer, Dorade (*Pagrus* spp.)		Porgy, Sea Bream	Silver Seabream, Japanese Seabream, Genuine Porgy		Japanese seabream
Pagrus pagrus	Pagro	Pargo		Sack-Brasse		Porgy	Seabream, Red Porgy, Porgy		Red porgy
Polysteganus coeruleopunctatus			Denté à points bleu						Blueskin seabream
Pterogymnus laniarius			Panga de l'Atlantique S-E Spare panga			Porgy			Panga seabream
Rhabdosargus globiceps			Sargue de l'Atlangique S.-E.	Stumpfnase, Weiße					White stumpnose
Rhabdosargus sarba	Sarago dorato		Sarguedorée					Tarwhine	Goldlined seabream
Sarpa salpa	Salpa	Salema	Saupe	Goldstriemen					Salema
Sparidentex hasta								SobaityBream	Sobaity seabream
Sparus aurata	Orata	Dorada		Gold-Brasse			Gilthead Bream	Bream	Gilthead seabream
Spondyliosoma cantharus	Tanuta	Chopa	Griset, Doradegrise	Meer-Brasse Streifen-Brasse, Dorade					Black seabream
Stenotomus caprinus						Porgy	Shiner, Seabream, Porgy,Longspined Porgy		Longspine porgy
Stenotomus chrysops						Porgy, Scup	Scup,Porgy		Scup

Table 1 SM. Official Trade Names of the species of commercial interest belonging to the Sparidae family according to the lists of Italy (Ministerial Decree of the Italian Minister of Agriculture, Food and Forestry (MIPAAF) of 27th March 2002 and subsequent integrations), Spain (Resolución de 22 Marzo 2011 de la Secretaría General del Mar), France (http://www.economie.gouv.fr/dgccrf/Consommation/Etiquetage-des-produits/Produits-de-la-

mer-et-d-eau-douce/Listes-des-denominations-commerciales), Germany (http://www.fischinfo.de/pdf/HANDELSBEZEICHNUNGEN_%28DEUTSCH%29.pdf), United Kingdom (Food Standard Agency of United Kingdom), USA (US Food and Drug Administration (USFDA), Regulatory Fish Encyclopedia (RFE), 2012), Canada (Canadian Food Inspection Agency, CFIA Fish List, 2012), Australia (Australia Government, Seafood Services Australia Ltd Fishery Research Development Corporation). Moreover, the FAO English names are reported ([a]Aquatic Sciences and Fisheries Information System (ASFIS) http://www.fao.org/fishery/collection/asfis/en).

[a]For all species of the family Sparidae except *Boops boops* the legal name is Sea bream or Porgy;

[b]Trade denomination assigned to the species;

NR = Not Reported.

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Species	Institution	Number of samples	Full-DNA barcoding region (655bp)	Mini DNA barcoding region (139bp)	Provenience (FAO Area)
Acanthopagrus australis	Australian Museum, Sydney, NSW, Australia	1	1	-	81
Acanthopagrus berda	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	4	4	-	61
	Museum of Natural Science, Louisiana State University Baton Rouge, LA, USA	1	1	-	71
Acanthopagrus bifasciatus	Department of Biotechnology and Biosciences University of Milan Bicocca Milan, Italy	3	0	0	51.1
Acanthopagrus butcheri	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	57.5.2
	Australian Center for Applied Acquaculture Research Challenger Institute of Technology Fremantle Freemantle, WA, Australia	7	7	-	57
	Australian Museum, Sydney, NSW, Australia	1	1	-	81
Acanthopagrus latus	Fisheries Research Laboratory, Mie University Mie, Japan	2	2	-	61
	Center for Molecular Biodiversity Research National Museum of Nature and Science Tsukuba, Ibaraki, Japan	3	3	-	
	This study	1	1	-	
Acanthopagrus pacificus[a]	Center for Molecular Biodiversity Research National Museum of Nature and Science Tsukuba, Ibaraki, Japan	3	2	1	61
Acanthopagrus palmaris	Australian Museum, Sydney, NSW, Australia	1	1	-	57
Acanthopagrus schlegelii[a]	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	4	3	1	61
	Fisheries Research Laboratory, Mie University Mie, Japan	2	2	-	
	Kanagawa Prefectural Museum of Natural History Odawara, Kanagawa, Japan	1	0	0	
Acanthopagrus sivicolus[a]	Center for Molecular Biodiversity Research	3	3	-	61

	National Museum of Nature and Science Tsukuba, Ibaraki, Japan				
Archosargus probatocephalus	Biodiversity Institute, University of Kansas Lawrence, KS, USA	2	1	0	31
	North Carolina Museum of Natural Sciences Raleigh, NC, USA	1	1	-	
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	1	1	-	
	Florida Museum of Natural History, Genetic Resources Repository, University of Florida Gainesville, FL, USA	1	1	-	
	Mississippi Museum of Natural Science Jackson, MS, USA	1	0	1	
Archosargus rhomboidalis	Biodiversity Institute, University of Kansas Lawrence, KS, USA	2	1	0	31
Argyrops bleekeri	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	1	1	-	61
	Center for Molecular Biodiversity Research National Museum of Nature and Science Tsukuba, Ibaraki, Japan	2	2	-	
	Department of Ichthyology American Museum of Natural History New York, NY, USA	1	1	-	
Argyrops filamentosus	South African Institute for Aquatic Biodiversity Grahamstown, South Africa	2	2	-	51.8
Argyrops spinifer	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	61
	South African Institute for Aquatic Biodiversity Grahamstown, South Africa	5	3	0	51.8
	This study	2	2	-	
Argyrozona argyrozona[a]	FishWeights Cape Town, South Africa	3	3	-	51.8
Boops boops	Department of Zoology, George S. Wise Faculty of Life Science Tel Aviv University Tel Aviv, Israel	3	3	-	37.2.2
	Wholesale fish market of Scoglitti Ragusa, Italy	2	1	0	
Calamus arctifrons	Academy of Natural Sciences, Ichthyology	1	0	0	31

	Drexel University Philadelphia, Pennsylvania, USA				
	Florida Museum of Natural History , Genetic Resources Repository, University of Florida Gainesville, FL, USA	5	2	1	
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	1	1	-	
Calamus bajonado	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	31
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	4	3	0	
Calamus brachysomus	Institution of Oceanography, University of California La Jolla, CA, USA	1	1	-	77
	Centro de Investigaciones Biologicas del Noroeste La Paz, México	1	1	-	77
Calamus calamus	University of Kansas - Biodiversity Institute, Dyche Hall Lawrence, KS, USA	3	3	-	31
	Florida Museum of Natural History –Genetic Resources Repository, University of Florida Gainesville, FL, USA	1	0	0	
Calamus leucosteus[a]	US FDA Center for Food Safety and Applied Nutrition College Park, MD, USA[b]	1	NS		31
	Biodiversity Institute, University of Kansas Lawrence, KS, USA	3	1	0	
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	2	2	-	
Calamus nodosus	Biodiversity Institute, University of Kansas Lawrence, KS, USA	2	1	1	31
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	3	3	-	
Calamus penna	Fish and Wildlife Research Institute St. Petersburg, FL, USA	2	1	1	31
Calamus pennatula	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	31

Calamus proridens	Fish and Wildlife Research Institute St. Petersburg, FL, USA	4	1	1	31
	Florida Museum of Natural History, Genetic Resources Repository, University of Florida Gainesville, FL, USA	1	0	0	
Cheimerius nufar	South African Institute for Aquatic Biodiversity Grahamstown, South Africa	5	4	1	51.6 51.8
Chrysoblephus cristiceps[a]	FishWeights Cape Town, South Africa	3	3	-	51.8
Chrysoblephus gibbiceps	FishWeights Cape Town, South Africa	1	1	-	51.8
Chrysoblephus laticeps[a]	FishWeights Cape Town, South Africa	3	3	-	51.8
Chrysoblephus puniceus[a]	School of Environment & Life Sciences, University of Salford Salford, United Kingdom	1	1	-	51
Crenidens crenidens[a]	Australian Museum Sydney, NSW Australia	1	1	-	51
Dentex angolensis	California Academy of Sciences San Francisco, CA, USA	3	1	1	34.3.1 34.3.4
	This study	2	2	0	34
Dentex canariensis	Aquatic Animal Health Laboratory, College of Veterinary Medicine Michigan State University East Lansing, MI, USA	1	0	0	34.3.1
	This study	1	1	0	34
Dentex congoensis	California Academy of Sciences San Francisco, CA, USA	1	0	0	34.3.1
Dentex dentex	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	37
	This study	4	3	1	37.1.3
		1	1	0	34
Dentex gibbosus	Aquatic Animal Health Laboratory, College of Veterinary Medicine Michigan State University East Lansing, MI, USA	1	1	-	34.3.1
Dentex macrophthalmus	Department of Zoology, George S. Wise Faculty of Life Science Tel Aviv University Tel Aviv, Israel	3	3	-	37.3.2
Dentex maroccanus	Department of Zoology, George S. Wise Faculty of Life Science	3	2	0	37.3.2

	Tel Aviv University Tel Aviv, Israel				
Dentex spariformis	Australian Museum Sydney, NSW Australia	1	1	-	81
Diplodus annularis	Department of Zoology, George S. Wise Faculty of Life Science Tel Aviv University Tel Aviv, Israel	3	3	-	37.3.2
	This study	2	2	-	
Diplodus argenteus	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	31
Diplodus bellottii	Aquatic Animal Health Laboratory, College of Veterinary Medicine Michigan State University East Lansing, MI, USA	1	0	0	34.3.1
Diplodus cervinus	South African Institute for Aquatic Biodiversity Grahamstown, South Africa	2	0	2	51.8
	Department of Ichthyology, American Museum of Natural History New York, NY, USA	1	0	0	Unknown
Diplodus cervinus hottentotus	Institution of Oceanography, University of California La Jolla, CA, USA	1	1	-	47
	Biodiversity Institute, University of Kansas Lawrence, KS, USA	1	1	-	47.2.2
Diplodus holbrookii	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	2	0	0	31
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	3	2	0	
Diplodus noct	Department of Biotechnology and Biosciences, University of Milan - Bicocca Milan, Italy	2	2	-	51.1
	Australian Museum Sydney NSW Australia	1	1	-	51
Diplodus puntazzo	This study	5	4	1	37.1.3
Diplodus sargus	Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University Tel Aviv, Israel	3	2	1	37.3.2
	Museu Nacional de História Natural e da Ciência Lisboa, Portugal	1	0	0	27 IXa

	This study	3	2	1	37.1.3
Diplodus vulgaris	Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University Tel Aviv, Israel	3	3	-	37.3.2
	This study	3	3	0	37.1.3
Evynnis cardinalis[a]	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	8	2	3	61
Evynnis tumifrons	Graduate School of Biosphere Science, Hiroshima University Hiroshima, Japan	2	2	-	61
	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	3	3	-	
Lagodon rhomboides	US FDA Center for Food Safety and Applied Nutrition College Park, MD, USA[b]	1	NS		31
	Biodiversity Institute, University of Kansas Lawrence, KS, USA	3	3	-	
	Florida Museum of Natural History, Genetic Resources Repository, University of Florida Gainesville, FL, USA	1	1	-	
	Museum of Natural Science, Louisiana State University Baton Rouge, LA,USA	1	1	-	
Lithognathus mormyrus	School of Environment & Life Sciences, University of Salford Salford, United Kingdom	1	1	-	37.1.1
	Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University Tel Aviv, Israel	3	2	0	37.3.2
	This study	3	3	-	
Oblada melanura	School of Environment & Life Sciences, University of Salford Salford, United Kingdom	1	0	1	37.1.2
	This study	10	2	5	37.1.3
Pachymetopon aeneum[a]	FishWeights Cape Town, South Africa	3	3	-	51.8
Pagellus acarne	Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University Tel Aviv, Israel	3	3	-	37.2.2
	Wholesale fish market of Scoglitti Ragusa, Italy	3	2	0	

Pagellus bellottii	Aquatic Animal Health Laboratory, College of Veterinary Medicine Michigan State University East Lansing, MI, USA	1	1	-	34.3.1
Pagellus bogaraveo	Wholesale fish market of Scoglitti Ragusa, Italy	3	3	-	37.2.2
	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	37.1.3
	This study	4	4	-	37.1.1
Pagellus erythrinus	School of Environment & Life Sciences, University of Salford Salford, United Kingdom	1	-	1	37.1.2
	This study	5	4	0	37.1.3
Pagrus africanus	Departamento de Oceanografia e Pescas – Universidade dos Açores, Açores, Portugal	1	0	1	34.3.2
Pagrus auratus	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	77
	Seafood and Marine Extracts, Plant & Food Research Nelson Nelson, New Zealand	6	6	-	81
	Cawthron Institute, Nelson, New Zealand	1	1	-	
Pagrus auriga	Aquatic Animal Health Laboratory, College of Veterinary Medicine Michigan State University East Lansing, MI, USA	1	1	-	34.3.1
	This study	1	1	-	
Pagrus caeruleostictus	Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University Tel Aviv, Israel	3	2	1	37.3.2
	California Academy of Sciences San Francisco, CA, USA	2	2	-	34.3.1 34.1.3
	This study	2	1	0	37.1.3
Pagrus major	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	4	3	1	61
	Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan	2	2	-	
Pagrus pagrus	Wholesale fish market of Scoglitti Ragusa, Italy	3	2	1	37.2.2
	US FDA Center for Food Safety and Applied Nutrition College Park, MD, USA[b]	1	NS		31

	This study	5	3	2	37.1.3
Pterogymnus laniarus	FishWeights Cape Town, South Africa	3	3	-	51.8
Rhabdosargus haffara	Department of Biotechnology and Biosciences University of Milan - Bicocca Milan, Italy	1	1	-	51.1
Rhabdosargus holubi[a]	Australian Museum Sydney, NSW Australia	1	1	-	47
Rhabdosargus sarba	Biodiversity Research Center, Academia Sinica Taipei, Taiwan	2	0	2	61
	Fisheries Research Laboratory, Mie University Mie, Japan	2	2	-	
	Kanagawa Prefectural Museum of Natural History Odawara, Kanagawa, Japan	1	0	0	
	Australian Museum Sydney, NSW Australia	1	1	-	81
Sarpa salpa	Mercato Ittico Scoglitti Ragusa, Italy	2	2	-	37.2.2
	This study	3	3	-	37
Sparus aurata	This study	5	5	-	37.1.3
Spondyliosoma cantharus	Museu Nacional de História Natural e da Ciência Lisboa, Portugal	1	0	0	27 IXa
	This study	5	5	-	37.1.3
Stenotomus caprinus	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	31
	Biodiversity Institute, University of Kansas Lawrence, KS, USA	1	1	-	
	Fish and Wildlife Research Institute St. Petersburg, FL, USA	2	2	-	
Stenotomus chrysops	Biodiversity Institute, University of Kansas Lawrence, KS, USA	4	4	-	21.6
	Academy of Natural Sciences, Ichthyology Drexel University Philadelphia, Pennsylvania, USA	1	0	0	21.6B
	North Carolina Museum of Natural Sciences	1	1	-	31

	Raleigh, NC, USA				
	Herpetology and Ichthyology, Division of Vertebrate Zoology Yale Peabody Museum of Natural History New Haven, CT, USA	1	1	-	21.6A
	US FDA Center for Food Safety and Applied Nutrition College Park, MD, USA[b]	1	NS		21
Viridiidentex acromegalus[a]	Departamento de Oceanografia e Pescas – Universidade dosAçores Açores, Portugal	2	1	1	34.3.2

Table 2 SM. Reference samples collected in the study, with the indications of the Institutions, the geographical origin and the number of full and mini barcode obtained. [a]Species not considered in the International Official Trade lists; [b]DNA samples only used for testing the amplification performance of primers; NS: Not Sequenced.

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Code	Place of collection	Label information			Product	bp	Species identification			
		Market name	International accepted name	Scientific name			BOLD Species Level Barcode Records	MI	GenBank	MI
MS1	Market	Dentale indiano	Santer seabream	*Cheimerius nufar*	Whole	655	***C. nufar***	**99.54**	***C. nufar***	**99**
						139	***C. nufar***	**100**	***C. nufar***	**100**
MS2-MS3	Market	Dentice atlantico	Angolan dentex	*Dentex angolensis*[a]	Fillets	655	***D. angolensis*** ***D. macrophthalmus*** ***D. maroccanus***	**100** **99.84** **99.84**	***D. angolensis*** ***D. macrophthalmus***	**100** **98**
						139	***D. angolensis*** ***D. macrophthalmus*** ***D. maroccanus*** ***D. canariensis***	**100** **99.28** **99.28** **98.55**	***D. angolensis*** ***D. macrophthalmus***	**100** **99**
MS4	Market	Dentice	Dentex	NR	Whole	655	***D. canariensis***	**100**	***D. canariensis***	**100**
						139	***D. canariensis*** ***D. macrophthalmus*** ***D. maroccanus*** ***D. angolensis***	**100** **99.28** **99.28** **98.55**	***D. canariensis*** ***D. macrophthalmus***	**100** **99**
MS5	Market	Dentice	Dentex	NR	Whole	655	**No match**		***Cheimerius nufar***	**95**
						139	***C. nufar*** ***D. gibbosus***	**98.55** **98.55**	***Cheimerius nufar***	**98**
MS6-MS7	Market	Dentice rosa	Santer seabream	NR	Whole	655	***C. nufar***	**99.08**	***C. nufar***	**99**
						139	***C. nufar***	**100**	***C. nufar***	**100**
MS8	Market	Dentice rosa	Santer seabream	NR	Whole	139	***C. nufar***	**100**	***C. nufar***	**100**
MS9	Restaurant	Dentice	Dentex	NR	Whole	655	***D. dentex***	**100**	***D. dentex***	**99**
						139	***D. dentex***	**100**	***D. dentex***	**99**
MS10	Restaurant	Mormora	Sand steenbras	*L. mormyrus*	Whole	606	***L. mormyrus***	**100**	***L. mormyrus***	**100**
						139	***L. mormyrus***	**100**	***L. mormyrus***	**100**
MS11	Market	Occhiata	Saddled seabream	*O. melanura*	Whole	139	***O. melanura*** ***D. capensis*** ***D. vulgaris*** ***D. sargus*** ***D. bellottii*** ***D. puntazzo*** ***D. sargus* subspecies** ***D. noct***	**100** **99.28** **99.21** **98.55** **98.55** **98.55** **98.55** **98.55**	***D. sargus*** ***D. argenteus*** ***D. holbrookii***	**99** **98** **98**
MS12	Market	Occhiata	Saddled seabream	*O. melanura*	Fillets	655	***D. sargus*** ***D. capensis*** ***D. sargus* subspecies** ***D. argenteus*** ***D. holbrookii***	**100** **99.54** **99.39 -98.46** **98.16** **98.15**	***D. sargus*** ***D. sargus kotschyi*** ***D. holbrookii*** ***D. argenteus***	**100** **99** **98** **98**

						139	***D. capensis*** **100** ***D. sargus kotschyi*** **100** ***D. sargus*** **100** ***D. bellottii*** **100** ***D. sargus*** **subspecies** **100** ***D. noct*** **100** ***D. holbrookii*** **99.28** ***D. argenteus*** **99.28** ***D. cervinus*** **99.05** ***D. fasciatus*** **98.55**	***D. sargus*** **100** ***D. argenteus*** **99** ***D. holbrookii*** **99** ***D. cervinus*** **99**	
MS13	Market	Occhiata	Saddled seabream	*O. melanura*	Fillets	655	***D. vulgaris*** **100** ***D. sargus*** **99.69** ***D. prayensis*** **98.73** ***D. fasciatus*** **98.62**	***D. sargus*** **99** ***D.vulgaris*** **99**		
						139	***D. sargus*** **100** ***D. prayensis*** **99.28** ***D. puntazzo*** **99.28** ***D. fasciatus*** **99.28** ***O. melanura*** **98.41**	***D. sargus*** **100**		
MS14	Restaurant	Occhiata	Saddled seabream	NR	Whole	655	***O. melanura*** **99.69**	***O. melanura*** **95**		
						139	***O. melanura*** **99.21** ***D. vulgaris*** **98.41**	***O. melanura*** **97** ***D. sargus*** **97**		
MS15	Market	Orata	Gilthead seabream	*S. aurata*	Fillets	655	***S. salpa*** **100**	***S. salpa*** **100**		
						139	***S. salpa*** **100**	***S. salpa*** **100**		
MS16-MS17	Restaurant	Orata	Gilthead seabream	*S. aurata*	Fillets	655	***S. aurata*** **100**	***S. aurata*** **100**		
						139	***S. aurata*** **100**	***S. aurata*** **100**		
MS18-MS19	Market	Orata	Gilthead seabream	*S. aurata*	Fillets	655	***S. aurata*** **100**	***S. aurata*** **100**		
						139	***S. aurata*** **100**	***S. aurata*** **100**		
MS20	Market	Orata	Gilthead seabream	NR	Whole	655	***S. aurata*** **99.85**	***S. aurata*** **99**		
						139	***S. aurata*** **100**	***S. aurata*** **100**		
MS21	Market	Pagello	Sea Bream	NR	Whole	655	***P. acarne*** **100** *O. melanura* 100	***P. acarne*** **99** *O. melanura* 99		
						139	***P. acarne*** **100** *O. melanura* 100	***P. acarne*** **100**		
MS22	Market	Pagello atlantico	Red pandora	*P. bellottii*	Whole	*655*	***P. bellotii*** **100** *P. pagrus* (3 seq.) 99.53 ***P. natalensis*** **99.21**	***P. bellotii*** **99** ***P. natalensis*** **99**		
						139	***P. bellotii*** **100** *P. pagrus* (3 seq.) 98.55	***P. bellotii*** **100** ***P. natalensis*** **98**		
MS23	Market	Pagello	Common	*P. erythrinus*	Whole	655	***P. erythrinus*** **100**	***P. erythrinus*** **100**		

		fragolino	pandora				*O. melanura* (2 seq.)	99.19	*O. melanura*	99
						139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
MS24	Market	Pagello	Seabream	NR	Whole	655	***P. erythrinus*** *O. melanura* (2 seq.)	**99.85** 99.19	***P. erythrinus*** *O. melanura* (2 seq.)	**99** 99
						139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
MS25-MS26-MS27	Market	Pagello	Seabream	NR	Filletts	655	***P. erythrinus*** *O. melanura* (2 seq.)	**99.85** 99.19	***P. erythrinus*** *O. melanura* (2 seq.)	**99** 99
						139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
MS28-MS29	Restaurant	Pagello	Seabream	NR	Whole	655	***P. erythrinus*** *O. melanura* (2 seq.)	**100** 99.19	***P. erythrinus*** *O. melanura* (2 seq.)	**100** 99
						139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
MS30	Restaurant	Pagello	Seabream	NR	Whole	655	*P. pagrus* ***C. nufar***	100 **99.23**	***C. nufar***	**99**
						139	***C. nufar*** *P. pagrus*	**100** 100	***C. nufar***	**100**
MS31	Market	Pagro	Redbanded seabream	*P. auriga*	Whole	655	***P. auriga***	**100**	***P. auriga***	**100**
						139	***P. auriga***	**100**	***P. auriga***	**100**
MS32-MS33	Market	Pagro	Bluespotted seabream	*P. caeruleostictus*	Whole	655	***P. caeruleostictus***	**100**	***P. caerulosticus***	**100**
						139	***P. caeruleostictus***	**100**	***P. caerulosticus***	**100**
MS34-MS35 – MS36	Market	Pagro	Seabream	NR	Whole	655	*P. pagrus* (1seq.) ***C. nufar***	100 **99.23**	***C. nufar***	**99**
						139	***C. nufar*** *P. pagrus* (1seq.)	**100** 100	***C. nufar***	**100**
MS37-MS38	Market	Pagro rosa indo pacifico	NR	NR	Whole	655	***A. spinifer***	**100**	***A. filamentosus***	**96**
						139	***A. spinifer*** ***A. blekeeri***	**100** **98.55**	***A. spinifer*** ***Porcostoma dentata*** ***A. filamentosus***	**99** **98** **98**
MS39	Market	Pagro reale	King soldier bream	*A. spinifer*	Whole	592	***A. spinifer***	**100**	***A. spinifer***	**100**
						139	***A. spinifer*** ***A. blekeeri***	**100** **98.72**	***A. spinifer*** ***P. major*** ***E. japonica***	**100** **98** **98**
MS40	Market	Pagro reale	King soldier bream	*A. spinifer*	Whole	139	***A. spinifer*** *A. bleekeri* (1seq.) *E. tumifrons*	**100** 98.72 98.15	***A. spinifer***	**100**
MS41-MS42-MS43	Market	Pagro	Seabream	NR	Whole	655	***P. caeruleostictus***	**100**	***P. caeruleostictus***	**99**
						139	***P. caeruleostictus***	**100**	***P. caeruleostictus***	**100**
MS44	Restaurant	Salpa	Salema	NR	Whole	655	***Spicara maena***	**100**	***Spicara maena***	**100**
						139	***Spicara maena***	**100**	***Spicara maena***	**100**
MS45	Restaurant	Salpa	Salema	NR	Whole	655	***Spicara maena***	**100**	***Spicara maena***	**100**

							Spicara flexousa (1 seq.)	99.84		
						139	***Spicara maena*** *Spicara flexousa* (1 seq.)	**100** 100	***Spicara maena***	**100**
MS46	Market	Salpa	Salema	*S. salpa*	Whole	655	***S. salpa***	**100**	***S. salpa***	**100**
						139	***S. salpa***	**100**	***S. salpa***	**100**
MS47	Restaurant	Salpa	Salema	*S. salpa*	Whole	655	***S. salpa***	**100**	***S. salpa***	**100**
						139	***S. salpa***	**100**	***S. salpa***	**100**
MS48	Market	Sarago sparaglione	Annular seabream	NR	Whole	655	***D. annularis***	**100**	***D. annularis***	**99**
						139	***D. annularis***	**100**	***D. annularis***	**100**
MS49	Market	Sarago sparaglione	Annular seabream	NR	Fillets	655	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. fasciatus***	**99.67** **99.53** **98.75** **98.75**	***D. sargus*** ***D. vulgaris***	**99** **99**
						139	***D. sargus*** ***D. vulgaris*** ***D. prayensis*** ***D. puntazzo*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.26** **99.26** **99.26** **98.52**	***D. sargus*** ***D. cervinus***	**100** **98**
MS50	Market	Sarago pizzuto	Sharpsnout seabream	*Diplodus puntazzo*	Whole	655	***D. puntazzo***	**100**	*D. labrax* ***D. puntazzo***	99 **96**
						139	***D. vulgaris*** ***D. puntazzo*** ***O. melanura*** ***D. sargus***	**100** **100** **99.21** **98.55**	***D. sargus*** *D. labrax*	**99** 98
MS51	Market	Sarago	Seabream	NR	Whole	655	***D. sargus*** ***D. capensis*** ***D. sargus* subspecies** ***D. noct*** ***D. holbrooki*** ***D. argenteus***	**100** **99.55** **99.39-98.46** **99.23** **98.16** **98.16**	***D. sargus*** ***D. holbrookii*** ***D. argenteus***	**100** **98** **98**
						139	***D. sargus*** ***D. capensis*** ***D. bellotii*** ***D. sargus* subspecies** ***D. noct*** ***D. holbrooki*** ***D. argenteus*** ***D. cervinus*** ***D. cervinus hottentotus*** ***D. fasciatus***	**100** **100** **100** **100** **100** **100** **99.28** **99.28** **99.05** **98.55**	***D. sargus*** ***D. argenteus*** ***D. holbrookii*** ***D. cervinus***	**100** **99** **99** **99**

								98.55		
MS52	Market	Sarago	Seabream	*D. vulgaris*	Whole	655	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. fasciatus***	**100** **100** **98.92** **98.92**	***D. sargus*** ***D. vulgaris***	**100** **99**
						139	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. puntazzo*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.28** **99.28** **99.28** **98.41**	***D. sargus***	**100**
MS53	Market	Sarago sparaglione	Annular seabream	NR	Whole	655	***D. annularis***	**100**	***D. annularis***	**99**
						139	***D. annularis***	**100**	***D. annularis***	**99**
MS54	Restaurant	Sarago	Seabream	NR	Whole	655	***D. puntazzo***	**100**	*D. labrax* ***D. puntazzo***	99 **96**
						139	***D. vulgaris*** ***D. puntazzo*** ***D. sargus*** ***D. capensis***	**100** **100** **98.89** **98.89**	***D. vulgaris*** ***D. sargus*** *D. labrax*	**100** **100** 99
MS55	Restaurant	Sarago	Seabream	NR	Whole	654	***D. puntazzo***	**99.85**	*D. labrax* ***D. puntazzo***	99 **95**
						139	***D. vulgaris*** ***D. puntazzo*** ***O. melanura*** ***D. sargus***	**100** **100** **99.21** **98.55**	***D. sargus*** *D. labrax*	**99** 98
MS56	Restaurant	Sarago pizzuto	Sharpsnout seabream	NR	Whole	655	***D. puntazzo***	**100**	*D. labrax* ***D. puntazzo***	99 **96**
						139	***D. vulgaris*** ***D. puntazzo*** ***O. melanura*** ***D. sargus***	**100** **100** **99.22** **98.55**	***D. sargus*** *D. labrax*	**99** 98
MS57	Restaurant	Sarago	Seabream	NR	Filletts	655	***D. sargus*** ***D. vulgaris*** ***D. prayensis*** ***D. fasciatus***	**100** **100** **98.92** **98.92**	***D. sargus*** ***D. vulgaris***	**100** **99**
						139	***D. sargus*** ***D. vulgaris*** ***D. prayensis*** ***D. puntazzo*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.28** **99.28** **99.28** **98.41**	***D. sargus***	**100**

MS58	Market	Tanuta	Black seabream	*S. cantharus*	Whole	655	***S. cantharus***	**99.84**	***S. cantharus***	**99**
						139	***S. cantharus*** ***S. emarginatum***	**100** **99.28**	***S. cantharus***	**99**

Table 3SM. Results of the IDs analysis (BOLD) and of the BLAST analysis (GenBank) of market samples (MS), with the information reported on the label. Mislabeled samples are highlighted with a grey background. [a] Sequences not available on both databases; [b] Sequences not available in Genbank; MI: Max Identity.

Primer name	Sequence code	Amp. Lenght (bp)	Ref.
LCO1490	GGTCAACAAATCATAAAGATATTGG	708	Folmer, 1994
HC02198	TAAACTTCAGGGTGACCAAAAAATCA		
FishF1	TCAACCAACCACAAAGACATTGGCAC	703/706	Ward, 2005
FishF2	TCGACTAATCATAAAGATATCGGCAC		
FishR1	TAGACTTCTGGGTGGCCAAAGAATCA		
FishR2	ACTTCAGGGTGACCGAAGAATCAGAA		
COIF-ALT	ACAAATCAYAARGAYATYGG	698	Mikkelsen, 2006
COIR-ALT	TTCAGGRTGNCCRAARAAYCA		
FF2d	TTCTCCACCAACCACAARGAYATYGG	707	Ivanova, 2007
FR1d	CACCTCAGGGTGTCCGAARAAYCARAA		
FISH-BCL	TCAACYAATCAYAAAGATATYGGCAC	706	Baldwin, 2009
FISH-BCH	TAAACTTCAGGGTGACCAAAAAATCA		
COI-Fish-F	TTCTCAACTAACCAYAAAGAYATYGG	709	Kochzius, 2010
COI-Fish-R	TAGACTTCTGGGTGGCCRAARAAYCA		
FISHCOILBC_ts	**CACGACGTTGTAAAACGA**CTCAACYAATCAYAAAGATATYGGCAC	705	Handy, 2011
FISHCOIHBC_ts	**GGATAACAATTTCACACAGG**ACTTCYGGGTGRCCRAARAATCA		
SPACOIREV	**GGATAACAATTTCACACAGG**ACTTCYGGGTGNCCRAARAATCA	705*	This study
REVshort1	**GGATAACAATTTCACACAGG**GGYATNACTATRAAGAAAATTATTAC	192*	This study

Table 4SM. Universal primers for the amplification of the *COI* gene from fish (Armani et al, 2012c with modification). * The length refers to the amplicon generated using the forward FISHCOILBC_ts

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Species name (morphological identification)	BOLD	NCBI	COI fragment (bp)	Species identification (BLAST)			
				BOLD Species Level Barcode Records	Max identity	GenBank	Max identity
Acanthopagrus australis	SPA239-14.COI-5P	Still waiting	655	***A. australis***	**100**	***A. australis***	**100**
			139	***A. australis***	**100**	***A. australis***	**100**
Acanthopagrus berda	SPA202-13.COI-5P	Still waiting	655	***A. berda***	**100**	***A. berda***	**98**
	SPA003-13.COI-5P	KJ012251	139	***A. berda*** ***A. pacificus***	**100** **98.55**	***A. berda***	**99**
	SPA002-13.COI-5P	KJ012252					
	SPA004-13.COI-5P	KJ012253					
	SPA203-13.COI-5P	KJ012254					
Acanthopagrus butcheri	SPA208-13.COI-5P	KJ012255	655; 653	***A. butcheri***	**100**	***A. butcheri***	**100**
	SPA207-13.COI-5P	KJ012256	139	***A. butcheri***	**100**	***A. butcheri*** ***A. schlegelii*** ***A. berda***	**100** **99** **98**
	SPA206-13.COI-5P	KJ012257					
	SPA205-13.COI-5P	KJ012258					
	SPA211-13.COI-5P	KJ012259					
	SPA210-13.COI-5P	KJ012260					
	SPA209-13.COI-5P	KJ012261					
	SPA240-14.COI-5P	Still waiting					
Acanthopagrus latus	SPA006-13.COI-5P	KJ012262	655	***A. latus***	**100**	***A. latus***	**99-100**
	SPA005-13.COI-5P	KJ012263	139	***A. latus***	**100**	***A. latus***	**100**
	SPA008-13.COI-5P	KJ012264					
	SPA007-13.COI-5P	KJ012265					
	SPA009-13.COI-5P	KJ012266					
	SPA010-13.COI-5P	KJ012267					
Acanthopagrus pacificus[b*]	--	HG937802	139	***A. pacificus*** ***A. berda***	**100** **100**	***A. berda***	**99**
	SPA189-13.COI-5P	KJ012269	583	***A. pacificus*** *A. berda* (2 seq.)	**99.83** 99.83	***A. berda***	**97**
	SPA022-13.COI-5P	KJ012268	655	***A. pacificus*** *A. berda* (2 seq.)	**100** 100	***A. berda***	**97**
			139	***A. pacificus*** ***A. berda***	**100** **100**	***A. berda***	**99**
Acanthopagrus palmaris[a]	SPA242-14.COI-5P	Still waiting	655	***A. berda***	**98.05**	***A. berda***	**98**
			139	***A. pacificus*** ***A. berda***	**100** **99.28**	***A. berda***	**99**
*Acanthopagrus schlegelii**	SPA024-13.COI-5P	KJ012273	655	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **99.85**	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **99**

			139	***A. schlegelii*** ***A. schlegelii schlegelii*** ***A. butcheri***	**100** **99.28** **98.55**	***A. schlegelii*** ***A. schlegelii schlegelii*** ***A. butcheri***	**100** **100** **99**
	SPA029-13.COI-5P SPA027-13.COI-5P SPA025-13.COI-5P SPA028-13.COI-5P	KJ012270 KJ012271 KJ012272 KJ012274	655	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **100**	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **100**
			139	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **100**	***A. schlegelii*** ***A. schlegelii schlegelii*** ***A. butcheri***	**100** **100** **98**
	--	HG937803	139	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **100**	***A. schlegelii*** ***A. schlegelii schlegelii*** ***A. butcheri***	**100** **100** **98**
Acanthopagrus sivicolus[a*]	SPA032-13.COI-5P SPA031-13.COI-5P SPA030-13.COI-5P	KJ012275 KJ012276 KJ012277	655	***A. schlegelii*** ***A. schlegelii schlegelii***	**99.85-100** **99.85-100**	***A. schlegelii*** ***A. schlegelii schlegelii***	**99-100** **99-100**
			139	***A. schlegelii*** ***A. schlegelii schlegelii***	**100** **100**	***A. schlegelii;*** ***A. schlegelii schlegelii***	**100** **100**
Archosargus probatocephalus	SPA011-13.COI-5P SPA012-13.COI-5P SPA013-13.COI-5P SPA014-13.COI-5P	KJ012278 KJ012279 KJ012280 KJ012281	655	***A. probatocephalus***	**100**	***A. probatocephalus***	**99-100**
			132-139	***A. probatocephalus***	**100**	***A. probatocephalus***	**100**
	--	HG937804	138	***A. probatocephalus***	**100**	***A. probatocephalus***	**100**
Archosargus rhomboidalis	SPA023-13.COI-5P	KJ012282	655	***A. rhomboidalis***	**100**	***A. rhomboidalis***	**100**
			139	***A. rhomboidalis***	**100**	***A. rhomboidalis***	**100**
Argyrops bleekeri[b]	SPA016-13.COI-5P SPA018-13.COI-5P SPA204-13.COI-5P SPA017-13.COI-5P	KJ012283 KJ012284 KJ012285 KJ012286	655	***A. bleekeri*** ***A. spinifer***	**100** **99.38-99.69**	***A. spinifer***	**99; 100**
			139	***A. bleekeri*** ***A. spinifer***	**100** **99.85-100**	***A. spinifer***	**99; 100**
Argyrops filamentosus	SPA019-13.COI-5P SPA020-13.COI-5P	KJ012287 KJ012288	655	***A. filamentosus***	**100**	***A. filamentosus***	**100**
			139	***A. filamentosus***	**100**	***A. filamentosus***	**100**
Argyrops spinifer			645-655	***A. spinifer***	**100**	***A. spinifer***	**100**
	SPA035-13.COI-5P SPA033-13.COI-5P SPA034-13.COI-5P	KJ012289 KJ012290 KJ012293	139	***A. spinifer*** ***A .blekeeri***	**100** **98.72**	***A. spinifer*** ***P. major*** ***E. japonica*** ***E. cardinalis*** ***P. edita*** ***P. auratus***	**100** **98** **98** **98** **98** **98**
			655	***A. spinifer***	**99.69; 99.85**	***A. filamentosus***	**96**
	SPA191-13.COI-5P SPA190-13.COI-5P	KJ012291 KJ012292	139	***A. spinifer*** ***A. blekeeri***	**99.28** **98.55**	***A. spinifer*** ***P. dentata*** ***A. filamentosus***	**99** **98** **98**

*Argyrozona argyrozona**	SPA236-14.COI-5P SPA237-14.COI-5P SPA238-14.COI-5P	Still waiting Still waiting Still waiting	655 139	***A. argyrozona*** ***A. argyrozona***	**99.84; 100** **100**	***A. argyrozona*** ***A. argyrozona***	**99; 100** **100**
Boops boops	SPA036-13.COI-5P SPA119-13.COI-5P SPA037-13.COI-5P SPA038-13.COI-5P	KJ012294 KJ012295 KJ012296 KJ012297	654-655 139	***B. boops*** *O. melanura* (2 seq.) ***B. boops*** *O. melanura* (2 seq.)	**100** 99.67-99.84 **100** 100	***B. boops*** *O. melanura* (2 seq.) ***B. boops***	**100** 99 **100**
Calamus arctifrons[a]	SPA041-13.COI-5P SPA039-13.COI-5P SPA232-13.COI-5P -- --	KJ012298 KJ012299 KJ012300 HG937805 --	655 139 139 --	**No match** **No match** **No match** --		***C. brachysomus*** ***C. penna*** ***C. brachysomus*** ***C. penna*** ***C. calamus*** ***C. brachysomus*** ***C. penna*** ***C. calamus***	**93** **93** **96** **96** **96** **96** **96** **96**
Calamus bajonado[b]	SPA043-13.COI-5P SPA042-13.COI-5P SPA044-13.COI-5P	KJ012301 KJ012302 KJ012303	655 139	***C. bajonado*** ***C. bajonado***	**99.54** **99.28**	***Calamus* sp.** ***Calamus* sp.**	**99** **99**
Calamus brachysomus	SPA045-13.COI-5P SPA243-13.COI-5P	KJ012304 Still waiting	655; 654 139	***C. brachysomus*** ***C. brachysomus*** ***C. nodosus*** ***C. leucosteus*** ***C. calamus*** ***C. penna***	**100** **100** **100** **99.28** **99.22** **98.55**	***C. brachysomus*** ***C. brachysomus*** ***C. calamus*** ***C. penna*** ***Calamus* sp.**	**100** **100** **99** **99** **99**
Calamus calamus	SPA046-13.COI-5P SPA047-13.COI-5P SPA048-13.COI-5P	KJ012305 KJ012306 KJ012307	655 139	***C. calamus*** ***C. calamus*** ***C. nodosus*** *C. brachysomus* (1seq.)	**100** **100** **99.28** 100	***C. calamus*** ***C. calamus*** ***Calamus* sp.** ***C. brachysomus***	**100** **100** **100** **99**
Calamus leucosteus[b*]	SPA049-13.COI-5P SPA050-13.COI-5P SPA051-13.COI-5P	KJ012308 KJ012309 KJ012310	655 139	***C. leucosteus*** ***C. leucosteus*** ***C. brachysomus*** ***C. nodosus*** ***C. calamus***	**100** **100** **100** **99.15** **98.45**	***C. brachysomus*** ***C. brachysomus*** ***C. penna*** ***C. calamus***	**94** **99** **98** **98**
Calamus nodosus[b]	SPA235-14.COI-5P SPA056-13.COI-5P SPA055-13.COI-5P SPA054-13.COI-5P	Still waiting KJ012311 KJ012312 KJ012313	655 139	***C. nodosus*** ***C. nodosus*** ***C. brachysomus*** ***C. calamus***	**100** **100** **100** **99.28**	***Actinopterygii* spp.** ***Calamus* spp.** ***C. calamus*** ***C. calamus*** ***Calamus* spp.** ***C. brachysomus***	**99** **98** **98** **99** **99** **99**

				C. leucosteus	**98.55**	***C. penna***	**98**
	--	HG937806	139	***C. nodosus*** ***C. brachysomus*** ***C. calamus*** ***C. leucosteus***	**100** **100** **99.28** **98.55**	***C. calamus*** ***Calamus* spp.** ***C. brachysomus*** ***C. penna***	**99** **99** **99** **98**
Calamus penna	--	HG937807	139	***C. penna***	**99.28**	***C. penna*** ***C. brachysomus***	**99** **98**
	SPA053-13.COI-5P	KJ012314	655	***C. penna***	**99.54**	***C. penna***	**99**
			139	***C. penna***	**99.28**	***C. penna*** ***C. brachysomus***	**99** **98**
***Calamus proridens*[a]**	SPA058-13.COI-5P	KJ012315	655	***C. leucosteus***	**99.23**	***Actinopterygii* spp.**	**97**
			139	***C. leucosteus*** ***C. pennatula***	**100** **100**	***Actinopterygii* spp.** ***Calamus* sp.**	**100** **98**
	--	HG937808	139	***C. leucosteus*** ***C. pennatula***	**100**	***Actinopterygii* spp.** ***Calamus* sp.**	**100** **98**
Cheimerius nufar	SPA062-13.COI-5P SPA060-13.COI-5P SPA064-13.COI-5P SPA061-13.COI-5P	KJ012316 KJ012317 KJ012318 KJ012319	655	***C. nufar*** *P. pagrus* (1 seq.)	**100** 98.92	***C. nufar***	**100**
			139	***C. nufar*** *P. pagrus* (1 seq.)	**100** 100	***C. nufar***	**100**
	--	HG937809	139	***C. nufar*** *P. pagrus* (1 seq.)	**100** 100	***C. nufar***	**100**
*Chrysoblephus cristiceps**	SPA259-14.COI-5P SPA260-14.COI-5P SPA261-14.COI-5P	Still waiting Still waiting Still waiting	655	***C. cristiceps***	**100**	***C. cristiceps***	**99**
			139	***C. cristiceps*** ***P. dentata*** ***C. laticeps***	**100** **99.26** **98.89**	***C. cristiceps*** ***P. dentata*** ***C. laticeps***	**100** **99** **98**
Chrysoblephus gibbiceps	SPA244-14.COI-5P	Still waiting	655	***C. gibbiceps***	**99.53**	***C. gibbiceps***	**99**
			139	***C. gibbiceps***	**98.55**	***C. gibbiceps***	**99**
*Chrysoblephus laticeps**	SPA255-14.COI-5P SPA256-14.COI-5P SPA257-14.COI-5P	Still waiting Still waiting Still waiting	655	***C. laticeps***	**100**	***C. laticeps***	**100**
			139	***C. laticeps*** ***C. cristiceps***	**100** **98.89**	***C. laticeps*** ***C. cristiceps***	**100** **98**
*Chrysoblephus puniceus**	SPA065-13.COI-5P	KJ012320	655	***C. puniceus***	**100**	***C. puniceus***	**99**
			139	***C. puniceus***	**100**	***C. puniceus***	**100**
*Crenidens crenidens**	SPA258-14.COI-5P	Still waiting	655	***C. crenidens***	**98.73**	***C. crenidens***	**98**
			139	***C. crenidens***	**100**	***C. crenidens***	**98**
***Dentex angolensis*[a]**	SPA067-13.COI-5P SPA192-13.COI-5P SPA193-13.COI-5P	KJ012321 KJ012323 KJ012324	655	***D. macrophthalmus***	**99.84**	***D. macrophthalmus***	**98**
			139	***D. macrophthalmus***	**99.28**	***D. macrophthalmus***	**99**
	--	HG937810	139	***D. macrophthalmus*** ***Spicara alta***	**100** **98.55**	***D. macrophthalmus***	**99**

Dentex canariensis[a]	SPA120-13.COI-5P	KJ012325	655	**No match**		***D. macrophthalmus***	**95**
			139	**No match**		***D. macrophthalmus***	**99**
Dentex dentex	SPA123-13.COI-5P SPA194-13.COI-5P SPA124-13.COI-5P SPA126-13.COI-5P	KJ012326 KJ012327 KJ012328 KJ012329	655	***D. dentex***	**98.85; 100**	***D. dentex***	**99**
			139	***D. dentex***	**99.28; 100**	***D. dentex***	**99**
	--	HG937811	139	***D. dentex***	**99.22**	***D. dentex***	**99**
Dentex gibbosus[a]	SPA222-13.COI-5P	KJ012330	655	**No match**		***P. caerulosticus***	**93**
			139	***P. acarne*** ***V. acromegalus***	**98.55** **98.55**	***C. nufar***	**98**
Dentex macrophthalmus	SPA069-13.COI-5P SPA071-13.COI-5P SPA070-13.COI-5P	KJ012331 KJ012332 KJ012333	655	***D. macrophthalmus***	**99.69; 99.84**	***D. macrophthalmus***	**98**
			139	***D. macrophthalmus***	**100**	***D. macrophthalmus***	**96**
Dentex maroccanus[a]	SPA132-13.COI-5P SPA131-13.COI-5P	KJ012334 KJ012335	655	***D. macrophthalmus***	**99.84; 100**	***D. macrophthalmus***	**98**
			139	***D. macrophthalmus*** ***Spicara alta***	**100** **98.55**	***D. macrophthalmus***	**100**
Dentex spariformis[b]	SPA253-14.COI-5P	Still waiting	563	***D. spariformis***	**100**	***D. tumifrons***	**94**
			139	***D. spariformis***	**100**	***D. tumifrons***	**95**
Diplodus annularis	SPA076-13.COI-5P SPA078-13.COI-5P SPA196-13.COI-5P SPA195-13.COI-5P SPA077-13.COI-5P	KJ012336 KJ012337 KJ012338 KJ012339 KJ012340	562-655	***D. annularis***	**99.53-100**	***D. annularis***	**99**
			139	***D. annularis***	**100**	***D. annularis***	**99; 100**
Diplodus cervinus	--	HG937812 HG937813	139	***D. cervinus*** ***D. cervinus hottentotus*** ***D. fasciatus*** ***D. sargus*** ***D. sargus* subspecies** ***D. bellottii*** ***D. vulgaris***	**100** **100** **100** **99.26** **98.55-99.17** **98.55** **98.41**	***D. cervinus*** ***D. sargus*** ***D. argenteus*** ***D. holbrookii***	**100** **99** **98** **98**
Diplodus cervinus hottentotus	SPA130-13.COI-5P SPA129-13.COI-5P	KJ012341 KJ012342	655	***D. cervinus*** ***D. fasciatus*** ***D. cervinus hottentotus***	**100** **99.54; 99.69** **99.54**	***D. cervinus***	**99; 100**
			139	***D. cervinus hottentotus*** ***D. cervinus*** ***D. fasciatus*** ***D. sargus* subspecies** ***D. bellotti*** ***D. vulgaris***	**100** **100** **100** **98.55-99.26** **98.5598.55** **98.41**	***D. cervinus*** ***D. sargus*** ***D. argenteus*** ***D. holbrookii***	**100** **99** **98** **98**

Diplodus holbrookii	SPA128-13.COI-5P SPA127-13.COI-5P	KJ012343 KJ012344	655	***D. holbrookii*** *Haemulon aurolineatum* ***D. argenteus***	**99.02; 98.86** 98.46; 98.77 **98.62; 98.46**	***D. holbrookii*** ***D. argenteus*** ***D. sargus***	**99** **98; 99** **98**
			139	***D. holbrookii*** *Haemulon aurolineatum* ***D. sargus* subspecies** ***D. argenteus*** ***D. bellottii***	**99.28** 99.28 **98.55-99.28** **99.28** **98.55**	***D. holbrookii*** ***D. argenteus*** ***D. sargus***	**99** **99** **99**
***Diplodus noct*[a]**	SPA133-13.COI-5P SPA134-13.COI-5P SPA254-14.COI-5P	KJ012345 KJ012346 Still waiting	655	***D. sargus* subspecies** ***D. holbrookii*** ***D. argenteus***	**98.62-99.69** **98.46** **98.16**	***D. sargus* subspecies** ***D. holbrookii*** ***D. argenteus***	**99** **98** **98**
			139	***D. sargus* subspecies** ***D. bellottii*** ***D. holbrookii*** ***D. argenteus*** ***D. cervinus*** ***D. cervinus hottentotus*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.28** **99.28** **99.05** **98.55** **98.55** **98.55**	***D. sargus*** ***D. holbrookii*** ***D. argenteus*** ***D. cervinus***	**100** **99** **99** **99**
Diplodus puntazzo	SPA108-13.COI-5P SPA111-13.COI-5P SPA110-13.COI-5P SPA009-13.COI-5P	KJ012347 KJ012348 KJ012349 KJ012350	655	***D. puntazzo***	**98.52-98.92**	*Dicentrarchus labrax* ***D. puntazzo***	99 **99**
			139	***D. vulgaris*** ***D. puntazzo*** ***O. melanura*** ***D. sargus***	**100** **99.28** **99.21** **98.55**	***D. sargus*** *Dicentrarchus labrax*	**99** 98
	--	HG937814	139	***D. vulgaris*** ***D. puntazzo*** ***O. melanura*** ***D. sargus***	**100** **99.28** **99.21** **98.55**	***D. sargus*** *Dicentrarchus labrax*	**99** 98
Diplodus sargus (sargus)	SPA114-13.COI-5P SPA113-13.COI-5P SPA117-13.COI-5P SPA116-13.COI-5P	KJ012351 KJ012352 KJ012353 KJ012354	655	***D. sargus* subspecies**	**98.46-100**	***D. sargus* subspecies** ***D. holbrookii*** ***D. argenteus***	**99-100** **98** **98**
			139	***D. sargus* subspecies** ***D. bellottii*** ***D. holbrookii*** ***D. argenteus*** ***D. cervinus*** ***D. cervinus hottentotus*** ***D. fasciatus***	**100** **100** **99.28** **99.28** **99.05** **98.55** **98.55**	***D. sargus*** ***D. argenteus*** ***D. holbrookii*** ***D. cervinus***	**100** **99** **99** **99**

				O. melanura	**98.41**		
	--	HG937815 HG937816	139	***D. sargus* subspecies** ***D. bellottii*** ***D. holbrookii*** ***D. argenteus*** ***D. cervinus*** ***D. cervinus hottentotus*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.28** **99.28** **99.05** **98.55** **98.55** **98.41**	***D. sargus*** ***D. argenteus*** ***D. holbrookii*** ***D. cervinus***	**100** **99** **99** **99**
Diplodus vulgaris	SPA138-13.COI-5P SPA140-13.COI-5P SPA135-13.COI-5P SPA136-13.COI-5P SPA139-13.COI-5P	KJ012355 KJ012356 KJ012357 KJ012358 KJ012360	655	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. fasciatus***	**99.84; 100** **99.69; 100** **98.73; 98.92** **98.62; 98.92**	***D. sargus*** ***D. vulgaris***	**99; 100** **99**
			139	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. puntazzo*** ***D. fasciatus*** ***O. melanura***	**100** **100** **99.28** **99.28** **99.28** **98.41**	***D. sargus***	**100**
	SPA137-13.COI-5P	KJ012359	655	***D. sargus*** ***D. vulgaris***	**99.37** **99.23**	***D. sargus*** ***D. vulgaris***	**99** **99**
			139	***D. vulgaris*** ***D. sargus*** ***D. prayensis*** ***D. puntazzo*** ***D. fasciatus***	**100** **99.28** **98.55** **98.55** **98.55**	***D. sargus***	**99**
*Evynnis cardinalis**	--	HG937817 HG937818 HG937819	139	***E. cardinalis*** ***E. tumifrons*** ***P. edita*** ***P. major*** ***P. auratus*** ***A. spinifer***	**100** **100** **100** **98.55** **98.55** **98.55**	***E. cardinalis*** ***E. japonica*** ***P. edita*** ***P. major*** ***P. auratus*** ***A. spinifer***	**100** **100** **100** **99** **99** **98**
	SPA144-13.COI-5P SPA145-13.COI-5P	KJ012361 KJ012362	655	***E. cardinalis*** ***E. tumifrons*** ***P. edita***	**100** **100** **99.69**	***E. cardinalis*** *E. japonica* (1 seq.) ***P. edita***	**100** 99 **99**
			139	***E. cardinalis*** ***E. tumifrons*** ***P. edita*** ***P. major***	**100** **100** **100** **98.55**	***E. cardinalis*** ***E. japonica*** ***P. edita*** ***P. major***	**100** **100** **100** **99**

				Pagrus auratus ***A. spinifer***	**98.55** **98.55**	***P. auratus*** ***A. spinifer***	**99** **98**
Evynnis tumifrons	SPA146-13.COI-5P	KJ012364	583	***E. tumifrons***	**100**	***E. tumifrons***	**99**
			67	<80		***E. tumifrons*** ***D. macrophthalmus***	**100** **98**
	SPA150-13.COI-5P SPA147-13.COI-5P	KJ012363 KJ012365	655	***E. tumifrons***	**99.69; 100**	***Dentex tumifrons* (syn. *E. tumifrons*)**	**99**
	SPA148-13.COI-5P SPA149-13.COI-5P	KJ012366 KJ012367	139	***E. tumifrons*** ***D. spariformis***	**100** **98.55**	***E. tumifrons***	**100**
Lagodon rhomboides	SPA155-13.COI-5P	KJ012368	655	***L. rhomboides***	**100**	***L. rhomboides***	**100**
	SPA152-13.COI-5P SPA153-13.COI-5P	KJ012370 KJ012371	139	***L. rhomboides***	**100**	***L. rhomboides***	**100**
	SPA156-13.COI-5P SPA154-13.COI-5P	KJ012369 KJ012372	520; 540	***L. rhomboides***	**100**	***L. rhomboides***	**100**
Lithognathus mormyrus	SPA221-13.COI-5P	KJ012373	655	***L. mormyrus***	**99.65-100**	***L. mormyrus***	**99;100**
	SPA220-13.COI-5P SPA079-13.COI-5P SPA151-13.COI-5P SPA197-13.COI-5P SPA219-13.COI-5P	KJ012374 KJ012375 KJ012376 KJ012377 KJ012378	139	***L. mormyrus***	**100**	***L. mormyrus***	**100**
Oblada melanura	SPA157-13.COI-5P	KJ012379	655	***O. melanura***	**99.52**	***O. melanura***	**95**
			139	***O. melanura*** ***D. sargus* subspecies** ***D. vulgaris*** ***D. bellottii*** ***D. puntazzo***	**100** **98.55-99.28** **99.21** **98.55** **98.55**	***D. sargus*** ***D. argenteus*** ***D. holbrookii***	**99** **98** **98**
	--	HG937820 HG937821 HG937822 HG937823 HG937824	139	***O. melanura*** ***D. sargus* subspecies** ***D. vulgaris*** ***D. bellottii*** ***D. puntazzo***	**100** **98.55-99.28** **99.21** **98.55** **98.55**	***D. sargus*** ***D. argenteus*** ***D. holbrookii***	**99** **98** **98**
	--	HG816028	139	***O. melanura*** ***D. capensis*** ***D. vulgaris***	**99.28** **98.55** **98.41**	***D. sargus***	**98**
	SPA198-13.COI-5P	KJ012380	655	***O. melanura***	**99.69**	***O. melanura***	**95**
			139	***O. melanura*** ***D. vulgaris***	**99.21** **98.41**	***O. melanura*** ***D. sargus***	**97** **97**
*Pachymetopon aeneum**	SPA250-14.COI-5P SPA251-14.COI-5P	Still waiting Still waiting	655	***P. aeneum***	**100**	*Paracaesio sordida* (1 seq.) ***P. aeneum***	100 **99**

	SPA252-14.COI-5P	Still waiting	139	***P. aeneum***	**100**	***P. aeneum*** *P. sordida* (1 seq.)	**100** 100
Pagellus acarne	SPA159-13.COI-5P SPA082-13.COI-5P SPA080-13.COI-5P	KJ012382 KJ012383 KJ012385	601; 655	***P. acarne*** *Oblada melanura* (1 seq.)	**100** 98.84; 99.3	***P. acarne*** *Oblada melanura* (2 seq.)	**99** **99**
			139	***P. acarne*** *Oblada melanura* (1 seq.)	**100** 100	***P. acarne***	**99; 100**
	SPA083-13.COI-5P SPA081-13.COI-5P	KJ012381 KJ012384	655	***P. acarne*** *O. melanura* (1 seq.)	**99.45; 100** 99.31; 100	***P. acarne***	**99; 100**
			66; 69	<80 bp		***P. acarne***	**100**
Pagellus bellottii	SPA162-13.COI-5P	KJ012386	655	***P. bellotii*** ***P. pagrus*** ***P. natalensis***	**99.84** **99.53** **99.21**	***P. bellotii*** ***P. natalensis***	**99** **99**
			139	***P. bellotii*** ***P. pagrus***	**100** **98.55**	***P. bellotii*** ***P. natalensis***	**100** **98**
Pagellus bogaraveo	SPA225-13.COI-5P	KJ012387	655	***P. bogaraveo***	**99.85; 100**	***P. bogaraveo***	**99; 100**
	SPA166-13.COI-5P SPA223-13.COI-5P SPA164-13.COI-5P SPA224-13.COI-5P SPA163-13.COI-5P	KJ012388 KJ012390 KJ012391 KJ012392 KJ012393	139	***P. bogaraveo***	**100**	***P. bogaraveo***	**100**
	SPA165-13.COI-5P	KJ012389	557	***P. bogaraveo***	**99.82**	***P. bogaraveo***	**99**
Pagellus erythrinus	--	HG937825	139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
	SPA176-13.COI-5P SPA177-13.COI-5P	KJ012394 KJ012395	655	***P. erythrinus*** *O. melanura* (2 seq.)	**99.85; 100** **99.19; 99.35**	***P. erythrinus*** *O. melanura* (2 seq.)	**99; 100** **99**
	SPA174-13.COI-5P SPA175-13.COI-5P	KJ012396 KJ012397	139	***P. erythrinus***	**100**	***P. erythrinus***	**100**
Pagrus africanus[a]	--	HG937826	139	***P. edita***	**98.1**	***P. major*** ***E. japonica*** ***E. cardinalis*** ***P. edita*** ***C. auratus***	**98**
Pagrus auratus	SPA212-13.COI-5P SPA218-13.COI-5P	KJ012398 KJ012399	655	***P. auratus*** ***P. major***	**99.85; 100** **99.54**	***P. auratus*** ***P. major***	**99; 100** **99**
	SPA217-13.COI-5P SPA216-13.COI-5P SPA215-13.COI-5P SPA213-13.COI-5P SPA214-13.COI-5P	KJ012400 KJ012401 KJ012402 KJ012403 KJ012404	139	***P. auratus*** ***P. major*** ***E. cardinalis*** ***E. tumifrons*** ***P.edita***	**100** **100** **98.89** **98.55** **98.55**	***P. major*** ***P. auratus*** ***E. japonica*** ***E. cardinalis*** ***E. tumifrons***	**100** **100** **99** **99** **99**

				A. spinifer	**98.55**	***P. edita*** ***A. spinifer***	**99** **98**
Pagrus auriga	SPA161-13.COI-5P	KJ012405	655	***P. auriga***	**99.85**	***P. auriga***	**99**
	SPA226-13.COI-5P	KJ012406	139	***P. auriga***	**100**	***P. auriga***	**100**
Pagrus caeruleostictus	SPA167-13.COI-5P SPA171-13.COI-5P SPA172-13.COI-5P SPA168-13.COI-5P SPA170-13.COI-5P	KJ012407 KJ012408 KJ012409 KJ012410 KJ012411	655	***P. caeruleostictus***	**99.82; 99.83; 99.84; 100**	***P. caeruleostictus***	**99; 100**
			139	***P. caeruleostictus***	**99.07; 100**	***P. caeruleostictus***	**99; 100**
	--	HG937827	139	***P. caeruleostictus***	**100**	***P. caeruleostictus***	**99**
Pagrus major	SPA181-13.COI-5P SPA183-13.COI-5P SPA178-13.COI-5P SPA179-13.COI-5P SPA182-13.COI-5P	KJ012412 KJ012413 KJ012414 KJ012415 KJ012416	655	***P. major*** ***P. auratus***	**100** **100**	***P. major*** ***P. auratus***	**99; 100** **99**
			139	***P. major*** ***P. auratus*** ***E. cardinalis*** ***E. tumifrons*** ***P. edita*** ***A. spinifer***	**100** **100** **98.89** **98.55** **98.55** **98.55**	***P. major*** ***P. auratus*** ***E. japonica*** ***E. cardinalis*** ***P. edita*** ***A. spinifer***	**100** **100** **99** **99** **99** **98**
	--	Still waiting	139	***P. major*** ***P. auratus*** ***E. cardinalis*** ***E. tumifrons*** ***P. edita*** ***A. spinifer***	**100** **100** **98.85** **98.52** **98.52** **98.52**	***P. major*** ***C. auratus*** ***E. japonica*** ***E. cardinalis*** ***P. edita*** ***A. spinifer***	**100** **100** **99** **99** **99** **98**
Pagrus pagrus	--	HG937828 HG937829 HG937830	139	***P. erythrinus*** ***O. melanura*** ***P. pagrus***	**100** **100** **99.28**	***P. pagrus*** *P. auratus* (2 seq.)	**99** 99
	SPA101-13.COI-5P SPA102-13.COI-5P SPA103-13.COI-5P SPA104-13.COI-5P SPA106-13.COI-5P	KJ012417 KJ012418 KJ012419 KJ012420 KJ012421	655	***P. pagrus*** *O. melanura*	**99.84; 100** 99.84; 100	*O. melanura* ***P. auratus*** ***P. pagrus***	100 **99** **99**
			139	***P. pagrus*** ***P. erythrinus*** ***O. melanura***	**100** **100** **100**	***P. pagrus*** ***P. auratus*** ***E. japonica*** ***E. cardinalis*** ***P. edita*** ***A .spinifer***	**100** **100** **99** **99** **99** **98**
Pterogymnus laniarius	SPA247-14.COI-5P	Still waiting	643; 655	***P. lanarius***	**99.69-100**	***P. lanarius***	**99**
	SPA248-14.COI-5P SPA249-14.COI-5P	Still waiting Still waiting	139	***P. lanarius***	**100**	***P. lanarius***	**99**

Rhabdosargus haffara	SPA227-13.COI-5P	KJ012422	655	***R. haffara*** *S. aurata* (1 seq.)	**99.82** 99.85	***R. haffara***	**99**
			139	***R. haffara*** *S. aurata* (1 seq.)	**100** 100	***R. sarba***	**96**
*Rhabdosargus holubi**	SPA246-14.COI-5P	Still waiting	652	***R. holubi***	**100**	***R. holubi***	**99**
			139	***R. holubi***	**99.28**	***R. holubi***	**99**
Rhabdosargus sarba	--	HG937831 HG937832	139	***R. sarba*** ***R. haffara*** ***R. globiceps***	**100** **100** **98.85**	***R. sarba*** ***R. globiceps*** ***A. berda***	**100** **98** **98**
	SPA186-13.COI-5P SPA233-13.COI-5P	KJ012423 KJ012424	655	***R. sarba***	**99.85; 100**	***R. sarba***	**99; 100**
			139	***R. sarba*** ***R. haffara*** ***R. globiceps***	**100** **100** **98.85**	***R. sarba*** ***R. globiceps*** ***A. berda***	**100** **98** **98**
	SPA245-14.COI-5P	Still waiting	655	***R. sarba***	**100**	***R. sarba***	**100**
			139	***R. sarba***	**100**	***R. sarba***	**100**
Sarpa salpa	SPA085-13.COI-5P SPA084-13.COI-5P SPA199-13.COI-5P SPA087-13.COI-5P SPA086-13.COI-5P	KJ012425 KJ012426 KJ012427 KJ012428 KJ012429	655	***S. salpa***	**99.85; 100**	***S. salpa***	**99**
			139	***S. salpa***	**99.85; 100**	***S. salpa***	**99; 100**
Sparus aurata	SPA074-13.COI-5P SPA200-13.COI-5P SPA072-13.COI-5P SPA075-13.COI-5P SPA073-13.COI-5P	KJ012430 KJ012431 KJ012432 KJ012433 KJ012434	655	***S. aurata***	**100**	***S. aurata***	**100**
			139	***S. aurata***	**100**	***S. aurata***	**100**
Spondyliosoma cantharus	SPA099-13.COI-5P SPA201-13.COI-5P SPA097-13.COI-5P	KJ012435 KJ012436 KJ012438	569; 643; 655	***S. cantharus***	**100**	***S. cantharus***	**99**
			139	***S. cantharus*** ***S. emarginatum***	**100** **99.28; 98.55**	***S. cantharus*** ***S. emarginatum***	**99** **98; 99**
	SPA096-13.COI-5P	KJ012439	655	***S. cantharus***	**100**	***S. cantharus*** ***S. emarginatum***	**100** **98**
			139	***S. cantharus*** ***S. emarginatum***	**100** **99.28**	***S. cantharus*** ***S. emarginatum***	**100** **99**
	SPA098-13.COI-5P	KJ012437	547	***S. cantharus***	**100**	***S. cantharus***	**99**
			69	<80 bp		***S. cantharus*** ***S. emarginatum***	**100** **99**
Stenotomus caprinus[b]	SPA090-13.COI-5P SPA089-13.COI-5P SPA088-13.COI-5P	KJ012440 KJ012441 KJ012442	655	***S. caprinus*** ***S. chrysops***	**99.69- 100** **99.85; 100**	***S. chrysops*** *C. penna* (1 seq.)	**99; 100** 99
			139	***S. caprinus*** ***S. chrysops***	**100** **100**	***S. chrysops*** *C. penna* (1 seq.)	**100** **100**

Stenotomus chrysops	SPA095-13.COI-5P SPA234-13.COI-5P SPA091-13.COI-5P SPA092-13.COI-5P SPA093-13.COI-5P SPA094-13.COI-5P	KJ012443 KJ012444 KJ012445 KJ012446 KJ012447 KJ012448	655	***S. chrysops*** ***S. caprinus***	**100** **99.69**	*C. penna* (1 seq.) ***S. chrysops***	100 **99**
			139	***S. chrysops*** ***S. caprinus***	**100** **100**	***S. chrysops*** *C. penna* (1 seq.)	**100** 100
Viridiidentex acromegalus[b*]	SPA187-13.COI-5P	KJ012449	655	***V. acromegalus*** ***P. acarne***	**100** **100**	***P. auriga***	**92**
			139	***V. acromegalus*** ***P. acarne***	**100** **100**	***Porcostoma dentata*** ***A. spinifer***	**97** **97**
	--	HG937833	139	***V. acromegalus*** ***P. acarne***	**100** **100**	***Porcostoma dentata*** ***A. spinifer***	**97** **97**

Table 5SM: The results of the IDs analysis on BOLD and of the BLAST analysis on GenBank for the full and for the mini DNA barcode. The BOLD codes and the NCBI access number are reported when available (no code is assigned in BOLD to sequences <200bp). The species not reported in **bold type** have been considered originating from incorrectly identified or mislabeled specimens. When two or more values of MI are reported they are referred to a range (if separated by a -) or to different MI retrieved (if separated by a semicolon ;). [a] No sequences were available for this species in consulted databases; [b]No sequences were available for this species in Genbank database;*Species not considered in the International Official Trade lists; *D. sargus* subspecies: *D. sargus ascensionis, D. sargus capensis, D. sargus helenae; D. sargus kotschyi, D. sargus lineatus, D. sargus sargus.*

Problematic sequences	Misidentified with	Possible explanation
Boops boops, *Pagellus acarne*, *Pagellus erythrinus*, and *Pagrus pagrus*	*O. melanura*	The probable misidentification of these sequences was already supposed by Keskin & Atar, (2013). However, a 7% mean genetic distance between our sequences and those of Keskin & Atar, (2013) highlights a remarkable intraspecific variation within the specimens of O. melanura. The reliability of our morphological identification is supported by the fact that our sequences show a mean identity value of 99.7% with other private sequences available on BOLD. Interesting to note that, while our specimens were collected in the Western part of the Mediterranean Sea, those analyzed by Keskin & Atar, (2013) came from the Eastern Mediterranean. Similar values of intraspecific divergence have been reported for the most diverse fish groups, and often attributed to cryptic species (April, Mayden, Hanner & Bernatchez, 2011; Ward, Holmes & Yearsley, 2008).
Rhabdosargus haffara	*S. aurata*	Considering the different geographical origin of the two species, it could be possible that the specimen identified as *S. aurata* was a misidentified specimen of *R. haffara* migrated through the Suez Canal (Golani, 1992).
Acanthopagrus spp	*Acanthopagrus* spp	There have been many re-descriptions within this genus and currently 15 species and 2 subspecies are recognized (Hsu *et al*., 2011).
A. pacificus	*A. berda*	Misidentification of specimens or identification based on previous classification, considering that *A. pacificus*, very similar in overall appearance to *A. berda*, has been recently re-described as a new species (Iwatsuki, Kume, & Yoshino, 2010).
A. schlegelii, *A. schlegelii schlegelii*, *A. sivicolus*	*A. schlegelii*, *A. schlegelii schlegelii*, *A. sivicolus*	Closely related species belonging to the “black seabream complex” (Hsu et al., 2011).
Pagrus major	*P. auratus*	As suggested by Tabata & Taniguchi (2000) they might be two subspecies,
D. sargus subspecies	*D. sargus* subspecies	Close phylogenetic relationship of the genus *Diplodus*, which includes 13 species and 11 subspecies (Summerer, Hanel & Sturmbauer, 2001).

Table 7SM. Possible explanation of the main cases of misidentification during comparison on BOLD and GenBank.

References

1. April, J., Mayden, R. L., Hanner, R., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America´s freshwater fishes. PNAS, 108(26), 10602–10607.
2. Golani, D. (1992). *Rhabdosargus haffara* (Forsskål, 1775) and *Sphyraena flavicauda* Rüppell, 1833—new Red Sea immigrants in the Mediterranean. *Journal of Fish biology*, 40(1), 139-140.
3. Hsu, T. H., Guillén Madrid, A. G., Burridge, C. P., Cheng, H. Y., & Gwo, J. C. (2011). Resolution of the *Acanthopagrus* black seabream complex based on mitochondrial and amplified fragment-length polymorphism analyses. *Journal of fish biology*, 79(5), 1182-1192.
4. Iwatsuki, Y., Kume, M., & Yoshino, T. (2010). A new species, *Acanthopagrus pacificus* from the Western Pacific (Pisces, Sparidae). *Bulletin of the National Science Museum*, 36, 115-130.
5. Summerer, M., Hanel, R., & Sturmbauer, C. (2001). Mitochondrial phylogeny and biogeographic affinities of sea breams of the genus *Diplodus* (Sparidae). *Journal of Fish Biology*, 59(6), 1638-1652.
6. Tabata, K., & Taniguchi, N. (2000). Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. *Fisheries Science*, 66(1), 9-18.

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