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Title: DNA barcoding reveals chaotic labeling and misrepresentation of Cod (鳕, Xue) products sold on the Chinese market

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Abstract: The increasing rate of seafood frauds, especially in the case of highly priced species, highlights the need of verifying the identity of fish products. This paper describes the application of DNA barcoding to the identification of 52 products commercialized with the Chinese term 鳕 (Xue, Cod) in supermarkets (Nanjing and Shanghai) and in the online market. Considering the lack of harmonization around the definition of Cod, the mislabeling rate was assessed according to three increasingly stringent definitions: Cod meaning Gadiformes species; Cod meaning Gadus spp.; Due to the fact that the term "Cod" does not mean any specific species, since a qualifier ("Atlantic", "Pacific" or "Greenland") should be added to refer to Gadus morhua, G. macrocephalus or G. ogac, respectively. Results highlighted a very high mislabeling rate, which exceeded 60% even with the less stringent definition. Interestingly, only 42.3% of samples were Gadiformes, while the other were Perciformes, Pleuronectiformes or toxic Tetraodontiformes species. Economic, ecological and health issues arising from the misuse of the term Cod are discussed in the light of the leading role of China in the seafood worldwide industry and of the increased national consumption of marine species.

Dear Editor,

we would like to submit the following manuscript for possible publication:

DNA barcoding reveals chaotic labeling and misrepresentation of Cod (鳕, Xue) products sold on the Chinese market

In the era of food trade globalization, seafood products generally arrive to destination after changing hands several times. Thus, also considering that the residual morphological characteristics of processed seafood are inadequate for the identification, the substitution of high-quality species with less expensive ones become quite easy. In this context, the accurate labeling of seafood species plays an important role in protecting consumers and benefits the stocks conservation by combating the Illegal, Unreported and Unregulated (IUU) fishing.

Seafood traceability has reached high standards level in Western countries, and in particular in the European Union. On the countrary, in China, where the incomes' growth has determined dramatic changes in the food consumption patterns towards premium marine species, such as Cod, most of the specific standards in force are non-mandatory and an official standardized system for seafood naming is still absent.

Considering that Cod products are among the most investigated and mislabeled species on international markets and the chaos affecting the Chinese nomenclature system for seafood, in the present wotk DNA barcoding was applied for the identification of 52 products commercialized with the denomination 鳕 (*Xue*) (alone or in combination with other terms) in supermarkets in Nanjing and Shanghai, and on Chinese online retailers. To the best of our knowledge, this is the first work that takes into consideration seafood products from e-commerce. In particular, considering the lack of harmonization around the definition of Cod at the international level, the mislabeling rate was assessed considering three increasingly strict definitions: Cod indicating Gadiformes species; Cod meaning *Gadus* spp.; Cod not referable to any species, since the qualifiers "Atlantic", "Pacific" or "Greenland" should be added for *Gadus morhua*, *G. macrocephalus* or *G. ogac*, respectively.

Results highlighted an impressive mislabeling, which exceeded 60%, even with the less stringent definition. Interestingly, only 42.3% of the samples were identified as belonging to Gadiformes (Gadidae and Macrouridae), while the others were Perciformes (Nototheniidae), Pleuronectiformes (Pleuronectidae) or toxic Tetraodontiformes (Tetraodontidae). Interestingly, Cod products were substituted not only with less valuable species, but also with potentially toxic species banned from the market and even with high valued species suffering of overexploitation. The results suggest the possible use of seafood renaming as a way to recycle IUU product on legal market. Therefore, in the light of the role of China in the seafood worldwide industry and of the increased national consumption of marine species, economic, ecological and health issues arise from the misuse of the term Cod.

We declare that the manuscript is an original contribution that has not been published elsewhere in the same form and that is not currently under consideration elsewhere.

Best regards

Andrea Armani

Dear Editor,

We are sending you back the revised version of the manuscript entitled "*DNA barcoding reveals chaotic labeling and misrepresentation of Cod (鳕, Xue) products sold on the Chinese market*". Thank you for considering the manuscript for publication after major revision. The manuscript has been implemented according to the revision proposals of the reviewers.

Reviewer #1: Manuscript Number: FOODCONT-D-01015

Title: DNA barcoding reveals chaotic labeling and misrepresentation of Cod (Xue) products sold on the Chinese market

Xiong et al investigated cod mislabeling from the Chinese market and also from e-commerce, revealing a fraud rate of 62.7% to 86%.,depending on the definition of "Cod".

The paper is well written and presents important results regarding mislabeling in a region not yet analyzed.

Regarding methods, I find difficult to understand the definitions used to classify mislabeling, in particular the definition number 3 (Page 9, line189) "3) Cod not referred to any species, since a qualifier (Atlantic, Pacific or Greenland) should be added to in order to identify G. morhua, G. macroephalus or G. ogac, respectively." Could you clarify this criterion?
We tried to clarify the definitions used to classify mislabeling, both in the abstract (line 31-33) and in Materials and methods (line 243-251). With this definition we want to point out that, as a matter of fact, the term cod alone does not allow to identify any specific species, unless an adjective (Atlantic for *Gadus morhua*, Pacific for *Gadus macrocephalus*, Greenland for *Gadus ogac*) is added.

Results and Discussion

Lines 202-218: reads as background information, maybe it should be transferred to the introduction section.
This part has been moved to the introduction section and integrated with the information already reported (line 71-86).

Line219-228: This section is of less concern. Suggest removing it from the manuscript.
This part has been maintained according to the suggestion of the other reviewer who particular appreciated the DNA quality and quantity evaluation (line 259-269).

Splitting results into Full barcode and mini barcodes made the results section hard to read. Briefly mention that X samples were not amplified using traditional barcode primers and X were recovered using your mini-barcode approach.
We are not sure if we have correctly interpreted you request. We have slightly modified the section to clarify the results (line 270-274).

Line267: Which 3 MDB are you mentioning? Use sample numbers or something else to identify your samples. See comments above
The same codes used in Table 3 have been added (line 312).

Lines 271-298: Reads as background information and it's too long.
Lines 334-344- Reads as background information.
These parts have been moved to the introduction section, shortened and integrated with the information already reported (line 87-139).

Lines 311-313: This paragraph seems to me as a resume of your results and it is lost in between a long discussion text.
The paragraph has been shortened (line 329-330) and the paragraph has been reorganized according to the Reviewer 2. In particular, considering also your suggestion (referred to moving section 271-298 to the introduction), the discussion has been improved.

Lines 381-393: The section Geographical origin is not important to the manuscript context.
Line 433-442: Another Geographical origin section? What's the difference between both sections, with exactly the same name?
The first section, under the paragraph "*3.2 Evaluation of the label: denomination and origin*" reports the information available on the labels and on the webpages about the geographical origin of the fish species. The other section (3.3.2) with the same name, under the paragraph "*3.3 Comparison between label information and molecular results*" refers to the comparison of the geographical area of distribution of the fish species molecularly identified with that reported on the label and on the webpage of product. The beginning of the second section (3.3.2) has been modified in order to clarify the aim of the section (lines 419-428).
Thus, we prefer not to remove the first section.

Results and Discussion is too long and hard to follow. Suggest resuming it and describing the main finds and discussing it accordingly.
The section Results and Discussion has been shortened as requested by removing all the background information. Moreover, it has been revised to make it more comfortable for the reading.

Reviewer #2: Summary and General Comments:
This manuscript describes a market survey carried out using DNA barcoding to identify market samples sold as Cod on the Chinese market. Samples included fresh, frozen, and roasted items purchased both in-store and online. DNA barcoding was carried out using a standardized segment of the COI gene as well as a shorter segment referred to as a mini barcode. In general this is a good paper and it seems like the authors did a good job with their work—though the manuscript itself can be improved. I have specific suggestions below.

Abstract:
Line 33: The word impressive usually means something positive or good, but mislabeling is negative and bad. Instead of impressive level of mislabeling, I recommend high level of mislabeling. This occurs at other places in the manuscript, too.
Impressive has been replaced with very high throughout the manuscript.

Introduction:
In general, the Introduction is well-written and makes important points. I just have minor changes listed below.

Line 48 and elsewhere in the manuscript: The word countries does not need a capital letter C.
The capital C has been removed.

Line 65: It is better to say caused instead of determined
Done.

Lines 83-100 can all be the same paragraph instead of having lines 83-85 as a separate paragraph.
This section has been modified according to the reviewer 1.

Line 101: Documentable instead of documental.
We think that documental is the correct word since it refers to the "papers/documents" accompanying the goods. However, to make it clearer we add the word "System" (line 140).

Lines 101-107: Since you are using the standardized 655 bp segmentof the COI gene originally established by Hebert et al., it is best to cite some of their work—these original papers established that this particular segment of the COI gene can be used to discriminate species. For example: Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; and de Waard, J.R. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B. 2003a, 270, 313-321. And also: Hebert, P.D.N.; Ratnasingham, S.; and de Waard, J.R. Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proc. R.Soc. Lond. B. 2003b, 270, S96-S99.
The references cited at line 144-145 refer to the application of the DNA barcoding to the seafood compart. However, the work that you suggested has been added as reference (line 143).

Lines 115 - 116: This study enabled understanding of which species………would be better than allowed to understand
The sentence has been modified according to the suggestion (line 155-156).

Materials and Methods:
Line 129: I see that you cited a reference, but please briefly describe the DNA extraction procedure. Briefly is okay since you cited a reference.
A brief description of the method has been added (line 168-179).

Lines 129-137: The authors did a good job adding the extra steps of checking their DNA quality with Nanodrop and gels.
Thanks for the comment. This is a standard procedure in our lab.

Lines 140-145: What was the concentration of magnesium ions in the PCR? I guess magnesium was in the 10x buffer? Magnesium is important for PCR performance so it is best to report it.
Yes, the Magnesium was in the 10x buffer. The final concentration (1.5mM) has been reported in the test (line 192-193).

Line 153: How were amplicons purified? Were they purified from the gel or cleaned up by some other method? Also please briefly describe how sequencing was carried out: what kind of sequencer was used?
All this information has been added in the text (line 205-208).

Lines 155-159: Please provide sequences for both the forward and reverse primers for the mini DNA barcode. I looked at the Armani 2015(b) paper, and I do not see the primer sequences there either. Also please say where the mini-barcode is: is it part of the COI gene? Is it part of the full DNA barcode region?

The forward primer used for the amplification of the mini DNA barcoding is the same used for the amplification of the Full DNA barcoding (Handy et al., 2011). The sequence of the reverse primer has now been reported in the text and the sentence has been modified to clarify the procedure (line 211-213). The mini barcode belongs to the *COI* gene as stated in the title of the paragraph: "*2.4 Amplification and sequencing of the mini-COI barcode (MDB)*"and corresponds to the first 192bp at the 5' of the gene. The primer sequence of the REVshort1 is available in the Table 4SM of the paper Armani 2015(b) :

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

Results and Discussion:

Line 122: Please give a range of concentrations obtained from the Nanodrop.

This information has been reported in the text (line 262-263).

Lines 224-227 and lines 233-235: I am not convinced that not proper cold conditions for unprocessed frozen products would degrade DNA enough to fully interfere with PCR from the full DNA barcode.

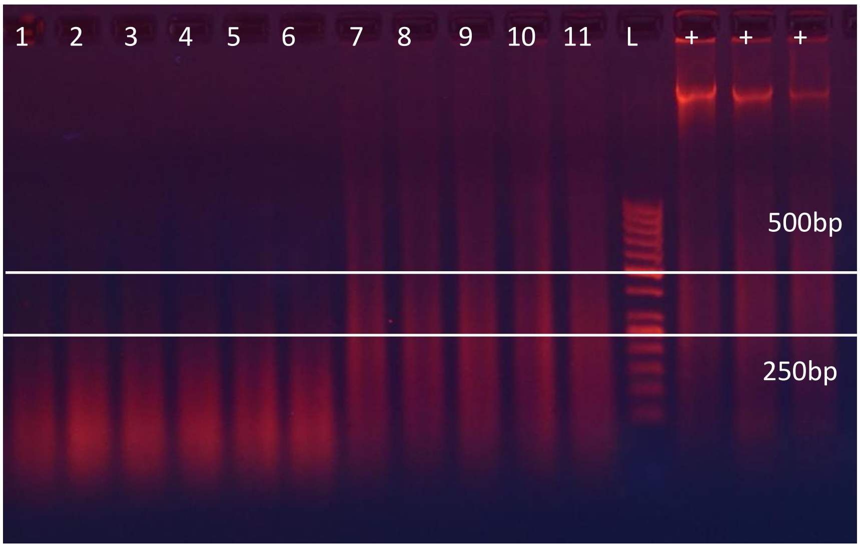
This is not the first time that we encountered this issue with unprocessed fresh/frozen products and other authors also observed the same problem. In fact, in previous articles we reported that:

"*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,_Alvarez, &Cotano, 2013) The reduced amplificability of the DNA extracted from the cooked products agrees with the observed degradation patterns* (Armani et al., 2015 b).

"*It is interesting to note that we also confirmed the high level of degradation already observed in case of fresh/frozen products (Armani et al., 2015; Lamendin et al., 2015*" (Armani et al., 2015 a).

Finally, in a survey currently carried on in our lab, we found a lot of highly degraded sampled of DNA extracted from sushi products. On the basis of our experience, we have hypothesized that this could be due to repeated cycles of freezing and thawing.

I would really like see the gels for all of these samples—both the gel for DNA quality/degradation and the gel for PCR products from the full DNA barcode. It would be valuable to include all these gels in the paper because that will allow the reader to compare the DNA quality and PCR products for each sample. Most people who do DNA barcoding do not run gels to check DNA degradation, so it will be useful to have this information published.
We think that it is not useful and feasible (since we did not think to include the pictures in the manuscript we do not have good quality photos with all the samples together) to include all the pictures of both the gel for DNA quality/degradation and the gel for PCR products from the full DNA barcode in the manuscript. We think that it is enough to describe it in the text as part of a quality control procedure. In fact, on the basis of our experience, we can state that not always the degradation level of DNA reflect its amplificability in term of base length. On the countrary, the degradation assessed by gel electrophoresis can explain amplification failures. This can be very useful because, otherwise, amplification failures could be attributed to other issues (i.e. inibhitors) that should be investigated. For the moment we send you the run of the degraded samples that we repeated to verify the level of degradation. If you think it necessary, we can include this picture in the manuscript.



1-2: ON1, 3-4:ON5, 5-6:ON10, 7-11: other DNA samples amplified with FDB. +: positive control of DNA extracted from fresh fish muscle.

Lines 236-241: Please give more information about the quality of the sequences obtained and the revision process used to analyze the raw data. What were the % high quality bases? In addition to the average length of the barcodes, please give the range of lengths.
The revision process used to analyze the raw data was that proposed by Handy et al., 2011 and a sentence has been added In Materials and Methods (line 219-220). The requested information has been reported in the text (line 278-283).

It looks to me like the mini-DNA barcode did not help you identify any species and did not improve on the full DNA barcode. Line 229 says that a full DNA barcode was obtained from 48 samples, and line 243 says those 48 gave species ID. Lines 267-270 say the mini DNA barcodes did not give any matches in databases above 96%. I am not sure that this is a problem with reference sequences. If the mini DNA barcode is within the full DNA barcode, the sequences should be in the databases as part of full DNA barcodes. Maybe the mini-DNA barcode is not long enough to give good species ID.

This is true, in this work the utilization of the MDB did not help us identify any species. Overall we get 51 sequences from the DNA of the 52 samples analyzed (48 FDB and 3 MDB). The MDB were obtained from the DNA samples not amplifiable with FDB. We stated that the lack of identification could be related to the absence of reference sequences on the basis of our previous experience. In fact, usually the MDB retrieve high identity value when compared with databases (see in particular Table 3SM in Armani et., 2015 a and Table 1SM in Armani et al., 2015b).

Lines 257-266, Tables 2 and 3: In order to get better ID from the databases, check the sequences in these databases to see if they came from vouchered museum specimens and try the ID using only sequences from vouchered, morphologically identified specimens. See if the sequences have publications to go with them. For some of the samples that had more than one match, it might be because something had the wrong name in the database.

The comparison on BOLD was performed using the Species Level Barcode Records that does not take into consideration unvalidated libraries and records without species level identification. This information has been added on the text (line 287). As reported, the raw data were analyzed according to the revision process used by Armani et al. (2015b) to solve ambiguous results due to the presence of some unreliable sequences in the databases. In particular, considering the high number of "ambiguous" results we further investigate the issues 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).

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: the barcode of life data system. MolecularEcology Notes, 7, 355e364. www.barcodinglife.org.

Costa, F. O., Landi, M., Martins, R., Costa, M. H., Costa, M. E., Carneiro, M., et al. (2012). A ranking system for reference libraries of DNA barcodes: applicationto marine fish species from Portugal. PloSone, 7(4), e35858.

Lines 311-333, Lines 345-375, lines 381-393, lines 433-442: Shorten these sections a lot! They repeat information from the tables and make the discussion very long.

The old section 311-333 now corresponds to line 329-339; the old section 345-375 now corresponds to line 340-364; the old section 381-393 now corresponds to line 370-379.

The old section 433-442, which now corresponds to line 419-428, has not been shortened because some information has been added to respond to Reviewer 1.

Overall, the discussion has been shortened also following the suggestion of Reviewer 1.

Lines 471-585: This part of the discussion has good information on how these species are fished, how they end up as bycatch, exploitation etc. I think the parts that repeat from tables (see above) can be shortened or removed from the discussion, but these last few pages (lines 471-585) should be kept in the manuscript.

We revised this part however we found it very difficult to shorten it without losing important information.

Figure 1: I like this Figure. It is helpful to visualize what species were found.

DNA barcoding reveals chaotic labeling and misrepresentation of Cod (鳕, Xue) products sold on the Chinese market

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Abstract

The increasing rate of seafood frauds, especially in the case of highly priced species, highlights the need of verifying the identity of fish products. This paper describes the application of DNA barcoding to the identification of 52 products commercialized with the Chinese term 鳕 (*Xue*, Cod) in supermarkets (Nanjing and Shanghai) and in the online market. Considering the lack of harmonization around the definition of Cod, the mislabeling rate was assessed according to three increasingly stringent definitions: Cod meaning Gadiformes species; Cod meaning *Gadus* spp.; Due to the fact that the term "Cod" does not mean any specific species, since a qualifier ("Atlantic", "Pacific" or "Greenland") should be added to refer to *Gadus morhua*, *G. macrocephalus* or *G. ogac*, respectively.

Results highlighted a very high mislabeling rate, which exceeded 60% even with the less stringent definition. Interestingly, only 42.3% of samples were Gadiformes, while the other were Perciformes, Pleuronectiformes or toxic Tetraodontiformes species. Economic, ecological and health issues arising from the misuse of the term Cod are discussed in the light of the leading role of China in the seafood worldwide industry and of the increased national consumption of marine species.

Keywords

DNA barcoding, Seafood mislabeling, Xue, Cod, Gadiformes, Pufferfish

1. Introduction

In the era of food trade globalization, seafood products generally arrive to destination after changing hands several times. These complex pathways, which often involve developing countries, make traceability difficult. Moreover, the residual characteristics of processed seafood products are inadequate for a morphological identification. In this scenario, the substitution of high-quality species with less expensive ones, or the abuse of generic and vernacular seafood names that may confuse consumers become quite easy (Cawthorn, Duncan, Kustern, Francis, & Hoffman, 2015; Di Pinto et al., 2013; Miller & Mariani, 2010). For the aforesaid reasons, the accurate naming and labeling of seafood species plays an important role in protecting consumers from frauds. Moreover, traceability could benefit the stocks conservation by combating the Illegal, Unreported and Unregulated (IUU) fishing.

In order to ensure food authenticity, many countries have constructed a legal framework for the management of the whole seafood supply chain. The European Union (EU), currently considered the global leader in food traceability (Charlebois, Sterling, Haratifar, & Naing, 2014), has established several compulsory information (such as the scientific name; the corresponding commercial denomination, according to the official list proposed by each member state; the production method; the catch/farm area and the category of fishing gear) which should be reported on the seafood products (Regulation EU No 1379/2013).

In China, income growth has recently caused dramatic changes in the food consumption patterns (Hu et al., 2014; Lam, Remais, Fung, Xu, & Sun, 2013). The domestic demand for seafood has markedly increased over the last decades, making China the largest fish consumer in the world (Villasante et al., 2013). Chinese consumers have begun to purchase a diversified basket of products, choosing premium marine species (Rabobank International, 2012; Hu et al., 2014).

Such changes are particularly evident in Nanjing and Shanghai, the main cities of the Yangtze River Delta region, one of the most industrialized and urbanized areas in China and in the world (Nanjing Municipal Bureau of Statistics, 2015; Shanghai Municipal Bureau of Statistics, 2015; National Bureau of Statistics of the People's Republic of China, 2015). The high income level, combined with a large inflow of tourists and with the fact that seafood is more available and consumed along the coast, result in a stronger consumers' demand in this area (Rabobank International, 2012). Even though supermarkets represent one of the main channels to get access to seafood products (Hu, Reardon, Rozelle, Timmer, & Wang, 2004), the e-commerce is experiencing a very rapid growth in China. In 2013, the Chinese online retail market became the biggest worldwide in terms of sale volume (China Internet Network Information Center, 2014) and in 2014 China's online sales for seafood have increased by 106% (http://www.undercurrentnews.com/2014/10/06/china-seafood-leader-sees-infinite-opportunity-in-domestic-e-commerce/). This emerging market greatly enhanced the access of the public to exotic seafood.

Food labeling in China is regulated by a multitude of laws, regulations and standards. However, inconsistencies among them often make it difficult to identify applicable labeling requirements. The main regulation enforced to ensure the food safety is the Food Safety Law of the Peoples' Republic of China of 2009 (United States Department of Agriculture, 2009). Following this regulation, the vertical national standard GB7718-2011 "*General rules for the labeling of prepackaged foods*" (Chinese National Standard GB 7718-2011) and the General Order No. 123 of 2009 on *"foods produced (sub-packaged) and distributed within the borders of the People's Republic of China"* (China General Order No. 123 of 2009) have been issued to regulate labelling of food products. According to the compulsory national standard GB 7718-2011 (Chinese National Standard GB 7718-2011), the name of the product and the ingredient list are the main source of information on fish identity for prepackaged products. The Decision General Order No. 123 of 2009 (China General Order No. 123 of 2009) refers to all food categories and applies to food products sold in bulk. Both regulations state the necessity to follow related standards, if available, to select the appropriate name of the product. However, most of the specific standards in force for seafood labeling and traceability are non-mandatory (Xiong et al., under review). For what concerns the online market, the webpage plays an important role in informing the consumers. Currently, in China no provisions about e-commerce business are illustrated in the Food Safety Law (United States Department of Agriculture, 2009) and the responsibility among different actors are not clearly defined. As a consequence, the draft of the newly revised Food Safety Law (not yet issued) points out the need to strengthen the control on operators of the online markets (http://www.npc.gov.cn/npc/xinwen/lfgz/flca/2014-12/29/content_1891935.htm).

Among the marine fishery products newly available on the Chinese market, Cod is one of the most appreciated and nowadays widely affordable (Rabobank International, 2012). In a broad sense, the term “Cod” generally refers to fish of the family Gadidae and to related species within the order Gadiformes (Xiong et al., under review). However, more specific denominations are often requested for the identification of the three species belonging to *Gadus* spp. (see section 3.3.1). In China, the word 鳕 (*Xue*), alone or in combination with other terms, is usually employed for the commercialization of Cod (Table 1). However, with the exception of the guideline issued by the Centre for Food Safety of the Hong Kong Government (Centre for Food Safety of Hong Kong, 2007) no specific legislation or standards regulate the labeling of these products in mainland China. In fact, a detailed system for seafood labeling is still absent in China as well as an official naming system comparable to those adopted by the EU countries (Xiong et al., under review).

This guideline pointed out that the term Xue is used in Chinese as a common name for fish species that do not even belong to the order Gadiformes, such as the Sablefish *Anoplopoma fimbria* (Scorpaeniformes, Anoplopomatidae), the Patagonian toothfish *Dissostichus eleginoides* and the Antarctic toothfish *D. mawsoni* (Perciformes, Nototheniidae). The aforesaid document recommends the use of the term Cod only for the species of the order Gadiformes, while, if used for the other 3 species (*A. fimbria*, *D. eleginoides* and *D. mawsoni*), it must be further specified by reporting the scientific name or the common name recommended by the FAO. Interestingly, this document had been issued to face the widespread custom to replace Cod with toxic oilfish (Centre for Food Safety of Hong Kong, 2007). In fact, it is quite difficult for most of the Chinese Food Business Operators (FBOs) and consumers to recognize the real Cod, especially when the shape of the fish has been modified by processing. Thus, since the term *Xue* attracts the buyer, suggesting that the products are made of Cod, misrepresentation of this denomination is recurrent. In fact, economic and health issues have already been reported in this Country, due to the commercialization of fake Cod products (Fang, 2011; Li et al., 2013).

To support the documental traceability of seafood products many DNA based methods have been proposed. Nowadays, the DNA barcoding of a ~655bp region of the mitochondrial cytochrome c-oxidase I (*COI*) gene (Full DNA Barcoding, FDB) (Hebert, Cywinska, Ball, & deWaard, 2003), is among the most used approaches (Armani et al., 2015a; Carvalho, Palhares, Drummond, & Frigo, 2015; Di Pinto et al., 2013; Cawthorn et al., 2015). In addition, the utilization of a Mini DNA barcoding of 139bp (MDB) has recently been shown to be a feasible alternative (Armani et al., 2015b).

In this work, considering that Cod products are among the most investigated and mislabeled species on international markets (Table 1SM), the increased interest of a large part of the Chinese population in marine species and the chaos affecting the Chinese nomenclature system for seafood (Xiong et al., under review), FDB and MDB was applied for the identification of 52 products commercialized with the term 鳕 (*Xue*) (alone or in combination with other terms) in supermarkets in Nanjing and Shanghai and online. To the best of our knowledge, this is the first work that takes into consideration seafood products from e-commerce. This study enabled understanding which species are currently sold using the term *Xue* in China and evaluating the accuracy of the employed designations.

2. Materials and method

2.1 Sample collection

Fifty-two samples showing the term 鳕 (Xue) (alone or in combination with other terms) (Table 1) were collected from the Chinese market. In particular, 34 of them were bought in supermarkets in the city of Nanjing and Shanghai and included 4 fresh, 17 frozen and 13 roasted products (Table 2). Another 18 frozen samples were acquired from two e-commerce giants in China (Table 3). The samples were temporary stored in absolute ethanol and labeled with an internal code. Once arrived in the lab, they were stored at -20°C until further analysis.

2.2 DNA extraction and evaluation of DNA fragmentation by gel electrophoresis.

Total DNA extraction was performed following the method of Armani et al. (2014) slightly modified by adding proteinase K. Briefly, 200 mg of tissue, 10 steal beads, 200 µl of lysis buffer and 20 µl of proteinase K (Eurcolone, Wetherby, UK) were put in a 2-ml tube and placed on a T-shaker (60 °C for 30 and 60 min at 1,500 rpm). At the end of the milling step, the samples were centrifuged (15,000×g for 2 min) and the collected supernatant was placed in a clean tube. The proteins were precipitated adding 0.5 volumes of 4M sodium acetate, pH 8.3. After an incubation step (5 min at RT) the mixture was centrifuged (15,000×g for 5 min). The DNA was precipitated with 0.6 volumes of isopropanol molecular biology grade (SERVA Electrophoresis

GmbH, Heidelberg, Germany), washed once in 70% (v/v) ethanol molecular biology grade (SERVA Electrophoresis GmbH, Heidelberg, Germany) and once in 100% ethanol, air-dried, and resuspended in deionized sterile water. DNA quality and concentration was determined by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, US).

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

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

The DNA samples were amplified using the universal primers proposed by Handy et al. (2011), tailed as proposed by Steffens, Sutter & Roemer (1993), for the amplification of a FDB of the *COI* gene. The PCR reactions were performed in a final reaction volume of 20 µl, containing 2 µl of a 10× buffer with 15mM of $MgCl_2$ (final concentration 1.5mM) (5Prime, Gaithersburg, USA), 100 µM of each dNTP (Euroclone, Pavia, Italy), 100 nM of each primer, 25 ng/mL of BSA (New England BIOLABS® Inc. Ipswich, MA, USA), 1.25 U of PerfectTaq DNA Polymerase (5Prime, USA), 100 ng of DNA and DNase free water (5Prime, USA). The amplification program involved an initial denaturation step at 94°C for 3 min, followed by 45 cycles at 94°C for 30s, 53°C for 30s and 72°C for 35s and final extension at 72℃ for 10 min. Five μL of PCR products, stained with GelRed™ Nucleid Acid Gel Staining 10,000× water solution (Biotium, Hayward, CA, USA) in 0.5×TBE buffer, were checked by electrophoresis on a 2% agarose gel (GellyPhorLE, EuroClone, UK). By visualizing on a UV transilluminator, the presence of the expected amplicons was assessed by a comparison with the standard marker SharpMass™ 50-DNA ladder (Eur oClone, S.p.A.—Life Sciences Division, Pavia, Italy). PCR products were purified from the reaction mix using QIAquick PCR Purification Kit (Qiagen, Shanghai, China), following the manufacturer's instructions, and then sent to the company GenScript (Nanjing, China) for sequencing using ABI 3730 DNA sequencer (Applied Biosystems Division, Foster City, USA).

2.4 Amplification and sequencing of the mini-COI barcode (MDB)

The DNA of the samples that failed the amplification of the FDB region was submitted to the amplification of a ~190bp MDB region (139bp without primers) with the forward primer FISHCOIBCL (Handy et al. 2011) and the reverse primer REVshort1 (5'-GGYATNACTATRAAGAAAATTATTAC-3'), tailed as proposed by Steffens et al. (1993). The PCR was performed following Armani et al. (2015b). All the PCR products were purified and sequenced as reported in section 2.3.

2.5 Post-sequencing data analysis

The sequences obtained were visualized, aligned and edited using Clustal W in BioEdit version 7.0.9 (Hall, 1999) Fine adjustments were manually made after visual inspection. In particular, the revision process used was that proposed by Handy et al. (2011). The generated *COI* sequences were analyzed using the Identification System

(IDs) on BOLD (Species Level Barcode Records) (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) and using the Basic Local Alignment Search Tool (BLASTn) on GenBank, (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A top match with a sequence similarity of at least 98% was used to designate potential species identification. Since the *COI* sequences obtained in this study were not derived from voucher samples or expert-identified fish specimens, the sequences were submitted neither to GenBank nor to BOLD.

2.6 Label analysis and evaluation of the accuracy of seafood designation

The label was analyzed in the light of the current Chinese legislation (Chinese National Standard GB 7718-2011; China General Order No. 123 of 2009). The Chinese ideogram indicating the name of the purchased product was translated by a Chinese native speaker with the aid of an on-line translation tool (https://translate.google.it/) and then verified consulting the FAO Fisheries and Aquaculture Statistics and Information Service (ASFIS) list (http://www.fao.org/fishery/collection/asfis/en), FishBase (http://www.fishbase.org/comnames/scriptlist.php?showAll=yes&script=Chinese) and the Latin-Chinese Dictionary of Fish Name (http://fishdb.sinica.edu.tw/eng/chinesequer1.php). The Chinese name was then compared with the name reported in the ingredient list (for supermarket products) or with further information available in the webpage (for online samples). Data on the received products were also checked for those purchased online.

Moreover, the scientific names retrieved from the sequences analysis after consulting BOLD and GenBank were compared with the denominations reported on the labels and the mislabeling rate was calculated considering three increasingly strict definitions of Cod: 1) Cod meaning Gadiformes species; 2) Cod meaning *Gadus* spp.; 3) Cod not meaning any specific species, since a qualifier (“Atlantic”, “Pacific” or “Greenland”) should be added in order to refer to *Gadus morhua*, *G. macrocephalus* or *G. ogac*, respectively. Finally, the indication of the catch area was assessed and compared with the distribution range of the molecularly identified species given by FishBase.

3. Results and discussion

The worldwide increasing rate of frauds, affecting in particular highly priced species such as Cod products (Table 1SM), attests the importance of verifying seafood identity. Currently, only a few studies applying DNA barcoding to identify Cod products sold on the Chinese market exist (Shen et al., 2014; Li et al., 2013). These surveys found misrepresentation rates reaching 100% and substitution with potentially toxic species, such as *Lagocephalus* spp. (Table 1SM).

3.1 Molecular analysis

3.1.1 DNA extraction and evaluation of DNA fragmentation by gel electrophoresis. The total DNA was successfully extracted from all the samples and showed good values of quantity (mean concentration=1414 ng/µl, ranging from 104 to 2966 ng/µl) and quality (average value of 260/280=2.14 and average value of 260/230=2.03). Even though food processing, such as heat exposure, low pH and drying could induce

DNA degradation, the electrophoretic analysis of DNA showed partial fragmentation mainly in the case of DNA samples extracted from unprocessed products (data not shown). This could be explained considering that, as observed in previous studies, not proper cold conditions during transportation and handling may result in DNA degradation also in case of frozen products (Armani et al., 2015a).

3.1.2 Amplification and sequencing. 48 out of the 52 sampled products yielded a FDB (overall amplification success 92.3%). In particular, it was possible to obtain a FDB from 97% of the supermarket products, while only from 83.3% of the online samples. Of the remaining 4 samples that failed to amplify a FDB, 3 produced a MDB and 1 sample was never amplifiable. This result confirms the outcomes of the electrophoretic analysis of total DNA (see section 3.2.1) and the usefulness of MDB in case of degraded DNA (Armani et al., 2015a,b).

All the PCR products gave readable sequences (overall rate of sequencing success 98.1%). The sequences' length and quality were analyzed first on the raw data and then after trimming at the 5' and 3' end, according to the criteria described in Handy et al. (2011). The overall average length of the raw sequences was 705 (699-720bp) for FDB and 203 (201-207bp) for MDB, while the average length of the trimmed sequences was ~635 (ranging from 485bp to 655bp) for FDB and ~135 (134-137bp) for MDB.

3.1.3 Comparison with the databases. The raw data were analyzed according to the revision process used by Armani et al. (2015b) to solve ambiguous results due to the presence of some unreliable sequences in the databases.

By using the IDs analysis on BOLD (Species Level Barcode Records), a maximum species identity in the range of 98–100% was obtained for 48 FDB sequences (94.1%). Of these, 25 (52.1%) were unambiguously identified at species level: 13 out of the 33 supermarket samples (39.4%) and 12 of the 18 online products (66.7%), respectively. The analysis of the remaining 23 sequences (47.9%) produced overlapping values of identity between 98 and 100% within the genus *Macrourus*, *Lagocephalus* and *Atheresthes*and among *Coryphaenoides acrolepis* and *Albatrossia pectoralis* (Table 2 and 3). These ambiguous results did not allow to precisely identify such samples. The impossibility of discriminating among several species of *Lagocephalus* has already been related to incomplete reference coverage in the database (Cohen et al., 2009). As for *A. pectoralis*, the close genetic relation with the genus *Coryphaenoides* has already been observed by Roa-Varón & Ortí (2009). Similar issues concern the Gadidae *G. morhua* and *Theragra chalcogramma* (Armani et al., 2015a). However, these latter 3 samples were considered identified as *G. morhua* on the basis of the higher max identity value retrieved for this species and of the specific identification obtained on GenBank.

All 48 FDB sequences which presented a maximum species identity in the range of 98–100% (94.1%) by BOLD analysis retrieved the same result when analyzed by BLAST. Totally, 28 products were unambiguously identified: in addition to the same 25 products identified in BOLD, the species *Macrourus carinatus* could also be unambiguously identified at the species level, thus resulting in a higher proportion of identified samples (58.3%). For the remaining 20 FDB, the occurrence of high matching with more than one species, prevented specific identification (Table 2 and 3). In conclusion, the identification results obtained using BOLD and GenBank showed a similar discrimination power (52.1% and 58.3%, respectively).

Finally, the 3 MDB (from samples ON1, ON5 and ON15) did not match any reference barcode in BOLD and retrieved a top match of maximum identity of 96% on GenBank, hence not allowing specific identification. The issue is likely related to the lack of reference sequences, has already discussed (Armani et al., 2015a, b).

3.2 Evaluation of the label: denomination and origin

3.2.1 Denomination declared on the label. A lack of harmonization around the definition of Cod also exists at the international level, where it is often used for all the *Gadus* species, such as in Spain and in Germany (Xiong et al., under review) or, in a broader sense, for fish of the family Gadidae, including *G. macrocephalus*, *G. morhua* and *G. ogac*, *Melanogrammus aeglefinus*, *Merlangius merlangus*, *Micromesistius poutassou*, *Pollachius pollachius* and *Pollachius virens* (Di Pinto et al., 2013). On the contrary, the ASFIS list and Fishbase, as well as the UK list (Xiong et al., under review) require a specific name for each species: Atlantic Cod for *G. morhua*, Pacific Cod for *G. macrocephalus* and Greenland Cod for *G. ogac*. The utilization of three distinct names is also proposed by the Latin-Chinese Dictionary of Fish Name (http://fishdb.sinica.edu.tw/eng/chinesequer1.php), which has been developed with the aim of providing a standardized nomenclature for fish species in China.

Totally 34 samples were collected from supermarkets (24 prepackaged and 10 in bulk). Details on the declared names of the prepacked supermarket samples are reported in Table 2. For 79.2% of the products, the name matched with the commercial or the scientific name in the ingredient list. Inconsistencies were found for 5 products (20.8%) labeled as Cod (Table 2). Regarding the bulk products, the fish identity could only be obtained from the bill board and the names reported are shown in Table 2. Overall, despite the high rate of correspondence between the product name and the ingredient list in prepacked products, we observed the misuse of the term Cod in 6 supermarket products, for which species completely different from Gadiformes (*P. alitivelis*), generic terms used to design other fish categories (Flatfish indicating Pleuronectiformes), or fake names (Flat cod, Water cod) were reported (Table 1).

Concerning the 18 products purchased online (7 prepackaged and 11 in bulk), the name declared on the main webpage is reported in Table 3. A few discrepancies were found investigating the specific information in the product page: one Silver cod product reported 南极银鳕鱼 (*Nanji Yin Xue Yu*, Antarctic cod), 1 Water cod reported Cod and 1 Cod product reported Atlantic cod. While in this latter case the denomination used refers to *G. morhua* and is not misleading, the denomination Antarctic cod is a vernacular term referring to *D. mawsoni* (http://www.fishbase.org/comnames/CommonNamesList.php?ID=7039&GenusName=Dissostichus&SpeciesName=mawsoni&StockCode=7377). The fake term Water cod (Table 1) can lead to confusion. For 1 product labeled as Silver cod, another Chinese denomination was also available (裸盖鱼, *Luo Gai Yu*, Sablefish). In other 3 products labeled as Silver cod or Cod, the Latin name *A. fimbria*, *D. eleginoides* and *G. macrocephalus/G. morhua* were reported. While in the Cod products the appropriate scientific names were indicated, the association between Silver cod and *D. eleginoides* was incorrect. In fact, even though the English term Silver cod does not refer to any species, the corresponding Chinese name (银鳕, *Yin Xue*) refers to *A. fimbria* (http://zh.wikipedia.org/wiki/銀鱈) (Table 1). The correct English name for this species, which is also referred to as Black cod, Blue cod and Coal cod, is Sablefish (http://www.fishbase.org/summary/ANOPLOPOMA-FIMBRIA.html). As mentioned, the corresponding Chinese name is 裸盖鱼 (*Luo Gai Yu*).

In addition, the information on the received package was compared to those available online. 39% of the samples did not report any label, while the remaining 61% reported the same fish denomination of the title of the webpage and/or the webpage description. In one product, not corresponding English (Toothfish) and Chinese (银鳕, *Yin Xue*, Silver cod) names were found (Table 3).

Overall, these results highlight how the absence of a standardized naming system for the commercialization of seafood can result in a great chaos of denominations that deceive the consumers. In particular, the use of improper names could give the false impression of a large availability of fish species, which, on the contrary, already suffer from overexploitation (see section 3.5).

3.2.2 Geographical origin declared on the label. Overall, 38.5% of the products did not show any indication regarding the origin. Interestingly, all of them were purchased in supermarkets. The country of origin was reported for 50% of the products, all of which were imported (Table 2 and 3), while only 25% of the products specified the capture area. Again, a great difference was observed between supermarket and online products: only 2 supermarket samples (5.8%) presented this latter indication (Bering Sea for both of them) (Table 2), which on the contrary was available for 11 online samples (61.1%). Of these, however, 1 sample generically indicated Atlantic Ocean and 5 products reported inconsistent declarations of different and distant capture areas in the webpage (Table 3).

The United Nations Food and Agriculture Organization (FAO) *Codex Alimentarius* requires the identification of the country of origin for all food products (http://www.fao.org/docrep/005/Y2770E/y2770e02.htm). However, considering that this could be deceptive for consumers, in Europe the indication of the capture area is mandatory for seafood (Council Regulation EC No 104/2000). Such information is particularly important for the fishery sector, considering that the processing country is often different from the source country.

3.3. Comparison between label information and molecular results

3.3.1 Denominations. Considering the above mentioned confusion on the term Cod (Section 3.2.1), the correspondence between the product name and the species identified by molecular analysis was assessed considering three different and increasingly strict definitions, according to different conventions existing in different areas of the world (see Section 2.6).

Therefore, the overall mislabeling rate rose from 62.7% (definition 1), to 82.3% (definition 2) and to 86.3% (definition 3). Incorrect label declarations were found in 63.6%, 88% and 88% (according to definition 1, 2, or 3, respectively) of the supermarket samples and in 61%, 72.7% and 83.3% of the samples purchased online.

Regarding the 31 samples labeled as Cod, several issues were found and the mislabeling rate widely changed according to the definition used. In particular, even considering a broad definition including all Gadiformes in the term Cod, 58% of the samples were mislabeled, since they were replaced with species belonging to different orders, such as *D. eleginoides*, different species of Flatfish and even Pufferfish of the genus *Lagocephalus*. The mislabeling rate rose to 100% if considering the most stringent definition.

Regarding the 11 samples labeled as Silver cod (银鳕), 100% of them were found to be mislabeled. In fact, while 银鳕 indicates *A. fimbria*, 8 products were identified as *D. eleginoides* (4 from the supermarket and 4 from the online market) and 3 as *D. mawsoni* (all from the supermarket).

Other issues were found for the 2 products labeled as Flat cod (SM2) and Water cod (ON10): they were identified as other species of Gadiformes (*M. carinatus* and *A. pectoralis*/*C. acrolepis*, respectively), but considered mislabeled, since the terms Flat cod and Water cod do not refer to any species. The remaining products, presenting more specific denominations, were correctly labeled (Table 4).

Our results highlighted a very high mislabeling rate, which, even using the most tolerant approach, exceeded 60%. This rate was similar to the mislabeling rate found by other authors (Table 1SM) in previous survey on Cod. In particular, with the exception of Di Pinto et al. (2013), who found a mislabeling rate of 100% in battered Cod chunks, the highest mislabeling rates were always reported in China and Hong Kong.

3.3.2 Geographical origin. The comparison between the declared geographical information and the range of the species identified by DNA barcoding was possible only for the 8 products which reported a capture area and were identified to the species level. The geographical origin reported on 5 products corresponded to the range of distribution of the identified species. A product reporting North Atlantic was identified as *A. stomias*, which is not present in this area (Fig. 2). Finally, for 2 online samples, for which two different capture area were reported, the identified species is distributed only in one of the two areas. It is interesting to note that all the samples not reporting indication on the origin were mislabeled and, among these, 9 samples (47%) were identified as *Lagocephalus* spp.

3.4 Species commercialized under the name Xue (alone or in combination with other terms).

Financial incentives are the strongest motivation to rename fish with more appetizing titles or as a high-priced species. Often, fish are given an entirely new name (similar to that of an already popular fish) to boost sales (Jaquet & Pauly, 2008) enhancing the marketability and the value of noncommercial fish species that were previously unknown to most consumers. Other than economic loss for consumers, the mislabeling of fish species could affect their sustainable exploitation. In fact, the promotion of a legal and sustainable seafood trade also passes through a consistent naming and labelling of seafood (Jacquet & Pauly, 2008; Rumbold, Engel, & Axelrad, 2011; Barendse & Francis, 2015).

Through the DNA barcoding technique, chaotic labeling of Cod products on the

Chinese market was highlighted. The collected products commercialized with the term *Xue* (alone or in combination with other terms) were identified to belong to 4 different orders: Gadiformes (n=22, 43.1%), Perciformes (n=12, 23.5%), Tetraodontiformes (n=9, 17.6%) and Pleuronectiformes (n=8, 15.7%) (Fig. 1). The main economic, environmental and health implications associated to the trading of these species were analyzed considering their commercial appealing on the international market, their conservation status and their toxic potentiality.

3.4.1. Gadiformes, Perciformes and Pleuronectiformes: economic and ecological implications. Of the 22 products identified as belonging to the Order Gadiformes, 11 (50%) were identified as belonging to the Gadidae family: 3 were *G. morhua*, 5 *T. chalcogramma*, 2 *P. virens* and 1 *M. aeglefinus*. The other 11 (50%) samples were identified as belonging to the Macrouridae family (*M. carinatus*, *C. acrolepis* or *A. pectoralis*) (Fig. 1). Depending on the definition of Cod used, the mislabeling of the products identified as Gadiformes varied from 13.6% to 68%. While incorrect labeling was found for *G. morhua* and *T. chalcogramma*, all products identified as *M. aeglefinus* and *P. virens* were correctly labeled.

The four species of the Gadidae family found in this study are commercially important species threatened by overexploitation. *G. morhua* (Atlantic cod) had been caught for centuries in the North Atlantic fisheries, but overexploitation from 1970s led to a dramatic decline in most source areas of the world (http://www.fao.org/fishery/species/2218/en). In fact, the species is listed as vulnerable in the IUCN Red List of Threatened Species (http://www.iucnredlist.org/details/8784/0). *T. chalcogramma* (Alaska pollock) is a species closely related to *G. morhua*. While in the past it was only used for animal feed, due to the restrictions on Cod fishing it has now become an important food resource for human (http://www.fao.org/fishery/species/3017/en). Alaska pollock is harvested in one of the largest industrial fishery in the world, while the related species *P. virens* (Atlantic pollock) is harvested with other ground fish species. In fact, this species was traditionally a bycatch and directed fishing started only in the 1980s. Since the 1970s, both pollock have experienced a gradual decrease of the catch volume and they are now subjected to strict harvest restrictions and other conservation efforts (http://www.fishwatch.gov/seafood_profiles/species/pollock/species_pages/atlantic_pollock.htm). High fishing pressure also occurs for *M. aeglefinus*, another widely commercialized fish included in the IUCN Red List (http://www.iucnredlist.org/details/13045/0).

Besides the four species of Gadidae, other 11 products were identified as belonging to Macrouridae (*M. carinatus*, *C. acrolepis* or *A. pectoralis*), ground fish known as Grenadiers, captured mainly as by-catch in deep-sea fisheries. Many species of this family are small and unpalatable, thus they are discarded or processed as fishmeal (Devine et al., 2012). However, due to the increased human demand, more and more species are intended for the market. *A. pectoralis* (Giant grenadier) is abundant in the northern Pacific Ocean and represents a large part of the bycatch in fisheries for *A. fimbria* and *R. hippoglossoides* (Clausen & Rodgveller, 2010). Similarly, *M. carinatus* is taken as bycatch in fisheries targeting Hake, squid and Patagonian toothfish (Devine et al., 2012).

Regarding Pleuronectiformes, 2 samples were identified as *R. hippoglossoides* and 3 as *Atheresthes* spp. Other 3 samples, for which only an MDB was available, were generically identified as flatfish (Fig. 1). These products were all sold under the generic name of Cod, while none of the products marketed as Flat cod was identified as a flatfish (Table 4).

R. hippoglossoides is a slow growing and valuable species, which can only tolerate low exploitation. Also for this species, conservation efforts have been launched and it is included in the seafood red list of Greenpeace International (http://www.greenpeace.org/international/en/campaigns/oceans/seafood/red-list-of-species/). *A. evermanni* and *A. stomias* are abundant species of the North Pacific Ocean. They are not as valuable as *R. hippoglossoides* and do not have such a great conservation pressure (Datsky, Yarzhombek, & Andronov, 2014). Thus, while the commercialization of *Atheresthes* spp. under the name of Cod can support a voluntary mislabeling due to the marked different value of this two species, doubts arise from the substitution of Cod with R. *hippoglossoides*. In our opinion, the commercialization under the term *Xue* of these species may be explained with the fact that they are ground fish that may be caught together with Cod or Cod related species using bottom trawling (Fig. 2). This hypothesis may explain why in this study the substitution of Cod not only occurred with low value species, but also with high priced species.

In this study, 2 species of Perciformes were found, namely *D. eleginoides* (n=9) and *D. mawsoni* (n=3) (Fig. 1). Only 1 product, identified as *D. eleginoides,* was sold as *Xue Yu* while all the remaining products were sold as *Yin Xue* (Table 1 and 4). Both species are slow-growing large fishes of the Antarctic Ocean of great commercial appeal (http://www.ccamlr.org/en/fisheries/toothfish-fisheries). In the mid-1990s, IUU fishing was widely reported for *Dissostichus* spp. (Agnew, 2000) thus, nowadays, Toothfish fishing is managed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (http://www.ccamlr.org/en/fisheries/toothfish-fisheries). Moreover, several fisheries have been independently certified as sustainable by the Marine Stewardship Council (MSC) (Pierre, 2013), despite a recent report about the misuse of MSC certification for *Dissostichus* sp. (Marko, Nance, & Guynn, 2011). Most of the Patagonian toothfish is harvested in distant waters of Antarctica, frozen onboard factory vessels and shipped several weeks to several months later (https://swfsc.noaa.gov/uploadedFiles/Operating_units/AERD/Fish/Chilean_Sea_Bass_fact_sheet.pdf), complicating the traceability. Thus, the commercialization of these species under a false name put into light a probable collateral flow that allow the recycling of illegal product on the market. Importation of IUU seafood from China has already been reported (Pramod, Nakamura, Pitcher & Delagran, 2014) This hypothesis is supported by their geographical distribution, which is completely different from the geographical range of *G. morhua* (Fig. 2), and their high economic value. Considering that, the Patagonian toothfish is one of the most valuable fish species on the US, Japanese and European markets, (http://thefishproject.weebly.com/iuu-fishing-of-the-patagonian-toothfish.html) and that it was one of the most replaced fish on the US market (Warner, Timme, Lowell, & Hirshfield, 2013), the substitution of Cod with Patagonian toothfish seems economically disadvantageous. This occurrence can probably be explained taking into consideration the lack of demand by Chinese consumers for this particular species.

Other than the commercial issues, it is important to emphasize that deep-water populations, such as *R. hippoglossoides* and *Dissostichus* sp., and ecosystems may be even more vulnerable to disruption than those of the continental shelf. In fact, deep-water species are slow growing, mature late and thus slow recovering from over-exploitation (Haedrich et al., 2001; Sumaila et al., 2015).

3.4.2 Tetraodontiformes: economic and health implications. Nine products were identified as *Lagocephalus* spp. (Tetraodontidae, Tetraodontiformes), a genus that includes toxic species known as Puffer fishes. Even though the system was not able to distinguish among *L. spadiceus*, *L. inermis*, *L. gloveri* and *L. wheeleri*, concerns arise from the possible presence of Tetrodotoxin, a neurotoxin with violent paralyzing effect. Despite the muscle is usually non-toxic or weakly toxic, cross-contamination with poisonous organs, such as the liver or the skin, can occur if the fish are not properly prepared. Moreover, toxicity may vary among species and individuals, according to physiological and environmental factors (Mosher & Fuhrman, 1984).

Incidents related to poisonous Pufferfish occurred in Brazil (de Souza Simões, Mendes, Adão, & Junior, 2014), Taiwan (Huang et al., 2014) and USA (Cohen et al., 2009). Many countries have issued specific rules for the commercialization of these species. While in Europe they are banned from the market (Regulation EC No 854/2004), in Asian countries, such as Taiwan, some species are commercialized. In China mainland the commercialization of puffer fish was prohibited by the "*Sanitary management for fishery products*" in 1990 (http://www.moh.gov.cn/mohzcfgs/s3576/200804/29459.shtml). However this rule was canceled in 2010 and updates have not been promulgated until now. Despite the removal of the ban, the commercialization of Pufferfish in China is still strictly controlled due to the health risk. However, as mentioned, products containing undeclared Pufferfish are still present on the market (Table 1SM).

In the present study, all the products identified as *Lagocephalus* sp. were commercialized under the generic name Cod (Table 4). Clearly, in this case the substitution could not be unintentionally due to the obvious morphological differences between Tetraodontiformes and Gadiformes and to the different geographical area of distribution (Fig. 2). Thus, the possibility to recycle non marketable potentially toxic species could enormously increase the economical profit.

4. Conclusions

In this study, a very high rate of mislabeling has been highlighted in seafood products sold on the Chinese market with the term *Xue* (alone or in combination with other terms). Our results suggest that the absence of a standardized nomenclature together with the unfamiliarity of Chinese FBOs and consumers to the new marine species create the ideal scenario for perpetuating economic and health frauds favoring the misrepresentation of Cod products. In fact, other than *Gadus* spp., other species of Gadiformes, but also Perciformes, Pleuronectiformes and Tetraodontiformes species have been marketed under the name *Xue*. The species found present ecological issues due to their overexploitation or health implications for the consumers due to their toxic potential. Thus, considering the key role of China in the world's seafood industry, the implementation of a traceability system for the seafood supply chain, also supported by molecular analysis, is strongly auspicable. Finally, considering that consumers' behavior is fundamental for the conservation of marine species, the utilization of fake or deceptive denomination compromises the ability of even the most knowledgeable people to make informed choice.

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Figure caption

Figure 1. Composition of Cod products analyzed in this work by DNA barcoding

Figure 2. Geographical distribution (on the basis of the native range reported by Fishbase) of the species most frequently used to replace Cod products in this study.

***Highlights (for review)**

DNA barcoding was used to evaluate the identity of fish sold as 鳕 (*Xue*=Cod)

Fish samples were purchased in supermarkets (Nanjing and Shanghai) and online

An impressive mislabeling rate, always standing over 60%, was discovered

Perciformes, Pleuronectiformes and toxic Pufferfish were sold under the name *Xue*

Economic, ecological and health issues arise from the misuse of the term Cod

Table

Table 1 Chinese denominations reported on the codfish samples collected in this study with the corresponding pinyin, English translation and scientific name.

Chinese name	鳕鱼	银鳕	黑线鳕	绿青鳕	大西洋鳕	细鳞壮鳕	阿拉斯加狭鳕	扁鳕	水鳕
Chinese pinyin	*Xue Yu*	*Yin Xue*	*Hei Xian Xue*	*Lv Qing Xue*	*Da Xi Yang Xue*	*Xi Lin Zhuang Xue*	*A La Si Jia Xia Xue*	*Bian Xue*[c]	*Shui Xue*[c]
English name[a]	Cod	Silver cod/ Sablefish[b]	Haddock	Saithe	Atlantic cod	Giant grenadier	Alaska Pollock	Flat cod	Water cod
FishBase/ASFIS valid name	--	*Anoplopoma fimbria*	*Melanogrammus aeglefinus*	*Pollachius virens*	*Gadus morhua*	*Albatrossia pectoralis*	*Theragra chalcogramma*	--	--

[a]the Chinese name was literally translated to English by a Chinese native speaker also with the aid of an online translation tool (https://translate.google.it/) and then verified consulting the ASFIS list (http://www.fao.org/fishery/collection/asfis/en), FishBase (http://www.fishbase.org/search.php) and the Latin-Chinese Dictionary of Fish Name (http://fishdb.sinica.edu.tw/eng/chinesequer1.php).

[b] 银鳕 (*Yin Xue*) literally means Silver cod, which in English does not refer to any species. In Chinese *Yin Xue* refers to *A. fimbria* (http://zh.wikipedia.org/wiki/銀鱈). The correct English name for this species is Sablefish (http://www.fishbase.org/summary/ANOPLOPOMA-FIMBRIA.html).

[c]*Bian Xue* (扁鳕) and *Shui Xue* (水鳕) were literally translated by a Chinese native speaker as Flat cod and Water cod and they do not correspond to any species or commercial denomination.

Table

Table 2 Product information (product presentation, product state, geographical origin, product name, denominations in the ingredient list) and comparison with the identification results based on cytochrome *c* oxidase I (COI) of the 34 supermarket samples

Code	Product presentation	Product state	Geographical origin	Product name: Chinese name, pinyin, English translation and additional names[a]	Commercial (Chinese name, pinyin, English translation) or scientific name[a] in the ingredient list	BOLD ID System	BLAST NCBI (Max id.)
SM1	in bulk	fresh	USA (no other specifications)	鳕鱼 *Xue Yu* Cod	--	*Dissostichus eleginoides* 100-98.16%	*Dissostichus eleginoides* 100-99%
SM2	in bulk	fresh	Russia (no other specifications)	扁鳕 *Bian Xue* Flat cod	--	*Macrourus carinatus* 100-99.53% *Macrourus holotrachys* 100-97.98% *Macrourus whitsoni* 98.44-98.14%	*Macrourus carinatus* 100-99%
SM3	prepackaged	frozen	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Macrourus carinatus* 100-99.53% *Macrourus holotrachys* 100-97.95% *Macrourus whitsoni* 98.41-98.1%	*Macrourus carinatus* 100-99%
SM4	prepackaged	frozen	Country of origin: Russia	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Macrourus carinatus* 100-99.52% *Macrourus holotrachys* 100-97.94% *Macrourus whitsoni* 98.41-97.94%	*Macrourus carinatus* 100-99%
SM8	prepackaged	frozen	Country of origin: Alaska	鳕鱼 *Xue Yu* Cod	比目鱼 *Bi Mu Yu* Flatfish	*Atheresthes stomias* 100-99.04% *Atheresthes evermanni* 98.45% (1 seq.)	*Atheresthes stomias* 100-99% *Atheresthes evermanni* 99%
SM9	prepackaged	frozen	Country of origin: Alaska	鳕鱼 *Xue Yu* Cod	比目鱼 *Bi Mu Yu* Flatfish	*Atheresthes evermanni* 99.83% (1 seq.) *Atheresthes stomias* 98.58-97.76%	*Atheresthes evermanni* 99% *Atheresthes stomias* 99-98%
SM10	prepackaged	frozen	Country of origin: USA	银鳕 *Yin Xue* Silver cod	银鳕 *Yin Xue* Silver cod	*Dissostichus mawsoni* 100%	*Dissostichus mawsoni* 100%
SM11	prepackaged	frozen	Country of origin: France	银鳕 *Yin Xue* Silver cod	银鳕 *Yin Xue* Silver cod	*Dissostichus mawsoni* 100-99.84%	*Dissostichus mawsoni* 100-99%
SM12	prepackaged	frozen	Country of origin: Russia Capture area: Bering Sea	细鳞壮鳕 *Xi Lin Zhuang Xue* Giant grenadier *Albatrossia pectoralis*[a]	细鳞壮鳕 *Xi Lin Zhuang Xue* Giant grenadier *Albatrossia pectoralis*[a]	*Albatrossia pectoralis* 100% *Coryphaenoides acrolepis* 100-99.38% *Coryphaenoides longifilis* 99.21% (1 seq.)	*Albatrossia pectoralis* 100-99% *Coryphaenoides acrolepis* 100-99% *Coryphaenoides longifilis* 99% (1 seq.)
SM13	prepackaged	frozen	Country of origin: Russia Capture area: Bering Sea	细鳞壮鳕 *Xi Lin Zhuang Xue* Giant grenadier *Albatrossia pectoralis* [a]	细鳞壮鳕 *Xi Lin Zhuang Xue* Giant grenadier *Albatrossia pectoralis*[a]	*Albatrossia pectoralis* 100% *Coryphaenoides acrolepis* 100-99.38% *Coryphaenoides longifilis* 99.21% (1 seq.)	*Albatrossia pectoralis* 100-99% *Coryphaenoides acrolepis* 100-99% *Coryphaenoides longifilis* 99% (1 seq.)
SM14	in bulk	frozen	Not indicated	鳕鱼 *Xue Yu* Cod	--	*Coryphaenoides acrolepis* 100-99.37% *Albatrossia pectoralis* 100% *Coryphaenoides longifilis* 99.21% (1 seq.)	*Albatrossia pectoralis* 100% *Coryphaenoides acrolepis* 100-99% *Coryphaenoides longifilis* 99% (1 seq.)
SM15	in bulk	fresh	Country of origin: Chile	银鳕 *Yin Xue* Silver cod	--	*Dissostichus eleginoides* 100-98.79%	*Dissostichus eleginoides* 100-99%

SM16	in bulk	fresh	Country of origin: Chile	银鳕 *Yin Xue* Silver cod	--	*Dissostichus eleginoides* 100-98.8%	*Dissostichus eleginoides* 100-99%
SM17	in bulk	frozen	Not indicated	鳕鱼 *Xue Yu* Cod	--	*Albatrossia pectoralis* 99.84% *Coryphaenoides acrolepis* 99.84-99.21% *Coryphaenoides longifilis* 99.04% (1 seq.)	*Albatrossia pectoralis* 99% *Coryphaenoides acrolepis* 99% *Coryphaenoides longifilis* 99% (1 seq.)
SM18	in bulk	frozen	Not indicated	鳕鱼 *Xue Yu* Cod	--	*Albatrossia pectoralis* 99.84% *Coryphaenoides acrolepis* 99.84-99.21% *Coryphaenoides longifilis* 99.04% (1 seq.)	*Albatrossia pectoralis* 99% *Coryphaenoides acrolepis* 99% *Coryphaenoides longifilis* 99% (1 seq.)
SM19	in bulk	frozen	Not indicated	银鳕 *Yin Xue* Silver cod	--	*Dissostichus eleginoides* 100-98.97%	*Dissostichus eleginoides* 100-99%
SM20	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM21	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM22	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	no sequences obtained	no sequences obtained
SM23	in bulk	frozen	Not indicated	银鳕 *Yin Xue* Silver cod	--	*Dissostichus eleginoides* 100-98.79%	*Dissostichus eleginoides* 100-99%
SM24	prepackaged	frozen	Country of origin: Norway	绿青鳕 *Lv Qing Xue* Saithe[a]	绿青鳕 *Lv Qing Xue* Saithe *Pollachius virens*[a]	*Pollachius virens* 100-99.53%	*Pollachius virens* 100-99%
SM25	prepackaged	frozen	Country of origin: Norway	绿青鳕 *Lv Qing Xue* Saithe[a]	绿青鳕 *Lv Qing Xue* Saithe *Pollachius virens*[a]	*Pollachius virens* 100-99.67%	*Pollachius virens* 100-99%
SM26	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM27	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM28	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Gadus chalcogrammus*[b] 100-99.83%	*Gadus chalcogrammus*[b] 100-99%
SM29	in bulk	frozen	Country of origin: Malaysia	银鳕 *Yin Xue* Silver cod	--	*Dissostichus mawsoni* 100-99.84%	*Dissostichus mawsoni* 100-99%

SM30	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod *Plecoglossus altivelis*[a]	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM31	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod *Plecoglossus altivelis*[a]	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM32	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.78% *Lagocephalus inermis* 100-99.84% *Lagocephalus gloveri* 100-91.01% *Lagocephalus wheeleri* 100-91.23%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM33	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus spadiceus* 100-93.43% *Lagocephalus inermis* 100-99.83% *Lagocephalus gloveri* 100-90.91% *Lagocephalus wheeleri* 100-91.08%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 100-99% *Lagocephalus gloveri* 99%
SM34	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Gadus chalcogrammus*[b] 100-99.65%	*Gadus chalcogrammus*[b] 100-99%
SM35	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Lagocephalus wheeleri* 100-99.53% *Lagocephalus* cf. *spadiceus* 99.84% *Lagocephalus gloveri* 99.68% *Lagocephalus spadiceus* 99.68-93.46% *Lagocephalus inermis* 99.51%	*Lagocephalus wheeleri* 100-99% *Lagocephalus spadiceus* 99% *Lagocephalus gloveri* 99%
SM36	prepackaged	frozen	Not indicated	鳕鱼 *Xue Yu* Cod Pollock[a]	水鳕 *Shui Xue* Water cod	*Gadus chalcogrammus*[b] 99.79-99.17%	*Gadus chalcogrammus*[b] 99%
SM37	prepackaged	roasted	Not indicated	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Coryphaenoides acrolepis* 100-99.37% *Albatrossia pectoralis* 100% *Coryphaenoides longifilis* 99.21% (1 seq.)	*Albatrossia pectoralis* 100-99% *Coryphaenoides acrolepis* 100-99% *Coryphaenoides longifilis* 99% (1 seq.)

In grey are the products mislabeled according to the most stringent definition of the term Cod, which has been considered not corresponding to any species (see Section 3.3.1)
[a]in some cases an English or a Latin name was additionally declared
[b]valid name *Theragra chalcogramma*

Table

Table 3 Product information (product presentation, product state, geographical origin, product name, denominations in the webpage and on the received product) and comparison with the identification results based on cytochrome *c* oxidase I (COI) of the 18 online samples

Code	Product presentation	Product state	Geographical origin	Product name: Chinese name, pinyin, English translation	Commercial (Chinese name, pinyin, English translation) and Scientific name[a] in the webpage description	Product name (Chinese name, pinyin, English translation) and additional names[a] on the received product	BOLD ID System	BLAST NCBI (Max id.)
ON1	in bulk	frozen	Area of origin: Chile Captured in Alaska/Chile[c]	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	No label	No match	*Reinhardtius hippoglossoides* 96% *Photostomias guernei* 96%
ON2	prepackaged	frozen	Area of origin:Antartic	银鳕 *Yin Xue* Silver cod	南极银鳕 *Nan Ji Yin Xue* Antartic cod	银鳕 *Yin Xue* Silver Cod Tooth fish[a]	*Dissostichus eleginoides* 100-98.8%	*Dissostichus eleginoides* 100-99%
ON3	in bulk	frozen	France	银鳕 *Yin Xue* Silver cod	银鳕 *Yin Xue* Silver cod *Anoplopoma fimbria*[a]	银鳕 *Yin Xue* Silver cod	*Dissostichus eleginoides* 100-98.79%	*Dissostichus eleginoides* 100-99%
ON4	prepackaged	frozen	Capture area: North Pacific Ocean	阿拉斯加鳕鱼 *A La Si Jia Xue Yu* Alaska pollock	阿拉斯加鳕鱼 *A La Si Jia Xue Yu* Alaska pollock	阿拉斯加鳕鱼 *A La Si Jia Xue Yu* Alaska pollock	*Gadus chalcogrammus*[b] 100-99.8%	*Gadus chalcogrammus*[b] 100-99%
ON5	in bulk	frozen	Area of origin: Chile Captured in Alaska/Chile[c]	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	No label	No match	*Reinhardtius hippoglossoides* 96% *Photostomias guernei* 96%
ON6	in bulk	frozen	Captured in Chile/Alaska[c]	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	No label	*Reinhardtius hippoglossoides* 100-99.36%	*Reinhardtius hippoglossoides* 99%
ON7	prepackaged	frozen	Capture area: North Atlantic	大西洋鳕鱼 *Da Xi Yang Xue* Yu Atlantic cod	大西洋鳕鱼 *Da Xi Yang Xue Yu* Atlantic cod	大西洋鳕鱼 *Da Xi Yang Xue Yu* Atlantic cod	*Gadus morhua* 100-99.33% *Gadus chalcogrammus*[b] 98.63-98.48%	*Gadus morhua* 99%
ON8	in bulk	frozen	Area of origin: Alaska	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	No label	*Reinhardtius hippoglossoides* 100-99.36%	*Reinhardtius hippoglossoides* 100-99%
ON9	prepackaged	frozen	Area of origin: Norway	黑线鳕 *Hei Xian Xue* Haddock	黑线鳕 *Hei Xian Xue* Haddock	黑线鳕 *Hei Xian Xue* Haddock	*Melanogrammus aeglefinus* 99.84-99.37%	*Melanogrammus aeglefinus* 99%
ON10	in bulk	frozen	Area of origin: Russia	水鳕 Shui Xue Water cod	鳕鱼 Xue Yu Cod	No label	*Coryphaenoides acrolepis* 100-99.37% *Albatrossia pectoralis* 100% *Coryphaenoides longifilis* 99.2% (1 seq.)	*Albatrossia pectoralis* 99% *Coryphaenoides acrolepis* 99% *Coryphaenoides longifilis* 99% (1 seq.)

ON11	in bulk	frozen	Area of origin: France Captured in Antartic/North Atlantic[c]	银鳕 *Yin Xue* Silver cod	银鳕 *Yin Xue* Silver cod	No label	*Dissostichus eleginoides* 100-98.96%	*Dissostichus eleginoides* 100-99%
ON12	in bulk	frozen	Area of origin: France	银鳕 *Yin Xue* Silver cod	银鳕 *Yin Xue* Silver cod *Dissostichus eleginoides*[a]	银鳕 *Yin Xue* Silver cod	*Dissostichus eleginoides* 100-98.96%	*Dissostichus eleginoides* 100-99%
ON13	prepackaged	frozen	Area of origin: Russia	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Coryphaenoides acrolepis* 100-99.37% *Albatrossia pectoralis* 100% *Coryphaenoides longifilis* 99.2% (1 seq.)	*Albatrossia pectoralis* 99% *Coryphaenoides acrolepis* 99% *Coryphaenoides longifilis* 99% (1 seq.)
ON14	in bulk	frozen	Captured in Atlantic	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Coryphaenoides acrolepis* 100-99.37% *Albatrossia pectoralis* 100% *Coryphaenoides longifilis* 99.2% (1 seq.)	*Albatrossia pectoralis* 99% *Coryphaenoides acrolepis* 99% *Coryphaenoides longifilis* 99% (1 seq.)
ON15	in bulk	frozen	Area of origin: Canda Captured in Norh Atlantic	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	No label	No match	*Reinhardtius hippoglossoides* 96% *Photostomias guernei* 96%
ON16	in bulk	frozen	Area of origin: Norway Captured in North Atlantic	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod	*Atheresthes stomias* 99.84-98.88%	*Atheresthes stomias* 99%
ON17	prepackaged	frozen	Captured in FAO61 FAO27	鳕鱼 *Xue Yu* Cod	鳕鱼 *Xue Yu* Cod *Gadus macrocephalus-Gadus morhua*[a]	鳕鱼 *Xue Yu* Cod	*Gadus morhua* 100-99.35% *Gadus chalcogrammus*[b] 98.51%	*Gadus morhua* 100-99%
ON18	prepackaged	frozen	Captured in North Atlantic Ocean	鳕鱼 *Xue Yu* Cod	大西洋鳕鱼 *Da Xi Yang Xue Yu* Atlantic Cod	大西洋鳕鱼 *Da Xi Yang Xue Yu* Atlantic Cod Atlantic Cod [a]	*Gadus morhua* 100-99.34% *Gadus chalcogrammus*[b] 98.65-98.51%	*Gadus morhua* 100-99%

In grey are the products mislabeled according to the most stringent definition of the term Cod, which has been considered not corresponding to any species (see Section 3.3.1)
[a]in some cases an English or a Latin name was additionally declared
[b]valid name *Theragra chalcogramma*
[c]inconsistencies about the declared capture area

Table

Table 4 Summarized results of the comparison between label information and molecular results

<table>
<tr><th rowspan="2">Chinese name
Pinyin
English name</th><th rowspan="2">Molecular identity</th><th colspan="3">Number of samples</th><th colspan="3">Mislabeling</th></tr>
<tr><th>Total</th><th>Supermarket</th><th>Online market</th><th>Cod = Gadiformes</th><th>Cod = Gadus spp.</th><th>Cod not referable to any specific species</th></tr>
<tr><td rowspan="9">鳕鱼
Xue Yu
Cod</td><td>Gadus morhua</td><td>2</td><td>0</td><td>2</td><td>V</td><td>V</td><td>X</td></tr>
<tr><td>Theragra chalcogramma</td><td>3</td><td>3</td><td>0</td><td>V</td><td>X</td><td>X</td></tr>
<tr><td>Macrourus carinatus</td><td>2</td><td>2</td><td>0</td><td>V</td><td>X</td><td>X</td></tr>
<tr><td>Coryphaenoides sp.
Albatrossia pectoralis</td><td>6</td><td>4</td><td>2</td><td>V</td><td>X</td><td>X</td></tr>
<tr><td>Dissostichus eleginoides</td><td>1</td><td>1</td><td>0</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Atheresthes stomias</td><td>3</td><td>2</td><td>1</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Reinhardtius hippoglossoides</td><td>2</td><td>0</td><td>2</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Unidentified flatfish</td><td>3</td><td>0</td><td>3</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Lagocephalus sp.</td><td>9</td><td>9</td><td>0</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td rowspan="2">银鳕
Yin Xue
Silver cod</td><td>Dissostichus mawsoni</td><td>3</td><td>3</td><td>0</td><td colspan="3">X</td></tr>
<tr><td>Dissostichus eleginoides</td><td>8</td><td>4</td><td>4</td><td colspan="3">X</td></tr>
<tr><td>细鳞壮鳕
Xi Lin Zhuang Xue
Giant grenadier</td><td>Albatrossia pectoralis
Coryphaenoides sp.</td><td>2</td><td>2</td><td>0</td><td colspan="3">V</td></tr>
<tr><td>绿青鳕
Lv Qing Xue
Saithe</td><td>Pollachius virens</td><td>2</td><td>2</td><td>0</td><td colspan="3">V</td></tr>
<tr><td>扁鳕
Bian Xue
Flat cod</td><td>Macrourus carinatus</td><td>1</td><td>1</td><td>0</td><td colspan="3">X</td></tr>
<tr><td>水鳕
Shui Xue
Water cod</td><td>Theragra chalcogramma</td><td>1</td><td>0</td><td>1</td><td colspan="3">X</td></tr>
<tr><td>阿拉斯加狭鳕
A La Si Jia Xia Xue
Alaska Pollock</td><td>Theragra chalcogramma</td><td>1</td><td>0</td><td>1</td><td colspan="3">V</td></tr>
<tr><td>大西洋鳕
Da Xi Yang Xue
Atlantic cod</td><td>Gadus morhua</td><td>1</td><td>0</td><td>1</td><td colspan="3">V</td></tr>
</table>

黑线鳕 *Hei Xian Xue* Haddock	*Melanogrammus aeglefinus*	1	0	1	V

Mislabeled products are highlighted in grey and indicated with a X, while correctly labeled products are indicated with a V

Figure
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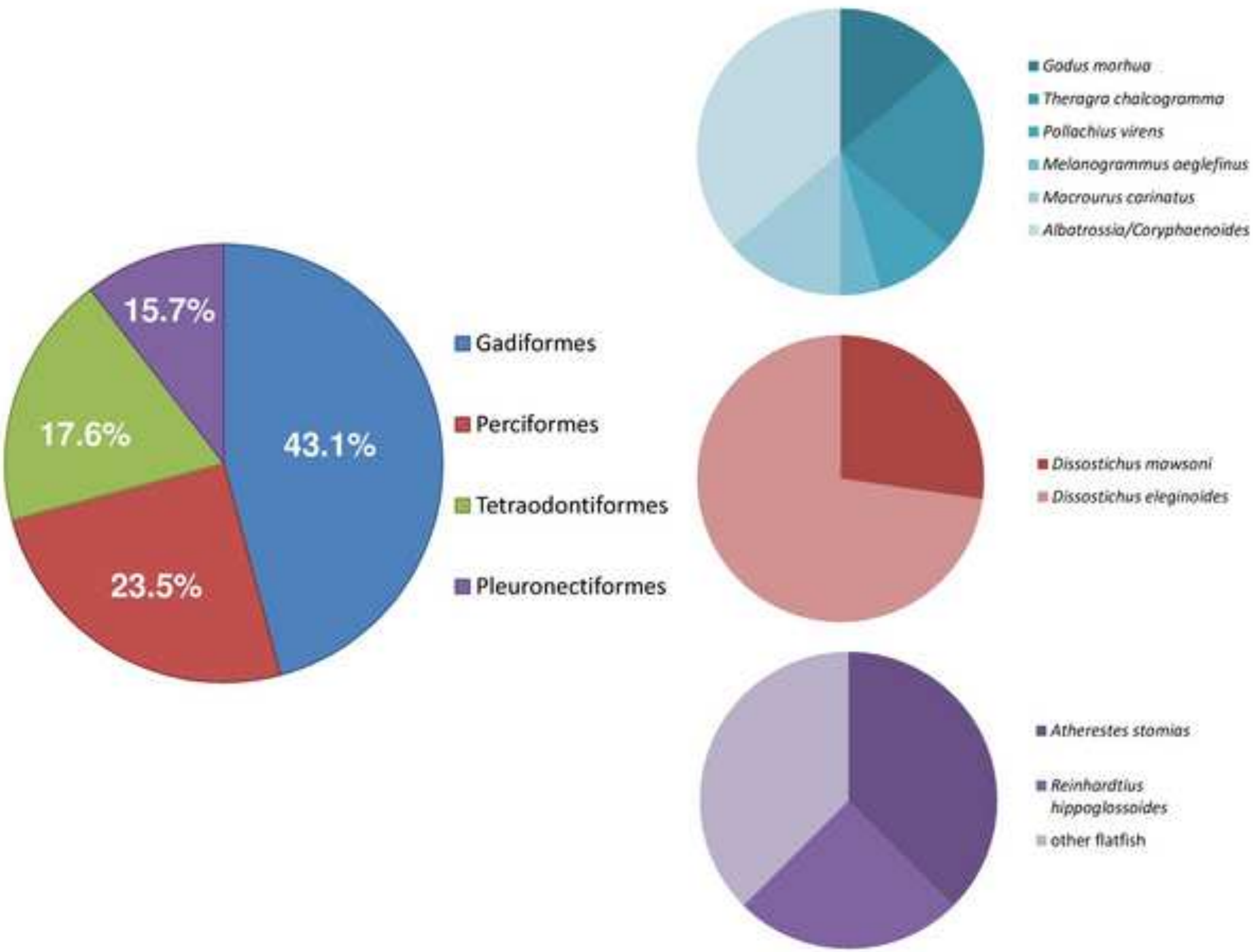

Figure
Click here to download high resolution image

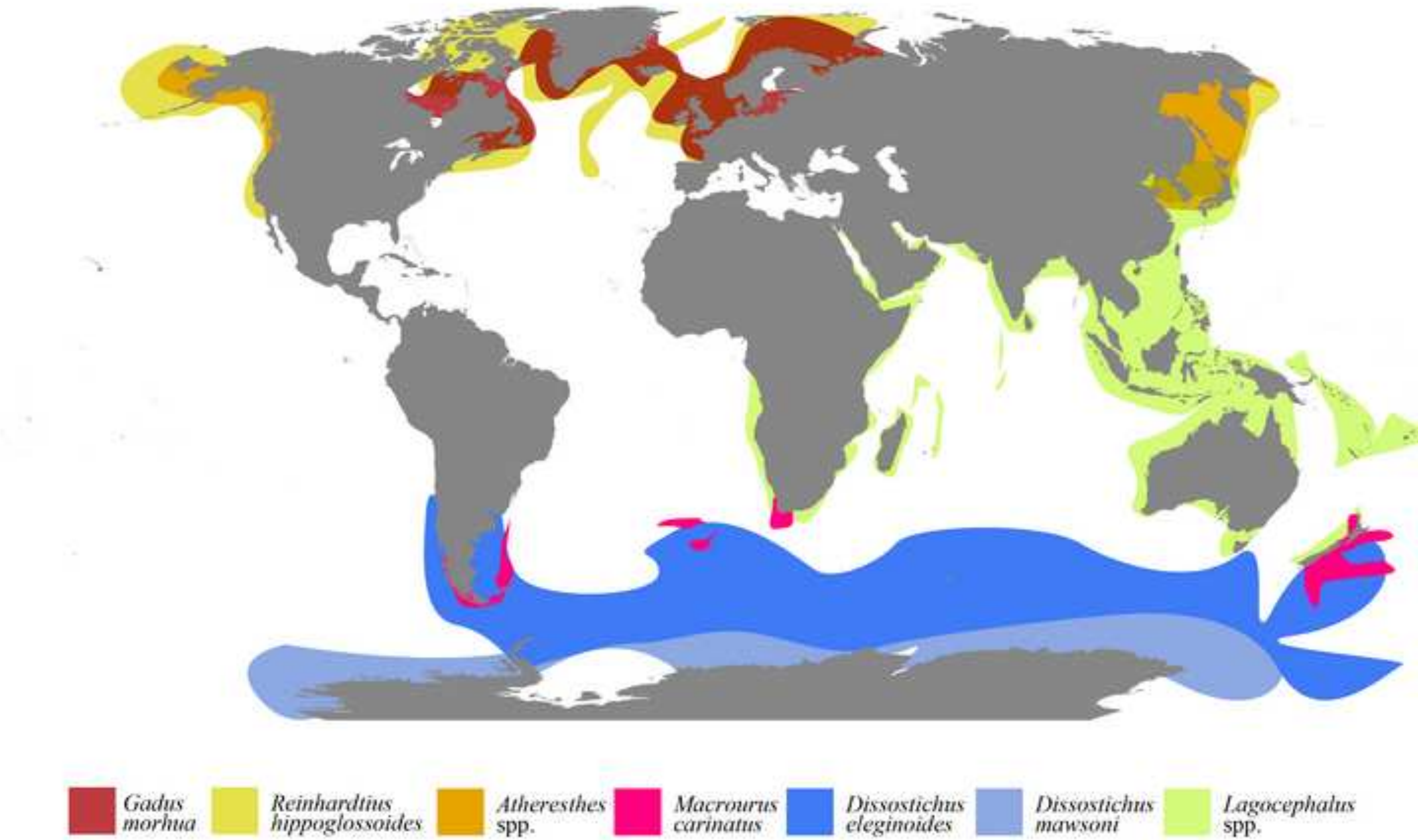

Table 1SM Studies on mislabeling of cod products

Reference	Sampling area	Analytical method	Product name	Sample number	Species found (n, if available)	Mislabel %
Helyar et al., 2014	United Kingdom	DNA barcoding (COI FDB)	Whitefish processed products	386	***Gadus macrocephalus*** ***Gadus morhua*** ***Melanogrammus aeglefinus*** ***Theragra chalcogramma*** ***Pangasius hypophthalamus*** ***Merluccius paradoxus*** ***Micromesistius poutassou***	5.66%
Oceana, 2014	Copenaghen	DNA barcoding (COI FDB)	Cod	120	***Melanogrammus aeglefinus*** ***Pollachius virens***	18%
Mariani et al., 2014	Ireland	DNA barcoding (COI FDB)	Cod	24[a]	*Gadus morhua* (14) ***Pollachius virens* (9)** ***Melanogrammus aeglefinus* (1)**	42%
Shen et al., 2014	China	DNA barcoding (COI 821bp)	Roasted cod fillet	7	***Lagocephalus lunaris*** (7)	100%
Di Pinto et al., 2013	Italy	DNA barcoding (COI FDB)	"Baccalà"[b]	70 dried salted cod fillets	*Gadus macrocephalus* *Gadus morhua* ***Pollachius virens*** ***Brosme brosme***	30%
				40 battered cod chunks	***Pollachius virens*** ***Brosme brosme***	100%
Li et al., 2013	China	DNA barcoding (COI FDB)	Roasted cod fillets	7	***Liparis tanakai* (4)** ***Saurida sp.1*(1)** ***Lagocephalus lunaris* (3)**	100%
			Frozen Cod fillet	7	*Theragra chalcogramma* (5) *Pollachius virens* (1) ***Dissostichus eleginoides* (1)**	14.3%
			Frozen sablefish fillet	1	***Dissostichus eleginoides***	100%
			Frozen Alaska pollock fillet	1	*Theragra chalcogrammus*	0%
			Frozen Greenland cod fillet	1	*Gadus morhua*	0%
Warner et al., 2013	USA	DNA barcoding (COI FDB)	Atlantic cod/scrod cod	116	*Gadus morhua* ***Gadus macrocephalus* (11)** ***Hippoglossus stenolepis* (Pacific Halibut) (1)** ***Sciaenops ocellatus* (Red drum) (1)** ***Urophycis tenuis* (White hake)(1)**	28%
			Pacific cod		*Gadus macrocephalus* ***Gadus morhua* (4)** **Striped pangasius (*Pangasius hypophthalmus*)(1)**	

					Yelloweye rockfish **(*Sebastes ruberrimus*) (1)** **Threadfin slickhead (*Talismania bifurcata*) (1)** **Tilapia (*Oreochromis niloticus*)(1)**	
			Rock cod		**Redbanded rockfish (*Sebastes babcocki*)(1)** **Olive rockfish (*Sebastes serranoides*)(1)** **Chilipepper rockfish (*Sebastes goodei*)(1)** **Madai (*Opistognathus hopkinsi*)(1)** **Striped pangasius (*Pangasius hypophthalmus*)(2)**	
Hwang et al., 2012	Taiwan	Direct sequencing (Cytb 452bp) and PCR-RFLP	Cod steaks	41	*Gadus macrocephalus (21)* *Gadus morhua (7)* ***Ruvettus pretiosus (7)*** ***Reinhardtius hipoglossoides (6)***	32%
Miller and Mariani, 2010	Ireland	DNA barcoding (COI FDB)	cod	131	***Pollachius virens*** ***Pollachius pollachius*** ***Argentina silus*** ***Melanogrammus aeglefinus*** ***Merlangius merlangius*** *Gadus morhua*	28.2%
Ling et al., 2008	Hong Kong	PCR-sequencing (COI, Cytb, 16S, 12S)	Cod fish	2	***Ruvettus pretiosus*** **(2)**	67%
			Canada cod fish	1	***Ruvettus pretiosus***	
			Canada silver cod	1	***Ruvettus pretiosus***	
			Codfish (Oilfish, *Ruvettus pretiosus*)	1	***Ruvettus pretiosus***	
			White cod fish	1	***Ruvettus pretiosus***	
			Yellow cod fish	1	***Ruvettus pretiosus***	
			Canada black cod	3	*Anoplopoma fimbria* (3)	
			Siver cod fish	2	***Reinhardtius hipoglossoides*** **(1)** *Gadus morhua* (1)	

[a]The number is referred to the samples purchased in "fish and chips" shops. Other 42 samples purchased in supermarkets were correctly labeled (according to the denomination "cod"), but no specific data are reported.
[b]According to the Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008, baccalà can be obtained exclusively from *G. macrocephalus* (Pacific cod) and *G. morhua* (Atlantic cod)

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