Multiple fish species identified from China's roasted *Xue Yu* fillet products using DNA and mini-DNA barcoding: Implications on human health and marine sustainability

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Abstract

Roasted *Xue Yu* fillet is among the most common fish products in China and the market appealing can be reflected by its high price that occasionally exceeds 300 RMB/kg in local supermarket. However, due to the lack of harmonization around the definition of *Xue Yu*, as well as the disability of visual inspection for transformed fish products, China's roasted *Xue Yu* fillet products are quite deep in the scandal of species adulteration. The objective of this study is to apply DNA and mini-DNA barcoding for the species identification of 153 roasted *Xue Yu* fillet products, on behalf of 30 brands, collected from 16 cities of China. The mislabeling rate was assessed according to three increasingly stringent definitions: 1) *Xue Yu* meaning Gadiformes species; 2) *Xue Yu* meaning Gadidae species; 3) *Xue Yu* meaning *Gadus* spp.

Results highlighted a very high mislabeling rate, which reached 58% even with the least stringent definition. Only 42% of the samples were identified as belonging to Gadiformes, while the others were Scorpaeniformes, Tetraodontiformes and Lophiiformes. Moreover, the implications on human health and marine sustainability were also discussed, given the identification of poisonous *Lagocephalus* spp. from 37 samples and the China's rising consumption of marine resources.

Keywords: Roasted *Xue Yu* fillet products; DNA and mini-DNA barcoding; Species mislabeling; Pufferfish; Marine conservation

1 Introduction

The catalytic growth of scientific literature dealing with seafood traceability during the last five years demonstrated that seafood species adulteration has emerged as a very common issue prevailing on the global market

(Armani, Guardone, Castigliego, et al., 2015; Cawthorn, Duncan, Kastern, Francis, & Hoffman, 2015; Chin, Bakar, Zainal Abidin, & Mohd Nor, 2016; Günther, Raupach, & Knebelsberger, 2017; Mu Oz-Colmenero et al., 2015; Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015; Wen et al., 2016; Xiong et al., 2015; Zhao et al., 2013). Several reasons accounting for this fraudulent conduct include the rising global seafood trade, particularly of processed seafood products (Armani, Guardone, La Castellana, Gianfaldoni, Guidi & Castigliego, 2015), the depleted fishery resources (Marko et al., 2004; Miller & Mariani, 2010), as well as the absence of policies governing seafood labeling and adequate enforcement in some countries (Barendse & Francis, 2015; Miller, Jessel, & Mariani, 2012; Xiong et al., 2016). In addition to the financial loss, seafood species substitution has also been considered as a great threat to human health and even the protection of depleted species (Armani, Guardone, Castigliego, et al., 2015; Bornatowski, Braga, & Vitule, 2013; Cline, 2012; Triantafyllidis et al., 2010).

Recent advancements in molecular biology contributed a lot for suppressing seafood species adulteration. In particular, the power of DNA in discriminating even closely-related seafood species has been validated (Armani, Castigliego, & Guidi, 2012; Rasmussen & Morrissey, 2008). Over the past decades, many DNA-based identification methods have been developed and DNA barcoding of a ~655 bp region of the mitochondrial *cytochromec-oxidase* I (COI) gene (Full DNA Barcoding, FDB) is among the most used approaches (Galimberti et al., 2013; Hebert, Ratnasingham, & de Waard, 2003). In addition, the utilization of a mini DNA barcoding (MDB) of less than 600 bp has recently been proved to be a feasible alternative for the identification of highly processed foods (Armani, Guardone, Castigliego, et al., 2015; Shokralla et al., 2015).

Roasted fish fillet (Fig. 1) is among the most common fish products in China. It is generally obtained from boneless fish fillet through a series of processing steps: soaking in seasoning for 1–2 h, pre-drying below 55 °C for 9–14 h followed by roasting at 150–250 °C for less than 10 min, and finally rolling to obtain the soft texture (Hsieh et al., 2010; Zhao, 2006). In particular, given the great popularity and preference with *Xue Yu* in Chinese public (Xiong et al., 2016), an increasing number of roasted fish fillets are now sold under the name *Xue Yu*. The market appealing of roasted *Xue Yu* fillet products can be reflected by its high price that occasionally exceeds 300 RMB per kg in local supermarket (Table 1).

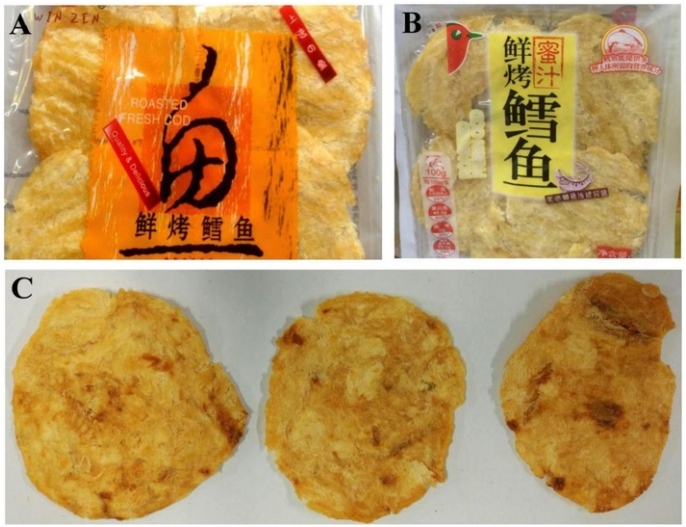

Fig. 1 Presentation of the purchased products: single package (A, B) and multiple slices (C).

alt-text: Fig. 1

Table 1 Sample information and the comparison with the identification results.

alt-text: Table 1

Sampling place		Brand	Price[a] (RMB/kg)	Species declared			Sample code	Species identified	
Province/province-level municipality	City			Chinese name (pinyin)	English name	Scientific name		NCBI Genbank database	BLOD database
Shanghai	Shanghai	YZ 1	270	*Xue Yu*	Cod	NR	NJ1-NJ20	*Lagocephalus spadiceus* 98–99% *Lagocephalus wheeleri* 99%	*Lagocephalus spadiceus* 98–99.42% *Lagocephalus wheeleri* 99.87%

								Lagocephalus inermis 98%	*Lagocephalus inermis* 98.22%
		YZ 2	296	*Xue Yu*	NR	NR	NJ21-NJ22	*Lagocephalus spadiceus* 98–99% *Lagocephalus wheeleri* 99% *Lagocephalus inermis* 98%	*Lagocephalus spadiceus* 98.33–99.4% *Lagocephalus wheeleri* 99.27% *Lagocephalus inermis* 98.48%
Zhejiang	Zhoushan	YZ 3	196.7	*Xue Yu*	NR	NR	NJ23-NJ27	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.45–100%
	Ningbo	YZ 4	128	*Xue Yu*	Cod	NR	NJ28-NJ35	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.07–100%
		YZ 5	165.7	*Xue Yu*	NR	NR	NJ36-NJ38	*Liparis* sp. 93%	No match
							NJ39-NJ41	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.24–100%
		YZ 6	115	*Xue Yu*	NR	NR	NJ42-NJ46	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.77–100%
	Wenzhou	YZ 7	105	*Xue Yu*	NR	NR	NJ47-NJ51	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.56–100%
Fujian	Zhangzhou	YZ 8	310	*Xue Yu*	NR	*Plecoglossus altivelis*	NJ52-NJ57	*Lagocephalus spadiceus* 98–99% *Lagocephalus wheeleri* 99% *Lagocephalus inermis* 98%	*Lagocephalus spadiceus* 98–99.37% *Lagocephalus wheeleri* 99.17% *Lagocephalus inermis* 98.22%
		YZ 9	239	*Xue Yu*	NR	NR	NJ58-NJ60	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.33–100%
	Xiamen	YZ 10	87.9	*Xue Yu*	NR	NR	NJ61-NJ65	*Liparis* sp. 96%	No match
Liaoning	Dalian	YZ 11	260	*Xue Yu*	NR	NR	NJ66-NJ69	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.35–100%
		YZ 12	186	*Xue Yu*	NR	NR	NJ70-NJ72	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.93–100%
		YZ 13	196.7	*Xue Yu*	Cod	NR	NJ73-NJ77	*Lophius litulon* 99%	*Lophius litulon* 99.75%
	Dandong	YZ 14	75	*Xue Yu*	NR	NR	NJ78-NJ82	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.84–100%
Shandong	Qingdao	YZ 15	200	*Xue Yu*	NR	NR	NJ83-NJ84	*Liparis* sp. 95%	No match
							NJ85	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.01–100%
							NJ86-NJ87	*Lophius litulon* 99%	*Lophius litulon* 99.02%
		YZ 16	158	*Xue Yu*	NR	NR	NJ88-NJ89	*Liparis* sp 96%	No match
		YZ 17	238	*Xue Yu*	NR	NR	NJ90-NJ94	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.78–100%
		YZ 18	218.8	*Xue Yu*	NR	NR	NJ95-NJ98	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.89–100%
	Yantai	YZ 19	145	*Xue Yu*	NR	NR	NJ99-NJ103	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.23–100%
		YZ 20	62	*Xue Yu*	NR	NR	NJ104-NJ110	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 99.25–100%

	Zibo	YZ 21	117.6	*Xue Yu*	NR	NR	NJ111-NJ115	*Liparis* sp. 95%	No match
Hubei	Wuhan	YZ 22	161.9	*Xue Yu*	Cod	NR	NJ116-NJ120	*Lagocephalus spadiceus* 98–99% *Lagocephalus wheeleri* 99% *Lagocephalus inermis* 98%	*Lagocephalus spadiceus* 98–99.74% *Lagocephalus wheeleri* 99.86% *Lagocephalus inermis* 98.28%
Sichuan	Chengdu	YZ 23	137.5	*Xue Yu*	Codfish	NR	NJ121-NJ125	*Liparis* sp. 95%	No match
Jiangsu	Nanjing	YZ 24	160	*Xue Yu*	NR	NR	NJ126-NJ130	*Liparis* sp. 96%	No match
		YZ 25	150	*Xue Yu*	Cod	NR	NJ131-NJ132	*Liparis* sp. 96%	No match
		YZ 26	71.9	*Xue Yu*	NR	NR	NJ133-NJ137	*Liparis*sp. 95%	No match
	Lianyungang	YZ 27	80	*Xue Yu*	NR	NR	NJ138-NJ139	*Theragra chalcogramma* 99–100%	*Theragra chalcogramma* 98.39–100%
		YZ 28	97.5	*Xue Yu*	NR	NR	NJ140-NJ144	*Lagocephalus inermis* 98% *Lagocephalus spadiceus* 98% *Lagocephalus wheeleri* 98%	*Lagocephalus spadiceus* 98–99.02% *Lagocephalus wheeleri* 99.57% *Lagocephalus inermis* 98.72%
Guangdong	Jieyang	YZ 29	90	*Xue Yu*	NR	NR	NJ145-NJ149	*Liparis* sp. 95%	No match
		YZ 30	150	*Xue Yu*	NR	NR	NJ150-NJ153	*Liparis* sp. 96%	No match

Words in grey are the samples identified using mini-DNA barcoding.

[a] Although the price was all described as RMB/kg, the roasted *Xue Yu* fillet products are generally sold as 40–150 g per package.

The term *Xue Yu*, in a broad sense, generally refers to fish of the family Gadidae and to related species within the order Gadiformes (Xiong et al., 2015). However, since specific provisions for the labeling of fishery products and a standardized seafood nomenclature in China are still not available (Xiong et al., 2016), there is still not a harmonization around the definition of *Xue Yu*. In this circumstances, producers and distributors are tempted to use species even beyond Gadiformes for the preparation of roasted *Xue Yu* fillet products (Li et al., 2013).

The situation could become even worse as the residual characteristics of roasted *Xue Yu* fillet products are often inadequate for a morphological identification. Our previous work identified several different species from thirteen roasted *Xue Yu* fillet products collected on Chinese market (Xiong et al., 2015). More interesting is that all six samples from Shanghai and all three samples from Zhangzhou, Fujian province were identified at the genus level as pufferfish, *Lagocephalus* spp., while the other three samples from Qingdao, Shandong province, either failed the amplification or were identified as *Theragra chalcogramma* and *Coryphaenoides acrolepis*/*Albatrossia pectoralis*. Only one sample was collected in Wuhan, Hubei province, and was identified as *T. chalcogramma*.

In order to assess the extent of misrepresentation and substitution with roasted *Xue Yu* fillet products occurring on this market and to investigate the possible reasons behind it, in this work we enlarged the number of sample collection to 153, on behalf of 30 brands, from 16 cities of China. FDB and MDB were employed for species authentication. This work enabled the understanding of which species are currently used in the preparation of roasted *Xue Yu* fillet products and facilitated the protection of consumers' health and even the support of marine sustainability.

2 Materials and method

2.1 Samples collection

One hundred and fifty-three packages of roasted *Xue Yu* fillet products on behalf of 30 brands were purchased from local markets in province of Liaoning, Shandong, Jiangsu, Zhejiang, Fujian, Guangdong, Hubei and Sichuan, as well as the province-level municipality of Shanghai in China (Table 1). Each package was brought to the laboratory and labeled with an internal code. The information reported on the label were registered and a visual inspection of the product content was performed by morphological analysis. All samples were stored at −20 °C for the next molecular analysis.

2.2 Molecular analysis

2.2.1 DNA extraction and gel electrophoresis

Total DNA extraction was performed following the method of Armani et al. (2014). DNA quality and concentration were determined using a NanoDrop ND-2000C spectrophotometer (NanoDrop Technologies, Wilmington, DE, US). For each sample, a standard working concentration of 100 ng/μl was prepared.

One thousand nanograms of the total DNA were electrophoresed on 1% agarose gel (BiowestRegular Agarose G-10, Shanghai, China) stained with ethidium bromide, and visualized via Molecular Imager® Gel Doc™ XR System (BIO-RAD, California, US). DNA fragment size was estimated by comparison with the standard 100bp DNA Ladder (Vazyme, Nanjing, China).

2.2.2 PCR amplification and sequencing

The DNA samples were firstly amplified using the universal primers proposed by Handy et al. (2011), for the amplification of a FDB of the COI gene. All the PCR were performed in a final volume of 20 μl containing 1 μl of a 10 × PCR buffer (Takara, Nanjing, China), 100 ng of DNA, 100 mM of each dNTP, 100 nM of each primer, 1U Perfect *Taq* DNA Polymerase and DNase free water applying a 35 cycles protocol (94 °C for 30s, 53 °C for 20s, 72°Cfor 30s) preceded by an initial activation at 94 °C for 3 min and followed by a final elongation step at 72 °C for 10 min.

The DNA of the samples that failed the amplification of the FDB region was submitted to the amplification of a ~226 bp MDB region with the primer set Mini-SH-E (Shokralla et al., 2015). PCR was repeated using the same amount of DNA and other reagents as in FDB amplification. The reaction was carried out following a 35 cycles protocol (94 °C for 40s, 46 °C for 60s, 72 °C for 30s).

All the PCR products (5 μl) were checked on a 1.8% agarose gel (Biowest Regular Agarose G-10, Shanghai, China) stained with ethidium bromide and the presence of fragments of the expected length was assessed by a comparison with the standard 100 bp DNA Ladder (Vazyme, Nanjing, China). The samples that presented the expected amplicon were sent for sequencing to the company GenScript (Nanjing, China) for purification and sequencing using ABI 3730 DNA sequencer (Applied Biosystems Division, Foster City, USA).

2.2.3 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. 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 then cross-referenced 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 (Barbuto et al., 2010). 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.

2.3 Seafood label analysis and comparison with the molecular result

A preliminary analysis of the label for all samples found that the standard for the product preparation all reported the recommended industry standard SC/T 3302-2010 "*Roasted fish fillet*" (SC/T 3302-2010). As required, the label preparation should follow the compulsory national standard GB 7718-2011 ˝*General rules for the labeling of prepackaged foods*" (GB 7718-2011). Therefore, the label evaluation was conducted according to the standard GB 7718-2011.

For analyzing the accuracy of the reported information, particularly the fish identity, the scientific names retrieved from the sequences analysis after consulting BOLD and GenBank were compared with the denominations reported on the labels. Since a standardized seafood nomenclature as stipulated in EU is still absent (D Amico, Armani, Gianfaldoni, & Guidi, 2016; Xiong et al., 2016), the mislabeling rate was calculated considering three increasingly strict definitions of *Xue Yu*: 1) *Xue Yu* meaning Gadiformes species; 3) *Xue Yu* meaning Gadidae species; 2) *Xue Yu* meaning *Gadus* spp..

3 Results and discussion

3.1 Samples collection

The 153 packages of roasted *Xue Yu* fillet products analyzed in this study fall into two broad categories according to the collection area: the majority of 143 samples from coastal regions (including Liaoning, Shandong, Jiangsu, Shanghai, Zhejiang, Fujian and Guangdong) and the rest 10 samples from inland areas (including Hubei and Sichuan).

The more focus on coastal regions is mainly due to the fact that the coastal regions have always been China's major areas for processing aquatic products. According to China Fisheries Yearbook (in some cases called China Fishery Statistical Yearbook), compiled by Fisheries Bureau of the Ministry of Agriculture of People's Republic of China and representing the most authoritative source of fishery data in China (China Fisheries Yearbook, 2016), the six coastal provinces of Liaoning, Shandong, Jiangsu, Zhejiang, Fujian and Guangdong, as well as the province-level municipality of Shanghai, in 2014 totally yielded 17.48 million tons of aquatic products, accounting for 85.1% of the overall volume. In particular, 90.4% of the production is contributed by seawater products and this ratio exceeded 95% for Shandong, Liaoning, Zhejiang and Fujian.

3.2 Molecular analysis

3.2.1 DNA extraction and amplification

The total DNA was successfully extracted from all the 153 samples, with the concentration ranging from 589 to 2235 ng/μl, and the average value of $A_{260/280} = 2.10$. In particular, 37 isolated DNA were found partial fragmentation by the DNA electrophoresis analysis (data not shown). Similar results were also revealed in our previous work (Xiong et al., 2015). In fact, food processing is a quite complex process, often involving mechanical stress, high temperature, pH variations, enzymatic activities, and even fermentations, which could affect the primary structure of DNA and thus induce DNA degradation (Gryson, 2010; Lindahl, 1993).

The 116 good quality DNA extracts were successfully amplified to produce FDB, while MDB was amplified from the remaining 37 samples that failed to amplify a FDB. This result confirmed the outcomes of the electrophoresis analysis of total DNA.

In fact, DNA barcoding has been proved a particular effective tool in the authentication of fish-based commercial products (Galimberti et al., 2013). Moreover, in order to favor the investigation of fish species substitution, the US Food and Drug Administration (FDA) has include DNA barcode data into the Regulatory Fish Encyclopedia (Yancy et al., 2008). However, DNA fragmentation for some pretreated and processed seafood products could often fail the FDB amplification. An increasing number of studies started to develop MDB of <600 bp, and its robustness in species identification from moderately or highly processed samples has been widely discussed (Armani, Guardone, Castigliego, et al., 2015; Shokralla et al., 2015). Although (Armani, Guardone, Castigliego, et al., 2015) has identified a similar performance of the 135 bp of COI gene with FDB in analyzing market samples, this short fragment in our previous work 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 (Xiong et al., 2015). While Shokralla et al. (2015) made a full evaluation of the six mini-barcode primer pairs targeting short (127–314 bp) fragments of COI gene, and found that the primer set Mini-SH-E targeting 226 bp fragment present the highest success rate towards the 44 commercial samples.

3.2.2 Sequence analysis and comparison to the databases

All the PCR products gave readable sequences. A maximum species identity in the range of 98–100% was obtained for 95 FDB and 15 MDB in both BOLD and BLAST, while the rest 21 FDB and 22 MDB did not match any reference barcode in BOLD and retrieved a top match of maximum identity of 96% with *Liparis* sp. (Scorpaeniformes) in BLAST.

Specifically, by using the IDs analysis in BOLD, of the 95 identified FDB, 62 samples were unambiguously identified at species level, including 57 samples of *T. chalcogramma* (Gadiformes) and 5 samples of *Lophius litulon* (Lophiiformes). While the analysis of the remaining 33 sequences produced overlapping values of identity between 98 and 100% within the genus *Lagocephalus* (Tetraodontiformes) (Fig. 2). This is not the first finding that DNA barcode failed the species identification to the species level. The incapability to discriminate several species of *Lagocephalus* has already been related to incomplete reference coverage in the database (Cohen et al., 2009). While the close genetic relation within the barcoding region among several species could also fail the identification (Carvalho, Palhares, Drummond, & Frigo, 2015).

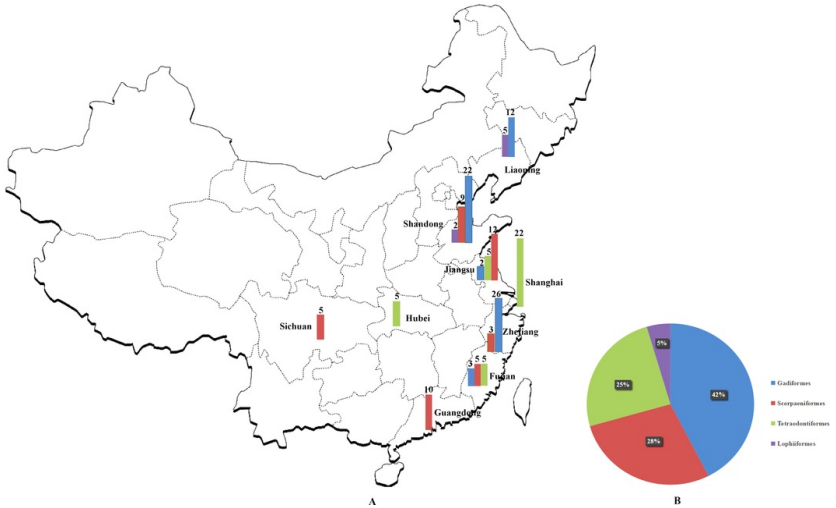

Fig. 2 Distribution of the analyzed products and of the molecularly identified species in relation to the provinces of origin of the products. Different column colours in (A) correspond with the same order illustrated in (B). The numbers on the top of the column (A) indicate the

identified samples.

alt-text: Fig. 2

With regards to the 15 identified MDB, 10 samples were unambiguously identified as *T. chalcogramma*, with the rest 5 samples producing the overlapping values of identity between 98 and 100% within the genus of *Lagocephalus*.

All the FDB and MDB sequences with maximum species identity in the range of 98–100% by BOLD analysis retrieved the similar results when analyzed by BLAST.

3.3 Label inspection: the denomination

Each package was found to be well prepared following compulsory national standard GB7718-2011 (GB 7718-2011), displayed the following information: the food name, ingredients table, net weight, manufacturer and/or distributor (name, address and contact information), date of production and expiration, storage conditions, food production license number, the code of the standard for the product preparation and any other information required by some specific standards and regulations.

With particular attention to the fish species identity, all the 153 packages reported *Xue Yu* in the ingredient table. In addition, the English name of "cod" or "codfish" were also available for 45 samples (45/153, 29.4%) (Table 1). Finally, 5 samples (5/153, 3.5%) displayed also the scientific name of *Plecoglossus altivelis* on the package (Table 1).

In fact, "cod" is a generic name for many species. The US FDA seafood list recommends the use of "cod" as the acceptable market name and/or common name for *Arctogadus borisovi, A.glacialis, Boreogadus saida, Eleginus gracilis, Gadus* spp., *Paranotothenia magellanica* (http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?s et = seafoodlist). While in UK, according to the fish list, the commercial designation of "cod" can only be used for *Gadus* species (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/236702/pb14027-uk-commercial-designation-fish-list.pdf). However, specific regulations/guidelines to specify certain species to "cod" are still not available in China (Xiong et al., 2016). In addition, the name of "codfish" also generally does not refer to some specific species. Therefore, the additional name of "cod" or "codfish" on the packages of roasted *Xue Yu* fillet products could not assist the species identification.

Finally, *Plecoglossus altivelis* is actually a fish species belonging to the order of Osmeriformes. Its presence on the package of roasted *Xue Yu* fillet products risk to amplify the confusion on *Xue Yu*.

3.4 Comparison between the molecular analysis and label inspection

Given the above mentioned disputes on the definition of *Xue Yu*, the correspondence between the product name and the species identified by molecular analysis was assessed considering three different and increasingly strict definitions. Overall, the mislabeling rate ranged from 58% (definition 1 and 2) to 100% (definition 3). This is quite comparable with our previous investigation (Xiong et al., 2015).

Actually, as has been mentioned in the Introduction, seafood species adulteration has become a global issue. One of the most effective measures towards this fraudulent conduct is the release of a standardized fish list that assign a specific commercial name to each fish species available on the market (Barendse et al., 2015; Xiong et al., 2016). A more stringent method is mandatory requirement of the preparation of the specific commercial name, the scientific name and the production method on the label (D'Amico et al., 2016). The European Union (EU), currently considered the global leader in food traceability (Charlebois, Sterling, Haratifar, & Naing, 2014), has already made a great work on this part. It 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) that should be reported on the seafood products (Regulation EU No 1379/2013). The recent efforts in legislation has been considered a great positive impact to the apparent sudden reduction of seafood mislabeling in Europe (Mariani et al., 2015).

However, in China, despite some important improvements, its fishery sector still suffers from great legislative and managerial shortcomings. Particularly, a specific provisions for the labeling of fishery products and an official reference list of seafood trade names are still absent (Xiong et al., 2016). The derived confusion has been widely reported, even in some scientific publications: Li et al. (2013) decided the correct labeling of *T. chalcogramma* for *Xia Xue* products, as well as *T. chalcogramma* and *Pollachius virens* for *Xue Yu* products; while Min et al. (2015) reported the correct labeling of *T. finnmarchica* for *Xia Xue* products.

3.5 Implications on human health and marine sustainability

3.5.1 Human health

The health implications were mainly due to the identification of *Lagocephalus* spp., a genus that includes toxic species known as pufferfish, from 37 samples. Similar adulteration has also been revealed by Li et al. (2013) and Armani, Guardone, Castigliego, et al. (2015). In particular, two poisoning incidents were reported by Cohen et al. (2009), due to mislabeling pufferfish as monkfish.

In fact, pufferfish generally contain tetrodotoxin (TTX) which is a potent neurotoxin that blocks sodium channels and affect the neuronal transmission in skeletal muscles. People affected would experience muscle weakness or paralysis and potentially death (Hwang & Noguchi, 2007). Moreover, this toxin is thermostable and cannot be decomposed during food preparation such as washing, cooking in high temperatures, freezing and drying (Lago, Rodríguez, Blanco, Vieites, & Cabado, 2015). A recent five-years count by Panão, Carrascosa, Jaber, and Raposo (2015) found globally 430 cases of intoxication and 52 deaths associated with pufferfish consumption.

Since a specific antitoxin towards TTX is still not available, the avoidance of pufferfish consumption is strictly advised. Moreover, the current EU legislations (Regulation (EC) 853/2004; Regulation (EC) 854/2004) even forbid the sale of all the fish belonging to the family Tetraodontidae. While in the US, the legal importation of pufferfish can only be conducted from one single certified Japanese exporter (http://www.fda.gov/InternationalPrograms/Agreements/MemorandaofUnderstanding/ucm107601.htm). Despite all this, TTX-related poisoning incidents were still continuously reported and a considerable proportion of accidental exposures have been attributed to fraudulent labelling (Armani, Guardone, Castigliego, et al., 2015; Cohen et al., 2009).

With regards to China, the policies on pufferfish commercialization are full of twists and turns towards progress. On one side, the tastiness and tenderness of the fish meat make pufferfish a delicacy in Chinese cuisine. On the other side, the frequently reported poisoning incidents facilitated the promulgation of a department rule "*Measures for the management of aquatic products hygiene*" in 1990, specifying the prohibition of pufferfish circulation (http://www.moh.gov.cn/mohzcfgs/s3576/200804/29459.shtml). Although, along with the great improvements with pufferfish aquaculture, the ban was abolished in 2010, an official statement to release pufferfish processing had not been stipulated until 2016 (http://www.moa.gov.cn/govpublic/YYJ/201612/t20161229_5421120.htm). Moreover, the release is strictly limited to the species of *Takifugu obscurus* and *T. rubripes*, aquacultured by certified farmers. However, *Lagocephalus* spp. were not specified and thus still should not be allowed to circulate on Chinese market. Therefore, a reasonable explanation for the identified *Lagocephalus* spp. could be that sellers deliberately mislabeled roasted *Lagocephalus* spp. fillet as *Xue Yu* fillet in order to overcome consumers resistance and also to cater to the public taste.

3.5.2 Marine sustainability

Marine sustainability could be undermined by the fact that mislabeled roasted *Xue Yu* fillet risk to create a false perception of great market availability and healthy stock status with Gadiformes species, including *G. morhua* that is listed as vulnerable in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (http://www.iucnredlist.org/details/8784/0). This issue has already be revealed by Miller et al., (2010) which suggested that mislabeling may hamper efforts to allow depleted cod stocks to recover.

In fact, global seafood sourcing networks had been expanding, from the coastal waters off North Atlantic and West Pacific to the waters in the Southern Hemisphere and into the high seas (Swartz, Sala, Tracey, Watson, & Pauly, 2010). The production of the world's marine fisheries reached a peak of 86.4 million tonnes in 1996 and have since exhibited a general downward trend, with the share of fish stocks within biologically sustainable levels declining from 90% in 1974 to 68.6% in 2013 (FAO, 2016). The situation could be even worse as the fisheries data assembled by FAO were found far less than the actual ones and the global catch has been declining much more strongly (Pauly & Zeller, 2016).

Under this circumstance, consumers are increasingly looked to promote sustainable fisheries production, by aligning their food choices with the sustainability criteria (Jacquet & Pauly, 2007; McClenachan, Dissanayake, & Chen, 2016). A variety of seafood-related campaigns, ranging from ecolabeling to the explicit boycott of certain products, have been launched, with the aim to educate consumers about the environmental effects of the products' consumption so as to catalyze a change in purchasing behavior. However, such conservation strategies depend critically on the accurate seafood species labeling, since fish products mislabeled the name of overfished species, such as *G. morhua* and *Lutjanus campechanus*, would create a false perception of great market availability and healthy stock status with these species (Logan, Alter, Haupt, Tomalty, & Palumbi, 2008). Conversely, in order to overcome consumers resistance, vulnerable species or Illegal, unreported, unregulated (IUU) fished species sold under the name of non-threatened species would also compromises the efforts to marine conservation (Bornatowski et al., 2013).

Following the meteoric economic rise, China has also become the greatest fish consumers in the world and its daily fish intake increased fourfold in the 1961–2011 period (Villasante et al., 2013). The rising fish consumption also motivated an increasing conservation concern, particularly on the marine ecosystems that supply its market (Eriksson et al., 2015). In particular, Fabinyi (2015) found that the exploitation of live reef food fish to meet consumer demand in China heavily drove fish stock declines and ecological problems in Philippines. Therefore, changing forms of seafood consumption in China has been widely considered a fundamentally important challenge for global marine sustainability (Fabinyi, 2016).

Last years have seen a range of measures encouraging sustainable consumption in Chinese seafood market. However, since specific provisions for the labeling of fishery products and a standardized seafood nomenclature in China are still not available (Xiong et al., 2016), seafood label available on the market may be prone to lead to confusion and even fraud (Wen et al., 2016; Xiong et al., 2015), thus compromising the ability of Chinese consumers to make informed choices when buying seafood.

4 Conclusion

Currently, seafood authentication and the veracity of seafood labels represent one of the most pivotal concerns for Chinese seafood industry. In this work, DNA and mini-DNA barcoding method was employed to assess the labeling accuracy of roasted *Xue Yu* fillet products commercialized on Chinese market. Our results confirmed the reliability of FDB and MDB in the identification of processed seafood products. Meanwhile, a high rate of mislabeling

rate even referring to the least stringent definition of *Xue Yu* was highlighted. Moreover, the implications on human health due to the identification of *Lagocephalus* spp. and on marine sustainability, particularly the vulnerable *G. morhua*, were analyzed. Finally, we recommended the construction of a legal framework for the management of the whole seafood supply chain and adequate enforcement with the stringent enforcement of regulations in China.

Conflicts of interest

The authors have no conflict of interest to declare.

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Highlights

- Roasted fish fillet products sold as *Xue Yu* in China were collected.
- DNA and mini-DNA barcoding revealed an alarming misrepresentation rate of 58%.
- Implications on human health and marine conservation have been discussed.

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