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Molecular Identification of Dried Shellfish Products Sold on the Market Using DNA Barcoding

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Abstract The dried shellfish products with rich nutrients and low-calorie content are favorite food in China, especially in coastal areas. However, the species of dried shellfish products in the market are usually unknown, as the taxonomic features were removed during the production process. This study described the application of DNA barcoding technique to the identification of 100 dried shellfish (scallop, squid, octopus and cuttlefish) products in markets. Samples were authenticated by comparing mitochondrial cytochrome oxidase subunit I (COI) gene and 16S ribosomal RNA (16S rRNA) gene sequences with public reference taxonomic databases. The results showed that all the 100 products can be identified at species level. Sixty four scallop adductor products were processed using the bay scallop, *Argopecten irradians*, and one was from Portuguese oyster, *Crassostrea angulata*. All the 27 squid, 2 cuttlefish and 6 octopus products were produced by the Jumbo flying squid, *Dosidicus gigas*. The neighbour-joining tree is in agreement with the results of DNA barcoding analysis. The 64 scallop samples formed one *A. irradians* cluster, leaving Sca65 clustered with the reference oyster sequence *C. angulata* (MH997922). All the 35 cephalopod (squid, octopus and cuttlefish) samples formed a *D. gigas* cluster. This investigation revealed a low variety of dried shellfish products sold on the market, and highlighted the high rate of mislabeling and species substitution. Our present work provides a practical method for tracing and authenticating shellfish products.

Key words dried shellfish product; DNA barcoding; species identification; mislabeling; species substitution

1 Introduction

Molluscs, the second most diverse phylum of life (Appeltans *et al*., 2012), is an important seafood resource for human consumption all over the world. The worldwide production of molluscs was 23.76 million tonnes in 2018 and about 16.33 million tonnes were produced by China, representing over 68.7% of the global production (FAO, 2018). Using shellfish as a protein source might be more sustainable than fish because the environmental impacts of shellfish production are relatively low (Crawford *et al*., 2003). The dried shellfish products (abalone, cephalopod, bivalve) are popular seafood in Asia over many centuries (Chan *et al*., 2012; Wen *et al*., 2017a, 2017b), as they are convenient for storage and transportation (Wen *et al*., 2018).

However, dried shellfish products are often sold in pieces (cephalopods) or without shells (bivalves) (Galal-Khallaf *et al*., 2016), which is difficult for morphological identification, paving the way for mislabeling species. Moreover, they usually arrive to market after several times of changing hands, making their traceability very difficult. So both consumers and merchants may be confused about the detailed species of dried shellfish products. The accurate naming and labeling of dried shellfish products is important for protecting consumers' right. Although the food labeling is regulated by a series of laws, regulations and standards, China is still in a developing stage of seafood traceability (D'Amico *et al*., 2014). The Food Safety Law of the Peoples' Republic of China of 2009 (Regulation No.9 of February 28, 2009) is the main regulation enforced to ensure food safety. However, most of the specific regulations in force for seafood labeling and traceability are non-mandatory (Xiong *et al*., 2016). This may result in food safety and quality issues, increasing the general concerns of consumers (Ren and An, 2010; Liu *et al*., 2013; Kevin *et al*., 2015; Xiong *et al*., 2016; Guo *et al*., 2019).

When taxonomic features were removed during the production process, DNA-based technique is usually proposed to identify food products at the species level. DNA barcoding is a promising tool and has been widely applied in species identification of both raw materials and processed foods sold on the market including meat and seafood (Galimberti *et al*., 2013; Armani *et al*., 2015a, 2015b; Khaksar *et al*., 2015; Okuma and Hellberg, 2015; Quinto *et al*., 2016; Too *et al*., 2016; Xiong *et al*., 2016). Currently, DNA barcoding based on mitochondrial cytochrome oxidase

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subunit I (COI) gene and 16S ribosomal RNA (16S rRNA) gene has been successfully applied for identification of Mollusca species, which can be used to reveal commercial and health issues in shellfish products (Galal-Khallaf *et al*., 2016; Ye *et al*., 2016; Zou and Li, 2016; Wen *et al*., 2017b).

The aim of this study was to identify a variety of dried scallop, squid, octopus and cuttlefish products sold on the market using mitochondrial COI gene and the 16S rRNA gene, which will evaluate the situation of dried shellfish products in market, improve the quality and safety of shellfish products regulatory and traceability, and protect consumers from frauds.

2 Materials and Methods

2.1 Sample Collection

In this study, a total of 100 samples representing a variety of processed products of dried shellfish (ready-to-eat, frozen, air-dried, smoked and grilled) were collected from 5 local supermarkets and 3 retail stores in Qingdao, Shandong Province (Table 1). Around 100 mg of tissue was taken aseptically from all the samples and preserved in absolute alcohol and kept at −20℃ until further processing. Each sample was labeled with a unique code and the details of the product (date of purchase, labeled species name and

Table 1 List of species identification results using the BOLD identification engine and NCBI GenBank in this study

Products	Sample code	Type of processing	BLAST	BOLD	Species identified	Common name
Scallop	Sca1	Ready-to-eat	99%		*Argopecten irradians*	Bay scallop
	Sca2	Ready-to-eat	100%		*Argopecten irradians*	Bay scallop
	Sca3	Ready-to-eat	100%		*Argopecten irradians*	Bay scallop
	Sca4	Ready-to-eat	99%		*Argopecten irradians*	Bay scallop
	Sca5	Ready-to-eat	99%		*Argopecten irradians*	Bay scallop
	Sca6	Ready-to-eat	99%		*Argopecten irradians*	Bay scallop
	Sca7	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca8	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca9	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca10	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca11	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca12	Frozen	99%		*Argopecten irradians*	Bay scallop
	Sca13	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca14	Frozen	99%		*Argopecten irradians*	Bay scallop
	Sca15	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca16	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca17	Frozen	99%		*Argopecten irradians*	Bay scallop
	Sca18	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca19	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca20	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca21	Frozen	98%		*Argopecten irradians*	Bay scallop
	Sca22	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca23	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca24	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca25	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca26	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca27	Frozen	100%		*Argopecten irradians*	Bay scallop
	Sca28	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca29	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca30	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca31	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca32	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca33	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca34	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca35	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca36	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca37	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca38	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca39	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca40	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca41	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca42	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca43	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca44	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca45	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca46	Air-dried	100%		*Argopecten irradians*	Bay scallop

(*to be continued*)

(*continued*)

Products	Sample code	Type of processing	BLAST	BOLD	Species identified	Common name
Scallop	Sca47	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca48	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca49	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca50	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca51	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca52	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca53	Air-dried	99%		*Argopecten irradians*	Bay scallop
	Sca54	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca55	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca56	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca57	Air-dried	99%		*Argopecten irradians*	Bay scallop
	Sca58	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca59	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca60	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca61	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca62	Air-dried	99%		*Argopecten irradians*	Bay scallop
	Sca63	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca64	Air-dried	100%		*Argopecten irradians*	Bay scallop
	Sca65	Air-dried	99%		*Crassostrea angulata*	Portuguese oyster
Squid	Squ1	Smoked	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ2	Smoked	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ3	Smoked	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ4	Ready-to-eat	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ5	Ready-to-eat	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ6	Ready-to-eat	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ7	Ready-to-eat	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ8	Ready-to-eat	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ9	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ10	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ11	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ12	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ13	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ14	Grilled	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ15	Roasted	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ16	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ17	Roasted	99%	99.69%	*Dosidicus gigas*	Jumbo flying squid
	Squ18	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ19	Roasted	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ20	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ21	Roasted	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ22	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ23	Roasted	99%	99.69%	*Dosidicus gigas*	Jumbo flying squid
	Squ24	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ25	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Squ26	Rroasted	99%	99.85%	*Dosidicus gigas*	Jumbo flying squid
	Squ27	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
Octopus	Oct1	Ready-to-eat	99%	99.69%	*Dosidicus gigas*	Jumbo flying squid
	Oct2	Grilled	99%	99.69%	*Dosidicus gigas*	Jumbo flying squid
	Oct3	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Oct4	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Oct5	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Oct6	Roasted	99%	100%	*Dosidicus gigas*	Jumbo flying squid
Cuttlefish	Cut1	Smoked	99%	100%	*Dosidicus gigas*	Jumbo flying squid
	Cut2	Roasted	99%	99.69%	*Dosidicus gigas*	Jumbo flying squid

region) were linked with this code for easy retrieval and cross checking the data (Nagalakshmi *et al*., 2016).

2.2 DNA Extraction and PCR Amplification

Total DNA was extracted from each individual of a sample (30 mg) by using the TIANamp Marine Animals DNA Kit (TIANGEN, China) in accordance with the manufacturer's instructions. DNA concentrations were measured using NanoDrop 2000 Spectrophotometer from Thermo Scientific. Two primer pairs of LCO-1490/HCO-2198 and

16Sar/16Sbr were respectively applied for cephalopods and scallop samples in this study. The short fragment of COI gene was amplified by PCR with primers LCO-1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HCO-2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al*., 1994). Another short fragment of 16S rRNA gene was amplified by PCR with primers 16Sar (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16Sbr (5'-CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi, 1996).

The PCR amplification reactions were performed in a total volume of 50 μL. Each reaction mixture contained 1 μL of 100 ng template DNA, 2 μL each primer (10 μmol L^{-1}), 5 μL of 10 × PCR buffer (Mg^{2+} plus) (TaKaRa, Japan), 4 μL High Pure dNTPs mixture (2.5 mmol L^{-1} each) (Beijing TransGen Biotech Co., Ltd.), and 0.2 μL Ex Taq DNA polymerase (5 U μL^{-1}) (TaKaRa, Japan), and water to a total volume of 50 μL for each sample. DNA amplifications were performed on the BIO-RAD T100 Thermal Cycler (made in Singapore) with the thermal cycler parameters as follows: 94℃ for 2–3 min; 35 cycles of 94℃ for 30 s, 48℃–52℃ for 30–40 s and 72℃ for 1 min; and one cycle of 10 min at 72℃ followed by a holding temperature 4℃.

The products of PCR amplification were analyzed by 1.5% agarose gel electrophoresis at 96 V for 45 min. The lengths of fragments were determined by comparing with the 100 bp DNA marker (TaKaRa, Japan). PCR products were viewed on a 1.5% agarose gel stained with ethidium bromide by gel imaging and analysis system, which showed evident DNA fragmentation.

2.3 DNA Sequencing and Species Identification

Amplified products were purified with AxyPrep™ DNA Gel Extraction Kit (Axygen, USA), then sequencing was completed on an ABI Prism™ 3730xl DNA sequencer by Beijing Genomics Institute. The sequences were analyzed with the Seqman program from DNASTAR (http://www.DNASTAR.com). The partial gene sequences obtained for each sample were manually assembled using Gene Runner software v.3.0 and were end-trimmed to a homologous region to avoid sequencing errors using Clustal X (Thompson *et al*., 1997). For the COI gene, approximately 670 bp partial sequences were obtained. For the 16S rRNA gene, approximately 510 bp partial sequences were obtained. The Open Reading Frames (ORF) of the resulted COI sequences and 16S rRNA sequences were predicted by NCBI ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) and it confirmed the absence of pseudogenes. The end-trimmed homologous sequences were compared for their similarity with NCBI GenBank database (http://www.ncbi.nlm.nih.gov) and taxonomic reference database of BOLD (http://www.boldsystems.org/) using the Basic Local Analysis Search Tool (BLAST) and the Identification System (ID's), respectively. The highest similarity of the queried sequence with the database sequences was determined, and the sequences that have 98%–100% similarity with database sequences were identified as the respective species. Furthermore, reference sequences for all the species labeled on products were downloaded from NCBI GenBank and BOLD databases. Except for a few reference sequences, most of the reference sequences have associated publications there by confirming the accuracy of expected species name. The correct assignment of individuals to species was performed by calculating the expected value of reference sequences' identities.

The results obtained from the comparison were then verified by Neighbor Joining (NJ) clustering analysis using MEGA 6 (Tamura *et al*., 2013) with Kimura-two parameter (K2P) distance model. Reference sequences of mitochondrial 16S rRNA gene (*Crassostrea angulata* (MH997922), *Argopecten irradians* (AF526234)) and mitochondrial COI gene (*Dosidicus gigas* (MK336957)) were collected from GenBank. Confidences in dendrograms were assessed by the bootstrap method with 1000 replications.

2.4 Comparison of the Product Description with the Identified Species

When the species identified by DNA barcoding did not match with the seafood category (scallop, squid, cuttlefish and octopus) declared for that product, the samples were regarded as misdescribed products.

3 Results and Discussion

3.1 PCR Amplification and Sequencing of COI Gene and 16S rRNA Gene

With primers of LCO-1490/HCO-2198 and 16Sar/16Sbr, the predicted DNA fragments of COI and 16S rRNA genes were successfully amplified with PCR from 100 samples. DNA barcoding is currently used in various fields, including conservation, food science and cryptic species identification. Due to its high nucleotide variability and relatively fast evolution rate, the COI gene is widely used for species identification (Hebert *et al*., 2003). However, in spite of the COI primers, they have failed to amplify PCR products in different marine organisms, such as venus clams and scallops (Chen *et al*., 2011; Feng *et al*., 2011). Consequently, we chose the COI gene and 16S rRNA gene. PCR amplification of all the dried shellfish products showed good values of quality and quantity after agarose gel electrophoresis analysis. After purification and bi-directional sequencing of the PCR products, clear and clean sequences were obtained. The sequences from 100 samples in this study have been submitted to the GenBank database under accession numbers KY446704-KY446804.

3.2 Species Identification and Comparison with Label Information

The obtained COI and 16S rRNA genes were compared with sequences available in BOLD (COI) and GenBank (COI and 16S rRNA) databases (Table 1). In the analysis, all the 100 products can be allocated to a species (100%). When comparing our results with the records in GenBank, each species showed high values of intraspecific homology, while 98%–100% of them are among scallop spe-

cies, 99% are among octopus, cuttlefish and squid species, respectively. By using the IDs analysis in BOLD, the species identity values of ≥99% were obtained for all octopus, cuttlefish and squid samples. The analysis showed that the 65 commercial scallop products belonged to two species. 64 scallop samples were identified to the Bay scallop, *Argopecten irradians*, and one (Sca65) was identified to Portuguese oyster, *Crassostrea angulata*. All the 27 squid, 2 cuttlefish and 6 octopus products belonged to the Jumbo flying squid, *Dosidicus gigas*.

The neighbour-joining analysis results were in agreement with the results of DNA barcoding (Fig.1). All the 64 scallop samples formed one cluster (*A. irradians*). Sca65 and the reference oyster sequence *C. angulata* (MH997922) clustered together, clearly differentiated from *A. irradians*. All the 35 *D. gigas* formed one cluster with very short branch lengths, indicating high similarity in all the analyzed cephalopod sequences.

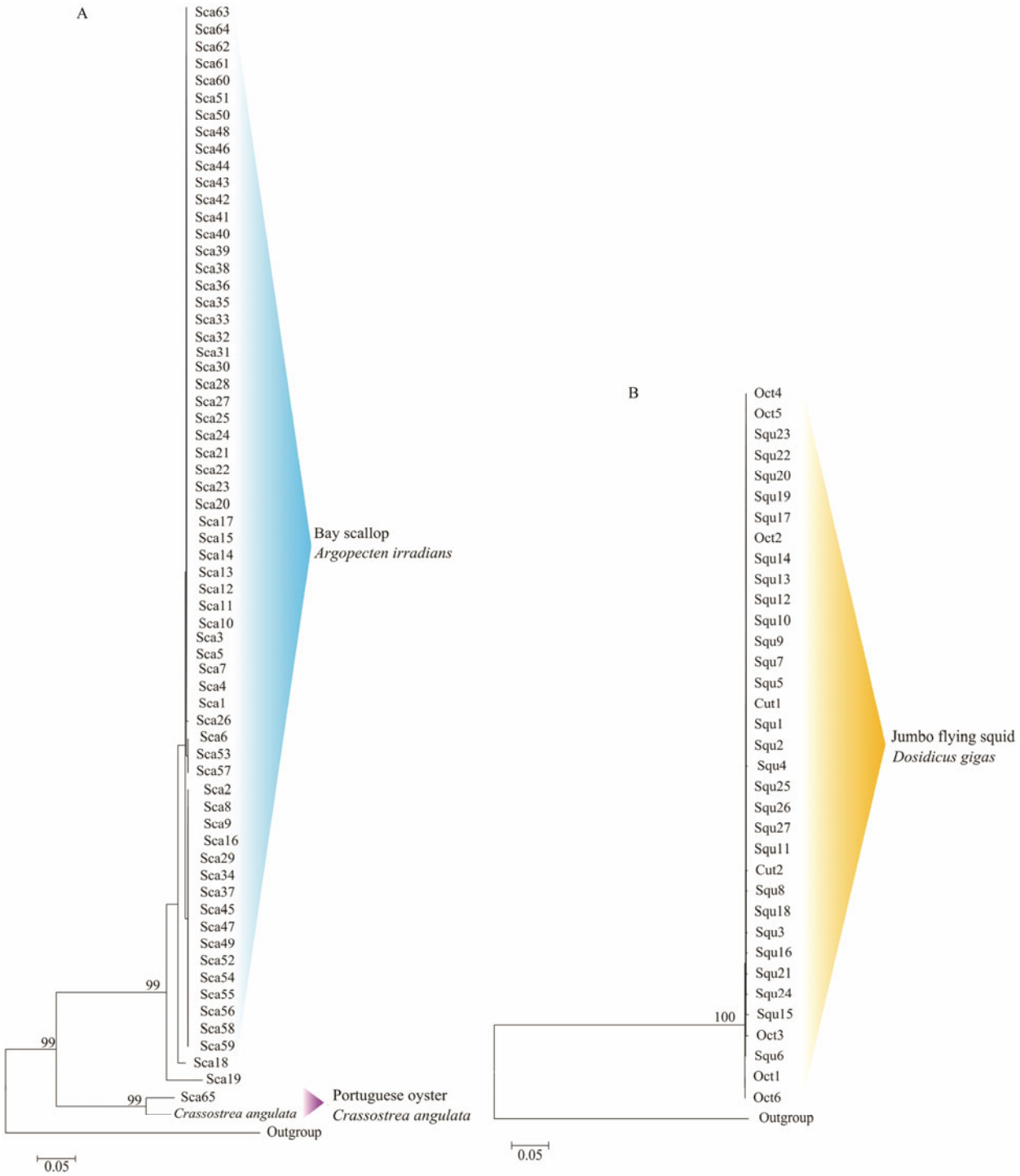

Fig.1 K2P distance Neighbour-Joining trees of (A) 16S rRNA gene sequences from 65 scallop samples, and (B) COI gene sequences from 35 cephalopod (27 squid, 2 cuttlefish and 6 octopus) samples. The codes are referred to Table 1. The bootstrap values are indicated at branches (only bootstrap values above 80% are shown). Scale bars refer to a distance of 0.05 nucleotide substitutions per site.

3.3 Evaluate the Situation of Dried Shellfish Products on the Market

Recently, several studies have reported the application of DNA barcoding for species identification in shellfish products in China, such as oyster, clam and mussel (Wen *et al*., 2017b), scallop (Wen *et al*., 2018), cephalopod (Wen *et al*., 2017a; Shi *et al*., 2020). They revealed the multiple species composition with the commercial squid products in China. Furthermore, Wen *et al*. (2017a) pointed out two cases of misdescription, involving shredded cuttlefish and octopus which were identified as the Jumbo flying squid, *D. gigas*. Our investigation evaluated a new situation of dried scallop products on the markets, which indicated that almost all dried scallop products were identified as the Bay scallop *A. irradians*. One scallop adductor muscle sample was identified as oyster species *C. angulata*, indicating the mislabeling of the scallop adductor products.

Our results highlighted the single species composition of squid products in the market. We pointed out eight cases of misdescription (22.9%), involving 2 cuttlefish and 6 octopus products which were all identified as the Jumbo flying squid, *D. gigas*. This rate of misdescription is much higher than the previous results revealed by Wen *et al*. (2017a), in which 2.1% of the analyzed cephalopod samples were incorrectly labeled. The misdescription of the dried cuttlefish and squid products indicated the seafood species substitution on the market.

3.4 Characterization of the Products Identified at Species Level

The result showed that the dried scallop products on the market were dominated by the Bay scallop *A. irradians*. According to FAO Fisheries and Aquaculture-Fishery Statistical Collections (http://www.fao.org/fishery/statistics/global-production/en), total world scallop production is 2.89 million tonnes in 2018, and 1.93 million tonnes are produced by China, which represents 66.78% of the global production. The Bay scallop *A. irradians* was first introduced to China from the United States for aquaculture in 1982 (Zhang *et al*., 1986). Because of its fast growth and short culture time, *A. irradians* has become one of the most dominant cultured scallops in China, accounting for more than half of the scallops production in China (Song *et al*., 2006).

In this work, all dried squid products were Jumbo flying squid *D. gigas*. The total world squid production is 2.21 million tonnes in 2018, and 0.09 million tonnes are from China, representing 4.07% of the global production (FAO Fisheries and Aquaculture). The Jumbo flying squid, *D. gigas*, is mainly distributed in the eastern tropical Pacific Ocean and the coastal waters of western South America (Arkhipkin *et al*., 2015). Now this species has been found in the waters of the China Sea (Chen *et al*., 2008). In 2001, after the first resources survey in the high sea of Peru and Costa Rica, the Chinese squid jigging industry start the commercial production of *D. gigas*, and now it has become the target of Chinese distant water fishing (Chen *et al*., 2008), and its annual catch reached 346200 tonnes in 2018 (http://www.fao.org/fishery/statistics/global-production/en). In this study, our investigation indicated that the dried products of the Jumbo flying squid become the most popular seafood in the marketplace of Qingdao.

3.5 Implications for Seafood Traceability

Mislabeling was a common phenomenon in commodity circulation, which have been attributed to several factors, such as economic stimulus, human error, incorrect identification and insufficient cleaning techniques of equipment that multiple species are ground on (Kane and Hellberg, 2016). In addition, the food business operators and official authorities are not familiar with marine species, leading the mislabeling of shellfish products. Therefore, assessing the labeling accuracy of seafood products is meaningful and necessary for seafood traceability in the whole production supply chain.

In present study, DNA barcoding showed powerful identification performance, which allows a rapid and definitive authentication of dried shellfish products that lack morphological characteristics. Some previous studies also examined some other seafood samples collected from markets by DNA barcoding successfully. For instance, Galal-Khallaf *et al.* (2014) reported that 33% species substitution for Nile perch and basa fillets in the Egyptian seafood market were identified with the standard DNA barcode method. Carvalho *et al*. (2011) investigated a freshwater catfish species in Brazilian markets using DNA barcoding and found a high rate of mislabeling. In addition, previous study has pointed out that a large number of aquatic species at distinct life stages (eggs, fry and adults) can also be identified by DNA barcoding (Carvalho *et al*., 2015). Thus, DNA barcode method may discourage the substitution in the seafood market and lead to a significant reduction in seafood mislabeling, which may be an effective tool on authenticating and tracing the seafood product.

4 Conclusions

In the present study, we identified 100 dried shellfish (scallop adductor muscle, squid, octopus and cuttlefish) products sampled in markets of Qingdao, China using mitochondrial COI and 16S rRNA genes. The results showed that the 100 products can be allocated to three species. Sixty four scallop adductor products were processed from *A. irradians* (Bay scallop) and one was from *C. angulata* (Portuguese oyster). All the 27 squid, 2 cuttlefish and 6 octopus products belonged to the Jumbo flying squid, *Dosidicus gigas*. The neighbour-joining tree analysis showed agreement with the results of DNA barcoding. This study indicated that the dried shellfish products on the market are with low variety. It also highlighted a high mislabeling and species substitution rate in the shellfish productsin some markets. Therefore, DNA barcoding can be employed for tracing and authenticating the dried shellfish products in the market.

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