



# Molecular tools for assuring human health and environment-friendly frozen shellfish products in the United Arab Emirates markets

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## ABSTRACT

Shellfish consumption in the United Arab Emirates (UAE) exceeds local supply and frozen fish and seafood products are imported to fill the gap. To determine the species in frozen shellfish brands on the UAE markets, 95 frozen samples were subjected to PCR amplification and sequencing of the hypervariable region of the 16S rDNA. This identified 11 different shrimp species and two squid species in the frozen shellfish packs. About 40% of calamari brands contained peanut worm, cattle, and rat 16S rDNA. Also, most shellfish species analyzed had low nucleotide diversity, including two shrimp species (*Litopenaeus vannamei* and *Metapenopsis barbata*), which had very limited genetic diversity, low raggedness, and an absence of population expansion. Species misnaming, substitution, overexploitation, origin misreporting, and low genetic diversity were found across frozen UAE shellfish samples analyzed, suggesting inspection and monitoring of frozen seafood sold in UAE markets would be appropriate.

## 1. Introduction

Seafood products are the most internationally traded food commodities and have complex difficulties in tracing stages of their supply chain (Kroetz et al., 2020). The world supply of fish and seafood has increased almost three-fold from about 54 million tons in 1965 to ca. 180 million tons in 2018 (FAO A Quarterly Update On World Seafood Markets Retrieved from <http://www.fao.org/3/ca4185en/ca4185en.pdf> January 2019, 2018). Seafood represents 17% of the total protein intake per person globally (Christiansen, Fournier, Helleman, & Volckaert, 2018), and food products should be labelled correctly. Therefore, methodologies for authentication and traceability are important to reduce the human health impacts.

The United Arab Emirates (UAE) imports >70% of its seafood. In 2015, the UAE imported 624 million USD of fish products and exported about 100 million USD (Fishery & Country, 2016). The UAE has a substantial amount of re-exportation of fish and fishery products (68 million USD in 2015) whose origins are imports from neighboring countries, especially the Sultanate of Oman. The UAE is also an approved exporting country to the European Union (Fishery & Country, 2016). According to the data of the Environment Agency of Abu Dhabi (EAD, 2018, 2019), the UAE fishing sector represented 0.12% of the country's gross

domestic product (GDP), i.e., >450 million USD. The UAE imports fulfill 72% of the country's seafood requirements, and the local fishing sector contributes only 27%. For the shellfish, the UAE Federal Customs Authority Monthly Electronic Statistical (2017) reports that crustaceans, mollusks, and other aquatic invertebrates are imported at 71 million kg/year with a value of 207 million USD; the exported weight was only 5 million kg with a value of 16 million USD.

Shellfish consumption by United Arab Emirates (UAE) inhabitants is remarkably high and exceeds the local fisheries output. Thus, imported frozen shellfish are common in supermarkets and fish markets. Shellfish are often sold as processed products. The expansion of seafood international trade without accurate inspection methodologies can lead to seafood fraud and misrepresentation in the frozen markets; this is increasingly prevalent (Galal-Khallaf, Ardura, Borrell, & Garcia-Vazquez, 2016). Processed seafood and packed products can be changed or replaced either partially or completely (Ortea et al., 2012). This triggers various concerns regarding consumer's economic rights, health, habits, and religious ethics (Bottero & Dalmaso, 2011). Therefore, detecting processed and/or packed species is necessary for reducing fraud and health risks (Haile et al., 2008).

Authentication of seafood using PCR and sequencing of short, standardized DNA fragments gained wide popularity due to the high stability

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of DNA under different food processing conditions. After being introduced by Hebert, Cywinska, Ball, and deWaard (2003), sequencing of DNA barcodes continued to represent a rapid and cheap protocol for accurate species identification (Zhang, Luo, & Bhattacharya, 2017). This approach provided key insights for the identification of correct species in different markets resolving many issues related to mislabeling, misnaming, and even illegal/unpleasant substitutions of species (Do et al., 2019; Xiong et al., 2016, 2019; Abdelbaset-Donya, Hamza, Mohammed-Geba, & Galal-Khallaf, 2020). Moreover, molecular phylogenies appended to DNA barcoding contributed to accurate species identification (Galal-Khallaf et al., 2017, 2019; Domingues, Garrone-Neto, Hilsdorf, & Gadig, 2019). Sequencing of the shellfish 16S rDNA inter-specific hypervariable region was shown to be a good marker to differentiate shellfish species (Kang et al., 2015; Galal-Khallaf et al., 2016). This work is the first study to assess the species present in frozen seafood packs in the UAE markets. We applied DNA fingerprinting and phylogenetic analysis to the resulting sequences. The results show the real identity of species used in this industry in the UAE markets and sheds light on the importance of the application of molecular markers in the traceability of food products that lack external morphological characteristics.

## 2. Materials and methods

### 2.1. Sampling

A total of 95 samples of frozen and processed shellfish were collected from four different supermarkets including one international market, one regional supermarket chain, and two local markets in three UAE emirates; Dubai, Sharjah, and Ajman (Fig. 1). Shellfish samples included 49 shrimp samples and 46 squid and calamari rings as summarized in Table 1. The collected shellfish samples were frozen and morphologically unrecognizable. The labels indicated only the common names and country of production of each type. Approximately 100 mg of the muscle tissue from each shellfish sample, i.e., squid and calamari rings and abdominal musculature of shrimps were removed using a pair of sharp scissors and placed in 1.5 mL sterile tubes containing 96% ethanol and stored at  $-20^{\circ}\text{C}$ . Ethanol-preserved shellfish samples were then shipped to the Molecular Biology and Biotechnology Laboratory of the Zoology Department in the Faculty of Science of Menoufia University in Egypt for subsequent genetic analysis.

### 2.2. DNA extraction

Total DNA was extracted from 10 mg of each shellfish sample using Chelex® 100 (Merck, Madrid, Spain) sodium form (Walsh, Metzger, & Higuchi, 1991). Briefly, a small amount of tissue was transferred to 500  $\mu\text{L}$  of Chelex suspension (10%) combined with 3  $\mu\text{L}$  of proteinase K (400  $\text{U mL}^{-1}$ , Cat. No. 3115887001, Merck KGaA, Darmstadt, Germany). The tubes were incubated for 90 min at  $65^{\circ}\text{C}$  with shaking every 15 min. The tubes were then boiled at  $100^{\circ}\text{C}$  for 20 min. Finally, aliquots of DNA were stored at  $4^{\circ}\text{C}$  for analysis.

### 2.3. PCR and sequencing of 16 s rDNA mitochondrial fragments

The universal primers described by Palumbi (1996) were used for the amplification of shellfish mitochondrial 16 S rDNA. The primers applied were 16SA (5'-ATGTTTTTGATAAACAGGCG-3') and 16SBr (5'-CCGGTCTGAACCTCAGATCACGT). Both primers were obtained from Macrogen Inc., Seoul, South Korea. The amplification reactions were performed in a total volume of 25  $\mu\text{L}$ . The reactions mixture contained 2  $\mu\text{L}$  of template DNA, 0.5  $\mu\text{M}$  of each primer, 12.5  $\mu\text{L}$  of  $2 \times$  of COSMO PCR RED Master Mix (Cat. No. W1020300X, Willowfort, Birmingham, UK), and completed to 25  $\mu\text{L}$  using PCR-grade water. The PCR reactions were carried out as follows: a preheating step at  $95^{\circ}\text{C}$  for 5 min, 35 cycles of amplification (1 min at  $95^{\circ}\text{C}$  for denaturation, 30 sec at  $52^{\circ}\text{C}$  for annealing and 45 sec at  $72^{\circ}\text{C}$  for extension for some reactions or annealing at  $55^{\circ}\text{C}$  for 30 sec, extension for 1 min at  $72^{\circ}\text{C}$  for other reactions, and a final 7 min extension step at  $72^{\circ}\text{C}$  for all to confirm the complete extension of all PCR fragments. As mentioned before, there were different annealing temperatures and extension times (Table 1). All PCR assays were done in the Thermal Cycler TC512 (Techne, UK).

After the PCR amplification, mitochondrial fragments of the 16S rDNA were separated on 1% agarose gel electrophoresis stained with 10  $\text{mg mL}^{-1}$  ethidium bromide (Cat. No. E7637, Merck KGaA, Darmstadt, Germany). Here, a 1 Kbp molecular ladder (Cat No. SM0314, ThermoFisher Scientific, USA) was applied to assess the resulting amplicon sizes. PCR amplicons were visualized by placing the agarose gel on a UV transilluminator (Transilluminator Ti 1, Biommetra, Germany). The PCR products were then sequenced using conventional Sanger sequencing (Macrogen, Inc. (Seoul, Korea).



**Fig. 1.** UAE map showing its location in the Arabian Peninsula between the Arabian Gulf and the Gulf of Oman, as well as the frozen shellfish markets' sampling sites (red circles Dubai, Ajman, and Sharjah). Map Source: Google Maps, photo credits are shown below the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

Label information, number of total samples with it is declared name and species identification of frozen commercial shellfish that collected from UAE markets (international, national, and regional) with clarification of the IUCN status.

Brand	Sample code	Declared name	Species identified	BLAST Percentage identity	Common name	Purchased from	Origin on label	Natural geographical range	Aquaculture Production	Depth	IUCN STATUS
A	N = 7 (A01-A05, A07-A08)	SHRIMP	<i>Metapenaeopsis barbata</i>	99%	whiskered velvet shrimp-Red Rice Prawn	International supermarket	UAE	Indo-Pacific	No	2–219 m	Not evaluated (or not listed?)
	N = 1 (A06)		<i>Trachypenaeus curvirostris</i>	99.02%	Southern rough shrimp	International supermarket	UAE	Indo-West Pacific, Arabian Gulf, Red Sea	No	13–300 m	Not evaluated
B	N = 8 (B01-B08)	SHRIMP	<i>Parapenaeopsis stylifera</i>	98.22%	Kiddi shrimp	Regional supermarket	UAE	Indo-West Pacific, Arabian Gulf	No	20–90 m	Not evaluated
C	N = 2 (C01, C03)	SHRIMP	<i>Metapenaeopsis andamanensis</i>	100%	Rice velvet shrimp	National supermarket	UAE	Indo-West Pacific, Arabian Gulf, Red Sea, Mediterranean Sea	No	150–350 m	Not evaluated
	N = 2 (C02, C04)		<i>Penaeopsis jerryi</i>	99.20%	Gondwana shrimp	National supermarket	UAE	Indo-West Pacific	No	600–650 m	Not evaluated
D	N = 2 (D01, D03)	SHRIMP	<i>Heterocarpus chani</i>	99.41%	NON	International supermarke	UAE	South China Sea and India	No	Deep sea (250–300 m)	Not evaluated
	N = 1 (D02)		<i>Penaeopsis jerryi</i>	99.60%	Gondwana shrimp	International supermarke	UAE	Indo-West Pacific	No	Deep sea?	Not evaluated
	N = 1 (D04)		<i>Plesionika quasigrandis</i>	98.98%	NON	International supermarke	UAE	Eastern Indian Ocean	No	Deep sea?	Not evaluated
E	N = 9 (E01-E09)	SHRIMP	<i>Litopenaeus vannamei</i>	100.00%	Pacific white shrimp-prawn	National supermarket	Vietnam	Pacific Ocean	Yes: Indo-Pacific	0–72 m	Not evaluated
F	N = 4 (F02, F03, F08-F010)	SHRIMP	<i>Metapenaeopsis barbata</i>	99%	whiskered velvet shrimp-Red Rice Prawn	National supermarket	UAE	Indo-Pacific	No	2–219	Not evaluated
	N = 1 (F04)		<i>Parapenaeopsis hardwickii</i>	100.00%	spear shrimp	National supermarket	UAE	Indo-Pacific	No	5–90 m	Not evaluated
	N = 1 (F05)		<i>Metapenaeopsis palmensis</i>	100.00%	southern velvet shrimp	National supermarket	UAE	Indo-Pacific	No	5–100 m	Not evaluated
	N = 1 (F07)		<i>Megokris granulatus</i>	99.57	Coarse shrimp	National supermarket	UAE	Indo-West Pacific, Arabian Gulf, Red Sea	No	5–81 m	Not evaluated
G	N = 7 (G01-G07)	Calamari	<i>Todarodes pacificus</i>	99.80%	Japanese flying squid	International supermarket	UAE	Pacific Ocean	No	0–500 m	least concern
H	N = 2 (H01, H02)	Squid Rings	<i>Todarodes pacificus</i>	99.80%	Japanese flying squid	International supermarket	UAE	Pacific Ocean	No	0–500 m	least concern
	N = 2 (H03, H05)		<i>Phascolosoma esculenta</i>	100%	Peanut worm	National supermarket	UAE	south-eastern China	Yes: China	benthic, no references	Not evaluated
	N = 2 (H04, H06)		<i>Bos taurus</i>	98%	cattle	National supermarket	UAE	Cosmopolitan		terrestrial	Not evaluated
	N = 1 (H07)		<i>Rattus norvegicus</i>	99%	Norwegian rat	National supermarket	UAE	Cosmopolitan		terrestrial	Not evaluated
I	N = 3 (I02, I04, I05)	Squid Rings	<i>Todarodes pacificus</i>	99.80%	Japanese flying squid	International supermarket	UAE	Pacific Ocean	No	0–500 m	least concern
	N = 1 (I03)		<i>Phascolosoma esculenta</i>	100%	Peanut worm	Interational supermarket	UAE	south-eastern China	Yes: China	benthic, no references	Not evaluated
J	N = 7 (J01-J07)	Calamari	<i>Uroteuthis edulis</i>	99	swordtip squid	Interational supermarke	Vietnam	Indo-West Pacific, Arabian Gulf, Red Sea	No	30–170 m	Data deficient
K	N = 9 (K01-K09)	Calamari	<i>Todarodes pacificus</i>	99.80%	Japanese flying squid	National supermarket	UAE	Pacific Ocean	No	0–500 m	least concern

## 2.4. Species identification and population genetic analyses

The resulting 16S rDNA sequences were manually edited using Chromas 2.6.6 software to trim the sequence ends. For sequence-based species identification, the resulting sequences were compared to GenBank database (<http://www.ncbi.nlm.nih.gov/>) employing the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). To ensure a high level of species assignment, cut-off values of >98% for identity were used for species identification. To infer the status of genetic population diversity within the populations from where the identified species were captured, and whenever the number of samples from each species exceeded 5, the 16S rDNA sequences from each species were individually aligned using ClustalW (Thompson, Higgins, & Gibson, 1994) integrated to Mega 6.06 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The alignment was uploaded to the software DNAsp6 (Rozas et al. 2017) to determine the existing haplotypes in addition to key population analysis such as the haplotype diversity index (Hd), nucleotide diversity index (Pi), F<sub>s</sub> and Tajimás D neutrality statistics for population expansion, as well as R2 statistic of Ramos-Onsins and Rozas (Ramos-Onsins & Rozas, 2002) and the raggedness index (r).

## 2.5. Phylogenetic analyses

In order to confirm the BLAST and/or BOLD-based species identification using Bayesian inference (BI)-based phylogenetic analysis, 16S rDNA reference sequences for the species identified herein were retrieved from the GenBank database. Clustal W algorithm (Thompson et al., 1994) incorporated in Mega X software (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) was applied for aligning all sequences obtained in the current study and their GenBank references. The alignment was uploaded as a nexus file MrBayes 3.2.1 software (Ronquist et al., 2012). Four MCMCs (Markov Chains Monte Carlo) chains were analyzed for 10 million (ngen = 10,000,000) generations saving a tree each 1000 generations. The subsequent analyses were carried out after assuring an average standard deviation of split frequencies below 0.001. The number of burn-ins was identified using Tracer 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Tracer 1.7 showed that 25% of the saved trees were discarded as burn-in. This information was transferred to MrBayes 3.2.1. for constructing the summarized tree, which was then viewed using the Interactive Tree of Life online algorithm (iTOL: <http://itol.embl.de/>).

## 3. Results

For all samples, the labels of the brands did not give any information regarding the common names or the scientific name of the species contained in the packs. They only wrote, “shrimp” on the shrimp packs and “calamari- squid” on calamari packs. Seventy four out of 95 samples gave good and non-overlapping sequences. The 16S rDNA sequences were about 505 bp in average length among all shellfish samples. Comparisons with the GenBank database resulted in matches with a similarity of 98% to 100%.

### 3.1. Molecular identification of shrimp species

Eleven different shrimp species were detected in the six brands (Table 1)—all were submitted to the GenBank database. These species were *Metapenaeopsis barbata* (GenBank accession numbers MT573887–MT573890), *M. andamanensis* (acc. No. MT573900), *M. palmensis* (acc. No. MT573906), *Litopenaeus vannamei* (acc. No. MT573895), *Parapenaeopsis stylifera* (acc. No. MT573891–MT573894), *P. hardwickii* (acc. No. MT573905), *Trachypenaeus curvirostris* (acc. No. MT573898, MT573899), *Penaeopsis jerryi* (acc. No. MT573896, MT573897), *Heterocarpus chani* (acc. No. MT573901), *Plesionika quasigrandis* (acc. No. MT573903), and *Megokris granulatus* (acc. No. MT573904). These

species belong to two different families, i.e., Penaeidea and Pandalidae. The distribution of different species is shown in Table 1. The brand (A) contained the whiskered velvet shrimp *M. barbata* in 50% of its samples while the remaining 50% belonged to the Southern rough shrimp *T. curvirostris* (Fig. 2). Brand B was pure, i.e., 100%, of the Kiddi shrimp *P. stylifera*. Brand C had 50% rice velvet shrimp (*M. andamanensis*, 99.8–100% identity) and 50% Gondwana shrimp (*P. jerryi*, 99.2–99.61% identity). Brand D contained three different species of shrimps—representing 50% of the total samples was *H. chani* (99.41% identity) while the other two species were 25% each: *P. jerryi* (99.61% identity) and *P. quasigrandis* with (98.98% identity). Brand E samples were 100% Pacific white shrimp *L. vannamei*. Brand F had five different species: *M. barbata* (55.6%), *T. curvirostris* (11.1%), spear shrimp *P. hardwickii* (11.1%), southern velvet shrimp *M. palmensis* (11.1%), and the coarse shrimp *M. granulatus* (11.1%). GenBank identities for all species were between 99% and 100% (Table 1). The phylogenetic analysis was completely in agreement with BLAST comparison. All species were clustered with their GenBank references from different areas of the world with high bootstrap support (Fig. 4).

### 3.2. Molecular identification of calamari species

Across the five calamari brands, two different cephalopod species belonging to two families were common among all packs: Japanese flying squid *Todarodes pacificus* (Ommastrephidae) (acc. No. MT573907) and the swordtip squid *Uroteuthis edulis* (Loliginidae) (Table 1, Fig. 3) (acc. No. MT573913). Twenty-three of the 34 frozen calamari samples that gave the best 16S rDNA sequence chromatograms (~61%) were identified as *T. pacificus* while the 7 of the 34 samples were *U. edulis* (~23%).

## 4. Genetic diversity and population criteria within species

Genetic diversity parameters (Table 2) exhibited wide variations among the populations from where the identified shellfish species were captured. Five species were analyzed for genetic diversity: *M. barbata*, *P. stylifera*, *L. vannamei*, *T. pacificus*, and *U. edulis*. *U. edulis* exhibited the highest haplotype diversity (Hd), i.e. 100%, while *L. vannamei* exhibited the least Hd, i.e., 0. Nucleotide diversities (Pi) in general were very low, i.e., between 0.01 and 0.02 % but the cephalopod *U. edulis* and the shrimp *M. barbata* showed a higher Pi value than all other species identified, i.e., 5% and 2%, respectively. *Parapenaeopsis stylifera* and *T. pacificus* were the only species that showed potentials for recent population expansion as inferred from the negativity of Tajimás D and F<sub>s</sub> and F<sub>s</sub> neutrality statistics and the low values of R2 and r statistics. The most common shrimp samples in the frozen packs, i.e., *Metapenaeopsis barbata*, showed very high raggedness and highly positive F<sub>s</sub> and D values. *L. vannamei* showed no genetic diversity at all (Table 2).

### 4.1. Species substitution and products' misdescription

The highest percentage of misdescription in all shellfish samples tested, and calamari brands specifically, was found in the brand (H) where the presence of unreported species attained 80% of the pack's contents (Fig. 3). Surprisingly, two samples in this brand were identified as the peanut worm (*Phascolosoma esculenta*, with 98.18–100% sequence identify; another two samples were from cattle (*Bos taurus*, 98.59–98.72% identify) and a single sample was 100% identical to the common brown rat (*Rattus norvegicus*). Only 20% of that brand was identified as *T. pacificus*, i.e., one out of the seven samples in brand H. The peanut worm could also be identified in 20% of samples of the brand (I) and came from an international market. Non or least 16S rDNA sequence overlapping could be identified for the three non-shellfish species obtained in the calamari packs (Fig. 5). Regarding the purity of species present in each brand, 100% of the samples from the brands G and K were identified as *T. pacificus*. Likewise, brand (J) contained a

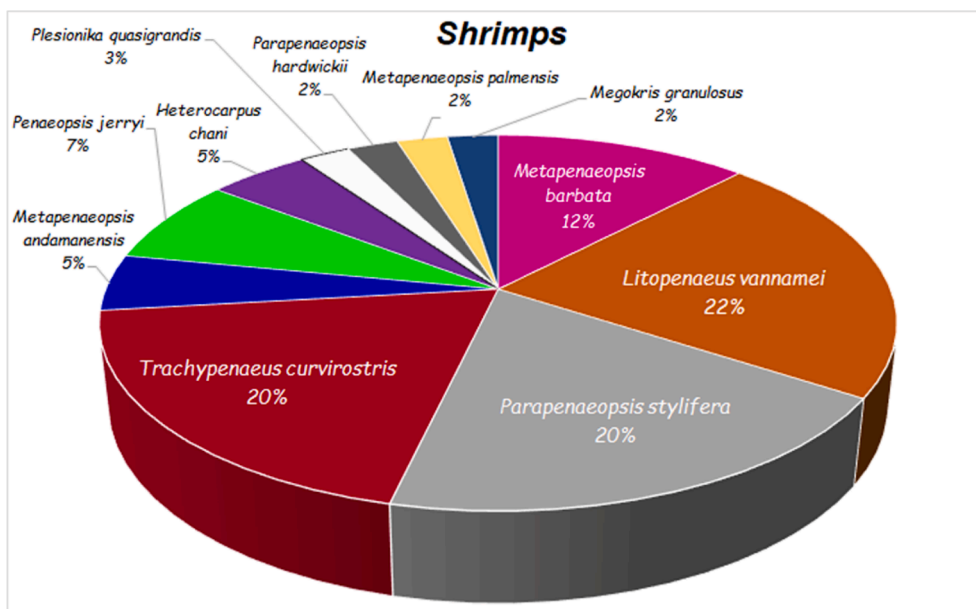


Fig. 2. Percentages of shrimp species identified to the species level (>98% GenBank sequence identity) in the six shrimp brands (A–F) purchased from the international, regional and national supermarket in Dubai, Sharjah, and Ajman principalities of the UAE.

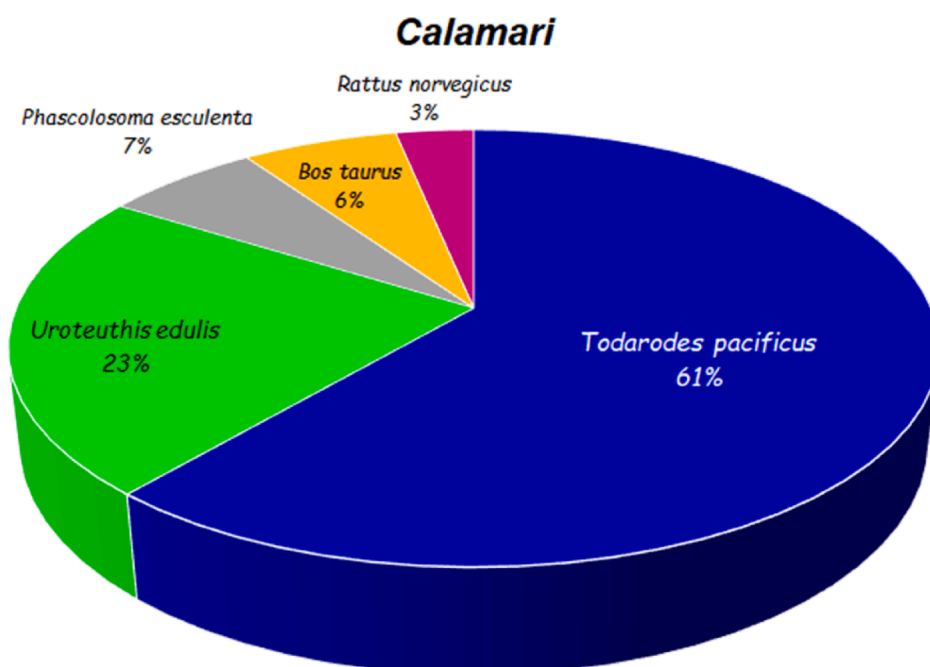


Fig. 3. Percentages of organisms identified to the species level (>98% GenBank sequence identity) in the five squid brands (G–K) purchased from the international, regional, and national supermarket in Dubai, Sharjah, and Ajman principalities of the UAE.

single cephalopod species (*U. edulis*).

#### 4.2. Origin mislabeling

*M. barbata* is concentrated in Japan, China, Taiwan, Thailand, Malay, Indonesia, and India (Dore, 2012). *Parapenaeopsis hardwickii* is mainly distributed in the Indo-West Pacific from Pakistan to Japan (TZENG, 2004); it is a very abundant and highly valued species in the East China Sea (ECS) and waters adjacent to Taiwan (Tzeng, 2007). *Parapenaeopsis stylifera* is distributed from Indian and Ceylon waters through Malaysian waters to Indonesia and Borneo (George, 1973). *Metapenaeopsis*

*palmensis* is mainly distributed in India, Singapore, Malaysia, Thailand, Vietnam, Indonesia, Philippines, Taiwan, Japan, New Guinea, Western Australia, Darwin, Queensland, New South Wales, and Australia (Chanda, 2014). *Metapenaeopsis andamanensis* is concentrated in Indo-West Pacific: India, Malaya, South China Sea, Japan (Kurup, Rajasree, & Venu, 2008). *Penaeopsis jerryi* is distributed in the Western Indian Ocean i.e., Yemen, India, and Somalia (Chanda, 2017). *Trachypenaeus curvirostris* is found in the Eastern Mediterranean, Natal, South Africa to Tanzania, Red Sea, Madagascar, Yemen to Arabian Gulf, India, Sri Lanka, Malaysia, Indonesia, Gulf of Tonkin, China, Hong Kong, Taiwan, Philippines, Japan, Korea, New Guinea, and Australia (Chanda, 2018).

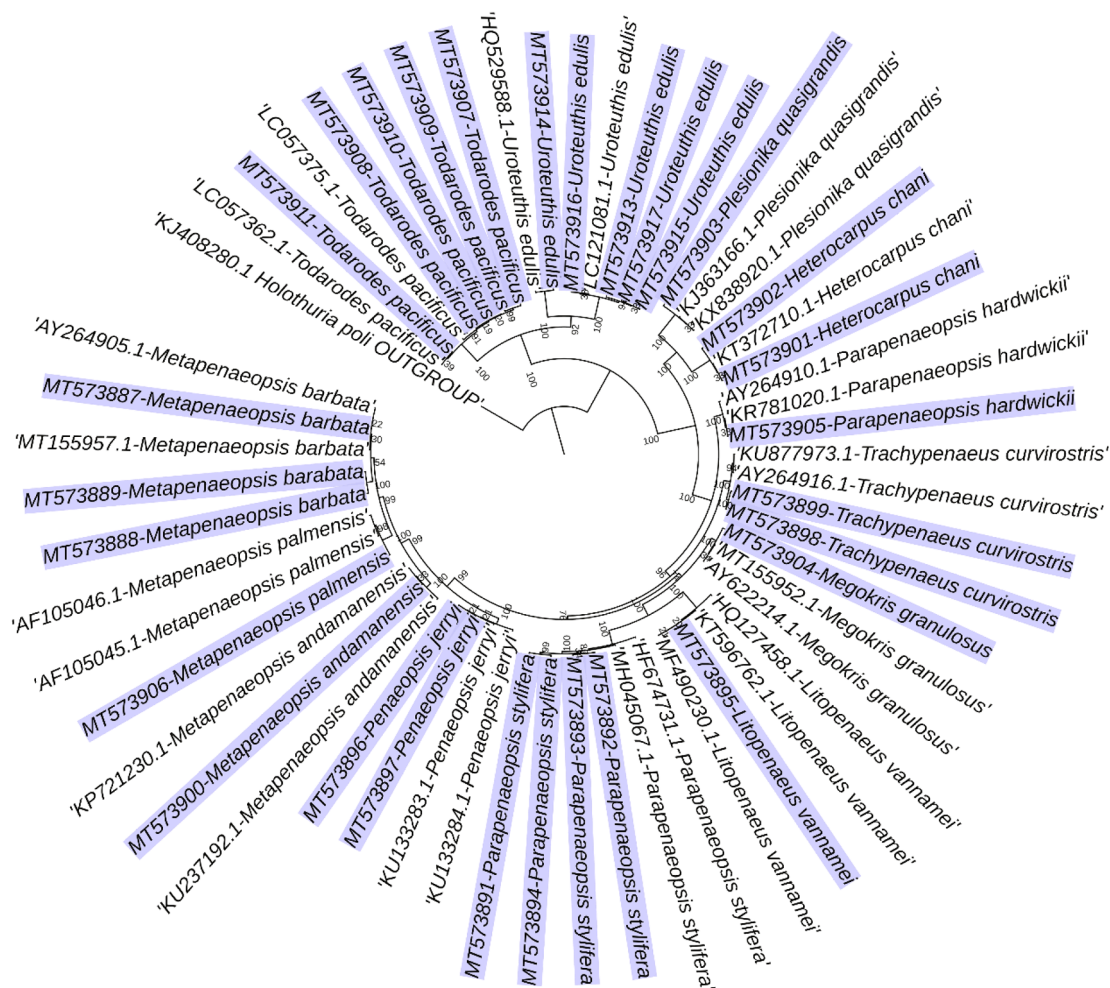


Fig. 4. Molecular phylogenetic tree using Maximum-Likelihood tree method based on partial 16 S rRNA gene sequences obtained from UAE shellfish samples in the current study and their GenBank reference sequences. Grey background refers to the species found in the UAE frozen shellfish packs analysed in the current study.

Table 2

Population parameters of the five species in seafood packs whose number of samples obtained were found to be more than five.

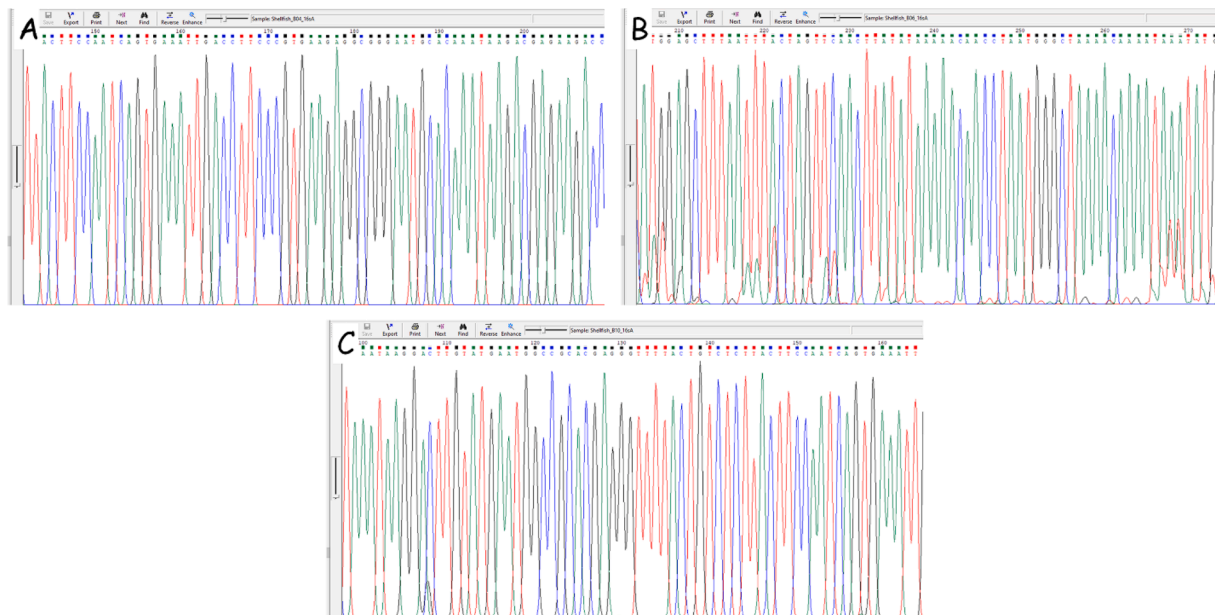
	n	h	Hd	Pi	Fs	D	R2	r
<i>Metapenaeopsis barbata</i>	10	3	0.6	0.016	6.43	1.26	0.21	0.36
<i>Parapenaeopsis stylifera</i>	8	4	0.64	0.002	-1.38	-0.81	0.16	0.06
<i>Litopenaeus vannamei</i>	9	1	0	0	0	0	0	0
<i>Todarodes pacificus</i>	19	5	0.39	0.001	-2.4	-2.05*	0.12	0.19
<i>Uroteuthis edulis</i>	6	6	1	0.05	0.15	-1.11	0.22	0.13

n: total number of samples, h: Number of Haplotype, Hd: Haplotype diversity, Pi: Nucleotide diversity, Fs: Fu's statistic, D: Tajima's statistic (Asterisk\* refers to statistical significance,  $P < 0.01$ ), R2: Ramos-Onsins and Rozas statistic, r: Raggedness statistic.

*Megokris granulosis* is broadly distributed in the East Coast of India (Chanda, 2017). *Litopenaeus vannamei* is native to the eastern Pacific Ocean from the Mexican state of Sonora to as far south as northern Peru. The calamari species used in the analyzed brands of the UAE markets include only one species (*U. edulis* (i.e., 50%)) native to the UAE. This species is relatively abundant in the Indo-Pacific from the north (southern Sea of Japan and the East China Sea) to tropical regions (the Java Sea and coastal waters of Indonesia, Malaysia, and Thailand) and as far south as the waters off northern Australia (Yamaguchi, Kawakami, & Matsuyama, 2015). The other species (*T. pacificus*) belongs to other areas in the worlds that are not related to the Arabian Gulf environment. *T. pacificus* is distributed all around Japan and its neighboring waters, extending from the northern part of the Kurile Islands south to Hong Kong. This squid is one of the most commercially important cephalopods in Japan (Watanabe, Sakurai, Segawa, & Okutani, 1996).

## 5. Discussion

The application of genetic markers for authentication of seafood market products is a theme of growing interest in the Arabian Gulf and the Sea of Oman. This area has an extensive interest and history in fisheries and their products. The area is a hub for international trade especially through maritime means, and the UAE is a plausible target for seafood market adulterations. To the best of our knowledge, this study is one of the few to deal with this research area in the UAE. The morphological similarities in some fresh fishes and the absence of morphological characteristics from frozen products make it crucial to provide adequate molecular clues for species identification. The 16 S rDNA is an efficient marker for discrimination among different shrimp species. For examples, Wilwet, Jeyasekaran, Shakila, Sivaraman, and Padmavathy (2018) could efficiently use it to differentiate *L. vannamei*,



**Fig. 5.** Sequence chromatograms for the three non-shellfish species identified using 16S rDNA sequencing in the current study, i.e. the peanut worm *Phascolosoma esculenta* (A), the rat *Rattus norvegicus* (B) and the cattle *Bos taurus* (C).

*Penaeus monodon*, *P. semisulcatus*, and *Fenneropenaeus indicus*. Moreover, Sharma, Watts, and Singh (2020) used this gene for discrimination of the penaeids *L. vannamei*, *P. duorarum*, *P. monodon*, *L. setiferus*, and *Pleoticus muelleri*. Similarly, 16 S rDNA-based methodologies elucidated clear success in identifying different cephalopod species included in seafoods. For example, it could be used to detect the flying squid *Dosidicus gigas* from cephalopod mixtures (Ye et al., 2017) authenticate the common cuttlefish (*Sepia officinalis*) in seafood products in the Italian markets (Maggioni et al., 2020), and many others. Hence, sequencing of the 16 S rDNA of frozen shellfishes was expected to provide success in accurate species identification.

Here, all analyzed shellfish samples exhibited very limited labelling, which apparently led to a high degree of species misreporting. Species substitution, mixing different species, and origin misreporting were all found to different degrees in the frozen products. Some substitutions included the presence of un-accepted species for the general gastronomy in the country, i.e., the presence of tissues belonging to the peanut worm *P. esculenta* and the common rat *R. norvegicus*. The peanut worm is an edible marine invertebrate and special dish in China with beneficial healthy characteristics (Chen, Lu, & Ye, 2009; Wu et al., 2020). The rat is considered an offensive and completely unaccepted meat source in the UAE and the entire Middle Eastern region. The presence of cattle traces instead of calamari is less offensive than the rat and the peanut worm but is still a clear form of fraud.

Pardo et al. (2018) reported that the mislabeling percentage in the cephalopod category was higher than that in other categories in seafood products including fish, crustaceans, and bivalves. Sarmiento, Pereda, Ventolero, and Santos (2018) identified a pig (*Sus scrofa*) in the squid and shrimp balls. There were several other confirmed cases for the use of pork intestines as 'imitation calamari' especially in the USA (Holbrook, 2013; Wong, 2016; Dickau, 2019). Our study added peanut worm, rat, and beef to the animal list that can be stealthily used to substitute for calamari rings. However, possible unintentional processing errors can lead to the introduction of these non-shell fish species into the shellfish packs, and this possibility cannot be completely excluded. In either cases, intentional or unintentional addition of those species requires more inspections at different stages of storage, processing, or handling to reduce food safety risks (Cavin et al., 2016; Gizaw, 2019).

A major problem that precludes the proper management of cephalopod fisheries in the Sea of Oman and the Arabian Gulf is the lack of

data about cephalopods fauna and their biology. This is partly attributed to the strong swimming ability and net avoidance behavior of these marine animals. In the Arabian Gulf, one of the squid species we identified was not recorded, i.e., *T. pacificus*. Therefore, the case of *T. pacificus* represents a clear origin mislabeling. In contrast, *U. edulis* was correctly labelled in relation to the origin because this species was recorded as native to Vietnam (Allcock, Zheng, Nabhitabhata, & Taite, 2019). Origin mislabeling accounted for 50% of cephalopod species identified in the current study then. Origin mislabeling was more severe for shrimp: 71.43% of shrimp samples were mislabeled in their origins. Of the 11 shrimp species that were found, only *T. curvirostris*, *P. stylifera*, and *M. andamanensis* and *M. granulosis* were confirmed in the Arabian Gulf and/or the Sea of Oman. All other species were neither sourced nor produced in the UAE.

This study also raises concerns related to the genetic status of the populations from where the identified shellfish species were obtained. Nucleotide diversities were in general very low for all species. This may suggest one of two possibilities. The first is that the fishing of these species comes from areas that are geographically very close and hence do not hinder gene flow. The second possibility is that these species were fished from stocks that suffer low genetic diversity, i.e., bottle-necked populations where some species showed very low haplotype diversity. *L. vannamei* had no genetic diversity at all despite being a complete shrimp brand (E) that was totally based upon it. Concomitantly, several studies pointed to a severe reduction in genetic diversity of *L. vannamei* especially in aquaculture and hatchery stocks in comparison to the species wild population (Perez-Enriquez, Hernández-Martínez, & Cruz, 2009; Knibb, Giang, Premachandra, Ninh, & Domínguez, 2020). Hence, the origin of *L. vannamei* in shellfish packs was analyzed in the current study and may be related to aquaculture-produced stocks. In contrast to *L. vannamei*, *M. barbata* showed high levels of genetic diversity and population expansion in its native ranges (Chu et al., 2011; Chu, Wang, Huang, Lin, and Tzeng, 2012). However, *M. barbata* samples present in the analyzed frozen packs showed completely different situation, besides having signs of raggedness and limited diversity. This may refer to the fishing of this species from bottle-necked populations.

In conclusion, the application of DNA fingerprinting appeared to be crucial for assuring authenticity and traceability in the UAE seafood markets. Pack labelling inadequacy and removal of outer morphology induced some cases of species substitution. This problem can be avoided

through continuous screening of seafood markets especially through molecular fingerprinting. Moreover, the application of DNA barcodes in the current study has revealed some insights regarding the original populations from which the shellfishes were fished. This, besides the identification of species substitution and origin mislabeling, strongly recommends continuous molecular-based inspection of UAE imported shellfishes for the protection of both human health and natural biodiversity.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Ethical approval

The study protocol was approved by the Animal Ethics Committee of the Zoology Department, Faculty of Science, Menoufia University, Egypt that bases mainly upon the directive 2010/63/EU of the European Commission regarding the use of animals for experimentation.

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