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Research Article

Molecular Fish Species Identification of Commercial Traditional Seafood Products in the Malaysian Market

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ABSTRACT

Seafood is a vital source of protein, multivitamins, and essential fatty acids, particularly omega-3. However, seafood fraud is becoming a growing threat to food safety in Malaysia. This study reports on the molecular identification of fish species in various seafood products collected from different regions in Malaysia. DNA barcoding employing a ~500 bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene was used to identify 46 samples. Successfully amplified and identified samples accounted for 48% of the analysed fish products. *Sardinella* species were most commonly used in traditional Malaysian products, especially in the east coast states. Interestingly, we found regional variations in fish species used for the same traditional products, highlighting the diversity of local preferences and resource availability. DNA barcoding with the COI marker was found to be effective for the rapid identification of fish species in processed foods, which could enhance food safety and consumer confidence. Although no toxic fish substitutions were detected, the results highlight the need for more comprehensive labelling practices in the Malaysian seafood market. This study emphasises the potential of molecular techniques in addressing food fraud concerns and improving seafood traceability.

Key words: DNA barcoding, food safety, processed fish products, seafood mislabelling

INTRODUCTION

Fish is one of the most vital sources of protein globally, offering high nutritional value and a renewable supply (Chin *et al*., 2016). Over 100 million tonnes of fish are consumed worldwide annually, providing approximately 500 million consumers with at least 20% of the average animal protein intake (Thilsted *et al*., 2016). The food industry has embraced fish as a major commodity, with more than 45% of fish-based products traded globally (FAO 2014). The increase in fish trade has raised concerns about the authenticity of fish-based products and food safety (Adibah *et al*., 2020). Processed fish products are particularly susceptible to mislabelling due to the absence of morphological features that are useful for identification (Chin *et al*., 2016).

Mislabelled fish products have become a global issue because manufacturing companies take advantage of it by replacing expected food with cheaper sources. Fish species substitution has been reported worldwide, including in the Americas (Hanner *et al*., 2011; Willette *et al*., 2017; Spencer *et al*., 2019), Europe (Bréchon *et al*., 2016; Harris *et al*., 2016), Asia (Chang *et al*., 2016; Chin *et al*., 2016; Nagalakshmi *et al*., 2016; Md-Zain *et al*., 2018; Adibah *et al*., 2020), the Middle East and North Africa (Galal-Khallaf *et al*., 2014). These substitutions may pose a serious health risk to consumers, especially when nontoxic fish species are replaced by toxic ones (Williams *et al*., 2020). Severe food poisoning incidents have been linked to species, such as the pufferfish, scombroid fish, oily fish, and ciguatoxin-containing fish species (Cohen *et al*., 2009; Armani *et al*., 2015; Adibah *et al*., 2020).

For the past two decades, DNA barcoding has emerged as a powerful tool for global species identification (Hebert *et al*., 2003). The cytochrome oxidase subunit I (COI) gene has been widely adopted as a DNA barcoding marker for animal identification (Hebert *et al*., 2003). This technique has been successfully applied across various taxa, including mammals (Luo *et al*., 2011; Nagarajan *et al*., 2020), birds (Pasha, 2020; Zou *et al*., 2020), reptiles (Dhar *et al*., 2020; Zangl *et al*., 2020), amphibians (Koroiva *et al*., 2020; Zangl *et al*., 2020), insects (Siozios *et al*., 2020) and fish (Chin *et al*., 2016; Md-Zain *et al*., 2018; Ali, *et al*., 2020; Zainal Abidin *et al*., 2021; 2024). In the seafood industry, DNA barcoding has also enabled the detection of mislabelled products, species substitutions, and illegal trade, as well as in wildlife forensics (Staffen *et al*., 2017; Adibah *et al*., 2020; Liu *et al*., 2020).

Our study focuses on identifying traditional fish products in the Malaysian market, which undergo various processing methods, including boiling, drying, salting, smoking, marinating, fermenting, mincing, and/or powdering (Soon-Eong & Sen-Min, 2002). The notable traditional fish products in Malaysia include *belacan* (shrimp paste), *budu* (anchovy sauce), *cincalok* (fermented shrimp), *pekasam* (fermented fish), and *ikan kering* (dried fish). Many of these products lack complete descriptions

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and proper labelling, which could impose food safety risks to consumers with health concerns.

Therefore, the main objective of the present study is to identify the fish species in traditional products and validate product labelling using the universal mitochondrial DNA barcoding gene, the cytochrome c oxidase I (COI) gene. This approach aims to enhance food safety, improve consumer awareness, and contribute to the authenticity of traditional Malaysian seafood products. Through the use of DNA barcoding techniques, we aim to recommend a reliable method of identifying fish species in processed seafood products, ultimately supporting both consumer protection and the integrity of the seafood industry.

MATERIALS AND METHODS

Sample collection and DNA analyses

A total of 46 seafood-based product samples were collected from various markets across Malaysia (Table 1). These products included both factory-produced and locally made seafood items. Traditional local products, such as *keropok lekor* (fish crackers/sausage), *satar* (spiced fish cake), *cincalok,* and *budu*, were selected to evaluate the efficacy of DNA barcoding in identifying fish or other aquatic species in processed foods. Before the DNA extraction, the samples were thoroughly rinsed and soaked overnight in distilled water to remove potential inhibitors that could interfere with the amplification process. After preparation, the samples were labelled and stored at −20°C until further analysis.

The DNA extraction was performed using three commercial kits selected based on the characteristics and condition of each sample, that is, innuPREP DNA Mini Kit (Analytik Jena, Germany), innuPREP Forensic Kit (Analytik Jena, Germany), and QIAamp DNA Investigator Kit, following the manufacturer's protocol. The use of multiple extraction kits allowed us to address the diverse nature of the samples and maximise the success rate of DNA isolation from these processed fish products. The DNA purity and concentration were quantified with a microvolume UV spectrophotometer (Quawell Q300, Quawell, CA) and retained at −20°C until further use. A ~500 bp fragment of the mitochondrial COI gene was PCR-amplified using universal barcode primers by Hebert *et al*. (2003): LCO1490 (5′ GGT CAA ATC ATA AAG ATA TTG G 3′) and HCO2198 (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′). The PCR amplification was performed in a 25 µL reaction comprising Mastermix MyTaq™ Red Mix (Bioline), ddH20, DNA template, and primer (forward and reverse). The thermal cycling conditions were as follows: predenaturation at 95°C for 1 min, followed by 30 cycles of 95°C denaturation for 30 sec, 51.3°C, 54.8°C, and 48.7°C annealing for 30 sec, extension at 72°C for 10 sec, and final extension at 72°C for 10 min. Negative controls lacking a template were included. The amplified PCR products were visualised by 2% agarose gel electrophoresis. Successful amplicons were purified and Sanger-sequenced unidirectionally by a commercial provider (Apical Scientific Sdn. Bhd.) using the ABI PRISM 3730XL automated sequencer and the ABI PRISM BigDye terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA), respectively.

Sequence analyses and phylogenetic validation

The chromatogram traces from each sequenced sample were visually inspected before alignment in BioEdit Sequence Alignment Editor 7.2.5 software. High-quality sequences were then subjected to similarity searches using the GenBank BLASTn tool with default parameters. Species identification was based on a sequence similarity threshold with reference sequences in the GenBank database. The proofread sequences were aligned using MEGA 7.0 ClustalW multiple alignments (Kumar *et al*., 2016). To validate species identifications and assess the discriminatory power of the COI marker, phylogenetic analyses were conducted using two complementary methods: neighbour-joining (NJ) and maximum parsimony (MP). The NJ tree was constructed using the Kimura two-parameter (K2P) distance model. The tree bisection and reconnection method, with the addition of a 50% consensus majority rule concept, was used for the MP tree (Swofford, 2002). For both trees, 1,000 bootstraps were used to test the stability of the tree topologies . These analyses serve as quality control measures to detect potential misidentifications and provide additional confidence in BLAST-based taxonomic assignments. Genetic distances were calculated using the Kimura 2-parameter (K2P) model in MEGA 7.0. Pairwise distances were categorized by taxonomic level (within species, within genus, and within family) to assess the presence of barcode gaps. Mean distances and standard errors were calculated for each category. The phylogenetic framework also helps evaluate evolutionary relationships among identified species, supporting the reliability of molecular identification in processed seafood products where morphological features are absent.

RESULTS

Molecular species identification

DNA extraction was successful for all 46 samples (100% success rate), though subsequent PCR amplification varied depending on DNA quality and the extraction kit used. Only 48 % (*n*=22 samples) yielded positive PCR products (Table 2). The sequence analysis using BioEdit and subsequent BLAST searches in GenBank revealed high sequence identities (>90%) for most samples, indicating a reliable species identification. However, two samples presented challenges: sample C with an 89.43% identity and sample OD with an 80.18% identity. Among the 22 successfully amplified samples, three species were identified with 100% sequence similarity: the Javan Ilisha (*Ilisha pristigastroides*), striped catfish (*Pangasignodon hypophthalamus*), and the scaly whipray (*Brevityron walga*) . Overall, our analysis identified 15 species belonging to 15 genera across eight families.

Phylogenetic analyses

The 22 sequences obtained from our samples were aligned with the reference sequences from GenBank (Table 3) to construct phylogenetic trees using both the NJ and MP methods. Final aligned sequences ranged from 500 to 520 bp after quality trimming and primer removal. *Turrum fulvoguttatum* (HQ956545.1) was used as an outgroup based on its distant evolutionary relationship to the identified fish species. The NJ tree (Figure 1) shows a taxonomically coherent clustering, with species grouped according to established family relationships, confirming the accuracy of BLAST-based identifications. Bootstrap support varied across clades: very high support (95-99%) for Clupeidae and Dasyatidae families confirms robust species discrimination, while moderate support (70-74%) for deeper nodes and weak support (55-66%) for some interfamily relationships reflect expected

limitations of COI for deep divergences.

The clear separation of fish families (Clupeidae, Pangasiidae, Cyprinidae, Dasyatidae) validates that the COI marker can effectively discriminate between species commonly found in processed Malaysian seafood. The phylogenetic analysis revealed three distinct evolutionary lineages: (1) marine pelagic species (Clupeidae) forming a well-supported monophyletic group, with Sardinella species showing minimal divergence suggesting recent radiation; (2) freshwater Cyprinidae clustering exclusively in fermented products, indicating convergent utilization based on physiological properties; and (3) cartilaginous fishes (Dasyatidae) as a distant family, confirming the broad taxonomic coverage of traditional seafood products. The congruence between the NJ (Figure 1) and MP (Figure 2) methods strengthens confidence in the quality of sequence data and taxonomic assignments and supports the reliability of DNA barcoding in detecting potential species substitution in commercial products.

Table 1. List of fish-based products from various markets around Malaysia.

No	Code sample	Locality	Type of sample
1	B1	Selangor	Fish balls
2	B2	Selangor	Fish balls
3	B3	Selangor	Fish balls
4	C	Kelantan	Fish crackers
5	F1	Selangor	Fillet
6	F2	Selangor	Fillet
7	F3	Selangor	Fillet
8	G	Perak	*Pekasam*/ Fermented fish
9	H	Kedah	*Pekasam*/ Fermented fish
10	I	Kedah	*Pekasam*/ Fermented fish
11	J	Terengganu	*Satar*
12	K	Terengganu	Satay
13	L1	Sarawak	*Pekasam*/Fermented fish
14	L2	Sarawak	*Pekasam*/ Fermented fish
15	L3	Sarawak	*Pekasam*/ Fermented fish
16	L4	Sarawak	*Pekasam*/ Fermented fish
17	L5	Sarawak	*Pekasam*/ Fermented fish
18	M	Selangor	Dried fish
19	OA	Selangor	Fish crackers
20	OB	Selangor	Fish crackers
21	OC	Kelantan	Fish crackers
22	OD	Selangor	Fish snacks
23	OE	Selangor	Popia
24	OF	Selangor	*Otak-otak*/ Spiced fish cake
25	OG	Selangor	Fish cocktail
26	OH	Selangor	Bean curd
27	OI	Kelantan	Satay
28	OJ	Selangor	Canned fish
29	OK	Selangor	Canned fish
30	OL	Kelantan	*Budu*/ Anchovy sauce
31	OM	Kelantan	*Budu*/ Anchovy sauce
32	ON	Selangor	Fish sauce
33	P	Terengganu	*Keropok lekor*/ Fish sausage
34	PB	Sabah	Dried fish
35	PS	Selangor	Dried fish
36	Q	Terengganu	*Satar*
37	R	Terengganu	Fish puffs
38	S	Terengganu	*Keropok lekor*/ Fish sausage
39	T	Terengganu	*Satar*
40	U	Terengganu	*Keropok lekor*/ Fish sausage
41	V	Sarawak	*Keropok lekor*/ Fish sausage
42	W	Sarawak	*Keropok lekor*/ Fish sausage
43	X	Sarawak	Dried fish
44	Y	Kelantan	Satay
45	Z	Sarawak	Fish crackers
46	CC	Malacca	Cincalok

Genetic distance analysis

K2P distance analysis confirmed hierarchical taxonomic structure with minimal intrageneric divergence (≤0.15%) but substantial family-level differentiation (mean = 0.25%). The 4-fold increase in mean distance from genus (0.05%) to family (0.25%) level, coupled with zero distances among some *Sardinella fijiensis* samples, demonstrates both the discriminatory power and resolution limits of COI barcoding. This clear genetic discontinuity at family boundaries validates our phylogenetic clustering and confirms reliable identification despite extensive processing of these traditional seafood products.

Table 2. DNA extraction method successfully amplified according to each sample *Mini Innuprep DNA* (IM), *Innuprep Forensic* (IF), and *QIAamp DNA Investigator* (Qiv) kit.

No.	ID sample	Type	DNA Extraction Method			Amplification
			IM	IF	Qiv	
1.	B1	Fish balls	+			+
2.	B2	Fish balls	+			+
3.	B3	Fish balls	+			+
4.	C	Fish crackers			+	+
5.	F1	Fillet	+			
6.	F2	Fillet	+			
7.	F3	Fillet	+			+
8.	G	*Pekasam*			+	
9.	H	*Pekasam*			+	+
10.	I	*Pekasam*			+	
11.	J	*Satar*	+			+
12.	K	Satay	+		+	
13.	L1	*Pekasam*	+			+
14.	L2	*Pekasam*			+	+
15.	L3	*Pekasam*	+			+
16.	L4	*Pekasam*	+			+
17.	L5	*Pekasam*			+	+
18.	M	Dried fish			+	
19.	OA	Fish crackers			+	
20.	OB	Fish crackers			+	
21.	OC	Fish crackers			+	
22.	OD	Fish snacks			+	+
23.	OE	Spring roll			+	
24.	OF	*Otak- otak*			+	+
25.	OG	Fish cocktail	+			
26.	OH	Bean curd			+	
27.	OI	Satay			+	
28.	OJ	Canned fish			+	
29.	OK	Canned fish			+	
30.	OL	*Budu*		+		
31.	OM	*Budu*		+		
32.	ON	Fish sauce		+		
33.	P	*Keropok lekor*	+			+
34.	PB	Dried fish	+			+
35.	PS	Dried fish			+	+
36.	Q	*Satar*			+	+
37.	R	Fish puffs			+	+
38.	S	*Keropok lekor*	+			
39.	T	*Satar*			+	
40.	U	*Keropok lekor*	+			+
41.	V	*Keropok lekor*			+	+
42.	W	*Keropok lekor*			+	
43.	X	Dried fish			+	
44.	Y	Satay			+	+
45.	Z	Fish crackers			+	+
46.	CC	Cincalok	+			

Table 3. Fish species in commercial food identified using BLASTn in GenBank

No.	Code Sample	Types of products	Name on the label	Identified species	GenBank Accession No.	Similarity percentage (%)
1.	B1	Fish balls	Fish	*Escualosa thoracata*	EF607377.1	90.32
2.	B2	Fish balls	Fish	*Ilisha pristigastroides*	MH325641.1	100
3.	B3	Fish balls	Fish	*Johnius carouna*	MH230988.1	93.26
4.	C	Fish crackers	Fish	*Sardinella fijiensis*	NC_044472.1	89.43
5.	F3	Fillet	Dory fish	*Pangasianodon hypophthalmus*	MH194534.1	100
6.	H	*Pekasam*	n.a	*Osteochilus hasseltii*[1]	JF915632.1	97.69
7.	J	*Satar*	n.a	*Upeneus* sp.	GU673190.1	99.85
8.	L1	*Pekasam*	n.a	*Osteochilus hasseltii*[1]	JF915633.1	95.38
9.	L2	*Pekasam*	n.a	*Hampala macrolepidota*	KT001059.1	97.25
10.	L3	*Pekasam*	n.a	*Leptobarbus hoevenii*	AP011286.1	99.28
11.	L4	*Pekasam*	n.a	*Puntius orphoides*[2]	JF915642.1	99.27
12.	L5	*Pekasam*	n.a	*Anematichthys apogon*[3]	AP011250.1	97.42
13.	OD	Fish snacks	Fish	*Callanthias japonicus*	AP017451.1	80.18
14.	OF	*Otak- otak*	Fish	*Sardinella fijiensis*	NC_044472.1	99.40
15.	P	*Keropok lekor*	Fish	*Sardinella jussieu*	KY315392.1	96.86
16.	PB	Dried fish	n.a	*Himantura walga*[4]	KM072995.1	100
17.	PS	Dried fish	n.a	*Neotrygon kuhlii*	KU497981.1	99.02
18.	Q	*Satar*	n.a	*Sardinella fijiensis*	NC_044472.1	96.92
19.	R	Fish puffs	n.a	*Sardinella jussieui*	KY964441.1	98.98
20.	U	*Keropok lekor*	n.a	*Sardinella fijiensis*	NC_044472.1	99.42
21.	V	*Keropok lekor*	n.a	*Opisthopterus tardoore*	AP017945.1	97.05
22.	Y	Satay	n.a	*Sardinella fijiensis*	NC_044472.1	97.02

n.a.: not available
Following the taxonomic revision described in Fricke *et al*. (2024):
[1] Valid as *Osteochilus vittatus* (Valenciennes, 1842)
[2] Valid as *Systomus orphoides* (Valenciennes 1842)
[3] Valid as *Cyclocheilichthys apogon* (Valenciennes 1842)
[4] Valid as *Brevitrygon walga* (Müller & Henle 1841)

Table 4. K2P divergence values from analysed specimens with increasing taxonomic levels. SE standard error

Category	n	Min	Maximum (%)	Mean (%)	SE (%)
Within species	11	0.00	0.15	0.06	0.02
Within genus	10	0.00	0.15	0.05	0.02
Within family	28	0.00	0.39	0.25	0.02

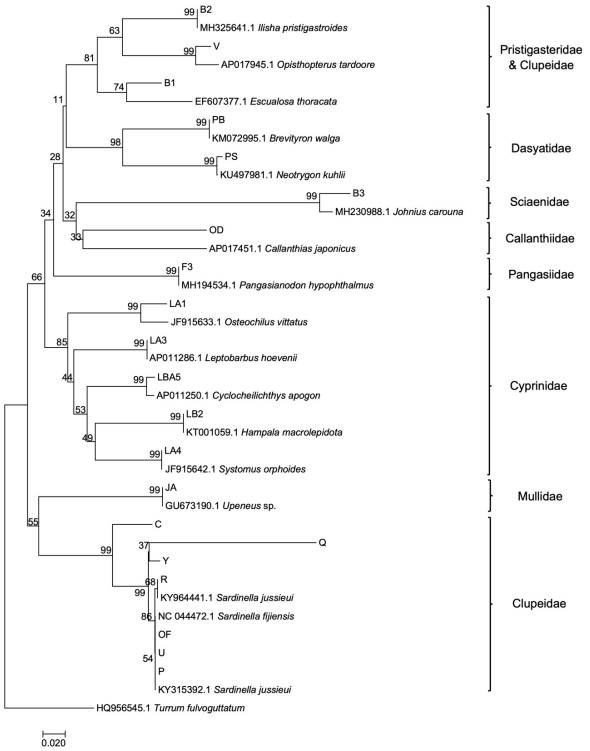

Fig. 1. NJ phylogeny tree for the COI sequence using a K2P model with 1,000 replications. The bootstrap value is shown above the tree branch.

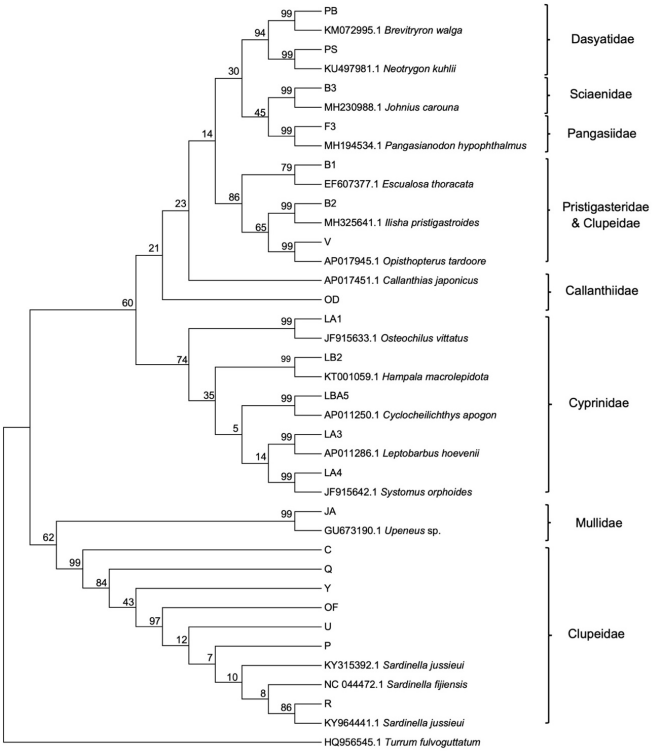

Fig. 2. MP phylogeny tree for the COI sequence using heuristic search with 1,000 repetitions. The bootstrap value is shown above the tree branch.

DISCUSSION

Despite the successful DNA extraction from all 46 samples, only 22 (48%) were successfully amplified using the universal mitochondrial COI gene marker. This success rate of 48% highlights the challenges associated with DNA barcoding in processed seafood products. The factors contributing to these difficulties include DNA degradation during processing, the presence of PCR inhibitors, and limitations in reference databases (Armani *et al*., 2015; de Boer *et al*., 2015). The inhibitory compounds in the samples can interfere with PCR by reducing or inhibiting the DNA polymerase activity (Di Pinto *et al*., 2007). Previous studies indicate that DNA extracted from processed foods is often degraded by various processing methods, such as steaming, cooking, and mixing with sauces (Armani *et al*., 2015; Chin *et al*., 2016). The poor DNA quality not only impedes amplification but also affects the identification efficiency of the DNA barcoding process (de Boer *et al*., 2015). These findings align with previous studies, such as those of Adibah *et al*. (2020) and Chin *et al*. (2016), who reported similar challenges in processed food products. While DNA extraction was successful for all samples, subsequent PCR amplification success varied due to DNA quality and processing-related degradation.

The BLAST searches in GenBank yielded high sequence identities (>90%) for most of our samples, indicating a reliable species identification. However, two samples posed a problem: sample C with an 89.43% identity and sample OD with an 80.18% identity. These lower similarity percentages raised concerns about the reliability of species identification in these samples. The discrepancy was particularly pronounced in sample OD, which was tentatively identified as a yellowtail redfish (*Callanthias japonicus*). This identification was particularly unlikely because *C. japonicus* has a limited distribution in the Northwest Pacific, particularly in southern Japan and the East China Sea, and has never been recorded in Malaysian waters (Froese & Pauly, 2023). The unexpected presence of *C. japonicus* in our samples warrants further investigation. Several factors can contribute to this anomalous result, including a potential misidentification caused by the limited reference sequences in the Genbank database , the presence of a closely related, but undescribed species, or the rare occurrence of *C. japonicus* outside its known range. Alternatively, this can indicate a case of species substitution or mislabelling in the seafood supply chain.

The high sequence similarities (>90%) and phylogenetic clustering patterns, combined with successful species discrimination, demonstrate the effectiveness of the COI marker for this application. The universal COI barcode is widely used in classifying major taxonomic groups, including fish, mammals, amphibians, and reptiles (Staats *et al*., 2016). However, it is important to note that this primer may not be universally effective for the identification of all species (Parveen *et al*., 2016). The broad applicability of COI barcodes can occasionally lead to a reduced accuracy in distinguishing closely related species (Zainal Abidin *et al*., 2021; 2022; 2024). Despite these limitations, our study successfully identified almost half of the fish products at the species level through the DNA barcoding analysis, with only one exception (i.e., sample J identified at the genus level as *Upeneus* sp.). This high success rate highlighted the potential of DNA barcoding as a powerful tool for seafood authentication in the Malaysian market.

The genetic distance analysis provides quantitative validation for COI barcoding in processed Malaysian seafood. The exceptionally low mean K2P distances within species (0.06%) and genus (0.05%) levels, including zero distances between geographically distant *Sardinella fijiensis* samples, indicate high gene flow and recent diversification in these commercially important species. The 4-fold increase in genetic distance from genus to family level (0.05% to 0.25%) creates a distinct "taxonomic gap" that enables reliable identification despite DNA degradation from traditional processing methods. Most

importantly, the clear barcode gap between maximum intrageneric (0.15%) and minimum interfamily distances (0.21%) ensures unambiguous species assignment when morphological features are absent. This hierarchical distance pattern, combined with consistent family-level clustering (e.g., Cyprinidae in pekasam, Clupeidae in keropok lekor), demonstrates that COI barcoding can effectively authenticate processed seafood products and support food safety monitoring in Malaysia, where traditional identification methods are impossible.

The genus *Sardinella* was found to be the most commonly used fish in traditional Malaysian fish products, accounting for 32% (*n*=7) of the samples analysed. These sardine-based products were particularly prevalent on the east coast of Peninsular Malaysia (e.g., Terengganu & Kelantan). Sardines serve as the main ingredient in popular traditional foods, such as *keropok lekor* (fish sausage), fish satay, and fish crackers, which are staples of the East Coast culinary tradition. The widespread use of sardines in these products reflects both their local abundance and cultural significance. Worldwide, *Sardinella* is distributed across tropical and subtropical seas, including the Mediterranean Sea and the Black Sea (Froese & Pauly, 2023). The identification of Sardinella as an important ingredient in Malaysian seafood emphasises the interaction between local culinary traditions, fisheries management, and marine ecology. This finding underscores the need for sustainable fishing practices and accurate labelling to ensure the long-term viability of both the fishing industry and the traditional food products that rely on these species.

The DNA barcoding analysis also revealed significant regional variations in the fish species used for traditional Malaysian seafood products. For example, longfin herring (*Opisthopterus tardoore*) is used for *keropok lekor* (fish sausage) from Sarawak, while sardines (*Sardinella* spp.) are commonly used in Terengganu. This diversity reflects the adaptation of traditional recipes to local fish availability and preference. The *keropok lekor* industry, which is mainly centred in the east coast states (Terengganu, Kelantan, & Pahang), uses several species for its production, namely, dorab wolf-herring (*Chirocentrus dorab*), goldstripe *Sardinella* (*Sardinella gibbosa*), golden threadfin bream (*Nemipterus virgatus*), sulphur goatfish (*Upeneus sulphureus*), and round scad (*Decapterus maruadsi*) (Wan-Md-Hatta, 2015).

In the production of *pekasam* (fermented fish), our findings indicate that only freshwater fish from the Cyprinidae family are used. For example, Nilem carp (*Osteochilus vittatus*) was used in the sample obtained from Kedah, while the samples from Sarawak contained the five following species: Nilem carp (*O. vittatus*), Hampala barb (*Hampala macrolepidota*), Hoven's carp (*Leptobarbus hoevenii*), Javaen barb (*Systomus orphoides*), and beardless barb (*Cyclocheilichthys apogon*). *Pekasam* production is concentrated in the north of Peninsular Malaysia, particularly in Perlis, Kedah, and Perak. The species commonly used in pekasam production also include tilapia (*Oreochromis mossambica*), spotted gourami (*Trichogaster trichopterus*), catfish (*Clarias batracus*), swamp barb (*Puntius brevis*), and snake head (*Channa striatus*) (Huda, 2012).

Our analysis revealed significant labelling discrepancies in the traditional seafood products examined. Most of the packages lacked specific information on the fish type and were often simply labelled as 'fish'. This problem was particularly evident in samples from street vendors, where the ingredient lists were often incomplete. This makes it difficult to compare the fish species specified by the manufacturer with the DNA barcoding results. Only one sample, F3, labeled as 'Dory fish' fillet, contained specific species information . Our DNA barcoding analysis confirmed that this was the Creme dory (*Pangasianodon hypophthalmus*) from the Pangasiidae family. *Pangasianodon hypophthalmus* is a freshwater fish commonly found in Southeast Asia and one of the most valuable species in aquaculture (Hedayati & Tarkhani 2014). Its low unsaturated fatty acid content makes it popular in the food industry (Strobel *et al*., 2012). These findings highlight the urgent need for improved labelling practices for Malaysian seafood products to increase transparency, facilitate informed consumer choices, and ensure food safety compliance.

Food labelling plays a crucial role in providing consumers with reliable and accurate information about their purchases. In Malaysia, the 1985 food regulations specifically require that common names be used in the ingredient lists instead of scientific names to improve consumer understanding. However, this well-intentioned regulation has inadvertently created opportunities for unscrupulous manufacturers to commit food fraud by substituting species (Bénard-Capelle *et al*., 2015). Documented cases include the substitution of valuable gourami for less profitable lizardfish (Adibah *et al*., 2020), and, more alarmingly, the substitution of pufferfish for poisonous monkfish (Cohen *et al*., 2009) and squid for poisonous pufferfish (Armani *et al*., 2015). These deceptive practices not only undermine consumer confidence in the seafood industry but also pose a serious health risk to the public (Adibah *et al*., 2020).

The DNA barcoding analysis of traditional Malaysian seafood products in this study revealed that no toxic fish species were found, indicating that the products tested are safe to consume, despite incomplete labelling. This result underlines the potential of DNA barcoding as a powerful tool for improving food safety and authenticity in the seafood industry. Accurate food labelling is not only for regulatory compliance, but is also an educational tool for promoting a healthy lifestyle (Souza *et al*., 2016) and a cost-effective means of providing important nutritional information to consumers (Miller & Cassady, 2015). Given our findings and the broader context of food safety, we recommend that labelling regulations be strengthened, stricter enforcement measures be taken, and advanced technologies (e.g., DNA barcoding) be incorporated into routine food safety assessments. These steps are essential in ensuring consumer safety, maintaining confidence in the seafood industry, and promoting informed food choices among the Malaysian public.

CONCLUSION

The extensive processing-induced morphological changes in seafood products and inadequate labelling practices in the Malaysian market have made conventional morphology-based fish species identification challenging. Our study demonstrated the efficacy of DNA barcoding, specifically utilising the mitochondrial COI gene as a reliable method for identifying fish species in processed commercial seafood products. The success of this approach in distinguishing species in various traditional Malaysian products emphasised its potential as a powerful tool for improving food safety and authenticity in the seafood industry. While the universal COI marker has proven to be effective, the development of fish-specific COI markers can potentially provide even more accurate results. The application of DNA barcoding techniques provides a rapid and accurate means of identifying fish species, thereby addressing critical issues, such as meat origin ambiguity, food fraud, and labelling inconsistencies that are prevalent in the food industry. The integration of DNA barcoding into routine food safety assessment and regulatory frameworks

can significantly improve consumer protection, support conscious eating, and promote greater transparency in the seafood supply chain. This study forms the basis for further research and policy development aimed at ensuring the integrity and safety of Malaysia's diverse and culturally significant seafood.

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ETHICAL STATEMENT

This project was conducted according to the relevant national and international guidelines and did not involve any endangered or protected fish species. All seafood products were bought from the local markets throughout Malaysia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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