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DNA Barcoding Revealed Mislabeling of Imported Seafood Products in Thailand

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Abstract: Seafood mislabeling threatens customer rights and causes economic loss worldwide. The information on seafood misrepresentation in Thailand is still lacking, and the investigation and monitoring program must be well established. This study investigated the mislabeling status of imported seafood in Thailand using the DNA barcoding technique. A total of 45 imported seafood products from five distributors were included. Scientific, common, local, and market names of seafood samples were obtained from FAO and Fishbase databases. DNA was extracted, and PCR was performed using a universal primer targeting the *COI* gene. Species of each sample were identified with over 98% similarity based on *COI* sequence analysis. DNA sequence revealed 11 mislabeled samples. Among substituted species, *Pangasianodon hypophthalmus* and *Thunnus maccoyii* were found to be endangered species according to IUCN status. Products obtained from Brand-C showed the highest mislabeling rate (42.85%). The phylogenetic analysis adopted with the TIM2+F+I+G4 model showed the sequenced DNA similar to the NCBI database reference sequence. Overall, mislabeled products of imported seafood were found at the rate of 24.44%, suggesting that strict surveillance for seafood substitution should be implemented in Thailand.

Keywords: DNA barcode; seafood species authentication; *COI* gene; mislabeled seafood

Key Contribution: The DNA barcoding analysis of seafood products retailed in Thailand unveiled a mislabeling prevalence of 24.44%, with two samples identified as endangered species based on the IUCN classification. Notably, the whole fish emerged as the category most significantly affected by mislabeling.

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1. Introduction

Seafood is a commodity susceptible to fraud due to several factors, such as the similarities in morphology across species, the growing demand of international trade, complex supply networks, or high demand but a shortage of specific species [1–3]. The most common seafood fraud is product mislabeling, in which fish with high economic value are replaced by a lower monetary value counterpart [4]. This scenario typically happens with closely related marine species due to the similarity in flavor, texture, and appearance [5]. Moreover, seafood mislabeling has been considered a growing issue of concern since it has been reported in several countries, such as Canada, Spain, Taiwan, Greece, Italy, France, and Bulgaria [6–11]. Consumption of mislabeled products may consequently impact economic and ecological phenomena and raise food safety issues, such as the high risk of dangerous species adulteration or allergy-related problems [12].

In Thailand, the Food and Drug Administration of Thailand (FDA), along with the National Bureau of Agricultural Commodity and Food Standards (ACFS), is responsible

for monitoring the national standard of processed foods, agricultural commodities, dairy, and fishery products [13]. However, the regulations of Thailand do not involve the strict declaration of the scientific name of seafood products on the label [8]. Therefore, the surveillance of the integrity of the seafood market, as well as the investigation of seafood mislabeling, is challenging in Thailand. This is particularly true for imported seafood products, mostly sold as processed commodities without morphological features. Moreover, the surveillance of seafood substitution has not been established. In addition, there is only one study assessing seafood mislabeling, which leads to the lack of information on the prevalence of seafood mislabeling in Thailand [8]. This may consequently result in violence against customer rights.

DNA barcoding is a standard method widely used for species identification until now [14], resulting in the identifying differences between closely related species [15]. Typically, mitochondrial genes are employed as a marker for animal identification since they are found in all living organisms, making them ubiquitous markers. Moreover, mitochondrial DNA provides many advantages, such as the intrinsic ability to resist degradation and high copy number compared to nuclear DNA [16]. Specifically, the cytochrome C oxidase subunit I (*COI*) gene is a typical marker in animal metabarcoding due to its comprehensive taxonomic coverage in verified databases, including NCBI GenBank and BOLD [17].

Despite seafood playing a vital role in Thailand, seafood mislabeling exposed to customers remains unclear. Therefore, this study aimed to investigate the current misrepresentation status of imported seafood products sold or distributed in Thailand using DNA barcoding. The universal primers targeting 700 bp of the *COI* gene were used for PCR amplification of isolated DNA from seafood samples.

2. Materials and Methods

2.1. Sample Collections and Preparation

A total of 45 seafood samples, including five samples of whole fish, one sample of the fish collar bone, one sample of sliced fish, 36 fish fillet samples, and two crustacean samples, were purchased randomly from various online and offline markets in Thailand. The criteria for selected samples are as follows: (1) All samples must be imported; (2) all samples should provide the common names/scientific names of species used on the labels; (3) all samples should be sold in processed formats, such as fillets, slices, deshelled, or deheaded products; (4) all samples were randomly purchased based on the availability in the markets (online and offline markets) without brand consideration. Only one sample was smoked before being frozen, while the remaining samples were unprocessed and subsequently frozen. The samples were collected and transferred to the laboratory at 4 °C. Subsequently, the samples were ground to achieve uniformity, and a portion of 50–100 mg was collected, packed in a zip-lock bag, and kept at –80 °C. All samples were assigned with a unique identification name for reference. The relevant information for each sample, including species, name of packaged products, processing method, brand, and importer name, was recorded. The samples were categorized into three groups based on their packaging and labeling attributes.

2.2. Scientific Name Identification

The scientific name of each product was determined by common names appearing on the packages by using the information based on The Food and Agriculture Organization (FAO) (https://www.fao.org/fishery/en/species (accessed on 15 June 2023)) and FishBase [18] database. The scientific names were assigned to all possible species for the products labeled with market names (such as snow fish), relying on the consensus local data.

2.3. DNA Extraction and Quality Evaluation

The DNA extraction was carried out using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. In brief, roughly 30 mg of seafood

samples were excised with a scalpel and grounded, followed by the addition of lysis buffer and proteinase K solution. The mixture was incubated at 56 (±1) °C for cell lysis. The spin column was used for DNA binding with a silica spin filter during centrifugation. Ethanolic wash buffer was used to remove the contaminants, and the purified DNA was eluted with 50 µL of elution buffer. DNA quality was assessed using the NanoDrop Lite Plus Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA purity was considered good when the A260/A280 ratio range of 1.8–2.2 and the A260/A230 ratio range of 1.8–2.0 were obtained. The DNA samples were stored at −20 °C until use. The agarose gel electrophoresis technique was then used to determine the integrity of the DNA.

2.4. Polymerase Chain Reaction (PCR) and Sequencing

The polymerase chain reaction (PCR) was performed using Mastercycler nexus—PCR Thermal Cycler (Eppendorf, Hamburg, Germany) with a reaction volume of 20 µL. AllTaq Master Mix Kit (Qiagen, Hilden, Germany) was used for PCR preparation. The universal primers targeting 700 bp of the *COI* gene were used for PCR amplification of isolated DNA from seafood samples [19]. The sequences are as follows: forward primer: 5′-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3′ and reverse primer: 5′-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3′. The M13(-21) forward (5′-TGTAAAACGACGGCCAGT-3′) and M13(-27) reverse (5′-GGAAACAGCTATGAC-3′) tails were added for the sequencing of the PCR products. Additionally, universal primers targeting 18S rRNA (size 141 bp) were used as an endogenous control. The sequences of 18S rRNA primer are as follows: forward sequence: 5′-GGTAGTGACGAAAAATAACAATACAGGAC-3′ and reverse sequence: 5′-ATACGCTAT-TGGAGCTGGAATTACC-3′ [13].

The PCR reactions were prepared using 1X AllTaq buffer, containing 0.4 µM of each forward and reverse primer and 50–100 ng of DNA template. The PCR condition targeting *COI* started with an initial denaturation step at 95 °C for 2 min. Subsequently, a series of 30 PCR cycles was applied, each consisting of denaturation at 95 °C for 5 s, annealing at 50 °C for 45 s, and extension at 72 °C for 10 s. The final extension was performed at 72 °C for 10 min. For 18S rRNA primers, PCR amplification was initiated with denaturation at 95 °C for 2 min, followed by a series of 30 PCR cycles, each consisting of denaturation at 95 °C for 5 s, annealing at 59 °C for 30 s, and extension at 72 °C for 10 s and the final extension step at 72 °C for 5 min. The PCR results were observed using a 2% agarose gel electrophoresis, which was subsequently stained with a 1% ethidium bromide solution and observed under ultraviolet light. The amplified PCR product underwent purification using the PureLink Quick PCR purification kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Sanger sequencing was conducted for all PCR amplicons by outsourced services (U2Bio Co., Ltd., Seoul, Republic of Korea).

2.5. Data Analysis

The DNA chromatogram was edited using FinchTV version 1.4.0 (Geospiza, WA, USA). The edited DNA sequences were analyzed and compared via the nucleotide BLAST [20]. The criteria used to identify species are as follows: (1) All species identified by BLAST (including substituted species) were assigned to the samples based on the identical species name found in the top twenty BLAST search results with 98% sequence similarity [21]; (2) mislabeling was declared when the nucleotide BLAST result of the data from DNA barcoding does not provide the similarity of 98% or above on the DNA sequence expected as the single species identified on the seafood labels; (3) for the species under umbrella names (such as Alaska sole, Arctic cod, or Engawa), mislabeling was declared when the species identified by BLAST with 98% similarity or above does not match any scientific names of species under this umbrella. In general, the BLAST E-value indicates the number of alignments with scores are equivalent to or greater than expected in a database.

The conservation status of the samples was determined by identifying the IUCN status on the International Union for Conservation of Nature (IUCN) database (https:

//www.iucnredlist.org/ (accessed on 27 September 2023)) using scientific names obtained from BLAST analysis. The *COI* sequences were aligned using Clustal W implemented in MEGA version 11.0.3 [22]. The phylogenetic tree was constructed using IQ-TREE version 1.6.11 [23] with 1000 ultrafast bootstraps. The TIM2+F+I+G4 substitution model was selected using ModelFinder [24]. Pontoh's pygmy seahorse (*Hippocampus pontohi*—NCBI accession no: MH645136.1) was used as an outgroup in this analysis.

3. Results

3.1. Seafood Samples and Package Labels

All samples were categorized into different groups according to their package labels. There were three major groups of labels found in the seafood products obtained involving (1) labels with scientific names and common names on the package (Figure 1A), (2) products with only transparent packages and the common name on them (Figure 1B), and (3) products vacuum sealed without any labeling on the product (Figure 1C). The information on seafood samples used in this study can be found in Table 1. Specifically, six samples provided the scientific name on the product package, including two crustacean samples (ASS-N5 and RS-N5) and four fish samples (AOAA-N6, AOAO-N6, RTMS-N6, and AS-N1). Moreover, five samples were packed in vacuum-sealed packages without the mentioned details on the scientific names, including horse mackerel (HM-N1, HM-N2), green-eyed fish (GEF-N1), capelin (CAP-N3), Engawa (ENG-N5), and Tsubugai (TSB-N5/N4). Therefore, the scientific names of these samples were mentioned as inconclusive. The remaining samples were packed in transparent packaging, mentioning only the product names.

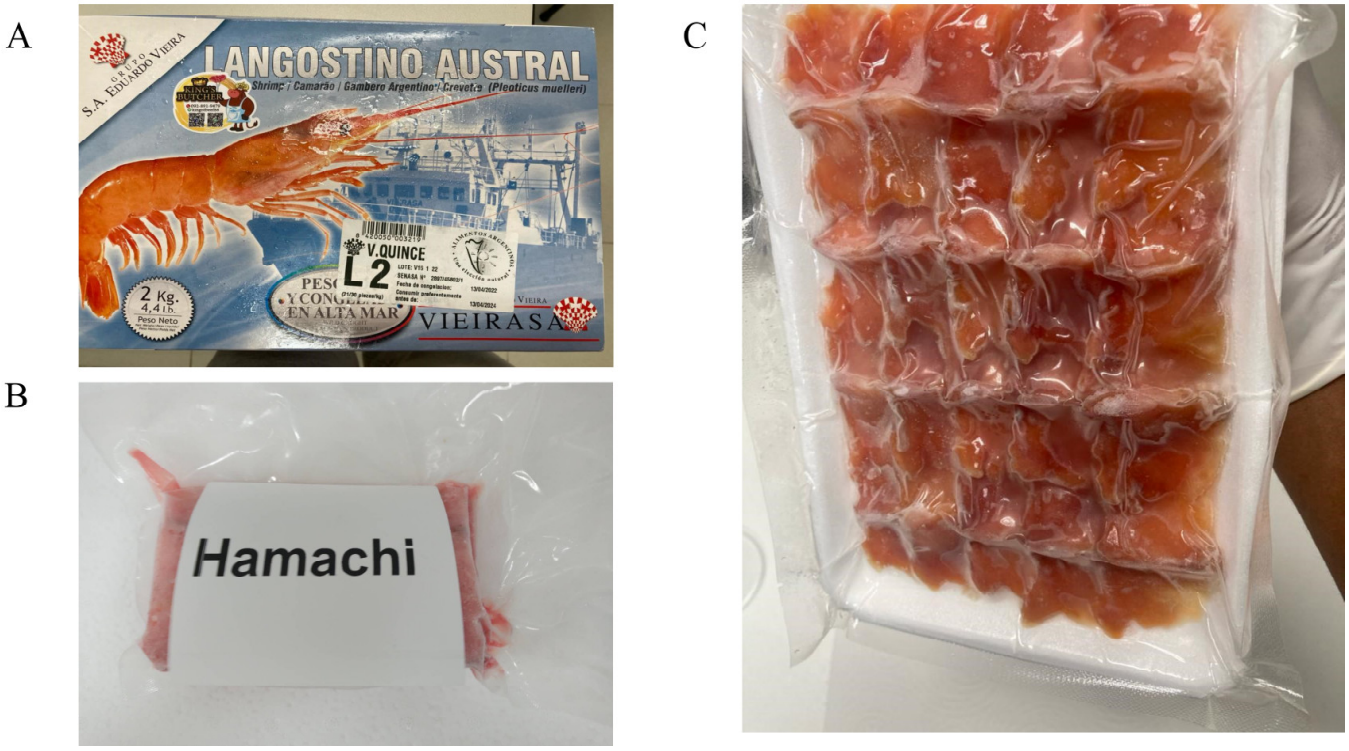

Figure 1. Examples of seafood packages obtained in this study. (**A**) A product labeled with a scientific name and common name. (**B**) Products are packed in a transparent bag with only a common name printed on paper. (**C**) Vacuum-sealed product without labeling.

Table 1. List of all seafood samples with brands, types, and distributors of samples.

No	Seafood Samples	Product Type	Packaged Name	Sample Name/ID	Sample Type	Brand/Distributors
1	Fish	Frozen	Trout (fjord)/rainbow trout	Tr	Fillet	Brand-D
2	Fish	Frozen	King salmon	KS	Fillet	Brand-D
3	Fish	Frozen	Sockeye salmon	SS	Fillet	Brand-D
4	Fish	Frozen	Japanese amberjack (Hamachi)	Hm	Fillet	Brand-A
5	Fish	Frozen	Alaska flathead sole	AFS-N1	Fillet	Brand-B
6	Fish	Frozen	Yellow tail collar meat	YTF-N1	Collar bone/head	Brand-B
7	Fish	Frozen smoked	Atlantic salmon (Norwegian) smoked black pepper	AS-N1	Fillet	Brand-C
8	Fish	Frozen	Horse mackerel—aji fry breaded	HM-N1	Whole fish	Brand-C
9	Fish	Frozen	Greeneye fish (Mehikari)	GEF-N1	Whole fish	Brand-C
10	Fish	Frozen	Kanetuka (Alaska pollock)	KAN-N2	Fillet	Brand-C
11	Fish	Frozen	Sockeye salmon (Alaska)	SS-N2	Fillet	Brand-C
12	Fish	Frozen	Halibut	HAL-N2	Fillet	Brand-C
13	Fish	Frozen	Horse mackerel—dried aji hiraki	HM-N2	Whole fish	Brand-C
14	Fish	Frozen	Alaska pollock	ALP-N2	Fillet	Brand-C
15	Fish	Frozen	QR code (cobia)	QR	Fillet	Brand-C
16	Fish	Frozen	Atlantic salmon	AS-N2	Fillet	Brand-C
17	Fish	Frozen	Snow fish (fillet)	SNF-N3	Fillet	Brand-C
18	Fish	Frozen	Cod fish (fillet)	COD-N3	Fillet	Brand-C
19	Fish	Frozen	Hirame fillet	HIR-N3	Fillet	Brand-C
20	Fish	Frozen	Capelin	CAP-N3	Whole fish	Brand-A
21	Fish	Frozen	Halibut	HAL-N3	Fillet	Brand-A
22	Fish	Frozen	Snow fish (fillet)	SNF-N3a	Fillet	Brand-A
23	Fish	Frozen	Snow fish (kimiri)	SNF-K-N3	Fillet	Brand-A
24	Fish	Frozen	Red tilapia	RT-N3	Whole fish	Brand-D
25	Fish	Frozen	Hallibut fillet	HAL-N3a	Fillet	Brand-D
26	Fish	Frozen	Snow fish fillet	SNF-N3b	Fillet	Brand-D
27	Fish	Frozen	Dory fish	PD-N3	Fillet	Brand-D
28	Fish	Frozen	Arctic cod	A-COD-N3	Fillet	Brand-D
29	Fish	Frozen	Black cod	B-COD-N3	Fillet	Brand-D
30	Fish	Frozen	Pangasius dory	PD-N5	Fillet	Brand-B
31	Fish	Frozen	Fish sashimi hiramasa	FSM-N5	Fillet	Brand-B
32	Fish	Frozen	Engawa slice	ENG-N5	Fillet	Brand-B
33	Shrimp	Frozen	Argentina sweet shrimp	ASS-N5	Whole shrimp	Brand-B
34	Shrimp	Frozen	Red shrimp (akeabi)	RS-N5	Whole shrimp	Brand-B
35	Fish	Frozen	Tsubugai sashimi grade	TSB-N5	Fillet	Brand-B

Table 1. *Cont.*

No	Seafood Samples	Product Type	Packaged Name	Sample Name/ID	Sample Type	Brand/Distributors
36	Fish	Frozen	Fish sashimi hamachi	FSP-N5	Fillet	Brand-B
37	Fish	Frozen	Alaska sole portion	ASP-N6	Fillet	Brand-B
38	Fish	Frozen	Akami akami	AOAA-N6	Fillet	Brand-B
39	Fish	Frozen	Akami otoro	AOAO-N6	Fillet	Brand-B
40	Fish	Frozen	Alaska pollack portion	APP-N6	Fillet	Brand-B
41	Fish	Frozen	Tuna steak	TS-N6	Fillet	Brand-B
42	Fish	Frozen	Tuna head to tail	THT-N6	Fillet	Brand-B
43	Fish	Frozen	Dried shima hokke hiraki (atka mackerel)	SHH-N6	Fillet	Brand-B
44	Fish	Frozen	Red-tailed mystus slice	RTMS-N6	Slice	Brand-E
45	Fish	Frozen	Redtail catfish	RTC-N6	Fillet	Brand-C

3.2. Seafood Mislabeling and IUCN Status Identifications

Polymerase chain reaction (PCR) revealed the appearance of a single DNA band of *COI* amplicon in the gel for each sample, indicating the adequate amplification of 700 bp of *COI* amplicon (Figure 2). After the identification of the scientific names of each sample, seven samples were found to be ambiguous products, including snow fish, Arctic cod, Engawa slices, Tsubugai sashimi, and Alaska sole portion (Table 2). These common market names can refer to more than one species of marine animals. Sanger sequencing revealed ten samples, which were mislabeled or substituted, including *Limanda aspera* (yellowfin sole), *Oncorhynchus mykiss* (rainbow trout), *Trachurus trachurus* (horse mackerel), *Oncorhynchus kisutch* (coho salmon), *Lepidocybium flavobrunneum* (escolar), *Atheresthes stomias* (arrowtooth flounder), *Reinhardtius hippoglossoides* (Greenland halibut), *Phractocephalus hemioliopterus* (redtail catfish), *Pangasianodon hypophthalmus* (iridescent shark), and *Oreochromis urolepis* (Wami tilapia) (Table 2). Moreover, two substituted samples were classified as endangered species in the IUCN red list, including *Pangasianodon hypophthalmus* and *Thunnus maccoyii*.

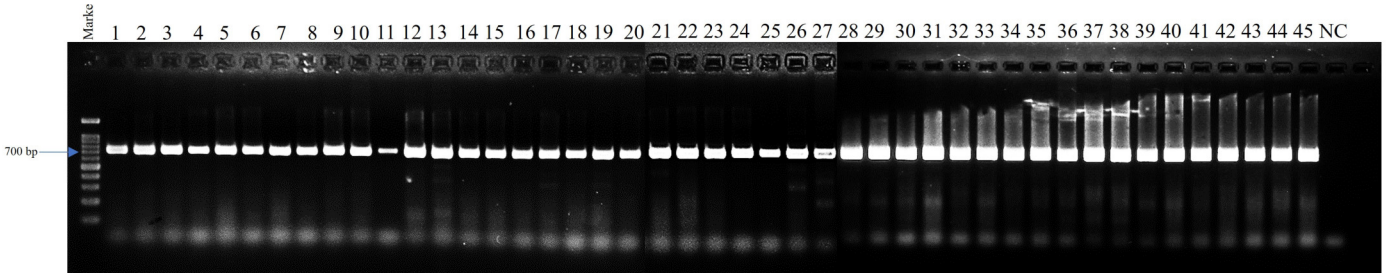

Figure 2. Agarose gel electrophoresis of *COI* amplicons of seafood samples. The PCR products of all samples demonstrated a size of 700 bp. Each sample is given with the reference no. 1–45 corresponding to those appearing in Table 1. Marker: 100 bp DNA ladder and NC: Negative control.

Table 2. Mislabeled seafood samples identified using the sequence of *COI* gene and BLAST analysis.

No.	Seafood Samples	Product Type	Packaged Name	Scientific Name	Remarks	Sample ID	BLAST Identified Results				Mislabeled	Brands	Identified Species	** IUCN Status of Identified Species
							Forward Sequence	Reverse Sequence	Identity Percentage	GenBank Accession No.				
1	Fish	Frozen	Trout (fjord)/rainbow trout	*Oncorhynchus mykiss*		Tr	✔	✔	99%	ON097695.1	No	Brand-D	-	NE
2	Fish	Frozen	King salmon	*Oncorhynchus tshawytscha*		KS	✔	✔	99%	OL457394.1	No	Brand-D	-	NE
3	Fish	Frozen	Sockeye salmon	*Oncorhynchus nerka*		SS	✔	✔	99%	KF278764.1	No	Brand-D	-	LC
4	Fish	Frozen	Japanese amberjack (Hamachi)	*Seriola quin-queradiata*		Hm	✔	✔	100%	OQ932939.1	No	Brand-A	-	LC
5	Fish	Frozen	Alaska flathead sole	*Hippoglossoides elassodon*		AFS-N1	X	X	98%	MH032456.1	Yes	Brand-B	*Limanda aspera*	LC
6	Fish	Frozen	Yellow tail collar meat	*Seriola quin-queradiata*		YTF-N1	✔	✔	99%	KU168711.1	No	Brand-B	-	LC
7	Fish	Frozen smoked	Atlantic salmon (Norwegian) smoked black pepper	*Salmo salar*		AS-N1	X	X	100%	KP013084.1	Yes	Brand-C	*Oncorhynchus mykiss*	LC
8	Fish	Frozen	Horse mackerel—aji fry breaded	*Trachurus japonicus*		HM-N1	✔	✔	100%	HM180926.1	No	Brand-C	-	NT
9	Fish	Frozen	Greeneye fish (mehikari)	*Chlorophthalmus albatrossis*		GEF-N1	✔	✔	99%	KP267656.1	No	Brand-C	-	LC
10	Fish	Frozen	Kanetuka (Alaska pollock)	*Gadus chalcogram-mus*		KAN-N2	✔	✔	100%	MW435131.1	No	Brand-C	-	NE
11	Fish	Frozen	Sockeye salmon (Alaska)	*Oncorhynchus nerka*		SS-N2	✔	✔	99%	KF278762.1	No	Brand-C	-	LC
12	Fish	Frozen	Halibut	*Reinhardtius hippoglos-soides*		HAL-N2	✔	✔	99%	MH032539.1	No	Brand-C	-	NT
13	Fish	Frozen	Horse mackerel—dried aji hiraki	*Trachurus japonicus*		HM-N2	X	X	99%	LR991652.1	Yes	Brand-C	*Trachurus trachurus*	Vu

Table 2. *Cont.*

No.	Seafood Samples	Product Type	Packaged Name	Scientific Name	Remarks	Sample ID	BLAST Identified Results				Mislabeled	Brands	Identified Species	** IUCN Status of Identified Species
							Forward Sequence	Reverse Sequence	Identity Percentage	GenBank Accession No.				
14	Fish	Frozen	Alaska pollock	*Gadus chalcogrammus*		ALP-N2	✔	✔	99%	MH035605.1	No	Brand-C	-	NE
15	Fish	Frozen	QR code (cobia)	*Rachycentron canadum*		QR	✔	✔	99%	KP410326.1	No	Brand-C	-	LC
16	Fish	Frozen	Atlantic salmon	*Salmo salar*		AS-N2	X	X	100%	OL457409.1	Yes	Brand-C	*Oncorhynchus kisutch*	LC
17	Fish	Frozen	Snow fish (fillet)	Inconclusive	Fish species with high oil are termed as snow fish. 1. *Anoplopoma fimbria* (Gindara) 2. *Dissostichus eleginoides* (Patagonian toothfish) 3. *Lepidocybium flavobrunneum* (Escolar)	SNF-N3	*Anoplopoma fimbria* (Gindara) ^	*Anoplopoma fimbria* (Gindara) ^	100%	JQ353976.1	No	Brand-C	-	NE
18	Fish	Frozen	Cod fish (fillet)	*Gadus morhua*		COD-N3	X	X	99%	HM007724.1	Yes	Brand-C	*Lepidocybium flavobrunneum*	Vu
19	Fish	Frozen	Hirame fillet	*Paralichthys olivaceus*		HIR-N3	X	X	99%	KF386420.1	Yes	Brand-C	*Atheresthes stomias*	NE
20	Fish	Frozen	Capelin	*Mallotus villosus*		CAP-N3	✔	✔	99%	FJ205579.1	No	Brand-A	-	NE
21	Fish	Frozen	Halibut	*Reinhardtius hippoglossoides*		HAL-N3	✔	✔	99%	MN893184.1	No	Brand-A	-	NT
22	Fish	Frozen	Snow fish (fillet)	Inconclusive	Fish species with high oil are termed as snow fish. 1. *Anoplopoma fimbria* (Gindara) 2. *Dissostichus eleginoides* (Patagonian toothfish) 3. *Lepidocybium flavobrunneum* (Escolar)	SNF-N3a	*Dissostichus eleginoides* ^	*Dissostichus eleginoides* ^	99%	NC_018135.1	No	Brand-A	-	NE

Table 2. *Cont.*

No.	Seafood Samples	Product Type	Packaged Name	Scientific Name	Remarks	Sample ID	BLAST Identified Results				Mislabeled	Brands	Identified Species	** IUCN Status of Identified Species
							Forward Sequence	Reverse Sequence	Identity Percentage	GenBank Accession No.				
23	Fish	Frozen	Snow fish (kimiri)	Inconclusive	Fish species with high oil are termed as snow fish. 1. *Anoplopoma fimbria* (Gindara) 2. *Dissostichus eleginoides* (Patagonian toothfish) 3. *Lepidocybium flavobrunneum* (Escolar)	SNF-K-N3	*Dissostichus eleginoides* ^	*Dissostichus eleginoides* ^	99%	NC_018135.1	No	Brand-A	-	NE
24	Fish	Frozen	Red tilapia	*Oreochromis niloticus*		RT-N3	X	X	100%	KU565854.1	Yes	Brand-D	*Oreochromis urolepis*	LC
25	Fish	Frozen	Hallibut fillet	*Reinhardtius hippoglos-soides*		HAL-N3a	✔	✔	99%	MN893184.1	No	Brand-D	-	NT
26	Fish	Frozen	Snow fish fillet	Inconclusive	Fish species with high oil are termed as snow fish. 1. *Anoplopoma fimbria* (Gindara) 2. *Dissostichus eleginoides* (Patagonian toothfish) 3. *Lepidocybium flavobrunneum* (Escolar)	SNF-N3b	*Dissostichus eleginoides* ^	*Dissostichus eleginoides* ^	99%	MW829388.1	No	Brand-D	-	NE
27	Fish	Frozen	Dory fish	*Pangasianodon hypophthal-mus*		PD-N3	✔	✔	100%	KR080263.1	No	Brand-D	-	EN
28	Fish	Frozen	Arctic cod	Inconclusive	Common Artic cod species: 1. *Boreogadus saida* (Polar cod) 2. *Gadus morhua* (Northeast Arctic cod)	A-COD-N3	*Gadus morhua* ^	*Gadus morhua* ^	99%	ON545931.1	No	Brand-D	-	Vu
29	Fish	Frozen	Black cod	*Anoplopoma fimbria*		B-COD-N3	✔	✔	99%	MG872572.1	No	Brand-D	-	NE

Table 2. *Cont.*

No.	Seafood Samples	Product Type	Packaged Name	Scientific Name	Remarks	Sample ID	BLAST Identified Results				Mislabeled	Brands	Identified Species	** IUCN Status of Identified Species
							Forward Sequence	Reverse Sequence	Identity Percentage	GenBank Accession No.				
30	Fish	Frozen	Pangasius dory	*Pangasianodon hypophthalmus*		PD-N5	✔	✔	99%	MW829646.1	No	Brand-B	-	EN
31	Fish	Frozen	Fish sashimi hiramasa	*Seriola lalandi*		FSM-N5	✔	✔	100%	LC178024.1	No	Brand-B	-	LC
32	Fish	Frozen	Engawa slice	Inconclusive	Engawa is part of flatfish species mainly: 1. *Paralichthys olivaceus* (Hirame) 2. *Pseudopleuronectes yokohamae* (Karei)	ENG-N5	X	X	99%	KF386350.1	Yes	Brand-B	*Reinhardtius hippoglossoides*	NT
33	Shrimp	Frozen	Argentina sweet shrimp	*Pleoticus muelleri*		ASS-N5	✔	✔	99%	MF490134.1	No	Brand-B	-	NE
34	Shrimp	Frozen	Red shrimp (akeabi)	*Pleoticus muelleri*		RS-N5	✔	✔	100%	KJ879318.1	No	Brand-B	-	NE
35	Fish	Frozen	Tsubugai sashimi grade	*Inconclusive*	Common whelks: 1. *Neptunea* spp., 2. *Buccinum undatum*, 3. *Babylonia japonica*	TSB-N5	X	X	100%	MW829378.1	Yes	Brand-B	*Reinhardtius hippoglossoides*	NT
36	Fish	Frozen	Fish sashimi hamachi	*Seriola quinqueradiata*		FSP-N5	✔	✔	100%	MK560632.1	No	Brand-B		LC
37	Fish	Frozen	Alaska sole portion	Inconclusive	Common Alaska sole: 1. *Pleuronectes quadrituberculatus* (Alaska plaice) 2. *Artheresthes stomias* (Arrowtooth flounder) 3. *Microstomus pacificus* (Dover sole) 4. *Hippoglossoides elassodon* (Flathead sole) 5. *Errex zachirus* (Rex sole) 6. *Pleuronectes bilineatus* (Rock sole) 7. *Limanda aspera* (Yellowfin sole)	ASP-N6	*Limanda aspera* ^	*Limanda aspera* ^	100%	KP975731.1	No	Brand-B	-	LC
38	Fish	Frozen	Akami akami	*Thunnus maccoyii*		AOAA-N6	✔	✔	100%	OU343215.1	No	Brand-B	-	EN

Table 2. *Cont.*

No.	Seafood Samples	Product Type	Packaged Name	Scientific Name	Remarks	Sample ID	BLAST Identified Results				Mislabeled	Brands	Identified Species	** IUCN Status of Identified Species
							Forward Sequence	Reverse Sequence	Identity Percentage	GenBank Accession No.				
39	Fish	Frozen	Akami otoro	*Thunnus maccoyii*		AOAO-N6	✔	✔	100%	JN086150.1	No	Brand-B	-	EN
40	Fish	Frozen	Alaska pollach portion	*Gadus chalcogrammus*		APP-N6	✔	✔	99%	MK216583.1	No	Brand-B	-	NE
41	Fish	Frozen	Tuna streak	All tuna species	All tuna species	TS-N6	✔	✔	99%	LN908910.1	No	Brand-B	*Thunnus albacares*	LC
42	Fish	Frozen	Tuna head to tail	All tuna species	All tuna species	THT-N6	✔	✔	99% 99%	MW817806.1 KF925362.1	No	Brand-B	*Thunnus* species.	
43	Fish	Frozen	Dried shima hokke hiraki (atka mackerel)	*Pleurogrammus monopterygius*		SHH-N6	✔	✔	100%	JQ354277.1	No	Brand-B	-	NE
44	Fish	Frozen	Red-tailed mystus slice	*Hemibagrus wyckioides*		RTMS-N6	X	X	99%	MF083116.1	Yes	Brand-E	*Phractocephalus hemioliopterus*	LC
45	Fish	Frozen	Redtail catfish	*Phractocephalus hemioliopterus*		RTC-N6	X	X	99%	MH119967.1	Yes	Brand-C	*Pangasianodon hypophthalmus*	NE

** Note: International Union for Conservation of Nature (IUCN); EN—endangered; Vu—vulnerable; NT—near threatened; LC—least concern; NE—not evaluated and could not be assessed. ✔—Correct forward and reverse sequence as per the package and scientific name identified initially with the reference database. X—Mismatched forward and reverse sequence as per the package and scientific name identified initially with the reference database. ˆ - Sequence which was identified from various near similar species or common names.

3.3. Seafood Mislabeling in the Aspects of Brands, Product Types, and Species

Different proportions of product mislabeling regarding retailers are demonstrated in Figure 3A. The rates of mislabeling were as follows: Brand-C exhibited the most remarkable mislabeling rate of 42.85%, followed by Brand-B, with a rate of 18.75%, and Brand-D, with the lowest rate of 11.11%. When considering the mislabeling rate based on types of seafood products, whole fish showed the highest rate of mislabeling (40%), followed by fillets (25%; Figure 3B). Inter-species substitutions were discerned among the sampled specimens, notably occurring within the same taxonomic families. For instance, various salmon species within the Salmonidae family, including coho salmon and rainbow trout, were identified, resulting in a mislabeling rate of 33% among the total salmon samples. Similarly, mackerel fish, affiliated with the Carangidae family, displayed substitution instances between Japanese horse mackerel and Atlantic horse mackerel. Furthermore, within the Cichlidae family, substitutions were particularly evident in tilapia, with Nile tilapia and Wami tilapia identified as among the detected substitutes. Additionally, instances of mislabeling were observed in fillet product packaging, where flathead sole was substituted with Yellowfin sole and Olive flounder with Arrowtooth flounder of the Pleuronectidae family, resulting in 100% mislabeling within these three families.

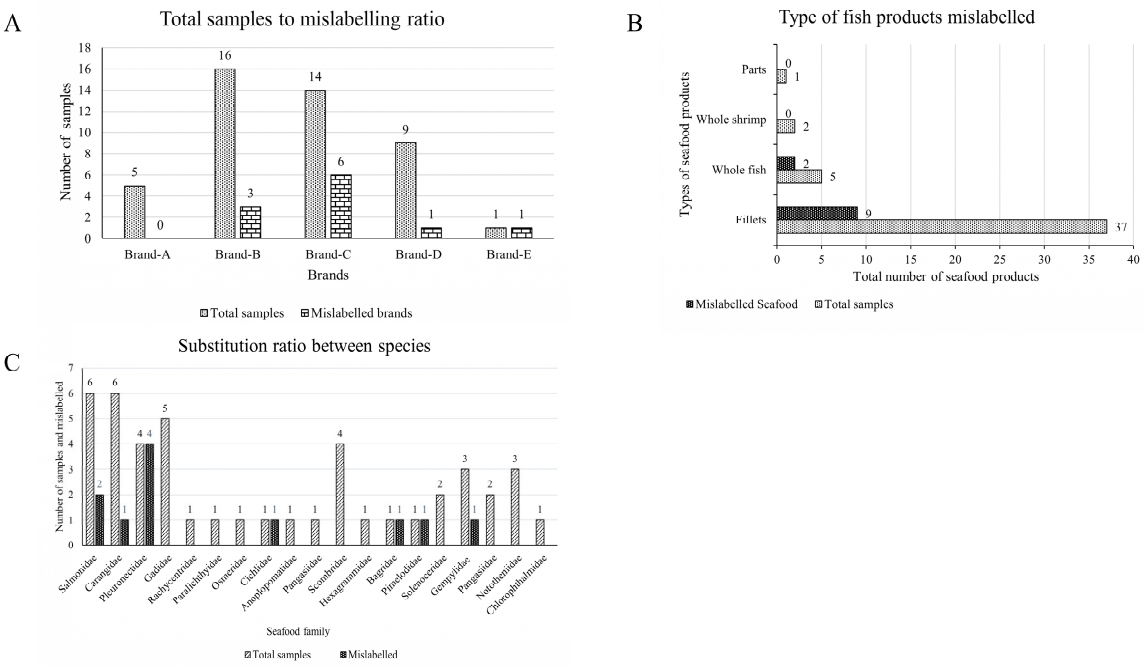

Figure 3. (**A**) The ratio of mislabeled products compared with total products from each brand. The mislabeling rates of Brand-C, Brand-B, and Brand-D were 42.85%, 18.75%, and 11.11%, respectively. For Brand-E, the percentage of mislabeling was 100%. (**B**) The ratio of the type of mislabeled seafood sample was computed relative to the total products purchased. (**C**) Total seafood samples were substituted and mislabeled between family and species levels.

3.4. Phylogenetic Comparison of Mislabeled Samples

A phylogenetic tree was constructed using barcode sequences obtained from mislabeled samples. Samples labeled as Engawa slice (ENG-N5) and Tsubugai sashimi grade (TSB-N5) showed the highest degree of similarity (96% and 99%, respectively) to the known reference sequence of Reinhardtius hippoglossoides (Figure 4). Similarly, the other seafood samples were identified to be incorrectly labeled on the package. The highest similarity of an organism obtained from phylogenetic analysis for each mislabeled sample was as follows (sample: species identified format): AFS-N1: *Limanda aspera*, HIR-N3: *Atheresthes stomias*, HM-N2: *Trachurus trachurus*, AS-N1: *Oncorhynchus mykiss*, AS-N2: *Oncorhynchus kisutch*, COD-N3: *Lepidocybium flavobrunneum*, RT-N3: *Oreochromis urolepis*, RTMS-N6: *Phrac-*

tocephalus hemioliopterus, and RTC-N6: *Pangasianodon hypophthalmus*. The pair mentioned above samples also exhibited high similarity (96–99%), signifying the mislabeling.

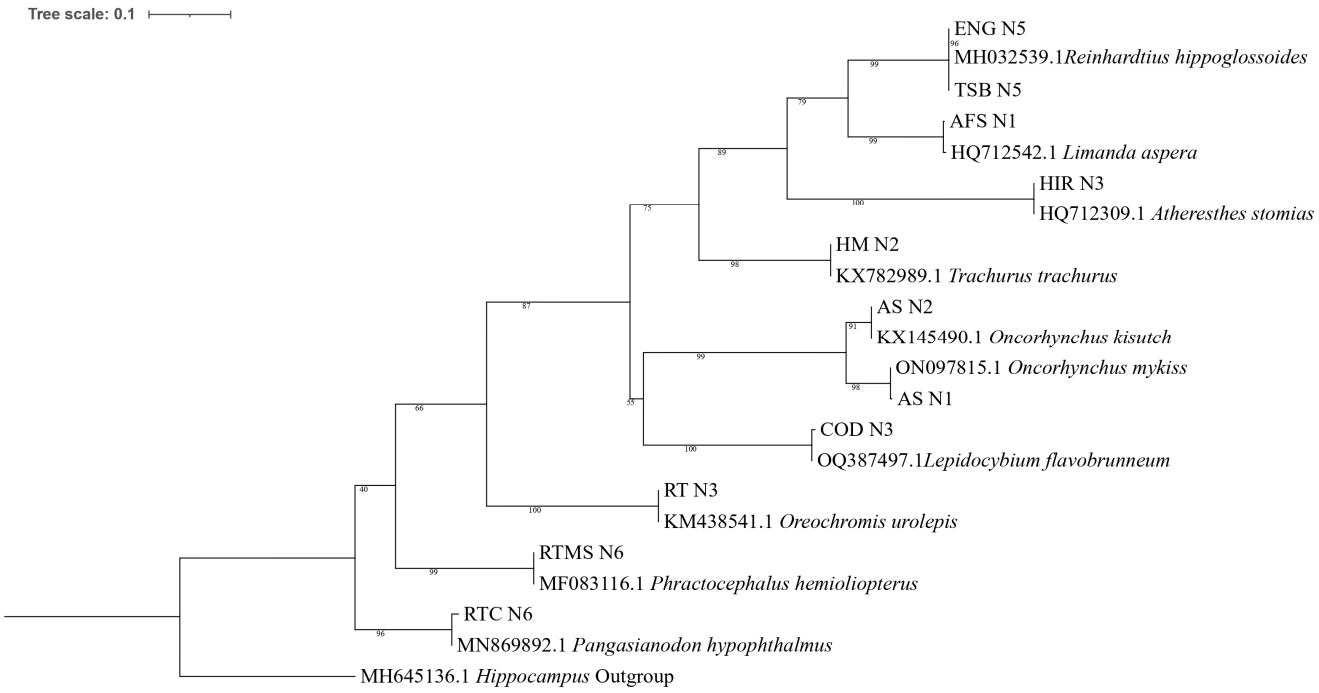

Figure 4. Rooted maximum likelihood phylogenetic tree constructed using the DNA sequence of mislabeled commercial fish products with the NCBI reference GenBank database. Bootstrap values are shown at each node. *Hippocampus* species was used as an outgroup.

4. Discussion

DNA barcoding is a standard method used for seafood authentication [8]. This method employed the conserved DNA sequence of mitochondrial genes, such as *COI*, Cytochrome b, or 16S rDNA, for species identification [8,25]. DNA barcodes of full-length *COI* genes were successfully amplified with more than 80% of seafood samples [8,26]. Additionally, the entire length of the *COI* gene primer with M13 tails was more precise and effective for sequencing than the conventional primers without M13 [19].

In this study, although most seafood products were successfully identified at the species level through DNA barcoding, determining the scientific names from the typical, market, and local names based on the product packaging labels was challenging because declaring the product species on the label is not compulsory in Thailand [8]. Some fish products share similar market names with other species, leading to customers' misconceptions about the species they require. For example, snow fish can refer to several kinds of oily white flesh fish species, such as *Anoplopoma fimbria* (gindara), *Dissostichus eleginoides* (Patagonian toothfish), or *Lepidocybium flavobrunneum* (escolar) [27]. This phenomenon was confirmed by the finding in this study, in which two species sold as snow fish showed obvious differences in the country of origin and price. Two different species were also identified under this market name, including *Anoplopoma fimbria* (gindara) and *Dissostichus eleginoides* (Patagonian toothfish), by DNA barcoding. Since proper labeling provides information that influences customers' decisions, a precise and complete label should be mandatory for the manufacturer [8].

The present study revealed the mislabeling of imported seafood products generally sold in Thailand. The information from FAO and Fishbase databases was used to find the scientific names of each product included in this study. Fishbase is a vast database on fish taxonomy, ecology, and biology. This database has been successfully applied to identifying marine species by many publications [21,28–30]. FAO, another website with

statistical databases, also provides reliable information on nutrition, food, agriculture, fisheries and aquaculture, and many sub-divisions of the FAO's mandate [31]. As a result, salmon was found to be the species with the highest rate of substitution, mostly misrepresented by *Oncorhynchus mykiss* (Rainbow trout, AS-N1) and *Oncorhynchus kisutch* (Coho salmon, AS-N2). This finding was in accordance with the previous study, in which the most common marine mislabeled species were *Salmo salar*, *Oncorhynchus mykiss*, *Oncorhynchus kisutch*, *Gadus chalcogrammus*, *Oreochromis niloticus*, and *Pangasianodon hypophthalmus* [8,32]. Although all these species are a member of the salmonids genera, they are different in terms of pricing and the quality of their meat [8,33].

The adulteration of health-threatening species is one of the issues of concern in the seafood mislabeling situation. DNA barcoding of imported seafood revealed the substitution of Atlantic cod (*Gadus morhua*; sample COD-N3) for escolar fish (*Lepidocybium flavobrunneum*), which may cause gastrointestinal illness, such as abdominal cramping, nausea, vomiting, and diarrhea [34] due to the high proportion of oil in their meat [27]. This oily fish was also reported to be used as a substitute for tuna species in Brazil [35,36]. Therefore, the surveillance of using escolar for substitution is crucial to prevent the occurrence of seafood-related illnesses.

Seafood misrepresentation does not involve only customer rights and transparency issues. The substitution has also affected the sustainability implications, particularly the replacement by the IUCN Red List of Threatened Species. In this study, two seafood products were substituted by threatened species, including horse mackerel (sample HM-N1) and redtail catfish (sample RTC-N6). Horse mackerel (Japanese horse mackerel or Aji) is a popular fishing species that is high in nutrition at a low price. However, DNA barcoding revealed the misrepresentation of Japanese horse mackerel replaced by Atlantic horse mackerel (*Trachurus trachurus*), which has been listed as "vulnerable" on the IUCN's red list of threatened species. Interestingly, although both horse mackerel are a member of the Trachurus genera, the distribution of each species is totally different in terms of geographical habitat. While Japanese horse mackerel is found in the Northwest Pacific Ocean from southern Japan, along the Korean Peninsula to the East China Sea, the distribution of Atlantic horse mackerel is around the Eastern Atlantic Ocean to South Africa and northward extending into the Mediterranean Sea and the Atlantic coasts of Europe [37]. In Spain, the bluefin tuna (*Thunnus thynnus*) has the highest substitution rate, with 58% substituted by other less expensive tuna species, such as yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) [36]. This implies the complexity of the seafood supply chain in addition to the morphological resemblance, which may contribute to mislabeling/misrepresentation for intentional or unintentional purposes [37].

Another threatened species found in this study was *Pangasianodon hypophthalmus*, commonly known as pangasius or striped catfish. This freshwater species belongs to the family *Pangasiidae*, which is often mislabeled with *Pangasius bocourti* as both species belonging to the same family [38]. *Pangasianodon hypophthalmus* has gained significant popularity and widespread consumption in Thailand due to its successful artificial breeding endeavors initiated in 1966. It is likely that the striped catfish is one of the species most involved in mislabeling since its white flesh makes an easy substitute for a variety of expensive white flesh fish species. For example, European perch (*Perca fluviatilis*) was reported to be substituted by *P. hypophthalmus* in Italy [9]. The replacement by using striped catfish was also found in snapper, grouper, sole, cod, and even sharks [39,40]. In Southeast Asian supermarkets, frozen striped catfish fillets are commonly mislabeled as a dory, probably as an inference to fishes under the John Dory fish family (Zeidae) [41].

As per the IUCN red list, *Pangasianodon hypophthalmus* is labeled as an endangered species which cannot be sold commercially. However, the consumption of endangered species is still documented in the vast seafood trade. Several endangered species have been illegally imported as fisheries products, such as narrow sawfish (*Anoxypristis cuspidata*) in Australia [42], bluefin tuna (*Thunnus thynnus*) in Belgium [43], and scalloped hammerhead (*Sphyrna lewini*) in Canada. *Thunnus maccoyii* (AOAA-N6 and AOAO-N6), known as

Southern bluefin tuna, has been designated as endangered by the IUCN red list since 1996. It was reported that stock abundance of Southern bluefin tuna dropped by 90% within 29 years in 1996. The population will not be extinct if the stock decreases by 90% within 30 years due to the long generation time for Southern bluefin tuna (15 years) [44]. However, it is still sold commercially despite being classified as endangered.

5. Conclusions

The species of a total of 45 seafood products, including fish and shrimp, were successfully identified using the DNA barcoding technique. The mislabeling rate is relatively high, about 24.44%, in which salmon showed the highest rate of misrepresentation. There are some products using generic and ambiguous terms as market names, such as snow fish, which have been used as a name for three fish species, including gindara, Patagonian toothfish, and escolar. Moreover, one product was substituted by vulnerable species listed in the IUCN Red List of Threatened Species. The universal primer used in this study was suitable for identifying natural and raw products such as fillets and less processed fish products. Distinguishing species and scientific names for each sample from common, market, and local names on product packaging labels is still required to conquer the mislabeling of seafood and products. It can be used by both governmental agencies and industry for seafood authentication monitoring, contributing to the transparency of the seafood market in Thailand and worldwide.

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