Product Authentication Using Two Mitochondrial Markers Reveals Inconsistent Labeling and Substitution of Canned Tuna Products in the Taiwanese Market

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Abstract: Fish of the tribe Thunnini represent a significant proportion of the stock caught by the fishing industry, with many of these fishes being collectively called tuna. However, only certain species can be used legally as an ingredient in canned tuna products, depending on regional food regulations. In Taiwan, only Thunnus species or Katsuwonus pelamis can be used as canned tuna. Here, we authenticated 90 canned tuna products, including 25 cat food samples, by sequencing two mitochondrial regions, 16S rRNA (16S) and the control region (CR). BLAST analysis revealed that Sarda orientalis, Euthynnus affinis, Auxis rochei, and Auxis thazard are all used as substitutes for legitimate tuna products. We found that 63.33% of investigated samples are true canned tuna, i.e., contain Thunnus species or skipjack tuna. We advocate that the Taiwanese government publishes an official standardized list of fishes, especially so that scientific, Chinese and vernacular names can be assigned unambiguously based on a “one species-one name policy”, thereby clarifying which species can be used in seafood products such as tuna. Furthermore, we feel that the large-scale and long-term monitoring of canned tuna products is warranted to fully assess the extent of tuna product adulteration in Taiwan.

Keywords: Thunnini; substitution; mislabeling rate; one species-one name; adulteration

1. Introduction

Approximately 17% of the global human population’s intake of animal protein in 2017 consisted of fish [1]. Although aquaculture satisfied about half of that consumption, wild-capture from oceans, lakes and rivers remains a mainstay of the global fishing industry. Among these wild-caught fishes, scombrids are particularly important fishery resources, especially species in the tribe Thunnini that constitute ~10% of the international seafood market [2,3]. In 2018, the global catch of Thunnini species represented ~7.9 million tons, 58% of which can be attributed to skipjack tuna (Katsuwonus pelamis) (in Chinese: 正鰹) and yellowfin tuna (Thunnus albacares) (in Chinese: 黃鰶) [1]. A large proportion of the Thunnini catch is destined for the canning industry [4,5].

The tribe Thunnini comprises five genera: Thunnus (in Chinese: 鮪屬), Katsuwonus (in Chinese: 正鰹屬), Auxis (in Chinese: 鱩屬), Euthynnus (in Chinese: 巴鰹屬), and Allothunnus (in Chinese: 細鰹屬). Fishes of this tribe can be generally termed “tuna”. For example, Auxis rochei is called bullet tuna (in Chinese: 圓花鰹), Euthynnus alletteratus is little tuna (in Chinese: 小鰹), and Allothunnus fallai is slender tuna (in Chinese: 細鰹). However, the Chinese translation of tuna is 鮪 in Taiwan or 金槍魚 in Mainland China, which specifically refers solely to Thunnus spp. Previously, many different scombrids were used as an ingredient in “canned tuna”, even if they did not belong to the tribe Thunnini.
For instance, *Sarda* (in Chinese: 鲷鰭屬) spp. were once widely used in canned tuna because they possess a similar taste and texture to it [6]. Importantly, a species of the tribe Thunnini may not always be used legally as a canned tuna product ingredient. Various legislative bodies have developed regulations that clearly define which species can be used in canned tuna products (Table 1). The Food and Agriculture Organization (FAO) and the federal government of the United States allow spotted tuna to be used as canned tuna, but that species is prohibited by Taiwanese and Japanese regulations. In general, fishes of the genus *Thunnus* and skipjack tuna are widely recognized as legal canned tuna species. To align with international standards, the Taiwan Food and Drug Administration allows skipjack canned tuna ingredient. Thunnus species is prohibited by Taiwanese and Japanese regulations. In general, fishes of the genus *Sarda* are used in canned tuna products, even though it does not belong to the genus *Thunnus*, but other “pseudo-tunas” can no longer be used legally as a canned tuna ingredient.

Table 1. Scientific, English common, Chinese common and vernacular names of scombrid fishes permitted by various legislative bodies as canned tuna or bonito products.

| Scientific Name | English Common Name | Chinese Name | Chinese Vernacular Name | Taiwan | FAO | USA | Japan | European Union |
|-----------------|---------------------|--------------|-------------------------|--------|-----|-----|-------|----------------|
| Thunnini tribe  |                     |              |                         |        |     |     |       |                |
| *Thunnus alibarua* | Albacore tuna | 長脅鰭 | 串仔、長鰭串、白肉串、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus albacares* | Yellowfin tuna | 黃鰭鰭 | 串仔、黃鰭串、黑肉鰭串、黃鰭串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus atlanticus* | Blackfin tuna | 黑鰭鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus obesus* | Bigeye tuna | 大目鰭 | 串仔、大眼鰭、大目串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus maccoyii* | Southern bluefin tuna | 南方黑鰭 | 串仔、大眼鰭、大目串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus thynnus* | Atlantic bluefin tuna | 大西洋黑鰭 | 串仔、大眼鰭、大目串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus orientalis* | Pacific bluefin tuna | 太平洋黑鰭 | 串仔、大眼鰭、大目串 | ✓      | ✓   | ✓   |       |                |
| *Thunnus tongol* | Longtail tuna | 長脅鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Katsuwonous pelamis* | Skipjack tuna | 正鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Auxis rochei* | Bullet tuna | 圈花鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Auxis thazard* | Frigate tuna | 圈花鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Euthynus affinis* | Kawakawa | 巴鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Euthynus allerus* | Little tunny | 小巴鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Euthynus lineatus* | Black skipjack tuna | 黑巴鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Allothunnus fallai* | Slender tuna | 派巴鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Sardina orientalis* | Oriental bonito | 東方鰭鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Sardina sarda* | Atlantic bonito | 鯖鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |
| *Sardinus chilensis* | Eastern Pacific bonito | 智利鰭鰭 | 串仔、長鰭串 | ✓      | ✓   | ✓   |       |                |

1. 107年度「鰭橋完整魚品標示管理體系計畫」—「宣稱鰭橋魚種標示說明會」，Taiwan; 2. Standard for canned tuna and bonito CXS 70-1981, Codex Alimentarius FAO-WHO; 3. Code of federal regulations CFR 21. Sec. 161.190, United State Food and Drug Administration, USA; 4. 水産物魚品及び水産物製品の日産規格, Japan; 5. European regulation (Council Regulation (EEC) No 1536/92) [7–11].
Seafood mislabeling is profuse worldwide [12–21]. Such mislabeling can be categorized into two types, i.e., deliberate or unintentional. Deliberate mislabeling primarily involves the substitution of high-priced fishes with low-priced ones for financial reasons, though the reverse scenario also arises occasionally, perhaps due to illegal fishing. Unintentional mislabeling occurs when morphologically similar fishes are misidentified, when the usage of vernacular names is not unified, or when product information is lost along the supply chain. Whatever the form of mislabeling, it ultimately entails consumer deception, public health risk, problems for fisheries management, and has religious implications (reviewed in Chang et al. [22]).

Traditional morphology-based identification is rarely applied to seafood because many products undergo physical (e.g., filleting) or chemical (e.g., smoking) processing before being sold. These aspects of food processing typically eliminate diagnostic morphological characters needed for species authentication. Fortunately, molecular authentication based on nucleic acid sequence similarity can overcome this limitation. DNA can be obtained from a tiny piece of tissue and it is more resistant to degradation and food processing. Therefore, DNA-based authentication is being widely employed to identify species in seafood [15,21,23–30].

The increasing global popularity of Japanese cuisine has markedly increased market demand for tuna, since *Thunnus* fishes are important elements of sashimi and sushi. The development of freezing technology and booming global trade in the early 1970s has transformed the bluefin tunas (*T. thynnus* (in Chinese: 大西洋黒鮪), *T. maccogii* (in Chinese: 南方黒鮪), and *T. orientalis* (in Chinese: 太平洋黑鮪)) from a cat food into a delicacy served at high-end restaurants [31]. Bluefin tunas are the most sought after of all *Thunnus* fishes, attaining the largest size and greatest price. However, increased consumption has also threatened their stocks, which are decreasing and the status of all three species is deemed Critical (IUCN). Today, regional fishery management organizations are responsible for managing and monitoring tuna fishing in order to keep it sustainable [32].

The soaring demand for *Thunnus* fishes, especially bluefin tuna, makes them very vulnerable to mislabeling. Previous molecular authentication studies on sushi reported that many fishes are used as substitutes for *Thunnus* species, including escolar (*Lepidocybium flavobrunneum*) (in Chinese: 鱸網帶鰆), salmon (*Salmo salar*) (in Chinese: 安大略鱒), banded rudderfish (*Seriola zonata*) (in Chinese: 環帶鰤), great amberjack (*Seriola dumerilii*) (in Chinese: 杜氏鰤), skipjack tuna, little tunny, as well as various shark species [14,17,18,20,33–37]. Furthermore, the value of different *Thunnus* species varies, prompting high-priced bluefin tuna or bigeye tuna (*T. obesus*) (in Chinese: 大目鮪) to be substituted for a cheaper species such as yellowfin tuna. Notably, enforcement of fishery management can drive reverse substitution, whereby high-priced bluefin tuna is sold as cheap yellowfin tuna or *Thunnus* fishes are labeled as skipjack tuna to enable market entry of illegal catch [33,38,39].

Although DNA-based methods are very powerful tools for authenticating fish products, food processing, and especially canning, can limit their applicability. To date, conventional DNA barcoding remains the most widely deployed authentication approach, whereby a ~650-bp region of the mitochondrial gene encoding for cytochrome c subunit I (COI) is sequenced as a bioidentification “barcode” [40,41]. However, the high heat treatment integral to the canning process largely degrades DNA into small fragments [42,43], so shorter fragments (or nested polymerase chain reaction, PCR) must be deployed for canned products [4–6,39,44,45], but a comprehensive investigation of canned tuna substitution in a particular region has not yet been conducted. In Taiwan, the mislabeling rate of tuna products varies according to the product type. Chang et al. [22] documented that all tuna-labeled meals produced at conveyor-belt sushi restaurants appear to truly come from *Thunnus* fishes, but Xiong et al. [46] and Hwang et al. [44] detected mislabeled Taiwan canned tuna products. Therefore, the goal of this study is to estimate levels of canned tuna product adulteration and to determine which species of scombrids are being marketed as canned product in the Taiwanese market.
2. Materials and Methods

2.1. Sample Collection

We purchased a total of 90 canned tuna products, belonging to 59 brands, from grocery stores or online, encompassing all major brands in Taiwan. Twenty-five of the collected samples represented canned cat food. Cans were selected if their label showed the Chinese word 鮪 (for tuna), if the company website claimed the product was made from Thunnus fishes, if the ingredients list contained Thunnus spp. or skipjack tuna, or if an image on the label indicated the can harbored Thunnus fishes. We recorded information, typically written in Chinese, on brand, manufacturer or importer, place of manufacture, labeling, and ingredients. If the cans were imported from the USA or Japan, the respective English or Japanese labels were also recorded (Table 2). The sampled cans were first photographed using a smartphone (Supplementary Information S1), and then a small quantity of the contents of each can was removed using autoclaved dissection tools, washed with 95% ethanol, before being preserved in 99.5% ethanol at −20 °C until DNA extraction. Some of the canned cat food products contained more than one type of meat, so potential Thunnus meat was selected based on color and texture.

2.2. Molecular Identification

DNA was extracted from each of the 90 tissue samples using DNA Extraction Kit S (Cat No./ID: GS100, Geneaid). PCR amplifications of the mitochondrial 16S rRNA fragment (16S) (85 bp) were performed in a mixture containing 5 ng template DNA, 12.5 µL of 2× Taq PCR MasterMix (GN-PCR201-01, Genomix), and 12.5 µmol of each forward and reverse primer. We used primers designed by Horreo et al. [47] and modified them by adding M13 primer to facilitate sequencing: Forward, M13F(-20)16S-HF (5′-GTA AAA CGA CGG CCA GTA TAA CAC GAG AAG ACC CT-3′); Reverse, M13R(-24)16S-HR1+2 (5′-AACAGCTATGACCATGCCCGGCGGTCCGCCCCA AC-3′). These primers were made up to a final volume of 25 µL using distilled water. If BLAST analysis (in the NCBI basic local alignment search tool) indicated that a sequenced 16S fragment belonged to Thunnus spp., we PCR-amplified a fragment of the mitochondrial control region (CR, approximately 236 bp) from the same DNA sample. CR amplification was conducted in a mixture containing 5 ng template DNA, 12.5 µL of 2× Taq PCR MasterMix (GN-PCR201-01, Genomix), and 12.5 µmol of each forward and reverse primer—Forward, Tuna-CR_F; Reverse, Tuna-CR_R1 or Tuna-CR_R2 [48]—made up to a final volume of 25 µL using distilled water. Thermal cycling began with one cycle at 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 s, 47–55 °C for 30 s, and 72 °C for 30 s and, finally, a single extension step at 72 °C for 7 min. PCR products were purified using a PCR DNA Fragment Extraction Kit (Geneaid, Taipei, Taiwan). The amplified mitochondrial fragments were subjected to Sanger sequencing, performed by Mission Biotech. (Taipei, Taiwan) using M13 sequencing primers. Primer sequences linked to the amplified fragments were trimmed before constructing the contigs using CodonCode Aligner. The mitochondrial sequences we generated in this study have not been submitted to GenBank as they do not come from voucher specimens.
Table 2. List of all canned tuna product samples authenticated by 16S rRNA BLAST and the results of neighbor-joining (NJ) analysis based on the mitochondrial control region (CR).

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|----------------------------|----------------------|---------------|---------------|---------------------|---------------------------------|----------------------|------------------------|------------------------|----------------|------------|----------------|
| 1   | T1          | 遠洋牌 | 遠洋冷凍食品               | Taiwan               | 鮪魚片        | Light tuna in oil | 鮪魚 | Thunnus spp.       | NO                  | Hap_F            | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccogii (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                      | |
| 2   | T53         | 遠洋牌 | 遠洋冷凍食品               | Taiwan               | 油漬鮪魚肉塊(煙仔虎) | 煙仔虎 | Skipjack tuna or oriental bonito | YES                | Hap_G            | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES NO                      | |
| 3   | T57         | 遠洋牌 | 遠洋冷凍食品               | Taiwan               | 玉米+鮪魚 | Tuna + sweet corn | 鮪魚 | Thunnus spp.       | NO                  | Hap_F            | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccogii (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                      | |
| 4   | T62         | 三興 | 恵昇食品                   | Taiwan               | SH油漬鮪魚(東方鰆) | Tuna in oil | 東方鰆 | Oriental bonito   | YES                | Hap_A            | Sarda orientalis (3) | NO NO                      | |
| 5   | T2          | 三興 | 恵昇食品                   | Taiwan               | SH水煮鮪魚(東方鰆) | Tuna in brine | 東方鰆 | Oriental bonito   | YES                | Hap_A            | Sarda orientalis (3) | NO NO                      | |
Table 2. Cont.

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|---------------------|--------------------------|---------------------------|----------------|-----------|-----------------|
| 6   | T3          | 好媽媽 | 東和食品                | Taiwan              | 鯖魚          | Tuna flakes in brine | 鯖魚               | Thunnus spp.                     | NO                  | Hap_C                     | Katsuwonus pelamis (7), Thunnus albacares (1) | YES          | YES       |                 |
| 7   | T52         | 好媽媽 | 東和食品                | Taiwan              | 陳妹鮪魚      | Tuna flakes in chili oil | 鮪魚               | Thunnus spp.                     | NO                  | Hap_F                     | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccgoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO           | YES       |                 |
| 8   | T4          | 好媽媽 | 東和食品                | Taiwan              | 三明治鮪魚 (煙仔虎) | Tuna sandwich | 烟仔虎 (鮪魚) | Skipjack tuna or oriental bonito (Thunnus spp.) | YES                  | Hap_A                     | Sarda orientalis (3) | ?            | NO        |                 |
| 9   | T49         | 好媽媽 | 東和食品                | Taiwan              | 無添加玉米鮪魚 Corn Tuna | Corn tuna | 烟仔虎 | Skipjack tuna or oriental bonito | YES                  | Hap_A                     | Sarda orientalis (3) | NO           | NO        |                 |
| 10  | T18         | 蘇澳區漁會 | 東和食品                | Taiwan              | 水煮鮪魚      | Canned boiled tuna | 鮪魚               | Thunnus spp.                     | NO                  | Hap_B                     | Euthynnus affinis (4), E. lineatus (1) | YES          | NO        |                 |
| 11  | T20         | 冬山河 | 東和食品                | Taiwan              | 三明治鮪魚 | 鮪魚          | Thunnus spp. | NO                          | Hap_B                      | Euthynnus affinis (4), E. lineatus (1) | YES          | NO        |                 |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haploype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|----------------------|-------------------------|-------------------------|---------------|------------|-----------------|
| 12  | T21         | 鮪拉鮮 | 東和食品 | Vietnam | 鮪魚罐頭 | 鮪魚 | Thunnus spp. | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO | |
| 13  | T5          | 紅鷹牌 | 活寶食品 | Taiwan | 鮪魚罐頭 | 鮪魚 | Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES | |
| 14  | T6          | 紅鷹牌 | 活寶食品 | Taiwan | 鮪魚罐頭 | 東方鰹魚 | Oriental bonito | YES | Hap_A | Sarda orientalis (3) | NO | NO | |
| 15  | T7          | 紅鷹牌 | 活寶食品 | Taiwan | 鮪魚罐頭 | 鮪魚 (鮪魚) | Thunnus spp. (Thunnus) | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccouui (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES | |
| 16  | T8          | 紅鷹牌 | 活寶食品 | Taiwan | 竹筍鮪魚 | 正當 (釣魚) | Skipjack tuna (Thunnini) | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO | |
| 17  | T9          | 紅鷹牌 | 活寶食品 | Taiwan | 鮪魚罐頭 | 鮪魚 | Thunnus spp. | NO | Hap_A | Sarda orientalis (3) | YES | NO | |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|---------------------------|----------------------|---------------|--------------|--------------------|----------------------------------|----------------------|------------------------|----------------------|----------------|-------------|-----------------|
| 18  | T54         | 紅鷹牌 | 活寶食品                 | Taiwan               | 紅鷹牌海底珍藏鰹魚片 | Slices tuna  | 正鰹（鰹族）    | Skipjack tuna (Thunnini)          | NO                   | Hap_A                  | Sarda orientalis      | YES            | NO           |                 |
| 19  | T55         | 紅鷹牌 | 活寶食品                 | Taiwan               | 洋蔥鮪魚        | Onion tuna   | 正鰹（鰹族）    | Skipjack tuna (Thunnini)          | NO                   | Hap_G                  | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES            | NO           |                 |
| 20  | T10         | 台糖   | 台糖                      | Taiwan               | 香筍鮪魚        | Tuna flakes  | 鮪魚、鰹魚（鰹族） | Thunnus spp., skipjack tuna       | NO                   | Hap_F                  | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccopy (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO             | YES          |                 |
| 21  | T46         | 台糖   | 台糖                      | Taiwan               | 台糖三明治鮪魚（油漬） | Tuna flakes  | 鮪鰹魚類       | Thunnus spp., skipjack tuna       | NO                   | Hap_H                  | Thunnus tonggol (1), T. obesus (1)             | NO             | YES          |                 |
| 22  | T61         | 台糖   | 台糖                      | Taiwan               | 台糖鮪魚片（油漬） | Tuna flakes  | 鮪魚、鰹魚（鰹族） | Thunnus spp., skipjack tuna       | NO                   | Hap_F                  | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccopy (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO             | YES          |                 |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|-----------------------------------|----------------------|------------------------|------------------------|----------------|-------------|------------------|
| 23  | T11         | 新東陽 | 新東陽                   | Taiwan              | 新東陽水煮鮪魚片 | 鮪魚、鰹魚     | Thunnus spp., skipjack tuna | NO                                | Hap_G                | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES NO                   |                      |             |                  |
| 24  | T12         | 愛之味 | 愛之味                   | Taiwan              | 愛之味鮪魚片       | 鮪魚          | Thunnus spp.         | NO                                | Hap_F                | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccocyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                   |                      |             |                  |
| 25  | T45         | 愛之味 | 愛之味                   | Thailand            | 獎贊三明治鮪魚   | 鮪魚          | Thunnus spp.         | NO                                | Hap_F                | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccocyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                   |                      |             |                  |
| 26  | T13         | 老船長 | 金將騰食品             | Taiwan              | 老船長特製鮪魚(煙仔虎) | 煙仔虎       | Skipjack tuna or oriental bonito | YES                                | Hap_A                | Sarda orientalis (3)                                                      | NO NO                   |                      |             |                  |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|----------------------|-------------------------|----------------------------|----------------|------------|-----------------|
| 27  | T56         | 老船長 金春勝食品 | Taiwan 肉仔焼き鮪魚 | Tuna flakes with bamboo shoots | 鮪魚 | Thunnus spp. | NO | Hap_F | Thunnus tonggol (101), Th. orientalis (4), Th. atlanticus (2), Th. thynnus (4), Th. albacares (14), Th. maccoyi (6), Th. obesus (4), Th. alalunga (1), Katsuwonus pelamis (1) | T. albacares | NO | YES |
| 28  | T14         | 新宜興 隆育企業 | Taiwan 水煮鮪魚 | Tuna in brine 鮪魚 | 鮪魚 | Thunnus spp., skipjack tuna | NO | Hap_H | Thunnus tonggol, Th. obesus | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| 29  | T47         | 新宜興 隆育企業 | Taiwan 新宜興三明治鮪魚 | Tuna sandwich 鮪魚類 | 鮪魚類 | Thunnus spp., skipjack tuna | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| 30  | T58         | 新宜興 隆育企業 | Taiwan 新宜興原味鮪魚片 | Tuna slice 鮪魚 | 鮪魚 | Thunnus spp., skipjack tuna | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| 31  | T15         | Viridis Vivus | Taiwan V V 鮪魚片 | Tuna slice 鮪魚 | 鮪魚 | Thunnus spp., skipjack tuna | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| 32  | T16         | 同榮 同榮實業 | Taiwan 同榮鮪魚片 | Tuna flake in oil 鮪魚 | 鮪魚 | Skipjack tuna or oriental bonito | YES | Hap_A | Sarda orientalis (3) | NO | NO |
Table 2. Cont.

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|----------------------|------------------------|------------------------|----------------|------------|----------------|
| 33  | T44         | 同榮 | 同榮實業            | Vietnam          | 三明治鰹魚 | 鰹魚         | Skipjack tuna   | Euthynnus affinis (4), E. lineatus (1) | YES                | NO                     |                        |                |            |                |
| 34  | T17         | 爭鮮 | 爭鮮        | Taiwan             | 油漬鰹魚 | 鰹魚.鰹魚 | Tuna flakes in oil | Thunnus spp., skipjack tuna | NO                  | Hap_H                 |                        |                |            |                |
| 35  | T19         | 藍海 | 旺來興       | Taiwan             | 三明治鰹魚 | 鰹魚.鰹魚 | Tuna in oil | Thunnus spp., skipjack tuna | NO                  | Hap_G                 | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) |                |            |                |
| 36  | T22         | KY   | 寬元行(進口)       | Vietnam             | 三明治鰹魚 | 鰹魚.鰹魚 | Shredded light tuna in oil | Thunnus spp., skipjack tuna | NO                  | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccyii (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) |                |            |                |
| 37  | T23         | 大海鰹魚 | 力達貿易        | Vietnam             | 鰹魚罐頭 | 鰹魚         | Thunnus spp. | NO                  | Hap_F                 |                        |                        |                |            |                |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|----------------------------|----------------------|---------------|---------------|---------------------|------------------------------------|---------------------|----------------------|----------------|------------|------------------|
| 38  | T24         | 紅龍  | 蔑富食品                   | Thailand             | 紅龍蒸煮三明治鰤魚 | 鰤魚           | Thunnus spp.        | NO                                | Hap_C               | Katsuwonus pelamis (7), Thunnus albacares (1) | YES           | YES        |                  |
| 39  | T25         | 金熊 | 洋華(進口商)                 | Indonesia           | 金熊三明治鰤魚   | 鰤魚           | Thunnus spp.        | NO                                | Hap_C               | Katsuwonus pelamis (7), Thunnus albacares (1) | YES           | YES        |                  |
| 40  | T27         | MACORO| 寬元行(進口商)                | Vietnam             | 每口變片狀三明治鰤魚 | Tuna flake in oil | 鰤魚、鰹魚        | Thunnus spp., skipjack tuna         | NO                  | Hap_H               | Thunnus tonggol (1), T. obesus (1) | NO           | YES        |                  |
| 41  | T28         | 南海洋| 力達貿易                    | Taiwan              | 油漬鰤魚(煙仔虎) | 煙仔虎         | Skipjack tuna or oriental bonito | YES                          | Hap_A               | Sarda orientalis (3)                           | NO           | NO         |                  |
| 42  | T29         | California Fresh | Unicord Public  | Thailand            | California Fresh油漬鰤魚片 | Skipjack tuna shredded in soybean oil | 鰤鰤魚類 (正鰤) | Thunnus spp., skipjack tuna (skipjack tuna) | NO                  | Hap_C               | Katsuwonus pelamis (7), Thunnus albacares (1) | NO           | YES        |                  |
| 43  | T30         | 慶全 | 老三林食品                  | Taiwan              | 慶全油漬鰤魚   | 鰤鰤魚肉       | Thunnus spp., skipjack tuna         | NO                                | Hap_A               | Sarda orientalis (3)                           | YES           | NO         |                  |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|-------------------|--------------------------------|-------------------|------------------------|---------------|------------|-----------------|
| 44  | T40         | 老三林 | 老三林食品             | Taiwan               | 油漬魚(煙熏魚) | 煙仔魚       | Skipjact tuna or oriental bonito | YES                          | Hap_A             | Sarda orientalis (3)  | NO           | NO         |                 |
| 45  | T31         | 三乃  | 三乃                    | Taiwan               | light meat tuna | 鮪魚         | Thunnus spp.               | NO                          | Hap_G             | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES          | NO         |                 |
| 46  | T32         | 雄哥標 | 雄哥企業*                | Thailand             | Tuna omega3 in extra virgin olive oil | 精選鮪魚 | Thunnus spp.               | NO                          | Hap_F             | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyii (6), T. oheuse (4), T. alalunga (1), Katsuwonus pelamis (1) | NO           | YES        |                 |
| 47  | T33         | 雄哥標 | 雄哥企業*                | Thailand             | Tuna chunks in extra virgin olive oil | 精選鮪魚 | Thunnus spp.               | NO                          | Hap_F             | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyii (6), T. oheuse (4), T. alalunga (1), Katsuwonus pelamis (1) | NO           | YES        |                 |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|-------------------|---------------------------------|---------------------|------------------------|--------------------------|----------------|-----------|----------------|
| 48  | T36         | 丸鯖魚夢工廠 | 山區海產加工廠          | Taiwan              | 黑鮪魚罐頭   | Bluefin tuna  | 黑鮪魚             | bluefin tuna                   | NO                  | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | ?            | YES       |
| 49  | T37         | 美鷹牌 | 逮捕企業(代理商)           | Thailand            | 鰹魚、鮪魚     | Thunnus spp., skipjack tuna | 鰹魚              | Thunnus spp.                      | NO                  | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO            | YES       |
| 50  | T41         | 丸漢堡 | 賢丸食品                   | Vietnam             | 鮪魚       | Thunnus spp.     | 鮪魚               | Thunnus spp.                      | NO                  | Hap_B                 | Euthynnus affinis (4), E. lineatus (1) | YES           | NO        |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|------------------|----------------------|---------------|-------------|----------------|
| 51  | T43         | 慶祥  | 慶祥食品 | Taiwan | 慶祥鮪魚罐頭 | 鮪魚類   | Thunnus spp. | skipjack tuna | NO | Hap_F     |              | NO         | YES          |
|     |             |       |                         |                      |               |               |                     | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuonius pelamis (1) |
| 52  | T48         | GERRN &SAFE | 永豐生技 | Taiwan | 橄欖油漬鮪魚(東方鰆) | 鮪魚(東方鰆) | Thunnus spp. (oriental bonito) | YES | Hap_A     | Sarda orientalis (3) | ?         | NO          |
| 53  | T42         | Kirkland signature | Costco | Fiji | kirkland signature 科克蘭 | Albacore | Chinese: 鮪魚/English: Albacore tuna | Chinese: Thunnus spp./English: albacore tuna | NO | Hap_D     | Thunnus obesus (3), T. thynnus (2), T. albacares (2), T. alalunga (3), T. orientalis (1) | NO         | YES         |
| 54  | T26         | マルハ | マルハニチロ株式会社 | Japan | 丸希鮪魚罐 | Tuna in soy sauce | Chinese: 金槍魚/Japanese: まぐろ | Chinese and Japanese: Thunnus spp. | NO | Hap_F     | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuonius pelamis (1) | NO         | YES         |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|---------------------------|----------------------|---------------|---------------|---------------------|----------------------------------|---------------------|-------------------------|-------------------------|-------------|-----------|----------------|
| 55  | T50         | 良好生活 | くらし良好 | Thailand | 生活良好 | 鮪魚罐(三人) | 鮪魚 / Japanese: きはだまぐろ | Chinese: 鮪魚 / Japanese: yellowfin tuna | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoupi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| 56  | T51         | 黄金口福(コウフ) | KODANML GROUP CO., LTD | Thailand | 黃金口福油三入黃 | 鮪魚罐 | 鮪魚 / Japanese: まぐろ | Chinese and Japanese: Thunnus spp. | NO | Hap_B | Euthynnus affinis (4), E. lineatus (1) | YES | NO |
| 57  | T59         | HOTEI  | ホテイフーズ | Thailand | HOTEI油漬鯖魚罐頂 | 黃鰭 / Japanese: かつお | Chinese: yellowfin tuna / Japanese: skipjack tuna | Chinese: yellowfin tuna / Japanese: skipjack tuna | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
| 58  | T65         | HOTEI  | ホテイフーズ | Japan | 豪德本堂鮪魚罐頂 | 鮪魚 / Japanese: きはだまぐろ | Chinese and Japanese: yellowfin tuna | Chinese and Japanese: yellowfin tuna | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoupi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
Table 2. Cont.

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|-----------------------------------|---------------------|------------------------|------------------------|-----------------|------------|-----------------|
| 59  | T60         | 今津   | 今津株式会社              | Thailand             | 今津鰹魚/玉未漬 | Chinese: 鰹魚/ Japanese: きはたまぐろ | Chinese: skipjack tuna/ Japanese: yellowfin tuna | NO                  | Hap_F                | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), Thunnus spp. | YES                  | YES        |                  |
| 60  | T34         | 極洋   | 極洋株式会社              | Thailand             | 極洋鰹魚罐頭-油漬 | Chinese: 鰹魚/ Japanese: まぐろ | Chinese: Japanese: Thunnus spp. | NO                  | Hap_F                | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), Thunnus spp. | NO               | YES        | T. albacares     |
| 61  | T35         | 極洋   | 極洋株式会社              | Thailand             | 極洋油漬鰹魚罐 | Chinese: 鰹魚/ Japanese: まぐろ | Chinese: Japanese: Thunnus spp. | NO                  | Hap_C                | Katsuwonus pelamis (7), Thunnus albacares (1) | YES              | YES        |                  |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|-------------------|----------------------------------|---------------------|-------------------------|------------------------|----------------|-----------|----------------|
| 62  | T38         | いなば食品 | Thailand                 | 稻葉鮪魚/鰹魚罐  | Light tuna    | Chinese: 鮪魚、鰹魚罐/Japanese:まぐろ | Chinese: Thunnus spp. and skipjack tuna/Japanese: Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | NO | YES |
| 63  | T39         | 伊藤食品 | Japan                    | 伊藤油漬鮪魚(金罐)  |               | Chinese: 鮪魚/Japanese:まぐろ | Chinese and Japanese: Thunnus spp. | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| 64  | T63         | Hagoromo | Japan                    | 一本釣頂級鮪魚罐  |               | Chinese: 鮪魚/Japanese: ひんなりまぐろ | Chinese: Thunnus spp./Japanese: albacore tuna | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
Table 2. Cont.

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|---------------------------------|----------------------|------------------------|------------------------|----------------|------------|-----------------|
| 65  | T64         | SSK   | 清水食品株式会社           | Japan                | 油浸鮪魚      | Chinese: 鮪魚/Japanese: きはたまぐろ | Chinese: Thunnus spp./Japanese: yellowfin tuna | NO  | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoupi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| 66  | B1B         | SEEDS | THAI UNION               | Thailand             | Hello Fresh 好鮮原汁湯罐 (清蒸鮪魚) | Tuna | 鮪魚 | Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
| 67  | B1C         | SEEDS | UNICORD                  | Thailand             | Tuna愛貓天然食(兩倍鮮嫩雞肉+白身鮪魚) | Chicken & Tuna light meat | 自身鮪魚, 雞肉 | Thunnus spp., chicken | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
| 68  | B2E         | SEEDS | UNICORD                  | Thailand             | Tuna愛貓天然食 | Chicken & tuna light meat | 雞肉, 自身鮪魚 | Chicken, Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
| 69  | B2F         | SEEDS | UNICORD                  | Thailand             | Tuna愛貓天然食 | Light tuna meat & shirasu | 自身鮪魚, 瑛仔魚 | Whitebait, Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
Table 2. Cont.

| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|-----------------------|---------------|---------------|---------------------|---------------------------------|---------------------|-----------------------------|--------------------------|---------------|------------|----------------|
| 70  | B2G         | SEEDS | THAI UNION Thailand      | Bistro Cat            | Tuna light meat + shrimp | Thunnus spp., shrimp | NO Hap_C            | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES |
| 71  | B1D         | YAMI | Hi-Q Food Thailand       | 健寶鰤魚蟹柳活力餐  | 鮭魚、蟹柳 | Thunnus spp., crab stick | NO Hap_B            | Euthynnus affinis (4), E. lineatus (1) | YES NO |
| 72  | B3A         | GOEN  | Pataya Food Thailand     | 御宴湯罐白身鰤魚、蟹柳  | 白身鰤魚、蟹柳 | Thunnus spp., chicken | NO Hap_C            | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES |
| 73  | B3B         | GOEN  | Pataya Food Thailand     | 御宴湯罐白身鰤魚、鮭魚  | 白身鰤魚、鮭魚 | Thunnus spp., salmon | NO Hap_C            | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES |
| 74  | B3F         | 元氣家族 | Pataya Food Thailand    | 元氣家族金罐鮭魚、鰤魚  | 鮭魚、鰤魚 | Thunnus spp., snapper | NO Hap_B            | Euthynnus affinis (4), E. lineatus (1) | YES NO |
| 75  | B3H         | 元氣家族 | Pataya Food Thailand    | 元氣家族金罐鮭魚、鮮蝦  | 鮭魚、鮮蝦 | Thunnus spp., shrimp | NO Hap_B            | Euthynnus affinis (4), E. lineatus (1) | YES NO |
| 76  | C1A         | 愛情貴族 | UNICORD Thailand        | CIH-C08白身鰤魚 & 牛肉 | 白身鰤魚、牛肉 | Thunnus spp., beef | NO Hap_E            | Katsuwonus pelamis (1) | YES YES |
| 77  | C1B         | 愛情貴族 | UNICORD Thailand        | CIH-C02白身鰤魚 & 鮭仔魚  | 白身鰤魚、鮭仔魚 | Thunnus spp., whitebait | NO Hap_C            | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES |
### Table 2. Cont.

| No. | Sample Code | Brand       | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------------|---------------------------|----------------------|---------------|---------------|---------------------|-----------------------------------|----------------------|-------------------------|--------------------------|---------------|------------|-----------------|
| 78  | C2B         | 每日貓罐 | 泛美力 (鼎食) | Taiwan               | 每日貓罐-鮪魚 + 蟹味絲湯 | 鮪魚、蟹味絲 | Thunnus spp., crab stick | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| 79  | C2C         | 每日貓罐 | 泛美力 (鼎食) | Taiwan               | 每日貓罐-鮪魚 + 巴沙魚湯 | 鮪魚、巴沙魚 | Thunnus spp., basa fish | NO | Hap_D | Thunnus obesus (3), T. thynnus (2), T. albacares (2), T. alalunga (3), T. orientalis (1) | NO | YES |
| 80  | C4A         | 鼎食貓罐 (鼎食) | 永慶企業有限公司 | Taiwan               | 鼎食貓罐(新鮮鮪魚 + 丁香魚) | 鮪魚、丁香魚 | Thunnus spp., clove fish | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|--------------------|---------------------------------|---------------------|-----------------------|-------------------------|---------------|-------------|----------------|
| 81  | C4C         | 鼎食貓罐 | 台湾 | 鼎食貓罐 (新鮮鮪魚+樱花蝦) | 鮪魚、櫻花蝦 | Thunnus spp., sakura shrimp | NO | Hap_F | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. mackiyyi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO | YES |
| 82  | C5A         | 怪獸部落 | 泰國 | 無膠無穀鮮肉煲-鮪魚片湯 | 鮪魚 | Thunnus spp. | NO | Hap_C | Katsuwonus pelamis (7), Thunnus albacares (1) | YES | YES |
| 83  | D1B         | YAMI亞米 | 泰國 | YAMI Platinum | 鮪魚白肉 | Thunnus spp. | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| 84  | D1C         | YAMI亞米 | 泰國 | YAMI Platinum | 鮪魚白肉 | Thunnus spp. | NO | Hap_G | Auxis thazard (6), A. rochei (7), Euthynnus affinis (1) | YES | NO |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label | Declared Ingredient | English Translation of Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|---------------|---------------------|---------------------------------|-----------------------|--------------------------|------------------------|----------------|------------|----------------|
| 85  | D2B         | TRIL GY | Real Pet Food Company    | Thailand             | 奇境無穀野生鰹魚燉湯 | 鰹魚          | Thunnus spp.       | NO                              | Hap_C                 | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES               |               |            |               |
| 86  | D2C         | O’KAT  | 黑豆國際有限公司        | Thailand             | 美喵人生無穀化毛餐 | 鰹魚          | Thunnus spp.       | NO                              | Hap_C                 | Katsuwonus pelamis (7), Thunnus albacares (1) | YES YES               |               |            |               |
| 87  | D2G         | Rico   | 聰奇寵物用品企業有限公司 | Taiwan              | 芝可-滋用副食鮮湯罐2號(鮪魚+鰹魚) | 鮪魚, 鰹魚, 鯖魚 | Thunnus spp., chicken, skipjack tuna | NO                              | Hap_F                 | T. tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoupi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                |               |            |               |
| 88  | E1E         | 樂妙貓 | サステナ株式会社         | Japan                | 獸界新3號-鮪.吻仔魚 | 鮪魚, 吻仔魚/Japanese:マグロ,しらす | Chinese and Japanese: Thunnus spp., whitebait | NO                              | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccoupi (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO YES                |               |            |               |
| No. | Sample Code | Brand | Manufacturer or Importer | Place of Manufacture | Chinese Label | English Label |Declared Ingredient | Inconsistent Labeling | 16S rRNA Haplotype Code | 16S BLAST (No. of Hits) | CR NJ Analysis | Mislabeled | True Canned Tuna |
|-----|-------------|-------|--------------------------|----------------------|---------------|--------------|-------------------|-----------------------|---------------------------|---------------------------|---------------|------------|-----------------|
| 89  | E2C         | 厚肉肉 | 厚肉肉（台湾）股份有限公司 | Taiwan              | 鮪魚、鯖魚      | Thunnus spp., salmon | NO                | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccocyti (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO             | YES        |                 |
| 90  | E2D         | 愛喜雅 | 愛喜雅株式会社            | Japan               | 鮪魚、鮭魚      | Thunnus spp., chicken, salmon | NO                | Hap_F                 | Thunnus tonggol (101), T. orientalis (4), T. atlanticus (2), T. thynnus (4), T. albacares (14), T. maccocyti (6), T. obesus (4), T. alalunga (1), Katsuwonus pelamis (1) | NO             | YES        |                 |
2.3. Data Analysis

Edited 16S sequences were first aligned using ClustalW in MEGA11 [49], and then the haplotypes were determined in DnaSP 6. Species identity for each 16S haplotype was achieved by comparing them (by BLAST) to reference sequences in the NCBI GenBank database. Following the approach of both Armani et al. [50] and Horreo et al. [51], only matches displaying full sequence coverage and 100% similarity, and with unambiguous species-level scientific names, were considered positive fish identifications. If more than one fish species was shown as a positive match, all of them were considered potential candidates (Table 2).

All CR sequences used in the study of Mitchell and Hellberg (2016) were downloaded to serve as reference sequences, and then our CR fragments and reference sequences were aligned in ClustalW. A neighbor-joining (NJ) analysis was then conducted based on Kimura two-parameter (K2P) distances and $1 \times 10^3$ bootstrapping replicates in MEGA11 [49]. According to the phylogenetic species concept [52], monophyly is a prerequisite for species recognition, so our specimens were authenticated based on the reference species with which they clustered and formed a monophyletic group (with high statistical support, i.e., bootstrapping value $\geq 70$) in the NJ phenogram.

2.4. Comparison of Analytical Results and Product Labels

We compared the molecular identification of each sample to the ingredient list of the sampled cans. Since a Taiwanese government-approved standard for common names of fishes does not exist, the English names of labeled Chinese names were ascertained from the fish database of Taiwan (https://fishdb.sinica.edu.tw/ (accessed on 26 October 2021)). Although the Chinese symbols 鰹鱼 could broadly refer to any species from the genera Auxis (in Chinese: 花鰤屬), Euthynnus (in Chinese: 巴鰤屬), and Katsuwonus (in Chinese: 正鰤屬), skipjack tuna is the only species generally termed 鰹鱼 and that can be used legally in Taiwan to make canned tuna (Table 1). Therefore, we assumed that if 鰹鱼 appeared on the ingredient list of a canned tuna product, it specifically represented skipjack tuna. Many of the imported products displayed labeling in Chinese and the language of source, but in those cases we exclusively relied on the Chinese label since Chinese is the only official language in Taiwan.

A sample was judged as displaying inconsistent labeling if the fish name in the ingredient list could not be linked unambiguously to a Thunnus species or skipjack tuna. It was then deemed mislabeled if the molecularly authenticated species it contained did not match the ingredient list on the label. Where a vernacular name used in an ingredient list refers to more than one species, a case of mislabeling was assigned when the molecularly authenticated species did not correspond to any fishes bearing that vernacular name. Finally, we determined a product as being true canned tuna if it contained Thunnus species or skipjack tuna.

3. Results

We observed inconsistent labeling in 11 of 65 canned tuna products destined for human consumption, but no such problem with cat food products. Inconsistent labeling reflected canned tuna products also claiming to be made from oriental bonito (Sarda orientalis) (in Chinese: 東方齒鰆) or displaying the ambiguous vernacular name 煙仔虎, which can refer to either skipjack tuna or oriental bonito (Figure 1, Table 2).
We successfully amplified the 16S fragment from all 90 samples, resulting in eight haplotypes (Supplementary Information S2). All haplotypes could be identified to species-level by BLAST analysis, but only haplotypes Hap_A and Hap_E specifically relate to oriental bonito and skipjack tuna, respectively. More than one species was identified by BLAST analysis for the remaining six haplotypes, but based on the number of BLAST hits we assume Hap_B represents kawakawa (*Euthynnus affinis*) (in Chinese: 巴鰹), Hap_C is skipjack tuna, Hap_D and Hap_F are *Thunnus* species, Hap_H is either longtail tuna (*T. tonggol*) (in Chinese: 長腰鰹) or bigeye tuna (*T. obesus*), and Hap_G is either bullet tuna or frigate tuna (*Auxis thazard*) (in Chinese: 庇花鰹).

Our BLAST analysis of 16S sequences revealed that 31 of our samples contained *Thunnus* fishes. However, the success rate of CR amplification from those samples was quite low (5/31; 16%). The aligned CR dataset (Supplementary Information S3) for NJ analysis is 256 bp in length and contains 47 taxa, including 159 variable sites and 131 parsimony-informative sites. The NJ analysis of CR sequences supports that samples T1, T7, and T56 are yellowfin tuna, and that sample T34 is longtail tuna, but we could not authenticate sample D2G based on its CR sequence (Figure 2).

Excluding the canned cat food samples that were all accurately labeled, 25 of the remaining 65 canned tuna products were mislabeled and a further three were potential mislabeling cases. Our BLAST analysis confirmed that sample T36 contained *Thunnus* fish, but did not reveal which species. We found the labeling of sample T48 to be misleading. In Chinese, the symbol “鰹” (for tuna) is never associated with oriental bonito, so it is unreasonable for the symbol for oriental bonito to be placed in parentheses following “鰹魚” (representing “tuna fish”) on the label for this sample. We observed a similar issue for sample T4, since neither skipjack tuna nor oriental bonito can be regarded as a type of “鰹” (*Thunnus* spp.). Since the ingredient statement on the label fails to clearly indicate which species is contained in the can, it is difficult to judge whether or not these two samples are mislabeled. Notably, many of the products identified as exhibiting inconsistent labeling were also found to be mislabeled. The mislabeling rate of canned products for human consumption was 38% (25/65). Mislabeling was even more pervasive among cat food products, with a rate of 68% (17/25). The main reason for this high mislabeling rate of cat food products is that many claim to contain *Thunnus* fishes but are in actual fact made from skipjack tuna. The overall mislabeling rate for the 90 tested products of this study is ~47% (42/90).

**Figure 1.** Taiwanese canned tuna products displaying inconsistent labeling. “鰹” (red arrows) in the Chinese labels declares both of these canned tuna products as legally being made from *Thunnus* fishes or skipjack tuna (*Katsuwonus pelamis*) (in Chinese: 正鰹). (a) Oriental bonito (*Sarda orientalis*) (in Chinese: 東方齒鰆) (white arrow) in the label indicates the product contains that species. (b) The Chinese vernacular name 魚仔虎 (black arrow) may represent both oriental bonito and skipjack tuna.
The Neighbor-joining (NJ) tree of the K2P model of 47 taxa inferred from 256 bp of mitochondrial control region (CR) sequences with 1000 bootstrapping replicates. Each terminal is labeled with the GenBank accession number or sample code. Bootstrapping values $\geq 70$ are indicated on the respective branches.

Figure 2. Neighbor-joining (NJ) tree of the K2P model of 47 taxa inferred from 256 bp of mitochondrial control region (CR) sequences with 1000 bootstrapping replicates. Each terminal is labeled with the GenBank accession number or sample code. Bootstrapping values $\geq 70$ are indicated on the respective branches.

Of the 65 human food products we tested, 37 (57%) legitimately contained either *Thunnus* fishes or skipjack tuna, and 20 out of 25 cat food products are true canned tuna. Overall, the proportion of true canned tuna products is about 63.33% (57/90) in this study.
4. Discussion

According to Article 28 of the Act Governing Food Safety and Sanitation in Taiwan, public labeling, promotion and advertisement of foods or food additives, cleansers, utensils, containers or packaging designated by the central competent authority shall not be false, exaggerated or misleading. The 11 cases of inconsistent labeling we identified among our 90 samples, which display “鯖” (for tuna) on their labels but also list scombrids other than *Thunnus* species or skipjack tuna as an ingredient, obviously mislead customers into believing these products contain true canned tuna. For this study, we solely relied on the information on Chinese labeling, but we also noted conflicting information between Chinese and source-language labeling of imported products. For example, the ingredient statement in Japanese of sample T42 clearly declares that it is made from albacore tuna (*T. alalunga*), but its Chinese label only states that it contains *Thunnus* fishes (in Chinese: 鮪魚). Similarly, the Japanese label of sample T59 indicates skipjack tuna as an ingredient (in Japanese: かつお), but its Chinese label specifies yellowfin tuna (in Chinese: 黃鰭鮪) (Table 2). Such conflicting labeling of imported products not only confuses consumers but may also circumvent legal controls.

The “one species-one name” policy is critical to the authentication of fishery products [38]. Clearly, usage of scientific names could enable investigators to easily judge if a product is mislabeled. Under European Union labeling regulations, including the species’ scientific name on fishery product labels is mandatory [53]. However, scientific names are not required on Taiwanese fishery products nor are such names familiar to the public. Xiong et al. [54] and Chang et al. [22] advocated the adoption of the Chinese-Latin Dictionary of Fish Names (https://fishdb.sinica.edu.tw/chi/chinesequer1.php (accessed on 26 October 2021)) as a standard list of fishes in Chinese corresponding to scientific nomenclature. This Dictionary indeed clarifies that the Chinese symbols 東方齒鰆 (in English: oriental bonito) correspond to *Sarda orientalis*, but it does not include other Chinese vernacular names. Thus, any official “one species-one name” standard should not only contain scientific and Chinese names, but also incorporate vernacular names.

Notably, our success rates for amplifying the two mitochondrial DNA fragments differed considerably—100% for 16S, but only 16% for CR. The canning process is known to damage DNA molecules, with Quinteiro et al. [43] documenting that most DNA segments extracted from canned tuna are <100 bp in length. Therefore, it is not surprising that amplification of the 85-bp 16S region was more successful than the 236-bp CR fragment (Binominal Generalized Logical Model, \( p < 0.01 \)).

Apart from haplotypes Hap_A and Hap_C, a single species was not identified by BLAST for the other 16S haplotypes. There are a number of possible reasons for that outcome. First, DNA degradation through the canning process limits molecular authentication based on longer sequences, such as via conventional DNA barcoding. Accordingly, shorter DNA segments must be targeted, but they contain less information and so are less likely to unambiguously assign a specific species [55]. Second, molecular identification based on mitochondrial sequences is very sensitive to gene flow and incomplete lineage sorting [25,56,57]. The tribe *Thunnus* comprises very closely related species, some of which display genetic introgression [58,59]. Consequently, though Hap_D, Hap_E, and Hap_H are all clearly form *Thunnus* fishes, their exact species identity remains unclear. Though conventional DNA barcoding can distinguish *Thunnus* fishes [60–62], it would be problematic to amplify the ~650 bp barcode from the degraded DNA of canned samples. Third, a reliable database is crucial to accurate DNA-based identification [63]. GenBank does not guarantee that deposited sequences display correct species names. For example, the BLAST result for Hap_C matches multiple sequences for skipjack tuna sequences and one for yellowfin tuna (GenBank accession number: KM055376), implying that accession KM055376 is very likely misidentified. Hence, as highlighted in a number of studies [22,64,65], a reliable and complete DNA reference database for authenticating seafood resources is sorely needed.
In this study, we found that many canned tuna products in Taiwan are made from oriental bonito, kawakawa, bullet tuna, or frigate tuna instead of legitimate *Thunnus* fishes or skipjack tuna. Although oriental bonito was never found in canned cat food products, the other three substituted fishes were identified in both human and cat food samples. These same four species have also been reported as illegitimate tuna substitutes in other studies [44,46,48,50,66,67]. Though istiophorid fishes have been reported as mislabeled *Thunnus* products in other studies [46,68], we did not detect them in this study.

Our NJ analysis of CR sequences, including five haplotypes generated in this study, further revealed that both yellowfin tuna and longtail tuna are used in canned tuna products. Yellowfin tuna is one of the commonest canned tunas [5,69], so it is not surprising that three of our five CR haplotypes clustered with yellowfin tuna sequences (sample T34 was identified as longtail tuna, and sample D2G could not be identified to species level). Our difficulties with amplifying the CR region mean that the specific *Thunnus* composition of canned tuna products in Taiwan remains unclear. Identifying canned tuna products to species level is important because certain *Thunnus* fishes have higher mercury levels [70], posing a human health risk. Therefore, mitochondrial regions other than CR, such as ATP synthase membrane subunit 8 (ATP8), ATP6, and COIII could be considered [71], or smaller CR fragments could be targeted.

We observed the terms 白身鰤魚 or 鰤魚白肉 commonly in the ingredient statements of our cat food samples (Table 2), reflecting the high mislabeling rate (17/25) among cat food products. However, most of the cat food samples (20/25) still represented true canned tuna, albeit not the species that might be expected. To date, there is no official definition for either of these two Chinese terms. They may be translated as “light tuna”, which often refers to yellowfin tuna or skipjack tuna, but could actually be any fishes mentioned in the Code of Federal Regulation Title 21 (CFR 161.190) and with flesh color in the Munsell color system ≥5.3 [48]. If those terms were to be officially recognized as translations of light tuna, then the mislabeling rate of cat food we report herein would be much lower (down to 8/25) (Binominal Generalized Logical Model, \( p < 0.01 \)). Accordingly, we implore the responsible authorities to clearly define the terms for use in canned product labeling.

Although we found that 63.33% of our samples are true canned tuna, this outcome may not reflect the actual adulteration level of canned tuna products in Taiwan. First, we selected only one small piece of tissue from each can, but the mixing of tuna species in cans has been found in the European market [4]. An assessment of how prevalent the mixing of tuna species is in cans in the Taiwanese market would be needed to determine how close our calculated adulteration level is to the real scenario. Second, we solely sampled major brands, so there are some that remain to be assessed, especially of cat food. Moreover, seasonality in scombrid catch may alter the species composition of adulterated canned tuna products. Thus, more comprehensive and long-term monitoring of the species composition of canned tuna products is needed.

5. Conclusions

We report an overall mislabeling rate of 46.67% among the 90 samples of this study, with 63.33% of sampled canned products being true canned tuna legitimately made from *Thunnus* fish or skipjack tuna. In many cases, the labels of the sampled canned tuna products would confuse customers as to what species they contain. Either they contain species such as oriental bonito that do not conform to Taiwanese legislation, or ill-defined terms such as 白身鰤魚 or Chinese vernacular names are used. We assert that a standard list of scientific names and their corresponding Chinese and vernacular names conforming to the “one species-one name” principle, as well as clear definitions of terms for use in canned tuna labeling, is crucial to tackling fish product adulteration. We found that ~37% of investigated canned tuna products contain illegitimate species. In many cases, the manufacturers have substituted so-called “pseudo-tunas”, such as oriental bonito, kawakawa, bullet tuna, and frigate tuna, for legal species, i.e., *Thunnus* species and skipjack tuna. Our study demonstrates that a pair of primers targeting a short segment (85 bp) of
16S performs well in amplifying DNA extracted from canned food samples. However, the limited information content provided by this short sequence hampered molecular identification to species level, especially given the close phylogenetic relationships and potential for gene flow among *Thunnus* species. Moreover, the CR fragment we targeted largely proved uninformative, likely owing to the extreme DNA fragmentation caused by high heat treatment during the canning process. Our previous study of seafood adulteration in conveyor-belt sushi restaurants revealed no case of tuna fraud in such establishments [22], so such adulteration appears to be more common in canned products. A large-scale and long-term monitoring study would help fully establish the extent of canned tuna fraud in the Taiwanese market.

**Supplementary Materials:** The following are available online at [https://www.mdpi.com/article/10.3390/foods10112655/s1](https://www.mdpi.com/article/10.3390/foods10112655/s1), Supplementary Information S1: The photos of all sampled canned tuna items, Supplementary Information S2: The 16S haplotypes, Supplementary Information S3: The aligned CR sequences for constructing NJ phylogenetic analysis.

**Author Contributions:** All authors contributed to the study conception and design. Conceptualization, C.-H.C. and Y.-C.W.; methodology, C.-H.C. and Y.-C.W.; software, Y.-T.K. and T.-T.H.; validation, C.-H.C., Y.-T.K. and T.-T.H.; formal analysis, Y.-T.K. and T.-T.H.; investigation, Y.-T.K. and T.-T.H.; resources, Y.-T.K. and T.-T.H.; data curation, Y.-T.K. and T.-T.H.; writing—original draft preparation, C.-H.C.; writing—review and editing, C.-H.C.; visualization, C.-H.C.; supervision, Y.-C.W.; project administration, C.-H.C. and Y.-C.W.; funding acquisition, C.-H.C. All authors have read and agreed to the published version of the manuscript.

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