Application of DNA barcoding to detect mislabeling of fish fillet products from Jabodetabek’s market

T A Widowati1, N Andayani1, 2 and A E Maryanto1, 2

1Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
2Biodiversity and Environmental Genomics Research Cluster, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: andayani@ui.ac.id

Abstract. Mislabeling of fish fillet product is one of the key issues in food safety and sustainability. Species identification is an important step of fish fillet traceability and DNA barcoding has been proved as a standard method. Forty seven 47 fish fillet products were collected from modern and traditional markets in the Jabodetabek area. DNA barcoding was used to analyze the compliance of the product label. This research also highlighted that near threatened (NT), Vulnerable (VU), endangered (EN), and critically endangered (CR) species considered to be facing a high risk of extinction have been used as a substitution in fish fillet product. The application of DNA mini-barcoding gives better resolution in species identification for commercial species. From this research, we found that there are some mislabeled fish fillet products, including blue shark meat that is listed as Near Threatened in IUCN Red List in one of the products,

Keywords: DNA labelling, mislabelling, fish fillet

1. Introduction
The conformity of labeling species in packaged foods is very important. Proper food labeling guarantees consumer safety. It is also necessary to have clear data on what species is used for these foods in order to support the sustainability of natural resources. Packaged food products used in this study are fish fillets. When the fish is intact, it will be easy to identify the fish species. It is different if the fish is only a fillet. It is difficult to identify a species of fish based on the appearance of the meat fillet because a lot of fish meat will look similar in the fillet form. Food traceability test often targets DNA as it is robust to processing associated with many food products [1].

DNA barcoding is one method that has particular promise for species identification in food. DNA barcoding uses a small fragment from a DNA sequence located within a standardized region of the genome to allow precise species identification [2]. For animal species, this method involves sequencing of a ~650bp fragment of the COI gene. In this study, we will use DNA Barcoding to study the accuracy of species labels on fish fillets sold in modern and traditional markets in Jabodetabek.

After identifying the species, we are going to check the status of the identified species on IUCN Red List to confirm the presence of species globally to support the sustainability of each fish species [3]. In the IUCN Red List, there are x level of species, there are Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct In the Wild, and Extinct.
2. Materials and method

2.1. Sample collection
A total of 47 samples were collected from the supermarket and traditional markets in 9 cities (North Jakarta, South Jakarta, East Jakarta, West Jakarta, Central Jakarta, Depok, Bekasi, Tangerang, and Bogor). Products were labeled fish fillet and non-labeled fish fillet from various brand. There are 4 samples from North Jakarta (JU), 9 samples from South Jakarta (JS), 5 samples from East Jakarta (JT), 4 samples from West Jakarta (JB), 6 samples from Central Jakarta (JP), 4 samples from Depok (D), 5 samples from Bekasi (BK), 5 samples from Tangerang (T), and 5 samples from Bogor (B). Samples are stored at -20 °C.

2.2. DNA extraction
The Chelex method is used for extracting DNA. Small cutlet of the sample placed in a 1.5 mL tube with 200 μL of 10 % Chelex Resin. Every tube was heated at 60 °C for 20 minutes, and then at 103 °C for 25 minutes [4]. Tubes get into vortex every 5 minutes of the heating process. In the end, tubes get 13000 rpm centrifugations for 2 minutes. Then supernatant is take in, a DNA isolate are preserve in -20 °C.

2.3. PCR
Approximately 655 bp were amplified from the 5' region of the cox1 gene from mitochondrial DNA using a different combination of two designed primer from Ward, et al. [5].
FishF1-5'TCAACCAACCACAAAGACATTGGCAC3'
FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'
The 25 μL PCR reaction mixes included 12.5 master mix, 0.25 μL of each primer, 9 μL of nuclease-free water and 3 μL of sample. The PCR profile is shown in figure 1.

2.4. Electrophoresis
PCR products were visualized on 1 % agarose gels with 10 % GelRed. Electrophoresis was running in 100 V for 20 minutes.

2.5. Species identification
DNA Sequencing results were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/) and BOLD (http://www.boldsystems.org/).

3. Results and discussion
Out of the 47 samples isolated and amplified, there were 33 samples seen in gel electrophoresis results. The PCR positive samples are JS3, JS4, JS5, JS6, JS9, JU2, JU3, JU4, B1, B2, B3, B4, B5, BK1, BK2, BK4, BK5, JT2, JT3, JT4, JT6, JB3, T2, T3, T4, T5, JP3, JP4, JP5, 1D, 2D, 3D, 4D and are shown in figure 2, figure 3, figure 4 and figure 5. Most likely, PCR produced 655 bp of DNA. The PCR results that have not been read on the gel were measured for concentration and purity using IMPLEN nanophotometer. The concentration range of samples are 48.5–715 ng/μL, and the purity range is 1.023–2.028. In samples with positive PCR results in gel electrophoresis, DNA sequencing will be continued to identify fish species. Then, DNA sequencing will be analyzed from 11 positive samples. The 11 samples that sequenced are from different brand, represent 30 samples. Of the 11 brands, 3 of the brands are fish fillets without species labels.

DNA barcoding is successful in identifying species from fish fillets. There are 19 fish fillet samples that species-labelled correctly. From table 1, we know that these 19 samples (JS9, JU4, B4, B2, D1, T3, BK2, B3, T5, JP4, D3, B5, JT4, BK4, BK5, JT6, JS5, JP5, D4) are from 8 different brands.
Figure 1. PCR profile

Figure 2. Electrophoresis Gel of D1, D2, D3, D4, JS3, JS4, JS5, JS6.

Figure 3. Electrophoresis Gel of JS1, JS2, JS3, JS4, JS5, JS6, JS7, JS8, JS9, B1, B2, JB3, B4, B5, JU1.

Figure 4. Electrophoresis Gel of JB4, JP2, JT4, BK2, JP6, JT2, JP4, JP3, BK1, T5, T4, T3, T2, T1, JU4.
Figure 5. Electrophoresis Gel of B3, JB1, JB2, JU2, JU3, BK3, BK4, BK5, JP1, JP5, JT1, JT3, JT5, JT6.

Table 1. Species identified from samples.

| Brand no. | Sample code | Species label on product | Species identified | Label | IUCN status |
|-----------|-------------|--------------------------|--------------------|-------|-------------|
| 1         | JS9, JU4, B4 | Tilapia                  | Oreochromis niloticus | +     | LC<sup>a</sup> |
| 2         | JB3, B1, BK1, JU2, T2, JP3, JS6, D2 | -                        | Prionace glauca     | —<sup>b</sup> | NT<sup>b</sup> |
| 3         | B2, D1      | Tilapia                  | Oreochromis mossambicus | +     | NT          |
| 4         | T3, BK2, B3, T5, JP4, D3 | Pangasius               | Oreochromis niloticus | —     | LC          |
| 5         | B5          | Dory                     | Pangasianodon hypophthalmus | —     | EN<sup>c</sup> |
| 6         | JT4, BK4    | Pangasius                | Diagramma picta     | —     | NT          |
| 7         | BK5, JT6, JS5 | Pangasius               | Pangasianodon hypophthalmus | +     | EN          |
| 8         | JT1         | -                        | Pangasianodon hypophthalmus | —<sup>b</sup> | EN          |
| 9         | JU3, T4     | -                        | Prionace glauca     | —<sup>b</sup> | NT          |
| 10        | JP5         | Pangasius                | Pangasianodon hypophthalmus | +     | EN          |
| 11        | D4          | Gindara                  | Lepidocybium flavobrunneum | +     | LC          |

<sup>a</sup> Least Concern  
<sup>b</sup> Near Threatened  
<sup>c</sup> Endangered  
<sup>d</sup> Mislabel  
<sup>e</sup> Correct Label

Figure 6 to figure 16 are the representative of the every brand. Brand number 1 (figure 6), which are JS9, JU4, B4, listed Tilapia fish fillet in their product. Brand number 1 is identified as *Oreochromis niloticus* that is listed as Least Concern in IUCN Red list. Brand number 2 (figure 7), which are JB3,
B1, BK1, JU2, T2, JP3, JS6, D2, are not the fish species listed in the product (non-labeled fish filet), and is identified as *Prionace glauca* which is listed as Near Threatened in IUCN Red List.

Brand number 3 (figure 8), which are B2, D1, are listed as Tilapia fish fillet in their product. Brand number 3 is identified as *Oreochromis mossambicus* that is listed as Near Threatened. Brand number 4 (figure 9), which are T3, BK2, B3, T5, JP4, D3, are listed as Pangasius fish fillet in their product but identified as *Oreochromis niloticus* that is listed as Least Concern. Brand number 5 (figure 10), which is B5, are listed as Dory fish fillet in their product but is identified as *Pangasianodon hypophthalmus* which is listed as Endangered.

Brand number 6 (figure 11), which are JT4, BK4, listed Pangasius fish fillet in their product but is identified as *Diagramma picta* that is listed as Near Threatened. Brand number 7 (figure 12), which are BK5, JT6, JS5, are listed as Pangasius fish fillet in their product. Brand number 7 is identified as *Pangasianodon hypophthalmus* that is listed as Endangered. Brand number 8 (figure 13), which is JT1, did not list fish species name in the product (non-labeled fish filet). Brand number 8 is identified as *Pangasianodon hypophthalmus* that is listed as Endangered.

Brand number 9 (figure 14), which are JU3, T4, did not list the fish species name in the product (non-labeled fish filet), is identified as *Prionace glauca* that is listed as Near Threatened in IUCN Red List. Brand number 10 (figure 15), which is JP5, is listed as Pangasius fish fillet in their product. Brand number 10 is identified as *Pangasianodon hypophthalmus* that is listed as Endangered. Brand number 11 (figure 16), which is D4, is listed as Gindara fish fillet in their product. Brand number 11 is identified as *Lepidocybium flavobrunneum* that is listed as Least Concern.

**Figure 6.** Brand number 1. The sample is from South Jakarta and collected on June 16th, 2019.

**Figure 7.** Brand number 2. The sample is from West Jakarta and collected on June 16th, 2019.

**Figure 8.** Brand number 3. The sample is from Bogor and collected on June 14th, 2019.

**Figure 9.** Brand number 4. The sample is from Tangerang and collected on June 14th, 2019.
Figure 10. Brand number 5. The sample is from Bogor and collected on June 14th, 2019.

Figure 11. Brand number 6. The sample is from East Jakarta and collected on June 15th, 2019.

Figure 12. Brand number 7. The sample is from Bekasi and collected on June 15th, 2019.

Figure 13. Brand number 8. The sample is from East Jakarta and collected on June 16th, 2019.

Figure 14. Brand number 9. The sample is from North Jakarta and collected on June 14th, 2019.

Figure 15. Brand number 10. The sample is from Central Jakarta and collected on June 15th, 2019.

Figure 17 shows that there are 45.45% samples are correctly labelled and 54.54% samples are mislabelled in this study. Tilapia is a group of freshwater fish, therefore brand number 1 and 3 are correctly labelled. Brand number 7, 10 and 11 are correctly labelled too because the species listed on the packaging is the same as the species identified. The result proves that brand number 4, 5, and 6 mislabelled the fish fillet because the species listed on the packaging does not match
Figure 16. Brand number 11. The sample is from Depok and collected on June 10th, 2019.

Figure 17. Result percentage of correct label and mislabel sample

the identified species. There also a misconception about pangasius and dory. It is assumed that they are the same fish or same group of fish, but pangasius is an aquaculture freshwater fish whereas dory is a saltwater fish.

Fish fillet from 3 brands (brand number 2, 8 and 9) that did not list the species are identified. One sample from one of the brands is identified as *Pangasianodon hypophthalmus* that is listed as Endangered and 10 samples from the other 2 brands are identified as Blue Shark (*Prionace glauca*), which is listed as Near Threatened species in IUCN Red List.

In Southern Brazil, molecular identification based on DNA barcoding (mtDNA, COI gene) is successful to show the evidence for mislabelling and trade of endangered species. They used the COI primers FishF2 and FishR2 for PCR process. All of the elasmobranch species are labelled as “cação” fish fillet. From the samples, high quality sequences ranged between 204 and 650 bases. From 63 samples collected, they found 20 different species. Considering IUCN criteria, 47% of the elasmobranch species found are threatened at the global level, while 53% are threatened and 47% are critically endangered in Brazil [6].

4. Conclusion
DNA barcoding can be a promising way to confirm whether a fish fillet product is mislabelled or not. This study successfully labelled the correct species of fish fillet product. Confirmation of the species
label in fish filet products can help in the conservation field to maintain the existence of a species in nature and can support the safety of consumers.

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References
[1] Frézal L and Leblois R 2008 Infect. Genet. Evol. 8 727-36
[2] Hebert P D, Ratnasingham S and de Waard J R 2003 Proc. R. Soc. Lond. B (Suppl.) 270 S96-9
[3] IUCN 2019 The IUCN Red List of Threatened Species available at www.iucnredlist.org
[4] Cardeñoso D, Hyde J and Caballero S 2014 PLoS ONE 9 e110193
[5] Ward R D, Zemlak T S, Innes B H, Last P R and Hebert P D N 2005 Phil. Trans. R. Soc. B 360 1847-57
[6] Almerón-Souza F et al. 2018 Front. Genet. 9 138