**BIODIVERSITAS**
Volume 19, Number 3, May 2018
Pages: 1035-1043

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190336

# Molecular identification of shark fins in Malaysian Borneo's local markets

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[1]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. 43600, Bangi, Selangor, Malaysia. Tel.: +60-389-213200, ♥email: abgbadd@ukm.edu.my, abgbadd1966@yahoo.com
[2]Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia. Pagoh Campus, 84000, Muar, Johor, Malaysia
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Manuscript received: 14 April 2018. Revision accepted: 14 May 2018.

**Abstract.** *Md-Zain BM, Abid-Kamal SNA, Aifat NR, Abdul-Latiff MAB, Mohd-Hashim A, Ampeng A, Yaakop S, Samat A. 2018. Molecular identification of shark fins in Malaysian Borneo's local markets. Biodiversitas 19: 1035-1043.* A molecular study was carried out to investigate the potential of the cytochrome c oxidase subunit I (COI) gene as a molecular marker for the genetic identification of shark fin samples that have gone through various preservation processes. A total number of 17 shark fin samples were collected from local markets in Sabah and Sarawak (Malaysian Borneo). The DNA sequences of the 17 samples were amplified by using polymerase chain reaction. The results from the analysis showed that, in the 17 sequences, there were 16 haplotypes present, with 244 sites from 688 bp of the sequences. For phylogeny analysis, tree topologies were reconstructed using the neighbor-joining (NJ) and maximum parsimony (MP) methods. DNA barcoding technique successfully identifies shark fins collected in local markets in Malaysian Borneo at species level employed during this study. Phylogenetic analysis showed that there were four clades that distinguish the four different orders present in the sample species. These clades had bootstrap values higher than 80. In addition, results indicated that 88.2% of the individuals are listed as endangered (*Lamiopsis tephrodes*, *Sphyrna mokarran*, and *Sphyrna lewini*), vulnerable (*Alopias pelagicus* and *Rhynchobatus australiae*), and near threatened (*Carcharhinus limbatus*, *Chiloscyllium griseum*, *Carcharhinus sorrah*, and *Carcharhinus brevipinna*), in the International Union for Conservation of Nature (IUCN) Red Data List.

**Keywords**: DNA barcode, phylogenetic relationships, phylogenetic tree, shark fin, species identification

## INTRODUCTION

In many places of Southeast Asia, sharks are intentionally captured; however, the whole body is seldom sold commercially (Clarke et al. 2006; Liu et al. 2013). Instead, sharks undergo a finning process, which is found to be a common practice among fishermen (Afonso et al. 2012). Finning is an unethical activity in which shark fins are cut off and their bodies disposed back into the sea. Later, the fins go through a drying process prior to being sold to wholesalers (Sembiring et al. 2014). Shark fins have been increasing in popularity; the demand is not only high in Chinese cuisine (Simoons 2001), but also in many Southeast Asian restaurants (Iloulian 2017). Alarming as the high demand may seem, complications arise in terms of the restriction and regulation of the shark trade. Most shark products sold are unlabeled, and their species is unknown (Castro et al. 1999). A way of overcoming this obstacle is using a molecular approach in which genetic markers are employed to identify the unknown identities of the shark fins being sold.

The mitochondrial cytochrome c oxidase subunit I (COI) gene is one of the most favored genetic markers for both DNA barcoding (Sultana et al. 2018) and phylogenetic studies (Ananthan and Murugan 2018), which require a gene to be capable of identifying species in certain taxonomic groups. Previous studies have found that COI can discriminate between closely allied species (Lissovsky et al. 2016). According to Hebert et al. (2003), high rates of sequence change in the COI gene in most animal groups reflect its success as a genetic marker in species identification. Many studies have been carried out for the species identification of seafood products through DNA barcoding (Fernandes et al. 2017; Günther et al. 2017; Nedunoori et al. 2017). Moreover, the uses of COI as a reliable genetic marker in shark species identification and phylogeny studies were fully supported (Gkafas et al. 2015; Vella et al. 2017).

This study applies a molecular approach for identifying unknown species of shark fins where morphological identification methods cannot be applied. Besides being able to identify species by comparing their genetic barcodes, the COI gene is suitable for taxonomic studies (Rosli et al. 2011; Syed-Shabthar et al. 2013; Ghazali et al. 2014) and may be able to resolve the phylogenetic relationships between the species of sharks identified from shark fin samples in Malaysian Borneo (Sabah and Sarawak); hence, this genetic marker has been chosen for the present study. This study demonstrates the COI gene's ability to identify shark fin specimens at the species level

based on samples collected from local markets in Malaysian Borneo (Sabah and Sarawak).

## MATERIALS AND METHODS

### Sample collection, DNA extraction, and polymerase chain reaction (PCR)

The samples were originally obtained from various local markets located in Sabah and Sarawak. There were three types of samples obtained, namely dried shark fins, salted shark fins, and fresh shark fins during the present investigation. DNA was extracted from 0.02–0.04 g of the shark fins using an Invisorb® Spin Tissue Mini Kit (Analytik Jena, Germany). The extraction steps were performed according to the protocol given, except for slight alterations in the initial steps for the dried and salted fin samples. These samples were soaked in dd$H_2O$ for 1 hour before being shredded; after this, the steps given in the protocol were followed.

Polymerase chain reaction (PCR) was employed to amplify the targeted locus of COI by using a Mastercycler® nexus (Eppendorf North America, Inc.). The primers used in this study were based on Ward et al. (2005) and Ivanova et al. (2007), as follows: VF2_tl GTA AAA CGA CGG CCA GTC AAC CAA CCA CAA AGA CAT TGG CAC and FR1d_tl CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A. PCR was performed by using Mastermix MyTaq Red Mix (Bioline) and involved a three-step PCR with the following conditions: pre-denaturation at 95°C for 1 min, 30 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, extension at 72°C for 10 s, and a final extension at 72°C for 10 min. The PCR cocktail used is shown in Table 1. The PCR products were sent to the 1st Base Laboratories Sdn Bhd (Malaysia) for sequencing.

### Sequence and phylogenetic analysis

The results of the sequence obtained from 1st Base Laboratories were in ABI format, and the chromatograms were displayed and examined using BioEdit (Hall 1990). The sequence similarity was compared with other fish mitochondrial genomes using the GenBank BLASTn application to confirm their species identity. Multiple alignments were performed using BioEdit's ClustalW to align the sequence results and ensure the forward and reverse DNA sequences were complementary. Later, sequence and phylogenetic analyses were performed by using DnaSP v6 (Rozas et al. 2017), PAUP 4.0b10 (Swofford 2002), and MEGA v6.0 (Tamura et al. 2013) to identify unique haplotypes and the neighbor-joining (NJ) and maximum parsimony (MP) tree reconstructions.

For construction of the NJ tree, the nucleotide compositions and sequence divergence within and between species were calculated by using the Kimura two-parameter (K2P) distance model (Kimura, 1980), with a bootstrap test of 5,000 replications. The tree bisection and reconnection (TBR) algorithm was used in the construction of the MP tree with the addition of a 50% consensus majority rule concept (Swofford 2002). The reconstructed MP tree underwent 1,000 replications to obtain the bootstrap confidence level.

## RESULTS AND DISCUSSION

COI sequences of ~750 bp in size were successfully obtained from 17 samples (10 from Sarawak and 7 from Sabah), out of a total of 24 samples analyzed in this study. A poor quality of DNA sequences from the remaining seven samples (Figure 1) was the main reason behind the sequences was exempted from this study to ensure accuracy in the analysis.

**Table 1.** PCR cocktail involved in DNA amplification

| Components | Final concentration | Volume (µL) |
|---|---|---|
| My Taq Red Mix | | 12.5 |
| Forward primer | 20 µmol | 1.0 |
| Reverse primer | 20 µmol | 1.0 |
| DNA template | | 3.0 |
| dd$H_2O$ | | 7.5 |
| Total | | 25 |


**Figure 1.** Chromatogram of the sequences obtained from shark fin samples. Chromatogram on top represent the quality of sequences used in the analysis, while chromatogram on bottom represent those deemed unacceptable

**Table 2.** Diversity of shark species detected from the shark fin specimens

| Sample type/ Code/ Locality | Species/ IUCN Status | Fin Specimen | Sample type/ Code/ Locality | Species/ IUCN Status | Fin Specimen |
|---|---|---|---|---|---|
| Fresh<br>F1<br>Sematan, Sarawak | *Sphyrna mokarran*<br>Endangered |  | Dried<br>D7<br>Semporna, Sabah | *Sphyrna lewini*<br>Endangered |  |
| Fresh<br>SF4<br>Sematan, Sarawak | *Sphyrna lewini*<br>Endangered |  | Dried<br>D8<br>Semporna, Sabah | *Sphyrna lewini*<br>Endangered |  |
| Dried<br>D5<br>Sematan, Sarawak | *Lamiopsis tephrodes*<br>Endangered |  | Dried<br>D9<br>Tawau, Sabah | *Sphyrna lewini*<br>Endangered |  |
| Fresh<br>SF3<br>Sematan, Sarawak | *Chiloscyllium griseum*<br>Near Threatened |  | Salted<br>M1<br>Kuching, Sarawak | *Rhynchobatus australiae*<br>Vulnerable |  |
| Salted<br>M2<br>Kuching, Sarawak | *Carcharhinus brevipinna*<br>Near Threatened |  | Fresh<br>F2B<br>Sematan, Sarawak | *Rhynchobatus australiae*<br>Vulnerable |  |
| Fresh<br>F2<br>Sematan, Sarawak | *Carcharhinus limbatus*<br>Near Threatened |  | Dried<br>D2<br>Semporna, Sabah | *Alopias pelagicus*<br>Vulnerable |  |
| Dried<br>SL1<br>Sematan, Sarawak | *Carcharhinus sorrah*<br>Near Threatened |  | DriedD4<br>Semporna, Sabah | *Alopias pelagicus*<br>Vulnerable |  |
| Dried<br>SL2<br>Sematan, Sarawak | *Carcharhinus sorrah*<br>Near Threatened |  | Dried<br>D3<br>Semporna, Sabah | *Loxodon macrorhinus*<br>Least Concern |  |
| Dried<br>S3<br>Tawau, Sabah | *Carcharhinus brevipinna*<br>Near Threatened |  | | | |

**Table 3.** Genetic distance among families based on Kimura-2-Parameter

| | Alopiidae | Carcharhinidae | Sphyrnidae | Rhynchobatidae | Hemiscylliidae |
|---|---|---|---|---|---|
| Alopiidae | - | | | | |
| Carcharhinidae | 0.162 | - | | | |
| Sphyrnidae | 0.168 | 0.106 | - | | |
| Rhynchobatidae | 0.236 | 0.235 | 0.227 | - | |
| Hemiscyllidae | 0.198 | 0.213 | 0.217 | 0.248 | - |

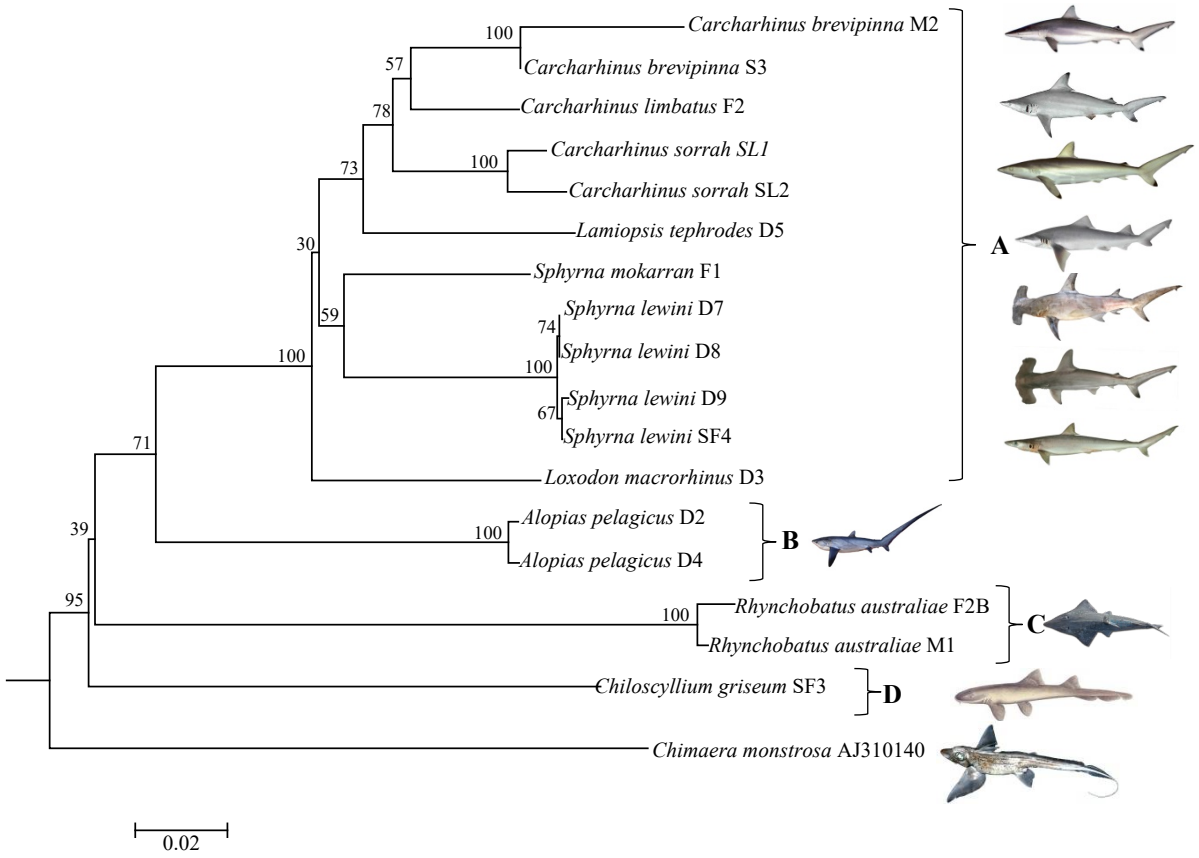


**Figure 2.** The Neighbour-Joining (NJ) phylogenetic tree of shark fin samples based on COI gene sequences. The numbers on the branches indicate bootstrap values

Dried and salted fin samples were extracted with a modified protocol to increase the success of DNA extraction. The sequence similarity searches using GenBank BLASTn validated all our sequences for sharks. These sequences indicated that of 688 bp of characters analyzed, 441 (64.09%) were conserved sites and 247 (35.90%) were variable sites, with 199 (28.92%) parsimony-informative characters. The sequence analysis revealed that thymine was the principal component of the sequences, at 33.2%, followed by adenine (26.5%) and cytosine (24.2%), and finally, guanine, at merely 16.2%.

Ten species were successfully identified from the samples, constituting five families, four of which represented sharks, namely Alopiidae (*Alopias pelagicus*), Carcharhinidae (*Carcharhinus brevipinna*, *Carcharhinus limbatus*, *Carcharhinus sorrah*, *Lamiopsis tephrodes*, and *Loxodon macrorhinus*), Sphyrnidae (*Sphyrna mokarran* and *Sphyrna lewini*) and Hemiscylliidae (*Chiloscyllium griseum*). One species of the wedgefish (Rhynchobatidae) family was also present in the collected fin samples, namely *Rhynchobatus australiae* (Table 2). Estimates of evolutionary divergence over sequence pairs between groups or the genetic distance of the sharks and wedgefish indicated that the families Hemiscylliidae and Rhynchobatidae were the most distant, at 0.248 (Table 3). These were followed closely by Rhynchobatidae–Alopiidae (0.236) and Rhynchobatidae–Carcharhinidae (0.235). Sphyrnidae–Carcharhinidae revealed the closest families, with a significantly lower genetic distance of 0.106.

The NJ phylogenetic tree yielded the optimal tree with the sum of branch length = 0.9921. Four distinct clades were successfully determined from the NJ tree, referred to as clades A, B, C, and D (Figure 2). The formation of clade A, as supported by a 100% bootstrap value, consisted of two families, Carcharhinidae and Sphyrnidae; these families were parallel, with the lowest genetic distance value between the two families. However, the subclades in clade A failed to reveal phylogenetic relationships between the genera *Carcharhinus*, *Lamiopsis*, *Sphyrna*, and *Loxodon*; this was attributed to the selection of the conserved COI locus. The remaining clades consisted solely of one family/genus, with clade B representing Alopiidae/*Alopias*, clade C comprising Rhynchobatidae/ *Rhynchobatus*, and clade D including Hemiscylliidae/ *Chiloscyllium*.

The MP phylogenetic tree was selected from the most parsimonious tree, with a tree length of 704 and a consistency index (CI) of 0.5277, retention index (RI) 0.6415, and composite index of 0.3691. Clade A, of the same family composition as that in the NJ tree, was

detected consistently in the MP tree, although the presence of polytomy was noted in the clade topology (Figure 3). Clades B (Alopiidae), C (Rhynchobatidae), and D (Hemiscylliidae), however, formed polytomy without distinctive clade formation, which resulted from the low number of representatives as compared with clade A and selection of conserved COI locus.

Haplotype analysis yielded 16 unique haplotypes, consisting of 244 polymorphic sites, which were then treated as barcoding sequences from the fin samples (Table 4). Four unique barcoding sequences were successfully determined for the five endangered samples collected in this study, specifically, three haplotypes for *S. lewini* (haplotypes 5, 6, and 11) and one haplotype for *S. mokarran* (haplotype 7). Six haplotypes were detected from the near threatened fin samples, namely one for *C. limbatus* (haplotype 8), *C. griseum* (haplotype 10), and two haplotypes each for *C. brevipinna* (haplotypes 13 and 14) and *C. sorrah* (haplotypes 15 and 16). Finally, two haplotypes were identified from *R. australiae* (haplotypes 9 and 12) and *A. pelagicus* (haplotypes 1 and 3), as well as one each from *L. macrorhinus* (haplotype 2) and *L. tephrodes* (haplotype 4).

## Discussion

Various molecular approaches have been utilized in identifying shark species, including multiplex PCR and DNA barcoding (Hoelzel 2001; Shivji et al. 2002; Holmes et al. 2009; Wong et al. 2009; Domingues et al. 2013; Sembiring et al. 2015). While using species-specific PCR primers, such as those presented by Abercrombie *et al*. (2005), in the multiplexed PCR can facilitate identification of multiple species in a processed product, all the primers for all the species in samples must be available. This represents a limitation to a current suite of available species-specific primers (Holmes *et al*. 2009); and the challenge is particularly great in samples of degraded DNA from processed products, such as dried or salted fins. The primers may not be 100% specific, or they may have annealed to the nuclear insertion of mtDNA (numts), which can compromise the identification process (Rosli et al. 2014; Abdul-Latiff et al. 2017).

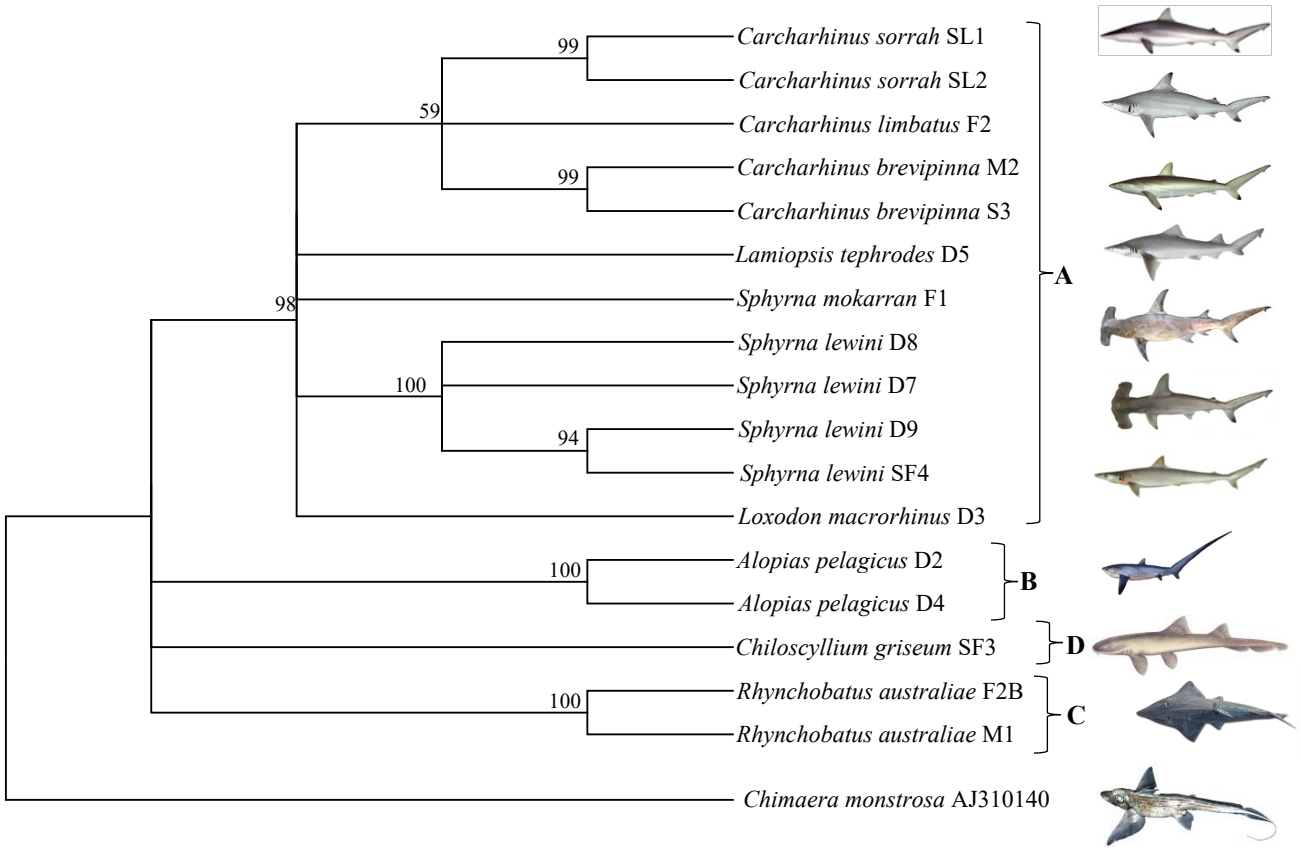


**Figure 3.** The Maximum Parsimony (MP) phylogenetic tree estimated using the TBR algorithm, and 1000 bootstrap replications 50% consensus rule was applied. Bootstrap values are shown on the branches

**Table 4.** Segregating sites (244 bp) in 688 bp segment of COI sequences defining 16 haplotypes. Haplotype 1-2 – Order Lamniformes; 3- 13 – Order Carcharhiniformes; 14 – Order Orectolobiformes; 15-16 – Order Rajiformes

| Hap | Sites |
|---|---|

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[              1 111 111 111 111 111 111 111 111 111 111 222 222 222 222 222 222 222 222 222 222 222 222 222 222 333 333 333 333 333 ]
[   11 111 223 444 455 556 666 667 777 888 899 990 011 122 233 344 455 666 777 778 889 999 000 011 222 333 344 556 666 667 778 888 888 999 999 000 112 222 333 444 ]
[  358 912 567 069 467 903 690 256 891 578 036 902 681 703 623 514 703 658 147 013 692 581 478 367 958 147 036 928 170 346 792 581 234 578 013 569 258 470 369 258 147 ]
#Hap_1 ACA TTT ATC TAG ACA CCA CCT ATC AGC TAG AGA ATA CAA TAC TTA TCC TAC ACT ATA CGG ATC ATA CAC GAT ATT AAC ATT AAT TCT ACC CTC CTT CTT TAC TCA CTA TAT CAC TTT AAT TTA
#Hap_2 ... ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#Hap_3 ..T .CC ..T ... ... ... TTC .C. ..T CC. .AT ..T T.G .GT .A. CT. CTT .T. ..G AAT ..T TC. T.T TC. ..A ..T .C. ... .T. ... ..A AA. ... CT. C.T ..C ..A ..A ... ..C C..
#Hap_4 ..T ..C ..T .C. ... ... T.C ... ..T CT. ..T ..T T.. .GT CA. ... CTT .T. GCG AAT ..T CC. T.T TC. ..C ... .CC ... ..C ... .CA .A. ..C CT. CTG ..C ..A ..A ..C ... ...
#Hap_5 ..T .CC ..T ... ... ... T.C ... ..T CT. GAT ... T.G .GT .A. C.. CTT .T. ... AAT ..T TC. T.. TTC ... ... .C. ... CTC ..T ..A AA. ... CC. ..C .CT ..A TGA ... ..G ...
#Hap_6 CTG CCC ..A ... ... ... T.C T.. ..T CT. GAT ... T.G .C. CA. C.. CTT .TC G.. AAT ..T TC. ... TC. ... ..T .CC .G. C.C ... ..A AA. .A. CT. C.C .CT ..A T.A ... ... ...
#Hap_7 ..T .CC ..T ... ... ... T.C T.. ..T CT. GAT ... T.G .G. CA. C.. CTT .TC G.. AAT ..T TC. ... TC. ... ..T .CC .G. C.C ... ..A AA. ... CT. C.C .CT ..A T.A ... ... ...
#Hap_8 ..C .CC ..T ... ... ... T.C ... ..T CT. .AT ..T T.. .GT .A. C.. CTT ..C G.. AAT G.T TC. T.T AT. ..A ..T .CC ..C CTC ..T ..A AA. T.A CT. ..T ..T ..A T.A C.. ..C C.T
#Hap_9 ..C ..C GAT ... ... ... T.. ..T ..T CC. GAT ..T T.. .GT .A. C.. CTT .TC G.. AAT G.T TC. T.T AT. ..A ..T .CC ..C CTC ..T ..A AA. T.. CT. C.T ..T ..A T.A C.. ... C.T
#Hap_10 ..T .CC ..T ..A ... ... TTC ... ..T CT. .A. ..T T.. .GT .A. .T. CT. .TC ..G AAT ..T T.G TG. TT. ... G.. GC. ... C.C G.T ..A AAC ... CC. C.. ..C .GA T.A ..C ... ...
#Hap_11 ..T .CA ..T ..A ... ... TTC ... ..T CT. .A. ..T T.. .GT .A. .T. CT. .TC ..G AAT ..T T.G TG. TT. ... G.. GC. ... C.C G.T ..A AAC ... CC. C.. ..C .GA T.A ..C ... ...
#Hap_12 ..T .CC ..T ..A ... ... TTC ... ..T CT. .A. ..T T.. .GT .A. .T. CT. .TC ..G AAT ..T T.G TG. TT. ... G.. GC. ... C.C G.T ..A AAC ... CC. C.. ..C .GA T.A ..C ... ...
#Hap_13 ..T .CC ..T ... ... ... TT. ... ..T CT. GA. ..C T.. .GT CA. ... CT. .T. GAG AAT ..T T.G T.. TT. ... ..T ..C ... C.C ..T ..A AA. ... CT. C.. ..T ..A T.A C.C ..C ...
#Hap_14 ..T ... ... C.. TT. .TT ... ... T.T C.A TAT G.C ..T CGT .A. CAT .T. ... GAG T.A ..T ... T.. ACC GCC .TT ... T.C ... .TA ... TAC T.A ... CT. .CT A.. TGA AAC .TC CAT
#Hap_15 ... .C. ..T CT. TAT GTG TGC C.. .AA CTA TA. .A. .T. C.. CGG .A. .CT GTC G.. AAC GCA C.G T.. CCC ... T.. .AC G.. C.C .TA TAT AA. ... CTT ATG GCT C.. T.T AA. GTG .AC
#Hap_16 ... .C. ..T CT. T.T GTG TGC C.. .AA CTA TA. .A. .T. C.. CGG .A. .CT GTC G.. AAC GCA C.G T.. CCC ... T.. .AC ... C.C .TA TAT AA. ... CTT ATG .CT C.. T.T AA. GTG .AC
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[  333 333 333 333 333 333 333 334 444 444 444 444 444 444 444 444 444 455 555 555 555 555 555 555 555 555 555 555 555 555 555 556 666 666 666 666 666 666 666 6]
[  455 555 666 677 778 888 899 990 000 011 112 223 334 444 566 677 777 888 900 000 111 222 333 333 444 444 455 556 667 778 889 999 990 001 122 233 333 344 455 568 8]
[  803 469 025 814 570 367 902 581 247 836 792 891 470 369 814 735 689 245 101 689 578 147 134 569 023 458 914 780 695 891 470 356 792 584 703 602 358 914 734 960 7]
#Hap_1 TAT GCT TAA TTA GAC TCT AGT TCT CCT TAT ATA TTA CAC CTT TTT ACC TCA CCC TAT TTG ATT AAA ATC TCT CAT TAC CCA ACT CCA ACA TTT AAC CAC AAT TAA CGA GTA ATT TTT CTG T
#Hap_2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .
#Hap_3 CG. A.C C.C ..G C.T .T. G.. ... .TC .T. G.. C.. T.. TCC ..C .A. A.C T.T ... ..T T.C T.T ... .T. .TC .TA .T. T.. AA. .T. CAC T.T .CT T.C CT. T.. .CT .C. ... ... .
#Hap_4 C.. A.. ..C ... C.T ..C ..C C.C ... .T. G.. CC. ..T .C. C.C .A. A.C T.T C.. ..T ... T.T GC. .T. .CC .CA .T. T.. AA. ... .AC T.. .TT C.. .TG T.. .C. .C. .C. ... .
#Hap_5 ... A.C ... ... C.T .T. ..C ... .TC .T. G.. ... T.T T.. ... .A. A.C T.. ... ..T T.. TGC ... .T. .CC .TA .T. C.. AG. .T. .A. T.. .CT C.. .T. T.. ... .C. ... ... .
#Hap_6 ... A.C C.. ... C.T .T. ... ... .TC .C. G.. C.. T.T T.C ..C .AA AAC TA. ... .AT CA. T.C ... AT. .CC ATA .T. C.. A.. .G. .A. TG. .TT T.. CT. .T. TC. GC. ... TAC .
#Hap_7 ... A.C C.. ... C.T .T. ... ... .TC .C. G.. C.. T.T T.C ..C .A. A.C T.. ... ..T C.. T.C ... .T. .CC .TA .T. C.. AG. .T. .A. T.. .TT T.. CT. ... .C. .C. ... ... .
#Hap_8 ... A.C ... ... C.T .T. ... ... ..C .T. G.. ... T.T T.. ... .A. A.C ... ... ..C T.. T.C ... ... .CC .CA .T. T.. AG. .T. .A. T.. .TT T.. .T. T.. ... .C. ... ... .
#Hap_9 ... A.C ..C ... C.T .T. ... ... ..C .T. G.. ... T.T T.. ... .A. A.C ... ... ... C.. T.C ... ... .CC .CA .T. T.. AG. .T. .A. T.. .TT T.. .T. T.. ... .C. ... ... .
#Hap_10 ... A.C ..C C.. C.T ... ... CT. ..C CC. GA. ... ..T TC. C.C GT. A.C T.T ... ..T C.C T.T .C. .A. .CC ..A .T. T.. AA. .T. CA. C.. .TT T.. .T. G.. ... .C. .C. ... .
#Hap_11 ... A.C ..C C.. C.T ... ... CT. ..C CC. GA. ... ..T TC. C.C GT. A.C T.T ... ..T C.C T.T .C. .A. .CC ..A .T. T.. AA. .T. CA. C.. .TT C.. .T. G.. ... .C. .C. ... .
#Hap_12 ... A.C ..C C.. C.T ... ... CT. ..C CC. GA. ... ..T TC. C.C GT. A.C T.T ... ..T C.C T.T .C. .A. .CC ..A .T. T.. AA. .T. CA. C.. .TT C.. .T. G.. ... .C. .C. ... .
#Hap_13 ... A.C C.T ... C.. .TC ..C C.. ..C .T. .C. CCG ..T TCC ..C ... A.T T.T C.. ..C T.. T.T ... .A. .CC .TA .T. C.. AA. .T. .A. C.. .TT T.. CT. A.. ... ... ... ... ... .
#Hap_14 ..A .T. ... .A. ..A .TC .A. ..C .AC ..A .C. ... .TT T.C C.. .A. A.. T.T .TC ..T C.C T.T ... .TA ..C .TA TAT TTA A.. GT. .AC ... T.T ... ... A.C ..T ..A .CC ... .
#Hap_15 CT. ... CTC .C. .CT A.. ..C CTA TA. ..A ..C ... ATT T.C .CC G.. A.. T.. CTC C.A ... T.. .CT .AC T.C ..A .A. T.A A.T ..C CA. T.T .CT .C. ..G G.. A.. .C. CC. ... .
#Hap_16 CT. ... CTC .CG .CT A.. ..C CTA TA. ..A ..C ... ATT T.C .CC G.. A.. T.. CTC C.A ... T.. .CT .AC T.C ..A .A. T.A A.T ..C CA. T.T .CT .C. ... G.. ... .C. CC. ... A
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The DNA barcoding method using the universal primers targeting the COI loci were utilized in this analysis and provided with significantly good stretches of COI sequences of ~750 bp. Barcoding success depends on low levels of sequence variation within species and much higher levels between species (Holmes et al. 2009). Barcodes can be submitted to publicly available databases, such as the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007) or National Center for Biotechnology Information (NCBI); the latter was employed in this study. The sequences obtained in this study were analyzed through GenBank BLASTn to verify the species of sharks in the fin samples. The total alignment scores from all the alignment segments, percentage of queries covered by the alignment to the database sequence, best expected value (E value) of all the alignments from the database sequence, and highest percent identity (Max ident) of all the query-subject alignments were the key parameters for ensuring the specificity of the DNA sequences (Abdul-Latiff et al. 2014, 2017).

The shocking finding in this study was that six collected fin samples were identified as endangered on the IUCN Red Data List, as they came from the species *L. tephrodes*, *S. mokarran*, and *S. lewini*. The remaining fin samples were listed as nearly threatened (six samples), including *C. limbatus*, *C. griseum*, *C. sorrah*, and *C. brevipinna*, and vulnerable (four samples), including *A. pelagicus* and *R. australiae*. Ironically, only one sample belonged to the least concern category, from the species *L. macrorhinus* (Table 3). In total, 35.3% of all fins came from shark species classified as endangered, 35.3% as near threatened, 23.5% as vulnerable, and merely 5.9% as least concern. Surprisingly, this is comparable to the results obtained by Sembiring et al. (2015), who utilized a similar approach in barcoding targeted shark fisheries in Indonesia.

Low support for clade reconstructions separating *C. brevipinna* and *C. limbatus* from *C. sorrah* are expected, as the analysis performed by Naylor et al. (2012) exhibited the same outcome by studying the NADH2 sequence data (1044 bp). The same distant relationships were observed by Vélez-Zuazo and Agnarsson (2011), whose study showed that the genus *Carcharhinus* is highly unstable, as the researchers discovered the inclusion of *Prionace* and *Nasolamia* in the same genus. The clade representing Carcharhinidae in this study portrayed a reliable tree topology in the family, as *Lamiopsis* has been proven to have closer relationships with *Carcharhinus* than *Sphyrna* (Iglésias et al. 2005; Vélez-Zuazo and Agnarsson 2011; Naylor et al. 2012). White et al. (2010) resurrected *L. tephrodes* as a different species from *L. temnickii*, as it differs in its dentition and morphological characteristics, as well as exhibiting substantial DNA sequence divergence in the mitochondrial marker ND2. However, we regard *L. tephrodes* and *L. temnickii* as belonging to a monotypic genus with two distinct populations, namely the broad fin shark and Borneo broadfin shark. While our low number of samples representing *A. pelagicus* perfectly distinguished *Alopiidae* from Carcharhinidae, Naylor et al. (2012) noted a lack of monophyly for the genus, consistent with analyses based on the whole mitochondrial genome and nuclear markers.

This data showed that it is not only sharks in the family Carcharhinidae that are being targeted and sold in the local markets of Sabah and Sarawak; rather, hammerhead sharks, which are members of the family Sphyrnidae, are also being sold. In addition, many wedgefish, or Rajiformes, are being sold and wrongly marketed as sharks. A major problem faced by the shark industry is the mislabeling of the shark species being marketed. A method of overcoming this situation is by training of the individuals involved in the fishing industry to monitor elasmobranches' that land from artisanal to industrial fisheries in supermarkets by incorporating genetic identification techniques (González-Wevaret al. 2015).

In 2011, the fisheries landings for Malaysia comprised 1,665,857 tons, with an estimated value of RM 9.38 billion; this contribution amounted to 1.1% of the gross domestic product (GDP). All fishing activities in Malaysia are governed by the Fisheries Act of 1985 and its regulations and fisheries management policies. Valid fishing gear and fishing vessel licenses are required for all vessels conducting any fishing activity. Licenses are issued based on the status of available fish stocks, mainly determined from research findings and fish landing data (Malaysia's NPOA-IUU 2013).

Due to the maximum exploitation of fish stocks in the coastal waters, a moratorium on new fishing licenses was imposed in 1982, except for those for fishing vessels of 70 GRT and above, for operating in Zone C2 (30 nautical miles up to the Exclusive Economic Zone) or deep-sea waters (Malaysia's NPOA-IUU 2013). Even with these regulations and regular monitoring, it has been claimed that Malaysia landed 231,212 tons of shark in 2002–2011, positioning it at the eighth highest country for shark fishing globally and accounting for 2.9% of the total global reported shark catch during that period (TRAFFIC 2013).

The main problem for ensuring that Malaysia has sustainable and legal shark fisheries is that it is not compulsory for the sharks to be landed as a whole organism, specifically, with their fins attached to their bodies. This has created a loophole that hinders the accurate identification of the species caught, which is a prerequisite for understanding the catches of various species, and therefore, determining sustainable harvest levels in Malaysia. Using the methods employed in this study would represent a breakthrough in managing illegal fisheries in this country.

In conclusion, this study successfully determined the diversity of sharks by evaluating the shark fins collected from the local markets of Malaysian Borneo, indicating that the DNA barcoding technique employed is an accurate, rapid approach for shark fin species identification. This study can further enhance the management of fisheries in Malaysia, especially the illegal shark industries, while simultaneously generating diverse data to be utilized in conservation and molecular systematic studies. This will also be useful in determining the origins of shark fins from various parts of the Malaysian waters, giving a larger pool of samples and also contributing to the construction of a DNA sequences library for barcoding purposes.

## ACKNOWLEDGEMENTS

The authors acknowledge Universiti Kebangsaan Malaysia and the Southeast Asian Fisheries Development Center (SEAFDEC), Malaysia, for providing necessary funding, facilities, and assistance. This research was supported by Grants FRGS/1/2015/SG03/UKM/02/1, GUP-2017-087, DLP-2013-006, AP-2015-004, and UKM-GUP-PLW-08-11-046.

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