

Species substitutions revealed through genotyping: Implications of traceability limitations and unregulated fishing

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Abstract

Trade flow of fisheries sector is very complex with several intermediaries and it is difficult to trace back the origin of a seafood commodity. Thus, traceability limitations could cause safety and quality issues in the final product delivered to the consumers. Decreased monitoring may also increase the possibilities of illegal, unreported and unregulated fishing practises. In global fisheries sector, a proper species identification system for identifying and preventing commercial frauds like species misrepresentation and illegal trade is mandatory. DNA analysis is a promising technique for food authentication as it provides increased specificity, sensitivity and reliable performance for accurate specimen identification and species confirmation. Mitochondrial cytochrome c oxidase subunit I gene (mtCOI) fragments represent one of the robust genetic marker for identification of specimens up to species level. It is a stable genetic marker which could be amplified from fresh, degraded, processed or cooked materials. India is having very limited regulations for preventing improper labelling of seafood items and ensuring authentication for traded fish and fishery products. This study focuses on the applicability of DNA barcodes over fish and fishery products traded at different sectors like local markets, supermarkets, restaurants etc. Samples collected from different stations of Ernakulam district (Kerala, S. India), were subjected to molecular analysis and COI sequences were developed. Among the 62 samples, 34 samples were identified as species substituents and the substitution rate was accounted up to 54.84%. In addition, trade of certain exotic/invasive and illegally cultured species were also confirmed. This study discusses the applicability of DNA barcoding in fisheries sector for preventing food fraud and suggests its implementation as a systematic regulatory programme conducted by governmental agencies for fishery stocks authentication.

Keywords: Fish and fishery products, Ernakulam district, DNA barcoding, COI, Species substitution

Introduction

With regard to the rise in global population, demand for meat products are concomitantly increasing across the globe (De Brito, Schneider, Sampaio, & Santos, 2015; FAO, 2012). Worldwide, fish constitutes up to 16.7% of the animal protein consumed by a person. Therefore, fish and fishery products may be considered as major players in satisfying this demand. Average world capita consumption of fish is reported to increase from 9.9 kg (during 1960s) to 19.7 kg in 2013 (FAO, 2016). In 2016, total fish production reached up to all time record of 171 million tonnes within which 88 percent was consumed by humans (FAO, 2018). Even though, aquaculture is partially satisfying this requirement, there exists high pressure over capture fisheries. Since seafood has dominated the diet of many people, it is considered as world's most traded food commodity with an estimated export value of \$142 billion (in 2016) (Kroetz, Donlan, Cole, Gephart, & Lee, 2018; Guardone et al., 2017; Asche, Bellemare, Roheim, Smith, & Tveteras, 2015). However, production from capture fisheries including inland and marine fisheries (nearly 90 million tonnes per year) has also reached a plateau (FAO, 2016). Overall, the 'inexhaustible' marine fish stock is confronting extreme fishing pressure for satisfying the insatiable human appetite for seafood and is in the verge of decline (Christiansen, Fournier, Hellemans, & Volckaert, 2018; Quaas, Reusch, Schmidt, Tahvonen, & Voss, 2015; Pauly & Zeller, 2016; Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003). Hitherto, there are numerous reports regarding the depletion of global fish stocks (up to 75%) and its deleterious effects over aquatic ecosystems (Cawthorn, Steinman, & Hoffman, 2013; Pauly, Watson, & Alder, 2005; Worm, Barbier, & Beaumont, 2006; FAO, 2009).

Because of the drastic increase in fish consumption rate, a considerable share of seafood should be imported by most of the countries for satisfying their domestic needs (Guardone et al., 2017; FAO, 2014; 2016). About 200 countries were reported to export fish and fishery products mainly for some developing countries (Abdullah & Rehbein, 2017). Since the trade flow of fisheries sector is very complex with several intermediaries, it is difficult to trace back the origin of a seafood commodity (Sterling & Chiasson, 2014). Disordered transferring of the fish and fishery products among intermediaries result in loss of traceability informations along the marketing chain, causing safety and quality issues to the final product delivered to the consumers (Sameera & Ramachandran, 2016). Increased seafood imports, decreased monitoring, difficulty in distinguishing cooked products and closely related species with similar taste and texture (in fresh and processed forms) opens an easy platform for substituting a high valued species with a low valued one (Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019; Buck, 2010). Thus, seafood is highly prone

to illegal practices like food frauds, causing deception in marketing (Johnson, 2013; Spink & Moyer, 2011). In addition, practise of Illegal, Unreported and Unregulated (IUU) fishing is also performed for satisfying the escalating demand for fish and fishery products (Helyar et al., 2014; Miller & Mariani, 2010; Sterling & Chiasson, 2014; Pramod, Nakamura, Pitcher, & Delagran, 2014).

Accounting all these factors, present scenario demands a proper species identification system in global fisheries sector for identifying commercial frauds like species misrepresentation/mislabelling (Ogden, 2008; Sameera. Ramachandran, Deepak, & Harikrishnan, 2019; Deepak et al., 2019). European Union (EU), the global leader in food traceability has rigorous regulations regarding seafood traceability and labelling to safeguard consumer benefit (Deepak et al., 2019; Vartak, Narasimmalu, Annam, Singh, & Lakra, 2015). EU legislation article 58 of EC 1224/2009 states that “all lots of fisheries and aquaculture products shall be traceable at all stages of production, processing and distribution, from catching or harvesting to retail stage” (EC 2009) (Charlebois, Sterling, Haratifar, & Naing, 2014). Since specimen identification and species confirmation are more cumbersome and demand scientific expertise, modern and innovative technologies could be implemented to ensure authenticity of traded products (Deepak et al., 2019; Dawnay, Ogden, McEwing, Carvalho, & Thorpe, 2007; Maldini, Marzano, Fortes, Papa, & Gandolfi, 2006).

In order to address the issue of seafood substitution, traditional (paper-based documentation and barcode scanners) as well as modern traceability techniques (fisheries forensic techniques and vibrational spectroscopy combined with chemometric methods) are used (Derrick & Dillion, 2004; Ogden, 2008; Power & Cozzolino, 2020). Among them, fisheries forensics based on molecular genetics represent one of the latest and advanced technique for identification of traded seafood (Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019; Christiansen, Fournier, Hellemans, & Volckaert, 2018; Lockely & Bardsley, 2000). Molecular genetics based on DNA analysis viz. DNA barcoding is a promising technique for food authentication as it provides increased specificity, sensitivity and reliable performance for accurate specimen identification, even in highly processed samples (Lockley & Bardsley, 2000; Lenstra, 2003; Wong & Hanner, 2008; Deepak et al., 2019). Usually, genetic markers are implemented in fisheries sector for specimen identification and species confirmation (Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019; Spencer & Bruno, 2019). Mitochondrial cytochrome c oxidase subunit I gene (COI) fragments represent one of the robust genetic marker for identification of specimens up to species level (Deepak, Nidhin, Anil Kumar, Pradeep, & Harikrishnan, 2015; Deepak et al., 2019;

Deepak & Harikrishnan, 2016; Sameera, Ramachandran, Deepak, & Harikrishnan et al., 2019; Ward, Zemlak, Innes, Last, & Hebert, 2005). COI fragments could be amplified from fresh, degraded, processed or cooked materials (Hanner, Becker, Ivanova, & Steinke, 2011; Wong & Hanner, 2008; Christiansen, Fournier, Hellemans, & Volckaert, 2018; Spencer & Bruno, 2019; Vartak, Narasimmalu, Annam, Singh, & Lakra, 2015; Helyar et al., 2014; Chin, Adibah, Hariz, & Azizah, 2016; Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019).

India, with a vast coastline represents one of the major exporter of seafood (Nagalakshmi, Annam, Venkateshwarlu, Pathakota, & Lakra, 2016). Estimated marine fish landings from the country was accounted up to 3.83 million tonnes in 2017 (CMFRI, 2018). Since people prefer both fresh water and marine fishes, demand for fish and fishery products are also high within the country (Nagalakshmi, Annam, Venkateshwarlu, Pathakota, & Lakra, 2016). With regard to this scenario, there exist little regulations for preventing improper labelling of seafood items and ensuring authentication for traded fish and fishery products (Vartak, Narasimmalu, Annam, Singh, & Lakra, 2015; Srinu & Padmavathi, 2016; Deepak et al., 2019). The necessity for an advanced tool to ensure food authentication, particularly in fisheries sector is important to ensure food safety (Vartak, Narasimmalu, Annam, Singh, & Lakra, 2015; Deepak et al., 2019). This study focuses on the applicability of DNA barcodes over fish and fishery products traded at different sectors like local markets, supermarkets, restaurants etc. Ernakulam district, one of the leading coastal district of Kerala state, the third largest producers of fish and fishery products in India (Shyam and Rahman, 2014; CMFRI, 2018) was selected for pursuing this study.

2. Materials and Methods

2.1 Specimen collection

A total of 67 samples were collected from different sources like local fish markets, hyper/super markets, retail outlets and hotels. Three samples were discarded because of their unacceptable/degraded condition and the remaining 64 samples were categorized according to their trading condition as fresh, frozen, chilled and cooked (for details, please refer Supplementary file 1). All samples were preserved at -20°C in a laboratory freezer (-20 °C) for conducting molecular studies

2.2 Genotyping

2.2.1 DNA extraction, PCR amplification and Gel electrophoresis

Total genomic DNA was extracted from the samples using DNeasy Blood and Tissue Kit (Qiagen), following the spin column protocol for purification of total genomic DNA from animal tissues. PCR reagent used was of Sigma Aldrich (ReadyMix™ Taq PCR Reaction Mix with MgCl²) and the machine used was Corbett gradient thermal cycler. Partial mitochondrial COI gene sequences from fish samples were amplified using the primer pair Fish F1 (5' TCAACCAACCACAAAGACATTGGCAC 3') and Fish R1 (5' TAGACTTCTGGGTGGCCAAAGAATCA 3') (Ward, Zemlak, Innes, Last, & Hebert, 2005). Thermal regime was selected according to modified Lakra et al. (2011) with an initial denaturation of 95 ° C for 2 min followed by 35 repeats of denaturation at 94 ° C for 40 sec, annealing at 45-55 ° C for 40 sec, extension at 72 ° C for 1 min 10 sec and a final extension of 72 ° C for 10 min. For crustaceans, COI region was amplified using the primer pair LCO1490-HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and thermal profile with an initial denaturation of 4 min at 95 ° C; followed by 10 cycles of denaturation at 95 ° C for 40 s, 40 ° C (annealing temperature) for 40s, extension at 72 ° C for 40 s; 25 cycles of 95 ° C for 40 s, 55 ° C annealing temperature for 40 s, 72 ° C for 40 s; and a final extension step at 72 ° C for 8 min.

PCR amplicons displaying intense bands following Agarose gel electrophoresis (1.2%) were outsourced for purification and sequencing.

2.2.2 Software analysis and validation of species authenticity

Protein coding COI gene sequences were translated online (<http://insilico.ehu.es/translate/>). Manual edition and compilation were done using BioEdit 7.0.9 (Hall, 1999). Alignment was performed using Clustal X (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). After a primary BLAST search using the developed sequences, >97% sequence similarity was fixed as a criterion for designating a particular sample to a corresponding species (Barbuto et al., 2010; Cawthorn, Steinman, & Hoffman, 2013; Deepak et al., 2019). Validation of results from the BLAST search were done by generating genetic distance based on Kimura 2 Parameter model using MEGA 5 (Tamura et al., 2011). Statistical analysis was conducted at 95% confidence interval. The proportion of species substitution rate was calculated and converted to percentage using RStudio 1.2.1335.

3. Results and discussion

Total genomic DNA extracted from the collected samples were successfully amplified using the mentioned COI primer pairs for fishes and crustaceans. PCR amplicons were purified and sequenced to obtain COI gene sequences ranging from 476 to 669 bp in length. Among the 64 samples, COI sequences were successfully amplified from 61 samples. Two chilled (mentioned as snapper and rays) and one cooked (Squid) sample did not amplify with the COI primer pairs. One case according to Sameera, Ramachandran, Deepak, & Harikrishnan (2019) i.e. *Piaractus brachypomus* as a substituent for *Parastromateus niger* was also incorporated in further analysis and the total samples were accounted as 62. Fig. 1 depicts total samples collected from different centres with their trading names.

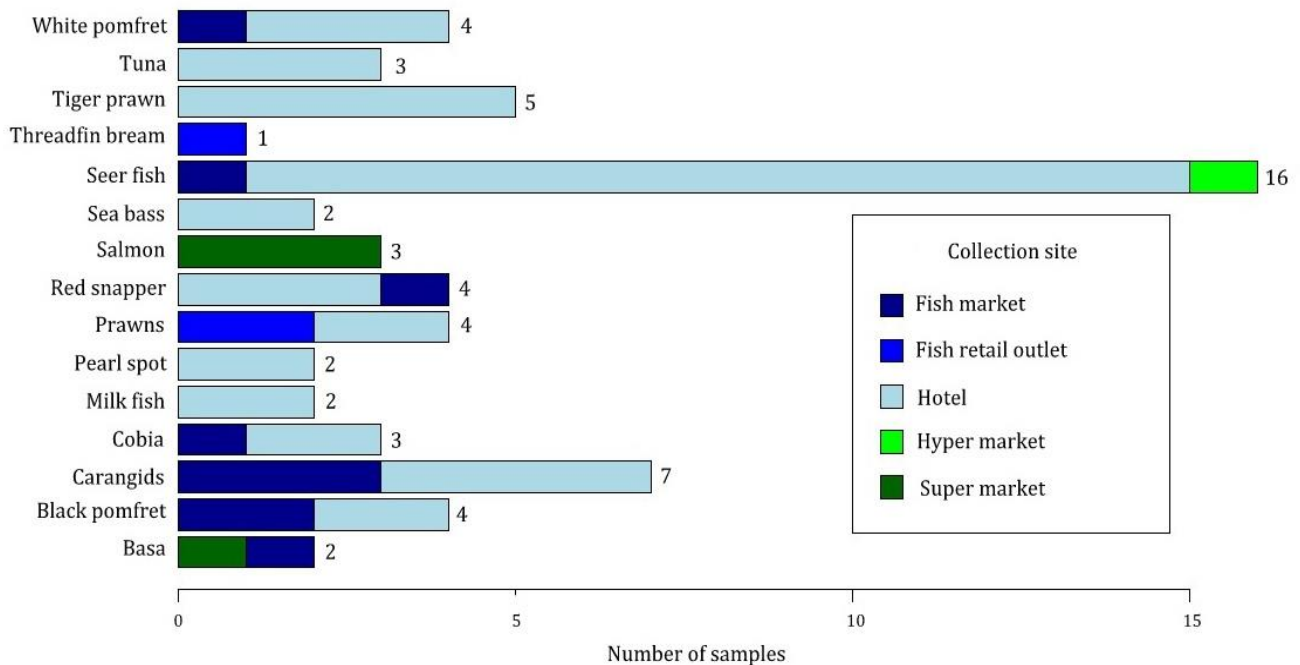


Fig. 1. Total samples collected from different sites and their trade names

A comparative approach with the public database revealed species identity of 60 samples with >99% similarity. However, two species substituents for prawn samples were identified to genus level with lower identity values (<92%). Among the 62 samples, 34 samples were identified as species substituents (Fig. 2). Rate of substitution was accounted up to 54.84%.

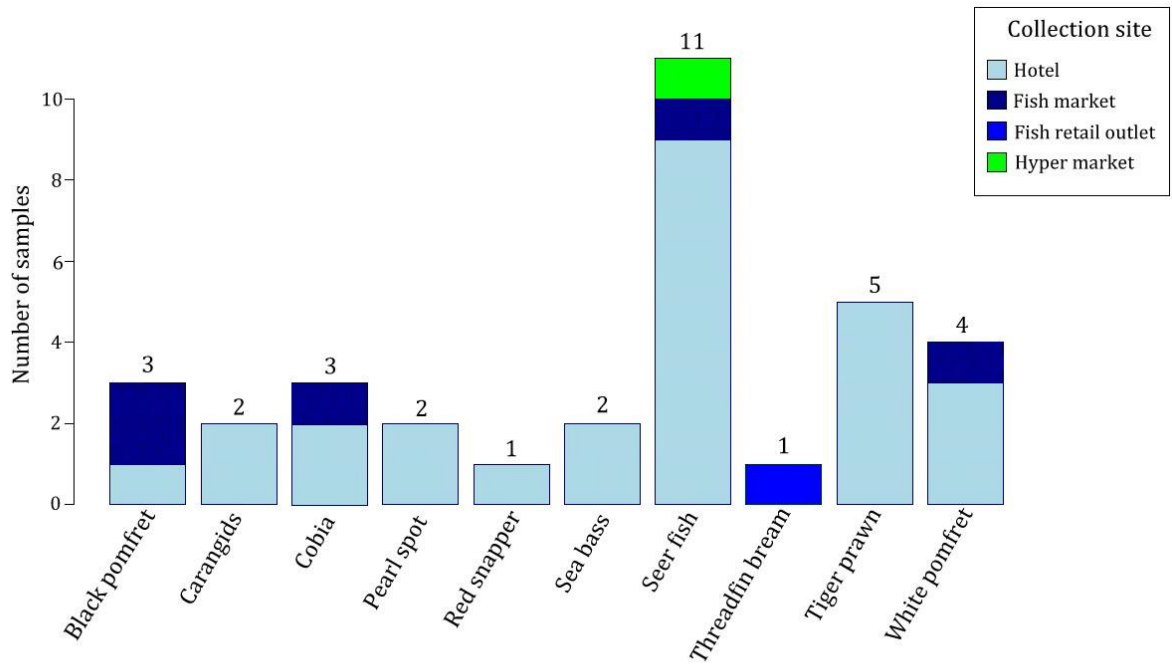


Fig. 2. Species substitution identified for samples (with trade names) collected from different sites
 Details regarding total samples collected, their trading names and identified substitutions are given in Table 1.

| Type of product | Collection site | Marketed as | Expected species | GenBank | | | Mislabelling status | Approx. interspecific genetic distance (%) |
|-----------------|--------------------|-----------------|----------------------------------|----------------------------------|----------------|------------------|---------------------|--|
| | | | | Species identified as | Similarity (%) | Accession Number | | |
| Fish steak | Supermarket | Red Snapper | <i>Lutjanus sp.</i> | <i>Lutjanus argentimaculatus</i> | 99.84 | MK328520 | No | na |
| Fish Kheema | Fish retail outlet | Threadfin bream | <i>Nemipterus japonicus</i> | <i>Parastromateus niger</i> | 100 | MF737197 | <u>Yes</u> | <u>21.6</u> |
| Fish Chunks | Fish market | Carangids | <i>Caranx sp.</i> | <i>Caranx ignobilis</i> | 100 | MF737192 | No | na |
| Fish fillets | Hypermarket | Basa | <i>Pangasiodon hypothalampus</i> | <i>Pangasiodon hypothalampus</i> | 99.79 | MG230659 | No | na |
| Fish chunks | Fish market | Carangids | <i>Caranx sp.</i> | <i>Caranx ignobilis</i> | 100 | MF737193 | No | na |
| Fish steaks | Fish market | White pomfret | <i>Pampus argenteus</i> | <i>Acanthurus mata</i> | 99.52 | MG230663 | <u>Yes</u> | <u>19.4</u> |
| Fish steaks | Fish market | Seer fish | <i>Scomberomorus commerson</i> | <i>Istiophorous platypterus</i> | 100 | MG230665 | <u>Yes</u> | <u>25.6</u> |
| Fish Fillets | Hypermarket | Salmon | <i>Salmo salar</i> | <i>Salmo salar</i> | 100 | MK295653 | No | na |
| Fish Chunks | Fish market | Cobia | <i>Rachycentron canadum</i> | <i>Xiphias gladius</i> | 100 | MK295656 | Yes | <u>24.8</u> |
| Fish steak | Fish market | Black pomfret | <i>Parastromateus niger</i> | <i>Platax teira</i> | 99.85 | MK301230 | Yes | <u>24.4</u> |

| | | | | | | | | |
|------------------------|--------------------|---------------|---------------------------------|---------------------------------|-------|-----------------|------------|-------------|
| Fish Steak | Hyper - market | Seer fish | <i>Scomberomorus commerson</i> | <i>Caranx ignobilis</i> | 100 | MF737 194 | Yes | <u>24.0</u> |
| Fish Chunks | Fish market | Carangids | <i>Caranx sp.</i> | <i>Caranx ignobilis</i> | 100 | MF737 195 | No | na |
| Small prawns (P and D) | Fish retail outlet | Prawns | <i>Penaeus vannamei</i> | <i>Penaeus vannamei</i> | 100 | MK30 1236 | No | na |
| Large prawns (P and D) | Fish retail outlet | Prawns | <i>Penaeus vannamei</i> | <i>Penaeus vannamei</i> | 100 | MK30 1237 | No | na |
| Fish fillets | Hyper - market | Salmon | <i>Salmo salar</i> | <i>Salmo salar</i> | 100 | MK29 5654 | No | na |
| Fish fillets | Super market | Basa | <i>Pangasiodon hypothalamus</i> | <i>Pangasiodon hypothalamus</i> | 99.79 | MG23 0660 | No | na |
| Fish fillets | Hyper - market | Salmon | <i>Salmo salar</i> | <i>Salmo salar</i> | 100 | MK29 5655 | No | na |
| Whole fish* | Market | Black pomfret | <i>Parastromateus niger</i> | <i>Piaractus brachipomus</i> | 100 | MF693 914-20 | <u>Yes</u> | <u>25.9</u> |
| Fish Curry | Hotel | Milk fish | <i>Chanos chanos</i> | <i>Chanos chanos</i> | 100 | MK30 1234 | No | na |
| Fish Masala | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Scomberomorus commerson</i> | 99.85 | MG23 0667 | No | na |
| Fish Curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Acanthocybium solandri</i> | 100 | MK30 1240 | <u>Yes</u> | <u>15.3</u> |
| Fish Curry | Hotel | Tuna | <i>Thunnus albacares</i> | <i>Thunnus albacares</i> | 99.85 | MK30 1243 | No | na |

| | | | | | | | | |
|--------------------|-------|---------------|--------------------------------|------------------------------------|-------|--------------|------------|-------------|
| Fish Curry | Hotel | Cobia | <i>Rachycentron canadum</i> | <i>Coryphaena hippurus</i> | 100 | MK32 8513 | <u>Yes</u> | <u>20.3</u> |
| Fish Curry | Hotel | Sea bass | <i>Latescalcarifer</i> | <i>Pristipomoides filamentosus</i> | 99.84 | MK32 8515 | <u>Yes</u> | <u>25.7</u> |
| Fish grill | Hotel | Pearl spot | <i>Etroplus suratensis</i> | <i>Oreochromis mossambicus</i> | 100 | MK32 8526 | <u>Yes</u> | <u>25.0</u> |
| Fish pepper masala | Hotel | White Pomfret | <i>Pampus argenteus</i> | <i>Trachinotus blochii</i> | 100 | MK32 8523 | <u>Yes</u> | <u>26.4</u> |
| Fish Masala | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Coryphaena hippurus</i> | 100 | MK32 8514 | <u>Yes</u> | <u>25.3</u> |
| Fish Curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Scomberomorus commerson</i> | 99.85 | MG23 0668 | No | na |
| Fish grill | Hotel | Red Snapper | <i>Lutjanus sp.</i> | <i>Lutjanus argentimaculatus</i> | 99.84 | MK32 8521 | No | na |
| Fish Curry | Hotel | Carangids | <i>Caranx sp.</i> | <i>Caranx sexfasciatus</i> | 100 | MK32 8518 | No | na |
| Prawns masala | Hotel | Tiger prawn | <i>Penaeus monodon</i> | <i>Heterocarpu s woodmasoni</i> | 100 | MG02 9398 | <u>Yes</u> | <u>28.1</u> |
| Prawns roast | Hotel | Tiger prawn | <i>Penaeus monodon</i> | <i>Acanthephyra sp.</i> | 91.19 | MG02 9402 | <u>Yes</u> | <u>33.9</u> |
| Fish Curry | Hotel | Carangids | <i>Caranx sp.</i> | <i>Epinephelus diacanthus</i> | 100 | MG23 0661 | <u>Yes</u> | <u>23.7</u> |
| Fish Curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Xiphias gladius</i> | 100 | MK29 | <u>Yes</u> | <u>21.8</u> |

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|---------------|-------|-------------|--------------------------------|------------------------------------|-------|--------------|------------|-------------|
| | | | | | | 5657 | | |
| Fish Curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Scomberomorus commerson</i> | 99.85 | MF737 199 | No | na |
| Fish fry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Acanthocybium solandri</i> | 100 | MK30 1241 | <u>Yes</u> | <u>15.3</u> |
| Fish Curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Istiophorous platypterus</i> | 100 | MG23 0666 | <u>Yes</u> | <u>25.6</u> |
| Chilli fish | Hotel | Cobia | <i>Rachycentron canadum</i> | <i>Acanthurus mata</i> | 99.52 | MG23 0664 | <u>Yes</u> | <u>26.5</u> |
| Prawns roast | Hotel | Tiger prawn | <i>Penaeus monodon</i> | <i>Aristeus sp.</i> | 83.60 | MG02 9408 | <u>Yes</u> | <u>26.5</u> |
| Fish fry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Acanthocybium solandri</i> | 100 | MK30 1242 | <u>Yes</u> | <u>15.3</u> |
| Fish masala | Hotel | Pearl spot | <i>Etroplus suratensis</i> | <i>Oreochromis mossambicus</i> | 100 | MK32 8527 | <u>Yes</u> | <u>25.0</u> |
| Prawns masala | Hotel | Tiger prawn | <i>Penaeus monodon</i> | <i>Heterocarpus woodmasoni</i> | 100 | MG02 9399 | <u>Yes</u> | <u>28.1</u> |
| Fish Curry | Hotel | Red Snapper | <i>Lutjanus sp.</i> | <i>Pristipomoides filamentosus</i> | 99.84 | MK32 8516 | <u>Yes</u> | <u>19.6</u> |
| Fish curry | Hotel | Tuna | <i>Thunnus albacares</i> | <i>Thunnus albacares</i> | 99.85 | MK30 1244 | No | na |
| Fish curry | Hotel | Tuna | <i>Thunnus albacares</i> | <i>Thunnus albacares</i> | 99.85 | MK32 8512 | No | na |

| | | | | | | | | |
|------------------|-------|---------------|--------------------------------|------------------------------------|-------|--------------|------------|-------------|
| Fishpepper roast | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Scomberomorus commerson</i> | 99.85 | MF737 200 | No | na |
| Fish curry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Scomberomorus commerson</i> | 99.85 | MF737 201 | No | na |
| Fish fry | Hotel | Black pomfret | <i>Parastromateus niger</i> | <i>Platax teira</i> | 100 | MK30 1231 | <u>Yes</u> | <u>24.4</u> |
| Fish curry | Hotel | Milk fish | <i>Chanos chanos</i> | <i>Chanos chanos</i> | 100 | MK30 1235 | No | na |
| Prawns curry | Hotel | Prawns | <i>Penaeus vannamei</i> | <i>Penaeus vannamei</i> | 100 | MK30 1238 | No | na |
| Prawns fry | Hotel | Prawns | <i>Penaeus vannamei</i> | <i>Penaeus vannamei</i> | 100 | MK30 1239 | No | na |
| Fish fry | Hotel | Sea bass | <i>Lates calcarifer</i> | <i>Pristipomoides filamentosus</i> | 99.84 | MK32 8517 | <u>Yes</u> | <u>25.7</u> |
| Fish fry | Hotel | Black pomfret | <i>Parastromateus niger</i> | <i>Parastromateus niger</i> | 100 | MF737 198 | No | na |
| Fish fry | Hotel | Carangids | <i>Caranx sp.</i> | <i>Caranx sexfasciatus</i> | 100 | MK32 8519 | No | na |
| Fish masala | Hotel | White Pomfret | <i>Pampus argenteus</i> | <i>Trachinotus blochii</i> | 100 | MK32 8524 | <u>Yes</u> | <u>26.4</u> |
| Fish grill | Hotel | White Pomfret | <i>Pampus argenteus</i> | <i>Trachinotus blochii</i> | 100 | MK32 8525 | <u>Yes</u> | <u>26.6</u> |
| Fish curry | Hotel | Red snapper | <i>Lutjanus sp.</i> | <i>Lutjanus argentimaculatus</i> | 99.84 | MK32 8522 | No | na |

| | | | | | | | | |
|---------------|-------|-------------|--------------------------------|---------------------------------|-----|--------------|------------|-------------|
| Fish fry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Istiophorous platypterus</i> | 100 | MK46 5053 | <u>Yes</u> | <u>25.6</u> |
| Chilli prawns | Hotel | Tiger prawn | <i>Penaeus monodon</i> | <i>Heterocarpus woodmasoni</i> | 100 | MG02 9400 | <u>Yes</u> | <u>27.8</u> |
| Fish Masala | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Caranx ignobilis</i> | 100 | MK31 5224 | <u>Yes</u> | <u>24.3</u> |
| Fish fry | Hotel | Seer fish | <i>Scomberomorus commerson</i> | <i>Caranx ignobilis</i> | 100 | MK31 5225 | <u>Yes</u> | <u>24.6</u> |
| Fish curry | Hotel | Carangids | <i>Caranx sp.</i> | <i>Epinephelus diacanthus</i> | 100 | MG23 0662 | <u>Yes</u> | <u>23.7</u> |

Table 1

Identification results based on cytochrome c oxidase I (COI) gene sequences for 62 samples from different sources. Species substitution cases are underlined

In total 24 different species belonging to 23 genera were accounted using COI gene. It could be noted that the highest rate of species substitution was witnessed under the trading name "seer fish" (which is expected to be a *Scomberomorus sp.* specifically *Scomberomorus commerson*). Among the 16 samples (14 from hotels; 1 from fish market; 1 from hypermarket) traded under the name "Seer fish (*Scomberomorus commerson*)", 11 were substituted with *Istiophorous platypterus*, *Caranx ignobilis*, *Acanthocybium solandri*, *Coryphaena hippurus* and *Xiphias gladius*. "Tiger prawns (*Penaeus monodon*)" (5 samples from hotels) represented another commodity which was completely substituted with species like *Heterocarpus woodmasoni*, *Acanthephyra sp.* and *Aristeus sp.* Similar case was observed in "White pomfrets (*Pampus argenteus*)" also were four samples (three from hotels; one from fish market) collected represented species like *Acanthurus mata* and *Trachinotus blochii*. Complete substitution was accounted for Cobia (*Rachycentron canadum*) also as three samples (two from hotel; one from fish market) traded were substituted with *Xiphias gladius*, *Coryphaena hippurus* and *Acanthurus mata*. For Black pomfrets (*Parastromateus niger*), three out of four samples (two from fish market and two from hotel) collected were substituted with *Platax teira* and *Piaractus brachypomus*. Complete substitution was identified for samples (two each) of "Pearl

spot (*Etroplus suratensis*)" and "Sea bass (*Lates calcarifer*)" collected from hotels. *Oreochromis mossambicus* was the substituent used for Pearl spot while Sea bass was substituted with *Pristipomoides filamentosus*. However, substitution rate was low among "Carangids (*Caranx sp.*)" since seven samples (three from fish retail outlet and four from hotels) collected contained only two substitutions (from hotels) using *Epinephelus diacanthus*. *Caranx ignobilis* and *C. sexfasciatus* represented the two major *Caranx* species identified using COI gene. Fish kheema (obtained from a fish retail outlet) of Threadfin bream expected to made of *Nemipterus japonicus* was identified to contain flesh of *Parastromateus niger*. Among the four Red snapper (*Lutjanus sp.*) samples (three from hotels and one from fish market) collected, one was substituted with *Pristipomoides filamentosus* while all the others represented *Lutjanus argentimaculatus*.

Higher number of species substitution was accounted in samples collected from hotels followed by fish markets, fish retail outlets and hypermarkets. However, no substitution was observed for samples collected from supermarkets. Since cooked products lack morphological features for species identification, practise of food fraud could be easily implemented in hotels and restaurants (Wong & Hanner, 2008; Deepak et al., 2019). In addition, mixing of low valued species substituents in processed products of high valued fishes like chunks, fillets, dressed frozen etc. (Sameera, Ramachandran, Deepak, & Harikrishnan, 2019) could be done in fish retail outlets, hyper and supermarkets. Even structurally similar species substituents could be used in whole fish trade (Sameera, Ramachandran, Deepak, & Harikrishnan, 2019; Smriti et al., 2017). A major threat related to such malpractices are the use of unauthorised/restricted/exotic species captured through unregulated fishing practices for intentional species substitution to increase profit for the dealer/seller causing loss to the customer (Helyar et al., 2014; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019). In this study, trade of certain species like *Acanthurus mata*, *Piaractus brachypomus*, *Platax teira*, *Pristipomoides filamentosus*, *Oreochromis mossambicus*, *Heterocarpus woodmasoni*, *Aristeus sp.*, *Acanthephyra sp.* and *Penaeus vannamei* were identified.

Acanthurus mata (Acanthuridae) represents a surgeonfish associated with reefs and corals which is reported to cause ciguatera in Hawaii (Chan, 2016). Even though presence of Acanthuridae members were detected through environmental DNA metabarcoding (CMFRI, 2019), this study provides the first confirmed report regarding the trade of an Acanthuridae member viz. *Acanthurus mata* in fish markets and hotels. *Piaractus brachypomus* or Red bellied Pacu is a well known exotic species traded as a species substituent for *Parastromateus niger* (Sameera, Ramachandran, Deepak, & Harikrishnan, 2019). Longfin batfish or *Platax teira* is another alien member accounted from fish

markets and hotels. It is a marine fish inhabiting tropical and temperate regions (Li et al., 2016; Liu et al., 2019). Usually, *Platax* species are considered for aquarium trade (Bilecenoglu & Kaya, 2006) because of its very long dorsal fins, anal and pelvic fins. Since Liu et al. (2019) estimated the nutritional compositions of this species, it could be considered as a food fish. However, this study has confirmed its use as a substituent for *Parastromateus niger*, which is having higher market value than *P. teira*. Another fish species in concern is *Pristipomoides filamentosus* or Crimson jobfish. Even though it is considered as a food fish (Ray, Mohapatra, Mishra, Yennawar, & Ghorai, 2017), its nutritional aspects are to be evaluated against *Lates calcarifer* and *Lutjanus sp.* for which it has been substituted.

Oreochromis mossambicus or Tilapia is considered within the top 100 invasive alien species on the planet and is listed in Global Invasive Species Database (Roshni, Renjithkumar, & Kurup, 2016; Laxmappa, Nagaraju, & Sharma, 2015). Its use as a species substituent to *Etroplus suratensis* or Pearl spot is confirmed in this study which could be a practise of intentional species substitution. In this study, *Heterocarpus woodmasoni*, *Aristeus sp.* and *AcanthePHYRA sp.* were identified as substituents for the high valued giant tiger prawn, *Penaeus monodon*. Even though congeners of genus *Heterocarpus* like *H. gibbosus* and *H. chani* are considered for trade, they possess only limited market acceptance with regard to giant tiger prawns. In addition, the utility of *H. woodmasoni* as an edible commodity is yet to be revalidated. In case of *Aristeus* and *AcanthePHYRA* members also, there exists confusions regarding the species. One more case identified apart from species substitution is the trade of *Penaeus vannamei*, exotic species introduced within the country for aquaculture purpose. According to previous reports, Indian black tiger shrimp export was threatened by the culture of *P. vannamei*. This has caused severe impact on country's tiger shrimp production causing higher competition with countries such as China and Thailand, the leaders in monodon farming (Regunathan & Kitto, 2011).

Now a days, species substitution, specifically intentional species substitution is commonly practised by dealers for acquiring additional profit. It is a clear case of consumer fraud in which species of lower market acceptance and commercial value are intentionally replaced for high valued fishes and fishery products, causing economic loss (Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019). In addition, vulnerable or overexploited species are also captured and traded in direct condition and as species substituents. These type of unregulated fishing practises are affecting fishing control and management systems to a great extent (Sameera, Ramachandran,

Deepak, & Harikrishnan, 2019). Impact of pathogens from imported species also remains as a serious matter of concern (Regunathan & Kitto, 2011).

4. Conclusion

Now a days, molecular methods are widely applied for identification of traded seafoods (Deepak et al., 2019; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019; Christiansen, Fournier, Hellemans, & Volckaert, 2018). Since DNA based technologies provide speed and precision to food traceability at affordable cost (Deepak et al., 2019; Christiansen, Fournier, Hellemans, & Volckaert, 2018; Bosona & Gebresenbet, 2013), they are widely used in different real-life purposes including fisheries assessment (Deepak et al., 2019). DNA barcoding serves as an efficient technique to rapidly determine the taxonomic group of a given organism (Hebert, Cywinska, Ball, & de Waard, 2003). Mitochondrial cytochrome c oxidase I subunit gene (COI) are useful for specimen identification and species confirmation in many cases, generating molecular sequence data from fresh, degraded, processed or cooked material (Deepak et al., 2019; Deepak & Harikrishnan, 2016; Sameera, Ramachandran, Deepak, & Harikrishnan, 2019). Hitherto, there are numerous reports by Wong & Hanner, 2008; Hanner, Becker, Ivanova, & Steinke, 2011; Filonzi, Chiesa, Vaghi, & Marzano, 2010; Jabado et al., 2015; Lockley and Bardsley, 2000; Gil, 2007; Ardura, Ana, Moreira, & Garcia-Vazquez, 2010; Xiong et al., 2016; Willette et al., 2017; Stern, Nallar, Rathod, & Crandall, 2017; Spencer & Bruno, 2019; Deepak et al., 2019 and Sameera, Ramachandran, Deepak, & Harikrishnan, 2019 regarding the utility of DNA barcoding in seafood authentication. Hence, this study also suggests the mandatory implementation of molecular techniques like DNA barcoding in Indian markets so that malpractices like food fraud in the form of species substitution/misrepresentation and unregulated fishing could be rectified. It is clear that proper traceability aids are the only means through which buyer and seller of fishery resources could be benefitted equally without disturbing the sustainability of fishery resources. The study recommends the implementation of DNA barcoding by government as a systematic regulatory programme to properly monitor the traded fishery stocks within the country.

Declaration of interest

The authors declare responsibility for the entire contents of this paper and have no conflicts of interest and there has been no significant financial support for this work that could have influenced its outcome.

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