*Journal of Sustainability Science and Management*
*Volume 17 Number 11, November 2022: 45-55*
*eISSN: 2672-7226*
*© Penerbit UMT*

# SPECIES CHECKLIST AND DNA BARCODING OF MARINE FISHES FROM PULAU TINGGI, JOHOR, MALAYSIA

MUHAMMAD ASYRAF AZAHAR[1], NUR AMIRA SHAFIQA ROSLI[1], NOR SHAHIDA AB RAHMAN[1], NUR SABRINA BADRUL HISHAM[1,2], SITI NAJIHAH SOLEHIN[1,2], 'AISYAH MOHAMED REHAN[3], MD-ZAIRI ZAINAL[4], MUHAMMAD ABU BAKAR ABDUL LATIFF[1,5,6], SEAH YING GIAT[7] AND KAMARUL RAHIM KAMARUDIN[1,2]*

*[1]Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Pagoh Higher Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia. [2]Centre of Research for Sustainable Uses of Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Pagoh Higher Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia. [3]Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), Pagoh Higher Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia. [4]Shaz Resort Pulau Tinggi Sdn. Bhd., Lot 44, Kampung Penaga, Pulau Tinggi, 86800 Mersing, Johor, Malaysia. [5]Environmental Management and Conservation Research Unit (eNCORe), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (Pagoh Campus), 84600 Muar, Johor, Malaysia. [6]Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. [7]School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. .*

**Corresponding author: kamarulr@uthm.edu.my*
*Submitted final draft: 26 April 2022* *Accepted: 18 May 2022* http://doi.org/10.46754/jssm.2022.11.006

**Abstract:** Since there was a lack of study on the diversity and genetics of marine fishes in Pulau Tinggi, Mersing, Johor could be found to date, this study was conducted to provide a baseline data of species checklist of marine fishes in Pulau Tinggi and to obtain DNA barcodes of the fish specimens using cytochrome c oxidase subunit I (COI) gene. Sample collection in this study was conducted starting from July to August 2019. Spinning rod (angling), hand-net (manual sampling) and gillnet (mid-water column) were used as sampling methods in this study. All specimens obtained in this study were first identified based on morphological features, followed by a molecular approach for unidentified individuals. The morphological and molecular classification proved that all 183 individuals of marine fishes obtained in this study belonged to 83 species, 58 genera, 36 families, 9 orders and 2 classes. Among 83 species obtained in this study, 74 (89%) possess important values for human use. According to IUCN Red List online platform, 22 species are classified as Not Evaluated (NE), one species is classified as Data Deficient (DD), 56 species are classified as Least Concern (LC), three species are classified as Near Threatened (NT) and the remaining one species is classified as vulnerable (VU). A total of seven bidirectional CO1 barcode sequences were obtained from seven species, six genera and six families. All the sequences obtained have significant E values, which were zero, with percent identity values being more than 98% and query coverage values being more than 89%. The blast results for all the sequences provided correct identification until the species level. For future research, this study has updated the latest checklist of marine fishes from Pulau Tinggi, Mersing, Johor, Malaysia.

Keywords: Pulau Tinggi, marine fishes, species checklist, DNA barcoding.

## Introduction

Malaysia, which falls under the Sundaland region, is also part of the South China Sea. Malaysia has been regarded as one of the biodiversity hotspots and listed as one of 17 megadiverse countries worldwide (Myers *et al*., 2000). The marine and coastal areas of Malaysia are thought to have a great diversity of marine life within its coral reefs, mangrove forests, mud flats, sea grass areas and sandy beaches (Mazlan *et al*., 2005). The first reference material listed 710 marine fish species from Malaysian waters and adjacent seas (Mohsin *et al*., 1996). The second edition book estimated 2,243 fish species inhabiting Malaysian waters (Ambak *et al*., 2010).

On the east coast of Peninsular Malaysia, which faces the South China Sea, particularly in Terengganu, about 441 species have been catalogued (Matsunuma *et al*., 2011). Another known marine and coastal area along the east coast of the Malay Peninsula is the East Johor Island Archipelago (EJIA) which is regarded as Taman Laut Sultan Iskandar (TLSI). One of the biggest islands in the TLSI is Pulau Tinggi (2°18'N; 104°07'E), which consists of well-developed coral reef ecosystems that may attract a variety of coral and rocky reef-associated fishes (Reef Check Malaysia, 2019). Even though some studies have been done to observe fish groups occupying coral reef areas in Pulau Tinggi, no documented study has been found on the richness and genetics of marine fishes on this island. This island has also been subjected to a growing tourism industry. Various development and activities on this island may strongly induce pressure directly or indirectly toward its local marine fish, thus, increasing the challenges for conservation efforts.

Many effective conservation actions depend on accurate species identification (Bakar *et al*., 2018). Over the past decade, DNA barcoding has played a facilitatory role in establishing a rapid, accurate and cost-effective system for identifying species including marine fishes (Zhang *et al*., 2011; Bingpeng *et al*., 2011). Arising integration of molecular and traditional taxonomic methods provides a powerful and standardized approach for marine species identification that helps fisheries and conservation programs (Hebert *et al*., 2003; Costa & Carvalho, 2007; Sheraliev & Peng, 2021; Ahmed *et al*., 2021). Accurate identification is important in determining the exact species richness present in an area, thus helping in the future study of species diversity in that area. For example, a previous study bore witness to the utility of DNA barcoding in revealing the overlooked diversity of Malaysian commercial snapper (*Lutjanus*) (Bakar *et al*., 2018). Accordingly, this study aimed to investigate the species checklist of marine fishes in Pulau Tinggi, Johor and to obtain the DNA barcodes of the fish specimens using the cytochrome c oxidase I (COI) mitochondrial DNA (mtDNA) gene.

## Materials and Methods

### *Procedures*

### *Study Sites*

Sample collection in this study was conducted in Pulau Tinggi, Johor (Figure 1), from July to August 2019. Pulau Tinggi (2°18'N; 104°07'E) is located about 30 kilometres southeast of Mersing, Johor on the east coast of Peninsular Malaysia, that faces the South China Sea (Azman *et al*., 2008; Amanda, 2018). Pulau Tinggi covered a total area of about 16 square kilometres and was gazetted as Marine Park in 1994 under the Fisheries Act 1985 (Amended 1993) (Azman *et al*., 2008). The white sandy beaches along the coastline of Pulau Tinggi are dotted with caves (Escobar *et al*., 2003). It is surrounded by clear turquoise sea waters supporting rocky, coral reefs, sea grasses and mangroves (Azman *et al*., 2008; Reef Check Malaysia, 2019). The interior part of the terrestrial ecosystem in Pulau Tinggi is mostly covered with secondary lowland dipterocarp rainforest but there is still part of the primary forest that remains virgin (Escobar *et al*., 2003). Petrographically, this island is dominated by a pyroclastic rock type that varies from millimetres to 5 centimetres in size (Ghani, 2006). Unpublished data for Ar-Ar whole rock dating indicated that the age of the Pulau Tinggi volcanic was approximately 85 million years ago (Roselee, 2014).

### *Marine Fish Sampling*

In this study, spinning rods (angling), small hand-net (manual sampling) and gillnet (mid-water column) were used to catch fish. Application of "catch and release" practice was incorporated in this study since there was the same morphospecies of fish caught during the sampling period. A spinning rod for bottom angling or line angling was used at the available jetty nearby Shaz Resort Pulau Tinggi while a


Figure 1: Pulau Tinggi, Mersing, Johor
Source: Google Earth Pro version 7.3.4.8248

floater was used during the angling along the beach. This method was performed when the tide was changing from low tide to high tide, both during the daytime and nighttime. The flesh of shrimp, squid and fish was used as the bait for this fishing method. Besides that, the fish that were trapped in the tide pools along the intertidal zone in front of Kampung Tanjung Balang were manually collected using a small hand net. This method was performed during low tide, where fish were usually trapped in temporary ponds created when sea water turns to low tide in the daytime and nighttime (White *et al*., 2015). Furthermore, a gill net of 15 m long and 1.2 m width with a mesh size of 1.5 inches was used to catch fish and was set up approximately 25 m from the shore in front of Kampung Tanjung Balang for about 6 hours during the daytime and nighttime sampling, once a week.

The fish collected from all gear types during the field study were sorted and identified based on the morphological features by referring to Matsunuma *et al*. (2011), Moore and Colas (2016), FishBase online platform and other available references prior to the molecular approach for unknown fish individuals.

### *DNA Barcoding*

DNA barcoding was done as a complementary method to morphological identification to confirm the species status of unknown individuals, especially for specimens with a deformed body. A portion of muscle tissue or a fin clip fragment from each fish specimen was dissected or removed with a sterile blade and preserved in 95% ethanol (Bingpeng *et al*., 2018). Total genomic DNA was extracted from each muscle tissue or fin clip sample using the Tissue Genomic DNA Extraction Mini Kit by Favorgen. The quality and quantity of the extracted total genomic DNA were determined through electrophoresis (on 1% agarose gel, with FloroSafe DNA Stain). The exTEN 2x PCR Master Mix (200 reactions) was used for polymerase chain reaction (PCR). Approximately 655 base pairs fragment of the 5′ end region of the protein-coding mitochondrial cytochrome c oxidase I (COI) gene was amplified using the primer set described by Ward *et al*. (2005) and Wang *et al*. (2012). PCR amplification was carried out in a thermal cycler, where the general protocol of the PCR kit (i.e. exTEN 2x PCR Master Mix) was followed, with annealing thermal condition at 54°C (Wang *et al*., 2012).

Amplified COI fragments were sent to the sequencing service provider, i.e., Apical Scientific Sdn. Bhd.) in suspension form to perform the bidirectional (forward and reverse) sequencing further. CO1 sequences were aligned using the Molecular Evolutionary Genetics Analysis software version 7.0 (MEGA7) (Kumar *et al*., 2016). Confirmation of species identity was done using nucleotide Basic Local Alignment Search Tool (BLAST) search that is available at National Center for Biotechnology Information (NCBI) online platform (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The COI sequences were then translated into amino acids and examined for the presence of stop codons prior to the GenBank submission. BankIt, a NCBI program was used to prepare the sequence data for GenBank submission. Each sequence was aligned and compared with corresponding sequences from the GenBank database to determine the reliability of the sequences.

## Results and Discussion

The morphological and molecular classification proved that all 183 individuals of coastal marine fishes recorded in this study represented the native fishes of the South China Sea with 83 species, 58 genera, 36 families and nine orders (Figure 2, Tables 1-2) (Mohsin *et al*., 1996; Randall *et al*., 2000). Among these, three species with nine individuals were classified as class Chondrichthyes while another 174 individuals represented the class Osteichthyes (i.e., the bony fishes), specifically the Teleostei (perfect bone) group.

A number of 24 families (66.7%) with 69 species (83.1%) were classified in order Perciformes, considered the highest taxonomic order in this study. Perciformes possess more than 9,200 species worldwide and it is not just the largest order in fish classification but also the largest order of all vertebrates (Moyle *et al*., 2004). In addition, the published reference material by Mohsin *et al*. (1996) also showed

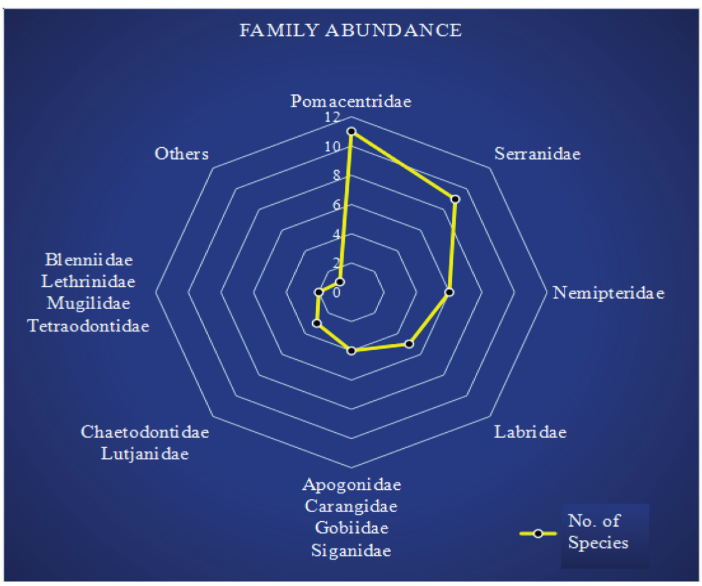


Figure 2: The family abundance of recorded marine fishes in Pulau Tinggi, Mersing, Johor

Table 1: Summary of ray-finned fishes in Pulau Tinggi, Mersing, Johor

| No. | Families | Scientific Name | Common Name | IUCN Status | Total Species |
|---|---|---|---|---|---|
| 1 | Pomacentridae | *Abudefduf bengalensis* | Bengal sergeant | LC | 11 |
| | | *Abudefduf sexfasciatus* | Scissortail sergeant | LC | |
| | | *Abudefduf sordidus* | Blackspot sergeant | LC | |
| | | *Abudefduf vaigiensis* | Indo-Pacific sergeant | LC | |
| | | *Amphiprion ocellaris* | Clown anemonefish | NE | |
| | | *Dischistodus fasciatus* | Banded damsel | NE | |
| | | *Dischistodus perspicillatus* | White damsel | NE | |
| | | *Dischistodus prosopotaenia* | Honey-head damsel | NE | |
| | | *Dischistodus pseudochrysopoecilus* | Monarch damsel | NE | |
| | | *Pomacentrus chrysurus* | Whitetail damsel | NE | |
| | | *Pomacentrus tripunctatus* | Threespot damsel | NE | |
| 2 | Serranidae | *Cephalopholis boenak* | Chocolate hind | LC | 9 |
| | | *Cephalopholis formosa* | Bluelined hind | LC | |
| | | *Epinephelus areolatus* | Areolate grouper | LC | |
| | | *Epinephelus coioides* | Orange-spotted grouper | LC | |
| | | *Epinephelus corallicola* | Coral grouper | LC | |
| | | *Epinephelus erythrurus* | Cloudy grouper | LC | |
| | | *Epinephelus fuscoguttatus* | Brown-marbled grouper | VU | |
| | | *Epinephelus quoyanus* | Longfin grouper | LC | |
| | | *Plectropomus maculatus* | Spotted coralgrouper | LC | |
| 3 | Nemipteridae | *Nemipterus furcosus* | Fork-tailed threadfin bream | LC | 6 |
| | | *Pentapodus bifasciatus* | White-shouldered whiptail | NE | |
| | | *Pentapodus trivittatus* | Three-striped whiptail | LC | |
| | | *Scolopsis ciliata* | Saw-jawed monocle bream | LC | |
| | | *Scolopsis margaritifera* | Pearly monocle bream | LC | |
| | | *Scolopsis monogramma* | Monogrammed monocle bream | LC | |
| 4 | Labridae | *Cheilinus chlorourus* | Floral wrasse | LC | 5 |
| | | *Choerodon anchorago* | Orange-dotted tuskfish | LC | |
| | | *Halichoeres chloropterus* | Pastel-green wrasse | LC | |
| | | *Hemigymnus melapterus* | Blackeye thicklip | LC | |
| | | *Thalassoma lunare* | Moon wrasse | LC | |

| | | | | | |
|---|---|---|---|---|---|
| 5 | Apogonidae | *Apogonichthyoides melas* | Black cardinalfish | NE | 4 |
| | | *Ostorhinchus chrysopomus* | Spotted-gill cardinalfish | NE | |
| | | *Ostorhinchus endekataenia* | Candystripe cardinalfish | NE | |
| | | *Pristicon trimaculatus* | Three-spot cardinalfish | NE | |
| 6 | Carangidae | *Carangoides bajad* | Orange-spotted trevally | LC | 4 |
| | | *Carangoides gymnostethus* | Bludger | LC | |
| | | *Caranx sexfasciatus* | Bigeye trevally | LC | |
| | | *Selar boops* | Oxeye scad | LC | |
| 7 | Gobiidae | *Amblygobius stethophthalmus* | Freckled goby | NE | 4 |
| | | *Gobiodon histrio* | Broad-barred goby | LC | |
| | | *Istigobius ornatus* | Ornate goby | LC | |
| | | *Valenciennea muralis* | Mural goby | NE | |
| 8 | Siganidae | *Siganus fuscescens* | Mottled spinefoot | LC | 4 |
| | | *Sigaus guttatus* | Orange-spotted spinefoot | LC | |
| | | *Siganus punctatus* | Goldspotted spinefoot | LC | |
| | | *Siganus virgatus* | Barhead spinefoot | LC | |
| 9 | Chaetodontidae | *Chaetodon octofasciatus* | Eightband butterflyfish | LC | 3 |
| | | *Chelmon rostratus* | Copperband butterflyfish | LC | |
| | | *Coradion chrysozonus* | Goldengirdled coralfish | LC | |
| 10 | Lutjanidae | *Lutjanus carponotatus* | Spanish flag snapper | NE | 3 |
| | | *Lutjanus fulviflamma* | Dory snapper | LC | |
| | | *Lutjanus russellii* | Russell's snapper | LC | |
| 11 | Blenniidae | *Blenniella bilitonensis* | Biliton blenniella | LC | 2 |
| | | *Salarias fasciaus* | Jewelled blenny | LC | |
| 12 | Lethrinidae | *Lethrinus lentjan* | Pink ear emperor | LC | 2 |
| | | *Lethrinus ornatus* | Ornate emperor | LC | |
| 13 | Mugilidae | *Ellochelon vaigiensis* | Squaretail mullet | LC | 2 |
| | | *Moolgarda seheli* | Bluespot mullet | NE | |
| 14 | Tetraodontidae | *Arothtron immaculatus* | Immaculate puffer | LC | 2 |
| | | *Arothtron mappa* | Map puffer | LC | |
| 15 | Caesionidae | *Caesio cuning* | Redbelly yellowtail fusilier | LC | 1 |
| 16 | Centrogenyidae | *Centrogenys vaigiensis* | False scorpionfish | NE | 1 |
| 17 | Diodontidae | *Diodon liturosus* | Black-blotched porcupinefish | NE | 1 |
| 18 | Echeneidae | *Echeneis naucrates* | Live sharksucker | LC | 1 |
| 19 | Gerreidae | *Gerres oyene* | Common silver-biddy | LC | 1 |
| 20 | Holocentridae | *Sargocentron rubrum* | Redcoat | LC | 1 |
| 21 | Kyphosidae | *Kyphosus cinerascens* | Blue sea chub | LC | 1 |
| 22 | Monacanthiae | *Acreichthys tomentosus* | Bristle-tail file-fish | LC | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 23 | Mullidae | *Upeneus tragula* | Freckled goatfish | LC | 1 |
| 24 | Paralichthyidae | *Pseudorhombus arsius* | Largetooth flounder | NE | 1 |
| 25 | Pegasidae | *Pegasus volitans* | Longtail seamoth | DD | 1 |
| 26 | Pempheridae | *Pempheris oualensis* | Blackspot sweeper | NE | 1 |
| 27 | Pomacanthidae | *Pomacanthus annularis* | Bluering angelfish | LC | 1 |
| 28 | Pseudochromidae | *Congrogadus subducens* | Carpet eel-blenny | NE | 1 |
| 29 | Scaridae | *Scarus ghobban* | Blue-barred parrotfish | LC | 1 |
| 30 | Sillaginidae | *Sillago aeolus* | Oriental sillago | NE | 1 |
| 31 | Soleidae | *Pardachirus pavoninus* | Peacock sole | LC | 1 |
| 32 | Synanceiidae | *Synanceia horrida* | Estuarine stonefish | LC | 1 |
| 33 | Terapontidae | *Terapon jarbua* | Jarbua terapon | LC | 1 |

Table 2: Summary of cartilaginous fishes in Pulau Tinggi, Mersing, Johor

| No. | Families | Scientific Name | Common Name | IUCN Status | Total |
|---|---|---|---|---|---|
| 1 | Dasyatidae | *Taeniura lymma* | Ribbontail stingray | NT | 1 |
| 2 | Carcharhinidae | *Carcharhinus melanopterus* | Blacktip reef shark | NT | 1 |
| 3 | Scyliorhinidae | *Atelomycterus marmoratus* | Coral catshark | NT | 1 |

Note: NE=Not Evaluated, DD=Data Deficient, LC=Least Concern, NT=Near Threatened, VU=Vulnerable

that the Perciformes were recorded as the highest order in their study, with 449 species classified into 60 families found in Malaysia and its neighbouring countries.

Regarding family abundance, the highest number of species in this study belonged to the family Pomacentridae (Damselfishes and Anemonefishes), with 11 species recorded throughout the sampling session. This was followed by the family Serranidae (Groupers) with nine species. According to Moyle *et al*. (2004), nearly 57% of all Perciformes species are dominated by just eight families, including Pomacentridae and Serranidae. In addition, Pomacentridae and Serranidae species are considered prolific fishes that can be found associated with tropical and temperate reefs as well as inshore environments (Moyle *et al*., 2004; Matsunuma *et al*., 2011). Besides that, Nemipteridae (Threadfin Breams and Monocle Bream) was considered the third largest family caught in this study with six species total. This was followed by five species from the family Labridae (Wrasses). Families that shared the same number of species were the Apogonidae (Cardinalfishes), Carangidae (Jacks), Gobiidae (Gobies) and Siganidae (Rabbitfishes) with four species each while Chaetodontidae (Butterflyfishes) and Lutjanidae (Snappers) shared three species in total for each family. Furthermore, four families possessed two species each, which were Blenniidae (Blennies), Lethrinidae (Emperors), Mugilidae (Mullets) and Tetraodontidae (Puffers). Another 22 families including the cartilaginous fishes had the least species recorded with each family showing only one species found throughout the sampling period.

Accordingly, FishBase online platform was used to assess the importance of marine fishes recorded in this study to humans. It shows that 74 species (89%) recorded in this study possess values important for human uses, either as wild fisheries products, commercial aquaculture, commercial ornamental fish, game fish or traditional medicine purpose or overlap between these categories. Among these, 63 species were recognized as wild fisheries products and classified into three ascending groups based on their importance, which were subsistence fishes (nine species), minor commercial fishes (25 species) and major commercial fishes (29 species). Besides that, nine fish in this study are also being recognized as aquaculture species. Furthermore, 37 fish recorded in this study are used as ornamental fish that act as decoration for public or private aquariums. Moreover, ten species were classified as game fish based on the FishBase online platform classification. Among all the fish species obtained in this study, only one species, *Pegasus volitans* (Longtail seamoth) was recognized as being used in Traditional Chinese Medicine (TCM) in dried form (Osterhage *et al*., 2016).

In addition, the conservation status of the fish species recorded in this study was assessed using The International Union for Conservation of Nature (IUCN) Red List online platform (https://www.iucnredlist.org/). A number of 22 species are classified as Not Evaluated (NE), one species is classified as Data Deficient (DD), 56 species are classified as Least Concern (LC), three species are classified as Near Threatened (NT) and the remaining one species is classified as vulnerable (VU). The single species categorized as DD is *P. volitans*, a rare species found on coastal marine and the species inhabits bays and estuaries with sandy, muddy, grassy bottoms. Three species classified as NT in this study are all from the cartilaginous fish group known as *Taeniura lymma* (Ribbontail stingray), *Carcharhinus melanopterus* (Blacktip reef shark) and *Atelomycterus marmoratus* (Coral catshark). *Epinephelus fuscoguttatus* (Brown-marbled grouper) is the only species recorded in this study with VU status. This species is relatively uncommon and typically forms transient spawning aggregations during a few months of the year.

Among the 83 species of marine fishes recorded in this study, 76 species (92.6%) were successfully identified based on the morphological features by referring to Matsunuma *et al*. (2011), Moore and Colas (2016), FishBase online platform, and others related references during the field study. However, another seven species (8.4%) were hard to be identified morphologically due to the limited knowledge of fish morphological taxonomy. Therefore, the remaining seven species were identified through DNA barcoding of COI.

A total of seven bidirectional COI barcode sequences were obtained from seven species, six genera and six families. The length of the sequences ranges from 669 to 714 base pairs. Table 3 shows the BLAST results of the COI barcode sequences obtained in this study containing the local extreme metrics (E value, Percent Identity and Maximum Score), total metrics (Total Score and Query Coverage), length of base pair, accession number of the corresponding sequence and also the species identity. All the sequences showed significant E values, which were zero. In terms of percent identity, the mean of similarity of the query sequences to the target sequences can be considered high with 99.59% (ranging from 98.47% to 100%) while the mean of query sequences covered by the target sequences was 94.57% by average (ranging from 89% to 100%). The nucleotide BLAST results provided correct identification until the species level. A morphological confirmation followed a nucleotide BLAST search that provided the species names.

## Conclusion

This study concluded that both objectives were successfully achieved based on the updated checklist of marine fish species inhabiting the inshore area of Pulau Tinggi, Mersing, Johor and the generation of DNA barcodes of the unknown fish specimens obtained using the cytochrome c oxidase I mitochondrial DNA gene. This study also suggested that DNA barcoding can close the gaps in morphologically-based identification systems. Along with the information from DNA

Table 3: Nucleotide Basic Local Alignment Search Tool (BLAST) results

| Code | Base Pairs | Max Score | Total Score | Query Coverage (%) | E Value | Percent Identity (%) | Accession No. of Corresponding Sequence | Species Identity |
|---|---|---|---|---|---|---|---|---|
| 006A | 708 | 1166 | 1166 | 92% | 0.0 | 98.93% | HQ564373.1 | *Pomacentrus tripunctatus* |
| 018A | 694 | 1277 | 1277 | 100% | 0.0 | 99.86% | KJ202140.1 | *Caranx sexfasciatus* |
| 037A | 714 | 1199 | 1199 | 91% | 0.0 | 99.85% | JN313083.1 | *Centrogenys vaigiensis* |
| 090A | 702 | 1166 | 1166 | 89% | 0.0 | 100% | KY849534.1 | *Moolgarda seheli* |
| 092A | 681 | 1149 | 1149 | 95% | 0.0 | 98.47% | KP194814.1 | *Pomacentrus chrysurus* |
| 096A | 681 | 1205 | 1205 | 95% | 0.0 | 100% | KJ937293.1 | *Pempheris oualensis* |
| 104A | 669 | 1236 | 1236 | 100% | 0.0 | 100% | AB890096.1 | *Pristicon trimaculatus* |

barcoding as the complement, an integrative taxonomic approach can be raised to identify marine fishes. Thus, the integration is beneficial in fisheries management and conservation of marine fish in the coastal region of Pulau Tinggi. This study also shows the importance of marine fish species that inhabit an inshore area of Pulau Tinggi to humans. The area can be vulnerable to the increasing trend of mass tourism and related activities such as establishing resorts, facilities and various water sports could cause acute and chronic effects on the coastal area in Pulau Tinggi if proper management and conservation are not in place. Therefore, the area needs a good management plan so that the marine fish resources will not be negatively affected and will be continuously available to support the livelihood of Pulau Tinggi local people.

## Acknowledgements

This project is funded by the Ministry of Higher Education Malaysia (MOHE) under the Malaysian Technical University Network (MTUN) grant scheme Vote K121 and Industrial Grant by Shaz Resort Sdn. Bhd. (UTHM-SHAZ-M004), both grants awarded to Associate Professor Ts. Dr. Muhammad Abdul Latiff Abu Bakar (UTHM) as the Principal Researcher. This research was supported by GPPS-UTHM-2018-H416 postgraduate grant by Universiti Tun Hussein Onn Malaysia, Malaysia. The authors acknowledge MOHE, Universiti Tun Hussein Onn Malaysia and Shaz Resort for providing the necessary funding, facilities and assistance.

## References

Ahmed, M. S., Datta, S. K., Saha, T., & Hossain, Z. (2021). Molecular characterization of marine and coastal fishes of Bangladesh through DNA barcodes. *Ecology and Evolution*, *11*(9), 3696-3709. https://10.1002/ece3.7355. PMID: 33976769; PMCID: PMC8093680.

Amanda, K. (2018). *Pulau Tinggi: The Most Peaceful & Relaxing Island in Johor. Taxi Singapore to Johor Bahru (JB) Malaysia*.

SGMYTAXI.com. https://www.sgmytaxi.com/pulau-tinggi/

Ambak, M. A., Isa, M. M., Zakaria, M. Z., & Ghaffar, M. A. (2010). *Fishes of Malaysia*. Universiti Malaysia Terengganu, Kuala Terengganu, xi, pp. 334.

Azman, B. A. R., Ramlan, O., Wan-Lotfi, W. M., Zaidi, C. C., & Othman, B. H. R. (2008). Seagrass biodiversity of Pulau Tinggi, Johor. In Mohamed C. A. R, Sahrani F. K., Ali M. M, Cob Z. C, & Ahmad N. (Eds). *Malaysia marine ecosystem: The studies of Johor Darul Takzim east coast. Volume 2 Research and Information Series of Malaysian Coasts.* Bangi, Selangor: Marine Ecosystem Research Centre (EKOMAR), Universiti Kebangsaan Malaysia. 53-57.

Bakar, A. A., Adamson, E. A., Juliana, L. H., Mohd, S. A. N., Wei-Jen, C., Man, A., & Md, D. N. (2018). DNA barcoding of Malaysian commercial snapper reveals an unrecognized species of the yellow-lined Lutjanus (Pisces: Lutjanidae). *PloS One*, *13*(9), e0202945. https://doi.org/10.1371/journal.pone.0202945

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., & Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. *PLOS ONE*, *13*(6), e0198109 https://doi.org/10.1371/journal.pone.0198109

Costa, F. O., & Carvalho, G. R. (2007). The barcode of life initiative: Synopsis and prospective societal impacts of DNA barcoding of fish. *Genomics, Society and Policy. 3*(2), 29.

Escobar, R. A., Grismer, J. L., Youmans, T. M., Wood, P. L., Kendall, S. D., Castro, J., Magi, T., Rasmussen, C., Szutz, T. R, & Hover, S. M. (2003). First report of the herpetofauna of Pulau Tinggi, Johor, West Malaysia. *Hamadryad*, *27*, 259-262.

Ghani, A. A. (2006). Batuan volkanik dari Pulau Tinggi dan Pulau Sibu Johor. Geological Society of Malaysia Bulletin, 52, 63-66.

Hebert, P. D., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321.

Kumar, S., Stecher, G., & Tamura, K., (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution. 33*(7), 1870-1874.

Matsunuma, M., Motomura, H., Matsuura, K., Shazili, N. A. M., & Ambak, M. A. (2011). *Fishes of Terengganu - East Coast of Malay Peninsula, Malaysia*. Malaysia: National Museum of Nature and Science, Universiti Malaysia Terengganu, and Kagoshima University Museum. (pp. 251).

Mazlan, A. G., Zaidi, C. C., Wan-Lotfi, W. M., & Othman, B. H. R. (2005). On the current status of coastal marine biodiversity in Malaysia. *Indian Journal of Marine Sciences, 34*(1), 76-87.

Mohsin, A. K. M., & Ambak, M. A. (1996). *Marine fishes and fisheries of Malaysia and neighbouring countries*. Serdang, Malaysia: University Pertanian Malaysia Press. 320-327.

Moore, B., & Colas, B. (2016). *Identification guide to the common coastal food fishes of the Pacific Islands region*. FAO. https://agris.fao.org/agris-search/search.do?recordID=XF2017001682

Moyle, P. B., & Cech, J. J. (2004). *Fishes: An introduction to ichthyology*. (5th ed.). San Francisco, CA: Prentice-Hall.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature. 403*(6772), 853.

Osterhage, D., Pogonoski, J. J., Appleyard, S. A., & White, W. T. (2016). Integrated taxonomy reveals hidden diversity in northern Australian fishes: A new species of seamoth (genus Pegasus). *PLOS ONE, 11*(3), e014941.

Randall, J. E., & Lim, K. K. P. (2000). A checklist of the fishes of the South China Sea. *The Raffles Bulletin of Zoology*, *8*, 569-667.

Reef Check Malaysia (2019). *RCM Annual Survey Report 2018* | Reef Check Malaysia. [online]. Retrieved April on 20, 2019, from: https://www.reefcheck.org.my/reports-downloads/annual-survey-reports/251-rcm-an nual-survey-report-2018

Roselee, M. H. (2014). *Petrology and geochemistry of the Teluk Ramunia volcanics, Southeastern Johor, Peninsular Malaysia; Implication for middle triassic tectonic*. [Doctoral dissertation, University of Malaya]. http://studentsrepo.um.edu.my/4800/1/Hatta_M.SC._(2014).pdf

Sheraliev, B., & Peng, Z. (2021). Molecular diversity of Uzbekistan's fishes assessed with DNA barcoding. *Scientific Reports, 11*, 16894. https://doi.org/10.1038/s41598-021-96487-1

Wang, Z. D., Guo, Y. S., Liu, X. M., Fan, Y. B., & Liu, C. W. (2012). DNA barcoding South China Sea fishes. *Mitochondrial DNA, 23*(5), 405-410.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences, 360*(1462), 1847-1857.

White, G. E., Hose, G. C., & Brown, C. (2015). Influence of rock-pool characteristics on the distribution and abundance of inter-tidal fishes. *Marine Ecology, 36*(4), 1332-1344.

Zhang, J., & Hanner, R. (2012). Molecular approach to the identification of fish in the South China Sea. *PLOS ONE, 7*(2), e30621. Retrieved on April 18, 2019, from: https://doi.org/10.1371/journal.pone.0030621