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DNA barcoding of commercially important marine fish species *Hipposcarus harid* from the central Red Sea, Saudi Arabia

by

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Abstract

In this study, the collected fish species were identified based on morphological observations and then evaluated by DNA barcoding. The COI gene has been recognized as a biological marker for fish species identification and the objective of this study is to analyze the variable region of the COI gene in subunit I. The mitochondrial cytochrome (COI) oxidase subunit I gene was analyzed as a suitable molecular maker for the identification of three specimens of the fish species *Hipposcarus harid*, widely distributed in the Red Sea. The COI gene sequences in the variable region revealed variations among the fish species. The COI gene sequences in the variable region were similar to the variable region of *Hipposcarus harid* collected from the Northern Red Sea and all three were named: *H. harid* H13, *H. harid* H2c, and *H. harid* H12. The identification of the fish species collected from the Red Sea in Saudi Arabia would help ichthyologists improve the management, conservation and monitoring of economically important long-nose parrotfish species in Saudi Arabia.

Key words: mitochondrial DNA, COI genes, marine fish, coral reef fish, fish population

1. Introduction

Coral reefs are one of the diverse marine habitats and are generally recognized as biodiversity hotspots throughout the world (Hubert et al. 2012). Among aquatic organisms, fish are the most important and fascinating organisms associated with coral reefs. However, due to a number of factors, including overfishing, habitat damage, and pollution, several coral reef fish species have become seriously endangered, as reported by Friedlander et al. (2018). A consistent assessment of coral reef fish biodiversity is essential for monitoring ecosystem habitats and developing conservation strategies (Dawson et al. 2011; Kar et al. 2022). Coral reef fishes are dominated by approximately 30 families, mostly from gobioids, chaetodontoids, acanthuroids, and perciform labroids. Many families differ ontogenetically, sexually or in terms of general phenotypic characters, making it difficult to identify organisms based on morphological characteristics (Radulovici et al. 2010; Shan et al. 2021). However, DNA barcoding is a molecular biology method that characterizes an organism based on the sequence of the mitochondrial cytochrome c oxidase I gene (COI gene; Hebert et al. 2003; Weigt et al. 2012). DNA taxonomy or DNA barcoding refers to the application of DNA sequence analysis to characterize fish species from other groups based on the COI gene sequence (Bektas et al. 2022). This method of analysis is simple, fast, and reliable for species identification and uses a short DNA fragment (Hollingsworth et al. 2011; Chakraborty et al. 2014; Antil et al., 2023). It is useful to characterize unknown species by analyzing them with a library of reference sequences, and the sequences show similarity within species and vary between species (Lakra et al. 2011). This molecular level identification is useful for identifying endangered species and helps protect these fish species (Valentini et al. 2009). Mitochondrial cytochrome c oxidase I gene sequences are molecular tools for identifying fish species based on sequences in the 5′ regions of the COI mitochondrial gene (Tavares & Baker2008; Schindel et al. 2011; Cermakova et al. 2023). *Hipposcarus harid* (Forsskål, 1775) is one of the species of parrotfish. Most species of fish from the genus *Hipposcarus* are found in benthic habitats. The presence of parrotfish has had a major impact on the structure of the benthic community and they are considered the main organisms causing bioerosion (Alwany et al. 2009). Parrotfish are harvested using pot fishing techniques and the gillnet method. They are the main fish stocks in small-scale fisheries on coral reefs. The population of parrotfish has an impact on the regeneration and erosion of coral reefs (Lokrantz et al. 2008; Charendoff et al. 2023). Parrotfish play a significant role in coral reef fisheries in Saudi Arabia and account for a major share in the annual catch. Between 2008 and 2016, an average annual catch of 364 tons of parrotfish was registered off the Red Sea coast of Saudi Arabia (FAO 2018). *H. harid* is one of the commercial parrotfish species and contributes a major share of the wild catch in Saudi Arabia. Based on alternating light and dark bands in hard structures, previous research has provided information on the growth rate of fish species (van Rooij et al. 1995; Shellem et al. 2023). In this study, we investigate the species *Hipposcarus harid* from the Red Sea in Saudi Arabia using molecular tools to provide insights into the availability of *H. harid* in this region.

2. Materials and methods

2.1. Sample collection

Fish were collected between March 2022 and June 2022 in the central Red Sea region (about 60 km south of Jeddah) of Saudi Arabia (21°27′22.5″N; 39°07′15.9″E), using mostly gill and cast nets. The collected fish were transported to the laboratory. Morphologically distinct species of the genus *Hipposcarus* were characterized based on taxonomic guides, labeled and photographed. Approximately 2 g tissue samples were excised from the fish and stored in ethanol (95%) at –80°C for further studies. All voucher specimens were deposited at King Abdulaziz University, Jeddah, Saudi Arabia.

2.2. DNA extraction and amplification

Total genomic DNA of the muscle sample was extracted using the DNeasy blood and tissue kit (Cat. No./ID: 69504, Qiagen, the Netherlands). Fragments of DNA specific to the mitochondrial CO1 gene were amplified using universal DNA primers: COI-Fish-F (*5′-TTCTCAACTAACCAYAAAGAYATYGG-3′*) and COI-Fish-R (*5′-TAGA CTTCT GGGTGGCCRAARAAYCA-3′;* Folmer et al. 1994). About 655 bp were amplified from the 5′ region of the cox1 gene from the mitochondrial DNA of the fish sample (Ward et al. 2005). PCR amplification was performed using 25 µl of a reaction mixture consisting of Taq polymerase (1.25 U), enzyme assay buffer (10 mM Tris-HCl, 0.1% Triton X-100, 50 mM KCl, 5 mM $MgCl_2$), 1 µM reverse and forward primers, dNTPs (0.2 mM) and purified DNA (50 ng). The PCR reaction was performed using a Thermocycler machine (MJ research PTC-100, MJ Research Inc., USA). The PCR conditions were as follows: 3 min of initial denaturation at 95°C,

30 cycles of denaturation for 45 s at 94°C, 1 min of annealing at 55°C, 45 s at 72°C for initial extension, and final extension at 71°C for 10 min.

2.3. CO1 gene sequencing and analysis

The amplified gene products were separated using a submarine horizontal electrophoresis system on 1.5% (w/v) agarose in Tris-acetate buffer with ethidium bromide (Trontelj et al. 2005). They were purified using the QIAquick PCR purification kit as instructed by the manufacturer (Qiagen, USA). The purified product was sequenced using the ABI Prism 310 genetic analyzer according to the manufacturer's instruction using the BigDye™ terminator cycle sequencing method.

2.4. Phylogenetic relationship analysis

The COI sequences were aligned pairwise using NCBI-BLASTN (version 2.2.5) and the PSI BLAST search tool (Altschul et al. 1997). Multiple sequence alignments were performed and variable site extraction of fish COI gene sequences obtained from Red Sea fish samples and the GenBank database were analyzed using MUSCLE 3.6 software (Thompson et al. 1994; Edgar2004). The COI gene sequences were deposited in the NCBI database and accession numbers were assigned. Phylogenetic trees were prepared using the neighbor-joining method. Bootstrap analysis was performed with 1000 replicates to analyze phylogenetic relationships. MEGA 6.0 software was used to analyze genetic distance, and identification was mainly based on homologous gene sequences obtained from the GenBank database.

3. Results and discussion

3.1. Morphological analysis of *H. harid*

The collected fish were morphologically identified and, the morphological appearance of *H. harid* (Fig. 1) was similar to other closely related groups. The body was flattened, oval, and elongated. The rostrum was very large and terminated in a short snout. On the dorsal side, the structure of the rostrum was concave. The maximum size of the rostrum was approximately 75 cm. The caudal fin was very prominent and slightly protruded from the body. The color of the fish was brownish-yellow with lighter edges. The edges of the scales were pink, and the eyes were orange-yellow. Moreover, the specific morphology may vary depending on environmental conditions. Environment is one of the important factors affecting morphological differences between populations. The color of fish varies depending on the environmental background (Macali et al. 2020).

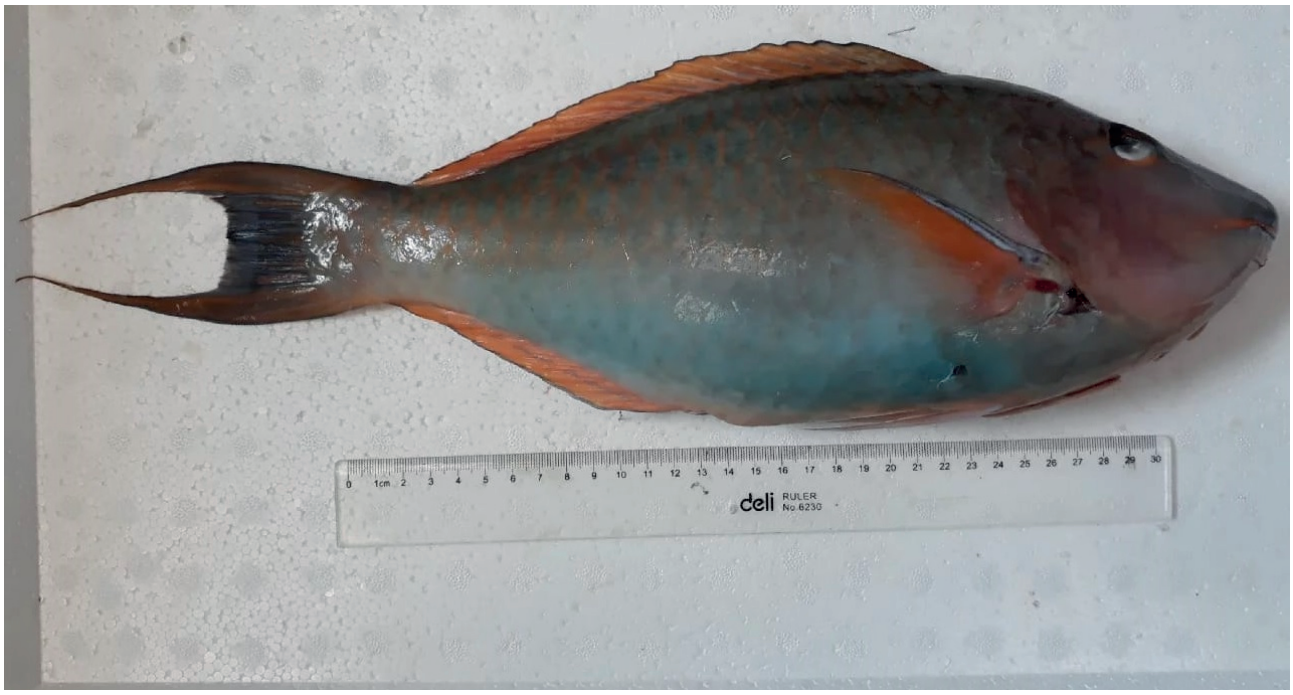

Figure 1

H. harid sample collected from the central Red Sea for this study.

3.2. COI sequence analysis

The COI gene sequences of the three samples were denoted as H2C, H12 and H13 for the analysis. The haplotype (H12) was obtained from three specimens of commercially important parrotfish from Saudi Arabian waters of the Red Sea. The sequence of sample H12 (haplotype) was submitted to NCBI GenBank (accession No. OQ674440). DNA barcoding has been found to be an effective method for distinguishing between marine fish species inhibiting different geographic locations. The COI gene sequences were edited and the final length of the 636 bp sequence was read. No mitochondrial pseudogenes were observed in this study, and no deletions, insertions or stop codons were found in the analyzed COI region. These pseudogenes may cause misidentification of organisms, because they were co-amplified in DNA barcoding in certain organisms. The COI sequences showed no differences between the three samples collected in the Red Sea. Characterizing organisms based on morphological properties using conventional methods is highly laborious, almost impossible to accomplish or may cause ambiguity. Thus, molecular characterization based on COI sequencing has proven to be a powerful classification tool to better identify economically important fish species. DNA barcoding is an accurate, fast, and reliable tool (Lakra et al. 2011).

The mitochondrial COI gene shows an increase level of conserved genes within genera and species, and some levels of genetic variation between different genera and species. The mitochondrial COI gene analysis was used for the identification of Mediterranean fishes (Karahan et al. 2017), Taiwan ray-finned fishes (Chang et al. 2017), Indian freshwater fishes (Chakraborty & Ghosh 2014), and Japanese marine fishes (Zhang & Hanner 2011). The selected primers applied in the present work proved effective in amplifying the target region without any insertions or deletions, revealing that DNA barcoding can be used as a gold standard method for characterizing organisms. The length of the mitochondrial gene sequence was about 636 bp. The sequences were free of any stop codons and did not contain any nuclear mitochondrial pseudogenes. In fish, the available number of COI genes is higher than the number of available pseudogenes. The conserved primers amplify the mitochondrial DNA more than nuclear mitochondrial pseudogenes, and no nuclear mitochondrial pseudogenes were reported to be available in fish. The nucleotide pair frequency analysis revealed more than 300 variable and conserved sites. The level of variation determined in the mitochondrial DNA can lead to demographic variations in fish species. The variation of COI bases revealed increased GC content compared to AT content. The variation in GC and AT content in the results of this study was similar to that of base pairs in fish species reported from Cuba (Lara et al. 2010), Canada (Hubert et al. 2008), and Australia (Ward et al. 2005). In this study, the illustration of C at the first codon position was the lowest compared to other "T" and "A" bases. In fish, base mutations altered codon sequences in mitochondrial genes. Species characterization of fish using DNA barcoding is based on both intraspecific and interspecific divergence.

DNA barcode analysis is used to identify boundaries to delineate species, which is related to divergence between closely related organisms within subspecies populations (Hebert et al. 2003; Chakraborty & Ghosh 2014). Moreover, there is still no unique method suggested for analyzing interspecies variation. Recently, the variation between maximum conspecific divergence and minimum congeneric divergence has been applied to identify the barcoding gap. This difference was highly useful for determining the average intra- and interspecific variations in the COI genes (Meier et al. 2008; Bhattacharjee et al. 2012). The amount of mitochondrial DNA variation determined in this study can lead to demographic differences in fish populations in Saudi Arabian waters. The present finding reveals that DNA barcoding is useful in identifying fish species. DNA barcoding-guided identification of fish species is useful for analyzing fish populations, diversity, management, conservation and monitoring of fish species (Takahara et al. 2013).

3.3. Identification of fish species using COI sequences

Molecular identification of fish species based on COI sequences depends on interspecific heterogeneity and intraspecific homogeneity of organisms under study. DNA barcoding is a useful method for identifying fish species and closely related organisms that share common DNA sequences. In this study, the amplified sequences were 626 bp long without stop codons, insertions and deletions, revealing they represented functional sequences. This revealed that nuclear DNA sequences were not amplified in our study, as in previous works (Zhang & Hewitt 1996). Multiple sequence analysis data revealed homologous sequences between the collected fish species and *H. harid* reported from Saudi Arabian waters of the Red Sea (Tables 1 and 2). The sequenced COI genes were analyzed with 15 COI gene sequences of closely related fish species. These include *H. harid* (MN560922),

Table 1

COI sequence of 1–326 bp of COI gene sequences at high polymorphic position. The sequenced COI sequences of fish samples collected from the Red Sea in Saudi Arabia were homologous and 100% similar to *H. harid*.

Accession No.	Variable site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Base number	59	65	77	89	92	98	146	164	188	200	206	230	233	278	281	282	284	305	317	320	326
MN560922		c	c	a	g	c	c	g	c	t	c	a	g	c	c	t	t	a	t	a	a	t
H13		c	c	a	g	c	c	g	c	t	c	a	g	c	c	t	t	a	t	a	a	t
H2c																						
H12																						

Table 2

COI sequence of 332–626 bp of COI gene sequences at high polymorphic position. The sequenced COI sequences of fish samples collected from the Red Sea in Saudi Arabia were homologous and 100% similar to *H. harid*.

Access. No.	Variable site	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
	Base number	332	335	341	344	353	365	368	380	401	428	461	497	512	515	542	548	575	582	584	605	614	626
MN560922		g	c	t	t	c	a	c	c	c	c	c	t	g	c	t	g	g	c	c	a	c	c
H13		g	c	t	t	c	a	c	c	c	c	c	t	g	c	t	g	g	c	c	a	c	c
H2c																							
H12																							

two species of the Red Sea coral cod, four specimens of *Plectropomus laevis*, three species of the leopard coral grouper, two species of the squaretail coral grouper, and three specimens of *Lethrinus mahsena* (JF493752, JF493750, and MF409569; Tables 3 and 4). None of the COI gene sequences in the variable region were similar except for *H. harid* (Tables 3 and 4). The results show the total number of variable sites for the Red Sea coral cod, *Plectropomus laevis*, the leopard coral grouper, and the Squaretail coral grouper. This method is useful for further studies of fish species and preparing DNA barcode databases. DNA barcoding can differentiate specific fish species and help identify fish eggs, carcass fragments and larvae. The DNA barcoding strategy has proven to be a novel method for characterizing fish species and specimens that are incomplete, damaged, or with various morphological changes (Fišer Pečnikar & Buzan 2014; Bingpeng et al. 2018). DNA barcoding is a highly complicated method that is useful for preparing a reference library of marine fishes. In the Red Sea, parrotfish are one of the major fishery resources for fisheries and are caught by pot fishing gear and gillnets. The declining parrotfish population due to overfishing can affect the dynamics of coral

Table 3

Analysis of COI gene sequences of fish species with other closely related fish species based on COI sequences (1–320 bp). Accession number MN560922 represents *H. harid* and these sequences are similar to the identified fish species.

Variable site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Base number	59	65	77	89	92	98	146	164	188	200	206	230	233	278	281	282	284	305	317	320
MN560922	c	c	a	g	c	c	g	c	t	c	a	g	c	c	t	t	a	t	a	a
Red Sea coral cod	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Red Sea coral cod	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Plectropomus laevis	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Plectropomus laevis	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Plectropomus laevis	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Plectropomus laevis	t	t	g	t	t	a	a	a	c	t	t	a	a	t	c	c	t	c	t	c
Leopard coral grouper	a	t	t	t	t	a	a	a	c	t	t	a	a	t	c	c	c	c	t	t
Leopard coral grouper	a	t	t	t	t	a	a	a	c	t	t	a	a	t	c	c	c	c	t	t
Leopard coral grouper	a	t	t	t	t	a	a	a	c	t	t	a	a	t	c	c	c	c	t	t
Squaretail coral grouper	t	t	g	t	a	a	a	a	c	a	t	a	a	t	c	c	c	c	t	t
Squaretail coral grouper	t	t	g	t	a	a	a	a	c	a	t	a	a	t	c	c	c	c	t	t
JF493752	a	t	t	a	a	g	a	a	c	t	c	a	a	g	c	c	c	c	g	t
JF493750	a	t	t	a	a	g	a	a	c	t	c	a	a	g	c	c	c	c	g	t
MF409569	a	t	t	a	a	g	a	a	c	t	c	a	a	g	c	c	c	c	g	t

Table 4

Analysis of COI gene sequences of fish species with other closely related fish species based on COI sequences (332–626 bp). Accession number MN560922 represents *H. harid* and these sequences are similar to the identified fish species.

Variable site	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Base number	326	332	335	341	344	353	365	368	380	401	428	461	497	512	515	542	548	575	582	584	605	614	626
MN560922	t	g	c	t	t	c	a	c	c	c	c	c	t	g	c	t	g	g	c	c	a	c	c
Red Sea coral cod	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Red Sea coral cod	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Plectropomus laevis	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Plectropomus laevis	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Plectropomus laevis	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Plectropomus laevis	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	t	t	a	t	t	g
Leopard coral grouper	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	a	t	a	t	t	a
Leopard coral grouper	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	a	t	a	t	t	a
Leopard coral grouper	a	a	a	c	g	a	c	t	t	a	t	t	c	a	a	c	a	a	t	a	t	t	a
Squaretail coral grouper	a	a	a	c	c	a	c	t	t	a	t	t	c	a	a	c	a	a	t	a	t	t	g
Squaretail coral grouper	a	a	a	c	c	a	c	t	t	a	t	t	c	a	a	c	a	a	t	a	t	t	g

reefs. This molecular method is useful for parrot fish stock assessment in marine ecosystems.

3.4. Genetic divergence analysis

A phylogenetic tree was constructed using COI sequences of 13 organisms (Fig. 2) belonging to two species from the genus *Hipposcarus* (*H. longiceps* and *H. harid*). The present finding showed that *H. harid* H12 sequences were clustered using *H. longiceps* (MN870054.1, OK347164.1, MW034073.1 and MW034072.1), *H. harid* (MN560922.1, MT888968.1, MT888969.1), *Scarus ghobban* (MW630741.1), and *Scarus rubroviolaceus* (MW034094.1). The phylogenetic relationship revealed that the COI sequence was similar to the sequences of *H. harid*. DNA barcoding analysis has been recognized as a novel and effective method to understand the phylogenetic relationships between organisms. This method was used to identify the phylogenetic relationships between marine fish (Xu et al. 2019), bagrid catfish (Zou et al. 2020), and sea bass *Lateolabrax maculatus* (Wang et al. 2017).

4. Conclusions

The present study showed that DNA barcoding has been useful in discriminating and identifying most ichthyofauna from the marine environment. The DNA barcoding approach has been recognized as a potential tool for species-level identification, especially for fish samples or specimens that are incomplete, damaged or consisting of various morphologically different stages, which otherwise would not possible for the identification of fish species. DNA barcoding can also serve as an effective tool for

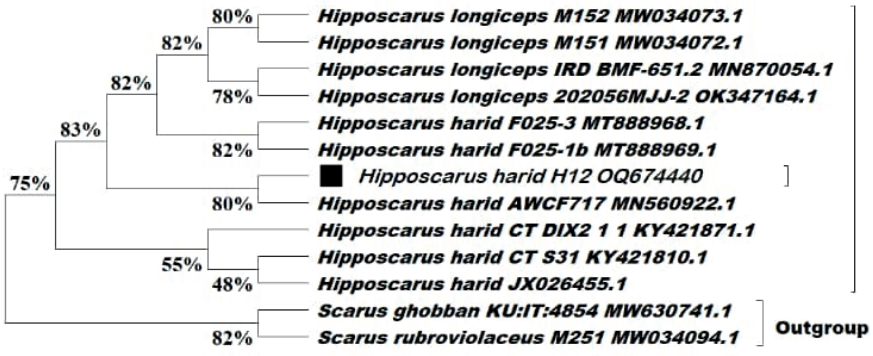

Figure 2

Neighbour-joining tree constructed from *H. harid* sequences (including two out-group sequences) of the COI gene. Numbers above the branches indicate neighbour-joining bootstrap percentages. Only bootstrap values >50% are shown in the tree below.

species identification if morphological identification is not possible. Thus, this kind of study helps establish a DNA barcode reference library for the Red Sea fish in Saudi Arabia, which could be used to identify unknown species using the database in the near future. Therefore, DNA barcoding research is useful in managing and monitoring fish diversity.

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References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389–3402. https://doi.org/10.1093/nar/25.17.3389 PMID:9254694

Alwany, M. A., Thaler, E., & Stachowitsch, M. (2009). Parrotfish bioerosion on Egyptian Red Sea reefs. *Journal of Experimental Marine Biology and Ecology, 371*, 170–176. https://doi.org/10.1016/j.jembe.2009.01.019

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R., & Toteja, R. (2023). DNA barcoding, an effective tool for species identification: A review. *Molecular Biology Reports, 50*(1), 761–775. https://doi.org/10.1007/s11033-022-08015-7 PMID:36308581

Bektas, Y., Aksu, İ., Kaya, C., Bayçelebi, E., & Turan, D. (2022). DNA barcoding and species delimitation of the genus *Oxynoemacheilus* (Teleostei: Nemacheilidae) in Anatolia. *Journal of Fish Biology, 101*(3), 505–514. https://doi.org/10.1111/jfb.15114 PMID:35607971

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

Bhattacharjee, M. J., Laskar, B. A., Dhar, B., & Ghosh, S. K. (2012). Identification and re-evaluation of freshwater catfishes through DNA barcoding. *PLoS One, 7*, e49950. Advance online publication. https://doi.org/10.1371/journal.pone.0049950 PMID:23166801

Cermakova, E., Lencova, S., Mukherjee, S., Horka, P., Vobruba, S., Demnerova, K., & Zdenkova, K. (2023). Identification of Fish Species and Targeted Genetic Modifications Based on DNA Analysis: State of the Art. *Foods, 12*(1), 228. https://doi.org/10.3390/foods12010228 PMID:36613444

Charendoff, J. A., Edwards, C. B., Pedersen, N. E., Petrovic, V., Zgliczynski, B., Sandin, S. A., & Smith, J. E. (2023, June 10). Variability in composition of parrotfish bite scars across space and over time on a central Pacific atoll. *Coral Reefs, 42*, 905–918. Advance online publication. https://doi.org/10.1007/s00338-023-02392-6

Chakraborty, C., Doss, C. G. P., Patra, B. C., & Bandyopadhyay, S. (2014). DNA barcoding to map the microbial communities: Current advances and future directions. *Applied Microbiology and Biotechnology, 98*(8), 3425–3436. https://doi.org/10.1007/s00253-014-5550-9 PMID:24522727

Chakraborty, M., & Ghosh, S. K. (2014). An assessment of the DNA barcodes of Indian freshwater fishes. *Gene, 537*(1), 20–28. https://doi.org/10.1016/j.gene.2013.12.047 PMID:24378233

Chang, C. H., Shao, K. T., Lin, H. Y., Chiu, Y. C., Lee, M. Y., Liu, S. H., & Lin, P. L. (2017). DNA barcodes of the native ray-finned fishes in Taiwan. *Molecular Ecology Resources, 17*(4), 796–805. https://doi.org/10.1111/1755-0998.12601 PMID:27717215

Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C., & Mace, G. M. (2011). Beyond predictions: Biodiversity conservation in a changing climate. *Science, 332*, 53–58. https://doi.org/10.1126/science.1200303 PMID:21454781

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research, 32*, 1792–1797. https://doi.org/10.1093/nar/gkh340 PMID:15034147

FAO. (2018). Fishery and aquaculture statistics. Global Capture Production 1950-2016 (FishstatJ). FAO Fisheries and Aquaculture Department, Rome. www.fao.org/fishery/statistics/software/fishstatj/en

Fišer Pečnikar, Ž., & Buzan, E. V. (2014). 20 years since the introduction of DNA barcoding: From theory to application. *Journal of Applied Genetics, 55*(1), 43–52. https://doi.org/10.1007/s13353-013-0180-y PMID:24203863

Friedlander, A. M., Donovan, M. K., Stamoulis, K. A., Williams, I. D., Brown, E. K., Conklin, E. J., DeMartini, E. E., Rodgers, K. S., Sparks, R. T., & Walsh, W. J. (2018). Human-induced gradients of reef fish declines in the Hawaiian Archipelago viewed through the lens of traditional management boundaries. *Aquatic Conservation, 28*, 146–157. https://doi.org/10.1002/aqc.2832

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299. PMID:7881515

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings. Biological Sciences, 270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218 PMID:12614582

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PLoS One, 6*(5), e19254. https://doi.org/10.1371/journal.pone.0019254 PMID:21637336

Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., & Bernatchez, L. (2008). Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One, 3*, e2490. Advance online publication. https://doi.org/10.1371/journal.pone.0002490 PMID:22423312

Hubert, N., Meyer, C. P., Bruggemann, H. J., Guérin, F., Komeno, R. J., Espiau, B., Causse, R., Williams, J. T., & Planes, S. (2012). Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. *PLoS One, 7*, e28987. https://doi.org/10.1371/journal.pone.0028987 PMID:22438862

Kar, C., Purkayastha, A., Limna Mol, V. P., & Sureshkumar, S. (2022). Comprehensive review and diversity analysis of coral-reef associated fishes from Southern India. *Regional Studies in Marine Science,* 56, 102657. Advance online publication. https://doi.org/10.1016/j.rsma.2022.102657

Karahan, A., Douek, J., Paz, G., Stern, N., Kideys, A. E., Shaish, L., Goren, M., & Rinkevich, B. (2017). Employing DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern Mediterranean, a hot-spot area for biological invasion. Journal for Nature Conservation. 36: 1–9. Advance online publication. https://doi.org/10.1016/j.jnc.2017.01.004

Lakra, W. S., Verma, M. S., Goswami, M., Lal, K. K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K. V., Ward, R. D., & Hebert, P. (2011). DNA barcoding Indian marine fishes. *Molecular Ecology Resources, 11*(1), 60–71. https://doi.org/10.1111/j.1755-0998.2010.02894.x PMID:21429101

Lara, A., Ponce de León, J. L., Rodríguez, R., Casane, D., Côté, G., Bernatchez, L., & García-Machado, E. (2010). DNA barcoding of Cuban freshwater fishes: Evidence for cryptic species and taxonomic conflicts. *Molecular Ecology Resources, 10*(3), 421–430. https://doi.org/10.1111/j.1755-0998.2009.02785.x PMID:21565041

Lokrantz, J., Nystrom, M., Thyresson, M., & Johansson, C. (2008). The non-linear relationship between body size and function in parrotfishes. *Coral Reefs, 27*, 967–974. https://doi.org/10.1007/s00338-008-0394-3

Macali, A., Semenov, A., Paladini de Mendoza, F., Dinoi, A., Bergami, E., & Tiralongo, F. (2020). Relative influence of environmental factors on biodiversity and behavioural traits of a rare mesopelagic fish, *Trachipterus trachypterus* (Gmelin, 1789), in a continental shelf front of the Mediterranean Sea. *Journal of Marine Science and Engineering, 8*(8), 581. https://doi.org/10.3390/jmse8080581

Meier, R., Zhang, G. Y., & Ali, F. (2008). The Use of Mean Instead of Smallest Interspecific Distances Exaggerates the Size of the "Barcoding Gap" and Leads to Misidentification. *Systematic Biology* 57(5): 809–13. Advance online publication. https://doi.org/10.1080/10635150802406343

Radulovici, A. E., Archambault, P., & Dufresne, F. (2010). DNA barcodes for marine biodiversity: Moving fast forward? *Diversity, 2*, 450–472. https://doi.org/10.3390/d2040450

Schindel, D. E., Stoeckle, M. Y., Milensky, C., Trizna, M., Schmidt, B., Gebhard, C., & Graves, G. (2011). Project description: DNA barcodes of bird species in the national museum of natural history, smithsonian institution, USA. *ZooKeys, 152*, 87–92. https://doi.org/10.3897/zookeys.152.2473 PMID:22287908

Shan, B., Liu, Y., Yang, C., Zhao, Y., Zhang, G., Wu, Q., & Sun, D. (2021). DNA barcoding of fish in mischief reef—Fish diversity of a reef fish community from Nansha Islands. *Frontiers in Marine Science, 7*, 618954. https://doi.org/10.3389/fmars.2020.618954

Shellem, C. T., Ellis, J. I., Coker, D. J., & Berumen, M. L. (2021). Red Sea fish market assessments indicate high species diversity and potential overexploitation. *Fisheries Research, 239*, 105922. https://doi.org/10.1016/j.fishres.2021.105922

Steinke, D., Zemlak, T. S., Boutillier, J. A., & Hebert, P. D. (2009). DNA barcoding of Pacific Canada's fishes. *Marine Biology, 156*(12), 2641–2647. https://doi.org/10.1007/s00227-009-1284-0

Takahara, T., Minamoto, T., & Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS One, 8*, e56584. Advance online publication. https://doi.org/10.1371/journal.pone.0056584 PMID:23437177

Tavares, E. S., & Baker, A. J. (2008). Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. *BMC Evolutionary Biology, 8*(1), 81. https://doi.org/10.1186/1471-2148-8-81 PMID:18328107

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research, 22*, 4673–4680. https://doi.org/10.1093/nar/22.22.4673 PMID:7984417

Trontelj, P., Machino, Y., & Sket, B. (2005). Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Molecular Phylogenetics and Evolution, 34*(1), 212–226. https://doi.org/10.1016/j.ympev.2004.09.010 PMID:15579394

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution, 24*(2), 110–117. https://doi.org/10.1016/j.tree.2008.09.011 PMID:19100655

van Rooij, J. M., Bruggemann, J. H., Videler, J. J., & Breeman, A. M. (1995). Plastic growth of the herbivorous reef fish Sparisomaviride—Field evidence for a trade-off between growth and reproduction. *Marine Ecology Progress Series, 122*, 93–105. https://doi.org/10.3354/meps122093

Wang, W., Ma, C., Chen, W., Zhang, H., Kang, W., Ni, Y., & Ma, L. (2017). Population genetic diversity of Chinese sea bass (*Lateolabrax maculatus*) from southeast coastal regions of China based on mitochondrial COI gene sequences.

Biochemical Systematics and Ecology, 71, 114–120. https://doi.org/10.1016/j.bse.2017.01.002

Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: Expanding taxonomic and geographic coverage. *PLoS One, 7*, e41059. https://doi.org/10.1371/journal.pone.0041059 PMID:22815912

Zhang, J. B., & Hanner, R. (2011). DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochemical Systematics and Ecology 39*(1), 31–42. Advance online publication. https://doi.org/10.1016/j.bse.2010.12.017

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 of London. Series B, Biological Sciences, 360*, 1847–1857. https://doi.org/10.1098/rstb.2005.1716 PMID:16214743

Xu, L., Van Damme, K., Li, H., Ji, Y., Wang, X., & Du, F. (2019). A molecular approach to the identification of marine fish of the Dongsha Islands (South China Sea). *Fisheries Research, 213*, 105–112. https://doi.org/10.1016/j.fishres.2019.01.011

Zhang, D. X., & Hewitt, G. M. (1996). Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution, 11*(6), 247–251. https://doi.org/10.1016/0169-5347(96)10031-8.

Zou, R., Liang, C., Dai, M., Wang, X., Zhang, X., & Song, Z. (2020). DNA barcoding and phylogenetic analysis of bagrid catfish in China based on mitochondrial COI gene. *Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis, 31*(2), 73–80. https://doi.org/10.1080/24701394.2020.1735379 PMID:32126856