Preliminary Assessment of Mitochondrial Genetic Diversity in *Epinephelus* spp. from Iraqi Marine Waters of the Arabian Gulf

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Abstract

The Arabian Gulf is an exceptional marine ecosystem subject to a range of harsh environmental conditions that can influence the genetic makeup of marine organisms. Grouper species of the genus *Epinephelus* are of ecological and economic importance in this ecosystem. Genetic studies of these species in the Iraqi marine environment are limited. This study presents a preliminary approach to estimating mitochondrial genetic diversity in *Epinephelus* species. DNA was extracted and sequenced, focusing on the mitochondrial control region (D-loop) adjacent to tRNA-Phe and a portion of 12S rRNA. Analysis of mitochondrial gene spacing (D-loop, tRNA-Phe, and 12S rRNA) revealed two distinct groups of *Epinephelus* species. Each group was characterized by short internode lengths and high sequence similarity. The groups were separated by intermediate gene spacing, and the high branch support values in the hierarchical tree indicated weak differentiation at the species level. This clustering analysis served as a graphical tool for revealing mitochondrial structure within a single species. Mitochondrial sequences revealed two genetically distinct clusters, indicating population genetic structure rather than species divergence. This genetic divergence may be due to local environmental conditions, geographic isolation, and reduced gene flow in grouper (*Epinephelus*) species in the Iraqi Arabian Gulf. This study provides preliminary mitochondrial genetic information for grouper species in the Iraqi region of the Arabian Gulf.

Keywords: *Epinephelus* spp., genetic diversity, mitochondrial DNA, Arabian Gulf, Iraqi marine waters

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Introduction

The Arabian Gulf is one of the most extreme marine environments globally, with high salinities and temperatures along with significant seasonal changes in both. Such punishing environmental conditions exert tremendous selective pressures on marine life and may dramatically shape genetic diversity, population structuring, and the evolutionary pace, especially in enclosed marginal seas (Sheppard *et al*., 2010; Burt *et al*. Molecular genetic work on marine fish from the northwestern part of the Gulf, particularly along the Iraqi coast, is relatively little in comparison to its ecologic and economic significance.

Groupers, *Epinephelus* species (Serranidae), are one of the most important reef-associated fishes in tropical and subtropical marine environments. As top, or macropredators, they are fundamental for the maintenance of trophic balance and ecosystem stability in coral reef and rocky reef ecosystems (Sadovy de Mitcheson *et al*., 2013). *Epinephelus* species are one of the most commercially important fishery resources and have been intensively fished by artisanal and industrial fisheries in the Indo-Pacific region, apart from their ecological significance (Coleman *et al.,* 2016). For this reason, several grouper stocks are more and more vulnerable to overfishing, habitat degradation, and climate-induced environmental alterations (especially for ecologically stressed areas, including the Arabian Gulf (Sadovy de Mitcheson *et al*., 2013).

Knowledge on the genetic diversity within populations of marine fishes is important for sustainable fisheries management and conservation. Genetic diversity is the foundation of population resilience and adaptability, as well as it contributes to long-term viability in a world with rapidly changing environments (Palsboll *et al.,* 2007). In marine organisms, molecular genetics approaches have thus become essential tools to determine population genetic structure, characterize management units, and estimate connectivity and gene flow (Avise 2000; Gaither *et al*., 2018).

Mitochondrial DNA (mtDNA) polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) markers have been broadly used in marine fish population genetics since they are maternally inherited, have high mutation rates, and lack recombination (Avise, 2000). Among these, the cytochrome c oxidase subunit I (COI) gene has been widely used in comparative genetic studies and to estimate biodiversity, whereas the mitochondrial control region (D-loop) is highly variable and an effective tool for detecting fine-scale population structure between different populations (Hebert *et al*., 2003; Craig *et al*., 2001). Other mitochondrial genes, for example, tRNA Phe, 12S rRNA, could be used to compensate for the lacking gene information owing to the conserved and variable regions; loci or loci combined produce the more complete gene pattern information (Lakra *et al*.,2015; Wang *et al*., 2020).

Previous molecular studies of the *Epinephelus* species in different parts of the Indo-Pacific identified marked genetic differentiation that correlated with geographical isolation, environmental heterogeneity, and restricted larval dispersal (Mabuchi *et al*., 2004; Ward *et al*., 2005). These are frequently observed patterns in reef-associated fish and may be due to past demographic processes, limited connectivity, or local selective pressures. But there are few genetic studies that exist on Iraqi marine populations of the Arabian Gulf, resulting in little information to infer population connectivity and local adaptation/management strategies for this region. There have been only a few molecular investigations on *Epinephelus* populations from the Arabian Gulf, and none were exclusively conducted on Iraqi marine waters. Based on this, the current study represents an initial evaluation of such variation among *Epinephelus* spp. obtained from the Iraqi marine waters of the Arabian Gulf by examination of the mitochondrial control region (D-loop), including the adjoined tRNA-Phe and incomplete 12S rRNA sequences. Through production of basic mitochondrial gene data, this work aims to build a foundation for a deeper insight into population-level genetic structuring among *Epinephelus* spp. and for further integrative research, fisheries management, and conservation efforts within this unique and poorly studied ecosystem.

Materials and Methods

Study Area and Sample Collection

Epinephelus spp. specimens were obtained from fish markets in Faw City, southern Iraq (fig 1), and from Iraqi marine waters of the Arabian Gulf over six months in 2024. The sampling area has a subtropical, hypersaline, and warm temperate climate, typical of the northwestern Arabian Gulf. Thirty specimens were selected based on external morphological characteristics (body shape and color) without prior species identification. Muscle samples were aseptically collected from each animal, preserved in 95-100% ethanol, and stored at -20°C for molecular analysis.

Figure 1: Sampling location of *Epinephelus* specimens collected from the coastal waters of Al-Faw, southern Iraq, located in the northwestern Arabian Gulf. The inset map shows the geographic position of the study area within Iraq.

DNA Extraction

Genomic DNA was extracted from 25-30 mg muscle samples using a commercial kit (Geneaid, Taiwan) following the manufacturer's protocol. DNA quality and integrity were assessed by electrophoresis on 1% agarose gels with ethidium bromide staining, while concentration and purity were determined spectrophotometrically.

PCR Amplification of Mitochondrial Markers

Mitochondrial genes, D-loop region (flanking tRNA-Phe and 12S rRNA), were employed as genetic markers. Thus, these markers are a mixture of conserved and variable regions and therefore can be used in population-based studies for genetic diversity. PCRs were conducted with 25 µL reactions using genomic DNA, primers (Table 1), PCR buffer, $MgCl_2$, dNTPs and Taq DNA polymerase. Thermal cycling consisted of an initial denaturation at 94°C for 1 min, and then a seven-cycle profile that allowed different annealing temperatures and fragment sizes to optimally cover allelic diversity but also facilitate application across related species. Contaminations were avoided by placing negative controls on every PCR plate.

Table 1: Primers used for amplification of mitochondrial D-loop (including tRNA-Phe & 12S rRNA) *Epinephelus* spp.

Target Gene	Primer Name	Primer Sequence (5′–3′)	Direction	Reference
D-loop (including tRNA-Phe & 12S rRNA)	DL-F	CCTGGCATTTGGTTCCTACTT	Forward	Ketchum *et al.*, 2016
D-loop (including tRNA-Phe & 12S rRNA)	DL-R	CAGTTTGTGCCTTGGCTTTC	Reverse	Ketchum *et al.*, 2016
COI	COI-F (FishF1)	TCAACCAACCACAAAGACATTGGCAC	Forward	Ward *et al.*, 2005
COI	COI-R (FishR1)	TAGACTTCTGGGTGGCCAAAGAATCA	Reverse	Ward *et al.*, 2005

DNA Sequencing and Sequence Editing

Positive PCR products were Sanger sequenced bidirectionally by a commercial provider. Resulting chromatograms were visually inspected, and low-quality regions were trimmed using BioEdit v7.2.5. Consensus sequences were then generated for each sample.

Sequence Alignment and Genetic Analysis

Edited sequences were aligned using ClustalW in MEGA X. BLASTn searches against NCBI GenBank confirmed mitochondrial origin and sequence similarity. All new mtDNA sequences were submitted to GenBank (accession numbers in results). Phylogenetic relationships were explored using a Neighbor-Joining (NJ) method from genetic distance matrices to identify genetic similarity patterns, not to determine definitive phylogenetic relationships.

Results

Sample Overview and DNA Amplification Success

Molecular analyses included thirty *Epinephelus* spp. specimens from Iraqi marine waters of the northwestern Arabian Gulf. These specimens showed external phenotypic variation in body shape and coloration (Figure 2), representing morphological diversity within the sampled population. This observation is descriptive and lacks taxonomic interpretation. High-quality genomic DNA was extracted from muscle tissues of all specimens. PCR amplification of the mitochondrial control region (D-loop), including adjacent tRNA-Phe and partial 12S rRNA, produced consistent ~450 bp products.

Figure 2: General phenotypic variation observed among representative *Epinephelus* spp. specimens collected from Iraqi marine waters of the Arabian Gulf.

Sequence Characteristics

Following removal of low-quality regions and resolution of ambiguous base calls, we obtained high-quality consensus sequences for all amplified samples. The final aligned sequences, approximately 450 bp for the D-loop and including tRNA-Phe and part of 12S rRNA, showed no sequencing errors, frame shifts, or aberrant profiles. As expected, nucleotide variation was detected in the mitochondrial control region, while partial 12S rRNA was highly similar across individuals. Overall sequence quality and consistency were high, with ≥ 80–90% paired-end read coverage for each sample.

Sequence Similarity and Database Comparison

NCBI GenBank BLASTn searches revealed all sequences to be of mitochondria origin, from *Epinephelus* species. There was a high level of nucleotide identity between the sequences assessed for analysis and those of *Epinephelus* species, with minimal variations for the different individuals. There were no unexpected patterns or linkages other than those within *Epinephelus* species.

Genetic Grouping and Clustering Patterns

Clustering analysis of mitochondrial **(D-loop, tRNA-Phe, and 12S rRNA)** genetic distances resolved the analyzed *Epinephelus* species into two mitochondrial clusters (Fig. 3). Each cluster exhibited low internal branch lengths and high sequence similarity, while the clusters were separated by moderate genetic distances. High clade support values in the hierarchical tree reflected weak divergence and lacked strong deep branches for species-level distinction. Therefore, the clustering pattern reflects intraspecies mitochondrial structuring rather than distinct phyletic lines. This clustering analysis served as a graphical tool to reveal intraspecies structuring.

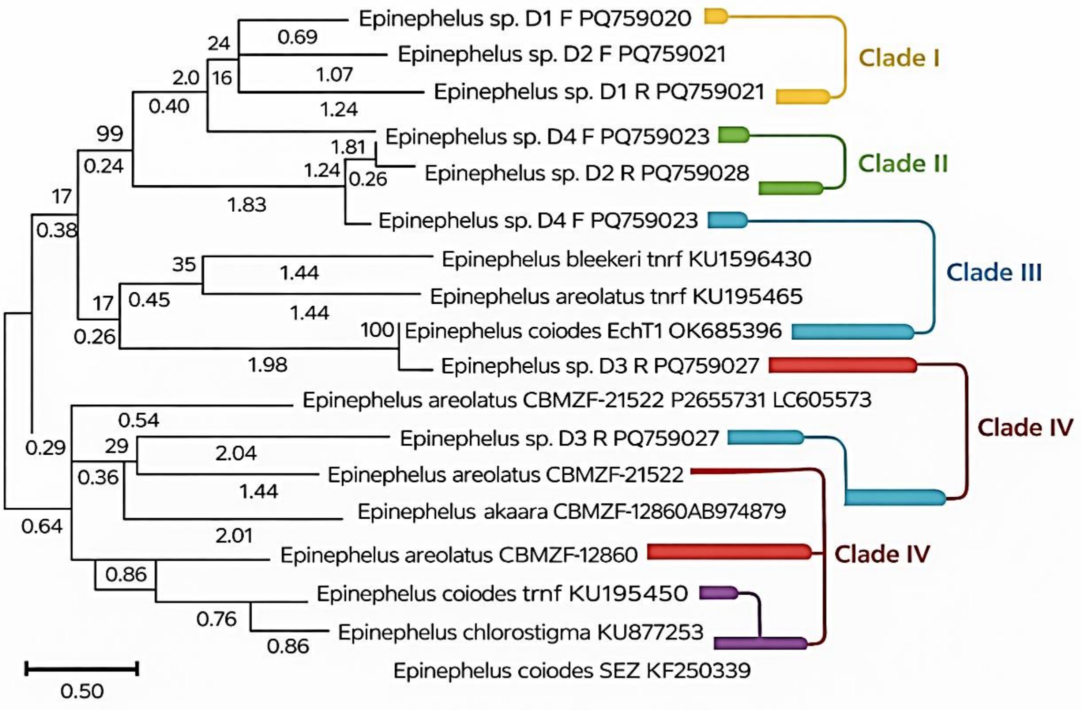

Figure 3: Neighbor-joining clustering of mitochondrial **(D-loop, tRNA-Phe, and 12S rRNA)** sequences reveals population-level genetic structure in *Epinephelus* spp. from Iraqi marine waters of the Arabian Gulf.

GenBank Accession Numbers

Mitochondrial D-Loop, tRNA-Phe and partial 12S rRNA regions sequences generated in this study have been submitted to NCBI GenBank (accession numbers in Table 2). This represents the first published mitochondrial genetic data for *Epinephelus* stocks from Iraqi marine waters of the Arabian Gulf.

Table 2: GenBank accession numbers for mitochondrial DNA sequences (D-loop, tRNA-Phe, and 12S rRNA) of *Epinephelus* spp. from Iraqi Arabian Gulf waters.

Sample ID	Target Region	GenBank Accession Number
D1	D-loop, tRNA-Phe, 12S rRNA	PQ759020
D2	D-loop, tRNA-Phe, 12S rRNA	PQ759021
D3	D-loop, tRNA-Phe, 12S rRNA	PQ759022
D4	D-loop, tRNA-Phe, 12S rRNA	PQ759023
D5	D-loop, tRNA-Phe, 12S rRNA	PQ759024
D1	D-loop, tRNA-Phe, 12S rRNA	PQ759025
D2	D-loop, tRNA-Phe, 12S rRNA	PQ759026
D3	D-loop, tRNA-Phe, 12S rRNA	PQ759027
D4	D-loop, tRNA-Phe, 12S rRNA	PQ759028
D5	D-loop, tRNA-Phe, 12S rRNA	PQ759029

Discussion

This study establishes a baseline for mitochondrial genetics of *Epinephelus* species in the Arabian Gulf off Iraqi marine waters, an area lacking molecular genetic data for grouper populations. Sequencing of the mitochondrial control region (D-loop), with flanking tRNA-Phe and partial 12S rRNA segments, revealed significant mitochondrial genetic variability and population-level genetic structuring, rather than species divergence.

The distribution of Iraqi samples (PQ accessions) within the two mitochondrial clades suggests maternal haplotype heterogeneity. This pattern, seen in many marine fishes, could arise due to demographic history, restricted dispersal ability, or the coexistence of multiple maternal lineages. But since the collection represented fish from markets rather than geographically separated, biologically distinct populations, it could have represented mixed local populations rather than fixed biological groups.

In Indo-Pacific reef fish, particularly groupers, mitochondrial lineage divergence tends, to a large extent, to be associated rather with population structure and ecology rather than classification and speciation processes (Mabuchi *et al.*, 2004; Ward *et al.*, 2005). Such divergence might be further enhanced within more extreme, semi-enclosed conditions, for example, within the Arabian Gulf, where factors like high salinity, high temperatures, large seasonality, and limited connectivity could all add to the accumulation of genetic divergence (Sheppard *et al.*, 2010; Burt *et al.*, 2011).

It should be noted that the haplotype similarity between the sequences obtained from PQ and reference accessions in GenBank is not to be considered conclusive for the identity of *Epinephelus* species. There are chances that *Epinephelus* species may share an identical mitochondrial haplotype because of ancestral polymorphism and gene flow. It is also possible that the genetic variation of Iraqi marine species is not correctly depicted in a public online database.

The identification of two mitochondrial genetic groups serves as a preliminary stepping stone in fisheries management. Though not sufficient to identify units for fisheries management, mitochondrial information presents fundamental preliminary information regarding haplotypeomes, population connectivity, and fishery impact in the marine habitat of Iraq. Large-scale analysis through nuclear genetics is necessary to grasp population structure adequately and ensure effective conservation, management, and conservation management of groupers in the Arabian Gulf.

Conclusions

The current research investigated the extent of mitochondrial genetic diversity for *Epinephelus* species in the Arabian Gulf and Iraqi coastal waters. Based on the results of assessing the genetic diversity of *Epinephelus* species from the Arabian Gulf, a fundamental genetic dataset is provided for *Epinephelus* species, a region where little molecular research has been conducted for marine fish species. Although the current research is limited in scale, relying solely on mitochondrial DNA, it offers a starting point for further research, including a larger sample size with a larger repertoire of nuclear markers, for studies in the Arabian Gulf examining grouper species connectivity.

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التقييم الأولي للتنوع الجيني للميتوكوندريا في أنواع أسماك الهامور (*Epinephelus* spp.) من المياه البحرية العراقية في الخليج العربي

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المستخلص

يُعدّ الخليج العربي نظاماً بيئياً بحرياً استثنائياً، يخضع لمجموعة من الظروف البيئية القاسية التي قد تؤثر على التركيب الجيني للكائنات البحرية. وتُعتبر أنواع الهامور من جنس *Epinephelus* ذات أهمية بيئية واقتصادية في هذا النظام البيئي. وتُعدّ الدراسات الجينية لهذه الأنواع في البيئة البحرية العراقية محدودة. تُقدّم هذه الدراسة منهجاً أولياً لتقدير التنوع الجيني للميتوكوندريا في أنواع *Epinephelus*. تم استخلاص الحمض النووي وتسلسله، مع التركيز على منطقة التحكم الميتوكوندريا (حلقة D) المجاورة لـ tRNA-Phe وجزء من 12S rRNA. كشف تحليل تباعد جينات الميتوكوندريا (حلقة D، وtRNA-Phe، و12S rRNA) عن مجموعتين متميزتين من أنواع *Epinephelus*. تميزت كل مجموعة بأطوال عقد قصيرة وتشابه عالٍ في التسلسل. وقد تم فصل المجموعتين بتباعد جيني متوسط، وأشارت قيم دعم الفروع العالية في الشجرة الهرمية إلى تمايز ضعيف على مستوى الأنواع. شكّل تحليل التجميع هذا أداةً بيانيةً للكشف عن بنية الميتوكوندريا داخل النوع الواحد. وكشفت تسلسلات الميتوكوندريا عن مجموعتين متميزتين وراثيًا، مما يشير إلى بنية وراثية سكانية بدلًا من تباين الأنواع. وقد يعود هذا التباين الوراثي إلى الظروف البيئية المحلية، والعزلة الجغرافية، وانخفاض تدفق الجينات في أنواع الهامور (*Epinephelus*) في الخليج العربي العراقي. تُقدّم هذه الدراسة معلومات وراثية أولية عن الميتوكوندريا لأنواع الهامور في المنطقة العراقية من الخليج العربي.

الكلمات المفتاحية: *Epinephelus* spp.، التنوع الجيني، الحمض النووي للميتوكوندريا، الخليج العربي، المياه البحرية العراقية.