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Original article

Phylogenetic Placement of Libyan *Mycteroperca rubra* Based on Mitochondrial COI Gene Sequences and Comparative Mediterranean Reference Data

Tarek Shoeib*[1], **Tawfeek Altawaty**[2], **Omar S. Alqabbasi**[2]

[1]*Department of Forensic Sciences, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya.*
[2]*Department of Molecular Diagnostics, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya*
Corresponding email. *tarek.shoeib@uob.edu.ly*

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ABSTRACT

The mottled grouper, *Mycteroperca rubra*, is a Mediterranean and eastern Atlantic serranid fish with ecological, fisheries, and conservation importance. In Libya, molecular data for this species remain limited, and its relationship with surrounding Mediterranean populations is poorly documented. This study examined the phylogenetic placement of one Libyan *M. rubra* mitochondrial COI sequence, KX255748.1, 661 bp, together with 11 Mediterranean GenBank reference sequences. Sequences were aligned and analysed in MEGA 10 using the Tamura 3-parameter model. Maximum Likelihood and Neighbor-Joining trees were reconstructed with 1000 bootstrap replicates. Pairwise genetic distances were calculated under the same model. The Libyan sequence was grouped within the main Mediterranean *M. rubra* lineage and showed very low distance from supported *M. rubra* references, especially two Spanish sequences, MH707832.1 and MH707833.1, with zero pairwise distance. Three nominal *M. rubra* records from Italy, Spain, and Tunisia formed a separate divergent lineage and are treated here as probable misidentified *Epinephelus costae*-like records requiring taxonomic rechecking. The Libyan sequence represents a distinct local COI haplotype branch within Mediterranean *M. rubra*. These findings support the value of COI barcoding for Libyan grouper taxonomy, reference library correction, and future conservation monitoring.

Introduction

Groupers are large predatory marine fishes that belong to the family Serranidae, subfamily Epinephelinae. They are important in rocky reef ecosystems because they are high-level predators, and they are also valuable to artisanal and commercial fisheries. Many groupers are biologically vulnerable because they grow slowly, mature late, may change sex during life, and may aggregate for spawning. These life-history traits can make populations sensitive to fishing pressure and habitat disturbance, even when the species appears locally common. Studies on grouper biology and conservation have repeatedly shown that correct species identification is essential before any serious ecological, fisheries, or conservation decision can be made [1-3]. The mottled grouper, *Mycteroperca rubra,* is distributed in the Mediterranean Sea and the eastern Atlantic. It is usually associated with rocky bottoms, reefs, and mixed rocky-sandy habitats. The *Mycteroperca rubra* has been less studied in the Mediterranean region than the dusky grouper, *Epinephelus marginatus*. *M. rubra* has been previously recognized in ecology as an animal that resides at the rock bottom. However, recent research emphasizes the importance of mating activities for the conservation of the species[4]. The species has also been reported from different Mediterranean localities, including the eastern Mediterranean, Tunisia, Lebanon, Turkey, Spain, Italy, and the Adriatic region [5, 6]. In Libya and adjacent North African waters, however, published molecular information remains limited. This creates a knowledge gap because Libya lies in a central Mediterranean position and may contain lineages that connect eastern, western, and southern Mediterranean populations. Traditional identification of groupers depends on external morphology, including body shape, color pattern, fin characters, jaw shape, and markings. This approach is useful, but it can be difficult when specimens are juveniles, damaged, processed, photographed underwater, or closely similar to related grouper species. Misidentification can also enter public sequence databases when the original voucher specimen is not checked carefully. Once a wrong name is attached to a sequence, the error can spread into later studies. This is not a minor problem. There are some authors who have warned that the DNA databases such as GenBank, could contain misclassification and taxonomic errors [7-10]. This point is especially important for groupers, because some species are morphologically similar and may occur in the same geographic region. DNA barcoding provides a practical tool for species identification. The mitochondrial cytochrome c oxidase subunit I gene, usually called COI or cox1, has

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become the standard animal barcode marker. COI has been widely used in fish identification and has been shown to work well for many marine fishes [6, 11, 12]. COI is not a complete solution to all taxonomic problems, but it is very useful when the aim is to confirm species identity, detect obvious misidentifications, or compare local samples with a regional reference library.

Phylogenetic trees are often used together with DNA barcoding. In this study, two common methods were used: Maximum Likelihood and Neighbor-Joining. Maximum Likelihood estimates the tree that best explains the observed sequence data under a chosen substitution model. Neighbor-Joining is a distance-based method that builds a tree from pairwise genetic distances. Both methods are useful when the aim is to see whether a query sequence falls within the expected species cluster or outside it. Bootstrap analysis provides a measure of branch support by resampling the alignment many times [13]. For COI data, the substitution model matters because mitochondrial sequences can show different rates of transitions and transversions, and can also show base composition bias [14]. The Tamura 3-parameter model is useful for this type of situation because it allows unequal nucleotide frequencies and different substitution patterns [15]. The present study focuses on one Libyan *M. rubra* COI sequence, KX255748.1, and compares it with 11 Mediterranean reference sequences. The main aims were: first, to place the Libyan sequence within a Mediterranean phylogenetic context; second, to compare the ML and NJ topologies; third, to estimate pairwise genetic distances between the Libyan sequence and reference sequences; fourth, to examine whether all nominal *M. rubra* GenBank records behave as true *M. rubra*; and fifth, to discuss the taxonomic and conservation meaning of the Libyan COI haplotype.

Materials and Methods

Study design and sequence dataset

This study used a comparative molecular phylogenetic design based on mitochondrial COI sequence data. The focal sequence was a Libyan *Mycteroperca rubra* sequence from GenBank, accession KX255748.1. The sequence length used in the analysis was 661 base pairs. The Libyan sequence was compared with 11 Mediterranean nominal *M. rubra* reference sequences from GenBank. The accession numbers and geographic labels were taken from the result file and included: KF564307.1 from Haifa, KJ709569.1 from Italy, KU739518.1 from Tunisia, KY176539.1 from Turkey, MH707831.1 from Spain, MH707832.1 from Spain, MH707833.1 from Spain, MW575709.1 from Tunisia, ON303317.1 from Lebanon, ON303318.1 from Lebanon, and ON303319.1 from Lebanon. Three additional congeneric sequences were used as outgroups in the tree figures: *Mycteroperca venenosa* JQ839850.1, *Mycteroperca bonaci* PQ347340.1, and JQ840175.1. These outgroups were used to root and visually separate the ingroup *M. rubra* lineages from more distant *Mycteroperca* species. The outgroup sequences were not included in the pairwise distance matrix, which was limited to the 12 nominal *M. rubra* records.

Sequence alignment and model selection

All sequences were treated as mitochondrial COI gene sequences. The dataset was trimmed to a common comparable region of 661 bp for the Libyan sequence and overlapping reference sequences. Sequence names were formatted to include accession number, species name, and locality. This was done to keep the phylogenetic tree readable and to allow direct interpretation of geographic placement. Sequences were aligned in MEGA 10 using the built-in multiple sequence alignment tools. The alignment was manually inspected after alignment to check for obvious gaps, stop codons, or frame-disrupting indels. Because COI is a protein-coding mitochondrial marker, unexpected stop codons or strong length differences may suggest sequencing error, nuclear mitochondrial copies, or incorrect sequence orientation. No such issue was observed in the final alignment used for analysis. The Nucleotide Substitution Model has been considered during MEGA 10. The preferred model for the COI data set was the Tamura 3-parameter model. This model was further considered for all three phases, including maximum likelihood tree construction, neighbor joining tree construction, and estimation of genetic distance. In other words, the use of the same model ensured comparability between different outputs. The Maximum Likelihood tree has been constructed in MEGA 10 using the Tamura 3-parameter substitution model. The robustness of branches was tested with 1000 bootstrap replicates. Bootstrap values were displayed beside branches in the final tree as generated by MEGA 10. Values above 70% were treated as strong support, while values below 70% were considered weak or moderate support and interpreted with caution.

Phylogenetic reconstruction and genetic distance analysis

The Neighbor-Joining tree was reconstructed in MEGA 10 using the Tamura 3-parameter model. The NJ method was used because it gives a simple distance-based view of relationships among the COI sequences and is useful for comparison with ML topology. Bootstrap support was assessed with 1000 replicates. As in

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the ML analysis, bootstrap values were displayed beside branches in the final tree. Pairwise genetic distances were calculated in MEGA 10 under the Tamura 3-parameter model. The distance matrix was exported and presented as (Table 1). The matrix included all 12 nominal *M. rubra* sequences: one Libyan sequence and 11 Mediterranean references. The matrix showed pairwise distances between every pair of sequences, including zero distances between identical or nearly identical haplotypes and high distances between divergent lineages. For summary interpretation, sequences were separated into two groups based on the tree topology and distance matrix. The first group was the supported core *M. rubra* lineage. This included KF564307.1, KU739518.1, KX255748.1, KY176539.1, MH707832.1, MH707833.1, ON303317.1, ON303318.1, and ON303319.1. The second group contained three divergent nominal *M. rubra* records: KJ709569.1 from Italy, MH707831.1 from Spain, and MW575709.1 from Tunisia.

Results

COI dataset composition

The final ingroup dataset contained 12 nominal *Mycteroperca rubra* COI sequences. One sequence was from Libya, KX255748.1, and 11 sequences were Mediterranean references from Haifa, Italy, Tunisia, Turkey, Spain, and Lebanon. The Libyan sequence was 661 bp and was labelled in the trees as KX255748.1 *Mycteroperca rubra* Libya. The analyses produced two phylogenetic trees and one pairwise distance matrix. The sequence list used for the pairwise distance matrix was as follows: KF564307.1 *Mycteroperca rubra* Haifa; KJ709569.1 *Mycteroperca rubra* Italy; KU739518.1 *Mycteroperca rubra* Tunisia; KX255748.1 *Mycteroperca rubra* Libya; KY176539.1 *Mycteroperca rubra* Turkey; MH707831.1 *Mycteroperca rubra* Spain; MH707832.1 *Mycteroperca rubra* Spain; MH707833.1 *Mycteroperca rubra* Spain; MW575709.1 *Mycteroperca rubra* Tunisia; ON303317.1 *Mycteroperca rubra* Lebanon; ON303318.1 *Mycteroperca rubra* Lebanon; and ON303319.1 *Mycteroperca rubra* Lebanon.

Maximum Likelihood phylogenetic analysis

The Maximum Likelihood tree reconstructed with the Tamura 3-parameter model placed the Libyan sequence KX255748.1 inside the main Mediterranean *M. rubra* lineage. The Libyan sequence is grouped with two Spanish sequences, MH707832.1 and MH707833.1. This shallow branch received moderate support in the ML figure, with a bootstrap value of 66. The larger main *M. rubra* cluster included sequences from Haifa, Tunisia, Libya, Turkey, Spain, and Lebanon, and received strong support at a deeper node, shown as 100 in the ML tree. A separate divergent cluster was recovered outside the main *M. rubra* lineage. This cluster included three nominal *M. rubra* sequences: KJ709569.1 from Italy, MH707831.1 from Spain, and MW575709.1 from Tunisia. In the ML tree, this divergent cluster was labelled as a misclassification lineage and was separated from the main Mediterranean *M. rubra* group. The cluster received strong bootstrap support, shown as 98 in the ML figure. The outgroup sequences were placed outside the ingroup *M. rubra* lineages.

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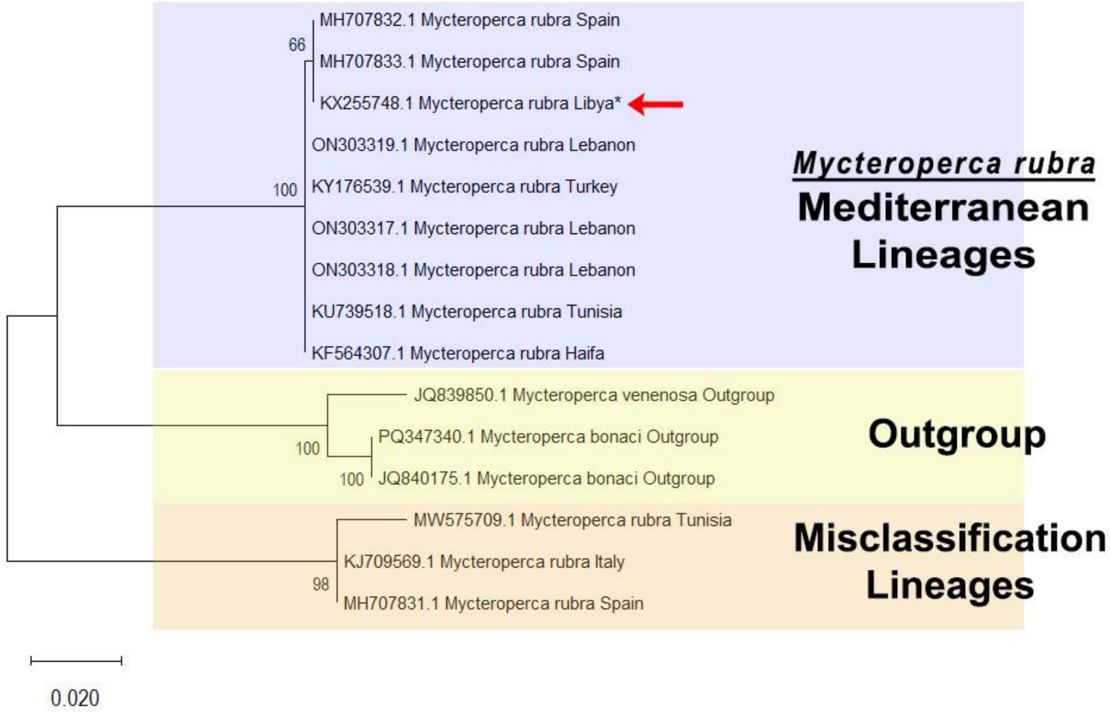

Figure 1. Maximum Likelihood phylogenetic tree of Libyan and Mediterranean nominal Mycteroperca rubra COI sequences reconstructed in MEGA 10 using the Tamura 3-parameter model. Bootstrap values were calculated from 1000 replicates and are shown beside branches as displayed by MEGA. The Libyan sequence KX255748.1 is indicated with a red arrow. The main Mediterranean M. rubra lineage includes the Libyan sequence and reference sequences from Haifa, Tunisia, Turkey, Spain, and Lebanon. Three nominal M. rubra sequences from Italy, Spain, and Tunisia form a separate divergent lineage labelled as probable misclassification lineages. Outgroup sequences include Mycteroperca venenosa and Mycteroperca bonaci.

Neighbor-Joining phylogenetic analysis

The Neighbor-Joining tree showed the same general pattern as the ML tree. The Libyan sequence KX255748.1 was placed within the main Mediterranean *M. rubra* lineage and clustered with MH707832.1 and MH707833.1 from Spain. The shallow Spanish-Libyan cluster showed a bootstrap value of 64 in the NJ figure. The main *M. rubra* cluster was supported by a bootstrap value of 100. The NJ tree also recovered the same divergent cluster seen in the ML tree. KJ709569.1 from Italy, MH707831.1 from Spain, and MW575709.1 from Tunisia were placed outside the main *M. rubra* lineage. This divergent cluster received strong support, with a bootstrap value of 100 at the main branch and 94 for the subcluster containing the Italy and Spain records. The NJ tree, therefore, agreed with the ML tree in separating the three divergent nominal *M. rubra* records from the core Mediterranean *M. rubra* group.

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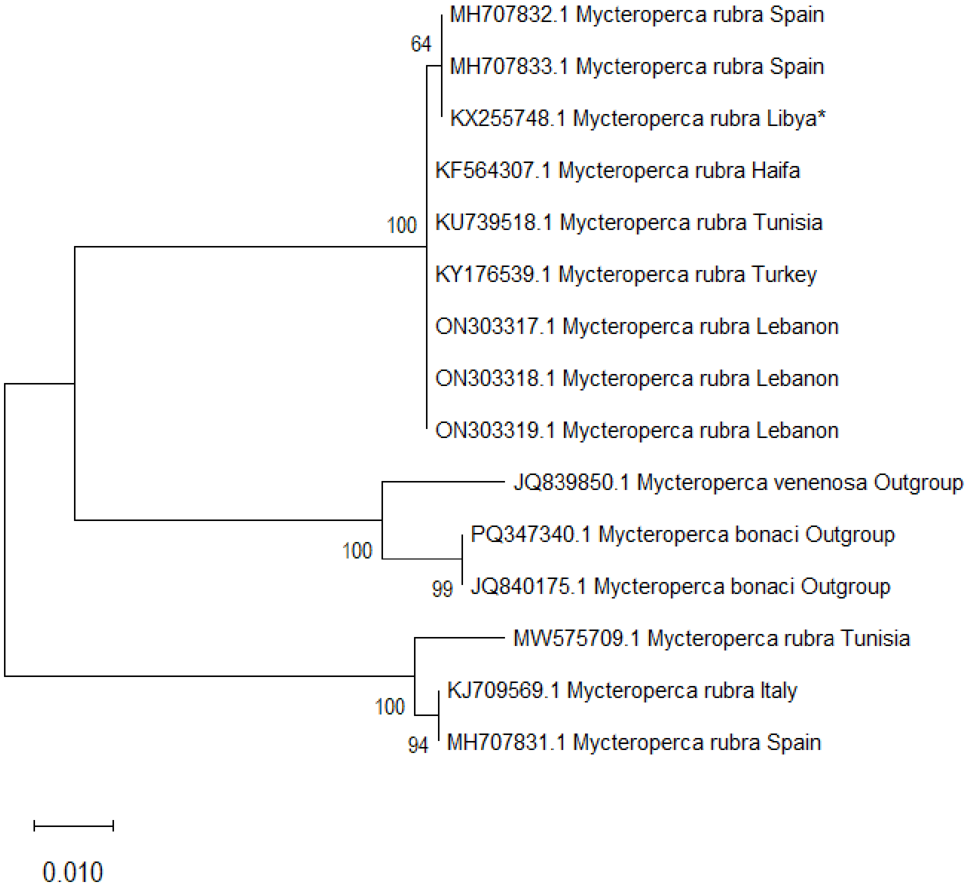

Figure 2. Neighbor-Joining phylogenetic tree of Libyan and Mediterranean nominal Mycteroperca rubra COI sequences reconstructed in MEGA 10 using the Tamura 3-parameter model. Bootstrap values were calculated from 1000 replicates and are shown beside branches as displayed by MEGA. The Libyan sequence KX255748.1 clusters with two Spanish sequences, MH707832.1 and MH707833.1, within the main Mediterranean M. rubra lineage. Three nominal M. rubra records from Italy, Spain, and Tunisia form a separate divergent lineage. Outgroups include Mycteroperca venenosa and Mycteroperca bonaci

Pairwise genetic distances and haplotype patterns

The Tamura 3-parameter pairwise distance matrix showed very low distances within the supported core *M. rubra* lineage and much higher distances between the core lineage and the three divergent nominal records. The Libyan sequence KX255748.1 had a distance of 0.0018 from KF564307.1 Haifa, 0.0018 from KU739518.1 Tunisia, 0.0018 from KY176539.1 Turkey, 0.0018 from ON303317.1 Lebanon, 0.0018 from ON303318.1 Lebanon, and 0.0018 from ON303319.1 Lebanon. It had zero distance from MH707832.1 Spain and MH707833.1 Spain. These values show that the Libyan sequence was almost identical or identical to several Mediterranean reference sequences within the supported *M. rubra* lineage. By contrast, the Libyan sequence showed much larger distances from the three divergent nominal *M. rubra* records. The distance from KJ709569.1, Italy, was 0.2175. The distance from MH707831.1, Spain, was 0.2175. The distance from MW575709.1 Tunisia was 0.2317. These distances were much higher than the values observed within the core *M. rubra* lineage. The supported core *M. rubra* lineage contained nine sequences. Pairwise distances inside this group ranged from 0.0000 to 0.0018, with a mean of approximately 0.0009. The three divergent nominal records had within-group distances from 0.0000 to 0.0153, with a mean of approximately 0.0102. Distances between the core *M. rubra* lineage and the divergent lineage ranged from 0.2099 to 0.2317, with a mean of approximately 0.2171. Across all 66 pairwise comparisons among the 12 nominal *M. rubra* records, distances ranged from 0.0000 to 0.2317, with a mean of approximately 0.0898. The haplotype pattern showed several repeated or shared COI haplotypes. One common haplotype included KF564307.1 Haifa, KU739518.1 Tunisia, KY176539.1 Turkey, ON303317.1 Lebanon, ON303318.1 Lebanon, and ON303319.1 Lebanon. These sequences showed zero pairwise distance among themselves. A second haplotype included KX255748.1 Libya, MH707832.1 Spain, and MH707833.1 Spain. These three sequences also showed zero pairwise distance among themselves. This placed the Libyan sequence in a distinct but very close haplotype branch within the main *M. rubra* lineage. A third haplotype included KJ709569.1 Italy and MH707831.1 Spain, which showed zero distance between each other. MW575709.1 Tunisia was close to this third haplotype, with a distance of 0.0153, but remained separate from it. These three sequences corresponded to the divergent lineage seen in both ML and NJ trees.

Table 1. Pairwise genetic distances among Libyan and Mediterranean nominal Mycteroperca rubra COI sequences were calculated in MEGA 10 using the Tamura 3-parameter model. The Libyan sequence is KX255748.1. Very low distances occur within the supported core M. rubra lineage, including the Libyan sequence. High distances separate the core lineage from three divergent nominal M. rubra records: KJ709569.1 Italy, MH707831.1 Spain, and MW575709.1 Tunisia

No.	**Sequence**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	KF564307.1 M. rubra Haifa												
2	KJ709569.1 M. rubra Italy	0.2099											
3	KU739518.1 M. rubra Tunisia	0.0000	0.2099										
4	KX255748.1 M. rubra Libya*	0.0018	0.2175	0.0018									
5	KY176539.1 M. rubra Turkey	0.0000	0.2099	0.0000	0.0018								
6	MH707831.1 M. rubra Spain	0.2099	0.0000	0.2099	0.2175	0.2099							
7	MH707832.1 M. rubra Spain	0.0018	0.2175	0.0018	0.0000	0.0018	0.2175						
8	MH707833.1 M. rubra Spain	0.0018	0.2175	0.0018	0.0000	0.0018	0.2175	0.0000					
9	MW575709.1 M. rubra Tunisia	0.2239	0.0153	0.2239	0.2317	0.2239	0.0153	0.2317	0.2317				
10	ON303317.1 M. rubra Lebanon	0.0000	0.2099	0.0000	0.0018	0.0000	0.2099	0.0018	0.0018	0.2239			
11	ON303318.1 M. rubra Lebanon	0.0000	0.2099	0.0000	0.0018	0.0000	0.2099	0.0018	0.0018	0.2239	0.0000		
12	ON303319.1 M. rubra Lebanon	0.0000	0.2099	0.0000	0.0018	0.0000	0.2099	0.0018	0.0018	0.2239	0.0000	0.0000	

Note. Empty cells represent the diagonal and upper triangle of the distance matrix.

Discussion

Phylogenetic placement of the Libyan sequence

The main result of this study is clear: the Libyan COI sequence KX255748.1 belongs within the supported Mediterranean *Mycteroperca rubra* lineage. Both tree-building methods gave the same broad result. The Maximum Likelihood tree and the Neighbor-Joining tree placed the Libyan sequence with Mediterranean *M. rubra* references rather than with the outgroups. The distance matrix also supported this result, because the Libyan sequence differed from the core *M. rubra* references by only 0.0000 to 0.0018 under the Tamura 3-parameter model. These distances are genetically very small and are consistent with conspecific COI variation. This result supports the molecular identity of the Libyan sequence as *M. rubra.* It also provides a useful Libyan COI reference for future studies. For a region such as Libya, where marine molecular reference data are still limited, even one verified sequence can be useful. It helps connect Libyan fish biodiversity to broader Mediterranean barcode libraries and can be used in later studies of fish markets, fisheries monitoring, environmental DNA, forensic seafood identification, and biodiversity surveys. The Libyan

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sequence did not appear as a deeply isolated lineage. Instead, it was very close to several Mediterranean sequences. This is expected for a marine fish that can disperse through larval stages and whose range extends across the Mediterranean and eastern Atlantic. Many marine fishes show low mitochondrial differentiation across wide geographic distances because there may be ongoing or historical gene flow among regions [16-18]. However, low COI divergence does not mean that all populations are ecologically identical. A single mitochondrial marker cannot measure local adaptation, demographic history, or nuclear gene flow. It only gives one maternal genetic view.

The Libyan haplotype branch and its relationship with Spain

The Libyan sequence showed zero pairwise distance from two Spanish sequences, MH707832.1 and MH707833.1. In both ML and NJ trees, these three records formed a shallow cluster within the main *M. rubra* lineage. This is one of the most important observations in the study. It means that the Libyan COI haplotype is either identical to, or indistinguishable from, a haplotype present in Spain in the analysed COI region. This finding can be interpreted in several ways. First, it may reflect historical connectivity between the western Mediterranean and North African populations. Second, it may reflect recent or ongoing gene flow through larval dispersal. Third, it may reflect retention of an ancestral haplotype that is shared across different Mediterranean regions. Fourth, it may be influenced by limited sampling, because only one Libyan sequence and a small number of reference sequences were included. For this reason, it is better to avoid saying that the Libyan fish definitely originated from Spain or that Spain is certainly the ancestor. COI alone cannot prove a geographic ancestor. A safer interpretation is that the Libyan sequence has a close COI affinity with two Spanish reference sequences and may share a recent maternal lineage or retained ancestral haplotype with western Mediterranean *M. rubra*.

Low divergence within the core Mediterranean lineage

The supported core *M. rubra* group showed extremely low pairwise distances, with a range from 0.0000 to 0.0018 and a mean of approximately 0.0009. This means that most reference sequences in the core group are identical or nearly identical over the analysed COI region. Such low variation is common in many marine fish barcode datasets. COI is usually good for species identification, but it may not always contain enough variation to resolve fine population structure. The shared haplotype including Haifa, Tunisia, Turkey, and Lebanon suggest that one COI haplotype may be widespread in the eastern and southern Mediterranean. The Libyan-Spanish haplotype branch may represent another very close haplotype. The difference between these two haplotypes is very small, about 0.0018. In a 661-bp fragment, this approximately corresponds to only one or a few nucleotide differences, depending on model correction. This level of difference is not enough to suggest species-level divergence. It is better treated as shallow intraspecific variation. For conservation, shallow variation still matters. If Libyan waters contain rare or under-sampled haplotypes, these should be documented before fisheries pressure or habitat change reduces local diversity. Conservation is not only about protecting species names; it is also about protecting local genetic variation and evolutionary potential [19, 20]. However, stronger conservation conclusions require more samples from the Libyan coast, including different landing sites, seasons, and size classes.

Divergent nominal records and probable misclassification

The strongest taxonomic result of the study is the separation of three nominal *M. rubra* sequences from the main *M. rubra* lineage. These were KJ709569.1 from Italy, MH707831.1 from Spain, and MW575709.1 from Tunisia. Both ML and NJ trees placed them outside the supported *M. rubra* cluster. The pairwise distances were also very high. Distances between the core *M. rubra* lineage and these three records ranged from 0.2099 to 0.2317, with a mean of about 0.2171. These values are far too high for normal intraspecific COI variation in the same fish species. This pattern strongly suggests that the three records are not true *M. rubra* COI sequences, at least not in the same sense as the supported core lineage. The tree labels identify them as misclassification lineages. Based on the study note, these sequences are interpreted as genetically corresponding to *Epinephelus costae* rather than *M. rubra*. This interpretation is biologically plausible because Mediterranean groupers can be difficult to identify, and public sequence databases can contain wrong names. However, because the trees did not include authenticated *Epinephelus costae* reference sequences, the statement should be written carefully. The most defensible wording is that these three nominal *M. rubra* records are probable misidentified records and are *E. costae*-like; final confirmation requires direct comparison with verified *E. costae* sequences and voucher specimens. This is important for future research. If misidentified sequences remain in GenBank or are used as references in later studies, they can cause wrong species assignments. For example, a true *M. rubra* query may appear very distant from a mislabeled *M. rubra* reference, or an *E. costae* specimen may be incorrectly assigned to *M. rubra*.

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Such errors affect biodiversity surveys, food authentication, fisheries statistics, and conservation planning. Previous studies have already warned that database errors can create error cascades in biological research [7, 8, 10] (Harris, 2003; Bortolus, 2008; Meiklejohn et al., 2019; Pentinsaari et al., 2020).

Taxonomic, forensic, and conservation implications for Libya

For Libya, the result has two taxonomic implications. First, the Libyan sequence KX255748.1 is consistent with true *M. rubra* and can be used as a Libyan reference barcode. Second, the study shows that regional reference libraries must be checked carefully before they are used for identification. If a Libyan researcher downloads all GenBank sequences labelled as *M. rubra* without checking their phylogenetic position, the dataset may include incorrect records. This can distort trees, distance estimates, and species assignment. A practical recommendation is that future Libyan studies should use a curated reference dataset. A curated dataset should include only sequences that cluster with verified *M. rubra*, have reasonable COI distances, and ideally have voucher specimens or published source information. Suspect records should not be removed silently; they should be flagged as questionable and tested against authenticated sequences of related species, especially *Epinephelus costae*, *Epinephelus marginatus*, *Epinephelus aeneus*, and other regional groupers. This is also relevant to forensic science. COI barcoding has clear forensic applications in fish identification. In seafood fraud, illegal trade, or disputed fish labeling, a wrong reference library can produce a wrong legal or regulatory conclusion [21, 22]. Therefore, molecular identification should combine COI, curated databases, voucher information, and, when necessary, nuclear markers. The conservation value of this study lies in documenting the Libyan COI identity of *M. rubra* and showing that the Libyan sequence belongs to a Mediterranean genetic background. The Libyan sequence is not deeply divergent, but it represents a local genetic record from an under-sampled coast. Under-sampled regions are important because the absence of data can look like the absence of diversity. Libya has a long Mediterranean coastline, and it may contain important habitats for groupers, including rocky reefs and coastal fishing grounds. More sampling is needed before the structure of Libyan *M. rubra* populations can be understood. The agreement between ML and NJ increases confidence in the main result. Both methods placed the Libyan sequence inside the core *M. rubra* lineage. Both methods also separated the three divergent nominal records. This is useful because ML and NJ are based on different principles. ML is model-based and evaluates the probability of the data under different trees, while NJ is distance-based and clusters sequences according to genetic distances. The shallow Libyan-Spanish branch had bootstrap support below 70 in both trees, so the exact arrangement of these very similar haplotypes should be interpreted cautiously. In contrast, the separation between the core *M. rubra* lineage and the divergent nominal records was strongly supported.

Conclusions

This study placed the Libyan *Mycteroperca rubra* COI sequence KX255748.1 within a Mediterranean phylogenetic framework using ML and NJ analyses under the Tamura 3-parameter model. The Libyan sequence was grouped within the supported core *M. rubra* lineage and showed very low genetic distance from Mediterranean references. It was identical across the analysed COI region to two Spanish sequences, suggesting a close maternal haplotype affinity with western Mediterranean records. Three nominal *M. rubra* sequences from Italy, Spain, and Tunisia formed a separate divergent lineage and are best treated as probable misidentified, *Epinephelus costae*-like records until confirmed by voucher-based and comparative analysis. The results support the usefulness of COI barcoding for Libyan grouper taxonomy and highlight the need for curated Mediterranean reference libraries. Future studies should include more Libyan samples, verified vouchers, additional mitochondrial and nuclear markers, and haplotype network analysis to better understand connectivity, local diversity, and conservation units of *M. rubra* in Libya.

Compliance with Ethical Standards

Disclosure of conflict of interest: The authors declare that they have no conflict of interest. Ethical approval: This study used publicly available GenBank sequence data and did not involve live animal handling, experimental procedures, or newly collected biological specimens. Therefore, ethical approval was not required.

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