BULLETIN OF THE IRAQ NATURAL HISTORY MUSEUM
Iraq Natural History Research Center & Museum, University of Baghdad
https://jnhm.uobaghdad.edu.iq/index.php/BINHM/Home
 Online ISSN: 2311-9799, Print ISSN: 1017-8678

Bull. Iraq nat. Hist. Mus.
(2026) 19 (1): 1-13. https://doi.org/10.26842/binhm.7.2026.19.1.0001

ORIGINAL ARTICLE

DNA BARCODING OF ABU-ZUMMAIR *MYSTUS PELUSIUS* (SOLANDER, 1794) (PISCES, SILURIFORMES, BAGRIDAE) FROM TIGRIS RIVER, IRAQ

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Received: 26 July 2025, Revised: 5 Dec. 2025, Accepted: 7 Dec. 2025, Published:20 June 2026

ABSTRACT

A total of three specimens of Tigris mystus *Mystus pelusius* (Solander, 1794) were collected from the Tigris River at the Gherai'at region in Baghdad Province in May 2024. Several species in the family Bagridae are rather morphologically similar, especially during the juvenile stage, which may pose difficulties in their identification. The present study aims to identify the fish and determine its ancestry at the molecular level. The mitochondrial gene (*cob*) functions as a barcoding gene. It included a designated primer set with PCR amplification to align a *cob* gene fragment with sequences in NCBI using BLAST. The molecular testing of current specimens of *M. pelusius* and the phylogenetic tree demonstrate that the *cob* gene is effective for the genetic identification of *M. pelusius*, with samples from India and Indonesia showing the closest lineage 86% to the original ancestor of *M. pelusius*. The results demonstrate that the chosen *cob* gene functions as a reliable marker for clarifying the origin of *M. pelusius* in the Iraqi aquatic ecosystem. This project constitutes an ongoing examination of fish in Iraqi aquatic environments. The mitochondrial gene *cob* was recorded for the first time to identify this fish, and the results were registered as references in the database (ID: PV535328, PV535329, PV535330) in the NCBI GenBank.

Keywords: Abu-zummair, Bagridae, Gene fragment, Gherai'at, Scaleless.

INTRODUCTION

Tigris mystus, or so called locally Abu-zummair *Mystus pelusius* (Solander, 1794) is a freshwater fish belonging to the family Bagridae. It is characterized by the existence of barbles and a scaleless body similar to other Silurid fishes (Coad, 2010; Hadi, 2023), which is why previous authors like Artedi (1738) and Linnaeus (1758) named it *Mystus*. This species inhabits the inland waters of Iraq, Turkey, Iran, and Syria. According to Mahdi and George (1969) and Al-Daham (1977), there are two species of *Mystus* in Iraq included *M. colvillii*

(Günther, 1874) and *M. pelusius*, which are both referred to locally as abu-zummair. Khalaf (1961) previously reported the existence of *M. pelusius* only. *M. colvillii* (Günther, 1874) is now considered a synonym of *M. pelusius* (Coad, 2010; Abed, 2016), in contrast to the suggestion made by Hora and Misra (1943) for the confirmation validity of *M. halepensis culvillii*, or described the specimen of Hora and Misra (1943) and described as *M. misrai* by Anuradha (1986). However, *M. misrai* resurrected by Freyhof and Yoğurtçuoğlu (2023) and considered it as valid species. The morphological similarity of some species in the family Bagridae, particularly at the juvenile stage, may make identification challenging. The genus *Mystus* Scopoli, 1777 is difficult to identify due to the morphological similarity and the lack of clear diagnostic features (Ferdous, 2013). Since DNA barcoding is a molecular-level study used to create a systematic identification key, it is superior to morphological identification. It can help correct misidentification in the field, improve the accuracy of species identification, create open-access databases, and increase technical expertise by enabling taxonomists to accurately sort specimens and highlighting divergent taxa that may represent new species (Hebert *et al*., 2003). The cytochrome b (*cob*) gene is one of the eleven proteins that make up complex III. The *cob* gene's sequence diversity makes it valuable within families and is commonly used to identify evolutionary relationships between different groups by locating mitochondrial DNA (Caine *et al*., 2006; Strüder-Kypke and Lynn, 2010).

Due to the paucity of research on bagrid fish broadly and this species specifically, the primary objective of the current study is to validate the identity of *M. pelusius* using molecular features, with phylogenetic analysis, and to improve genomic information about this fish which represents the only species of Bagridae in Iraq.

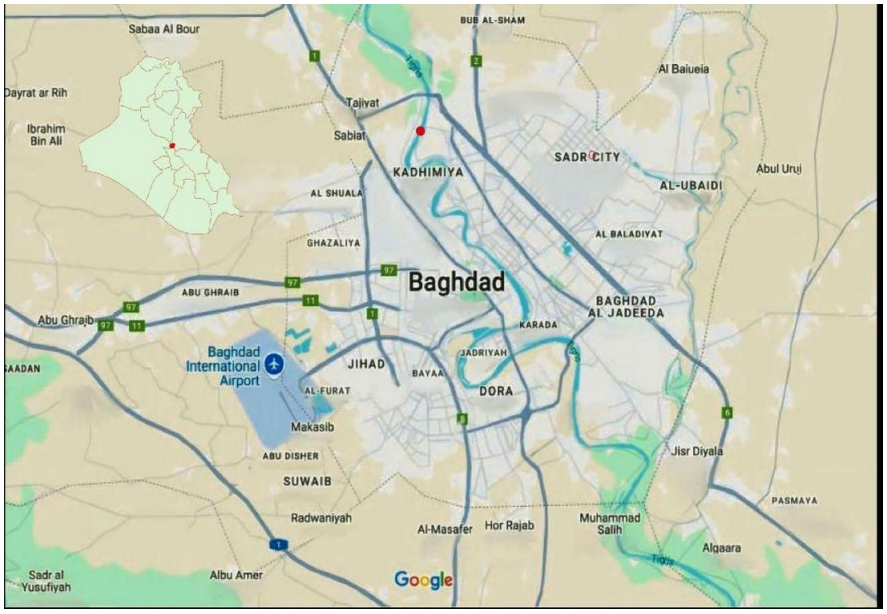
MATERIALS AND METHODS

Study area: The Gherai´at region is an old residential neighborhood on the northern side of Baghdad, along the Tigris River, which is known for its extensive agricultural activities, within coordinates of 33° 24' 31.7014" N latitude and 44°20'24.104" E longitude. The sampling location is depicted on the map of Iraq (Map 1).

Specimen collection: Three adult Abu-zummair *Mystus pelusius* were caught by fishermen using gill nets during May 2024. The fish were taken to the laboratory in the Iraq Natural History Research Center and Museum-University of Baghdad in a temperature-controlled container with crushed ice. Abu-zummair *Mystus pelusius* was morphologically identified according to Coad (2010), and their common names are classification were verified were made based on Froese and Pauly (2025) and Eschmeyer's Catalog of Fishes (Fricke *et al*., 2025).

DNA Extraction: Genomic DNA was isolated from three *M. pelusius* specimens separately using a DNA extraction kit (addbio / Korea, Cat. no. 10023) by cutting the right dorsal side of the pectoral fin (around 20 mg), then immediately storing it in absolute ethanol. Genomic DNA extraction was performed following the protocol of Sambrook and Russell (2001) then examined on a 1% agarose gel electrophoresis. A UV transilluminator was used to view the gel. NanoDrop (Quawell, USA) was used to assess 2 µl of each DNA sample at two

wavelengths (260 and 280 nm) in order to measure the concentration and purity of the extracted DNA for the *cob* gene.



Map (1): Sampling area (red spot).

Amplification and polymerase chain reaction PCR: Approximately, 508 bp were amplified from the 5' region of the *cob* gene using universal primers. The forward primer Fish F1 5' (CAAGCCTACGAAAAACMCAC) 3' and the reverse primer 5' Fish R1 (TCTACTGAGAAKCCRCCTCA) 3' were designed and selected by U Gene lab. A total reaction volume of 25 µl was prepared containing 3 µl DNA templates, 13 µl GoTaq® G2 Green Master Mix (Promega / USA), 1 µl for each primer and 7 µl double deionized water (ddH2O) in an Optimus 96G thermal Cycleras shown in Table (1).

As indicated in the Diagram (1), the temperature profile comprised an initial denaturation at 94ºC for 5 minutes, 35 cycles of 94ºC for 30 seconds, annealing at 54ºC for 30 seconds, extension at 72ºC for 1 minute, and a final extension of 72ºC for 5 minutes. The temperature was then held at 4ºC. For the analysis of the PCR results, 1.5% agarose gel electrophoresis was performed for 60 minutes at 70 volts. Sanger sequencing was used to sequence the PCR products on both strands at Macrogen (South Korea).

Table (1): Preparation of PCR Reaction compounds.

Reagents	Volume (µl)
Master Mix	13
Primer forward	1
Primer reverse	1
DNA template (30-100ng)	3
dd H2O (deionized water)	7
Total	25

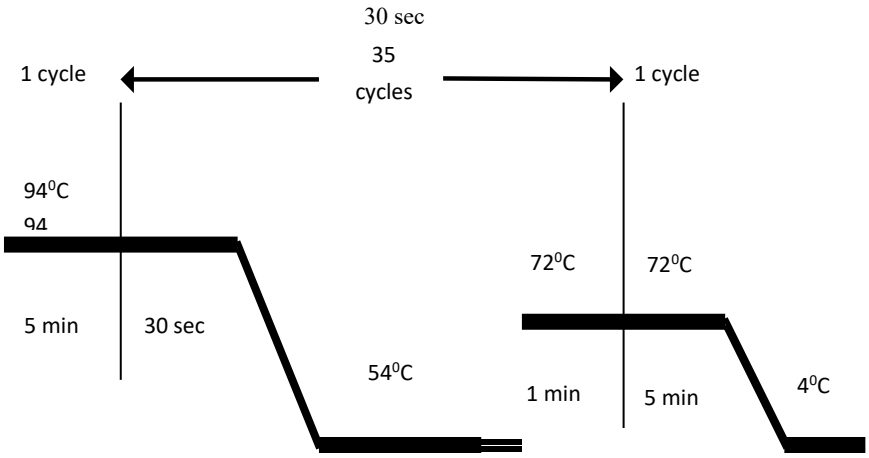


Diagram (1): The thermal cycling conditions of (*cob*) gene used for amplification.

Data analysis: The obtained amplicon has been sent to Macrogen in South Korea together with the forward and reverse primers. The data were evaluated utilizing Bioedit software and were presented as specialized files (Hall, 1999). The information from the genes of the same organism, is identified in the National Center for Biotechnology Information's (NCBI). GenBank which was also originally analyzed in various countries through the Basic Local Alignment Search Tool (BLAST), and was additionally compared with the alignment produced by sequencing results. It revealed an 86% similarity to another species of *Mystus*: *M. artfasciatus* Fowler, 1937 and *M. cavasius* (Hamilton, 1822). Using MEGA X: Molecular Evolutionary Genetics Analysis (Kumar *et al*., 2018). The phylogenetic tree of the species was created using the sequence data of the specimen being researched and compared with the species that had already been studied. Using the maximum likelihood (ML) method to calculate the nucleotide location value of variance, a molecular phylogenetic tree was produced. The precision of the generated phylogenies was determined utilizing the 500-repeat bootstrap method (Tamura *et al*., 2021).

RESULTS

A total of three mature, Abu-zummair *Mystus pelusius* were collected from the Tigris River of Baghdad Province. The length and weight are shown in Table (2).

Order: **Siluriformes**
Family: **Bagridae**
Mystus pelusius (Solander, 1794) (Pl. 1).
Common names: Tigris mystus, Abu-zummair
Synonyms: *Bagrus halepensis* Valenciennes, 1840
Macrones aleppensis Günther, 1864
Macrones colvillii Günther, 1874
Silurus pelusius Solander, 1794

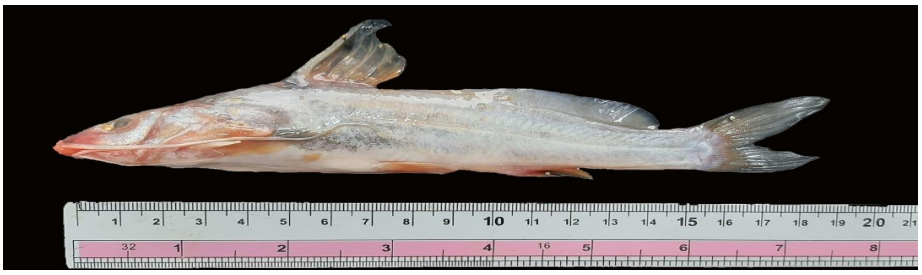


Plate (1): Abu-zummair *Mystus pelusius* in total length collected from Tigris River of Baghdad Province.

Table (2): The length and weight of *Mystus pelusius*.

Measurements	Fish 1	Fish 2	Fish 3
Weight (gm)	39.7	49.1	37.2
Total Length (TL) mm	222	229	190
Fork Length (FL) mm	202	203	165
Standard Length (SL) mm	191	192	155

The target gene of *Mystus pelusius* was PCR amplified, and the products were electrophoresed and visualized by a UV transilluminator. The result indicated that the size of amplified gene was 508 bp for the *cob* gene (Pl. 2). Sequencing of this gene was performed to identify the genotype of *M. pelusius* obtained from the Gherai´at region. As part of the prerequisites for the sequential approach and the application of PCR technology within the context of genetic analysis methodology, the sequence of a single gene was evaluated using both forward and reverse primers. The nucleotide alignment findings with the sequences in the GenBank indicated that identities varied to 86% with *Mystus* species (Tab. 3).

Partial cds and mitochondrial cytochrome b gene were matched with an identical sequence fragment marker accessible from the National Center for Biotechnology Information (NCBI) Gene Bank. Diagram (2), displays the fish specimens' partial sequencing and pairwise analysis. The current findings indicate an average of 508 bp at the 5' end for the *cob* mtDNA region among three specimens, and the optimal likelihood tree derived from partitioned maximum likelihood analysis revealed the genetic relationships among the examined samples, as illustrated in Diagram (3), displaying two sub-branches. One branch indicated that the local sample M1 (*M. pelusius*) formed a sister group to the local sample M2 (*M. pelusius*). A genetic relationship was identified among the local samples (M1 and M2) and the original

sequences of *M. atrifasciatus* Fowler, 1937 from India (KF862957) and *Mystus cavasius* (Hamilton, 1822) (OP588457.1) but the local sample (M3) showed genetic affinity with *Mystus bocourti* (Bleeker, 1864) (HQ257290) from Thailand that was derived from the same gene. Another branch showed that the local samples (M1, M2 and M3) exhibiting a resemblance to the conventional sequences of *Mystus* sp., *M. albolineatus* Roberts, 1994 and *M. vittatus* (Bloch, 1794) of the same gene from Indonesia (OM858672), Vietnam (PQ412709), and the United Kingdom (DQ119356) respectively.

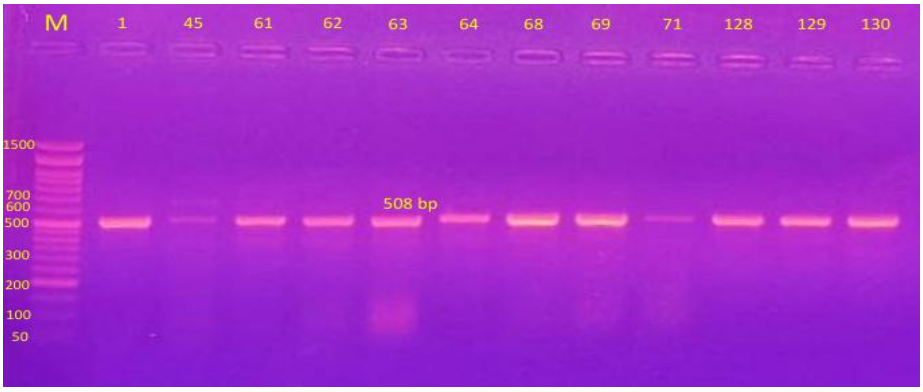


Plate (2): PCR products of the amplification of the partial region of the gene *cob* of *Mystus pelusius*.

Table (3): Identity results for *Mystus pelusius* our isolates with reference copy (NCBI).

Specimen ID	Accession Number	Percentage of Identity %	Country	Scientific Name	Reference (NCBI data)
M 1	PV535328	86.95	India	*Mystus atrifasciatus*	KF862957
		86.68	Indonesia	*Mystus* sp.	OM858672
M 2	PV535329	86.20	India	*Mystus cavasius*	OP588457.1
M 3		86.16	Vietnam	*Mystus albolineatus*	PQ412709
	PV535330 *Mystus pelusius*	85.90	Thailand	*Mystus bocourti*	HQ257290
		85.90	United Kingdom	*Mystus vittatus*	DQ119356

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                DQ119356-Mystus-vittatus-United-Kingdom
TTTTAACCGGACTATTCCTAGCCATACACTACACTTCTGATATTTCCACTGCCTTTTCAT      60
                  KF862957-Mystus-atrifasciatus-India
TCTTAACCGGACTCTTCCTAGCCATACACTACACCTCTGACGTCTCCACCGCCTTTTCAT      60
                    OP588459-Mystus-cavasius-India
TCCTAACTGGACTCTTCCTAGCCATACACTACACCTCAGACGTCTCCACCGCCTTTTCAT      60
                    PV535330-Mystus-pelusius-Iraq
TTTTAACCGGACTCTTCCTAGCCATACACTACACCTCAGATATCTCCACCGCCTTTTCAT      60
                    PV535328_Mystus_pelusius_Iraq
TTTTAACCGGACTCTTCCTAGCCATACACTACACCTCAGATATCTCCACCGCCTTTTCAT      60
                    PV535329-Mystus-pelusius-Iraq
TTTTAACCGGACTCTTCCTAGCCATACACTACACCTCAGATATCTCCACCGCCTTTTCAT      60
                  HQ257290-Mystus-bocourti-Thailand
TTTTAACAGGACTCTTCCTAGCCATACATTACACCTCAGACATTTCCACCGCCTTCTCAT      60
                    OM858672-Mystus-sp.-Indonesia
TCCTAACCGGACTCTTCCTAGCTATACACTACACCTCAGATATCTCCACCGCCTTTTCAT      60
                  PQ412709-Mystus-albolineatus-Vietnam
TTCTAACTGGACTCTTCCTAGCCATACACTACACCTCAGATATTTCTACTGCATTCTCAT      60
    *  **** ***** ******** ***** ***** ** **  * ** ** ** ** ****

                DQ119356-Mystus-vittatus-United-Kingdom
CCGTGGCCCACATTTGCCGAGACGTAAACTACGGCTGAGTCATCCGAAATCTTCACGCCA      120
                  KF862957-Mystus-atrifasciatus-India
CCGTAGCCCACATTTGCCGAGACGTAAACTACGGCTGAATCATCCGAAACCTGCACGCCA      120
                    OP588459-Mystus-cavasius-India
CCGTAGCCCACATCTGCCGAGATGTAAACTACGGATGAATTATCCGAAACCTGCACGCCA      120
                    PV535330-Mystus-pelusius-Iraq
CCGTAGCCCATATTTGCCGGGACGTAAACTCCGGCTGAGTTATCCGAAACCTACACGCCA      120
                    PV535328_Mystus_pelusius_Iraq
CCGTAGCCCATATTTGCCGGGACGTGAACTACGGCTGAGTTATCCGAAACCTACACGCCA      120
                    PV535329-Mystus-pelusius-Iraq
CCGTAGCCCATATTTGCCGGGACGTAAACTACGGCTGAGTTATCCGAAACCTACACGCCA      120
                  HQ257290-Mystus-bocourti-Thailand
CCGTGGCCCACATTTGCCGAGACGTAAATTATGGATGAGTTATCCGTAACCTGCACGCCA      120
                    OM858672-Mystus-sp.-Indonesia
CCGTAGCACACATTTGCCGAGACGTAAACTACGGATGAGTCATCCGAAATCTGCACGCCA      120
                  PQ412709-Mystus-albolineatus-Vietnam
CCGTAGCACATATTTGCCGAGACGTAAACTACGGATGAGTTATCCGAAACCTACATGCCA      120
    **** ** ** ** ***** ** ** ** *  ** *** * ***** ** ** ** ****

                DQ119356-Mystus-vittatus-United-Kingdom
ACGGAGCCTCCTTCTTCTTCATCTGTCTCTACCTACACATTGGGCGGGGTCTTTATTATG      180
                  KF862957-Mystus-atrifasciatus-India
ACGGAGCCTCTTTCTTCTTCATCTGTCTCTACTTGCACATTGGACGAGGCCTTTATTACG      180
                    OP588459-Mystus-cavasius-India
ACGGAGCCTCTTTCTTCTTCATTTGCATCTACCTACACATCGGACGAGGCCTCTACTACG      180
                    PV535330-Mystus-pelusius-Iraq
AAGGCGCCTCATTCTTCTTCATCTGCCTCTACATCCACATCGGACGGGGCCTCTACTATG      180
                    PV535328_Mystus_pelusius_Iraq
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Diagram (2): Pair wise alignment of partial cds, cytochrome b (*cob*) gene of *Mystus pelusius* Query is the study or sample sequence and subject is the GenBank sequence.

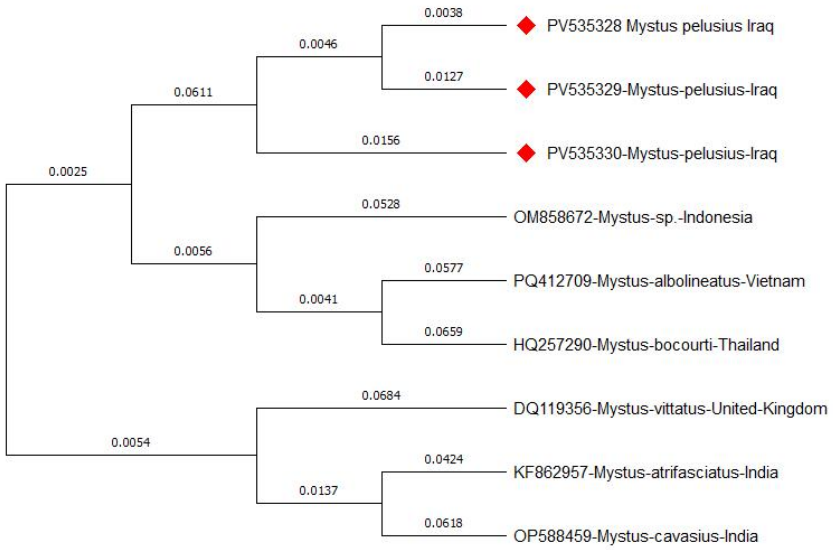

Diagram (3): Phylogenetic tree analysis of *Mystus pelusius* local samples resemble with other *Mystus* spp. in various countries, generated by using MEGA X with the Maximum Likelihood (ML) method and a bootstrap value of 500 replicates.

DISCUSSION

The external morphological traits were not studied in this study as a basis for traditional classification and identification. However, it is challenging to distinguish between different maturation stages of fish based solely on morphological traits (Victor *et al*., 2009). Consequently, mitochondrial DNA sequencing was used to validate the conventional identification and classification results. One of the essential techniques that facilitated rapid diagnosis of species was DNA sequencing. This study supports the notion that cob barcodes are an effective tool of identification of the species (Pons *et al*., 2006). The identification of individually separated freshwater fishes, larvae, etc., would obviously be possible with this technology, offering new resources for the practice of conservation and genetic forensics in these freshwater species. At the systematic position, *cob* barcodes offer an unusual and rapid method for determining the actual number of species with particular sets of diagnostic traits (Jong-Man, 2001). In order to identify species, the *cob* gene was PCR amplified using specially created primer pairs that demonstrated a strong complementarity to the conserved region. This finding was mostly consistent with prior research that employed universal primers as supplements to conserved regions within species' *cob* genes (Pons *et al*., 2006). The importance of freshwater fish in Iraqi water will be revealed by the registration of *M. pelusius* genes in the NCBI GenBank database. The molecular method was developed to offer high specificity and precision, as previous research on fish phylogeny utilized the mtDNA *cob* gene (Garg *et al*., 2009). The Tigris mystus fish used in this study were obtained, identified, and categorized using standard methods based on their outward appearance. Dependence on

morphological traits, a lack of taxonomic history, and confusion surrounding the origins of some species make it difficult to identify different species, which can make it difficult to identify native species accurately (Koutsikos *et al*., 2017).

Using mtDNA sequencing to determine the species variances, molecular data are crucial for the correct identifying native species (Garg *et al*., 2009). Species conservation is essential to prevent the loss of biodiversity. According to the current study, specimens of Tigris mystus from the Tigris River shared a common ancestor with those from India and Indonesia, as evidenced by the phylogenetic tree of the *cob* gene. The NCBI website listed 10 *M. pelusius* nucleotides worldwide, confirming the dearth of research on this species worldwide. The current study is considered to be the first to use the *cob* gene to study this species (NCBI, 2025). According to a phylogenetic study, *M. pelusius* is grouped with bagrid catfishes and other *Mystus* species. The new information on phylogenetic relationships and structural characterization is provided by this work. The chromosomal structure of *M. pelusius* from the Tigris River was studied by Al-Khayat and Sahan (2016) in Iraq. Using the cytochrome c oxidase subunit I (*COX1*) gene, Esmaeili *et al*. (2022) described *Mystus cyrusi* as a new species from southern Iran. Although this species shares many external appearance traits with *M. pelusius*, and *M. cyrusi* but it was *M. pelusius* was found to have the lowest molecular genetic distance (4.6%).

Kanthimathi *et al*. (2023) used the cytochrome c oxidase I (*COI*) gene to study the molecular characterization of three species of *Mystus*. They discovered that *Mystus montanus* (Jerdon, 1849) differs slightly from the other two species, while *Mystus gulio* (Hamilton, 1822) and *Mystus cavasius* (Hamilton, 1822) have similar sequence patterns and morphometric characteristics. The current results are consistent with the research of Roy *et al*. (2024), who used mitochondrial sequencing of *M. cavasius* in India. The Phylogenetic analysis shows that *M. cavasius* is grouped with bagrid catfishes and other *Mystus* species. The current work shows that DNA barcoding may be an effective method for identifying freshwater fish species, especially the species complex of small-sized species, and that *cob* barcoding could eventually be applied to ecology and systematics.

CONCLUSIONS

The results of this study serve as reference sequences in the NCBI database (ID: PV535328, PV535329, PV535330), and confirm the taxonomic identity of *M. pelusius* in Iraqi inland waters. It is also one of the first studies on freshwater fish in Iraqi water to deposit its findings in the NCBI GenBank using the *cob* gene. Given the limited research on silurid fishes in general and on this species in particular, our findings suggest that molecular data could be utilized for the categorization of related fish species and to provide genomic information about Iraqi fishes.

ACKNOWLEDGMENTS

We are deeply grateful to Prof. Dr. Hula Y. F. Al-Sadi, College of Science, University of Baghdad, Dr. Azhar A. Jaffar, Marshes Research Center, University of Thi-Qar for their important guidance as well as their contributions to the molecular studies, and Dr Ali T.

Yaseen, Marine Science Centre. University of Basrah for his contribution in illustrating the map.

CONFLICT OF INTEREST STATEMENT

"The author has no conflicts of interest to declare".

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ترميز الحمض النووي لسمكة أبو الزمير ***Mystus pelusius*** **(Solander, 1794) (Pisces, Siluriformes, Bagridae) في نهر دجلة، العراق**

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الاستلام: 2025/7/26، المراجعة: 2025/12/5، القبول: 2025/12/7، النشر: 2026/6/20

الخلاصة

جمعت ثلاثة نماذج من سمكة ابو الزمير *Mystus pelusius* (Solander, 1794) من نهر دجلة في منطقة الكريعات، شمال محافظة بغداد في ايار 2024. تتشابه العديد من الانواع التي تعود الى عائلة ابو الزمير Bagridae من الناحية المظهرية، خاصة في المرحلة اليافعة، مما يصعب تحديد تشخيصها. هدفت الدراسة الحالية الى تحديد هوية هذه السمكة واكتشاف سلفها الاصلي.

استخدم في هذه الدراسة جين المايتوكوندريا (*cob*)الرمز الشريطي وبادئ محدد وتم تضخيم PCR لمواءمة جزء من جين *cob* مع التسلسلات في قاعدة بيانات NCBI عبر تقنية BLAST، يشير التحليل الجزيئي لسمكة ابو الزمير وشجرة النسب التطورية إلى أن جين *cob* أكثر فعالية في تحديد الهوية الوراثية لهذا النوع، حيث تمثل العينات القادمة من الهند واندونيسيا السلالة الأقرب إلى السلف الأصلي لأبو الزمير. تشير النتائج إلى أن الجينات المختارة من *cob* تُعد مؤشرات فعالة لتوضيح أصل ابو الزمير في البيئة المائية العراقية. تمثل هذه الدراسة تحقيقاً متطورًا حول الأسماك في المياه العذبة العراقية، سُجل في الدراسة الحالية هذا الجين لأول مرة لتحديد هوية هذه السمكة وتم تثبيت النتائج كمرجع في قاعدة بيانات (ID: PV535328, PV535329, PV535330) ضمن بنك الجينات الخاص بقاعدة بيانات NCBI.