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.
(2025) 18 (4): 801-814. https://doi.org/10.26842/binhm.7.2025.18.4.0801

ORIGINAL ARTICLE

DNA BARCODING OF NORTH AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) (SILURIFORMES, CLARIIDAE) FROM TIGRIS RIVER, IRAQ

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Received: 1 May 2025, Revised: 2 Aug. 2025, Accepted: 5 Aug. 2025, Published:20 December 2025

ABSTRACT

The conservation for biodiversity in Iraqi freshwater environments is important to protecting native species from the environmental impacts of alien species. *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae) has been recognized as an alien species in Iraqi water bodies. This study aims to use molecular DNA to identify this catfish and trace its origins using. The DNA sequences of *C. gariepinus* were done using the mitochondrial DNA cytochrome c oxidase subunit 1 *(COI*) gene, and a specific primer set. The polymerase chain reaction (PCR) amplification was used to align the *COI* gene as a barcoding marker. After analysis, the sequences were compared with sequences in the National Center for Biology Information (NCBI) database using BLAST. Molecular analysis and genetic sequence reconstruction revealed that the *COI* gene is instrumental in the genetic identification of *C. gariepinus*. The phylogenetic tree indicated a close genetic link between the Iraqi samples and populations from China and North Korea, suggesting that these may represent the closest known lineages to the origin of this species in Iraq. The findings showed that the selected *COI* gene is a reliable indicator for tracking the origin of alien catfish populations in the Iraqi environment. This study contributes to the development of molecular detection of alien species in Iraq. The accession numbers LC868421 and LC868422 were employed to submit the sequences to the NCBI GenBank database.

Keywords: Commercial, Culture, Forward primer, Reproduction, Shwaka.

INTRODUCTION

North African catfish, *Clarias gariepinus* (Burchell, 1822), is a freshwater Clariid fish which occupies tropical and subtropical areas (Omitoyin, 2007; Barasa *et al.*, 2014). It is a commercial fish, and commonly cultured in beyond sub-Saharan Africa; This catfish is subjected to variation of physicochemical criteria including human interventions, harsh

ecological environment e.g. temperature and salinity, accordingly it leads to an imbalance in the ecosystem (Chandra Segaran *et al*., 2023).

Their indigenous distribution includes lakes, reservoirs, as well as rivers throughout sub-Saharan Africa, which additionally was imported to South America, Southeast Asia, and Europe (Truter *et al*., 2023). The distribution of African catfish in Syria is confined to a few warm springs, with a production rate gradually increasing. Semi-intensive culture is prevalent in earthen ponds, whereas intensive culture in cages is restricted to small number of farms located in large reservoirs (FAO, 2025). Iraq is identified to be a member of the regions where this catfish has been introduced (Bartley, 2006; Froese and Pauly, 2024). De Silva *et al*. (2009) revealed that about 12% of aquaculture productivity consisted of non-native species fish. Nevertheless, increasing anthropogenic activities will make tolerant non-native fish into an invasive species. Singh and Lakra (2011) concluded that in spite of exhibiting appealing cultural traits, typically diminish the availability of native species and invade lakes and rivers, thereby negatively impacting fish biodiversity and aquatic environments. Numerous studies have focused on its growth, reproduction, and physiology due to its high economic value (Nwani *et al*., 2014). However, there is inadequate research regarding the genetic characteristics about this catfish, particularly its mitochondrial genome (Barasa *et al.*, 2014).

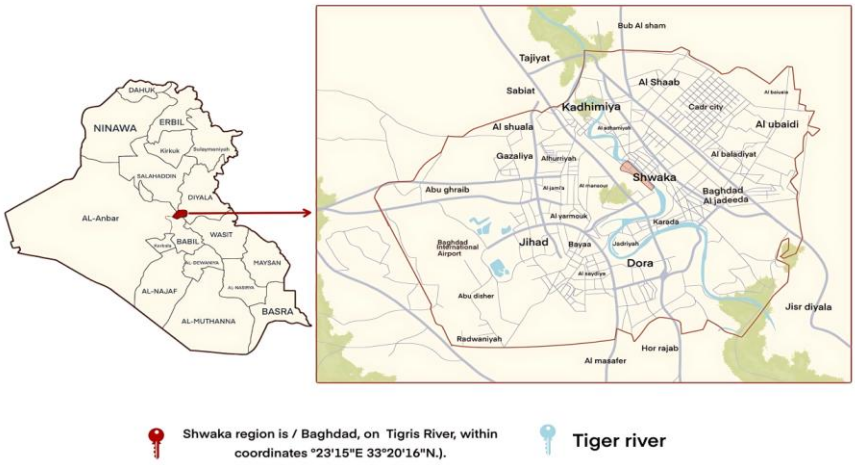
DNA analysis is a powerful tool for investigating species' genetic structure (Menezes *et al.*, 2012; Haldar and Nath, 2020). DNA barcoding was introduced as a swift, precise, automated, in addition to being globally accessible technique enabling species identification and differentiation (Hebert *et al.*, 2003). The procedure involves sequencing a short portion of a DNA barcode from an unidentified species; it would be compared with information from a barcode database from recognized species, consequently offering an alternate to morpho-taxonomy approach (Falade *et al.*, 2016). The vertebrate cytochrome c oxidase I (*COI*) gene shows a phylogenetic signal compared to numerous other genes (Boopathi *et al*., 2004; Strüder-Kypke and Lynn, 2010).

This study aims to use the *COI* gene to carry out a DNA identification with phylogenetic study of African catfish in Tigris River.

MATERIALS AND METHODS

Study area: The Shwaka Region is an old residential neighborhood next to Al-Karkh in the Baghdad, situated on both banks of the Tigris River, within coordinates 33°20′16″N, 44°23′15″E. Its location is shown on the map of Iraq (Map 1).

Sample collection: A total of two mature North African catfish, *Clarias gariepinus*, were collected by fishermen using gill nets in June 2024. The samples ranged in length from 550 to 630 mm and in body weight from 470 to 565 g. The fish were transported in a temperature-controlled container with crushed ice to the laboratory of the Iraq Natural History Research Center and Museum, University of Baghdad.



Map (1): Sampling areas.
(Designed by ArcGIS Online https://www.arcgis.com/index.html)

DNA Extraction: DNA was isolated from two specimens of *Clarias gariepinus*, using approximately 20 mg of muscle tissue from the right pectoral fin, which was immediately conserved within absolute ethanol (100%) with a DNA extraction kit (addbio/Korea, Cat. no. 10023). Genomic DNA was isolated according to Sambrook *et al.* (1989) then analyzed by 1% agarose gel electrophoresis, afterwards visualized using a UV transilluminator. The final concentration and purity of DNA extractions were assessed using NanoDrop by analyzing 2 µl of each DNA sample at two wavelengths (260 and 280 nm).

Amplification and polymerase chain reaction PCR: A fragment of approximately 455 base pairs was amplified from the 5' region of the *COI* gene using universal fish primers. The forward primer Fish F1 5’ (CTAGCAGGTGTCTCATCAATTCT) 3’ and the reverse primer Fish R1 5’ (GCTCGGGTGTCTACATCTATTC) 3’ are listed in Table (1).

The temperature profile involved the starting denaturation step around 94ºC about 5 minutes, afterwards undergoing 35 cycles of 94ºC for 30 seconds, 52ºC within 30 seconds, then 72ºC for 30 seconds, concluding with a final extension at 72ºC for 5 minutes followed by a hold at 4ºC. The PCR results were analyzed using 1.5% agarose gel electrophoresis at 70 volts throughout 60 minutes. The PCR products were transferred to Macrogen (Korea) for bidirectional sequencing using the Sanger method.

Table (1): Details of Polymerase Chain Reaction composition

Component	Component Volume
GoTaq Green Master mix	25 µL
F primer	2 µL
R primer	2 µL
Template DNA	4 µL
Nuclease-free water	17 µL
Total reaction volume	50 µL

Data analysis: The sequencing of the PCR products was sent to Macrogen / Korea using the forward and reverse primers. The findings have been analyzed via Bioedit software. Data of a similar organism's genomes located in the National Center for Biotechnology Information (NCBI) GenBank which had already been studied in numerous countries around the world utilizing the Basic Local Alignment Search Tool (BLAST), were compared through multiple sequence alignment. Sequencing results indicated 97–99% concordance with reference sequences by Molecular Evolutionary Genetics Analysis (MEGA X), and a molecular phylogenetic tree was constructed through Maximum Likelihood (ML) to estimate genetic variation among sequences. The degree of precision of the derived phylogenies was evaluated using the 500 bootstrap replicates (Kumar *et al*., 2018).

RESULTS

A total of two mature *Clarias gariepinus* were identified, following the classification established by Hadi *et al.* (2024).

Order: Siluriformes
Family: Clariidae
Clarias gariepinus (Burchell, 1822) (Pl. 1).
Common names: North African catfish.
Synonyms: *Silurus gariepinus* Burchell, 1822
Macropteronotus charmuth Lacepède, 1803
Clarias capensis Valenciennes, 1840.

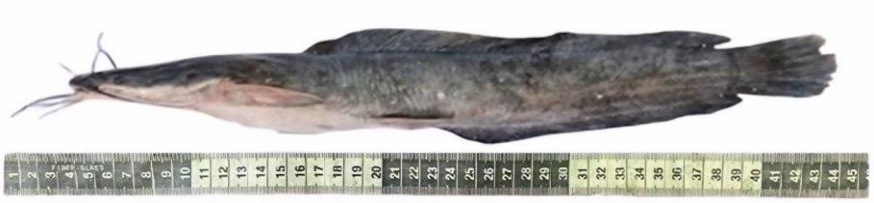


Plate (1): Lateral view of *Clarias gariepinus*.

The selected gene of *C. gariepinus* underwent PCR amplification, followed by electrophoresis of the products, which were subsequently visualized using a UV transilluminator. The final amplified product was 455 bp in size for the *COI* gene.

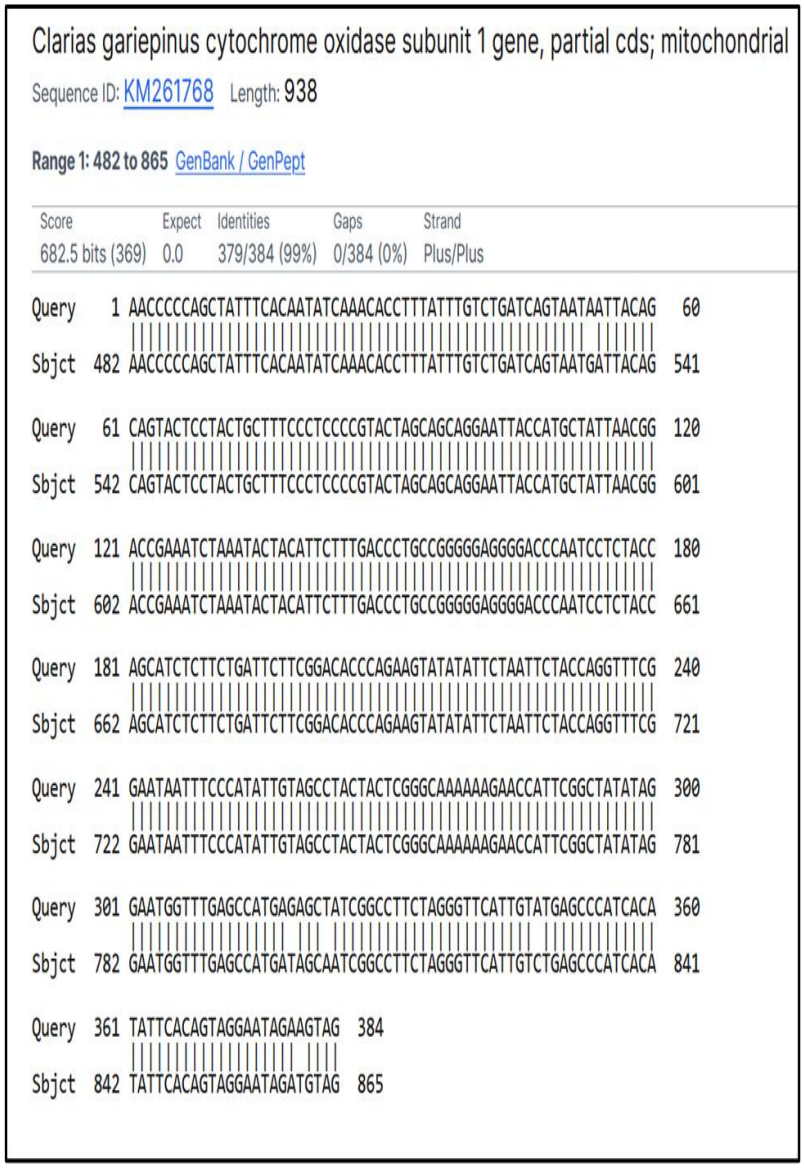
Sequencing of the *COI* gene was conducted to ascertain the genotype of *C. gariepinus* obtained from Baghdad. The examination of a gene's sequence involves the use of forward and reverse primers, which are essential components of the technique of sequencing. The findings from the nucleotide alignment showed 97 to 98% identity with *C. gariepinus* sequences in GenBank showing the identities varied from 97% to 98% with *C. gariepinus* (Tab. 2).

Partial cds of the mitochondrial gene *COI* were matched with the corresponding reference sequence accessible within the Gene Bank at the NCBI. Diagrams (1, 2) exhibited that the partial sequence in addition to the pairwise sequence comparison was performed on the sequenced samples.

The current findings revealed a total of 455 base pairs at the 5' end for the *COI* mtDNA region in two sequenced samples. The optimal phylogenetic tree was derived from partitioned maximum likelihood; the analysis illustrated the genetic relationships among the studied samples, as depicted in Diagram (3), showing two sub-branches. One branch showed that the local sample C1 (*C. gariepinus*) constituted a sister group to the local sample C2 (*C. gariepinus*). A genetic affinity was detected between the local samples (C1 and C2) and the reference sequences of *C. gariepinus* from North Korea (KM261768) and China (NC_027661), derived from the same gene. Another branch shown to be local samples (C1 and C2) exhibited a resemblance to the conventional sequencing of *C. gariepinus* for the identical gene from Hungary (KT809508.1), Germany (XM_053485204), United Kingdom (PQ197863.1), and Netherlands (XM_053485208.1).

Table (2): Sample IDs showing Similarity Searches in Sequence Alignment

Sample ID	Accession Number for our study	Percentage of Identity %	Country	Scientific Name	Reference copy (NCBI data)
C1	LC868421	98.72%	China	*Clarias gariepinus*	NC_027661
		98.72 %	North Korea	*Clarias gariepinus*	KM261768
		97.19 %	Hungary	*Clarias gariepinus*	KT809508.1
		97.19 %	Germany	*Clarias gariepinus*	XM_053485204
C2	LC868422	99.08%	United Kingdom	*Clarias gariepinus*	PQ197863.1
		99.23 %	China	*Clarias gariepinus*	NC_027661
		99.23 %	North Korea	*Clarias gariepinus*	KM261768.1
		97.19 %	Netherlands	*Clarias gariepinus*	XM_053485208.1



Clarias gariepinus cytochrome oxidase subunit 1 gene, partial cds; mitochondrial

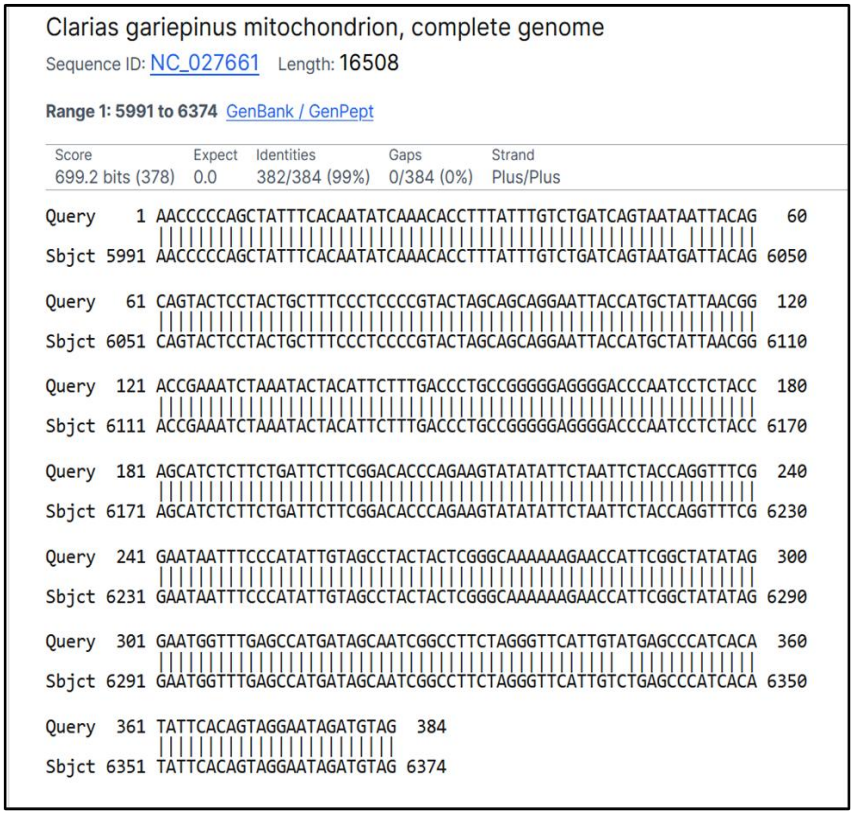
Sequence ID: KM261768 Length: 938

Range 1: 482 to 865 GenBank / GenPept

Score	Expect	Identities	Gaps	Strand
682.5 bits (369)	0.0	379/384 (99%)	0/384 (0%)	Plus/Plus

```
Query    1 AACCCCCAGCTATTTCACAATATCAAACACCTTTATTTGTCTGATCAGTAATAATTACAG   60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  482 AACCCCCAGCTATTTCACAATATCAAACACCTTTATTTGTCTGATCAGTAATGATTACAG  541

Query   61 CAGTACTCCTACTGCTTTCCCTCCCCGTACTAGCAGCAGGAATTACCATGCTATTAACGG  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542 CAGTACTCCTACTGCTTTCCCTCCCCGTACTAGCAGCAGGAATTACCATGCTATTAACGG  601

Query  121 ACCGAAATCTAAATACTACATTCTTTGACCCTGCCGGGGGAGGGGACCCAATCCTCTACC  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602 ACCGAAATCTAAATACTACATTCTTTGACCCTGCCGGGGGAGGGGACCCAATCCTCTACC  661

Query  181 AGCATCTCTTCTGATTCTTCGGACACCCAGAAGTATATATTCTAATTCTACCAGGTTTCG  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662 AGCATCTCTTCTGATTCTTCGGACACCCAGAAGTATATATTCTAATTCTACCAGGTTTCG  721

Query  241 GAATAATTTCCCATATTGTAGCCTACTACTCGGGCAAAAAAGAACCATTCGGCTATATAG  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  722 GAATAATTTCCCATATTGTAGCCTACTACTCGGGCAAAAAAGAACCATTCGGCTATATAG  781

Query  301 GAATGGTTTGAGCCATGAGAGCTATCGGCCTTCTAGGGTTCATTGTATGAGCCCATCACA  360
           |||||||||||||||||  ||| |||||||||||||||||||||| ||||||||||||||
Sbjct  782 GAATGGTTTGAGCCATGATAGCAATCGGCCTTCTAGGGTTCATTGTCTGAGCCCATCACA  841

Query  361 TATTCACAGTAGGAATAGAAGTAG  384
           ||||||||||||||||||| ||||
Sbjct  842 TATTCACAGTAGGAATAGATGTAG  865
```

Diagram (1): Pairwise alignment of partial coding sequences of the cytochrome c oxidase subunit 1 mitochondrial (*COI*) gene from *C. gariepinus* (C1) The Query is the specimen sequence, and the subject is the GenBank sequence.



Clarias gariepinus mitochondrion, complete genome

Sequence ID: NC_027661 Length: 16508

Range 1: 5991 to 6374 GenBank / GenPept

Score	Expect	Identities	Gaps	Strand
699.2 bits (378)	0.0	382/384 (99%)	0/384 (0%)	Plus/Plus

```
Query     1 AACCCCCAGCTATTTCACAATATCAAACACCTTTATTTGTCTGATCAGTAATAATTACAG   60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  5991 AACCCCCAGCTATTTCACAATATCAAACACCTTTATTTGTCTGATCAGTAATGATTACAG 6050

Query    61 CAGTACTCCTACTGCTTTCCCTCCCCGTACTAGCAGCAGGAATTACCATGCTATTAACGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6051 CAGTACTCCTACTGCTTTCCCTCCCCGTACTAGCAGCAGGAATTACCATGCTATTAACGG 6110

Query   121 ACCGAAATCTAAATACTACATTCTTTGACCCTGCCGGGGGAGGGGACCCAATCCTCTACC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6111 ACCGAAATCTAAATACTACATTCTTTGACCCTGCCGGGGGAGGGGACCCAATCCTCTACC 6170

Query   181 AGCATCTCTTCTGATTCTTCGGACACCCAGAAGTATATATTCTAATTCTACCAGGTTTCG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6171 AGCATCTCTTCTGATTCTTCGGACACCCAGAAGTATATATTCTAATTCTACCAGGTTTCG 6230

Query   241 GAATAATTTCCCATATTGTAGCCTACTACTCGGGCAAAAAAGAACCATTCGGCTATATAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6231 GAATAATTTCCCATATTGTAGCCTACTACTCGGGCAAAAAAGAACCATTCGGCTATATAG 6290

Query   301 GAATGGTTTGAGCCATGATAGCAATCGGCCTTCTAGGGTTCATTGTATGAGCCCATCACA  360
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  6291 GAATGGTTTGAGCCATGATAGCAATCGGCCTTCTAGGGTTCATTGTCTGAGCCCATCACA 6350

Query   361 TATTCACAGTAGGAATAGATGTAG  384
            |||||||||||||||||||||||
Sbjct  6351 TATTCACAGTAGGAATAGATGTAG 6374
```

Diagram (2): Pairwise alignment of partial cds, cytochrome *c* oxidase subunit 1 mitochondrial (*COI*) gene of *Clarias gariepinus* (C2) The Query is the specimen sequence, and the subject is the GenBank sequence.

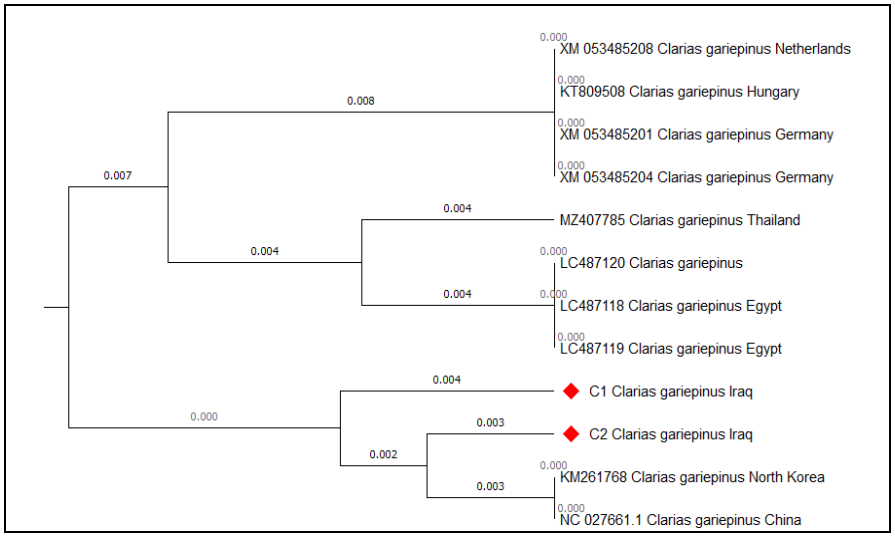


Diagram (3): Phylogenetic tree of *Clarias gariepinus* produced using partial sequences of the mitochondrial cytochrome c oxidase subunit I *(COI)* gene. The tree has been produced via the Maximum Likelihood (ML) method within MEGA X software, with 500 bootstrap replicates to assess node support. Local samples (C1 and C2) show high similarity to reference sequences from *C. gariepinus* originating from different countries.

DISCUSSIONS

Advancements in sequencing technology are becoming indispensable in ecology, evolution, and conservation through allowing swift species identification by DNA barcoding while markedly decreasing costs and time; mainly sequencing offers significant opportunities to biologists; it was aligned with developments in computing and sequencing technologies, which allowed for the rapid creation of a library of DNA barcodes applicable to all known species (Page, 2016; Gostel and Kress, 2022).

The present study demonstrated that the selected portions of the *COI* gene were chosen due to their extensive taxonomic representation in nucleotide databases. The *COI* gene has shown accuracy in studies of genetic variation and geographic distribution across numerous fish species. The findings have revealed that the slow mutation rate of the *COI* gene designates it as an optimal DNA marker for conducting genetic research (Antoniou and Magoulas, 2014). Many works have exhibited that DNA polymorphism for *C. gariepinus* is a little high (Galbusera, 1997; Barasa *et al*., 2014). Registration of *C. gariepinus* genes in the NCBI GenBank database will highlight the importance of monitoring exotic species in Iraq. The utilization of *COI* gene sequencing as a molecular marker was designed to provide high correctness and particularity, as prior research had looked at fish phylogeny with the *COI* gene in mtDNA. In this investigation, the *C. gariepinus* was caught, classified and identified, depending on its morphological features. In most cases using morphological

characteristics alone in the identification of fauna species is complex, absence of up-to-date taxonomic revisions, new combination and arising synonyms, according to continuous changes in nomenclature rules may prevent accurate identification of exotic species (Rosen and Bailey, 1963; Koutsikos *et al*., 2017). When utilizing mtDNA sequencing to fix their species differences, molecular data are definitive for identifying the alien species (Schories *et al*., 2009; Ho *et al*., 2016). The assessment of species is necessary for preventing biodiversity impairment.

A DNA barcoding is considered as powerful tool be in control of the proliferation of invasive species, the observation of freshwater habitat, and helping the rapid identification of alien fish globally. These genetic means improve management efficiency by helping in the reduction of repeated introductions along with the limiting or eradication of non-native fish populations (Agdamar and Tarkan, 2019).

According to this study, the phylogenetic tree of the *COI* gene in Iraqi *C. gariepinus* samples showed a high degree of genetic similarity to populations in China and North Korea, revealing a common ancestor. Van der Walt *et al*. (1993) uncovered significant evidence for inheritance with mutations in most cage populations when analyzing the genetic variety in *C. gariepinus*.

Popoola *et al.* (2014) stated that the gradual elimination of species genetically varied in nature as a result of hatchery-raised fish escaping or fries being released which may make *C. gariepinus* vulnerable to loss of genetic diversity and variability. Ezilrani and Christopher (2015) found that genetic variation among fish species enhances adaptation to changing environmental conditions, which may arise from spontaneous mutations or migration to genetically distinct populations. The present findings align with the study of Han *et al*. (2015), who applied the *COI* gene to characterize catfishes, specifically *C. gariepinus* in China. The complete mitochondrial genome sequence of *C. gariepinus* was sequenced using 17 primer pairs. Diyaware *et al*. (2018) studied the genetic variation regarding wild with farmed populations of *C. gariepinus* throughout Nigeria to enhance the species via selective breeding; DNA sequencing findings reveal a close genetic link between these strains. Chalermwong *et al*. (2023) studied the *COI*, cytochrome b (*Cytb*) genes, and D-loop sequences of 37 catfish species. The Cytb gene was determined as particularly suitable for differentiating catfish and can be considered a standard region for DNA barcoding due to its greater sequence variability.

CONCLUSIONS

This study represents the first to genetic investigation of the exotic fish African catfish in Iraqi freshwater ecosystem, with findings documented in the NCBI GenBank, performing as a reference sequence in the NCBI database (ID: LC868421, LC868422). The African catfish, is classified as *C. gariepinus*. The results confirmed the identity of the specimens as *C. gariepinus*, an exotic species now established in the different parts of Tigris River. DNA barcoding using the *COI* gene has demonstrated efficacy as a precise instrument for genetic identification, with the obtained sequences showing high similarity to reference populations

from China and North Korea, suggesting a possible shared lineage or introduction route. This work might be regarded as a new study on biodiversity and exotic fish species in the Tigris River. The significant expansion of *C. gariepinus* may pose a threat to indigenous fish species, alter the local ecosystem, and indicate rising levels of pollutants.

ACKNOWLEDGMENTS

We express our sincere gratitude to Prof. Dr. Hula Younis Fadhil Al-Sadi (College of Science, University of Baghdad) and Dr. Azhar A. Jaffar (Marshes Research Center, University of Thi-Qar) for their invaluable guidance and contributions to the molecular aspects of this study.

CONFLICT OF INTEREST STATEMENT

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

DECLARATION OF EDITORIAL INVOLVEMENT

The first and second authors declare that they serve as a member of the Editorial Board of this journal. The manuscript was processed strictly in accordance with the journal's standard editorial procedures, including assignment to independent peer reviewers and management by an editor with no conflict of interest. The authors had no involvement in the editorial handling or decision-making process.

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ترميز الحمض النووي لجري شمال افريقيا(Burchell, 1822) *Clarias gariepinus* (Siluriformes, Clariidae) في نهر دجلة، العراق

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الاستلام: 2025/5/1، المراجعة: 2025/8/2، القبول: 2025/8/5، النشر: 2025/12/20

الخلاصة

يُعد الحفاظ على التنوع الحيوي في النظم البيئية للمياه العذبة الداخلية في العراق أمرًا بالغ الأهمية لحماية الأنواع الأصلية من التأثيرات البيئية التي تسببها الأنواع الغريبة. وقد تم تحديد سمك جري شمال أفريقيا *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae) كنوع غريب في المسطحات المائية العراقية. هدفت هذه الدراسة إلى تحديد هذا النوع وراثيًا وتتبع أصله السلالي باستخدام الحمض النووي.

أُسْتُخْدِمَ جين الميتوكوندريا (*COI*) كجين ترميزي، فضلا عن مجموعة بادئات خاصة، وتقنية التضخيم بواسطة التفاعل التسلسلي للبوليميرا (PCR)، لتضخيم مقطع جين *COI* الذي تمت مواءمته لاحقًا مع تسلسلات موجودة في قاعدة بيانات NCBI باستخدام أداة BLAST.

بينت التحليلات الجزيئية وإعادة بناء الشجرة الوراثية، أن جين *COI* يُعد فعالًا لتحديد النوع وراثيًا، كما أشارت الشجرة الوراثية إلى وجود علاقة جينية وثيقة بين العينات العراقية وتلك التي تنتمي الى الصين وكوريا الشمالية، مما يشير إلى احتمالية أن تكون هذه التجمعات من أقرب السلالات المعروفة التي تمثل أصل النوع في العراق. وأظهرت النتائج أن جين COI المحدد يعد مؤشرا موثوقا لتتبع أصل مجموعات سمك السلور الغريبة في البيئة العراقية. وتُسهم هذه الدراسة في تطوير نظام الرصد الجزيئي للأنواع الغريبة في العراق، حيث تم تسجيل النتائج كمرجع في قاعدة بيانات NCBI (ID: LC868421, LC868422) ضمن بنك الجينات الخاص بـ NCBI.