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RESEARCH PAPER

DNA barcoding of some species of the genus *Capoeta* Valenciennes, 1842 from Kurdistan Region, Iraq.

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A B S T R A C T:

The genus *Capoeta* belongs to the Cyprinidae family and can be found in many of the inland waters of Middle Eastern nations. They are systematically complex species, many of which cannot be distinguished based on morphology. Genetic techniques like DNA barcoding may be used to identify species due to the limits of such species' morphological identification, although their genetic links have not yet been fully elucidated. The current study provides information on the genetic structure of three *Capoeta* species (*C. damascina*, *C. trutta*, and *C. umbla*) found in the Greater Zab River/ Bekhme Dam of the Kurdistan Region- Iraq. In order to confirm the identification of *Capoeta* species at the molecular level, the mitochondrial cytochrome oxidase I (COI) gene was sequenced. The interspecific genetic between species was (0.003) to (0.064). The maximum likelihood phylogeny indicates a consensus tree topology containing two branches, the first branch is divided into two subbranches including *C. damascina* and *C. umbla* and the second branch includes *C. trutta*. The results suggest that COI gene sequencing can be used to identify *Capoeta* species from streams in the Kurdistan Region, which is one of the first steps to improving the genetic understanding of *Capoeta* species.

KEY WORDS: DNA barcoding; *Capoeta*; Greater Zab River.
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1.INTRODUCTION :

The genus *Capoeta* Valenciennes, 1842 is a freshwater fish ranging from West Asia to East Europe, it is found in almost all freshwater bodies in the Kurdistan Region. (Bånårescu and Coad, 1991; Banarescu, 1999; Coad, 2010; Levin et al., 2012; Bektas *et al*., 2017 and Bektas *et al*., 2018). The genus *Capoeta* belongs to the largest freshwater fish family Cyprinidae. To date, nighty-six species and subspecies of the genus Capoeta have been identified (Froese and Pauly, 2022), and five species (*C. aculeata*, *C. barroisi*, *C. damascina*, *C. trutta*, and *C. umbla*) are identified in Iraq. (Abdullah, 2002; 2006; Abdullah *et al*., 2007; Coad, 2010; Abdullah, 2020; Abdullah *et al*., 2021, Agha, 2017; and Agha *et al*., 2021)

Genus *Capoeta* has compressed to the rounded and moderately elongated body, small to moderately large scales; an inferior, transverse mouth, barbels one pair in Iraq, dorsal fin, and anal fin are short (Coad, 2010). To identify fish species, there are several taxonomic methods. The morphological study is the traditional method, suitable and quick for the identification of species (Strauss and Bond, 1990 and Stein *et al*., 2014). It includes several characters, morphometric and meristic characters, and is commonly used in the species identification in the field (Waldman, 2005). The morphological similarities, particularly among species belonging to the same genus, were one of the main reasons for the confusion in the description of Iraqi fish fauna (Faddagh, *et al*., 2012). *Capoeta umbla* was once thought to be a

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synonym for *C. damascina* (Valenciennes, 1842) (Coad, 1991; 1996).

A global DNA-based barcode identification system applicable to all species provides a simple and universal means of identifying fish species and products. Genetic region part of mitochondrial DNA cytochrome c oxidase I gene, COI barcode scheme based on sequence diversity. It is based on the idea that genetic variation in DNA sequences varies more interspecies than intraspecies (Hebert *et al*., 2003; Fernandes *et al*., 2020). The COI gene has been designated as the standard region for DNA barcoding in animals because its short sequence makes it an efficient gene for amplification by the polymerase chain reaction (PCR) (Hebert *et al*., 2003; Hajibabaei *et al*., 2007; Lyra *et al*., 2017).

The present investigation aims to study the utility of a molecular technique as a DNA barcode approach for the identification of the *Capoeta* genus in the Kurdistan Region, Iraq. Especially several fish species have similar morphologies including the genus *Capoeta*, therefore using a molecular technique for identification of different species belonging to the genus *Capoeta* species.

2 MATERIALS AND METHODS

2.1 Study area:

The Greater Zab River is a river that roughly runs between Turkey and Iraq. It passes through Duhok and Erbil provinces, Iraq. The sampling region for this study was near the Bekhme Dam in the northeast of Erbil (36o42 12" N, 44o 16 43" E) (Fig. 1) (Wright, 2004).

2 .2 Sample collection and DNA extraction

A total of 77 *Capoeta* species including *C. damascina*, *C. trutta* and *C. umbla* were collected from a different region of Greater Zab by local fishermen twice monthly using different size gillnets (Fig. 2).

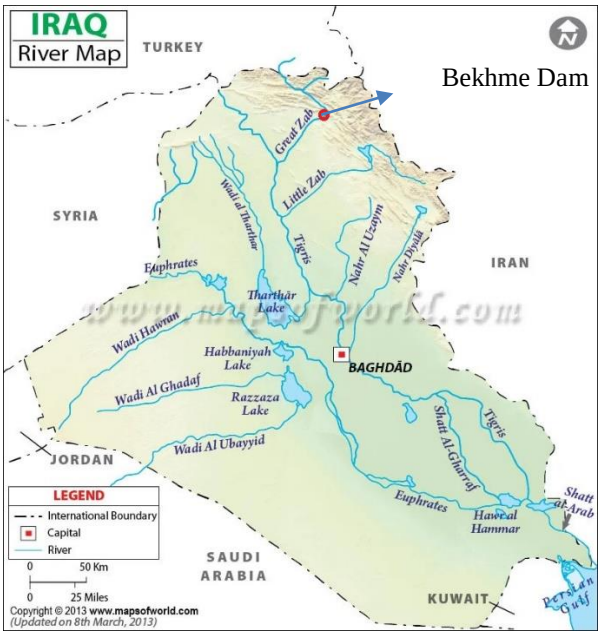

Figure 1. Map of the Iraq rivers (www.mapsofworld.com).

Samples were kept in a cool box and they were initially identified based on morphometric and meristic characters following Coad (1996), Esameili *et al*. 2016 and Froese and Pauly (2022). From 49 fish samples, approximately 70-90 mg of white muscle tissue with three repeats were removed from the left side of each specimen. The tissues were preserved in 99% ethanol. COI subunits were studied (Table 1). With the help of the Jena Bioscience Blood, Animal and Plant DNA Preparation Kit (Jena Bioscience GmbH. 07749 Jena Germany), whole genomic DNA (with three repeats from each fish sample) were extracted from muscle samples and stored in 18 º C in deionized distilled water. Approximately 615 bp in the 5' region of the COI gene were amplified using universal primers for Fish barcodes: primer FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3'), primer FishR1 (5'-TAGACTTCTGGTGGGGCAAAGAATCA -3') (Ward *et al*., 2005).

2.3 DNA amplification and polymerase chain reaction PCR

Partial PCR amplification of COI was performed in 50 µl reaction mixture. 25 µl 2x Taq DNA Polymerase Master Mix (AMPLIQON A / S Stenhuggervej 22); 18 µl water without DNase; 2

µl of each COI primer (FishF1 and FishR1) and 3 µl of DNA sample using a Bioresearch PTC-200 gradient thermocycler. The temperature profile included the initial denaturation at 95ºC for five minutes; followed by 35 cycles of 95ºC for 40 seconds, a primer annealing at 62ºC for 35 seconds, an extension at 72ºC for 1 minute, and a final extension of 72ºC for 10 min; and hold at 4ºC. PCR products were run on a 1.5% agarose gel, stained with ethidium bromide (0.5 g/ml) in TAE buffer, and seen on a Quantum-Capt ST4 UV system (Vilbert Lourmat, France). The UV-Vis NanoDrop 2000C spectrophotometer was used to determine the concentration of the purified PCR product (Thermo Fisher Scientific, USA).

2.4 DNA Sequencing and alignment

Partial COI gene samples of PCR products were sequenced using the ABI Prism Terminator sequencing kit from Macrogene (Applied Biosystem) in Korea. Finch TV programming software was used to process the COI chromatogram and verify the source call.

2.5 DNA sequence analysis

The Basic Local Alignment Search Tool (BLAST), a search engine that employs the sequence alignment method, has received and been given access to partial sequences of the COI gene. To locate sequences that are more similar to fish species, compare and align laboratory or query sequences with other biological sequences on the National Biotechnology Information Center (NCBI) website. Sequence divergences were computed using the Kimura two-parameter (K2P) distance model following sequence alignment (Kimura, 1980). Utilizing MEGA 11 (Molecular Evolutionary Genetic Analysis), a distance-based approach like maximum likelihood, the molecular phylogenetic tree was created. The bootstrap method with 100 repeats was used to assess the accuracy of the estimated phylogenies.

3. RESULTS AND DISCUSSION

A total of 77 specimens were identified morphological depending on morphometrics and meristic characters. measurements and meristic counts following Coad (2010) and Esmaeili *et al*. (2016) for identifying *Capoeta* species (*C. damascina*, *C. trutta*, and *C. umbla*) (Fig. 2) Using PCR, the mitochondrial cytochrome oxidase I (COI) region of the samples was effectively amplified, and each COI sequence obtained showed no stop codons, insertion, or deletions.

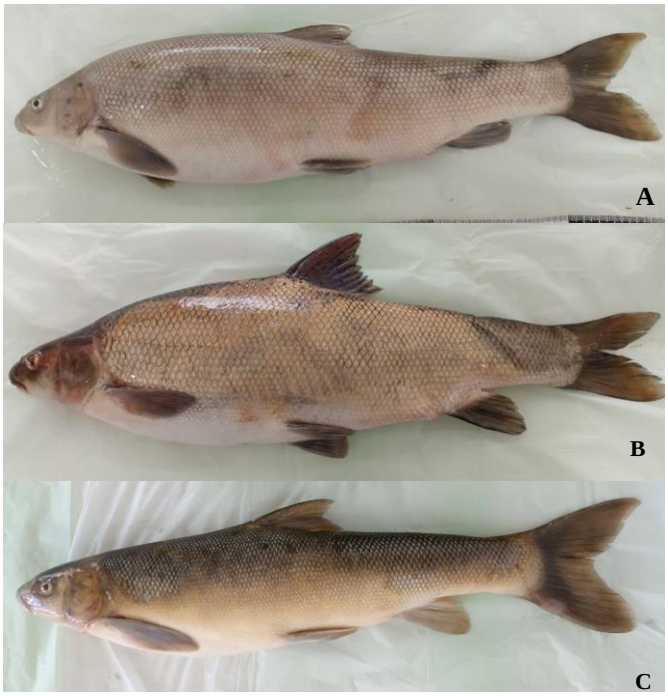

Figure 2. Photos showing *Capoeta damascina* (A; total length: 41 cm), *Capoeta trutta* (B; total length: 38 cm), and *Capoeta umbla* (C; total length: 40 cm).

The COI barcode obtained ranges from 600 bp to 632, with an average of 620 bp. The sequence analysis revealed that the average nucleotide frequencies represented as A=26.83%, T=26.77%, G=16,93% and C=29.47% (Table 2).

Table 1. partial COI gene sequences in NCBI after submission.

Taxon	Accession number	Query cover %	Identic n. %	Source
C. damascina	ON619601	100	98.2	*
C. damascina	ON619602	99	98.2	*
C. damascina	MW250386	99	98.2	#
C. damascina	MW250393	99	98.2	#
C. trutta	ON619599	100	100	*
C. trutta	ON619600	100	100	*
C. trutta	MW251738	99	99.7	#
C. trutta	MW250395	99	99.7	#
C. umbla	ON619603	100	100	*
C. umbla	ON619604	97	98.5	*
C. umbla	ON619605	100	100	*
C. umbla	ON619606	100	100	*

(*) current study, (#) Agha *et al*., (2021)

Kimura 2 paramater (K2P) was used for intraspecific and interspecific variations. The pairwise distances between *Capoeta* genus are shown in table (3). Intraspecific genetic diversity

within *C. damascina* was from zero to (0.003), within *C. trutta* zero to (0.003) and *C. umbla* was zero to (0.029). be zero when it was maximum within *C. umbla* samples (0.006). The minimum interspecific genetic distance was observed between *C. damascina* and *C. umbla* (0.003) when the maximum genetic distance was observed between *C. trutta* and *C. umbla* (0.064) and *C. trutta* and *C. damascina* (0.064).

Table 2. Nucleotide ratios from 12 Capoeta Genus COI sequences collected from the Greater Zab River/Bekhme Dam and Gwer

	Mean	minimum	maximum	s. error
T%	26.77	26.0	27.7	0.41667
A%	26.83	26.2	27.8	0.83900
G%	16.93	16.2	17.0	0.33333
C%	29.47	29.1	30.0	0.55675
GC%	46.44	45.5	47.2	0.13787

The maximum likelihood, shown in figure 3, was used to cluster the three species of *Capoeta* genus. While all the three species *C. damascina*, C. trutta and *C. umbla* depicted from a single branch, which is divided into two subbranches. In the first subbranch were detected *C. damascina* and *C. umbla* while *C. trutta* was detected in the second subbranch with a 100% bootstrap value.

experiences in traditional morphological classification and facilitates the computerization and standardization of species identification. The COI gene was found to be effective in identifying different species of *Capoeta* genus in this study. The molecular examination of three species of *Capoeta* were done by amplifying COI gene region and sequencing the amplicon. Based on the DNA sequencing and morphological characteristics results, it is clear and investigated that *C. umbla* is a separate species and not a synonym of *C. damascina*. In the Kurdistan region, there is a previous molecular study on *Capoeta* species, the results of *Capoeta* in this study are in agreement with (Ali and Abdullah 2019 and Agha *et al*. 2021). There were no additions or deletions found in the sequence, and no stop codons were detected during translation. The AT content (53.56 %) and GC content (46.44 %) of the COI sequence's base composition analysis are similar to those of DNA barcoding of Taiwan Strait fish species (Bingpeng *et al*., 2018) and DNA barcoding Cyprinidae (Agha *et al*., 2021). The current findings were consistent with those found in previous studies (Ozdemir, 2012; Bektas *et al*., 2018). Ward (2012) reported that the K2P-based intraspecies genetic distance was less

Table 3. Estimation of pairwise genetic distances between and within *Capoeta* species.

		1	2	3	4	5	6	7	8	9	10	11	12
ON619603	*C. umbla*	1											
ON619604	*C. umbla*	2	0.000										
ON619606	*C. umbla*	3	0.026	0.027									
ON619605	*C. umbla*	4	0.018	0.016	0.029								
MW250395	*C. trutta*	5	0.060	0.057	0.051	0.059							
MW251738	*C. trutta*	6	0.060	0.057	0.051	0.059	0.000						
ON619599	*C. trutta*	7	0.060	0.057	0.051	0.059	0.000	0.000					
ON619600	*C. trutta*	8	0.064	0.061	0.055	0.063	0.003	0.003	0.003				
MW250393	*C. damascina*	9	0.003	0.005	0.025	0.018	0.056	0.056	0.056	0.060			
MW250386	*C. damascina*	10	0.003	0.005	0.025	0.018	0.056	0.056	0.056	0.060	0.000		
ON619601	*C. damascina*	11	0.005	0.005	0.026	0.018	0.060	0.060	0.060	0.064	0.003	0.003	
ON619602	*C. damascina*	12	0.005	0.005	0.026	0.018	0.060	0.060	0.060	0.064	0.003	0.003	0.000

DNA barcoding technology is an evolutionary method for rapid, accurate species identification by utilizing small, standardized gene regions and a DNA sequence fragment shared by organisms that differ significantly. This technology alleviates the reliance on taxonomists' personal talents and

than 1% and rarely exceeded 2% in fish-containing taxa; estimates that the interspecies and intraspecies differences of K2P based on three *Capoeta* species are less than 1%. Intraspecific

between *C. damascina* and *C. umbla* was smaller than interspecific variations between *C. damascina*, *C. umbla* and *C. trutta*, the same as the results of (Ghanavi *et al*., 2016 and Bektas *et al*., 2018). The topologies of the COI barcode region sequencing data analysis's maximum likelihood trees, with a high bootstrap value (100) in the Greater Zab River/Bekhma Dam, in which *C. damascina* and *C. umbla* in one branch and *C. trutta* in another branch. The present results were in congruence with the molecular phylogeny of the Anatolia *Capoeta* species by Ozdemir (2013) and DNA barcoding of *Capoeta* (Bektas *et al*., 2018). As previously stated, the use of DNA barcoding has been demonstrated as a powerful tool for identifying marine and freshwater fish species from various geographic regions (Hubert *et al*., 2008; Mabragana *et al*., 2012; Paknejad *et al*., 2019). Molecular genetic techniques provide a powerful approach to solve taxonomic confusion and allow new insights into the relatedness and evolution of fish species (Pegg *et al*., 2006).

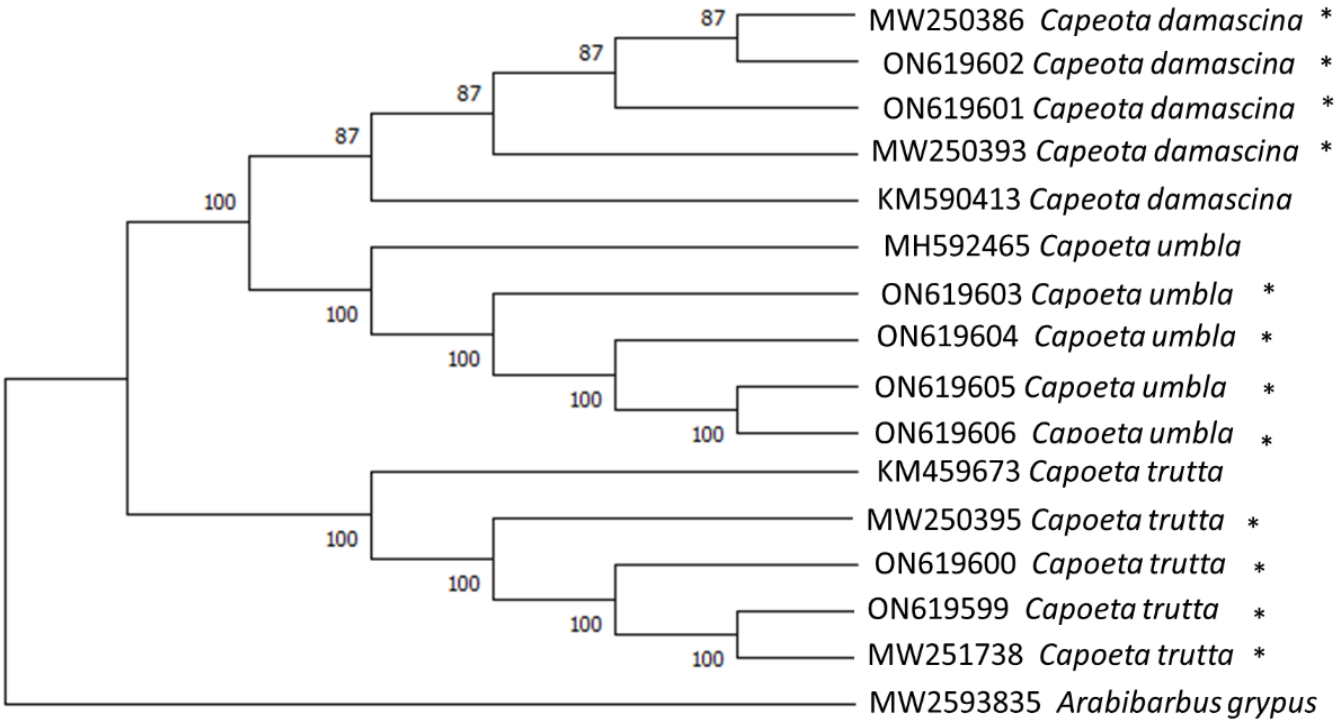

Figure 3. Phylogenetic tree of *Capoeta* genus form Iraq; Kurdistan Region (*) using Maximum Likelihood (NJ) method. *Arabibarbus grypus* was used as an out-group.

In conclusion, by using robust molecular techniques, the current study, it was successfully identified *Capoeta* species. The present study results imply that COI barcoding can be used as a practical strategy for resolving unambiguous identification and examining species whose identification is dependent on the morphology of the fish fauna of the Kurdistan Region/Iraq especially *Capoeta damacina* and *C. trutta* as well as *C. umbla*, especially since the color of this fish and some other morphological character can affect easily with environmental factors, the present finding is same to that mentioned by Stein *et al*. (2014).

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References

ABDULLAH, S. M. A. (2002). Ecology, taxonomy, and biology of some parasites of fishes from Lesser Zab and Greater Zab Rivers in North of Iraq. Ph.D. Dissertation, Baghdad University, 153 pp (In Arabic).

ABDULLAH, S. M. A. 2006. The Inhabitant Fishes in Dukan Lake in North of Iraq and Methods for Developing their Culturing. 2nd International Scientific Congress for Environments, South Valley University, Qena, Egypt/ 28-30 March. P. 68-78.

ABDULLAH, S. M. A., RAHIMO, Z. I. F. and SHWANI, A. A. 2007. The Inhabitant Fishes in Darbandikhan Lake in North of Iraq and Methods for Developing

their Culturing. Egypt Journal Aquatic Biology and Fisheries, 11(3), p. 1-7.

ABDULLAH, Y. S. 2020. Ultra-Morphological Structure and Molecular Characterization of Contracaecum larvae (Nematode) Parasitic of Some Fishes in Sulaimani Province, Kurdistan Region-Iraq. Ph.D. Dissertation, Sulaimani University.

ABDULLAH, Y. S., ABDULLAH, S. M. A. and RIDHA, H. H. 2021. Biodiversity of fishes in Sulaimani Province in Kurdistan Region, Iraq. Zanco journal of pure and applied sciences, 32(1), 39-44.

AGHA, G. F. (2017). Morphological and Molecular Identification of Some Inhabitant Fishes in Greater Zab River/ Aski-Kalak in Kurdistan Region, Iraq. M.Sc. Thesis Salahaddin University. BRYAN, J. E. 2002. *Bulbs*, Portland, Oregon, U.S.A., Timber Press.

AGHA, G. F., IBRAHIM, I. R. and ABDULLAH, S. M. A. 2021. DNA barcoding of the Cyprinidae from Greater Zab River Gwer in Kurdistan Region, Iraq. Plant Achieves, 21(2), 639-644.

ALI, M. K. and ABDULLAH, S. M.A. 2019. Morphological and molecular identification of Capoeta trutta (Cyprinidae) and Planiliz abu (Mugilidae) freshwater fish in Sulaimani Governorate, Iraq. Applied ecology and environmental research. 17(4). 7439-7451.

BåNåRESCU, P. and B.W. COAD, 1991. Cyprinids of Eurasia. p. 127-155. In I.J. Winfield and J.S. Nelson (eds.) Cyprinid fishes: Systematics, Biology, and Exploitation. Fish and Fish. Ser. 3, 667 p. Chapman and Hall, London.

BåNåRESCU, P. M. 1999. The Freshwater Fishes of Europe. Volume 5. Cyprindae 2. Part1: Rhodeus to Capoeta. AULA-Verlag, Wiebelsheim, xviii.P:427.

BEKTAS, Y., AKSU, I., KAYA, C. and TURAN, D. 2018. DNA Barcoding of genus Capoeta (Actinopterygii: Cyprinidae) from Anatolia. Turkish Journal of Fisheries and Aquatic Sciences, 19(9), 739-752.

BEKTAS, Y., TURAN, D., AKSU, I., ÇIFITCI, Y., EROGLU, O., KALAYCI, G. and BELDZ, Ç. 2017. Molecular Phylogenetic of genus Capoeta (Actinopterygii: Cyprinidae) in Anatolia, Turkey. Biochemical Systematics and Ecology, 70, 80-94.

BINGPENG, X., HESHAN, L., ZHILAN, Z., CHUNGUANG, W., YANGUO, W. and JIANJUN, W. 2018. DNA barcoding for identification of fish species in the Taiwan Strait. Plose ONE, 13(6): e0198109.

COAD, B. W. 1991. Fishes of Tigris-Euphrates Basin: A Critical Checklist. Syllogeus, 68, p. 49.

COAD, B. W. 1996. Zoogeographic of the Fishes of the Tigris- Euphrates Basin. Zoology in the Middle East, 13, 51-70.

COAD, B. W. 2010. Freshwater Fishes of Iraq. Pensoft Series Faunistica, 93, p: 274, 16 plates.

ESMAEILI, H. R., ZAREIAN, H., EAGDERI, S. and ALWAN, N. 2016. Review on the taxonomy of Tigris scraper, Capoeta umbla (Heckel, 1843) and its confirmation record from the Iranian part of Tigris River, Persian Gulf basin (Teleostei: Cyprinidae).FishTaxa, 1(1),35-44.

FADDAGH, M. S., HUSSAIN, N. A. and Al-BADRAN, A. I. 2012. DNA fingerprinting of Eight Cyprinid Fish Species of Iraqi Inland Waters Using RAPD-PCR Technique. Advances in Life Sciences, 2(2),9-16.

FERNANDES, T. J., AMARAL, J. S. and MAFRA, I. 2020. DNA barcode markers applied to seafood authentication: an updated review. Food sciences and nutrition, 61(22), 3904-3935.

FROESE, R., and D. PAULY. 2022. Fishbase. World Web Electronic Publication, www.Fishbase.com, (02/2022).

GHANAVI, H. R., GONZALEZ, E. G. and DOADRIO, I. 2016. Phylogenetic ralationships of freshwater fishes of the genus Capoeta (Actenoptergii, Cyprinidae) in Iran. Ecology and Evolution, 6(22), 8205-8222.

HAJIBABEI, M., SINGER, G. A., HEBERT, P. D. N. and HICKY, D. A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics, and population genetics. Trends in Genetics, 23(4),167-172.

HEBERT, P. D. N., CYWINSKA, A., BALL, S. L. and DEWAARD, J. R. 2003. Biological Identification Through DNA barcodes. Proceedings of the Royal Society. Biological Sciences, 270, 313-321.

HUBERT, N., HANNER, R., HOLM, E., MANDRAK, N. E., TAYLOR, E., BURRIDGE, M., WATKINSON, D., DUMOT, P., CURRY, A., BENTZEN, P., ZHANG, J., APRIL, J. and BENATCHEZ, L. 2008. Identifying Canadian freshwater fishes through DNA barcodes. PLOS ONE, 3, e2490.

KIMURA, M. J. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16 (2), 111-120.

LEVIN, B. A., FREYHOF, J., LAJBNER, Z., PEREA, S., ABDOLI, A., GAFFAROGLU, M., OZULUG, M., RUBENYAN, R. H., SALNIKOV, V. B. and DOADRIO, I. 2012. Phylogenetic relationships of the algae scraping cyprinid genus Capoeta (Teleostei, Cyprinidae). Molecular Phylogenetics and Evolution, 62,542-549.

LYRA, M. L.M., HADDAD, C. F. and AZERDO-ESPIN, A. L. 2017. DNA Barcoding: neotropical amphibians: polymerase chain reaction optimization and new COI primers. Molecular Ecology Resources,: 17(5), 966-988.

MABRAGANA, E., de ASTARLOA, M. D., HANNER, R., ZHANG, J. and CASTRO, M. G.. 2011. DNA barcoding identifies Argentine fishes from marine and brackish waters. PLOS ONE,6, e28655.

OZDEMIR, F. 2013. The revision of species and subspecies of the genus Capoeta (Teleostei. Cyprinidae) by using both the classical systematic and molecular systematic methods in Turkey. Ph.D Dissertation, Hacettepe University.

PAKNEJAD, H., JALALI, A., FADAKAR, D., ASKARI, G., FAROKHI, M. 2019. A DNA barcoding approach to identify species of Nemacheilidae: a complex family of cypriniforms fishes in Iran.

Iranian Journal of Animal Biosystematics, 15(1), 47-59.

PEGG, G. G., SINCLAIR, B., BRISKEY L. and ASPDEN, W. J. 2006. MtDNA barcode identification of fish larvae in the southern Great Barrier Reef, Australia. Sciences, 70(2), 7-12.

STEIN, E. D., MARTINEZ, M. C. STILES, S. MILLER, P. E. and ZAKHAROV, E. 2014. Is DNA Barcoding Actually Cheaper and Faster than Traditional Morphological Methods: Results from a Survey of Freshwater Bioassessment Efforts in the United States? PLOS ONE,9(4), e95525.

WALDMAN, J. R. 2005. Meristics in Stock Identification Methods: Applications in Fishery Science, p. 197-209. Ed. By CADRIN, S. X., FRIEDLAND, K. D., and WALDMAN, J. R.. Elsevier Academic Press, Amsterdam, p:719.

WARD, R., ZEMAK, T. INNES, B., LAST, P. and HUBERT, P. 2005. DNA barcoding Australia's fish species. Philosophical transactions of the royal society of London. Series B, Biological Sciences, 360, 1847-1857.

WARD, R, 2012. A case study for DNA barcodes pp. 423-439 in Kress W. J and Erickson D. L. eds. DNA barcodes: methods and protocols, methods in molecular biology. Spring science Business Media, LLC, New York, Fish-BOLL.858.

WRIGHT, H. E. 2004. Pleistocene Glaciation of Iraq. Quaternary Glaciations – Extent and Chronology, 3(2), 2015-216.