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ORIGINAL ARTICLE

MORPHOLOGICAL AND MOLECULAR STUDIES OF KAIS KINGFISH *CYPRINION KAIS* HECKEL, 1843 (PISCIES, CYPRINIFORMES, CYPRINIDAE) FROM THE MIDDLE OF IRAQ

Hind Dyia Hadi*♦, Abed Hassan Baraaj** and Atheer Hussain Ali***
*Iraq Natural History Research Centre and Museum, University of Baghdad, Baghdad, Iraq.
** Department of Biology, College of Science, Baghdad University, Iraq.
***Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.
♦Correspondence: e-mail**:** hinddhiaa86@gmail.com

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ABSTRACT

Cyprinidae species are the most abundant and widely distributed fish species in the inland waters of Iraq. Cyprinids are complex species, and it is difficult to identify them on the basis of morphology. Thus, the morphological characteristics must be achieved and confirmed by molecular analysis. Twenty specimens of *Cyprinion kais* Heckel, 1843 (Piscies, Cypriniformes, Cyprinidae) were collected from two localities at Tigris River in the middle of Iraq: five specimens from Al-Tharthar Lake, Saladin Province, and 15 specimens from Al-Zubaydiyah sub-district, Wasit Province.

The DNA sequences of *C. kais* were done using the mitochondrial DNA cytochrome b (*cytb*) gene. After analysis, the sequences were compared with sequences of other fish genera and species available in GenBank. The barcoding result (DNA sequencing) showed that it fit with *C. kais*. We conclude the results of the current study confirm that the validity of the morphological diagnosis of *C. kais*, using DNA sequencing analysis.

Keywords: Cyprinidae, Cytochrome, Fishes, Morphology, Tigris River.

INTRODUCTION

Kais kingfish *Cyprinion kais* Heckel, 1843, is a freshwater fish belonging to the largest family of freshwater fish Cyprinidae, and this species is distributed in the inland waters of Iraq, Turkey, Syria, and Iran. In Iraq, it is recorded from marshes such as Al-Chabaish, large rivers such as the Tigris, Shatt Al- Arab River, and Lesser Zab, smaller rivers such as the Khalis near Baghdad, in ponds such as those on Za'faraniyah fish farm south of Baghdad, and in reservoirs such as the Dukan Dam (Coad, 2010; Afrasiab *et al*., 2013; Froese and Pauly, 2023).

This fish is edible and a valuable species for sport fishing, and it could be used as an aquarium fish (Nasri *et al*., 2010). Despite its wide distribution and importance, few studies have been conducted on this species, due to its lower abundance, compared to *C. macrostomum*. Some researchers considered *C. kais* and *C. macrostomum* to be synonymous (Berg, 1949; Karaman, 1971), but Bianco and Banarescu (1982) indicated that they are two different species; as well as Coad (2010) used the most important characteristics to differentiate *C. kais* from *C. macrostomum*, the shape of the mouth is one such character, being narrower and more arched and the lateral lobes present in *C. kais*, while the mouth is broader and lacking lateral lobes in *C. macrostomum*. Freshwater groups are morphologically diverse, indicating their ability to adapt to local selection pressures, but marine groups are globally uniform, reflecting the stability of marine environments. In the case of the sympatric species *C. macrostomum* and *C. kais*, the strongest selective factor appears to be food competition, as all other environmental factors are the same (Nasri *et al*., 2018; Al-Thahaibawi *et al*., 2019).

The nature of the feed related to one species of fish determines the size and shape of the mouth of fish; the mouth shape, is a trophic character in *C. kais* (Gazwan *et al*., 2021; Coad, 2023); it can be considered as developmental plasticity as well as a few related morphological adaptations to feed on benthic invertebrates (the deeper body, the least head height, dorsal fin base length, dorsal-fin height, and longer pectoral-fin); but osteological, new genetic method, and morphological information rejected their synonymy (Daştan *et al*., 2012; Nasri *et al*., 2013; Coad, 2023).

To recognize fish species, there are numerous taxonomic methods, the morphological study being the conventional one, which is helpful, speedy, and includes many characters, morphometric and meristic characters, for species identification (Al-Janabi, 2014; Stein *et al*., 2014). The morphological similarities, particularly between species belonging to the same genus, have been one of the most common causes of confusion in recognizing the Iraqi fish fauna (Faddagh *et al*., 2012a). *C. kais* is morphologically similar to *C. macrostomum*, so molecular techniques are necessary to elucidate species identification in this case. With the improvement of new genetic advances that utilize information obtained from the molecular components of the cell, the mitochondria cytochrome b (*mt-cyb*) gene is one of 11 components of a group of proteins called complex III. Cytb is generally utilized as a locale of mitochondrial DNA to identify evolutionary connections between different groups, and because of its sequence diversity it is valuable inside families (Caine *et al*., 2006). There are a few uncertainties about the taxonomic and developmental status of *Cyprinion* species, and many authors considered the taxonomic status of Cyprininae species and genera with their genetic links to remain doubtful (Howes, 1982).

The aim of the current study is to confirm the identification of *C. kais* by combining the morphological and molecular characters.

MATERIALS AND METHODS

Study area

Two localities at the Tigris River were chosen to collect studied fish species as follows:

A. Al-Tharthar Lake

The biggest natural lake in Iraq; which lies within the middle of Iraq, around 120 km northwest of Baghdad, between the Tigris and the Euphrates rivers, within coordinates 33°58′N 43°11′E. The Lake; with an area ranging between 1875 - 2710 km^2 and a maximum depth of 68.4 m, is fed by the Tigris River; the water is discharged from the lake to the Euphrates River (Szczerbowski *et al.*, 2001; Zdanowski *et al.*, 2001).

B. Al- Zubaydiyah Sub-district

It is one of the sub-districts of the Al-Suwaira District in Wasit Province. Al- Zubaydiyah is located 50 km to the south of the city of Essaouira and 85 km to the north of the city of Kut, the capital of Wasit Province, southeastern Baghdad, within coordinates 32°45'46.6"N 45°10'48.7"E. The sampling localities are illustrated on the Iraqi map (Map.1).

Map (1): Sampling areas.

Specimens' collection

A total of 20 specimens of fishes, 17 specimens for morphology study, and three specimens for molecular study) were collected using gill nets by fishermen; during the period from June; 2022 until December 2022. Fishes were transported in a cool box with crush of ice to the laboratory of the Iraq Natural History Research Centre and Museum, University of Baghdad.

Morphological study

Each specimen is measured using 1-meter measuring board graduated in millimetres (mm) and a digital calliper, and the weight of each fish individual was done immediately by digital balance. The morphometric features were measured from the left side of each fish. The morphometric and meristic characters are measured according to those in Hubbs and Lagler (1964) as shown in Table (1). The specimens were photographed using a Samsung camera of Korean origin.

Table (1): Morphometric and meristic characters and their abbreviations.

No.	Morphometric characters	Abbreviated name
1	Total Length	TL
2	Fork Length	FL
3	Standard Length	SL
4	Head Length	HL
5	Head Height	HH
6	Snout Length	SnL
7	Eye Diameter	ED
8	Interorbital Distance	IOD
9	Postorbital Length	POL
10	Maximum Body Height	MAXH
11	Minimum Body Height	MINH
12	Caudal Peduncle Length	CPL
13	Dorsal Fin Length	DFL
14	Dorsal Fin Height	DFH
15	Pectoral Fin Length	PFL
16	Pelvic Fin Length	PFL
17	Pectoral-Pelvic Distance	PPD
18	Pelvic-Anal Distance	PAD
19	Anal Fin Length	AFL
20	Anal Fin Height	AFH
21	Pre Dorsal Length	PDL
22	Post Back Distance	PBD
23	Upper Caudal Fin Length	UCFL
24	Lower Caudal Fin Length	LCFL
25	Centre of Caudal Fin Length	CCFL
26	Caudal Peduncle Height	CPH
27	Mouth Width	MW
28	Prepectoral Length	PPL
29	Prepelvic Length	PVL
30	Preanal Length	PAL

31	Preanus Length	PASL
Meristic characteristics		
32	Scales of Lateral Line	SLL
33	Up Scales of Lateral Line	USLL
34	Down Scales of Lateral Line	DSLL
35	Dorsal Fin Spines	DFS
36	Dorsal Fin Rays	DFR
37	Anal Fin Spines	AFS
38	Anal Fin Rays	AFR
39	Pectoral Fin Rays	PFR
40	Pelvic Fin Rays	PVFR
41	Caudal Fin Rays	CFR
42	Barbels	NB
43	Scales around the least circumference of the caudal peduncle	SCP
44	Gill Rakers	GR

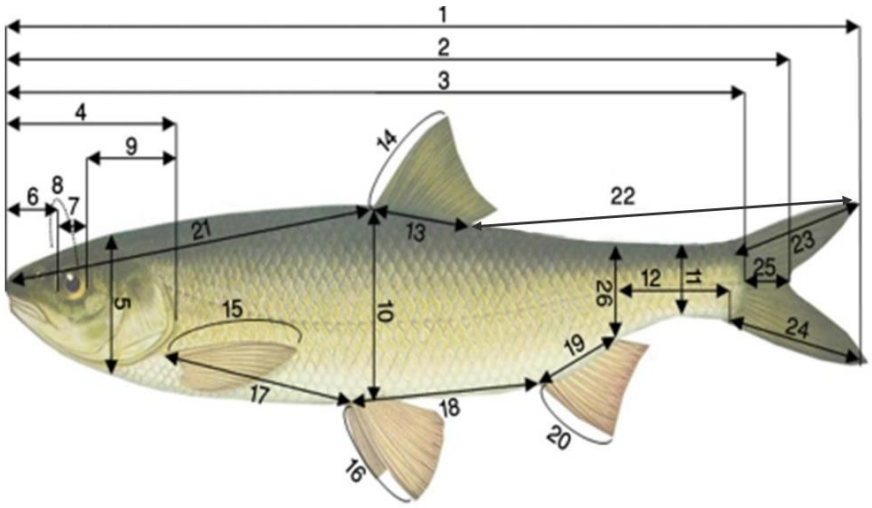

Plate (1): The morphometric measurements carried out of *C. kais*. [From Kashefi *et al*. (2012) with own authors modification].

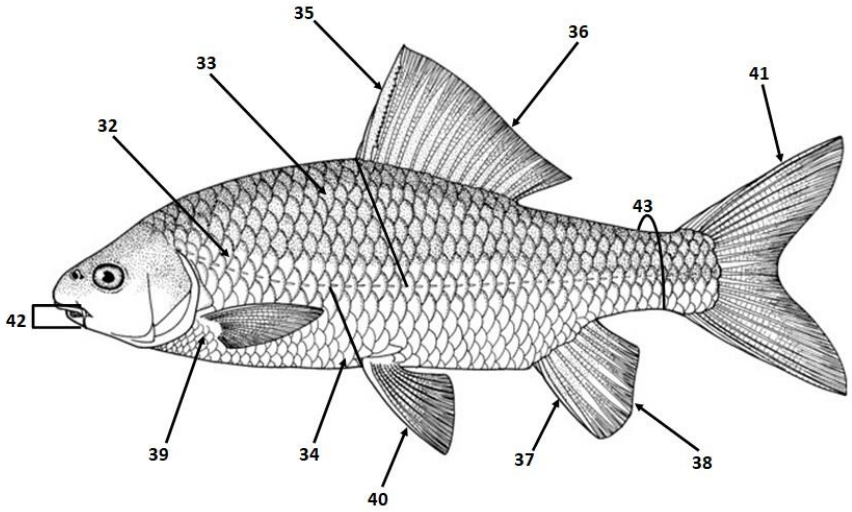

Plate (2): The meristic characters carried out on *Cyprinion kais* [From Coad (2010) with own authors modification]

DNA Extraction

Total DNA was extracted from the muscle dorsal side of each fish species, and specimens were preserved in absolute ethanol 100%, using the Genomic DNA Extraction Kit (addbio/Korea, Cat.no. 10023). Genomic DNA were extracted following Sambrook *et al*. (1989) and tested using electrophoresis on 1% agarose gel stained with ethidium bromide dye.

Amplification and polymerase chain reaction PCR

Approximately 508 bp were amplified from 5' region of the *cytb* gene using universal fish primers (design in this study). The forward primer FishF1 5' (CAAGCCTACGAAAAACMCAC) 3', and the reverse primer 5' FishR1 (TCTACTGAGAAKCCRCCTCA) 3'. Polymerase Chain Reaction PCR reactions are given as 50 µl total volumes containing 25 µl of 2xTaq DNA Polymerase Master Mix, 2 µl of each primer (FishF1 and FishR1), 17 µl free water, and 4µl of DNA template by Optimus 96G thermal cycler. The PCR thermal cycling conditions were: 94ºC for five min; 35 cycles of 94ºC for 30s, 52ºC for 30 s, 72ºC for 30 s; final extension of 72ºC for 5 min; and hold at 4ºC. The PCR products were then electrophoresed on 2% agarose gel stained with ethidium bromide dye. A ladder of 100 bp (promega) was utilized with this test. The profiles were tested on UV light Transilluminator and documented by photographing with Canon Camera with a gel documentation tool.

Sequencing of PCR product sent to (Macrogen / Korea) with forward primer. The results were in the form of special files that were evaluated with Bioedit software. Version 7; 2013; https://bioedit.software.informer.com/7.2/. The alignment and comparison of sequences generated from sequencing findings were also compared with data from the same organism genes that were found in the gene bank at the National Center for Biotechnology Information

(NCBI), which had previously been investigated in various nations across the world by using the Basic Local Alignment Search Tool (BLAST). The results of sequencing showed 99 - 100 % agreement with reference sequences. The phylogenetic tree of the species was drawn by using the sequence data of the samples under study and compared with the previously studied species using the MEGA X: Molecular Evolutionary Genetics Analysis (Kumar *et al*., 2018). The nucleotide location value of variance is calculated using the maximum likelihood (ML), and a molecular phylogenetic tree is generated. The bootstrap method with 500 repeats was used to assess the accuracy of the estimated phylogenies.

RESULTS

Morphology

The morphometric and meristic characters of *C. kais* are indicated in Tables (2, 3), to show the general morphology of Kais kingfish.

Table (2): The results of morphological characters for *Cyprinion kais*.

Characters	**min-max (Mean ±SD) in mm**	**Proportional measurements as expressed as a percentage of Standard Length or of Head length***
Weight	320- 584 (404.8± 74.5)	-
Total Length TL	121- 184 (143±1.71)	-
Fork Length FL	108-166 (128±1.62)	-
Standard Length SL	100-144 (116±1.42)	-
Maximum Body Height MAXH	33-47 (39.6 ± 4.4)	31.25-37.38 (34.32 ± 1.7)
Minimum Body Height MINH	9-15 (11.6 ± 1.5)	8.88-11.0 (10.2 ± 0.69)
Caudal Peduncle Length CPL	11-33 (15.4 ± 5.4)	10.9-24.4 (13.21 ± 3.59)
Dorsal Fin Length DFL	21-36 (28.7 ± 4.0)	21- 27.69 (24.80 ±1.71)
Dorsal Fin Height DFH	13-28 (22.1 ± 4.2)	12.59-22.1 (19.07± 2.30)
Pectoral Fin Length PFL	16-24 (19.9 ± 2.2)	14.58-20.58 (17.28 ± 1.33)
Pelvic Fin Length VFL	15-26 (18.7 ± 2.7)	14.52-19.60 (16.19 ± 1.34)
Pectoral-pelvic Distance PV	27-45 (34.5 ± 4.9)	26.73-33.0 (29.79 ± 1.64)
Predorsal distance	50-73 (60.9 ± 7.7)	47.61- 56.86 (52.58±2.65)
Prepelvic Distance PAD	52-75 (61.7 ± 7.2)	49.52-59.81 (53.38±2.45)
Prepectoral Distance PAD	22-34 (26.8 ± 3.6)	18.80-26.73 (23.26±2.10)
Preanal Distance PAD	76-113 (90.4 ± 10.8)	73.07-82.24 (78.11±2.47)
Pelvic-Anal Distance VA	23-38 (31.2 ± 4.1)	22.30-34.56 (27.45±2.82)

Anal Fin Length AFL	8-16 (11.9±2.2)	8.33-13.76 (11.04±2.30)
Anal Fin Height AFH	13-23 (18.3±3.3)	11.11-22.54 (15.91 ±3.18)
Preanus Distance	73-108 (85.3±9.8)	67.69-77.57 (73.73±2.70)
Post Back Distance POB	45- 78 (61.5±8.6)	42.88- 58.93 (50.90±4.99)
Upper Caudal Fin Length UCFL	23- 38 (29.1±4.4)	21.36-29.41 (25.16±2.25)
lower Caudal Fin Length DCFL	23-35 (27.6±3.9)	19.65-27.45 (23.92±2.01)
Center Caudal Fin Length CCFL	9-15 (12.6±3.7)	8.25-12.87 (10.97±1.29)
Caudal Peduncle Height	11-16 (14.2±1.4)	10.89-14.01 (12.32±1.02)
Head Length	22-32(25.4±3.1)	(HL/SL)*100
Head Height	15-26(18.4±2.7)	65.38-81.25 (72.36*±4.23)
Snout Length	6-11(7.8±1.3)	26.92-36.66 (30.84*±3.26)
Orbit diameter	4-6 (5.3±0.6)	17.24-25.00 (21.10*±2.46)
Eye Diameter	3-3.9 (3.5±8.8)	9.09-16.95 (12.35*±1.95)
Post Orbit Distance	9-15 (11.6±1.5)	39.28-52.17 (45.99*±3.29)
Between Eye Distance	7-11.2 (9.1±1.2)	31.03- 41.30 (35.96*±3.29)
Mouth width	5-9.4 (7.0±1.6)	20.83-33.91 (27.41*±4.07)

Table (3): The results of meristic characters for *C. kais*.

Meristic characters	**Min-max (Mean ±SD)**
Lateral Line Scales	36-39 (37.5 ±1.12)
Up Scales of Lateral Line	7-8 (7.5 ± 0.49)
Down Scales of Lateral Line	3-4 (3.5± 0.52)
Dorsal Fin Spines	1-1(1±0)
Dorsal Fin Rays	13-14 (13.5± 0.35)
Pectoral Fin Rays	12-13 (12.5± 0.41)
Anal Fin Spines	1-1(1±0)
Anal Fin Rays	7-8 (7.5± 0.26)
Caudal Fin Rays	16-18 (17.5±0.6)
Pelvic Fine Spines	1-1
Pelvic Fin Rays	8-8 (8±0)
Scales around the least circumference of the caudal peduncle	8-9 (8.5±0.5)
Gill rakers	11-12 (11.5± 0.52)

Plate (3): *Cyprinion kais* (Whole body) from Al-Tharthar Lake, Saladin Province.

Plate (4): Mouth of *Cyprinion kais* **(**ventral view).

Molecular study

PCR primers of the cytochrome b gene, and specific primers are successfully amplified, and gel electrophoresis is performed to show PCR amplification of the *cyto b*, which yielded 508 bp product. These amplifications are used for species diagnosis and give comparative data for developmental taxonomy studies and family development research at the species level. Sequencing of these genes is performed in order to determine the genotype of *C. kais* is collected from the Al-Zubaydia sub-district. The sequence of one gene examined forward and reverse primer is performed; this is part of the sequence process requirements and the use of PCR technology in the context of the method of genetic analysis. The results of nucleotide alignment with the sequences in the gene bank showed that the identities ranged from 99-100%. Partial cds, and *cytochrome b* gene mitochondria are compatible with the same sequence fragment marker, which is available at the Gene Bank in the National Center for Biotechnology Information (NCBI). Plate (5) showed a partial sequence and pair-wise analysis of the fish specimens.

The current results of a total 508 bp at the 5 ends for the *cytb* mtDNA region for three specimens were sequenced, and the best likelihood tree resulting from partitioned maximum likelihood analysis indicated that the tree of genetic relations between the studied specimens showed in Plate (6) that there are two sub-branches. The bootstrap value (BP) between these two branches is equal to 59%. One of the branches showed that the *C. kais* (Local sample T1)

formed a sister group with *C. macrostomum* from AF 180836.1 with a bootstrap value (BP) is equal to 54% and There was a similarity between local samples (T1), and *C. macrostomum* with standard sequences of *C. kais* of the same gene in France (AF180860.1) with a bootstrap value (BP) is equal to 33% and the another branched showed that the *C. kais* (Local sample - T_2) formed a sister group with *C. kais* (Local sample $-T_3$) with a bootstrap value (BP) is equal to 50% and there was a similarity between local samples (T1 and T2) with standard sequences of *Cyprinion* sp. of the same gene from Saudi Arabia (MT 157380.1) with a bootstrap value (BP) is equal to 33%.

Plate (5): Pair wise alignment of partial cds, cytochrome b (*cyt b*) gene of *C. kais* Query is the study or sample sequence and subject is the GenBank sequence.

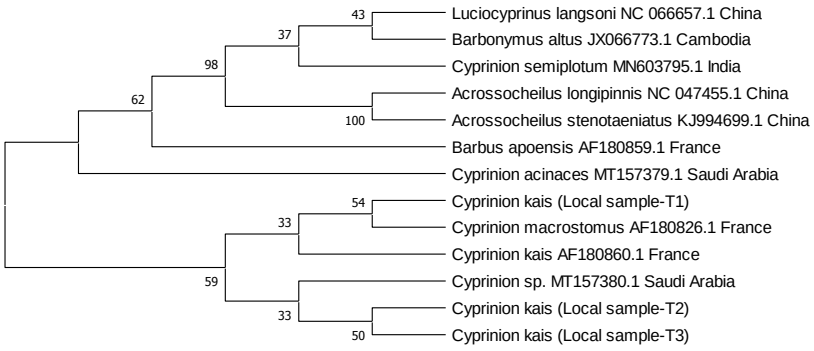

Plate (6): Phylogenetic tree analysis of *Cyprinion kais* local samples resemble for others in different countries; draw by MEGA X using Maximum like hood (ML) method with bootstrap value 500 repeats.

DISCUSSION

Morphology

Morphometric and meristic traits are practical tools of fish morphology for new species definition (Strauss and Bond, 1990) and species identification (Nelson *et al*., 2016). In addition, fish morphology can influence their swimming performance, reproductive, camouflage, and feeding activities, etc. (Sfakiotakis *et al*., 1999). The fish species in the current study belong to the family Cyprinidae. The description of the current specimens of *C. kais* similar to those explained by Coad (2010). The dorsal fin rays in this study are similar to those in Banarescu and Herzig-Straschil (1995), but the number of lateral line scales in the current study is lesser (36-39 vs 41-44), and the width of the mouth is expressed as percentage to the head length in this study is larger (22.7-29.3% vs 13.5-22.0%). As a comparison of some traits of *C. kais* in the current study, such as number of the anal-fin ray, the number of the dorsal-fin ray, the number of the pelvic- fin ray, lateral line scales, and gill rakers which are 7, 13-14, 8, 36-39, and 11-12 respectively which are in agreement with the results of Coad (2010), which are 7, 12-16, 8, 36-43, 8-12 respectively, the number of the pectoral-fin rays in this study is lower in the number (12-14 rays) with 14-18 rays in Coad (2010).

As a comparison of some features of *C. kais* in the current study, such as total length, standard length, body depth, head length, number of the dorsal-fin ray, number of the pelvic-fin ray, and number of the anal-fin ray are 14.3, 11.58, 3.9, 2.54, (13-14), 8, 7 respectively are in agreement with the results of Agha (2017), which are 15.7, 12.76, 3.84, 2.86, (13-14), 8,7 but the number of the scales of lateral line, and gill rakers in this study lesser (36-39 vs 39-40), and 11-12 vs 13-14 respectively.

The form of a mouth, dorsal fin ray, and pectoral fin ray in the current study are similar to the results of Nasri *et al*. (2013), but the standard length of *C. kais* in the current study is larger than the results of Nasri *et al*. (2013). The dorsal fin ray, pelvic fin ray, pectoral fin ray,

and gill rakers in this study are in agreement with the result of Kaya *et al*. (2016) but the lateral scale line of Iraqi specimens has lesser than that of Turkish specimens (36-39 vs 40-43). The numbers of the dorsal-fin ray, the anal-fin ray, the pelvic-fin ray, pectoral fin ray, lateral line scale, scale above lateral line, and scale below lateral line are 13-14, 7, 8, 12-13, 36-39, 7-8, and 3-4 respectively, are in agreement with the results of Nasri *et al*. (2018), which are 13, 7, 7-8, 12-13, 39, 7, and 4 respectively.

As a comparison of some features of *C. kais* in the current study, such as a small mouth with large lateral lobes, lateral line scales, the number of dorsal fin branched rays, number of the pelvic-fin rays, number of the pectoral fin rays, are: 36-39, 13-14, 8, 12-13 respectively which in agreement with the results of Coad (2023), which are 36-43, 12-16, 7-8, 12-18 respectively.

Molecular study

In the present study, we classified, and identified conventionally on the basis of external morphological traits. However, the various developmental stages of fish are difficult to identify by morphological characteristics. Therefore, the traditional identification and classification results were confirmed by mitochondrial DNA sequencing, and fortunately, the results matched and there was no confusion in the scientific nomenclature. DNA sequencing is one of the most important methods that contributed to the rapid diagnosis of species (Pons *et al*., 2006). The *cyto b* gene as a code for animal species identification (Hebert *et al*., 2004); especially the fish species, which have been attracting attention lately. This study demonstrated the effectiveness of the *cyto b* gene in identifying Cyprinidae species. Banarescu and Herzig-Straschil (1995) reported that there are some specimens that are to a certain degree intermediate between 'typical' *C. macrostomus* and *C. kais*, being closer to *C. macrostomus* based on some morphological traits; these specimens have mouth width as expressed as a percentage of head length and number of dorsal fin rays. This is similar to the current study, but DNA sequencing revealed and confirmed that the fish species is *C. kais*.

The present phylogenetic analysis showed that the *cyt b* genome sequencing results of *C. kais* showed a strong association with *C. macrostomum* and a sister group formed with a BP of 54%. These results agreed with Faddagh *et al*. (2012a), who determined that BP has 89 % common ancestry. Faddagh *et al*. (2012b) also determined that taxonomical status of some cyprinid species in Iraqi waters using the mitochondrial 16S rRNA gene: *Barbus kersin* (=*L. kersin*), *B. grypus* (=*L. grypus*), *B. barbulus* (=*L. barbulus*), *B. xanthopterus* (= *L. xanthopterus*), *Cyprinus carpio*, *B. sharpeyi* (=*M. sharpeyi*), and *C. luteus*, the results confirmed that the six fish species genetically belong to the Cyprinidae. Aziz (2015) used the same gene *cyto b* to identify nine species of Cyprinidae. The DNA sequencing result showed that all species belong to Cyprinidae, and the phylogenetic relationship degree with this family for *C. macrostomum*, *L. esocinus*, *C. trutta* and *L. xanthopterusa* was a BP of 90%, for *C. luteus* was a BP of 87%, for *grypus* (*A. grypus*) was a BP of 76%, and *C. regium*, *C. carpio* and *C. carassius* was a BP of 75%; this finding is also in agreement with the previous study (Zardoya *et al*., 1999), who found that the origin of *Barbus* sp. belongs to Cyprinidae.

Durand *et al*. (2002) used the *cyt b* gene to conduct phylogenetic and zoogeographical studies on *C. macrostomum* and *C. kais* in the Middle East Region and indicated that the level of difference between them is less than 0.4% among the studied cyprinids. In this study, the results are in agreement with Daştan *et al*. (2012), who used mtDNA PCR-RFLP based on pairwise comparison to study the phylogeographic and phylogenetic relationships among *C. kais*, *C. macrostomus*, and *Carasobarbus chantrei* and indicated that the divergence rates among haplotypes of fish populations from each river basin ranged from 0.023 to 7.35%. Yang *et al*. (2012) used *cyto b* gene as a molecular marker to identify *Barbus* sp. and *Carasobarbus* sp. DNA sequencing results indicate that there is a genetically close relationship among them, and both species belong to the Cyprinidae family, which in turn belongs to the order Cypriniformes.

CONCLUSIONS

In view of the results of the current study, the identification of *Cyprinion kais* is confirmed morphologically. The DNA sequencing results showed and confirmed the validity of the fish species and indicated that the correctly sequenced species were identified using *cyto b* gene. Locally, the number of freshwater native fishes that have been genetically studied reaches 29 out of 53 (54.7%) known species. As well, seven species out of 14 (50%) known species of Cyprinidae are genetically established.

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CONFLICT OF INTEREST STATEMENT

These results are a part of Ph. D. Dissertation, in the Department of Biology, College of Science, University of Baghdad for the first author. Further, the authors of this manuscript confirm no existence other relationship with any official institution.

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دراسة مظهرية وجزيئية لأسماك بنيني صغير الفم ***Cyprinion Kais*** **(Heckel, 1843) (Piscies, Cypriniformes, Cyprinidae) في وسط العراق**

هند ضياء هادي*، عبد حسن براج** و اثير حسين علي***

* قسم الاسماك، مركز بحوث و متحف التاريخ الطبيعي، جامعة بغداد، بغداد، العراق.

**قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

***قسم الاسماك و الثروة البحرية، كلية الزراعة، جامعة البصرة، البصرة، العراق.

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الخلاصة

تمتاز عائلة الشبوطيات Cyprinidae بانها تضم أكثر أنواع الأسماك وفرة، وهي موزعة في العديد من المياه الداخلية في العراق. تعتبر هذه الاسماك من الأنواع المعقدة تصنيفياً ومن الصعب تشخيصها مظهرياً. وبالتالي، يجب التاكد من صحة تشخيص الانواع باستخدام الفحص الجزيئي. في هذه الدراسة جمع 20 عينة من البنيني صغير الفم *Cyprinion kais* Heckel, 1843 (Piscies, Cypriniformes, Cyprinidae) من بحيرة الثرثار (5 عينة) التابعه الى محافظة صلاح الدين ومنطقة الزبيدية (15عينة) التابعه الى محافظة واسط، وسط العراق.

كان تسلسل الحمض النووي لـ *C. kais* عبارة عن جين (*cytb*) DNA cytochrome b. بعد التحليل ، تمت مقارنة التسلسلات مع متواليات الأجناس والأنواع السمكية الأخرى المخزنة في البنك العام. أظهرت نتائج تسلسل الحمض النووي أن الأنواع المدروسة تنتمي إلى هذا النوع. تؤكد نتائج الدراسة الحالية صحة التشخيص المظهري لسمكة *Cyprinion kais* وذلك باستخدام تحليل تسلسل الحمض النووي.