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A FURTHER DISTRIBUTION RECORD OF THE GENUS *COBITIS* (ACTINOPTERYGII: CYPRINIFORMES: COBITIDAE) IN IRANIAN INLAND WATERS WITH A NOTE ON ITS ZOOGEOGRAPHIC IMPORTANCE IN THE URMIA LAKE BASIN

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Abstract. This study reports the presence of the genus *Cobitis* in the Zarrineh River, Urmia Lake basin, Iran. Its taxonomy is clarified and zoogeography of Urmia basin is discussed. This study provides morphological characteristics, mtDNA COI barcode sequences of the collected *Cobitis* specimens and analyses phylogenetic relation within the members of this genus in Iran. The results revealed that morphometric and molecular (COI) characters of the collected *Cobitis* specimens are largely overlapping or completely identical with those of *Cobitis saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh et Esmaeili, 2017. Sequences of *C. saniae* from the Urmia Lake Basin clustered with published sequences of *C. saniae* showing a K2P genetic distance of 0.76% to the population from the Caspian Sea basin. This record shows a range extension of this species toward the western part of Iran. Based on first record of *C. saniae* from the Zarrineh River, the ichthyofauna of the Urmia Lake basin includes 30 species. The presence of *C. saniae* in Urmia Lake basins reveals the past connection of this basin with the Caspian Sea basin.

Keywords: taxonomy, morphology, spined loach, distribution

INTRODUCTION

Members of the genus *Cobitis* Linnaeus, 1758 represent one of the most widely distributed Palearctic fish groups, primary freshwater (Freyhof et al. 2018) with four valid species in Iran (Joulade-Roudbar et al. 2017, Eagderi et al. 2017a, 2017b; Freyhof et al. 2018), including *Cobitis linea* (Heckel, 1849) from the Kor River and upper Kol River drainages, *Cobitis avicennae* Mousavi-Sabet, Vatandoust, Esmaeili, Geiger et Freyhof, 2015, occurring in the Karkheh and Karun, two sub-tributaries of the Tigris River drainage, *Cobitis faridpaki* Mousavi-Sabet, Vasil′eva, Vatandoust et Vasil′ev, 2011 from the eastern part of the Iranian Caspian Sea and Namak Lake basins, and *Cobitis saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh et Esmaeili, 2017 from the western part of the Caspian Sea basin (Mousavi-Sabet et al. 2015, Eagderi et al. 2017a, 2017b, Esmaeili et al. 2018). The species of the genus *Cobitis* are identified based on diagnostic characters of the color pattern including black spots at the caudal-fin base and four longitudinal pigment zones on the flank (Gambetta's zones 1–4), sexual dimorphism (males have 1–2 Canestrini's scales on the dorsal surface of the anterior pectoral fin rays), suborbital spine morphology, barbel and mental lobe morphology (Menon 1992, Kottelat and Freyhof 2007). In addition to morphological identification, mitochondrial DNA (mtDNA) differentiation, known as DNA barcoding, plays an important role in taxonomic studies and phylogenetic inferences (Yang et al. 2010). The purpose of DNA barcoding, based on partial COI sequence, is to improve the identification of species and to discover new species, and it has been used successfully for delimitation of species in more than 90% of animal species studied (Ward et al. 2005, Hubert et al. 2008).

We collected spined loaches of the genus *Cobitis* from the Zarrineh River, Urmia Lake basin, Iran in 2016. Therefore, this study was aimed to report for the first-time presence of the genus *Cobitis* in Urmia Lake basin and clarify its taxonomic status by providing morphological characteristics, mtDNA COI barcode sequences and revealing its phylogenetic relation with

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the members of the genus *Cobitis* in Iran with a note on its zoogeographic importance.

MATERIALS AND METHODS

Five specimens of the genus *Cobitis* were collected from the Zarrineh River, Urmia Lake basin, Kurdistan Province, Iran in May 2016 by electrofishing device. After anesthesia, the fin clips of two specimens were fixed in 96% ethanol for molecular studies and all specimens were preserved in 5% buffered formaldehyde.

Morphological analysis. A total of 32 morphometric characteristics were measured by a digital caliper to the nearest 0.01 mm (Table 1), then percentage ratios of the morphometric characters in relations to SL and HL were calculated. All measurements were made point to point based on Kottelat and Freyhof (2007). The standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The percentage ratios of morphometric characters in relations to SL and HL were calculated. The terminology of the pigmentation pattern follows Kottelat and Freyhof (2007).

DNA extraction and PCR. DNA was extracted from the collected pectoral-fin tissues using a modified phenol-chloroform method (Sambrook et al. 1989). The COI gene was amplified using primers FCOI20 (5′-AACCTCTG TCTTCGGGGCTA-3′) and RCOI20III (5′-TTGAGCC TCCGTGAAGTGTG-3′) (Hashemzadeh Segherloo et al. 2012). Polymerase chain reaction (PCR) conditions were as follows: a 50 µL final reaction volume containing 5 µL of

Table 1

Morphometric data of *Cobitis saniae* from Urmia lake basin (IMNRF-UT-1093-16; $n = 5$) and Caspian Sea basin (IMNRF-UT-1093, $n = 15$)

	C. saniae Urmia Lake		*C. saniae* Caspian Sea	
Character	Range	Mean ± SD	Range	Mean ± SD
Standard length [mm]	52.3–70.7		49.2–85.6	
In percent of standard length				
Body depth at dorsal fin origin	15.1–18.1	16.2 ± 1.7	14.6–16.6	15.6 ± 0.8
Caudal peduncle depth	9.6–10.2	9.9 ± 0.3	9.1–11.0	10.0 ± 0.5
Predorsal length	50.9–51.6	51.3 ± 0.4	50.0–54.4	52.0 ± 0.8
Postdorsal length	34.3–40.8	36.8 ± 1.9	45.6–49.9	48.6 ± 1
Prepelvic length	50.1–54.5	52.2 ± 2.2	51.6–57.0	54.6 ± 2.0
Preanal length	78.4–79.5	79.0 ± 0.6	79.6–81.7	80.5 ± 0.6
Caudal peduncle length	12.7–17.3	14.7 ± 2.4	12.3–15.6	14.1 ± 1.1
Dorsal-fin base length	14.0–15.4	14.8 ± 0.8	12.5–16.6	13.4 ± 0.8
Dorsal-fin depth	4.4–7.0	6.2 ± 1.4	5.8–7.1	6.5 ± 0.4
Anal-fin base length	8.9–11.4	8.4 ± 2.3	10.9–12.8	11.7 ± 0.5
Anal-fin depth	10.7–14.3	12.3 ± 1.4	10.6–12.8	12.1 ± 0.9
Pectoral fin length	9.6–14.4	12.1 ± 1.2	10.6–12.8	11.4 ± 0.7
Pelvic fin length	12.2–16.9	14.8 ± 2.4	10.6–12.8	11.5 ± 1.1
Distance between pectoral and pelvic-fin origins	30.4–36.9	33.7 ± 3.3	32.6–36.6	34.0 ± 1.1
Distance between pelvic and anal-fin origins	26.4–28.1	27.2 ± 0.9	23.6–28.7	25.4 ± 1.6
Body width at dorsal fin origin	7.1–9.2	7.7 ± 0.8	8.9–11.0	9.9 ± 0.5
Caudal peduncle width	1.9–3.1	2.6 ± 0.6	1.6–3.0	2.2 ± 0.5
Head length (HL)	17.9–18.9	18.4 ± 0.5	16.4–21.0	18.0 ± 1.2
In percent of head length				
Snout length	29.8–42.4	36.3 ± 6.3	37.7–45.3	41 ± 3.6
Horizontal eye diameter	9.9–16.2	13.6 ± 3.3	12.3–17.5	13.3 ± 1.4
Postorbital distance	53.4–56.8	55.3 ± 1.7	48.3–58.4	51.6 ± 3.9
Head depth at nape	61.8–65.7	63.9 ± 1.9	62–76.1	68.4 ± 5.6
Head depth at eye	48.1–56.8	53.5 ± 4.7	54.0–63.6	59.2 ± 2.5
Dorsal head length	78.8–87.4	83.4 ± 4.3	77.8–91.0	83.2 ± 4.8
Head width at nape	46.6–51.5	48.8 ± 2.5	48.1–61.0	53.6 ± 5.1
Interorbital distance	21.1–26.3	24.2 ± 2.8	20.6–25.0	22.3 ± 1.9
Internasal distance	19.1–20.2	19.8 ± 0.6	15.1–20.3	17.5 ± 1.8
Mouth width	16.8–22.2	20.4 ± 3.1	16.6–22.5	20.4 ± 2.4
Inner rostral barbel length	11.0–17.9	15.3 ± 2.5	9.4–12.6	10.4 ± 1.5
Outer rostral barbel length	18.2–21.4	20.2 ± 1.8	9.7–16.2	12.8 ± 3.3
Maxillary barbel length	14.3–22.2	20.2 ± 2.0	9.6–18.1	12.5 ± 3.6

10X Taq polymerase buffer, 1 μL of (50 mM) $MgCl_2$, 1 μL of (10 mM) deoxynucleotide triphosphate (dNTP), 1 μL (10 μm) of each primer, 1 μL of Taq polymerase (5 U · μL^{-1}), 7 μL of total DNA and 34 μL of H_2O. Amplification cycles were as follows: denaturation for 10 min at 94°C; 30 cycles at 94°C for 1 min, 58.5°C for 1 min, 72°C for 1 min, and a final extension for 5 min at 72°C. PCR products were purified using a purification Kit (Expin Combo GP – mini; Macrogen incorporation, Korea). The PCR products were sequenced using the Sanger method by a robotic ABI-3130xl sequencer using the manufacturer's protocol. The forward and reverse primers were used for single-strand sequencing.

Molecular data analysis. We newly generated two DNA barcodes and the sequences were compared to the published *Cobitis* sequences using a basic local alignment search tool (BLASTn) (Altschul et al. 1990). The retrieved sequences of the other members of the genus *Cobitis* from the GenBank database (NCBI) following the blast search are shown in Table 2. Sequence data were aligned using BioEdit software. For phylogenetic reconstructions, the datasets were analyzed by Bayesian Inference (BI) using MrBayes 3.1.2 (Ronquist et al. 2012) and maximum likelihood (ML) method in IQTREE 1.6.0 (Hoang 2018). We determined the best-fit model of molecular evolution for the given data using the Bayesian information criterion scores (BIC) in ModelFinder (Kalyaanamoorthy et al. 2017).

MrBayes was run with 6 substitution types (nst = 6) and considered gamma-distributed rate variation across sites with a proportion of invariable sites (GTR + I). Four simultaneous Monte Carlo Markov Chains were run for 10 000 000 generations, sample frequency every 1000 generations, chain temperature 0.2. Log-likelihood stability was attained after 10 000 generations, and we excluded the first 1000 trees as burn-in. The remaining trees were used to compute a 50% majority-rule consensus tree. For ML analyses, we conducted heuristic searches (1000 runs) according to TPM2 + F + G4 model. The genetic distances were investigated based on Kimura two-parameter

Table 2

List of species used for molecular analysis and their GenBank accession number

Accession no	Species	Reference
Cobitis avicennae	KP050525	Mousavi-Sabet et al. 2015
Cobitis avicennae	KP050516	Mousavi-Sabet et al. 2015
Cobitis avicennae	KP050520	Mousavi-Sabet et al. 2015
Cobitis battalgili	KJ552796	Geiger et al. 2014
Cobitis battalgili	KJ552985	Geiger et al. 2014
Cobitis battalgili	KJ552834	Geiger et al. 2014
Cobitis elazigensis	KP050514	Mousavi-Sabet et al. 2015
Cobitis elazigensis	KP050527	Mousavi-Sabet et al. 2015
Cobitis elazigensis	KP050513	Mousavi-Sabet et al. 2015
Cobitis faridpaki	KY476339	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY476334	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY476337	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY476338	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY476336	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY646316	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY646317	Jouladeh-Roudbar et al. 2017
Cobitis faridpaki	KY646318	Jouladeh-Roudbar et al. 2017
Cobitis linea	KP050530	Geiger et al. 2014
Cobitis linea	KP050539	Geiger et al. 2014
Cobitis saniae	KP050518	Geiger et al. 2014
Cobitis saniae	KP050506	Geiger et al. 2014
Cobitis saniae	KY646319	Jouladeh-Roudbar et al. 2017
Cobitis saniae	KY646320	Jouladeh-Roudbar et al. 2017
Cobitis saniae	KY646321	Jouladeh-Roudbar et al. 2017
Cobitis saniae	KY646322	Jouladeh-Roudbar et al. 2017
Cobitis saniae	KP050528	Mousavi-Sabet et al. 2015
Cobitis saniae	KP050509	Mousavi-Sabet et al. 2015
Cobitis turcica	KJ552985	Geiger et al. 2014
Cobitis taenia	KJ128460	Eagderi et al. 2017b
Cobitis taenia	KM286524	Knebelsberger et al. 2015
Cobitis taenia	KJ128459	Eagderi et al. 2017b
Cobitis turcica	KJ553220	Geiger et al. 2014
Cobitis turcica	KJ552782	Geiger et al. 2014
Misgurnus fossilis	KM286765	Knebelsberger et al. 2015
Misgurnus fossilis	KM286763	Knebelsberger et al. 2015

(K2P) distances (Kumar et al. 2008) in Mega7 software (Kumar et al. 2016). Screening for diagnostic nucleotide substitutions relative to *Oryzias latipes* (Temminck et Schlegel, 1846) (NC_004387) was performed manually from the resulting sequence alignment in BioEdit software. As an outgroup, sequences of *Misgurnus fossilis* (Linnaeus, 1758) were retrieved from GenBank database (accession numbers: KM286763 and KM286765).

RESULTS

The general body shape of the collected *Cobitis* specimens is displayed in Fig. 1 and the morphological characteristics in Table 1. The morphometric characters of the *Cobitis* specimens from the Zarrineh River overlapped to those of *C. saniae* from the Sefid River, the Caspian Sea basin (Table 1) except postdorsal length (34.3–40.8 vs. 45.6%–49.9% SL) and outer rostral barbel length (18.2–21.4 vs. 9.7%–16.2% HL). In addition, *Cobitis* specimens of the Zarrineh Rivers bear a single lamina circularis on the pectoral fin in males with its base widely connected to pectoral-fin ray as *C. saniae* key character. The color patterns i.e., Gambetta's zones of the pigmentations of the Urmia lake basin specimens were similar to those of *C. saniae* with a single black spot on the upper caudal-fin base and type II pigmentations pattern of Z4 i.e., merged blotches not forming a dark stripe.

Fig. 1. (**A**) *Cobitis saniae* from the Zarrineh River, Urmia Lake basin, (**B**) *C. saniae* from the Sefid River, Caspian Sea basin (IMNRF-UT-1091-1, holotype), and (**C**) sampling station of *C. saniae* from the Zarrineh River, Urmia lake basin

Two phylogenetic approaches Bayesian Inference (BI) and Maximum Likelihood (ML), gave the same tree topologies and thus one is presented (Fig. 2). Our analysis of the COI sequence data clusters the newly found *Cobitis* population from the Zarrineh River with specimens of *C. saniae* from the Caspian Sea basin (Fig. 2). In addition, in our tree, Iranian members of the genus *Cobitis* including *C. saniae*, *C. faridpaki*, and *C. avicennae* into the *Cobitis taenia* Linnaeus, 1758 species group (Freyhof et al. 2018). Table 3 lists the mean genetic divergences found in the mtDNA COI sequences of the studied species. The generated DNA barcode sequences of this *Cobitis* population showed more than 99% similarity with the available sequences of *C. saniae*. In addition, it is distinguished by having two diagnostic nucleotide substitutions in the mtDNA COI barcode region from other members of the genus *Cobitis* in Iran (Table 4) and a minimum K2P genetic distance of 0.7% with *C. saniae* from the Caspian Sea basin.

DISCUSSION

Endorheic Urmia Lake basin, particularly its center wetland i.e., Urmia Lake is an important ecosystem in north-western Iran (Ghasemi et al. 2015). Drastic changes in the lake environment are suggested to be the consequences of aggressive regional water resources development plans, intensive agricultural activities, and upstream competition for water (AghaKouchak et al. 2015). Inflows of the lake are also under severe pressure due to the above-mentioned factors and lack of perspective management. Their ecological conditions are strongly affected, and the biodiversity of running waters is threatened (Ghasemi et al. 2015).

Ghasemi et al. (2015) reported 29 species belonging to 25 genera and 7 families from the Urmia Lake basin. However, there are some changes in the taxonomic status of some species that is worth to be mentioned here. From the superfamily Cobitoidea, three species of the family Nemacheilidae, including *Oxynoemacheilus bergianus* (Derjavin, 1934), *Oxynoemacheilus brandtii* (Kessler, 1877), and *Paracobitis malapterura* (Valenciennes, 1846) were reported (Ghasemi et al. 2015). Based on the recent works (Eagderi et al. 2018a), those of the reported *O. brandtii* from the Urmia lake basin were described as *Oxynoemacheilus elsae* Eagderi, Jalili et Çiçek, 2018. In addition, those of *Barbus* cf. *cyri* (see Jalili et al. 2015, Ghasemi et al. 2015, Nikmehr et al. 2016, Khaefi et al. 2017, Çiçek et al. 2018, Esmaeili et al. 2018) in this basin described as *Barbus urmianus* Eagderi, Nikmehr, Çiçek, Esmaeili, Vatandoust et Mousavi-Sabet, 2019 (see

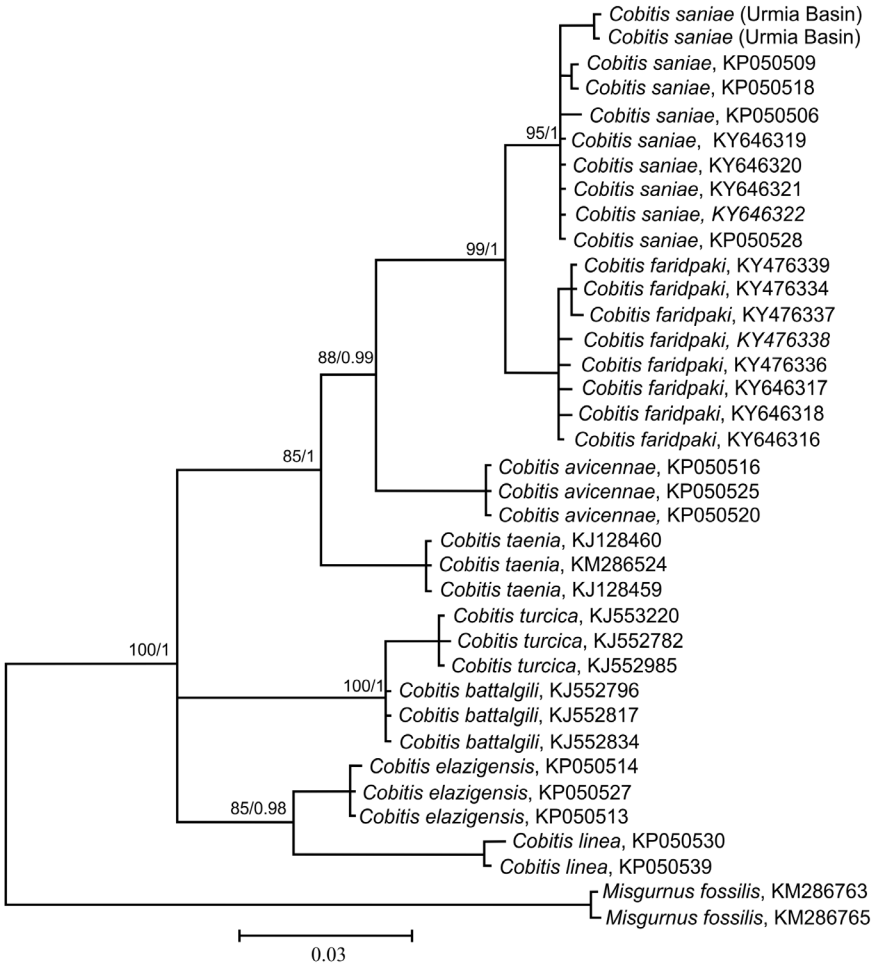

Fig. 2. Maximum likelihood estimation of the phylogenetic relations based on the mitochondrial COI barcode region; values at nodes correspond to ML bootstrap / BI posterior probability

Table 3

Estimates of average K2P genetic divergence over sequence pairs between Iranian *Cobitis* species

Species	No	1	2	3	4	5	6	7	8
C. linea	1								
C. elazigensis	2	5.6							
C. turcica	3	9.9	8.0						
C. battalgili	4	9.7	7.8	1.4					
C. taenia	5	11.3	8.2	9.6	8.9				
C. avecennae	6	13.6	9.3	9.8	10.0	5.2			
C. faridpaki	7	12.7	9.3	10.7	11.0	6.6	6.3		
C. saniae (Caspian) Sea)	8	13.0	10.1	11.2	10.9	6.2	6.4	2.4	
C. saniae (Urmia) Lake)	9	13.4	10.2	11.6	11.4	6.7	6.7	2.9	0.7

Table 4

Diagnostic nucleotide substitutions found in mtDNA COI barcode region of *Cobitis* species from Iran (those red are diagnostic nucleotide substitutions in the mtDNA COI barcode region of *C. saniae* from Urmia Lake basin)

	5535	5580	5598	5646	5672	5676	5679	5692	5701	5748	5755	5763	5778	5781	5788	5790	5820	5835	5838	5841	5844	5847	5850	5853	5856	5862	5889	5895	5902
C. linea	**A**	**T**	T	**C**	**A**	**T**	**T**	**C**	**G**	T	A	A	A	**C**	T	**G**	**T**	**T**	**T**	T	**T**	**C**	**T**	**G**	**T**	C	T	T	**T**
C. avicennae	C	C	**C**	T	T	C	C	T	A	**C**	**G**	A	A	T	**C**	A	C	C	C	**C**	C	A	A	**C**	C	**T**	T	**C**	C
C. faridpak	C	C	T	A	T	C	C	T	A	T	A	A	A	T	T	A	C	C	C	T	C	A	A	T	C	C	**A**	T	C
C. saniae (Caspian)	C	C	T	A	T	C	C	T	A	T	A	**G**	**G**	T	T	A	C	C	C	T	C	A	A	T	C	C	T	T	C
C. saniae (Urmia)	C	C	T	A	T	C	C	T	A	T	A	**G**	**G**	T	T	A	C	C	C	T	C	A	A	T	C	C	T	T	C

	5904	5907	5916	5929	5940	5952	5955	5957	5958	5961	5967	5970	5976	6001	6002	6024	6033	6042	6043	6048	6049	6051	6054	6057	6063	6069	6072	6078	6081
C. linea	**G**	C	**A**	**T**	**T**	**C**	A	**T**	**T**	T	**A**	**A**	**A**	**T**	T	**G**	T	**A**	**T**	**A**	T	**A**	**T**	T	**T**	**A**	T	**A**	**A**
C. avicennae	A	C	G	C	C	C	A	C	A	**C**	G	G	C	C	T	A	**C**	**G**	C	T	**C**	G	C	**C**	C	T	**C**	G	C
C. faridpak	A	C	G	C	C	C	**T**	C	A	T	G	G	C	C	T	A	T	**T**	C	T	T	G	C	T	C	T	T	G	C
C. saniae (Caspian)	A	**T**	G	C	C	C	A	C	A	T	G	G	C	C	T	A	T	**C**	C	T	T	G	C	T	C	T	T	G	C
C. saniae (Urmia)	A	**T**	G	C	C	C	A	C	A	T	G	G	C	C	**G**	A	T	**C**	C	T	**A**	G	C	T	C	T	T	G	C

Eagderi et al. 2019). Furthermore, those of previously reported *Rhinogobius similis* Gill, 1859 (see Eagderi and Moradi 2017), *Squalius orientalis* Heckel, 1847, and *Capoeta capoeta* (Güldenstädt, 1773) were distinguished as *Rhinogobius lindbergi* Berg, 1933 (see Eagderi et al. 2018b), *Squalius turcicus* De Filippi, 1865 (see Esmaeili et al. 2018), and *Capoeta sevangi* De Filippi, 1865 (see Zareian et al. 2018, Esmaeili et al. 2018), respectively. Therefore, based on the above-mentioned changes with the first record of *C. saniae* from the Zarrineh River, the ichthyofaunal of the Urmia lake basin is included 30 species.

Members of the genus *Cobitis* show interspecific variations in terms of morphological characters and color patterns (Eagderi et al. 2017a, 2017b, Freyhof et al. 2018). The presently reported study revealed that morphometric characters (Table 1) and color pattern of the *Cobitis* specimens from the Zarrineh River were largely overlapping with those of *C. saniae* from the Caspian Sea basin except post-dorsal and outer rostral barbel lengths. These morphological differences could be a result of phenotypic plasticity caused by different environmental conditions of their habitats (Poorbagher et al. 2017, Radkhah et al. 2017). In a species with a large distribution area as *C. saniae*, we expect some intraspecific morphological differences in different environmental conditions (Marcil et al. 2006).

The Middle East, species of the genus *Cobitis* had been reviewed recently by Freyhof et al. (2018), who recognized 30 species in four species groups. *Cobitis saniae* occurs from the Sefid River in Iran north-west to the Kura-Aras River system in Azerbaijan, Armenia, and Turkey (Eagderi et al. 2017a, 2017b, Freyhof et al. 2018). Furthermore, Freyhof et al. (2018) reported *C. saniae* from the Rioni River, the Georgian Black Sea basin. In the presently reported study, *C. saniae* sequences from the Urmia Lake Basin clustered with published sequences of *C. saniae* from the Caspian Sea basin with a K2P genetic divergence of 0.7%. The presently provided data constitute the evidence that the distribution range of this species reaches to the western part of Iran. Low genetic distance found between Urmia Lake and the Caspian Sea basin fall in an intraspecific range of distance among *Cobitis* species (Freyhof et al. 2018)

Despite many efforts to collect more specimens of the genus *Cobitis* in the Zarrineh River, we could able to collect only five specimens, however, these few records show its zoogeographic importance. The presence of *C. saniae* in the Caspian Sea basin (the Aras River) and Urmia Lake basins suggests their connection in the past due to geological events. Possible corridors between these basins

could be inter-drainage connections in the northern part of the Urmia Lake basin in Khoy (Ghara-Tappeh) via the Aras River, which drains into the Caspian Sea, and stream capture events occurring in the southeast of this basin, as the headwaters of some rivers draining to the Caspian Sea (e.g., Qezel-Ozan River) are located in the proximity to the south-eastern Urmia Lake basin (Zarrineh River) (Jalili et al. 2017, Khaefi et al. 2017). This conclusion is supported by geological reports indicating the origin of the modern Urmia Lake c. 10 000–30 000 years ago (Darvishzadeh 2007). In addition, the Lake Urmia was formed during the late Pliocene–Pleistocene and may have had a Pleistocene connection to the Caspian Sea (Coad 2019).

Materials used for morphological analyses. All from Iran: *Cobitis faridpaki*: IMNRF-UT-1016, 19, 37–68 mm SL; Mazandaran Province: Siah River at Ghaemshahr, Caspian Sea basin, 36°26′39.0″N, 52°53′43.6″E; August 2016. — IMNRF-UT-1015, 21, 51-90 mm SL; Mazandaran Province: Keselian River at Savadkoh, Caspian Sea basin, 36°12′19.1″N53°00′56.0″E; July 2015. — IMNRF-UT-1100, 11, 37–68 mm SL; Alborz Province: Karaj River at Asara, Namak Lake basin, 36°1′52″N 51°12′51″E; May 2016. — ZM-CBSU H2007, 20, 42–67 mm SL; Siah River, Ghaemshahr at Saru Kola village, 36°27′26.50″N, 52°53′28.75″E; 2014. — IMNRF-UT-1083, 5, 54.6–85.2 mm SL; Kurdistan Province: Zarineh River at Qeshlaq Pol, Urmia Lake basin, 36°05′09″N, 46°20′06″E; May 2016.

Cobitis saniae: IMNRF-UT-1018, 6, 38–53 mm SL; Gilan Province: Sefid River at Totkaboon, Caspian Sea basin, 36°53′27.3″N 49°30′42.0″E; July 2014. — IMNRF-UT-1091, holotype, 84.6 mm SL, Guilan Province: Bara Goor River a tributary of Sefid River, near Emamzadeh Hashem, Caspian Sea Basin, 37°00′11″N, 49°37′49″E; 26 January 2017. — IMNRF-UT-1091, 9 paratypes, 49.2–85.6 mm SL; Iran: Guilan Province: Bara Goor River a tributary of Sefid River, near Emamzadeh Hashem, Caspian Sea Basin, 37°00′11″N, 49°37′49″E; 26 January 2017.

Cobitis avicennae: IMNRF-UT-1096, 12, 71–115 mm SL; Kermanshah Province, Dinevar River at Hossein Abad, Tigris drainage, 34°33′16.6″N, 47°24′48.4″E; Aug 2016. — IMNRF-UT-1020, 1, 95 mm SL; Kermanshah Province; Dinevar River at Hossein Abad, Tigris drainage, 34°33′16.6″N, 47°24′48.4″E; Jun 2016.

Cobitis linea: ZM-CBSU H2090, 6, 53–79 mm SL; Fars Province: Ghadamgah spring at Dorudzan, Kor River basin, 30°14′19.65″N, 52°22′23.3″E; May 2013. — ZM-CBSU H2096, 6, 45–72 mm SL; Fars Province: Ghadamgah spring at Dorudzan, Kor River basin, 30°14′19.65″N, 52°22′23.3″E; August 2004.

Materials used for molecular analyses: *Cobitis saniae*: IMNRF-UT-1083-1-2, 2-fin clips; Kurdistan Province: Zarineh River at Qeshlaq Pol, Urmia Lake basin, 36.089810°N, 46.343340E°; May 2016, GeneBank Accession number (MW009104, MW009105).

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