FishTaxa (2017) 2(1): 48-61
E-ISSN: 2458-942X
Journal homepage: www.fishtaxa.com

Taxonomic status of the genus *Cobitis* Linnaeus, 1758 (Teleostei: Cobitidae) in the southern Caspian Sea basin, Iran with description of a new species

Soheil EAGDERI[1*], Arash JOULADEH-ROUDBAR[1], Pariya JALILI[1], Golnaz SAYYADZADEH[2], Hamid Reza ESMAEILI[2]

[1]Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.
[2]Ichthyology and Molecular Systematics Research Laboratory, Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran.
*Corresponding author: *E-mail: soheil.eagderi@ut.ac.ir*

Abstract

Members of the genus *Cobitis* in the southern Caspian Sea basin of Iran are found from the Atrak to Aras Rivers. Two species, namely *C. keyvani* and *C. faridpaki* had been already described from this distribution range. However, previous study revealed that *C. keyvani* is a junior synonym of *C. faridpaki*, therefore populations of the eastern part of the Sefid River are *C. faridpaki* and those of the western part of this basin represent an undescribed species misidentified as *C. keyvani* in previous studies. Here we describe and compare it with other species of this genus from Iran based on morphological and molecular (COI barcode region) characters.

Keywords: Freshwater fish, Morphology, COI, Spined loach, Sefid River.
Zoobank: urn:lsid:zoobank.org:pub:8E2059BD-3345-4FB1-A7D1-12FD62CE01AD
urn:lsid:zoobank.org:act:B5BA46A4-6ABE-4392-A558-DA54A08D270A

Introduction

Members of the genus *Cobitis* represent one of the most widely distributed Palearctic primary freshwater fishes (Sawada 1982; Coad 2017). They are found in Eurasia and Morocco (North Africa) and Southern Asia (Eschmeyer and Fong 2011). This genus has three valid species in Iran including *C. linea* Heckel, 1849 found in the Kor River and reported from the upper Kol River drainages, *C. avicennae* Mousavi-Sabet, Vatandoust, Esmaeili, Geiger & Freyhof, 2015 occurs in the Karkheh and Karun, two sub-tributaries of the Tigris, and *C. faridpaki* Mousavi-Sabet, Vasil'eva, Vatandoust & Vasil'ev, 2011 found in the eastern part of the Iranian Caspian Sea basin (Jouladeh-Roudbar et al. 2015, 2017).

Nalbant and Bianco (1998) recorded for Iran, *C. turcica* Hanko, 1925, described from central Anatolia at Eregli, Turkey. Therefore, they included *C. turcica* in the fauna of Iran based on two specimens from the Kor River near Persepolis (1 specimen, 81 mm SL, IZA 7829 and 1 specimen 72 mm SL, ISBB uncat., Kor River near the Persepolis, 30 May 1976, P. G. Bianco), and in conformity with their publication, Eschmeyer and Fricke (2011) expanded the range of this Turkish species to Iran. But B.W. Coad examined IZA 7829-30 specimens and identified them as *C. linea* (see Coad 2011). For a long time, spined loach populations from the Southern Caspian Sea basin were considered as *C. taenia* Linnaeus, 1758 or *C. satunini* Gladkov, 1935 (Saadati 1977; Kiabi et al. 1999; Esmaeili et al. 2010; Coad 2011) but recent studies revealed that the Southern Caspian Sea populations are distinct species: *C. faridpaki*, from the Siah River (Siahrud), and *C. keyvani*, from the Keselian River by Mousavi-Sabet et al. (2011, 2012, 2015). Mousavi-Sabet et al. (2015) distinguished *C. faridpaki* from *C. keyvani* by morphological characters and COI barcode region sequences. But, their molecular materials of *C. keyvani* was not from its type locality or the type river drainage. Recently, Jouladeh-Roudbar et al. (2017) confirmed that *C. faridpaki* and *C. keyvani* are indistinguishable by the morphological characters and molecular data based on the fresh specimens collected from their type localities, therefore *C. keyvani* was treated a junior synonym of *C. faridpaki*.

According to Jouladeh-Roudbar et al. (2017), the *Cobitis* populations in the eastern part of the Sefid River are *C. faridpaki* and those of the western part of this basin from the Sefid River to Aras River represent an undescribed species. Hence, the goal of this work is to describe the populations of the Sefid River, previously assigned as *C. keyvani*, as new distinct species.

Accepted: 9 February 2017; Published: 18 March 2017

Material and Methods

Sampling and morphologcal study: Fish specimens were collected by electrofishing from the Kargan and Sefid Rivers, the Caspian Sea basin. After anaesthesia, the collected specimens were fixed in 5% formaldehyde and stored in 70% ethanol. Fin clips were directly fixed and stored in 96% ethanol for molecular studies. Measurements were made using a digital caliper to the nearest 0.1 mm. All measurements were made point to point, never by projections. Methods for counts and measurements follow Kottelat and Freyhof (2007). Terminology of the pigmentation pattern follows Kottelat and Freyhof (2007). Standard length (SL) is measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle is 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 last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as "1½". For morphological comparisons of new species with *C. amphilekta*, *C. kellei*, *C. elazigensis*, *C. satunini* and *C. taenia*, Mousavi-Sabet et al. (2011, 2015) was considered.

To find if the counted characterization of the pigmentation pattern are significantly discriminated, a Discriminate Function Analysis (DFA) was used. Number of blotches in Z4 and Mid-dorsal pigmentation were the independent and fish species grouping variables. A chi-square was used to examine a significant difference between the grouping of the individuals based on their species and those from DFA. The analysis were performed in SPSS 16 software.

Osteological study: For osteological examination, 8 specimens of *Cobits* sp. (73-76 mm SL) collected from the Sefid River were cleared and stained with alizarin red S and alcian blue according to Taylor and Van Dyke (1972). Then, the cleared and stained specimens were studied using a stereo microscope (Leica MC5) and their skeletal elements were scanned by a scanner equipped with a glycerol bath (Epson V600). The scanned images were illustrated by CorelDrawX6 software. Nomenclature and abbreviation of skeletal elements follow Jalili et al. (2015a, b, c). Detailed descriptions of the osteological features of *C. faridpaki*, *C. linea* and *C. avicennae* have been provided by Jalili et al. (2015 a, b, c, 2016) for comparison.

DNA extraction and PCR: DNA was extracted from the fin clips using a Genomic DNA Purification Kit (#K0512; Thermo Scientific Corporation, Lithuania) following the manufacturer's protocol. The COI gene was amplified using primers FCOI20-(5'- AACCTCTGTCTTCGGGGCTA -3') and RCOI20III-(5'- TTGAGCCTC CGTGAAGTGTG -3') (Hashemzadeh Segherloo et al. 2012). Polymerase chain reaction (PCR) conditions were as follows: a 50 µl final reaction volume containing 5 µl of 10X Taq polymerase buffer, 1 µl of (50 mM) $MgCl_2$, 2 µl of (10 mM) deoxynucleotide triphosphate (dNTP), 1 µl (10 µm) of each primer, 1 µl of Taq polymerase (5 $U\mu l^{-1}$), 7 µl of total DNA and 33 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 purification Kit (Expin Combo GP – mini; Macrogen incorporation, Korea). The PCR products were sequenced using Sanger method by a robotic ABI-3130xl sequencer using manufacturer's protocol. The forward and revers primers were used to single strand sequencing.

Molecular data analysis: In this study, we considered sequences obtained in the previous studies and those which are deposited in the GenBank (Table 1). Sequences were aligned using Geneious software (Geneious v. 10.0.2, Biomatters, http://www.geneious.com/), and visually verified to maximize positional homology. Sequences of *Misgurnus fossilis* species were chosen as outgroup based on their phylogenetic relationship to genus *Cobitis* (Perdices and Doadrio 2001; Perdices et al. 2016). Uncorrected-p pairwise distances between species (Table 2) were calculated with Mega 6 (Tamura et al. 2013). A bootstrapping process was implemented with 1000 repetitions. As multiple tests, P-values were further adjusted by Bonferroni's correction (Rice 1989). Jmodeltest 2.1.4 (Darriba et al. 2012) selected HKY+G+I as the best evolutionary model. RAxML (Stamatakis 2006) implemented in Geneious software was used to estimate the maximum- likelihood (ML) tree. Bayesian inference was conducted with MrBAYES v. 3.2.2 (Ronquist et al. 2012). Two simultaneous analyses were run on $1.5*10^7$ generations, each with four MCMC chains sampling every 2000 generations. Convergence was checked on Tracer 1.6 (Rambaut and Drummond 2013). After discarding the first 10% of generations as burn-in, we obtained the 50% majority rule consensus tree and the posterior probabilities.

Abbreviations used: SL, standard length; HL, lateral head length; Z4, midlateral row of dark-brown blotches along the flank, bp, base pair; IMNRFI-UT, Ichthyological Museum of Natural Resources Faculty, University of Tehran. ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz.

Table 1. List of species used from GenBank including accession numbers.

No.	Species	Accession no.	No.	Species	Accession	No.	Species	Accession no.	No.	Species	Accession
1	KJ553298	*Cobitis arachthosensis*	26	KY476334	*Cobitis faridpaki*	51	KJ553005	*Cobitis ohridana*	76	KM286524	*Cobitis taenia*
2	KJ553219		27	KY476336		52	KJ552816		77	KJ128460	
3	KJ553181		28	KY476337		53	KJ552794		78	KJ128459	
4	KJ553088		29	KY476338		54	KJ553165	*Cobitis phrygica*	79	KJ553220	*Cobitis turcica*
5	KP050508	*Cobitis avicennae*	30	KY476339		55	KJ552900		80	KJ552985	
6	KP050516		31	KJ553203	*Cobitis hellenica*	56	KJ552845		81	KJ552782	
7	KP050525		32	KJ553094		57	KJ553154	*Cobitis pontica*	82	HQ600718	*Cobitis vardarensis*
8	KJ552834	*Cobitis battalgili*	33	KJ552940		58	KJ553118		83	KJ553280	
9	KJ552817		34	KJ553006	*Cobitis illyrica*	59	KJ553296	*Cobitis puncticulata*	84	KJ553250	
10	KJ552796		35	KJ552992		60	KJ552795		85	KJ553300	*Cobitis vettonica*
11	KJ553211	*Cobitis bilineata*	36	KJ553096	*Cobitis jadovaensis*	61	KJ553153	*Cobitis punctilineata*	86	KJ553242	
12	KJ553176		37	KJ552968		62	KJ552981		87	KJ553226	
13	KJ552762		38	KJ553147	*Cobitis levantina*	63	KJ552769		88	KJ553016	
14	KJ553073	*Cobitis bilseli*	39	KJ553104		64	KP050518	*Cobitis saniae*	89	KJ553193	*Cobitis zanandreai*
15	KJ553049		40	HQ536326	*Cobitis lutheri*	65	KP050506		90	KJ553015	
16	KJ552872		41	HQ536325		66	KP050528		91	KJ553001	
17	KJ553275	*Cobitis calderoni*	42	HQ536324		67	KP050509		92	KM286765	*Msisgurnus fossilis*
18	KJ553149		43	KJ553155	*Cobitis maroccana*	68	KY646319		93	KM286764	
19	KJ553130		44	KJ553110		69	KY646320		94	KM286763	
20	KR477018	*Cobitis elongatoides*	45	KJ553105		70	KY646321				
21	KR477017		46	KJ553099	*Cobitis meridionalis*	71	KY646322				
22	KR477016		47	KJ552999		72	KJ553059	*Cobitis splendens*			
23	KJ553207	*Cobitis evreni*	48	KJ553072	*Cobitis narentana*	73	KJ552919				
24	KJ552911		49	KJ553012		74	KJ553143	*Cobitis strumicae*			
25	KJ553276	*Cobitis fahireae*	50	KJ552915		75	KJ553048				

Results

According to the results, out of 644 bp of partial COI, 413 bp were conserved and 231 bp parsimony informative. Genetic distances between studied species are listed in Table 2. The Bayesian and ML analyses yielded similar topologies with well-supported taxa (Fig. 1). The reconstructed topology was also in agreement with previously published works (Mousavi-Sabet et al. 2015; Jouladeh-Roudbar et al. 2017). The results revealed the monophyly of the members of the genus *Cobitis* in the Caspian Sea basin as sister group of *C. avicennae*. In addition, DFA analysis of the characterization of the pigmentation pattern indicated that over 97% of individual (species) were correctly grouped based on the characteristics. The results revealed a significant different between *C. faridpaki* and *Cobitis* sp. from the Sefid River in terms of number of blotches in Z4 (P<0.05).

Table 2. Estimates of evolutionary divergence over sequence pairs between *Cobitis saniae* sp. nov. and other *Cobitis* species.

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Cobitis avicennae	1																											
Cobitis battalgili	2	8.39																										
Cobitis bilineata	3	10.97	10.97																									
Cobitis bilseli	4	7.66	3.00	10.20																								
Cobitis calderoni	5	9.32	8.51	11.13	8.56																							
Cobitis elazigensis	6	7.98	7.05	9.00	7.10	8.18																						
Cobitis elongatoides	7	5.37	7.97	10.97	7.43	9.81	7.64																					
Cobitis fahireae	8	6.21	8.54	10.97	7.97	9.81	7.98	3.74																				
Cobitis faridpaki	9	5.25	9.04	11.52	8.10	10.76	7.89	5.37	6.71																			
Cobitis hellenica	10	9.94	8.85	12.93	8.64	10.14	9.07	9.59	10.40	9.91																		
Cobitis illyrica	11	12.37	11.61	5.52	11.16	10.51	10.51	12.04	11.35	12.41	12.04																	
Cobitis jadovaensis	12	11.46	11.62	3.44	10.53	10.97	10.47	11.62	10.97	12.01	12.77	4.75																
Cobitis linea	13	10.97	7.99	9.95	8.84	9.68	4.48	9.77	9.04	10.36	10.54	10.23	10.68															
Cobitis lutheri	14	10.46	9.83	9.82	9.27	10.74	8.50	10.95	10.61	10.96	11.80	10.23	11.40	9.63														
Cobitis maroccana	15	10.40	9.73	11.57	9.01	9.32	10.31	9.86	10.51	11.75	11.75	12.54	13.04	10.27	10.66													
Cobitis narentana	16	10.71	10.04	3.11	9.78	10.80	8.86	11.06	10.77	11.24	12.63	5.45	3.87	8.95	10.14	11.96												
Cobitis neridionalis	17	8.45	7.94	10.94	7.10	8.55	6.57	7.72	7.94	7.56	7.30	10.95	10.74	9.60	10.33	10.32	11.05											
Cobitis ohridana	18	12.41	11.94	8.90	10.73	11.20	11.21	13.05	13.04	12.98	12.04	9.30	8.79	11.24	11.27	12.54	8.80	9.85										
Cobitis phrygica	19	8.85	5.28	11.78	4.45	9.00	7.36	8.29	9.32	8.82	8.54	11.70	12.11	9.04	10.46	10.04	11.28	8.14	11.62									
Cobitis pontica	20	6.22	7.69	11.13	7.58	9.17	7.53	2.78	4.09	6.38	9.82	11.54	11.78	9.41	10.37	9.77	10.64	7.62	13.68	9.00								
Cobitis puctilineata	21	9.69	6.64	11.64	7.47	9.59	8.81	9.53	10.08	10.06	10.31	12.25	12.01	10.13	9.62	10.46	11.35	9.23	11.71	7.97	9.39							
Cobitis saniae	22	5.29	8.98	11.44	7.61	10.35	8.65	5.64	6.33	2.33	10.42	12.06	11.77	10.69	10.89	11.27	11.21	8.51	13.29	9.00	6.30	9.70						
Cobitis splendens	23	5.43	8.23	11.13	7.97	10.14	7.67	2.28	4.66	5.90	9.94	12.21	12.11	9.83	10.92	10.66	11.44	7.61	13.78	8.85	2.78	9.77	6.20					
Cobitis taenia	24	4.83	7.64	11.14	7.53	9.00	7.54	3.74	4.68	5.95	9.51	12.29	11.79	9.43	10.34	9.82	11.43	7.84	12.81	9.19	3.77	9.36	5.58	3.74				
Cobitis turcica	25	8.31	1.14	10.53	3.22	8.89	7.28	8.51	9.09	8.90	8.78	11.17	11.19	8.26	10.08	10.13	9.66	7.70	11.69	5.82	8.24	6.75	9.23	8.78	8.18			
Cobitis vardarensis	26	6.68	8.80	12.17	8.70	9.16	7.93	4.93	6.63	6.67	10.61	13.11	12.66	10.07	10.46	10.87	11.49	8.70	13.09	9.83	5.78	10.10	6.90	5.80	5.09	8.73		
Cobitis vettonica	27	9.00	8.18	9.82	7.91	7.28	8.02	9.82	9.66	10.67	10.56	11.62	10.80	9.23	10.15	4.94	9.87	9.92	11.15	9.49	9.49	9.60	9.77	10.64	8.76	8.90	9.87	
Cobitis zanandreai	28	12.93	11.95	9.00	10.86	11.13	10.15	12.93	13.42	12.37	12.77	9.25	8.51	10.95	10.26	12.11	9.22	9.26	5.60	11.29	13.58	11.73	12.71	13.42	12.45	11.52	13.64	11.46

Cobitis saniae sp. nov.

(Figs. 2-6, Tables 3-4)

Holotype: IMNRF-UT-1091-1, Female, 84.6 mm SL, Iran: Guilan prov.: Bara Goor River a tributary of Sefid River, near Emamzadeh Hashem, Caspian Sea Basin, 37°00'11"N, 49°37'49"E, S. Eagderi & A. Jouladeh-Roudbar, 26 January 2017.

Paratypes: IMNRF-UT-1091, 9, 49.2-85.6 mm SL; data same as holotype.

Additional materials: IMNRF-UT-1017, 13, 42.5-83.2 mm SL, Iran; Guilan prov.: Sefid River, near Rostam Abad, Caspian Sea Basin, 36°53'34.9"N, 49°30'52.7"E, S. Eagderi, July 2015.

Diagnosis: *Cobitis saniae* is distinguished from other species of *Cobitis* in Iran and the region by the combination of the following characters (none unique to the species): a single lamina circularis on pectoral fin in males (vs. two in *C. elazigensis* and *C. linea*); a large and almost roundish or oval black spot on upper caudal-fin base (vs. very small narrow spot in *C. amphilekta*, a small comma-shaped spot in *C. avicennae*, elongated spot in *C. pontica*, two spots in *C. melanoleuca*), Z4 consisting of 13-23 dark-brown large blotches larger than eye diameter (vs. 12-17 distinct, large, dark-brown blotches in *C. avicennae*, 11-14 in *C. amphilekta*, 14-18 in *C. satunini*, 25-30 usually merged small spots, smaller than eye diameter, indistinct blotches in *C. kellei*); sub-dorsal scales with a small focal zone (vs. large in *C. amphilekta* and *C. taenia*), and having broaden and elongate horizontal part of the ectopterygoid. It is also distinguished from other *Cobitis* species in Iran by 2.33 to 10.69% divergence in the mtDNA COI barcode region.

Description: See Figures 2-5 for general appearance. Morphometric and meristic data are given in Tables 3-4. Body elongate and laterally compressed. Head and snout blunt. Interorbital space narrow and slightly convex.

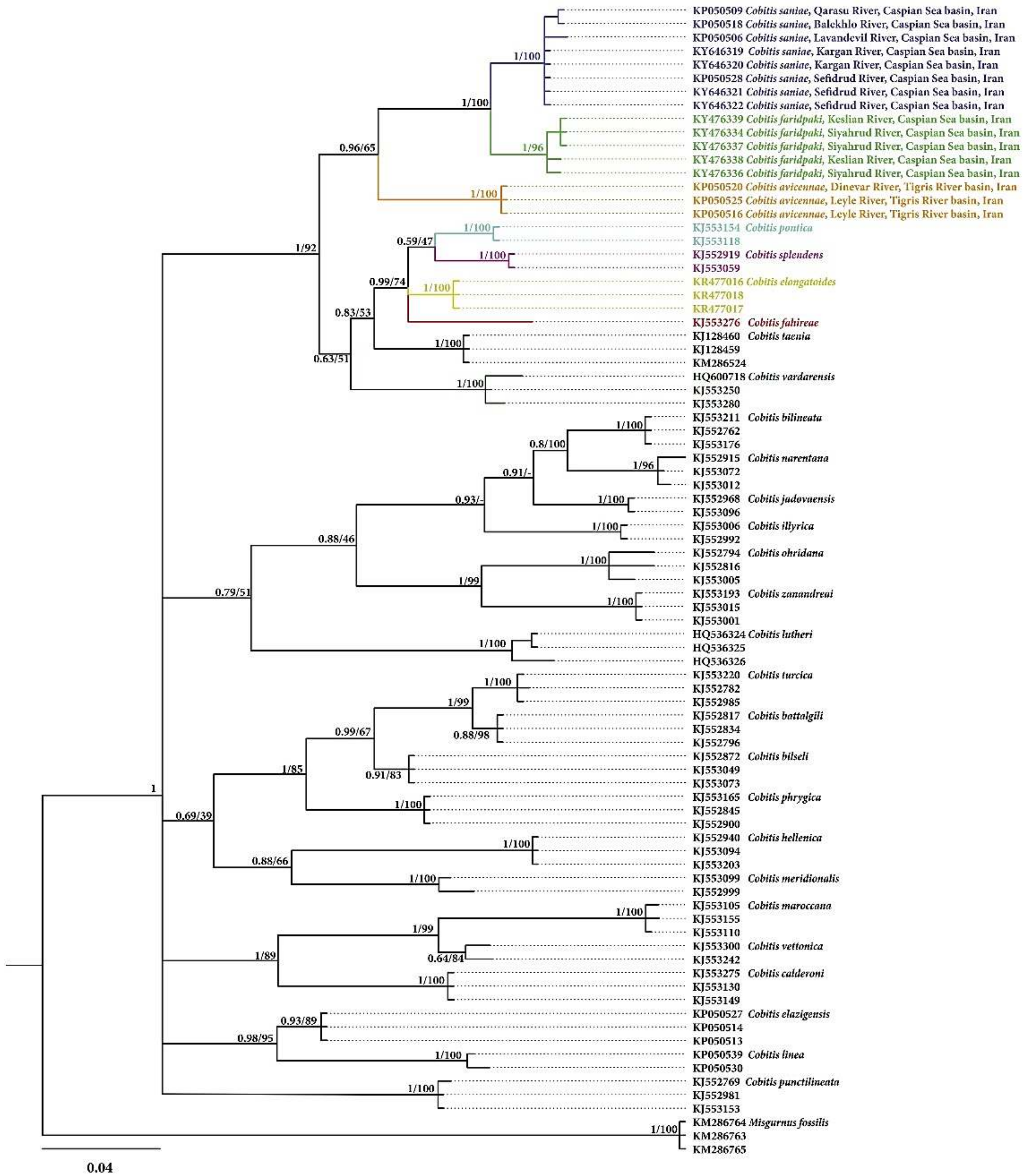

Figure 1. *Cobitis* genus; values at nodes correspond to BI posterior probability/ML bootstrap. Numbers before each species name corresponds to GenBank accession number on Table 1. Low posterior probability and bootstrap did not shown in clads node.

Eye relatively large. Caudal peduncle relatively compressed. A thin and incomplete crest above and below the caudal peduncle. Mouth small, inferior and arched; upper lip without any fold; lower lip with a small wrinkles on the surface, and divided with a median interruption (Fig. 6). Barbels short, mandibular barbel larger than rostral and maxillary barbels, rostral barbel reaching to base of maxillary barbel, maxillary barbel reaching to

Figure 2. *Cobitis saniae* sp. nov., IMNRF-UT-1091-1, holotype, female, 84.6 mm SL. Iran: Guilan prov.: near Emamzadeh Hashem, Bara Goor stream a tributary of Sefid River.

Figure 3. *Cobitis saniae* sp. nov., IMNRF-UT-1091-1, holotype, female, 84.6 mm SL. Iran: Guilan prov.: near Emamzadeh Hashem, Bara Goor stream a tributary of Sefid River.

beyond base of mandibular barbel and maxillary barbel reaching vertical of middle of eye. Dorsal-fin base equidistant from base of caudal fin and top of snout. Pelvic-fin origin below second or third branched dorsal-fin ray. Margin of dorsal fins roundish or almost straight and anal fin almost straight. Caudal fin truncate. Lateral line short, reaching pectoral-fin base. Body covered by small cycloid scales embedded in the skin. Sub-dorsal scales rounded, with reduced and eccentric clear focal zone, located in posterior region of scale with well-developed radii (Fig. 7).

Dorsal fin with 5(1), 6(5) or 7(4) ½ branched rays. Anal fin with 5½ branched rays. Caudal fin with 8+7, 8+8 branched rays. Pectoral fin with 7(7), 8(1) or 9(2) branched rays and pelvic fin with 5(1), 6(7) or 7(2) branched rays.

Sexual dimorphism: Males with a single lamina circularis at base of first branched pectoral-fin ray. Lamina circularis absent in females. Females larger than males, males with deeper dorsal and anal fins (14.6-16.6 and 12.6-12.8 vs. 12.5-14.4 and 10.9-12.3 %SL in female, respectively).

Coloration: Body light brown with a dark-brown pigmentation pattern organised in one mid-dorsal and four lateral zones (Z1-Z4). Flanks golden gloss, and belly and lower head yellowish-white in live specimens. Mid-dorsal pigmentation consisting 13-19 often fused, irregularly shaped blotches. Zones of Z1 and Z3 narrower than Z2 and Z4 (Fig. 2). Zone Z1 with many minute to small spots, reaching to caudal-fin base. Zone Z3 poor

Figure 4. *Cobitis saniae* sp. nov., paratypes. (A) IMNRF-UT-1091-2; 85.6 mm SL, (B) IMNRF-UT-1091-3; 71.8 mm SL, and (C) IMNRF-UT-1091-9; 55.1 mm SL.

Figure 5. *Cobitis saniae* sp. nov., paratypes. (A) IMNRF-UT-1091-2; 85.6 mm SL, (B) IMNRF-UT-1091-3; 71.8 mm SL, and (C) IMNRF-UT-1091-9; 55.1 mm SL.

Table 3. Morphometric of *Cobitis saniae* (IMNRF-UT-1091, n=10) with holotype.

Characters	Holotype	female		male	
		range	mean±SD	range	mean±SD
Standard length (mm)		65.2-85.6		49.2-71.8	
In percent of standard length					
Body depth at dorsal fin origin	15.6	14.6-16.3	15.6±0.6	14.8-16.6	15.7±0.9
Caudal peduncle depth	10.2	9.1-10.3	9.9±0.4	9.8-11.0	10.5±0.6
Predorsal length	53.1	50.0-54.4	52.8±1.7	51.1-52.5	52.0±0.8
Postdorsal length	49.0	45.4-49.9	47.4±1.6	48.1-49.2	48.6±0.6
Prepelvic length	53.2	51.6-55.2	53.9±1.3	53.0-57.0	54.6±2.1
Preanal length	79.6	79.6-81.7	80.5±0.8	79.6-80.6	80.1±0.5
Caudal peduncle length	13.1	12.3-14.4	13.7±0.8	13.4-15.6	14.7±1.2
Dorsal-fin base length	9.7	8.5-9.4	9.0±0.3	9.1-10.1	9.8±0.6
Dorsal-fin depth	12.8	12.5-14.4	13.4±0.8	14.6-16.6	15.3±1.1
Anal-fin base length	7.0	5.8-6.8	6.3±0.4	6.1-7.1	6.7±0.5
Anal-fin depth	11.2	10.9-12.3	11.7±0.5	12.6-12.8	12.7±0.1
Pectoral fin length	10.5	10.6-12.9	12.1±0.9	12.2-12.8	12.5±0.4
Pelvic fin length	9.8	10.6-12.4	11.4±0.7	10.8-12.8	11.6±1.0
Distance between pectoral and pelvic-fin origins	33.5	32.9-36.6	34.5±1.3	32.6-34.5	33.6±0.9
Distance between pelvic and anal-fin origins	26.3	26.0-28.7	27.2±1	23.6-26.4	25.4±1.6
Body width at dorsal fin origin	10.2	9.4-10.8	9.9±0.5	8.9-11.0	9.9±1.1
Caudal peduncle width	2.7	2.2-3.0	2.4±0.3	1.6-2.6	2.2±0.5
Head length (HL)	18.9	16.4-19.3	18.0±1.2	18.8-21	19.6±1.3
In percent of head length					
Snout length	41.8	40.9-45.3	43.1±1.7	37.7-44.9	41.0±3.6
Horizontal eye diameter	15.6	13.9-17.5	15.5±1.3	12.3-14.9	13.3±1.4
Postorbital distance	55.8	48.6-58.4	52.5±3.6	48.3-56.0	51.6±3.9
Head depth at nape	68.0	66.8-76.1	70.8±3.2	62.0-71.9	68.4±5.6
Head depth at eye	56.1	54.7-62.2	59.2±2.5	55.4-63.6	59.0±4.2
Dorsal head length	86.5	84.0-91.1	88.8±2.6	77.8-87.0	83.2±4.8
Head width at nape	57.6	47.0-61.0	56.9±5.2	48.1-58.1	53.6±5.1
Interorbital distance	22.8	20.9-25.0	22.6±1.5	20.6-24.4	22.3±1.9
Internasal distance	18.4	15.1-20.3	18.1±1.9	16.0-19.5	17.5±1.8
Mouth width	18.3	16.6-22.5	20.4±2.4	18.1-23.9	20.4±3.1
Inner rostral barbel length	9.6	10.0-12.6	11.1±1.0	9.4-12.1	10.4±1.5
Outer rostral barbel length	12.3	12.3-16.0	14.1±1.3	9.7-16.2	12.8±3.3
Maxillary barbel length	16.3	13.6-18.1	15.8±1.6	9.6-16.5	12.5±3.6

Table 4. Meristic data of *Cobitis saniae* (IMNRF-UT-1091, n=10) with holotype.

	female		male	
Characters	range	mode	range	mode
Blotches in Z4	13-23	21	16-17	16
Predorsal blotches	8-15	10	8-9	9
Postdorsal blotches	7-12	7	6-9	7
Branched dorsal-fin rays	5-7	7	6-6	6
Branched anal-fin rays	5-5	5	5-5	5
Pectoral-fin rays	7-9	7	7-8	7
Pelvic-fin rays	5-7	6	5-5	5
Caudal-fin rays	15-16	16	16-17	16

Figure 6. Ventral view of head. *Cobitis saniae* sp. nov., holotype, female, 84.6 mm SL.

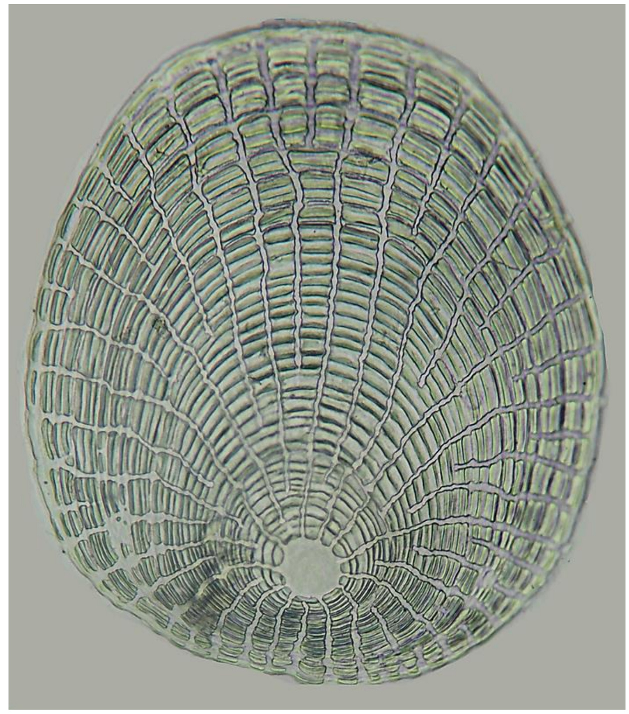

Figure 7. Caudal peduncle scale of *Cobitis saniae* sp. nov. paratype IMNRF-UT-1091-2, 85.6 mm SL.

developed with small, irregular spots and reduced after postdorsal fin reaching to anal-fin base. 13-23 blotch of Z4 usually round but sometimes squarish, blotches clear and separated but in some specimen can be poorly defined, especially on the anterior part, merged with each other in few specimens. Anterior flank blotches may combine to form short bars although their origin from spots can still be distinguishable. Number of blotches varies in juvenile fish. The color pattern of Z4 was different among specimens from different rivers and was accordingly classified into three types: type I, vey distinct and separated large blotches; type II, merged blotches not forming a dark stripe, and type III, merged blotches forming a dark-black stripe running from behind the head to vertical of anus or anal fin (Fig. 8). A large, roundish black spot on upper caudal-fin base. Dorsal and caudal fins with dark-brown spots or bars of pigment arranged in 3-5 rows. Fins yellowish/hyaline in live, hyaline in preserved specimens. Head splotchy with brown spots. A clear dark streak running from tip of snout to occiput, crossing to eye in some specimens and suborbital stripe is clear in some specimens (Fig. 9). Barbels whitish. Iris silvery, golden or orange.

Distribution and Habitat: *Cobitis saniae* like other *Cobitis* species remains buried in gravel and sand. They are mostly found in slow current parts of the rivers with sandy and muddy substance. This species stays hidden in thin sand and mud, or accumulated grass growths during the day, being nocturnal and often solitary. *Cobitis saniae* prefers clear running waters. Along the western part of the Caspian Sea basin, it is found in the lower reaches of rivers (Fig. 10). *Ponticola iranicus*, *Alburnoides samiii*, *Capoeta gracilis*, *Barbus cyri*, *Squalius turcicus* and *Luciobarbus capito* co-exists in type locality with *C. saniae*. *Cobitis saniae* knowns from most of rivers and stream between Sefid to Aras Rivers in southern Caspian Sea basin.

Etymology: The new species is named to Sania Eagderi, the daughter of first author, Dr. Soheil Eagderi.

Remarks: *Cobitis saniae* belongs to a group of *Cobitis* species having: I) a single lamina circularis on pectoral fin in males including *C. amphilekta*, *C. avicennae*, *C. faridpaki*, *C. kellei*, *C. melanoleuca*, *C. satunini* and *C. taenia*, (vs. two in *C. elazigensis* and *C. linea*); II) a single black spot on upper caudal-fin base including *C. amphilekta*, *C. avicennae*, *C. elazigensis*, *C. faridpaki*, *C. kellei*, *C. linea*, *C. satunini* and *C. taenia* (vs. two spots in *C. melanoleuca*) and III) a small focal zone in sub-dorsal scales including *C. avicennae*, *C. elazigensis*,

Figure 8. Color pattern variations of *Cobitis saniae*. (A) Type I from Talesh, (B) type II from IMNRF-1095-5, and (C) type III from the Siahbishe, Anzali wetland.

Figure 9. Gambetta's four zones of pigmentation (Z1-Z4) according to Gambetta (1934) and characteristic stripes on the head of *Cobitis saniae* sp. nov.: S1, Orbital stripe; S2, suborbital stripe; S3 and S4, stripes on opercle.

C. faridpaki, *C. kellei*, *C. linea*, *C. melanoleuca* and *C. satunini* (vs. large in *C. amphilekta* and *C. taenia*).

Cobitis saniae is similar to *C. faridpaki* in many morphological characters, but can be further distinguished from it by almost distinct S4 strip on opercle (vs. absent or reduced) and less blotches on Z4 in male (range, mode 16-17, 16 vs. 14-25, 25) and female (13-23, 21 vs. 14-33, 25).

Cobitis saniae is distinguished from *C. avicennae* by having a large, roundish or oval black spot on upper

Figure 10. Bara Goor River a tributary of Sefid River, Caspian Sea basin, type locality of *Cobitis saniae* sp. nov.

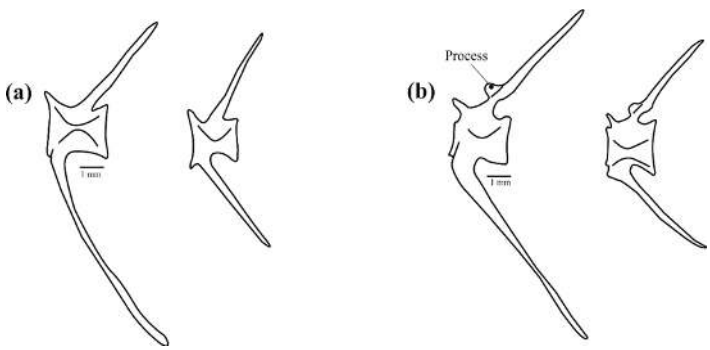

Figure 11. Lateral view of the vertebrae. (a) *Cobitis faridpaki* and (b) *Cobitis saniae* sp. nov.

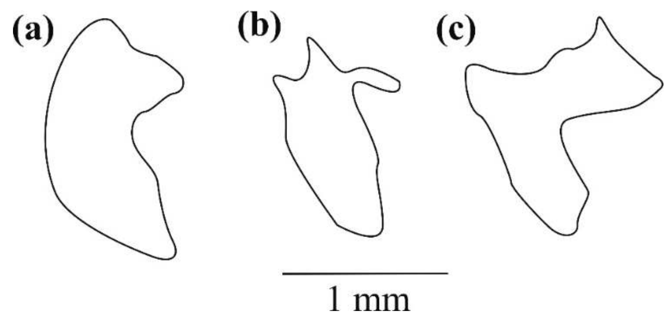

Figure 12. The lateral view of the ectopterygoid. (a) *Cobitis linae*, (b) *Cobitis faridpaki* and (c) *Cobitis saniae* sp. nov.

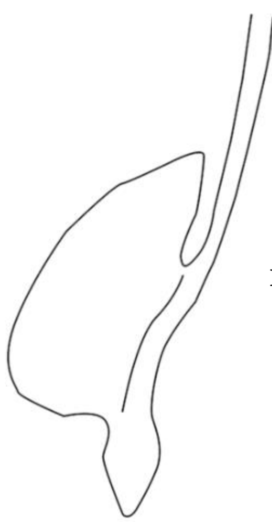

Figure 13. Lamina circularis of the *Cobitis saniae* sp. nov.

caudal-fin base (vs. a small, usually comma-shaped black spot), and a narrow incomplete dorsal and ventral crest on caudal peduncle (vs. absence crest on caudal peduncle).

Cobitis saniae is distinguished from *C. linea* by having a single lamina circularis on pectoral fin in males (vs. two), highly developed latero-caudal process of suborbital spine (vs. poor developed), presence of lateral process of suborbital spine (vs. absence of lateral process), dorsal and ventral keel at caudal peduncle more elevated (vs. poor developed), and more developed second Gambetta's zone (vs. Z2 poor developed, incomplete and narrow).

Based on the osteological characters, *C. saniae* is distinguished from *C. faridpaki* and *C. avicennae* by having an antero-ventral process of the neural spines (vs. absence) (Fig. 11). *Cobitis saniae* also bears broaden and elongate horizontal part of the ectopterygoid (vs. narrow and short one in other members of the genus *Cobitis* in Iran) (Fig. 12). In *C. saniae*, the junction of the lamina circularis and connected ray is wide (vs. short one of in *C. linea* and *C. faridpaki*) (Fig. 13). Finally, in *C. saniae*, the proximal tip of the first pterygiophore of the dorsal and anal fins is started at 16th and 30th vertebrae, respectively, (vs. 17th and 31st vertebrae, respectively, in *C. faridpaki*).

Comparative Materials:

Materials used in the morphological analyses: *Cobitis faridpaki*: — IMNRF-UT-1016, 19, 37-68 mm SL; Mazandaran prov.: Siah River at Ghaemshahr, Caspian Sea basin, 36°26'39.0"N 52°53'43.6"E; S. Eagderi & A. Jouladeh-Roudbar, Aug 2016. — IMNRF-UT-1015, 21, 51-90 mm SL; Mazandaran prov.: Keselian River at Savadkoh, Caspian Sea basin, 36°12'19.1"N53°00'56.0"E; S. Eagderi & A. Jouladeh-Roudbar, July 2015.

Cobitis saniae: — IMNRF-UT-1018, 6, 38-53 mm SL; Gilan prov.: Sefid River at Totkaboon, Caspian Sea basin, 36°53'27.3"N 49°30'42.0"E; S. Eagderi, July 2014.

Cobitis avicennae: — IMNRF-UT-1096, 12, 71-115 mm SL; Kermanshah prov., Dinevar River at Hossein Abad, Tigris drainage, 34°33'16.6"N 47°24'48.4"E; A. Soleymani, T. Hossein pour & A. Jouladeh-Roudbar, Aug 2016. — IMNRF-UT-1020, 1, 95 mm SL; Kermanshah prov.; Dinevar River at Hossein Abad, Tigris drainage, 34°33'16.6"N 47°24'48.4"E; S. Eagderi & A. Jouladeh-Roudbar, Jun 2016.

Cobitis linea: — ZM-CBSU H2090, 6, 53-79 mm SL; Fars prov.: Ghadamgah spring at Dorudzan, Kor river basin, 30°14'19.65"N 52°22'23.3"E; G. Sayyadzadeh, S. Mirghiasi & S. Ghasemian, May 2013. — ZM-CBSU H2096, 6, 45-72 mm SL; Fars prov.: Ghadamgah spring at Dorudzan, Kor river basin, 30°14'19.65"N 52°22'23.3"E; H.R. Esmaeili, V. Niknejad & Ebrahimi, August 2004.

Material used in the molecular genetic analysis: *Cobitis saniae*: — IMNRF-UT-1091-A, Guilan prov.: Bara Goor River a tributary of Sefid River, near Emamzadeh Hashem, Caspian Sea Basin, 37°00'11.0"N 9°37'52.0"E; GeneBank Accession numbers (KY646321, KY646322). — IMNRF-UT-1101-A, Guilan prov.: Kargan River at Talesh city, Caspian Sea Basin, 37°48'30.8"N 48°54'45.5"E; GeneBank Accession numbers (KY646319, KY646320).

Acknowledgments

Authors wish to thank Dr. H. Poorbagher and H.R. Ghanavi for data analysis and T. Hosseinpour for helping in laboratory. This study was financially supported by the University of Tehran and Shiraz University.

Literature cited

Coad B.W. 2011. Freshwater Fishes of Iran. Available at http:// *www.briancoad.com* (accessed on 12 April 2011).

Coad B.W. 2017. Freshwater Fishes of Iran. Available at http://www.briancoad.com (accessed on 12 July 2016).

Darriba D., Taboada G.L., Doallo R., Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772-772.

Eschmeyer W.N., Fong J.D. 2011. Pisces. In: Z.-Q. Zhang (Ed.). Animal biodiversity: An outline of higher level classification and survey of taxonomic richness. Zootaxa 3148: 26-38.

Esmaeili H.R., Coad B.W., Gholamifard A., Teimori A. 2010. Annotated checklist of the freshwater fishes of Iran. Zoosystematica Rossica 19(2): 361-386.

Esmaeili H.R., Coad B.W., Mehraban HR., Masoudi M., Khaefi R., Abbasi K., Mostavavi H., Vatandoust S. 2014. An updated checklist of fishes of the Caspian Sea basin of Iran with a note on their zoogeography. Iranian Journal of Ichthyology 1(3): 152-184.

Hashemzadeh Segherloo I., Bernatchez L., Golzarianpour K., Abdoli A., Primmer C.R., Bakhtiary M. 2012. Geneticd differentiation between two sympatric morphs of the blind Iran cave barb *Iranocypris typhlops*. Fish Biology 81(5): 1747-1753.

https://doi.org/10.1111/j.1095-8649.2012.03389.x

Jalili P., Eagderi S., Mousavi-Sabet H. 2015a. Descriptive osteology of the endemic spined loach *Cobitis linea* from Iran. Aquaculture, Aquarium, Conservation and Legislation, International Journal of the Bioflux Society (AACL Bioflux) 8(4): 526-534.

Jalili P., Eagderi S., Mousavi-Sabet H. 2015b. Descriptive osteology of *Cobitis avicennae* (Mousavi-Sabet et al., 2015). Iranian Journal of Ichthyology 2(1): 53-60.

Jalili P., Eagderi S., Mousavi-Sabet H., Mafakheri P. 2015c. Descriptive osteology of faridpaki spined loach, *Cobitis faridpaki* (Mousavi-Sabet et al., 2007) (Cypriniformes: Cobitidae) from southern Caspian Sea basin. Journal of Marine Biology 24: 37-70. (In Farsi)

Jalili P., Eagderi S., Pourbagher H. 2016. Phylogeny of the genus *Cobitis* (Linnaeus, 1758) in the southern Caspian Sea basin using osteological characters. The Forth Iranian Conference of Ichthyology, Ferdowsi University of Mashhad, 20-21 July 2016. pp: 90-96. (In Farsi)

Jouladeh-Roudbar A., Vatandoust S., Eagderi S., Jafari-Kenari S., Mousavi-Sabet H. 2015. Freshwater fishes of Iran; an updated checklist. Aquaculture, Aquarium, Conservation and Legislation, International Journal of the Bioflux Society (AACL Bioflux) 8: 855-909.

Jouladeh-Roudbar A., Eagderi S., Sayyadzadeh G., Esmaeili H.R. 2017. *Cobitis keyvani*, a junior synonym of Cobitis faridpaki (Teleostei: Cobitidae). Zootaxa 4244(1): 118-126.

https://doi.org/10.11646/zootaxa.4244.1.6

Kiabi B.H., Abdoli A., Naderi M. 1999. Status of the fish fauna in the South Caspian Basin of Iran. Zoology in the Middle East 18: 57-65.

Kottelat M., Freyhof J. 2007. Handbook of European freshwater fishes. Publications Kottelat. 646p.

Mousavi-Sabet H., Yerli S.V., Vatandoust S., Özeren S.C., Moradkhani Z. 2012. *Cobitis keyvani* sp. nova—a new species of spined-loach from south of the Caspian Sea basin (Teleostei: Cobitidae). Turkish Journal of Fisheries and Aquatic Sciences 12: 7-13.

https://doi.org/10.4194/1303-2712-v12_1_02

Mousavi-Sabet H., Vasil'eva E.D., Vatandoust S., Vasil'ev V.P. 2011. *Cobitis faridpaki* sp. nova—a new spined loach species (Cobitidae) from the Southern Caspian Sea basin (Iran). Journal of Ichthyology 51(10): 925-931.

https://doi.org/10.1134/S0032945211100055

Mousavi-Sabet H., Vatandoust S., Esmaeili H.R., Geiger M.F., Freyhof J. 2015. *Cobitis avicennae*, a new species of spined loach from the Tigris River drainage (Teleostei: Cobitidae). Zootaxa 3914(5): 558-568.

https://doi.org/10.11646/zootaxa.3914.5.4

Nalbant T.T., Bianco P.G. 1998. The loaches of Iran and adjacent regions with description of six new species (Cobitoidea). Italian Journal of Zoology 65: 109-123.

Perdices A., Doadrio I. 2001. The molecular systematics and biogeography of the European cobitids based on mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 19(3): 468-478.

Perdices A., Bohlen J., Šlechtová V., Doadrio I. 2016. Molecular evidence for multiple origins of the European spined loaches (Teleostei, Cobitidae). PloS one 11(1): e0144628.

Rambaut A., Drummond A.J. 2013. Tracer v1. 5 Available from http://beast. bio. ed. ac. uk/Tracer.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43(1): 223-225.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539-542.

https://doi.org/10.1093/bioinformatics/btg180

Saadati M.A.G. 1977. Taxonomy and distribution of the freshwater fishes of Iran. M.Sc. Thesis, Colorado State University, Fort Collins. 212 p.

Sawada Y., 1982 Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). Memoirs of the Faculty of Fisheries of Hokkaido University 28(2):65-223.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688-2690.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.

https://doi.org/10.1093/molbev/mst197

Taylor W.R., Van Dyke G.C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107-119.