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Article

Tashan Cave a new cave fish locality for Iran; and *Garra tashanensis*, a new blind species from the Tigris River drainage (Teleostei: Cyprinidae)

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Abstract

A new cave fish locality is reported for Iran, and the first true cave species is described from the Tigris River drainage. *Garra tashanensis*, new species, from Tashan Cave, the Tigris River drainage in Iran, is distinguished from its congeners by the combination of characters, including lacking pigment and eyes, having a well-developed round mental disc, two pairs of barbels, a well-developed rostral cap, no obvious pores on lateral line, and rare scales on anterior body. *Garra tashanensis* sp. nov. is further distinguished substantially in its DNA barcode region from the subterranean congeners (K2P nearest-neighbor distance of 10.4% to *G. lorestanensis* and 11.8% to *G. typhlops*).

Keywords: Khuzestan Province, *Iranocypris typhlops*, *Nemacheilus smithi*, Middle East.
Zoobank: urn:lsid:zoobank.org:pub:EEFF054B-DF6C-4F3F-9C69-1BED70CA145D
urn:lsid:zoobank.org:act:28D40360-4085-43CC-8BF2-B33B17297192

Introduction

After loaches (Teleostei: Cypriniformes: Cobitoidei) with 54 species, the family Cyprinidae with 47 species is the second largest group of subterranean fishes (Proudlove 2006, 2010; Kottelat 2012; Freyhof et al. 2016). Subterranean fishes in Iran: *Garra lorestanensis* Mousavi-Sabet and Eagderi, 2016, *G. typhlops* (Bruun and Kaiser, 1948) and *Eidinemacheilus smithi* (Greenwood, 1976) are characterized by lacking pigment and eyes. All these subterranean fishes are found in Loven Cave, a well-like pool, the natural outlet of a subterranean limestone system of the Zagros Mountains in the Ab-e Sirum or Ab-e Serum Valley near Tang-e Haft railway station in Lorestan Province, west Iran (Bruun and Kaiser 1948; Smith 1953; Movaghar 1973; Greenwood 1976; Coad 1996; Proudlove 1997; Sargeran et al. 2008; Mousavi-Sabet 2013; Jouladeh-Roudbar et al. 2015; Mousavi-Sabet and Eagderi 2016). However, *G. typhlops* is occasionally recorded from the Simarreh River drainage (Mahjoorazad and Coad 2009) with no further confirmation.

Iranian subterranean fishes are investigated in some aspects by several authors, and their taxonomic status is reviewed recently by Mousavi-Sabet and Eagderi (2016). Further, Hashemzadeh-Segherloo et al. (2016) reviewed the Iranian subterranean loach, *E. smithi*, by molecular approach, and transferred the species from the genus *Paracobitis* to a newly described genus i.e. *Eidinemacheilus*. In addition, the second subterranean nemacheilid loach species from the region, *E. proudlovei*, is described from subterranean waters in the Little Zab River drainage in Iraqi Kurdistan (Freyhof et al. 2016).

The members of the genus *Garra* in the Persian Gulf and Oman Sea basins are reviewed by Sayyadzadeh et al. (2015) and Esmaeili et al. (2016), who recognized six epigean species including *G. barreimiae*, *G. longipinnis*, *G. persica*, *G. rossica*, *G. rufa*, *G. variabilis*; two subterranean species including *G. typhlops* and *G. widdowsoni*, and described two new species including *G. mondica* (Sayyadzadeh et al. 2015) and *G. amirhosseini* from the region (Esmaeili et al. 2016).

Before summer 2016, the only known subterranean habitat for fishes in Iran was the Loven Cave in Lorestan Province. In summer 2016, there was some local reports about presence of a new cave fish locality in Khuzestan

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Province, southwestern Iran. Our field studies in the area confirmed that there can be considered as the second subterranean habitat for fishes in Iran. Our initial observations at the sampling site, showed that the fish can be a *Garra* species. Comparing the specimens with the other known subterranean and epigean *Garra* in the region, based on morphological and molecular data, it was revealed that the caught specimens from the second cave represent an unnamed species which is described here as new species.

Material and Methods

After anaesthesia, two specimens were fixed in 5% formaldehyde and then stored in 70% ethanol, and the two remain specimens fixed in 96% ethanol. The morphological measurements were made by a dial caliper and recorded 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). The terminology of the snout morphology and the oromandibular structures follow Stiassny and Getahun (2007) and Nebeshwar and Vishwanath (2013). Standard length (SL) was measured from the tip of the snout to the end of the hypural notch. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural notch, 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 noted as "1½". In the present study, the subterranean species from Iraq, *G. widdowsoni* and *E. proudlovei*, are compared based on original descriptions and available literatures (Hamidan et al. 2014; Sayyadzadeh et al. 2015; Freyhof et al. 2016).

DNA extraction and PCR: DNA was extracted from the fin clips of two specimens using a Genomic DNA Purification Kit (#K0512; Thermo Scientific Corporation, Lithuania) following the manufacturer's protocol. The COI gene was amplified using primers FishF1-(5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1-(5'-TAGACTTCTGGGTGGCCAA AGAATCA-3') (Ward et al. 2005). 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) $MgCl_2$,1 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 primer were used to single strand sequencing.

Molecular data analysis: The sequences were compared to published *Garra* sequences using (BLASTn) basic local alignment search tool (Altschul et al. 1990). Sequence data were aligned using MEGA6 software (Tamura et al. 2013). Sequences of COI gene were trimmed to the size of the smallest fragment, resulting in a dataset of 651 base pairs (bp). Modeltest (Posada and Crandall 1998), implemented in the MEGA 6 software (Tamura et al. 2013) was used to determine the most appropriate sequence evolution model for the given data, treating gaps and missing data with the partial deletion option under 95% site coverage cut-off. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern (Nei and Kumar 2000; Posada and Crandall 2001). Bayesian analyses of nucleotide sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because over parametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck and Ranala 2004). Each Bayesian analysis comprised two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies <0.01. Estimates of evolutionary divergence over sequence pairs between species

were conducted in Mega6 (Tamura et al. 2013). Analyses were conducted using the Kimura 2-parameter model (Kimura 1980). The rate variation among sites was modelled with a gamma distribution (shape parameter=1). Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. As outgroup, *Cyprinus carpio* sample was retrieved from GenBank database (accession number: JX983283).
Abbreviations used: SL, standard length. HL, lateral head length. GUIC, Collection of the Ichthyology Museum, Department of Fisheries Sciences, Faculty of Natural Resources, the University of Guilan, Guilan Province, Iran. VMFC, Vatandoust and Mousavi-Sabet Fish Collection, Tehran.

Results

We generated COI barcodes for 2 specimens of *G. tashanensis* sp. nov. collected from the Tashan Cave, Iranian part of the Tigris River drainage. Additional 42 sequences of 18 species have been downloaded from Genbank (Table 1). Tables 2 and 3 show the estimates of the average evolutionary divergence and nucleotide substitutions found in the mtDNA COI barcode region, which show between 1.32% and 19.07% K2P sequence divergence in their COI gene. Furthermore, *G. tashanensis* sp. nov. corresponds a distinct clade showing between 10.25% (*G. ghorensis*) and 15.53% (*G. rossica*) K2P sequence divergence in their COI barcode region with other members of the genus *Garra*.

Bayesian Inference (BI) and Maximum Likelihood (ML) gave the same topologies and thus one is presented (Fig. 1). The two different phylogenetic approaches produced almost identical tree topologies. The estimation of the phylogenetic relationships based on the COI barcode region places the used sequenced of the members of the genus *Garra* into 17 groups (Fig. 1). Two methods produced trees with three major lineages supported by high posterior probability clade (Fig. 1): (I) *G. mondica*, *Garra* sp. (Kol basin), *G. elegans*, *G. widdowsoni*, *G. persica*, *G. rufa*, *G. barreimiae*, *G. ghorensis*, *G. jordanica*, *G. typhlops*, *G. gymnothorax*, *G. lorestanensis*, *G. longipinnis*, (II) *G. tashanensis*, and (III) *G. rossica*, *G. nudiventris*, *G. variabilis* (Fig. 1).

***Garra tashanensis*, sp. nov.**
(Figs. 2- 6)
Holotype: VMFC GT-H, 22 mm SL; Iran, Khuzestan prov.: Tashan Cave, the Tigris River drainage, the Persian Gulf basin (30°51'91"N, 50°10'49"E), altitude 490 m, Y. Fatemi, R. Lashkarizadeh, A. Jamashi & F. Shojaei, 13 August 2016.
Paratypes: VMFC GT-P1to VMFC GT-P3, 3, 24 - 27 mm SL; the same data as holotype.
Diagnosis: *Garra tashanensis* sp. nov. can be distinguished from its congeners by lacking pigment and eyes (vs. presence of eyes and pigments in all epigean species), having a round mental disc (vs. absence of mental disc in *G. typhlops*; elliptical mental disc in *G. lorestanensis*), two pairs of barbels (vs. three pairs of barbels in the subterranean loaches, *E. smithi* and *E. proudlovei*; and one pair of barbels in *G. variabilis*), a well-developed rostral cap (rostral cap poorly developed in *G. lorestanensis*), and rare scales on anterior body (vs. body fully covered by scales or scales restricted to lateral midline in *G. widdowsoni*; naked body in *G. lorestanensis*). *Garra tashanensis* sp. nov. is also distinguished from all other congeners in the comparison group, by having four fixed, diagnostic nucleotide substitutions in the mtDNA COI barcode region. *Garra tashanensis* sp. nov. is also distinguished from the subterranean congeners by a K2P nearest-neighbor distance of 10.4% to *G. lorestanensis* and 11.8% to *G. typhlops*.
Description: See Figures 2-6 for general appearance and Table 4 for morphometric data of holotype and paratypes. Relatively stout species with wide head, moderately compressed laterally, more compressed posteriorly especially in caudal peduncle region. Body deepest at or slightly in the front of dorsal-fin base, depth decreasing towards caudal-fin base. Greatest body width at pectoral-fin base, body almost equally wide prior to

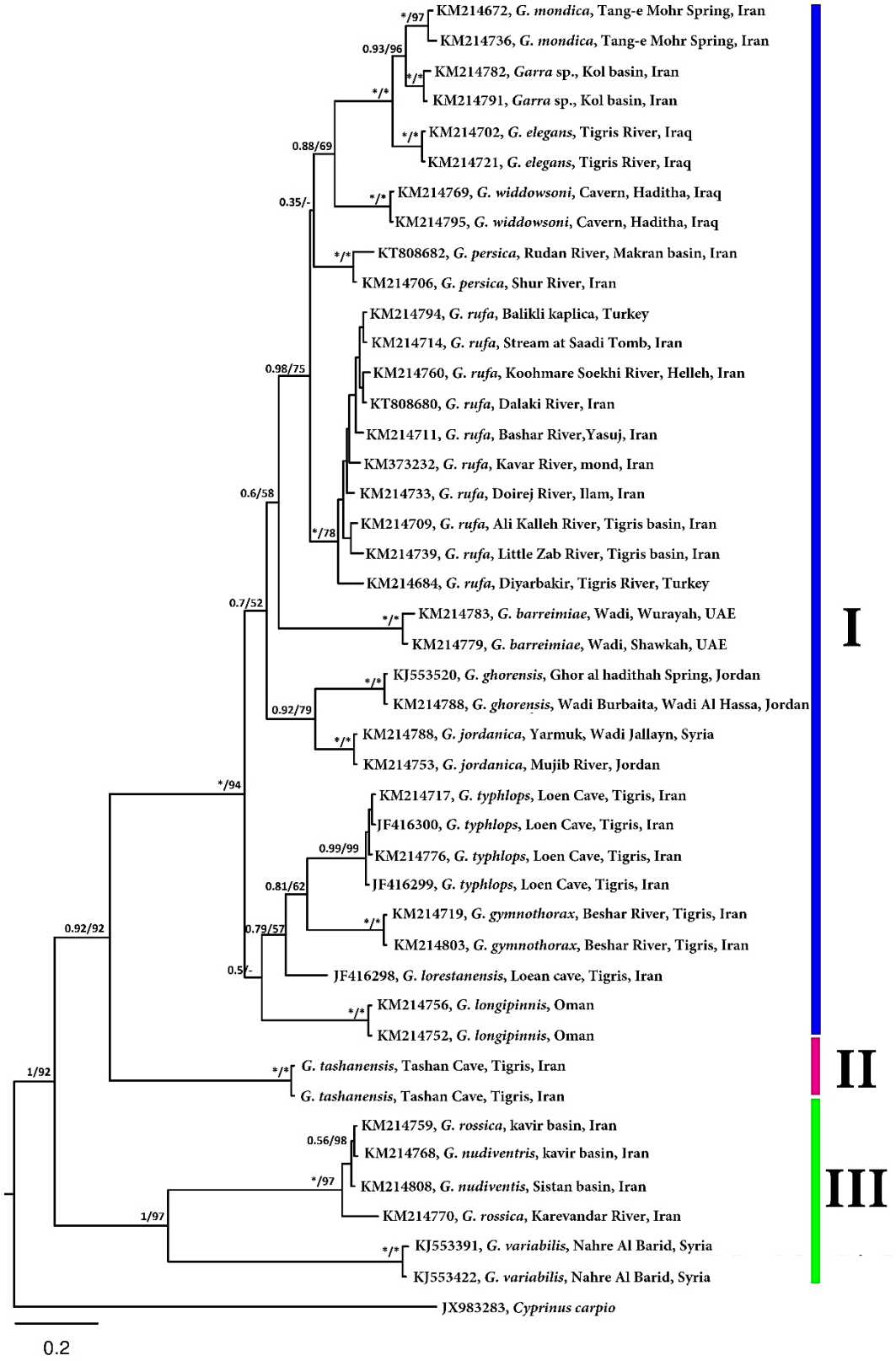

Figure 1. Values at nodes correspond to BI posterior probability/ML bootstrap. Asterisks (*) indicate 1 or 100 posterior probabilities support for the node. Dash (-) indicate branch does not exist.

Table 1. List of species used for molecular analysis for COI and GenBank Accession Number.

No.	Species	Accession No.	No.	Species	Accession No.
1	*G. barreimiae*	KM214783	23	*G. rufa*	KM214709
2	*G. barreimiae*	KM214779	24	*G. rufa*	KM214760
3	*G. elegans*	KM214702	25	*G. rufa*	KM214684
4	*G. elegans*	KM214721	26	*G. rufa*	KM214714
5	*G. ghorensis*	KJ553520	27	*G. rufa*	KM373232
6	*G. ghorensis*	KM214788	28	*G. rufa*	KM214733
7	*G. gymnothorax*	KM214719	29	*G. rufa*	KM214711
8	*G. gymnothorax*	KM214803	30	*G. rufa*	KM214739
9	*G. jordanica*	KJ553525	31	*G. rufa*	KT808680
10	*G. jordanica*	KM214753	32	*G. typhlops*	JF416299
11	*G. longipinnis*	KM214756	33	*G. typhlops*	JF416300
12	*G. longipinnis*	KM214752	34	*G. variabilis*	KJ553391
13	*G. lorestanensis*	JF416298	35	*G. variabilis*	KJ553422
14	*G. mondica*	KM214762	36	*G. widdowsoni*	KM214795
15	*G. mondica*	KM214736	37	*G. widdowsoni*	KM214769
16	*G. nudiventris*	KM214808	38	*G. tashanensis*	KY365750
17	*G. nudiventris*	KM214768	39	*G. tashanensis*	KY365751
18	*G. persica*	KM214706	40	*G. typhlops*	KM214717
19	*G. persica*	KT808682	41	*G. typhlops*	KM214776
20	*G. rossica*	KM214770	42	*Garra* sp.	KM214791
21	*G. rossica*	KM214759	43	*Garra* sp.	KM214782
22	*G. rufa*	KM214794	44	*Cyprinus carpio*	JX983283

Table 2. Estimates of the average evolutionary divergence between Iranian *Garra* species. All positions with less than 95% site coverage were eliminated.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*G. barreimiae*																
2	*G. elegans*	8.00															
3	*G. ghorensis*	8.50	7.37														
4	*G. gymnothorax*	7.97	6.58	7.75													
5	*G. jordanica*	7.08	6.40	3.78	6.38												
6	*G. longipinnis*	7.54	7.75	8.14	7.33	7.96											
7	*G. lorestanensis*	7.79	6.79	6.77	4.50	5.82	5.41										
8	*G. mondica*	7.97	1.83	7.31	6.88	6.39	7.60	7.23									
9	*G. nudiventris*	16.72	16.06	14.26	16.02	16.06	15.21	13.81	15.45								
10	*G. persica*	6.41	4.68	6.94	6.55	5.99	6.92	5.23	4.86	14.24							
11	*G. rossica*	17.21	16.34	14.77	16.29	16.34	15.74	14.32	15.81	0.71	14.44						
12	*G. rufa*	6.70	4.60	6.27	6.57	4.61	7.14	4.90	5.09	14.67	2.98	14.92					
13	*G. tashanensis*	13.81	12.00	10.25	12.66	11.55	11.49	10.44	11.74	15.13	10.84	15.53	11.30				
14	*G. typhlops*	7.97	7.37	6.36	4.31	5.99	6.34	3.78	7.53	12.43	5.78	12.91	5.83	11.81			
15	*G. variabilis*	19.07	18.79	15.05	16.58	15.85	16.53	13.15	18.87	13.04	15.03	13.30	16.28	14.84	13.42		
16	*G. widdowsoni*	7.48	4.33	7.37	7.37	6.01	8.16	5.63	4.49	16.85	4.31	17.13	3.62	11.78	6.97	17.69	
17	*Garra* sp.	8.08	1.82	7.45	7.25	6.09	7.43	6.87	1.32	15.76	4.76	16.03	4.63	10.75	7.25	18.18	4.23

dorsal-fin origin. Head relatively large, and slightly depressed. Dorsal head profile rising gently from the tip of snout, slightly convex, dorsal body profile almost straight from nape to dorsal-fin origin. Ventral profile more or less straight from pectoral to anal-fin origin. Caudal peduncle relatively shallow (caudal peduncle depth 11.8-13.1 %SL). Caudal peduncle length 1.2-1.4 times longer than its depth. Lateral line reduced, with no obvious pores. Rare scales (1-3 sales in studied specimens) on anterior body, around pectoral fin origin. Pharyngeal teeth and gill rakers did not examined, since the available specimens were small size and limited in number. Snout roundish; normally no obvious head tubercles (if presence), since the studied materials are small size; no obvious tubercle on transverse lobe, shallow transverse groove between transverse lobe and proboscis. No obvious tubercle on proboscis. Proboscis not elevated from depressed rostral surface. No obvious tubercle on lateral

Table 3. Parsim-Informative nucleotide substitutions found in mtDNA COI barcode region of *Garra* species.

	Nucleotide position relative to *Oryzias latipes* complete mitochondrial genome (AP004421)
	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 8 8 8
	0 2 3 3 3 4 5 5 6 6 7 8 8 9 9 9 9 1 1 1 2 3 4 4 5 6 7 7 7 8 8 9 9 0 0 0 1 2 3 4 5 6 6 7 8 8 8 9 9 0 0 1
Species	8 0 0 5 8 7 6 9 2 8 4 3 9 2 5 6 8 3 6 9 5 4 0 3 8 7 4 6 9 2 5 1 4 0 3 7 8 4 0 8 1 4 6 9 4 5 7 0 9 5 8 7
G. elegans	C C G T G C C C T A G G G G T T A C C C C T T T T A T G C G G G T T G C G A A T T T A T A T A A A C A G
G. gymnothorax	T C G T A T T C C A G G A A T T A T C C T T T T T A C A T G G A T T G C G A A T T C A T A C A A A T A A
G. lorestanensis	T C A T A T C C T G A G A A T T A C C C T T T T T A T A T A G A T T G C G A A T T C A C A C A A A T A A
G. nudiventris	T T G A A C C T T G A A A G C C G C C T C C T C C A C A T G A A C C A C A C G C C C T T G C T G C G T G
G. persica	C C G T A C C C T G G G A G T T A C C C C T T T T G C G C A G G T T G C G A A T T C A T A T A A A T A G
G. rossica	T T G A A C C T C G A A A G C C G C C T C C T C C A C A T G A G C C A T A C G C C C T T G C T G C G T G
G. tashanensis	T T A A A C C C G G A G A A T T A T T C C C C T T A C G C A G A C C G C A A G C T C A T A T A A C A T A
G. typhlops	T C G T A T T C T A A G A G T T A C C C T C T T T A C A T A G A T T A C A A A T T C A T A C A G A T A G
G. variabilis	T T A A A C C C T G A G A G T T A T T T T C C T C G C A T A G A T C A T A C A T T C T C A C T A C C T G
Garra sp. Kol	C C G T G C C C T A G G G G T T A C T C C T T T T A C G C G G G T T G C G A A T T T A T A T A A A C A G
	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
	8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 9 9 9 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1
	2 4 6 7 7 8 9 0 0 1 1 3 4 4 5 5 5 6 8 9 9 0 0 0 1 1 2 4 4 4 4 5 5 5 5 6 7 7 8 9 0 0 1 2 2 2 3 4 4 5 5
	9 4 2 4 7 6 8 1 7 3 6 1 0 3 2 7 8 4 2 1 7 0 3 9 2 5 7 2 3 5 9 1 4 7 8 9 2 8 2 6 2 5 1 0 3 6 2 4 7 3 4
G. elegans	A A T A A C C A T G A G C C C C C T C C A A C G A C C A T A T A A A C G C G C T C C T G G A A T C C C
G. gymnothorax	A G T A A C C A T G A G T T C C C C T T A A C G G T C A T A T G A A C A T G C T C C T G A A A T T C C
G. lorestanensis	G G T A G C C A T A G A C T C T C C T T A A C G G T C A T A C A A G C G T G C T C C T G A A A T T C C
G. nudiventris	G A A G A T T A C A T A C T T C T C C T G T T A A T T T C G C T C G T A T A T C T T C G A A T C C T T
G. persica	A A T A G C C G T A G A T T C C C T C T A A C A G T C A T A T A A G C A C G T T C C T G G A A T T C C
G. rossica	G A A G A T T A C A T A C T T C T C C T G T T A A T T C C A C T C G T A T A T C T T C G A A T C C T T
G. tashanensis	A A A A A C C A T A G A C T C T C T C C A C A G G C C A T A C A C A C A T A T T T C C A C G A T T C C
G. typhlops	G G T A G C T G T A A A C T C C C C T T A A C G G T C A T A T G T A C A T G C T C C T A A A A T T C C
G. variabilis	A G A A G T T A C A T A C T T T T T T T A C T T G T C C C G T A C G C A T G T C T T C G A G C T T C C
Garra sp. Kol	A A T A A C C A T G G A C C C C C T C C A A C G A C C A T A C A A A C G C G C T C C T A G A A T T T C

Figure 2. *Garra tashanensis*, VMFC GT-H, holotype, 22 mm SL; Iran: Tashan Cave.

surface of snout. Depressed rostral surface small and normally without tubercles, anterior arm of depressed rostral surface not reaching to base of rostral barbel, separating transverse lobe from lateral surface. Commonly no obvious groove between transverse lobe and lateral surface. Mouth surrounded by two pairs of barbels; rostral barbel antero-laterally located, maxillary barbel at corner of mouth, shorter than rostral barbel. Rostral cap well-developed, fimbriate, papillate on ventral surface. Upper lip present, upper jaw completely covered by rostral cap. Disc almost round, narrower than head width through roots of maxillary barbel; papillae on antero-median fold; well-developed groove between antero-median fold and central callous-pad narrow and deep, scattered medium sized papillae on latero-posterior flap; surface of central callous pad with sparsely arranged medium sized papillae.

Table 4. Morphometric data of *Garra tashanensis* sp. nov.

Morphometric character	holotype	paratype	paratype	paratype
Standard length (mm)	22	24	25	27
In percent of standard length				
Head length	25.8	26.1	25.0	25.6
Body depth at dorsal-fin origin	19.5	19.1	18.8	18.5
Prepectoral length	23.6	24.4	23.3	25.1
Predorsal length	49.6	51.5	50.6	51.4
Postdorsal length	36.3	32.4	36.3	33.2
Preanal length	72.8	74.6	74.7	74.9
Prepelvic length	54.3	54.9	55.1	55.6
Distance between pectoral and pelvic-fin origins	29.9	31.7	30.9	31.5
Distance between pelvic and anal-fin origins	18.1	18.5	18.8	19.0
Distance between vent and anal-fin origin	4.0	3.9	4.1	4.0
Depth of caudal peduncle	12.9	13.1	12.8	11.8
Length of caudal peduncle	17.8	15.7	16.9	15.5
Dorsal-fin depth	18.8	21.0	20.8	20.9
Anal-fin base length	6.6	8.4	6.9	7.5
Pectoral-fin length	19.4	18.1	19.3	19.1
Pelvic-fin length	13.1	15.0	13.8	14.9
In percent of head length				
Head depth at nape	64.5	62.8	60.3	59.5
Maximum head width	81.9	79.5	79.8	80.9
Inter-nasal width	27.0	28.4	28.1	29.0

Dorsal fin with 3 simple and 7½ branched rays. Anterior dorsal-fin origin located mid dorsum, or slightly posterior. Pelvic fin with 1 simple and 6-7 branched rays. Pelvic-fin origin behind a vertical of anterior dorsal-fin origin, about a vertical of mid dorsal-fin base. Pectoral fin with 1 simple and 12-14 branched rays. Pectoral fin reaching approximately 57-65% of distance from pectoral-fin origin to pelvic-fin origin. Anal fin with 5½ branched rays. Margin of dorsal fin straight or slightly concave, and margin of anal fin straight or slightly convex. Caudal fin with 9+8 branched rays. Caudal fin distinctly forked; tip of lobes pointed.

Colouration: In live specimens (Fig. 6) body is pinkish to red from the blood showing through the skin, although this species is almost entirely unpigmented. The gill filament area and lower part of head are bright red. The skin over the brain semi-transparent, so that the brain can be seen as a dark spot in some specimens. In preserved specimens (Fig. 2), body is yellowish-white. All fins are hyaline (in both live and preserved specimens).

Distribution and habitat: *Garra tashanensis* sp. nov., is known from the Tashan Cave, the natural subterranean limestone cave of the Zagros Mountains near Sarjusher Village (about 600 m away from the village) in Tashan region, the Tigris River drainage, the Persian Gulf basin, about 35 km away from Behbahan City, Khuzestan Province, southwestern Iran (Fig. 7). After the Loven Cave and Simarreh River, the Tashan Cave is the third known locality for subterranean species in Iran. According to presence of human traces in the cave, it seems that the Tashan Cave was known for indigenous people in the distant past. However, it seems that for unknown reasons the cave entrance was closed and the cave remains unknown for a long time, probably until 2016. In summer 2016, some local climbers/cavers reported a natural subterranean limestone cave near Behbahan City, which contains pools and live blind fish. Our observation showed that the only known entrance for the cave (may be not the main entrance), is very small about 2 Sq. m, which is not enough big for an effortless entry (Fig. 8). After the entrance, there is a narrow corridor with downward sloping. After about 120 m the corridor reaches to the main cave, the soffit of the area is about 7-8 m, the first pool is located in this area, at a depth of about 90

m underground. The first pool area is about 30 m^2, with a maximum depth of about 2 m (Fig. 9). Our initial estimate at the locality showed that there are at least 30-40 fish specimens in the pool (Fig. 10). After about 500 m away the first pool in the main cave, there is the second pool at a depth of about 95 m underground. The second pool area is about 20 m^2, with a maximum depth of about 2.5 m, the soffit of this area is about 10-12 m. Our initial estimate showed that there are at least 30-60 fish specimens in the second pool. Both pools were stagnant, no water flow was observed at the sampling time (Summer 2016). The only water inlet was the water droplets from the ceiling of the cave (Fig. 11). The air temperature inside the main cave was 20°C, and the water temperature was 17°C.

Figure 3. *Garra tashanensis*, VMFC GT-H, holotype, 22 mm SL; Iran: Tashan Cave.

Figure 4. *Garra tashanensis*, from above to below: VMFC GT-P1, 27 mm SL; VMFC GT-P2, 25 mm SL; VMFC GT-P3, 24 mm SL; paratypes; Iran: Tashan Cave.

Etymology: The species name *tashanensis*, treated as an adjective, is derived from Tashan region, where the Tashan Cave (the type locality of the new species) is located.

Remarks: The history of subterranean cyprinids in Iran is recently reviewed and the current taxonomic status of them is well-discussed by Mousavi-Sabet and Eagderi (2016). The present study confirmed the second subterranean ecosystem for cave fishes in Iran, which contains the fourth subterranean species for the country. All the three known subterranean fishes from Iran are described from the same locality (Loven Cave), but the fourth species is described from a different locality (Tashan Cave) with about 288 km directly away from the Loven Cave.

According to Freyhof et al. (2016), 10 species of troglomorphic fishes are known from the Middle East (7 Cyprinidae, 2 Nemacheilidae, 1 Cobitidae). The only true cave species - in the sense that they live in waters in caves - in the Middle East are *Garra* cf. *longipinnis* from the Omani Al Hoota Cave (Banister 1984, taxonomy follows Hamidan et al. 2014) and *Garra dunsirei*, also from Oman (Banister 1987). Therefore, *Garra tashanensis* sp. nov. is the third true cave species in the Middle East, and the first one in the Tigris River drainage and Iran. *Garra tashanensis* sp. nov. is differs from all the epigean members of the genus *Garra* by lacking pigment and eyes (vs. presence of eyes and pigments in all epigean species). It is distinguished from all the congeners by a K2P nearest-neighbor distance of 10.25% to *G. ghorensis* and 15.53% to *G. rossica*.

Figure 5. *Garra tashanensis*, from above to below: VMFC GT-P1, 27 mm SL; VMFC GT-P2, 25 mm SL; VMFC GT-P3, 24 mm SL; paratypes; Iran: Tashan Cave.

Figure 6. *Garra tashanensis*, VMFC GT-P1, paratype, 27 mm SL; Iran: Tashan Cave.

Garra tashanensis sp. nov. is distinguished from the subterranean *G. lorestanensis* (Fig. 12) by having rare scales on anterior body (vs. body completely scaleless), a well-developed rostral cap (rostral cap poorly developed in *G. lorestanensis*), rostral cap completely covered the upper jaw (partly covered in *G. lorestanensis*), and no obvious pores on lateral line (lateral line with 28-35 pores in *G. lorestanensis*).

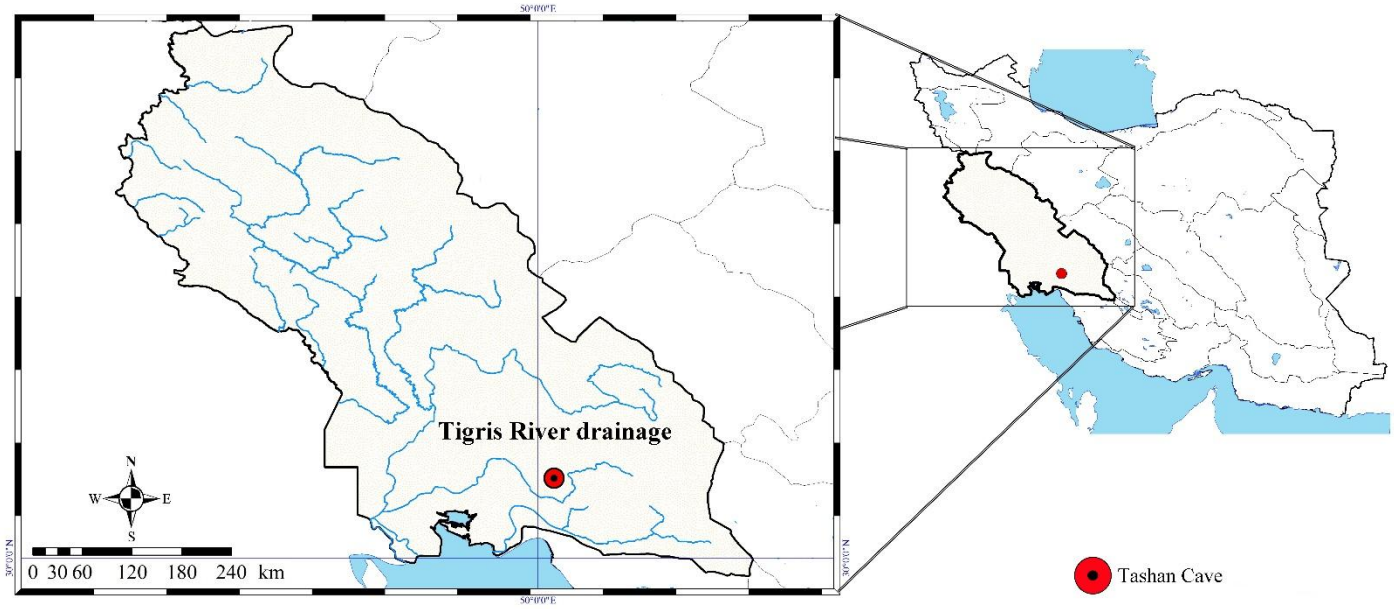

Figure 7. Map of Iranian part of the Tigris River basin, showing the Tashan Cave, the type locality of *Garra tashanensis* in Khuzestan Province, south-west Iran.

Figure 8. Tashan Cave, type locality of *Garra tashanensis*.

Figure 9. Tashan Cave, type locality of *Garra tashanensis*.

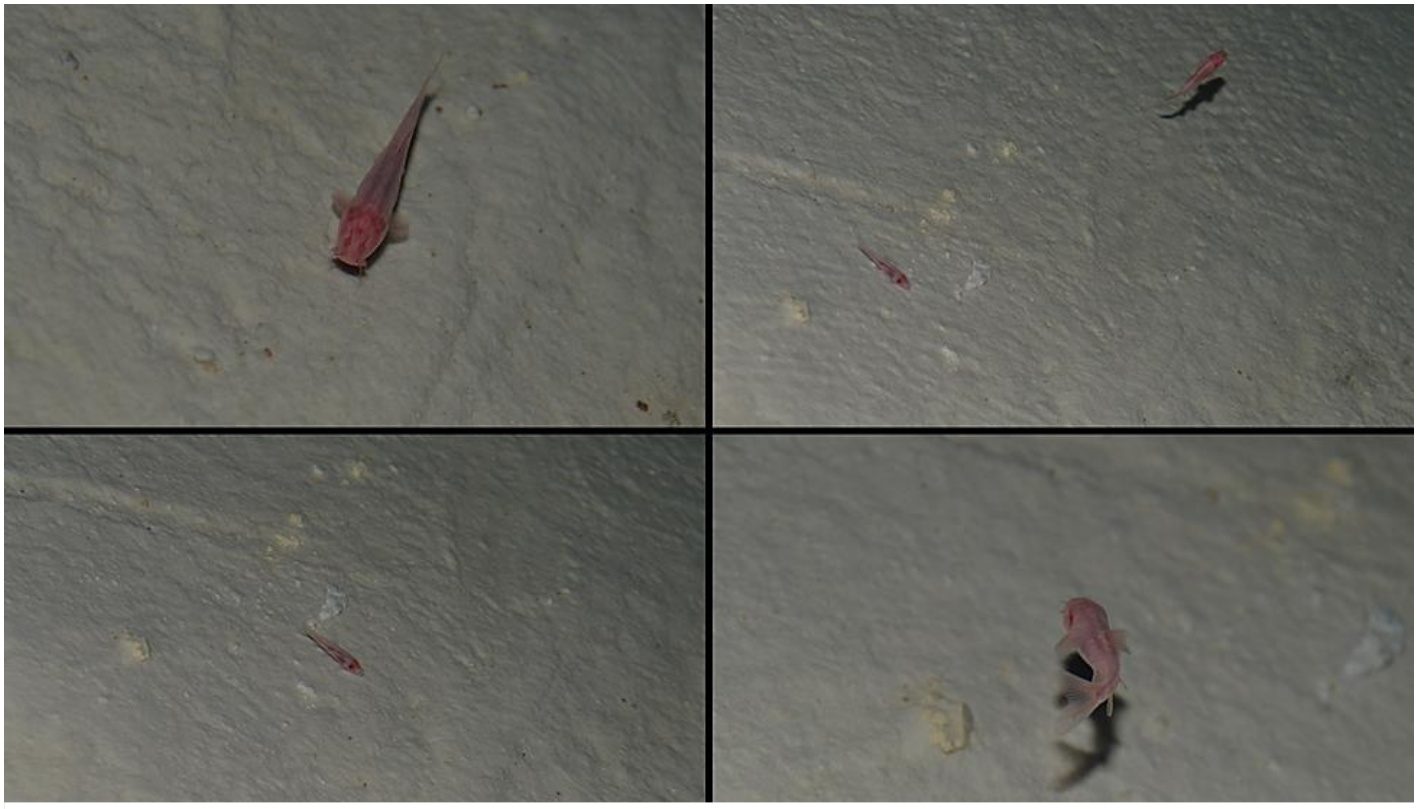

Figure 10. *Garra tashanensis* in its natural habitat, in the Tashan Cave (the type locality).

Figure 11. Tashan Cave, type locality of *Garra tashanensis*.

Figure 12. *Garra lorestanensis*, VMFC GL-H, holotype, 55 mm SL; Iran: Loven Cave.

Figure 13. *Garra typhlops*, VMFC GT01, 41 mm SL; Iran: Loven Cave.

Garra tashanensis sp. nov. is distinguished from the subterranean *G. typhlops* (Fig. 13), by having mental disc (vs. absence of mental disc; Fig. 14). *Garra tashanensis* sp. nov. furthers differs substantially in its DNA barcode from the subterranean congeners; K2P nearest-neighbor distance of 10.4% to *G. lorestanensis* and 11.8% to *G. typhlops*.

Garra tashanensis sp. nov. is distinguished from the subterranean *G. widdowsoni* found in the Euphrates basin, by having rare scales on anterior body (1-3 scales on anterior body in studied specimens vs. body fully covered by scales or scales restricted to lateral midline in *G. widdowsoni*).

Garra tashanensis sp. nov. is easily distinguished from the subterranean loaches, *Eidinemacheilus smithi* (Fig. 15) and *E. proudlovei* by having two pairs of barbels (vs. three pairs; Fig. 14).

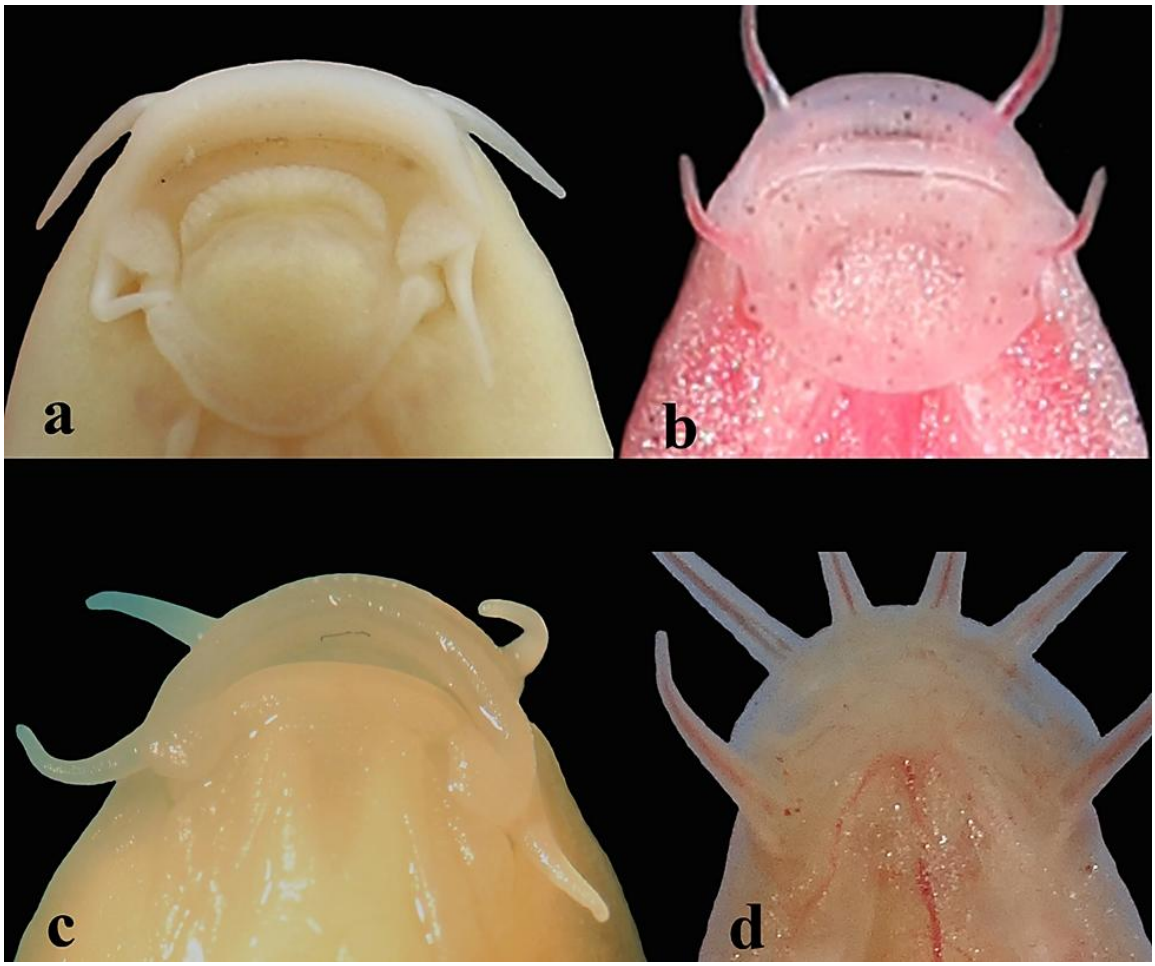

Figure 14. Head (ventral view) of: (A) *Garra lorestanensis*, VMFC GL-H, holotype, 55 mm SL, Iran: Loven Cave; (B) *Garra tashanensis*, VMFC GT-H, holotype, 22 mm SL, Iran: Tashan Cave; (C) *Garra typhlops*, VMFC GT01, 41 mm SL, Iran: Loven Cave; (D) *Eidinemacheilus smithi* VMFC PS1410, 44 mm SL, Iran: Loven Cave.

Figure 15. *Eidinemacheilus smithi*, VMFC PS1410, 44 mm SL; Iran: Loven Cave.

Comparative material: *Garra lorestanensis*: VMFC GL-H, holotype, 1, 55 mm SL. Iran, Lorestan prov.: Loven Cave, the Tigris River drainage, the Persian Gulf basin (33°04'39"N, 48°35'33"E), H. Mousavi-Sabet, A. Jouladeh-Roudbar & S. Vatandoust, 23 April 2014. - VMFC GL-P1 to VMFC GL-P3, paratypes, 3, 27.2-58.0 mm SL, the same locality as holotype, S. Eagderi, 17 June 2012. - GUIC GL-P1 and GUIC GL-P2, paratypes, 2, 31.6 and 45.1 mm SL, the same locality as holotype, S. Eagderi, 17 June 2012.

Garra typhlops: VMFC GT01, 1, 41 mm SL, Iran, Lorestan prov.: Loven Cave, the Tigris River drainage, the Persian Gulf basin (33°04'39"N, 48°35'33"E), H. Mousavi-Sabet, A. Jouladeh-Roudbar & S. Vatandoust, 23 April 2014. - VMFC GT02 to VMFC GT07, 6, 33.2 -64.0 mm SL, Iran, Lorestan prov.: Loven Cave, the Tigris River drainage, the Persian Gulf basin (33°04'39"N, 48°35'33"E), S. Eagderi, 17 June 2012.

Eidinemacheilus smithi: VMFC PS1410 to VMFC PS1411, 2, 41-44 mm SL, Iran, Lorestan Prov.: Loven Cave, the Tigris River drainage, the Persian Gulf basin (33°04'39"N, 48°35'33"E), H. Mousavi-Sabet, A. Jouladeh-Roudbar & S. Vatandoust, 23 April 2014.

Material for molecular analysis: VMFC DNA-1001, VMFC DNA-1002; Khuzestan prov.: Tashan Cave (30°51'91"N, 50°10'49"E) (GenBank accession numbers: KY365750 and KY365751).

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