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Taxonomic review of the cryptic *Barbus lacerta* species group with description of a new species (Teleostei: Cyprinidae)

Roozbehan KHAEFI[1], Hamid Reza ESMAEILI[*1], Matthias F. GEIGER[2], Soheil EAGDERI[3]

[1]Ichthyology and Molecular Systematics Research Laboratory, Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran.
[2]Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Adenauerallee 160, 53113 Bonn, Germany.
[3]Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.
*Corresponding author: *E-mail: hresmaeili@shirazu.ac.ir*

Abstract

The *Barbus lacerta* species group is reviewed. All species occur in the wider Euphrates and Tigris drainages, the endorheic Lake basins of Namak, Van and Urmia and the southern Caspian Sea basin. *Barbus cyri*, *B. lacerta* and *B. miliaris* are considered valid and one new species is described: *Barbus karunensis* from the Karun River drainage in Iran. *Barbus karunensis* is distinguished from the other species in the *B. lacerta* group by a well-developed middle pad of the lower lip, a short anal fin, a straight posterior anal-fin margin and 59-66+2-4 scales in the lateral line. We compared COI sequences for a total of 123 individuals from 29 Barbus species to test for mitochondrial monophyly of the *B. lacerta* species group, including DNA barcodes of 70 individuals from all four species of the *B. lacerta* group. The estimation of the phylogenetic relationships based on the DNA barcode region places the sequenced *Barbus* specimens into a largely taxonomical concordant cluster, but also indicates the presence of gene flow among a few species, most likely a consequence of geological events in the past.

Keywords: DNA barcoding, Freshwater fish, Middle East, Biodiversity, *Barbus karunensis*.
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Introduction

Diagnosis of species based only on morphological characters is sometimes impossible, hence using other characters obtained from molecular, biogeographical, behavioral and ecological studies have been suggested for consideration especially in pseudo-cryptic species (those species that can be recognized by accurately re-examining morphological differences) and fully cryptic species (those that cannot be recognized using morphological characters) within a species complex group (Dayrat 2005; DeSalle et al. 2005; Markmann and Tautz 2005; Valdecasas et al. 2008; Padial et al. 2010). Aquatic species, especially fishes, are known to contain many cryptic species (La Mesa et al. 2004; Griffiths et al. 2006). The closely related species in the *Barbus lacerta* group with few diagnostic morphological traits but genetic diversity, seems to be a case for cryptic taxonomic behavior based on previous published articles during the last 60 years and the present study (see Berg 1949; Almaça 1983, 1986; Karaman 1971; Bianco 1995; Saadati 1977; Motamedi et al. 2014).

The widespread *Barbus* populations from the southern tributaries of the Caspian Sea, Lake Namak and Urmia Lake basins, the Euphrates and Tigris drainages, and the Qweik endorheic basin in Syria have been previously identified as *B. lacerta* (Berg 1949; Coad 1995; Esmaeili et al. 2010). Berg (1949), Almaça (1983) and Coad (1995) recognized two subspecies in *B. lacerta* and *B. l. lacerta* from the Euphrates and Tigris drainages including the Qweik and *B. l. cyri* from the southern Caspian Sea basin.

Coad (1995) lists the following nominal species as synonyms of *B. lacerta*: *B. angustatus*, *B. armenicus*, *B. bortschalinicus*, *B. cyri*, *B. caucasicus*, *B. cyri* var. *chaldanica*, *B. scincus*, *B. sursunicus* and *Capoeta fundulus* var. *toporovanica*. *Barbus lacerta* and *B. scincus* were described by Heckel (1843) from the Qweik River in Aleppo and *B. scincus* is a synonym of *B. lacerta* (Coad 1991, 1995; Bănărescu and Bogutskaya 2003). Naseka and Bogutskaya (2009) recognize *B. cyri* as a valid species and as all other nominal species were described from the Kura and Aras drainage in the Caucasian Caspian Sea basin, they were treated as synonyms of *B. cyri*. *Barbus goktschaicus* from the Lake Sevan basin in Armenia is another *Barbus* species from the Aras

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drainage. It is treated as a valid species by Berg (1949) and Bogutskaya (1997), but both authors already mentioned its similarity to *B. cyri*. Levin et al. (2015) identified *B. goktschaicus* as a synonym of *B. cyri* and we follow this opinion. Three other species occur adjacent or within the distribution area of *B. lacerta* and *B. cyri*. These are *B. miliaris*, a nominal species found in the Lake Namak basin, *B. kosswigi* described from the Tigris River in Turkey, recently treated as a valid species in *Luciobarbus* by Fricke et al. (2007) and *B. ercisianus*, from the Lake Van basin in Turkey.

In this study, all species of the *B. lacerta* group are re-diagnosed and analysed for their morphological characters, colour patterns and molecular COI data. In combination, these data supported the view that four species are present in the studied region, one of them undescribed. Here, we describe the undescribed species, *Barbus karunensis* and discuss the taxonomic statues of other species of the *B. lacerta* group from its reported distribution area i.e. *B. ercisianus* and *B. kosswigi*.

Material and Methods

After anesthesia, the collected fishes were fixed in 10% formaldehyde and stored in 70% ethanol. Measurements were made with 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). 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 width of the upper lip is measured ventrally at the anterior tip of the lip. The scales in the lateral line are counted as total scales from the first scale on the flank to the last scale on the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as "1½". Several *Barbus* species show a sexual dimorphism of the shape and length of the anal-fin. Such a sexual dimorphism could not be observed in the material of the examined species of the *B. lacerta* group.

Abbreviations used: SL, standard length; HL, lateral head length; IMNRF-UT, Ichthyological Museum of Natural Resources Faculty, University of Tehran; FSJF, Fischsammlung J. Freyhof, Berlin; JNMP, Národni Museum, Natural History Museum, Praha; MNCN, Museo Nacional de Ciencias Naturales, Madrid; NMW, the Naturhistorisches Museum Wien; MZUT, the Istituto e Museo di Zoologia della R. Università di Torino; NRM, Naturhistoriska Riksmuseet, Department of Vertebrate Zoology, Ichthyology Section, Stockholm; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz; ZMH, Zoologisches Museum Hamburg, Hamburg. ZFMK:ICH:TIS, Zoologisches Forschungsmuseum A. Koenig, Bonn, Ichthyological Tissue Collection.

DNA extraction and PCR: Genomic DNA was extracted from fin clips or muscle tissue of fixed alcohol specimens using Macherey and Nagel NucleoSpin® Tissue kits following the manufacturer's protocol on an Eppendorf EpMotion® pipetting-roboter with vacuum manifold. The standard vertebrate DNA barcode region of the COI (cytochrome c oxidase subunit 1) was amplified using a M13 tailed primer cocktail including FishF2_t1 (5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC), FishR2_t1 5'CAGG AAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5'TGTAAAACGACGG-CCAG TCAACCAACCACAAAGACATTGGCAC) and FR1d_t1 (5'CAGGAAACAGCTATGACACCTCAGG-GTGTCCGAARAAYCARAA) (Ivanova et al. 2007). Sequencing of the ExoSAP-IT (USB) purified PCR product in both directions was conducted at Macrogen Europe Laboratories with forward sequencing primer M13F (5'GTAAAACGACGGCCAGT) and reverse sequencing primer M13R-pUC (5'CAGGAAACAGCTA TGAC).

Molecular data analysis: Data processing and sequence assembly was done in BioEdit 7.2.5 (Hall l999) and MEGA6 (Tamura et al. 2013), and the ClustalW algorithm used to create a DNA sequence alignment. No indications of unexpected stop-codons or nuclear copies of mitochondrial fragments occurred in any sequence; all generated DNA barcodes are deposited in the NCBI GenBank, and given with their respective accession numbers. The most appropriate sequence evolution model for the given DNA sequence alignment was determined with Modeltest (Posada and Crandall 1998) as implemented in the MEGA 6 software (Tamura et al. 2011), treating gaps and missing data with the partial deletion option and 95% site coverage cutoff. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern. According to Modeltest, the TN93 model (Tamura and Nei 1993) accounting for differences between transitions and transversions with discrete Gamma distribution best explained the COI alignment, and was used to model the evolutionary rate differences among sites (3 categories (+G, parameter = 0.1919)). We generated maximum likelihood phylogenetic trees with 10,000 bootstrap replicates to explore phylogenetic affinities. Bayesian inference (BI) was conducted to estimate phylogenetic relationships using MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003) and using Markov-chain Monte Carlo tree searches for 10 million generations with a sampling frequency of 1000. As appropriate outgroup to root the constructed phylogenetic relationship hypothesis, we included the only distantly related species *Luciobarbus mursa* from the Caspian Sea basin, and also molecular data for an additional 25 species of *Barbus* (Table 1). We used *Barbus barbus* HQ961091.1 whole COI sequence (652bp) for demonstrating the differences between different species of genus *Barbus* in Iran.

Material of outgroup species used in the molecular genetic analysis: *Barbus balcanicus*: ZFMK 51923–51924; Romania: Danube drainage, 44°53'10.4712"N 21°42'31.3056"E, GenBank accession number (MF106054, MF106055).

Barbus barbus: ZFMK 58045–58046; Germany: Rhine drainage, 50°48'10.512"N 7°10'28.884"E, GenBank accession number (KM286489, KM286494).

Barbus bergi: JNMP D1153–D1154; Bulgaria: Kamchiya drainage, 42°50'28.248"N 27°11'5.8236"E, GenBank accession number (MF106056, MF106057).

Barbus caninus: ZFMK 45986–45987; Italy: Po drainage, 44°38'30.498"N 7°18'36.7452"E, GenBank accession number (KJ553046, KJ553075).

Barbus carpathicus: ZFMK 52058–52059; Romania: Danube drainage, 47°43'15.366"N 24°27'6.5664"E, GenBank accession number (MF106058, MF106059).

Barbus ciscaucasicus: ZFMK:ICH:TIS: NB100; Dagestan: Sulak drainage, 42°32'2.5224"N 46°52'28.0704"E GenBank accession number (MF106061, MF106062). — ZFMK:ICH:TIS: NB101; Dagestan: Sulak drainage, 42°33'28.6848"N 46°43'4.8396"E, GenBank accession number (MF106063, MF106064).

Barbus cyclolepis: JNMP C1327; Bulgaria: Evros drainage, 41°51'45.1044"N 25°55'10.506"E, GenBank accession number (KJ553269). — JNMP D897; Bulgaria: Evros drainage, 41°58'20.2044"N 23°57'6.426"E, GenBank accession number (KJ552909).

Barbus euboicus: ZFMK 55768–55769; Greece: Evia drainage, 38°33'15.7284"N 24°3'52.1856"E, GenBank accession number (KJ553014, KJ553285).

Barbus haasi: MNCN A625–A626; Spain: Ebro drainage, 42°7'26.364"N 0°55'7.302"E, GenBank accession number (KJ553060, KJ553063).

Barbus kubanicus: ZFMK:ICH:TIS: NB128b, NB129b, NB133b, NB134b; Russia: Belaya drainage, 44°10'8.8428"N 40°8'51.2448"E, GenBank accession number (MF106111, MF106112, MF106113, MF106114).

Barbus macedonicus: ZFMK 50739–50740; Greece: Vardar drainage, 40°59'14.5932"N 22°33'29.052"E, GenBank accession number (KJ552849, KJ553186).

Barbus meridionalis: ZFMK 55599–55600; France: Rhone drainage, 45°29'8.563"N 4°47'34.116", GenBank accession number (KJ553042, KJ553260).

Table 1. Estimates of the evolutionary divergence between species expressed as number of base substitutions per site from averaging over all sequence pairs. Standard error estimates are shown above the diagonal. Analyses were conducted using the Kimura 2-parameter model and involved 132 mitochondrial COI sequences and 652 base pairs. All 3 codon positions were included and ambiguous positions were removed for each sequence pair.

	B. cyri	*B. balcanicus*	*B. barbus*	*B. bergi*	*B. caninus*	*B. carpathicus*	*B. ciscaucasicus*	*B. cyclolepis*	*B. lacerta*	*B. eubiocus*	*B. haasi*	*B. karunensis*	*B. kubanicus*	*B. macedonicus*	*B. meridionalis*	*B. miliaris*	*B. niluferensis*	*B. oligolepis*	*B._peloponnesius*	*B. pergamonesis*	*B. petenyi*	*B. plebejus*	*B. prespensis*	*B. rebeli*	*B. rionicus*	*B. sperchiensis*	*B. strumicae*	*B. tauricus*	*B. tyberincus*	*B. waleckii*	*L. mursa*
B. cyri																															
B. balcanicus	0.04																														
B. barbus	0.01	0.04																													
B. bergi	0.02	0.03	0.00																												
B. caninus	0.07	0.09	0.07	0.07																											
B. carpathicus	0.05	0.02	0.05	0.04	0.09																										
B. ciscaucasicus	0.02	0.05	0.02	0.02	0.07	0.06																									
B. cyclolepis	0.07	0.07	0.07	0.07	0.10	0.09	0.08																								
B. lacerta	0.01	0.04	0.02	0.02	0.07	0.05	0.03	0.07																							
B. eubiocus	0.06	0.08	0.06	0.06	0.10	0.09	0.07	0.07	0.07																						
B. haasi	0.04	0.05	0.04	0.04	0.07	0.05	0.04	0.08	0.04	0.08																					
B. karunensis	0.02	0.04	0.02	0.02	0.08	0.05	0.03	0.08	0.02	0.06	0.04																				
B. kubanicus	0.02	0.05	0.02	0.02	0.07	0.06	0.02	0.07	0.03	0.06	0.04	0.03																			
B. macedonicus	0.03	0.05	0.02	0.02	0.07	0.05	0.03	0.07	0.02	0.07	0.04	0.03	0.03																		
B. meridionalis	0.05	0.05	0.04	0.04	0.07	0.05	0.06	0.08	0.04	0.08	0.03	0.05	0.06	0.04																	
B. miliaris	0.03	0.06	0.04	0.04	0.08	0.06	0.04	0.09	0.03	0.08	0.06	0.03	0.04	0.05	0.07																
B. niluferensis	0.06	0.07	0.05	0.06	0.09	0.08	0.06	0.05	0.06	0.04	0.07	0.06	0.05	0.07	0.08	0.08															
B. oligolepis	0.02	0.04	0.01	0.00	0.07	0.04	0.02	0.08	0.02	0.06	0.04	0.02	0.02	0.02	0.04	0.04	0.06														
B._peloponnesius	0.07	0.08	0.06	0.07	0.10	0.09	0.08	0.07	0.07	0.01	0.08	0.07	0.06	0.08	0.08	0.08	0.05	0.07													
B. pergamonesis	0.07	0.07	0.06	0.07	0.09	0.09	0.08	0.06	0.07	0.05	0.08	0.06	0.06	0.07	0.08	0.08	0.04	0.07	0.05												
B. petenyi	0.04	0.06	0.04	0.04	0.07	0.06	0.05	0.07	0.05	0.04	0.05	0.04	0.04	0.05	0.06	0.06	0.04	0.05	0.05	0.05											
B. plebejus	0.03	0.05	0.02	0.02	0.07	0.05	0.03	0.07	0.03	0.07	0.05	0.03	0.02	0.03	0.05	0.05	0.06	0.02	0.08	0.08	0.05										
B. prespensis	0.05	0.06	0.04	0.05	0.08	0.07	0.06	0.06	0.05	0.04	0.07	0.05	0.05	0.06	0.06	0.07	0.05	0.05	0.05	0.06	0.04	0.05									
B. rebeli	0.04	0.06	0.04	0.05	0.09	0.07	0.05	0.07	0.05	0.05	0.06	0.04	0.04	0.05	0.07	0.07	0.05	0.05	0.05	0.05	0.03	0.05	0.04								
B. rionicus	0.02	0.04	0.00	0.00	0.07	0.05	0.02	0.07	0.02	0.06	0.04	0.02	0.02	0.02	0.04	0.04	0.06	0.01	0.07	0.07	0.04	0.02	0.05	0.05							
B. sperchiensis	0.05	0.06	0.05	0.06	0.08	0.07	0.06	0.07	0.05	0.07	0.06	0.06	0.06	0.07	0.06	0.07	0.06	0.06	0.07	0.07	0.06	0.06	0.05	0.06	0.06						
B. strumicae	0.06	0.08	0.06	0.06	0.09	0.08	0.07	0.05	0.06	0.05	0.06	0.07	0.06	0.06	0.07	0.08	0.05	0.06	0.05	0.05	0.04	0.07	0.06	0.05	0.06	0.07					
B. tauricus	0.02	0.03	0.00	0.00	0.07	0.04	0.02	0.07	0.02	0.06	0.04	0.02	0.02	0.02	0.04	0.04	0.06	0.00	0.07	0.07	0.04	0.02	0.05	0.05	0.00	0.06	0.06				
B. tyberincus	0.02	0.04	0.02	0.02	0.07	0.05	0.03	0.07	0.03	0.07	0.04	0.03	0.02	0.03	0.05	0.04	0.06	0.02	0.07	0.07	0.05	0.01	0.05	0.05	0.02	0.06	0.06	0.02			
B. waleckii	0.05	0.02	0.05	0.04	0.09	0.00	0.06	0.09	0.05	0.09	0.05	0.05	0.06	0.05	0.05	0.06	0.08	0.04	0.09	0.09	0.06	0.05	0.07	0.07	0.05	0.07	0.08	0.04	0.05		
L. mursa	0.10	0.11	0.10	0.10	0.12	0.11	0.11	0.12	0.10	0.11	0.10	0.10	0.10	0.10	0.11	0.12	0.10	0.10	0.12	0.11	0.11	0.10	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.11	

Barbus niluferensis: FSJF DNA-84; Turkey: Simav drainage, 39°58'49.134"N 28°25'23.6532"E, GenBank accession number (KJ553266, KJ552904).

Barbus oligolepis: FSJF DNA-122; Turkey: Simav drainage, 39°54'58.554"N 28°9'50.166"E, GenBank accession number (KJ553033). — FSJF DNA-1680; Turkey: Simav drainage, 38°57'49.482"N 35°14'35.9592"E, GenBank accession number (KJ552990).

Barbus peloponnesius: ZFMK 55775-55776; Greece: Alfios drainage, 37°38'17.7828"N 21°46'52.266"E, GenBank accession number (KJ552970, KJ552764).

Barbus pergamonensis: FSJF DNA-62; Turkey: Büyük Menderes drainage, 37°49.942"N 28°34.526"E, GenBank accession number (MF106152, MF106153).

Barbus petenyi: ZFMK 52017-52018; Romania: Danube drainage, 47°8'40.5276"N 25°51'54.2556"E, GenBank accession number (MF106154, MF106155).

Barbus plebejus: ZFMK 49755-49756; Italy: Po drainage, 44°56'20.58"N 7°36'25.956"E, GenBank accession number (KP902457, KP902431).

Barbus prespensis: ZFMK 55780, 50887; Greece: Aoos drainage, 40°2'11.7852"N 20°44'31.3692"E, GenBank accession number (KJ553204, KJ553007).

Barbus rebeli: JNMP A44, A46; Albania: Vani drainage, 41°46'40.0224"N 19°52'5.0124"E, GenBank accession number (KJ553140, KJ552802).

Barbus rionicus: FSJF DNA-1580; Turkey: Coruh drainage, 40°49'51.5712"N 41°32'27.2652"E, GenBank accession number (MF106156). — ZFMK:ICH:TIS: NB185; Georgia: Rioni drainage, 42°10'25.9788"N 42°25'46.8372"E, GenBank accession number (MF106157).

Barbus sperchiensis: ZFMK 55784-55785; Greece: Sperchios drainage, 38°49'45.6132"N 22°26'57.246"E, 2, GenBank accession number (KJ552987, KJ553093).

Barbus strumicae: ZFMK 50706-50707; Greece: Strymon drainage, 41°3'30.168"N 24°15'43.9848"E, GenBank accession number (KJ553191, KJ553281).

Barbus tauricus: ZFMK:ICH:TIS: NB29 and NB40; Ukraine: Salgir drainage, 45°6'25"N 34°42'53"E, GenBank accession number (MF106158, MF106159).

Barbus tyberinus: ZFMK 50388-50389; Italy: Tiber drainage, 43°28'45.7788"N 12°4'34.3344"E, GenBank accession number (KJ552866, KJ553254).

Barbus waleckii: ZFMK:ICH:TIS: Y399; Ukraine: Dniestr drainage, 49°32'8.2572"N 23°14'49.0812"E, GenBank accession number (MF106160).

Luciobarbus mursa: FSJF DNA-1776; Georgia: Kura drainage, 41°46'42.222"N 45°57'15.5844"E, GenBank accession number (MF106170). — FSJF DNA-145; Iran: Tajan drainage, 36°16'55.3908"N 53°14'16.026"E, GenBank accession number (MF106171, MF106172).

Results

We were able to generate DNA barcodes for a total of 70 *Barbus* specimens from all species of the *B. lacerta* group and 2 of *L. mursa*. Furthermore, we included DNA barcodes of 51 individuals of 26 other species of the genus *Barbus*. The estimation of the phylogenetic relationships based on the DNA barcode region places the sequenced *Barbus* into largely taxonomical concordant cluster (Fig. 1). The *B. lacerta* species group forms a monophyletic clade with four clades correspond to *B. cyri*, *B. lacerta*, *B. miliaris* and *B. karunensis.* A map with all records of all individuals of the *B. lacerta* species group considered in this study is shown in Figure 2.

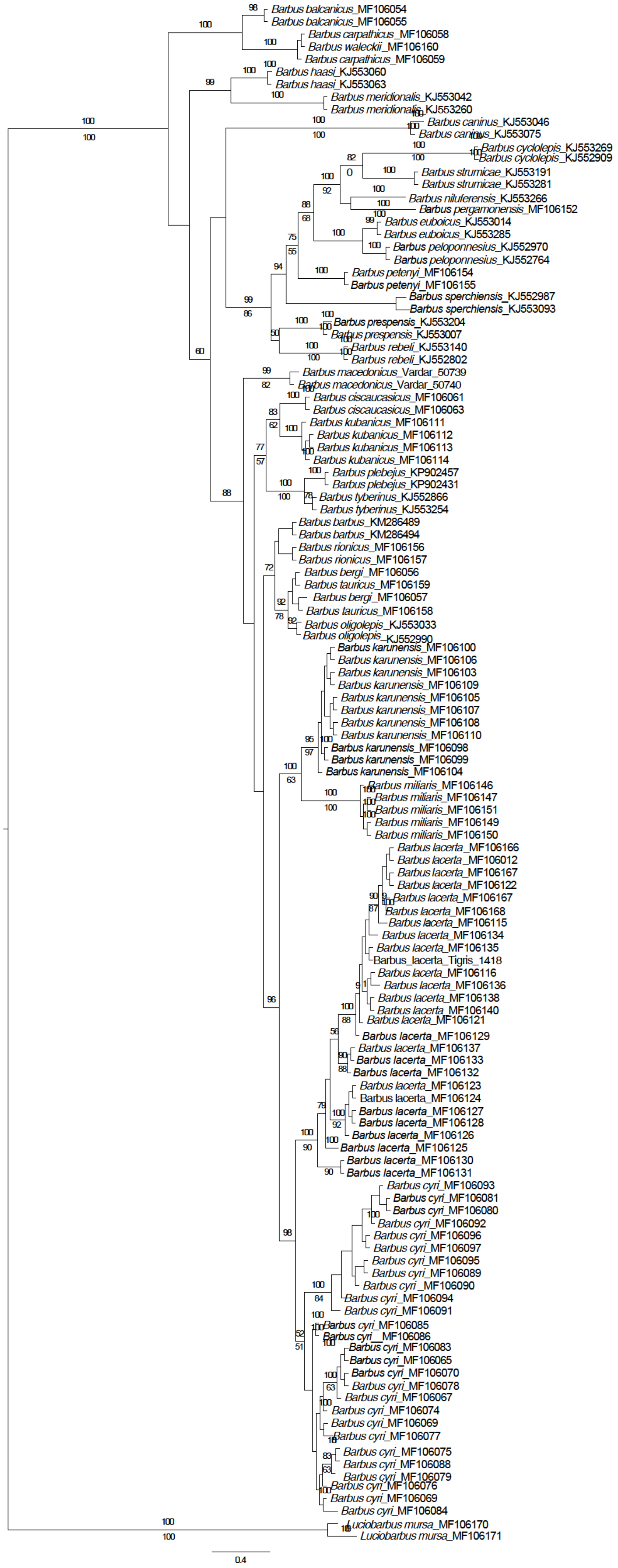
Barbus balcanicus_MF106054
Barbus balcanicus_MF106055
Barbus carpathicus_MF106058
Barbus waleckii_MF106160
Barbus carpathicus_MF106059
Barbus haasi_KJ553060
Barbus haasi_KJ553063
Barbus meridionalis_KJ553042
Barbus meridionalis_KJ553260
Barbus caninus_KJ553046
Barbus caninus_KJ553075
Barbus cyclolepis_KJ553269
Barbus cyclolepis_KJ552909
Barbus strumicae_KJ553191
Barbus strumicae_KJ553281
Barbus niluferensis_KJ553266
Barbus pergamonensis_MF106152
Barbus euboicus_KJ553014
Barbus euboicus_KJ553285
Barbus peloponnesius_KJ552970
Barbus peloponnesius_KJ552764
Barbus petenyi_MF106154
Barbus petenyi_MF106155
Barbus sperchiensis_KJ552987
Barbus sperchiensis_KJ553093
Barbus prespensis_KJ553204
Barbus prespensis_KJ553007
Barbus rebeli_KJ553140
Barbus rebeli_KJ552802
Barbus macedonicus_Vardar_50739
Barbus macedonicus_Vardar_50740
Barbus ciscaucasicus_MF106061
Barbus ciscaucasicus_MF106063
Barbus kubanicus_MF106111
Barbus kubanicus_MF106112
Barbus kubanicus_MF106113
Barbus kubanicus_MF106114
Barbus plebejus_KP902457
Barbus plebejus_KP902431
Barbus tyberinus_KJ552866
Barbus tyberinus_KJ553254
Barbus barbus_KM286489
Barbus barbus_KM286494
Barbus rionicus_MF106156
Barbus rionicus_MF106157
Barbus bergi_MF106056
Barbus tauricus_MF106159
Barbus bergi_MF106057
Barbus tauricus_MF106158
Barbus oligolepis_KJ553033
Barbus oligolepis_KJ552990
Barbus karunensis_MF106100
Barbus karunensis_MF106106
Barbus karunensis_MF106103
Barbus karunensis_MF106109
Barbus karunensis_MF106105
Barbus karunensis_MF106107
Barbus karunensis_MF106108
Barbus karunensis_MF106110
Barbus karunensis_MF106098
Barbus karunensis_MF106099
Barbus karunensis_MF106104
Barbus miliaris_MF106146
Barbus miliaris_MF106147
Barbus miliaris_MF106151
Barbus miliaris_MF106149
Barbus miliaris_MF106150
Barbus lacerta_MF106166
Barbus lacerta _MF106012
Barbus lacerta_MF106167
Barbus lacerta_MF106122
Barbus lacerta_MF106167
Barbus lacerta_MF106168
Barbus lacerta_MF106115
Barbus lacerta_MF106134
Barbus lacerta_MF106135
Barbus_lacerta_Tigris_1418
Barbus lacerta_MF106116
Barbus lacerta_MF106136
Barbus lacerta_MF106138
Barbus lacerta_MF106140
Barbus lacerta_MF106121
Barbus lacerta_MF106129
Barbus lacerta_MF106137
Barbus lacerta_MF106133
Barbus lacerta_MF106132
Barbus lacerta_MF106123
Barbus lacerta_MF106124
Barbus lacerta_MF106127
Barbus lacerta_MF106128
Barbus lacerta_MF106126
Barbus lacerta_MF106125
Barbus lacerta_MF106130
Barbus lacerta_MF106131
Barbus cyri_MF106093
Barbus cyri_MF106081
Barbus cyri_MF106080
Barbus cyri_MF106092
Barbus cyri_MF106096
Barbus cyri_MF106097
Barbus cyri_MF106095
Barbus cyri_MF106089
Barbus cyri _MF106090
Barbus cyri_MF106094
Barbus cyri_MF106091
Barbus cyri_MF106085
Barbus cyri__MF106086
Barbus cyri_MF106083
Barbus cyri_MF106065
Barbus cyri_MF106070
Barbus cyri_MF106078
Barbus cyri_MF106067
Barbus cyri_MF106074
Barbus cyri_MF106069
Barbus cyri_MF106077
Barbus cyri_MF106075
Barbus cyri_MF106088
Barbus cyri_MF106079
Barbus cyri_MF106076
Barbus cyri_MF106069
Barbus cyri_MF106084
Luciobarbus mursa_MF106170
Luciobarbus mursa_MF106171
0.4

Figure 1. Estimation of the COI sequence relationships based on the Bayesian method derived topology of 121 Barbus specimens. Numbers of branches (up to down) indicate posterior probabilities and bootstrap (BS) values (>50%) from the Maximum Likelihood, Neighbour joining methods based on 1000 pseudoreplicates. Node support values are only given for clades with BS>50%.

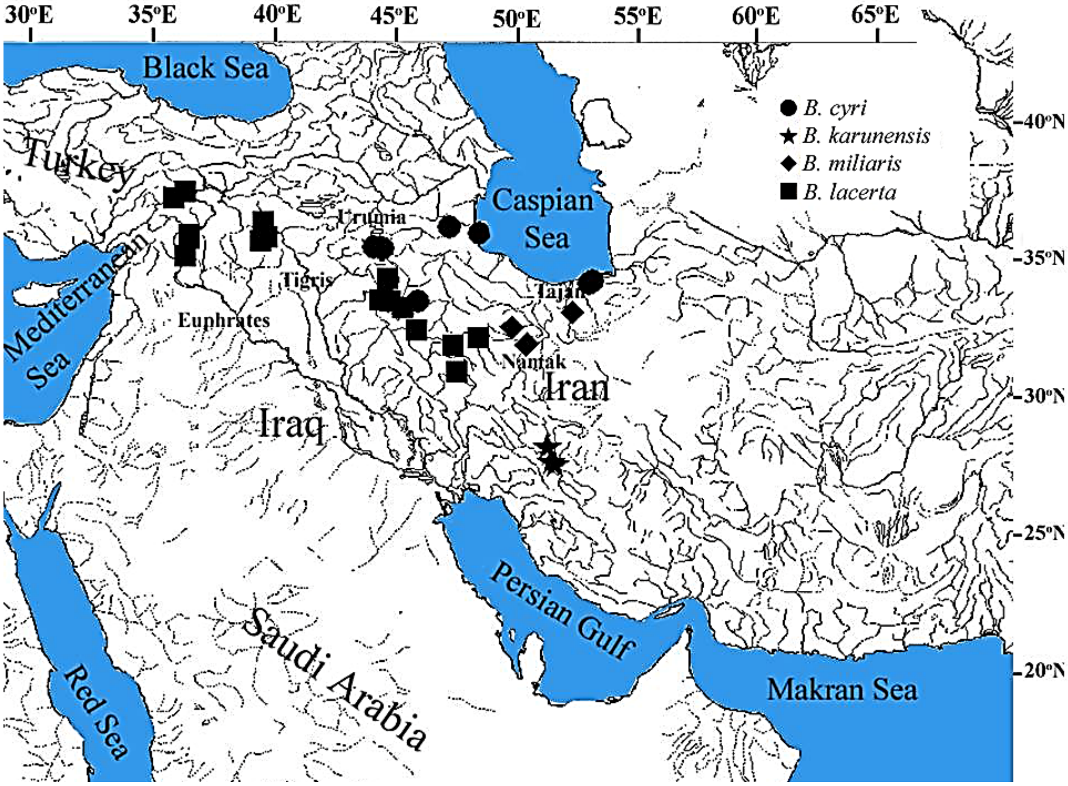

Figure 2. Distribution map of the studied *Barbus* species.

Barbus cyri De Filippi, 1865

(Figs. 3–4, Tables 2-3)

Barbus cyri De Filippi, 1865:358
Barbus caucasicus Kessler, 1877:102
Capoeta fundulus var. *toporovanica* Kamensky, 1897:83
Barbus armenicus Kamensky, 1899:80
Barbus cyri var. *chaldanica* Kamensky, 1899:62
Barbus bortschalinicus Kamensky, 1899:67
Barbus sursunicus Kamensky, 1899:76
Barbus angustatus Kamensky, 1899:88
Barbus goktschaicus Kessler, 1877:105

Material examined: FSJF 2201, 21, 95–157 mm SL; Iran: Mazandaran prov.: Tajan River below Shahid Rahaei Dam, about 30 km south of Sari, 36°16.886'N 53°14.311'E, GenBank accession number (MF106082, MF106083, MF106084 (DNA 162)). — FSJF 3247, 5, 30–41 mm SL; Iran: Ardabil prov.: Yalekhlou river north of Kalyan, 38°00'8.95"N 47°45'40"E, GenBank accession number (MF106087, MF106088 (DNA 2010)). — ZM-CBSU G1125, 24, 69–127 mm SL. Iran: Mazandaran prov.: Tajan River at Sari, 36°12'13.8"N 53°05'10.7"E. — ZM-CBSU G968, 12, 81–117 mm SL. Iran: East Azerbaijan prov.: Baranduz Chay River at Urmia, 37°24'59.95"N 45°08'56.34"E. — ZM-CBSU M488, Iran: West Azarbaeijan prov.: Ghara Chay River at Shahrchay at Bardehsue village, 5 km northwest of Silvana, 37°26'18.4"N 44°49'46.7"E. GenBank accession number (MF106091).

Figure 3. *Barbus cyri*, ZM-CBSU G1013, 116 mm SL, Iran: Guilan, Havigh, Havigh River.

Figure 4. *Barbus cyri*, ZM-CBSU G1130, 122 mm SL, Iran: Guilan, Sari, Tajan River.

Material for molecular genetic analysis: FSJF 2201, Iran: Mazandaran prov.: Tajan River below Shahid Rajaei Dam, about 30 km south of Sari, 36°16.886'N 53°14.311'E, GenBank accession number (MF106082, MF106083, MF106084 (DNA 162)). — FSJF 3247, Iran: Ardabil prov.: Yalekhlou River north of Kalyan, 38°00'8.95"N 47°45'40"E, GenBank accession number (MF106087, MF106088 (DNA 2010)). — FSJF DNA-1777, ZFMK:ICH:TIS: NB159, NB160, Georgia: Kura River drainage: Alazani River at road Liliani-Gurdzhaan, 41º46'02"N 45º55'27"E. GenBank accession number (MF106074, MF106075, MF106076). — FSJF DNA-2342, Turkey: Ardahan prov.: a stream 15 km east of Ardahan at Çıldır Hanak junction, 41°8'5.136"N 42°51'22.104"E, GenBank accession number (MF106078). — FSJF DNA-2353, DNA-2395; Turkey: Ardahan prov.: east coast of Çıldır Lake at Akçakale village, 41°4'53.292"N 43°17'42.432"E, GenBank accession number (MF106069). — FSJF DNA-2359, Turkey: Ardahan prov.: a stream between Göle and Ardahan near to Yiğitkonağı, 20 km before from Ardahan, 40°58'0.912"N 42°35'11.94"E, GenBank accession number (MF106079). — FSJF DNA-2360, Turkey: Kars prov.: Sırataşlar Stream at Sırataşlar village, 30 km North of Horasan, 40°17'26.016"N 42°17'47.436"E, GenBank accession number (MF106067). — ZFMK 66647, Turkey: Kars prov.: Bahceli Meydon Stream 16 km southeast of Kağızman, 40°03'44.7"N 43°16'13.4”E, GenBank accession number (MF106066). — ZFMK 66555, Turkey: Kars prov.: Digor Stream 12 km northeast of Kağızman, 40°13'20.5"N 43°15'12.8”E, GenBank accession number (MF106065). — ZFMK 66659, Turkey: Yalnizcom Stream 18 km southwest of Ardahan, 41°4'2.82"N 42°29'58.56”E, GenBank accession number (MF106077). — ZM-CBSU M489, Iran: West Azarbaijan prov.; Ghara Chay River at Shahrchay, 5 km west north of Silvana, 37°26'18.4"N 44°49'46.7”E, GenBank accession number (MF106092). — ZM-CBSU M64, Iran: Kordestan prov.: Seroudan River at Marivan-Saqqez road, 35°44'34.5"N 46°26'40.4”E, GenBank accession number (MF106089). — ZM-CBSU M65, Iran: Kordestan prov.: Seroudan River at Marivan-Saqqez

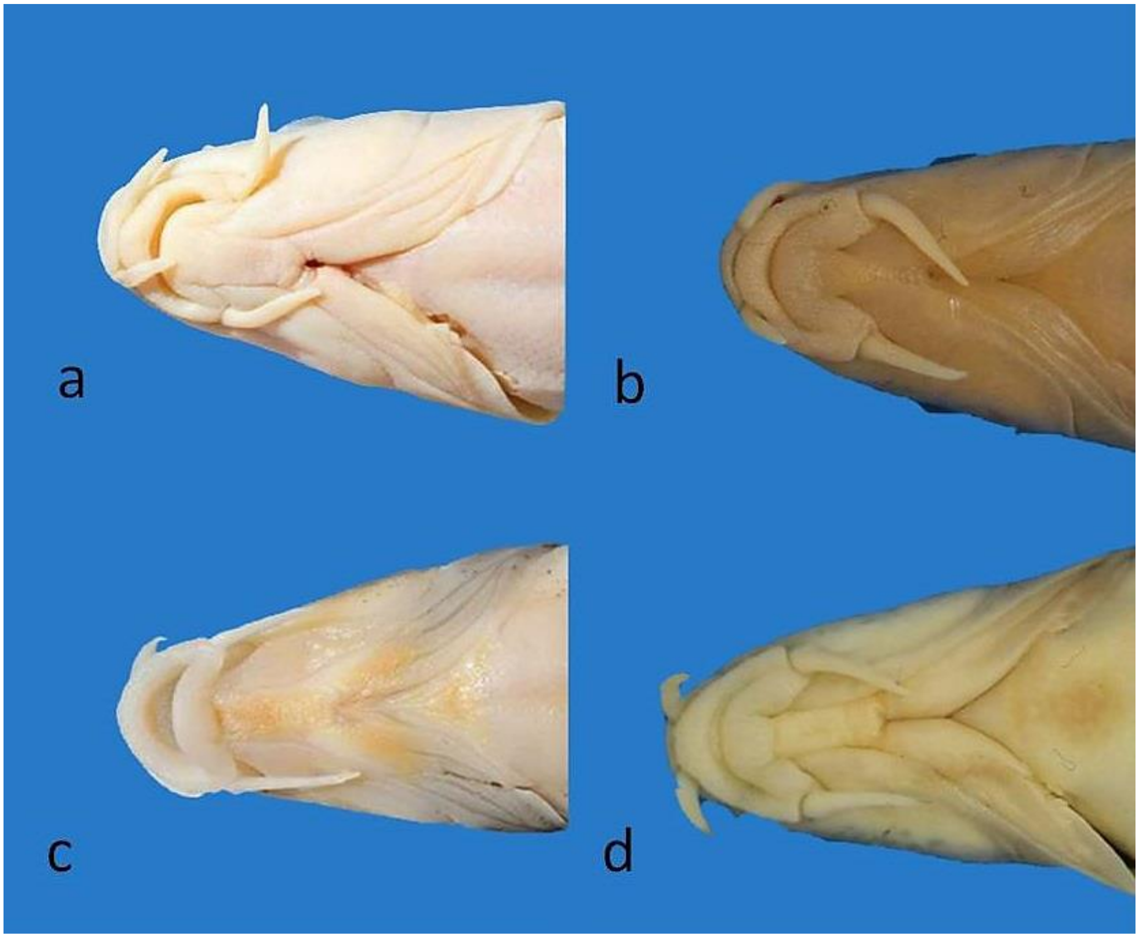

Figure 5. Ventral side of head of a. *B. cyri*, b. *B. lacerta*, c. *B. miliaris*, d. *B. karunensis*.

road, 35°44'34.5"N 46°26'40.4”E, GenBank accession number (MF106090). — ZM-CBSU M490, Iran: Giulan prov.: Karganrud River at Talesh City, 37°48'20.33"N 48°54'17.18”E, GenBank accession number (MF106070). — ZM-CBSU M1055-M1057, Iran: West Azarbaeijan prov.; Urmia-Ashnuye Road, Ghasemlu, 37°18'04.3"N 45°07'13.2”E, GenBank accession number (MF106093, MF106094, MF106095). — ZM-CBSU M1058-M1059, Iran: West Azarbaeijan prov.; Ashnuye, Godarkhosh River, 36°59'41.7"N 45°04'17.7”E, GenBank accession number (MF106096, MF106097). — IMNRF-UT-COIB-13, IMNRF-UT-COIB-14, Iran: Guilan prov.: Tootkabon River, tributary of Sefid River, 36°51'45"N 52°53'43, GeneBank Accession number (MF106085, MF106086). — IMNRF-UT-COIB-15, IMNRF-UT-COIB-16, Iran: Kurdistan prov.: Saqqez River, 35°50'13"N 46°24'49", GeneBank Accession number (MF106081, MF106080).

Diagnosis: *Barbus cyri* is distinguished from all other species of the *B. lacerta* group by having a convex posterior anal-fin margin (vs. straight), a wide upper lip (width of upper lip 7–9 %HL vs. 4–6 %HL). Other characters useful to identify *B. cyri* are: tip of anal-fin, when pressed to body, not reaching or reaching to middle of distance between base of last anal-fin ray and lower caudal-fin origin; 3–6 (mode 5) scale rows between tip of anal fin and base of caudal fin; length of caudal peduncle 1.7–2.3 times longer than deep; snout short, its length 44–47% of body depth at dorsal-fin origin; maxillary barbel 12–30 %HL, not reaching to posterior eye margin; gular region rectangular; 60–70% of posterior margin of last unbranched dorsal-fin ray covered with denticles; eye diameter 2.5–2.9 times in snout length; median pad of lower lip wide and shallow (Fig. 5); 50-66+2-4 total scales along lateral line and 24–34 (mode 29) predorsal scales. See below for details to distinguish *B. cyri* from the other species of the *B. lacerta* group. Morphometric data are provided in Table 2–3. Among all other species of *Barbus* as well as within the *B. lacerta* group, *B. cyri* is not characterized by any fixed diagnostic

Table 2. Morphometric and meristic data of *Barbus cyri* (ZM-CBSU G1125, 24 specimens from Iran: Mazandaran prov.: Tajan River at Sari City, Caspian Sea Basin).

	Barbus cyri Caspian Sea Basin			
	min	max	mean	SD
Total length (mm)	81.8	146.5	107.8	17.84
In percent of standard length				
Head length	22.0	25.0	23.6	075
Pre orbital distance	9.2	10.8	10.1	0.50
Post orbital distance	10.9	12.3	11.5	0.30
Inter orbital distance	7.9	10.7	8.7	0.60
Predorsal length	50.0	54.2	52.0	1.07
Postdorsal length	47.8	58.4	53.6	2.52
Dorsal-fin length	16.1	21.2	19.3	1.29
Dorsal-fin depth	11.0	13.6	12.3	0.82
Anal-fin length	16.8	22.2	19.3	1.55
Anal-fin depth	6.1	10.4	8.0	0.92
Preanal length	69.9	74.0	71.8	1.10
Pectoral-fin length	17.5	20.0	19.0	0.66
Pelvic-fin length	15.1	17.7	16.4	0.63
Minimum body depth	10.2	11.7	10.8	0.44
Maximum body depth	18.3	24.7	21.2	1.38
Distance between pectoral and Anal-fin	49.7	52.7	50.7	0.83
Distance between pectoral and pelvic-fin	24.4	31.9	26.8	1.53
Distance between pelvic and anal-fin	21.9	28.6	24.1	1.32
First barbel	2.6	6.0	4.0	0.63
Second barbel	3.0	7.3	5.9	0.87
In percent of head length				
Head depth	47.0	57.7	54	2.77
Pre orbital distance	40.0	46.4	42.8	1.84
Post orbital distance	46.4	53.1	48.7	1.40
Inter orbital width	32.5	43.1	36.9	2.44
Eye diameter	12.5	20.9	17.0	1.89
Maximum body length	78.4	99.3	89.9	5.70
Length of caudal fin	75.4	109.3	91.1	7.12
Mouth width	19.2	28.1	24.7	2.24
Meristic data				
Dorsal fin unbranched rays	3	3	3.0	0.00
Dorsal fin branched rays	7	8	7.8	0.38
Pectoral fin rays	14	16	15.0	0.69
pelvic fin rays	8	9	8.2	0.44
Anal fin unbranched rays	3	3	3.0	0.00
Anal fin branched rays	6	6	6.0	0.00
Lateral line scales	52	69	59.4	3.82
Caudal peduncle scale	23	28	25.3	1.29
Scale above lateral line	9	13	11.1	0.87
Scale below lateral line	8	12	9.9	0.93
Predorsal scales	24	34	28.7	2.42
Gill raker	8	10	9.2	0.56

nucleotide substitutions in the DNA barcode region studied.

Distribution: *Barbus cyri* is widespread in the southern Caspian Sea basin, where it occurs in the entire Kura-Aras drainage from the headwaters in Turkey to Azerbaijan and Iran. It is also found in rivers and streams south and east of the Kura until the very south-east of the Caspian Sea basin, where the species is found in the Atrak River drainage. *Barbus cyri* is also known from tributaries (Talkheh, Nazlu Chai, Tatavi and Zarrineh) of the hypersaline Urmia Lake basin (Fig. 2).

Table 3. Morphometric and meristic data of *Barbus cyri* from Urmia basin (ZM-CBSU G968, 12 specimens from Iran: East Azarbaeijan prov.: Baranduz Chay River at Urmia City, Urmia Basin).

	Barbus cyri Urumia			
	min	max	mean	SD
Total length (mm)	95.8	136.5	123.0	13.26
In percent of standard length				
Head length	22.5	25.3	23.6	0.89
Pre orbital distance	9.3	11.0	9.9	0.52
Post orbital distance	10.6	12.0	11.4	0.42
Inter orbital distance	8.6	10.2	9.2	0.56
Predorsal length	49.3	56.1	51.5	1.76
Postdorsal length	51.2	60.7	54.5	2.66
Dorsal-fin length	15.8	20	17.5	1.25
Dorsal-fin depth	11.3	13.9	12.2	0.63
Anal-fin length	15.5	18.7	16.7	0.91
Anal-fin depth	6.9	8.6	7.9	0.62
Preanal length	69.5	78.6	72.7	2.33
Pectoral-fin length	16.6	20.4	18.0	1.02
Pelvic-fin length	14.7	18.3	16.0	0.98
Minimum body depth	9.0	11.0	10.0	0.64
Maxmum body depth	18.4	21.3	19.5	0.88
Distance between pectoral and Anal-fin	48.9	57.2	51.2	2.27
Distance between pectoral and pelvic-fin	25.5	29.0	27.3	0.99
Distance between pelvic and anal-fin	21.4	25.8	23.4	1.16
First barbel	3.5	5.4	4.2	0.47
Second barbel	5.2	7.0	6.4	0.60
In percent of head length				
Head depth	50.9	578	54.1	2.19
Pre orbital distance	40.1	46.9	42	1.81
Post orbital distance	44.1	50.7	48.5	1.89
Inter orbital width	35.9	42.0	39.1	2.43
Eye diameter	15.8	21.5	18.5	1.76
Maximum body length	77.1	88.4	82.7	3.98
Length of caudal fin	72.9	94.5	84.1	6.37
Mouth width	22.1	29.6	25.9	2.54
Meristic data				
Dorsal fin unbranched rays	4	4	4.0	0.00
Dorsal fin branched rays	8	8	8.0	0.00
Pectoral fin rays	15	16	15.5	0.52
pelvic fin rays	8	10	9.1	0.70
Anal fin unbranched rays	4	4	4.0	0.00
Anal fin branched rays	5	6	5.3	0.47
Lateral line scales	60	76	66.8	4.35
Caudal peduncle scale	28	33	30.2	1.54
Scale above lateral line	12	15	14.1	0.94
Scale below lateral line	11	13	11.8	0.75
Predorsal scales	41	50	45.5	3.14
Gill raker	7	10	8.4	0.82

Barbus lacerta Heckel 1843

(Figs. 6-7, Table 4)

Barbus scincus Heckel, 1843:1049

Barbus plebejus ercisianus Karaman, 1971:204

Barbus plebejus kosswigi Karaman, 1971:206

Material examined: NMW 54227, 4 syntypes of *B. lacerta*, 4, 108–182 mm SL; Syria: Aleppo. — NMW 22272, 2 syntypes of *B. scincus*, 7.6, 146.7 mm SL; Syria: Aleppo. — NMW 54525-26, 1 syntype of *B. scincus* (1, 1) 158.8 mm SL; Syria: Aleppo. — ZMH 4209, holotype of *B. kosswigi*, 138 mm, SL. — ZMH 1159, 5 paratypes of *B. kosswigi*, 58.8-116 mm SL; Turkey. — ZMH 4208, holotype *of B. ercisianus*, 109 mm SL. — ZMH 3566, 12 paratypes *of B. ercisianus*, 40-145 mm SL; Turkey: Ercis. — ZMH 3567, 13 paratypes *of B. ercisianus*, 53-96 mm SL; Turkey: stream at road between Ercis and Patros. — FSJF 2543, 8, 86–100 mm SL; Turkey: Sivas prov.: Mancılık Stream 5 km west of Mancılık, about 30 km north of Gürün, 39°03'38.5"N 37°10'24.8"E, GenBank accession number (MF106115, MF106116, MF106117 (DNA-892)). — FSJF 2562, 2, 91–106 mm SL; Turkey: Diyarbakır prov.: Göksu Stream below Göksu Dam, south of Çınar at road from Diyabakır to Mardin, 37°41'33.8"N 40°26'52.4"E. — FSJF 2632, 2, 104–112 mm SL; Turkey: Adıyaman prov.: upper Göksu River, 5 km northeast of Gölbaşı, 37°50'12.9"N 37°41'05.4"E, GenBank accession number (MF106118, MF106119 (DNA-898)). — FSJF 2872, 18, 35–106 mm SL; Turkey: Elazığ prov.: upper Tigris River 5 km north of Maden, 38°24'58.3"N 39°39'11.2"E, GenBank accession number (MF106142, MF106143, MF106144, MF106145 (DNA-1418)). — FSJF 2894, 3, 78–118 mm SL; Turkey: Gaziantep prov.: tributary to Merzimen Stream south of Yavuzeli, 37°16'36.8"N 37°31'57.0"E. — FSJF 2906, 3, 105–114 mm SL; Turkey: Sivas prov.: stream Kangal under railway bridge at Çetinkaya, 39°15'05.7"N 37°37'08.0"E. — FSJF 2936, 11, 73–124 mm SL; Turkey: Sürgü Stream between Hamzalar and Kapıdere, 37°57'12.96"N 37°41'42.72"E. — FSJF 2943, 1, 100 mm SL; Turkey: Diyarbakır prov.: Spring of Pamuk at Kocaköy, 38°16'19.56"N 40°33'46.08"E. — FSJF 2948, 6, 62–115 mm SL; Turkey: Diyarbakır prov.: Bağlıca Stream betwenn Bismil and Tepe, 37°48'30.24"N 40°43'00.84"E. — FSJF 3346, 4, 73–148 mm SL; Iraq: stream north-west of Saburawa, a tributary of Tabin river, 35°50'01"N 45°06'16"E. — FSJF 3351, 8, 42–106 mm SL; Iraq: Kuna Massi Stream in Sevanja, 35°47.35'N 45°24.18'E. — FSJF 3364, 15, 61–105 mm SL; Iraq: Nalparez River, 35°34.24'N 45°51.78'E. — FSJF 3380, Iraq: Tabin River west of Zarbi, 35°48'06"N 44°58'47"E. — ZM-CBSU D111, 8, 57–139 mm SL; Iran: Lorestan prov.: Karkheh River at Kashkanrud 25 km west of Khoramabad, 33°35'14"N 47°52'55"E. — ZM-CBSU G964, 4, 104–136 mm SL. Iran: Kermanshah prov.: Leylehrud River at Shervineh west of Javanrud, 34°52'29"N 46°21'06"E. — ZM-CBSU J1688, 34, 36–126 mm SL; Iran: West Azarbaeijan prov.: Little Zab 10 km south of Piranshahr, 36°28'36.3"N 45°19'54.0"E.

Material for molecular genetic analysis: FSJF 2543, Turkey: Sivas prov.: Mancılık Stream 5 km west of Mancılık, about 30 km north of Gürün, 39°03'38.5"N 37°10'24.8"E, GenBank accession number (MF106115, MF106116, MF106117 (DNA-892)). — FSJF 2632, Turkey: Adıyaman prov.: upper Göksu River, 5 km northeast of Gölbaşı, 37°50'12.9"N 37°41'05.4"E, GenBank accession number (MF106118, MF106119 (DNA-898)). — FSJF 2872, Turkey: Elazığ prov.: upper Tigris River 5 km north of Maden, 38°24'58.3"N 39°39'11.2"E, GenBank accession number (MF106142, MF106143, MF106144, MF106145 (DNA-1418)). — FSJF DNA-2205, Iraq: Arbil prov.: Chami Rean River near Ziraran, a tributary to Great Zab, 41°8'5.136"N 42°51'22.104"E, GenBank accession number (MF106138, MF106139). — FSJF DNA-2206, Iraq: Sulaymānīyah prov.: Tabin River west of Zarbi, 35°48'06"N 44°58'47"E, GenBank accession number (MF106140, MF106141). — FSJF DNA-2371, Turkey: Sivas prov.: Tatlı Stream at Kabakçevliği village, 25 km east of Kangal, 39°18'4.716"N 37°40'27.552"E, GenBank accession number (MF106120). — FSJF DNA-2374, Turkey: Sivas prov.: Kangal Stream under railway bridge at Çetinkaya, 39°15'4.644"N 37°37'7.464"E, GenBank accession number: MF106121. — FSJF DNA-2390, Turkey: Erzurum prov.: Sırlı Stream at Sırlı village, 30 km north of Ilıca, 40°13'5.232"N 41°4'34.86"E, GenBank accession number (MF106122). — ZFMK 54811–54813; ZFMK 54815, Turkey: Ercis prov.: Deli Stream 10 km southwest of Ercis, 38°58'0.12"N 43°16'0.12"E, GenBank accession number (MF106166, MF106167, MF106168). — ZM CBSU M485–M487, Iran: West Azerbaijan prov.: Little Zab 10 km south of Piranshahr City, 36°28'36.3"N 45°19'54.0"E, GenBank accession number (MF106134, MF106135, MF106136). — ZM-CBSU M518, Iran: Hamedan prov.: Gamasiyab

Figure 6. Four syntypes of *Barbus lacerta* in the Naturhistorisches Museum Wien (NMW 54227). The Vienna card catalogue in 1997 lists one of NMW 54227 as the lectotype (54227-1, 181.6 mm SL) and 3 others as paralectotype (in courtesy of N. Bogutskaya, NMW).

Figure 7. *Barbus lacerta*, ZM-CBSU J1700 102 mm SL, Iran: "West Azerbaijan," Little Zab River, Tigris basin.

River at Dehno Village 30 km north of Malayer, 34°35'40.3"N 48°44'19.9"E, GenBank accession number (MF106123). — ZM-CBSU M519, Iran: Kermanshah prov.: Gamasiyab River at Garous 15 km southwest of Kangavar, 34°23'1.66"N 47°45' 8.37"E, GenBank accession number (MF106124). — ZM-CBSU M520, Iran: Kermanshah prov.; Paveh, Hajij, Sirvan, 35°07'3.76"N 46°15'24.34"E, GenBank accession number (MF106137). — IMNRF-UT-COIB-02, Iran: Kermanshah prov.: Dinevar River at Dastjerd Sofla, Karkheh drainage, 34°35'04"N 47°31'11"E, GeneBank Accession number (MF106126). — IMNRF-UT-COIB-03, Iran: Lorestan prov.: Kahman River, Karkheh drainage, 33°47'04"N 48°12'25"E, GeneBank Accession number (MF106125). — IMNRF-UT-COIB-06, IMNRF-UT-COIB-07, Iran: Kermanshah prov.: Sirvan River at Hajij, Tigris basin, 35°09'37"N 46°20'22"E, GeneBank Accession number (MF106132, MF106133). — IMNRF-UT-COIB-08, Iran: Kermanshah prov.: Leileh River at Kalash bakhan, Tigris drainage, 34°54'03"N 46°12'35"E, GeneBank Accession number (MF106129). — IMNRF-UT-COIB-09, IMNRF-UT-COIB-10, Iran: Kermanshah prov.: Dinevar River at Hosein Abad, Karkheh drainage, 34°33'16"N 47°24'48"E, GeneBank Accession number (MF106127, MF106128). — IMNRF-UT-COIB-11, IMNRF-UT-COIB-12, Iran: Lorestan prov.: Roudbar River, tributary of Bakhtiyari River, near Kazem Abad village, 33°08'17.8"N 49°40'43.9"E, GeneBank Accession number (MF106130, MF106131).

Diagnosis: *Barbus lacerta* is distinguished from all other species of the *B. lacerta* group by having a "almost" triangular gular region (vs. rectangular in other species of the *B. lacerta* group) (Fig. 5) and almost a longer anal fin. The tip of the anal fin, when pressed to the body, reaches beyond the middle of the distance between the base of the last anal-fin ray and the lower caudal-fin origin, often to the caudal-fin base (vs. not reaching to middle of the distance) and there are 0–3 (mode 2) scale rows between the tip of the anal fin and the base of the caudal fin (vs. 3–6, mode 5). *Barbus lacerta* is also distinguished from *B. cyri* by having a straight posterior anal-fin margin (vs. convex) and a narrow upper lip (its width 4–6 %HL vs. 7–9 % HL). Other useful characters to identify *B. lacerta* are: long snout, its length 46–56% of body depth at dorsal-fin origin; eye diameter 2.5–3.1 times in snout length; 52-66+2-4 total scales along the lateral line; 35–48 (mode 39) predorsal scales; 25–32 (mode 28) circumpeduncular scales; length of caudal peduncle 1.7–2.0 times longer than depth; 60–70% of posterior margin of last unbranched dorsal-fin ray covered with denticles and median pad at lower lip small (Fig. 5). See below for details to distinguish *B. lacerta* from the other species of the *B. lacerta* group.

Morphometric data are provided in Table 4. Compared to all other species of *Barbus*, there are no diagnostic nucleotides in the examined COI sequences, but within the *B. lacerta* group, *B. lacerta* is characterized by one fixed diagnostic nucleotide substitution in the DNA barcodes studied (Position 313).

Distribution: *Barbus lacerta* is widespread and found in smaller rivers and streams in the entire Euphrates and Tigris drainages. It is also found in the upper Karkheh, the tributary to the lower Tigris adjacent to the Karun. *Barbus lacerta* is also found in tributaries of Lake Van, an endorheic basin in Eastern Turkey and in adjacent Lake Nemrut. It occurred in the Qweik River but might be extirpated now. See Figure 2 for the distribution of this species.

Remarks: *Barbus lacerta* was described by Heckel (1843) from Aleppo in Syria, which is situated at the Qweik River. Unfortunately, we were not able to collect fresh materials of *B. lacerta* from the Qweik, an endorheic basin with headwaters in Turkey ending south of Aleppo in the desert. Jörg Freyhof visited Aleppo and the Qweik basin in 2008, but there was no water in the entire Syrian part of the catchment (pers. comm.). In Turkey, there are two very small streams, the Sünnep, and the Balıksuyu, which belong to the Qweik catchment. Dagli and Erdemli (2009) published about the fishes of the Balıksuyu without record of *B. lacerta*. Furthermore, Davut Turan and Erdogan Cicek (pers. comm.) informed us that he never found *B. lacerta* in these streams. S. Eagderi sampled the Sünnep and Balıksuyu during March 2017 without any record of *B. lacerta*. Heckel (1843) also described *B. scincus* from the Qweik, which was treated as synonym of *B. lacerta* by later authors (Coad 1991, 1995; Bănărescu and Bogutskaya 2003). The Qweik is inhabited by a fish fauna mostly made up of species also occurring in the Euphrates. The Qweik is believed to have been a tributary of the Euphrates until the early Holocene (Krupp 1985). There is one endemic species (*Oxynoemacheilus tigris*) and two species (*Pseudophoxinus zeregi*, *Garra variabilis*) shared with the Orontes, which is adjacent to the Qweik in the west. No *Barbus* species are known from the Orontes drainage. We have no doubt, that *B. lacerta* is the species found also in the Euphrates and the Tigris drainage.

Barbus ercisianus was found to be nested within the *B. lacerta* clade based on COI sequences. This species has been described as a subspecies of *B. plebejus* by Karaman (1971) from streams close to Ercis following to

Table 4. Morphometric and meristic data of *Barbus lacerta* (ZM-CBSU D111, 8 specimens from Iran: Lorestan prov.: Karkheh River at Kashkanrud near Khoramabad City, Persian Gulf Basin and ZM-CBSU G964, 4 specimens from Iran: Kermanshah prov.: Leylehrud at Shervineh near Javanrud City, Persian Gulf Basin).

	Barbus lacerta			
	min	max	mean	SD
Total length (mm)	70.0	183.2	123.7	31.30
In percent of standard length				
Head length	22.6	27.9	25.26	1.35
Pre orbital distance	10.0	12.0	10.6	0.62
Post orbital distance	11.5	13.6	12.4	0.67
Inter orbital distance	7.6	10.4	9.3	0.99
Predorsal length	50.3	55.7	52.5	1.69
Postdorsal length	46.4	59.1	53.0	3.31
Dorsal-fin length	16.2	21.8	19.6	1.69
Dorsal-fin depth	10.4	14.6	12.5	1.32
Anal-fin length	16.5	24.3	20.0	2.78
Anal-fin depth	6.0	9.1	7.6	0.85
Preanal length	72.0	76.8	74	1.78
Pectoral-fin length	16.4	19.6	17.6	1.10
Pelvic-fin length	14.9	17.4	15.9	0.63
Minimum body depth	10.1	11.2	10.5	0.35
Maxmum body depth	19.6	25.7	21.3	1.57
Distance between pectoral and Anal-fin	49.3	55.0	51.5	1.93
Distance between pectoral and pelvic-fin	23.3	31.3	27.0	1.88
Distance between pelvic and anal-fin	22.6	27.3	24.7	1.46
First barbel	3.5	6.1	4.9	0.89
Second barbel	4.4	9.3	6.7	1.31
In percent of head length				
Head depth	46.9	57.0	52.8	3.00
Pre orbital distance	39.1	44.7	41.9	1.78
Post orbital distance	46.3	52.8	49.3	1.85
Inter orbital width	31.7	40.5	36.7	3.34
Eye diameter	14.2	23.2	17.3	2.39
Maximum body length	72.8	92.3	84.3	5.37
Length of caudal fin	61.9	99.6	81.4	8.93
Mouth width	20.0	44.0	23.9	6.36
Meristic data				
Dorsal fin unbranched rays	3	4	3.4	0.51
Dorsal fin branched rays	7	8	7.9	0.28
Pectoral fin rays	14	18	15.7	0.95
pelvic fin rays	8	10	8.8	0.72
Anal fin unbranched rays	3	4	3.4	0.51
Anal fin branched rays	5	6	5.5	0.52
Lateral line scales	56	67	62.9	3.04
Caudal peduncle scale	25	32	28.5	2.47
Scale above lateral line	11	15	13.2	1.30
Scale below lateral line	10	13	11.0	1.08
Predorsal scales	35	48	40.5	3.95
Gill raker	9	10	9.5	0.52

Lake Van in Turkey. Lake Van is situated within the Euphrates watershed but has no actual connection to that river system. Karaman (1971) studied materials from the Caspian Sea basin to describe *B. lacerta*, which was also treated as a subspecies of *B. plebejus*. All populations from the Caspian Sea basin are here identified as *B. cyri*. Karaman (1971) differentiated *B. p. ercisianus* from *B. cyri* by having no or a very shallow predorsal keel (vs. keel well developed), thin barbels (vs. thick in *B. cyri*) and a very poorly ossified last unbranched dorsal-fin ray (vs. moderately or well ossified). Geldiay and Balik (2007: 394) copied these character states in the key given to differentiate Turkish *Barbus* species. Based on the materials examined for this study, the width of the barbels of *B. lacerta*, *B. cyri* and *B. ercisianus* as well as the ossification strength of the last unbranched dorsal-fin ray are quite variable and we found no difference in these characters between *B. lacerta*, *B. cyri* and *B. ercisianus*. As we found also no other differences between *B. ercisianus* and *B. lacerta*, therefore we treat *B. ercisianus* as a synonym of *B. lacerta*.

Barbus kosswigi was described as a subspecies of *B. plebejus* by Karaman (1971:206) from the upper Tigris drainage in Turkey. He distinguished *B. kosswigi* from *B. lacerta* by the head width, which is greater than the head depth in *B. kosswigi*, but equally or smaller in *B. lacerta*. We were not able to visit the type locality of *B. kosswigi* and no fresh material for DNA sequencing was available. Fricke (2007) placed this species in the genus *Luciobarbus* without comments. According to Jörg Freyhof, who examined the type series of *B. kosswigi* at ZMH, these fishes have very narrow lips and the pad on the lower lip forms a continuous structure with the gular tissue, a character state seen in several species of *Luciobarbus* (vs. mental pad separated from gular tissue by a deep groove in *Barbus*). In *B. lacerta*, the pad on the lower lip is always separated by a deep groove from the gular tissue. *Barbus kosswigi* has a short dorsal fin, with a quite flexible, poorly ossified last unbranched ray with a series of short serrae as it is typical for all species of the *B. lacerta* species group. All species of *Luciobarbus* in the Tigris (*L. esocinus*, *L. xanthopterus*, *L. barbulus*, *L. kersin* and *L. subquincunciatus*) have a very long, pointed, strongly ossified and strongly serrated last unbranched dorsal-fin ray. Except *L. subquincunciatus*, all species of *Luciobarbus* in the Tigris lack brown blotches, speckles or spots in adults, which are present only in juveniles. The pale-brown blotches, speckles or spots patterns are faded in juvenile *Luciobarbus* larger than 100 mm SL, while they are still visible in the types of *B. kosswigi*. Furthermore, *B. kosswigi* has a very long anal fin typical for *B. lacerta* (vs. anal fin very short in all *Luciobarbus* in the Tigris) and other *Barbus* species (e.g., *B. caninus, B. cyclolepis, B. euboicus, B. goktschaicus, B. haasi, B. macedonicus* and *B. peloponnesius*). We see no reason to place *B. kosswigi* in *Luciobarbus* because of the lip structure only, as it is very variable in *Barbus* and *Luciobarbus* species.

Barbus miliaris **De Filippi, 1863.**

(Fig. 8, Table 5)

Material examined: All from Iran. — MZUT 676, syntype, 1, 100 mm SL. — FSJF 3228, 1, 51 mm SL; Qom prov.: Qom River, about 40 km southwest of Qom, 34°21'11.25"N 50°32'52.66"E. GenBank accession number (MF106147, MF106148 (DNA-1991)). — FSJF 3459, 6, 121–239 mm SL; Markazi prov.: Qara Chai (Gharehchai) River, at Jalayer, 34°53'13.9"N 50°02'10.9"E. — ZM-CBSU G1101, 24, 70–97 mm SL; Markazi prov.: Qara Chai (Gharehchai) River, at Jalayer, 34°53'13.9"N 50°02'10.9"E.

Material for molecular genetic analysis: FSJF 3228, 1, 51 mm SL; Qom prov.: Qom River, about 40 km southwest of Qom, 34°21'11.25"N 50°32'52.66"E. GenBank accession number (MF106147, MF106148 (DNA-1991)). — ZM-CBSU M424; Markazi prov.: Saveh Dam, Qara Chai River, at Jalayer, 34°53'13.9"N 50°02'10.9"E, GenBank accession number (MF106149). — ZM-CBSU M434, Semnan prov.: Hableh River at 20 km northeast of Garmsar, 35°18'07"N 52°24'58"E, GenBank accession number (MF106146). — ZM-CBSU M425, Markazi prov.: Qara Chai (Gharehchai) River, 34°53'14.69"N 50°02'15.19"E, GenBank accession

Figure 8. Live specimen of *Barbus miliaris* ZM-CBSU G1040, 65 mm SL, Iran: Qom River, Emamzadeh Abdolah, Namak basin (Khaefi et al. 2017).

number (MF106150). — ZM-CBSU M426, Qom prov.: Qom River, about 35 km southwest of Emamzadeh Abdolah, 34°22'47"N 50°36'08"E, GenBank accession number (MF106151).

Diagnosis: *Barbus miliaris* is distinguished from the other species of the *B. lacerta* group by having more scales along the lateral line (69-87 (mode 77) vs. 50–66 (mode 57) in *B. cyri;* 52–66 (mode 61) in *B. lacerta* and 80–95% of the posterior margin of the last unbranched dorsal-fin ray covered with denticles (vs. 60–70%). *Barbus miliaris* is also distinguished from *B. cyri* by having more predorsal scales (37–47, mode 42 vs. 24–34, mode 29); a straight posterior anal-fin margin (vs. convex) and a narrower upper lip (its width 4–6 %HL vs. 7–9 %HL). *Barbus miliaris* is also distinguished from *B. lacerta* by the tip of the anal fin, when pressed to the body, reaching to about the middle of the distance between the base of the last anal-fin ray and the lower caudal-fin origin (vs. beyond, often to caudal-fin base). In *B. miliaris*, there are 3–6 (mode 4) scale rows between the tip of the anal fin and the base of the caudal fin (vs. 0–3), the maxillary barbel is longer (26–41 %HL vs. 12–32 %HL in other species) and does not reach to the posterior eye margin (vs. reaching in *B. lacerta*) and this species has a rectangular gular region (vs. triangular). Other useful characters to identify *B. miliaris* are: snout length 54–57% of body depth at dorsal-fin origin; eye diameter 2.3–2.6 times in snout length; length of caudal peduncle 1.7–2.2 times longer than deep; median pad of lower lip shallow (Fig. 5). See below for details to distinguish *B. miliaris* from *B. karunensis*. Morphometric data are provided in Table 5. The ventral side of the head is shown in Figure 16. Among all other studied species of the *Barbus*, *B. miliaris* is characterized by four fixed diagnostic nucleotide substitutions in the DNA barcode region studied (Positions 107, 301, 352, 508), and among the *B. lacerta* group by nine fixed diagnostic substitutions (Positions 107, 220, 238, 277, 301, 352, 463, 502, 508).

Distribution: *Barbus miliaris* is known from the Qom River drainage, a tributary of Namak Lake basin and from the Hableh River in the Kavir basin (Fig. 2).

Remarks: Morphological properties and characteristics were discussed by (Khaefi et al. 2017). Furthermore, our molecular data suggest, that *B. miliaris* and *B. cyclolepis* are only distantly related (Table 1).

Barbus karunensis, new species

(Figs. 9-11, Table 6)

Holotype: ZM-CBSU G1047, 102 mm SL; Iran: Kohgiluyeh and Boyer-Ahmad prov.: Bashar River at Talegah village 10 km north of Yasuj City, 30°47'27.5"N 51°25'13.3"E; R. Khaefi, G. Sayadzadeh and A. Khajehpanah, 16 Jan 2014.

Paratypes: ZM-CBSU G1038, 12, 47–121 mm SL; same data as holotype. — ZM-CBSU D11, 8, 77–116 mm SL; Iran: Kohkiluyeh and Boyer-Ahmad prov.: Bashar River at Yasuj, 30°40'56.2"N 51°31'94.8"E. — FSJF 2215, 10, 86–154 mm SL; Iran: Chaharmahal Bakhtiari prov.: Sangan Stream at Sangan, 31°15.692'N

Table 5. Morphometric and meristic data of *Barbus miliaris* (ZM-CBSU G1101, 24 specimens from Iran: Markazi prov.: Saveh Dam at Saveh City, Qara chai River, Namak Basin) (Khaefi et al. 2017).

	Barbus miliaris			
	min	max	mean	SD
Total length (mm)	84.7	117.5	104.3	8.65
In percent of standard length				
Head length	24	29.7	25.7	1.13
Pre orbital distance	9.8	14.0	11.1	0.80
Post orbital distance	10.5	13.2	11.9	0.60
Inter orbital distance	7.5	9.7	8.8	0.50
Predorsal length	49.3	55.4	51.8	1.63
Postdorsal length	52.3	58.7	55.7	1.60
Dorsal-fin length	18.4	22.6	20.7	1.25
Dorsal-fin depth	11.8	14.8	13.3	0.66
Anal-fin length	17.2	21.6	19.3	1.18
Anal-fin depth	6.8	9.1	7.9	0.61
Preanal length	70.2	74.8	72.8	1.15
Pectoral-fin length	16.5	20.7	19.8	1.05
Pelvic-fin length	16	19.7	18.5	0.96
Minimum body depth	9.9	11.4	10.5	0.38
Maximum body depth	17.5	21.9	20.1	1.08
Distance between pectoral and Anal-fin	45.1	50.3	47.9	1.21
Distance between pectoral and pelvic-fin	22.1	26.2	24.2	1.16
Distance between pelvic and anal-fin	21	25.8	23.6	1.03
First barbel	3.7	6.4	5.7	0.60
Second barbel	6.4	10.1	7.9	0.75
In percent of head length				
Head depth	46.7	60.3	52.6	3.03
Pre orbital distance	39.3	47.7	43.1	2.24
Post orbital distance	42.9	51.3	46.3	1.64
Inter orbital width	28.2	38.6	34.3	2.41
Eye diameter	15.9	21.8	18.4	1.23
Maximum body length	70.7	87.6	78.2	4.37
Length of caudal fin	72.2	96.1	87.4	5.47
Mouth width	12.9	28	24.1	2.97
Meristic data				
Dorsal fin unbranched rays	4	4	4.0	0.00
Dorsal fin branched rays	7	8	7.9	0.33
Pectoral fin rays	14	17	15.9	2.38
pelvic fin rays	8	10	9.0	0.68
Anal fin unbranched rays	4	4	4.0	0.00
Anal fin branched rays	5	5	5.0	0.00
Lateral line scales	71	90	80.0	5.11
Caudal peduncle scales	28	35	32.0	1.55
Scale above lateral line	13	17	15.5	1.05
Scale below lateral line	10	14	12.4	1.11
Predorsal scales	37	45	41.0	2.21
Gill raker	9	12	10.3	0.87

51°17.150'E, GenBank accession numbers (MF106100, MF106101, MF106102 (DNA-138)).

Material for molecular genetic analysis: FSJF 2215, Iran: Chaharmahal Bakhtiari prov.: Sangan Stream at Sangan, 31°15.692'N 51°17.150'E, GenBank accession numbers (MF106100, MF106101, MF106102 (DNA-138)). — ZM-CBSU M1351–M1354; M1375, Iran: Kohkiluyeh and Boyer-Ahmad prov.: Bashar River at Talegah Village, 30°47'27.5"N 51°25'13.5"E, GenBank accession numbers (MF106105, MF106106, MF106107, MF106108). — ZM-CBSU M453; Iran: Esfahan Prov.: Semirom River at Tange-Khoshk

Figure 9. *Barbus karunensis*, ZM-CBSU G1047, holotype, 101 mm SL, Iran: Kohgiluyeh and Boyer-Ahmad, Yasuj, Talegah, Bashar River.

(Bibiseyedan), 31°11'2.3"N 51°26'59.2"E. GenBank accession numbers (MF106103). — ZM-CBSU M454; Iran: Kohgiluyeh and Boyer-Ahmad prov.: Sheylaneh River at Kata Village, 31°11'1.18"N 51°16'16.5"E, GenBank accession numbers (MF106104). — ZM-CBSU M1112, ZM-CBSU M1113; Iran: Kohgiluyeh and Boyer-Ahmad prov.: Bashar River at Dehnoo village, 30°38'55.2"N 51°37'05.7"E, GenBank accession numbers (MF106109, MF106110). — IMNRF-UT-COIB-04, IMNRF-UT-COIB-05, Iran: Chaharmahal and Bakhtiari prov.: Beheshtabad River, Karoun drainage, 32°00'41"N 50°36'41"E, GeneBank Accession number (MF106098, MF106099).

Diagnosis: *Barbus karunensis* is distinguished from the other species of the *B. lacerta* group by having a well-developed middle pad of the lower lip (vs. poorly developed or absent) (Fig. 5). *Barbus karunensis* is distinguished from *B. cyri* by having a thin upper lip (upper lip width 4–6 %HL vs. 7–9 % HL), and from *B. lacerta* by having a shorter anal fin. The tip of the anal fin is not reaching or reaching to about the middle of the distance between the base of the last anal-fin ray and the lower caudal-fin origin when pressed to the body (vs. reaching beyond middle, often to caudal-fin base) and there are 5–9 (mode 6) scale rows between the tip of the anal fin and the base of the caudal fin (vs. 0–3, mode 2). *Barbus karunensis* is distinguished from *B. miliaris* by having 59-66+2-4 (mode 61) scales in the lateral line (vs. 69-87+3-5), 26–29 (mode 26) circumpeduncular scales (vs. 28–35); maxillary barbels not reaching the middle of the eye (vs. reaching beyond), and a longer snout (eye diameter 2.7–3.5 times in snout length vs. 2.3–2.6 or eye diameter 27-35% snout length vs. 23-26%).

Figure 10. *Barbus karunensis*, ZM-CBSU G1048, paratypes, a. 100 mm SL, b. 96 mm SL, c. 92 mm SL.

Figure 11. Live specimen of *Barbus karunensis*, ZM-CBSU G1048, 100 mm SL, Iran: Kohgiluyeh and Boyer-Ahmad, Yasuj, Talegah, Bashar River.

Among all other studied species of *Barbus*, *B. karunensis* is characterized by one fixed diagnostic nucleotide substitution in the DNA barcode region studied (Position 70), and among the *B. lacerta* group by two fixed diagnostic substitutions (Positions 70 and 166).

Description: For general appearance see Figures 9-11; mouth structure in Figure 5 and morphometric data are provided in Table 6. Small-sized and slender species. Body compressed laterally. Head deep and narrow. Body deepest at dorsal-fin origin, depth decreases towards middle of caudal peduncle. Caudal peduncle 1.0–2.4 times

Table 6. Morphometric and meristic data of *Barbus karunensis* (ZM-CBSU G1038, 13 specimens from Iran: Kohgiluyeh and Boyer-Ahmad prov.: Bashar River at Talegah Village near Yasuj City, Tigris Basin and ZM-CBSU D11, 8 specimens from Iran: Kohkiluyeh and Boyer-Ahmad prov.: Bashar River at Yasuj City, Tigris Basin).

	Barbus karunensis			
	min	max	mean	SD
Total length (mm)	47.5	116.4	84.8	27.18
In percent of standard length				
Head length	23.6	28.2	25.6	1.44
Pre orbital distance	10.3	12.7	11.2	0.67
Post orbital distance	10.5	13.1	11.8	0.55
Inter orbital distance	8.5	12.0	9.8	1.16
Predorsal length	49.2	55.0	52.1	1.67
Postdorsal length	10.5	13.1	11.8	0.55
Dorsal-fin length	16.8	21.8	19.3	1.26
Dorsal-fin depth	10.4	12.6	11.8	0.59
Anal-fin length	16.5	19.4	17.9	0.96
Anal-fin depth	7.1	8.6	7.7	0.44
Preanal length	68.3	73.9	71.4	1.51
Pectoral-fin length	16.3	21.5	18.4	1.39
Pelvic-fin length	14.7	17.7	16.3	1.03
Minimum body depth	9.1	19.9	10.6	2.55
Maxmum body depth	9.1	21.7	18.4	2.67
Distance between pectoral and anal-fin	46.1	53.2	48.1	1.76
Distance between pectoral and pelvic-fin	23.3	28.2	25.3	1.23
Distance between pelvic and anal-fin	19.8	24.6	21.8	1.26
First barbel	3.2	8.6	25.6	1.17
Second barbel	5.0	7.6	11.2	0.82
In percent of head length				
Head depth	51.5	64.3	55.1	3.10
Pre orbital distance	38.4	46.4	43.8	2.12
Post orbital distance	40.5	49.5	46.2	2.55
Inter orbital width	33.8	43.9	38.3	3.08
Eye diameter	15.2	22.6	18.8	2.62
Maximum body length	32.4	83.5	72.3	11.36
Length of caudal fin	71.6	100.1	81.5	6.98
Mouth width	19.0	25.1	22.4	1.93
Meristic data				
Dorsal fin unbranched rays	4	4	4.0	0.00
Dorsal fin branched rays	8	8	8.0	0.00
Pectoral fin rays	15	17	15.6	0.63
pelvic fin rays	8	10	8.9	0.53
Anal fin unbranched rays	3	4	3.8	0.43
Anal fin branched rays	5	5	5.0	0.00
Lateral line scales	60	70	63.1	2.70
Caudal peduncle scale	26	29	27.0	1.11
Scale above lateral line	12	15	12.8	0.89
Scale below lateral line	9	11	10.1	0.53
Predorsal scales	33	42	37.0	2.63
Gill raker	8	11	9.2	1.12

longer than deep. Triangular axillary scale at pelvic-fin base. Pelvic-fin origin below vertical of last unbranched to 1-3 branched dorsal fin ray. Caudal fin forked. Posterior dorsal- and anal-fin margins straight. Tip of anal fin, when pressed to body, not reaching or reaching to middle of caudal peduncle. Pectoral fin reaching approximately 50–65% distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin not reaching anus. Snout 51–91% of body depth at dorsal-fin origin. Gular region rectangular. Lips covered with papillae. Width of upper lip 4–6 %HL. Lower lip thicker than upper lip, with a well-developed median pad separated by a deep groove from adjacent gular tissue (Fig. 5). Rostral barbel short, not reaching nostril; maxillary barbel 19–32 %HL, just reaching before to the anterior half of eye. Largest known individual 154 mm SL.

Dorsal fin with 4 unbranched rays and 8½ branched rays, 65–80% of posterior margin of last unbranched dorsal-fin ray covered with denticles. Anal fin with 3–4 (mode 4) unbranched and 5 ½ branched rays. Pectoral fin with 15–17 (mode 16) rays. Pelvic fin with 8–10 (mode 9) rays. Lateral line with 59-66+2-4 (mode 64) scales. Scale rows between dorsal-fin origin and lateral line 12–15 (mode12). Scale rows between pelvic-fin origin and lateral line 9–11 (mode 10). Scale rows between tip of anal fin and base of caudal fin 5–9 (mode 6). Predorsal scales 33–42 (mode 39). Circumpeduncular scales 26–29 (mode 28) and 8–11 (mode 8) gill rakers on first gill arch.

Coloration: Overall coloration is brownish gray, with the shade being darker dorsally than laterally; almost entire body including head, dorsal and caudal fins and sometimes pectorals with numerous small, irregular dark-brown spots and medium sized blotches. The belly is yellowish and without spots. Barbels yellowish without brown spots. First barbel with some dark spots. Second barbel without black spots and yellowish.

Live specimens brown to yellow, flank dark-brown, belly yellowish white. Dorsal, pectoral, anal and pelvic fins brownish, caudal fin yellowish; numerous irregular dark-brown spots on back and flanks, and sometimes a few on anal, pelvic and pectoral fins, first barbel with a few black spots, second barbel yellowish without black spots.

Distribution: *Barbus karunensis* is known from the Karun River drainage in Iran (Fig. 2).

Etymology: The species is named for the Karun River, where the type locality is located. An adjective.

Discussion

The taxonomic and systematic history of *Barbus* was marked by a heated scientific debate, mainly based on morphological characters at the genus, species and subspecies level (see Berg 1949; Almaça 1983; Coad 1995; Bogutskaya 1997; Naseka and Bogutskaya 2009; Esmaeili et al. 2010; Levin et al. 2015). However, species boundaries are hard to delimit based on morphological characters. Hence, additional sources of information (e.g., molecular data, biogeography, behavior and ecology) are being implemented (Dayrat 2005) as an integrative taxonomy approach. DNA based data can aid delineating the boundaries between species, and also help to assign a correct taxonomy to closely related and cryptic species (Guo et al. 2016). This is reported here for widely distributed and recently isolated *Barbus* populations from the southern tributaries of the Caspian Sea, Namak Lake and Urmia basins and the Euphrates and Tigris drainages including the Qweik endorheic basin in Syria, which all have been previously considered as one species, *B. lacerta,* due to their morphological similarities.

Based on the presented DNA barcode data, the *B. lacerta* species group forms a monophyletic unit. Within this group, one of the clusters corresponds to *B. cyri* (Caspian Sea and Urmia Lake basin), which is sister group to all other Iranian species. Another unit corresponds well to *B. lacerta* (upper reaches of Tigris River drainage), and groups as sister to *B. karunensis* + *B. miliaris*. In addition, *B. miliaris* (endorheic Namak Lake basin) and *B. karunensis* (upper reaches of distinct Karun River drainage) are resolved as distinct mitochondrial lineages in the recovered topologies. Therefore, previous conclusions suggesting the presence of one species only (*B. lacerta*) in its wide geographically distribution can be refuted. A common hypothesis about complex and cryptic species groups is that such species are the result of recent speciation events, in which morphological characters and other diagnostic characters have not had enough time to be modified (Bickford et al. 2007). Recent allopatric speciations have been reported for several taxa (e.g., *Aphanius* species group) (Esmaeili et al. 2012; Esmaeili et al. 2014) and this may be in the case of *Barbus lacerta* group. According to Levin et al. (2011), *B. lacerta* diverged from *B. cyri* about 60,000 years ago. Hence, it can be considered as a young species pair with morphological similarities, and little genetic differentiation. The similarity found among *B. cyri* populations in the Caspian Sea basin (Aras, Kura, and Tajan River drainages) and Urmia Lake basins might indicate that gene flow occurred among them in the past due to geological events. This has been previously reported among fish species inhabiting the Aral, Caspian and Black Sea basins (e.g., *Salmo trutta*) based on haplotype similarities (Osinov and Bernatchez 1996). Possible corridors between the basins studied here could be interdrainage connections in the northern part of the Urmia Lake basin in Khoy (Ghara-Tappeh) via the Aras River, which drains into the Caspian Sea, or stream capture events occurring in the southeast of this basin, as the headwaters of some rivers draining to the Caspian Sea (e.g., Qezel Owzan River) are located in proximity to the south-eastern Urmia Lake basin (Zarrineh River) and both of these rivers are located in proximity to the north-eastern part of Tigris River drainages having *B. lacerta*. Based on the negligible sequence divergence and pronounced morphological similarities found among Iranian *Barbus* populations, it can be inferred that the time elapsing since their isolation has not been long. This conclusion is supported by geological reports indicating the origin of the modern Urmia Lake *c.* 10,000–30,000 years ago (Darvishzadeh 2007). Additionally, it was noted by Coad (2010) that the Lake Urmia was formed during the late Pliocene–Pleistocene and may have had a Pleistocene connection to the Caspian Sea. Saadati (1977) suggested two possible connections between the Caspian Sea and Urmia Lake basins, including a Pliocene–early Pleistocene connection, giving rise to endemic species of this basin and a late Pleistocene connection resulting in species that are similar or subspecifically distinct from their Caspian counterparts. Headwater stream capture might be another mechanism that could have allowed the introduction of some species from one basin to another. A number of Lake Namak River drainages in its northern part are located in proximity to the southern Caspian Sea basin. Moreover, some western rivers of the Namak Lake basin are very close to eastern headwaters of the Tigris River drainage too. These river captures and the close phylogenetic relationship of *B. miliaris* in the Namak Lake basin to *B. lacerta* in the upper reaches of Tigris River drainage, to *B. karunensis* (upper reaches of distinct Karun River drainage) and *B. cyri* from the Caspian Sea and Namak Lake basin may be of recent Pliocene origin of this endorheic basin which has already been proposed by Berg (1940). Defensible inferences might be derived by screening more samples and more molecular markers (D loop, microsatellite) from other rivers in these basins to provide new insights into historical relationships among *B. lacerta* cryptic species group in these parts of their range, which is subject of a new project.

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