



Natural Hybridization of *Luciobarbus barbulus* x *Luciobarbus kersin* and *Luciobarbus barbulus* x *Luciobarbus xanthopterus* in the Persian Gulf Basin

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Abstract

Among vertebrates, hybridization is common phenomena in cyprinid fishes particularly among closely related species/genera which share common habit and habitat (niche) requirements and desires. In this paper we report presence of two hybrids of *Luciobarbus barbulus* x *Luciobarbus kersin* and *Luciobarbus barbulus* x *Luciobarbus xanthopterus* from the Tigris River tributaries (the Persian Gulf basin), southwestern Iran. The hybrid specimens were identified on the basis of their intermediate morphological characteristics with the parent species and the position of them in the clade and close genetic distance with their maternal sequences. Our studies showed intermediate characteristics between putative hybrids and their parents. In the putative hybrid of *L. barbulus* and *L. kersin*, the general characteristics and morphometric measurements are similar to one parent especially paternal characteristics (*L. kersin*), although most of the meristic characteristics are similar to maternal characteristics (*L. barbulus*). The other putative hybrid between *L. barbulus* and *L. xanthopterus* show the general appearance for *L. barbulus* (the putative father) and some meristic characteristics were similar to *L. xanthopterus* (the putative mother). Low abundance of the hybrids shows that the hybridization might be a random event.

Keywords: Cyprinidae, hybrid, morphological characteristics, Persian Gulf.

Introduction

Natural hybridization is not almost a common event among most organisms, but it can happen when the spawning time/season and breeding ground, have been overlapping (Hubbs, 1955; Stoumboudi, Villwock, Selas, & Abraham, 1992). Among vertebrates, hybridization is common phenomena in cyprinid fishes particularly among closely related species/genera which share common habit and habitat (niche) requirements and desires (see Schwartz, 1972, 1981; Scribner, Page, & Bartron, 2001; Almodóvar, Nicola, & Elvira, 2008; Almodóvar, Nicola, Leal, Torralva, & Elvira, 2012; Gante, Doadrio, Alves, & Dowling, 2015; Witkowski et al., 2015), including members of the *Barbus* complex species (Berrebi, Cattaneo-Berrebi, & Le Brun, 1993; Almodóvar et al., 2008). Hybridizations can be increased due to human interference (anthropogenic disturbances) with aquatic environments e.g., introducing exotic fishes, changing river habitats and also aquaculture (Hubbs, 1955; Economidis & Sinis, 1988; Unver & Erkakan, 2005; Chunco, 2014; Wang & Bradburd, 2014; Young et al., 2016). To determine status of hybrids, both morphological and molecular approaches using

nuclear and mitochondrial markers have been implemented (see Liu, Gao, Yokogawa & Zhang, 2006; Ferrito et al., 2013; Masoudi et al., 2016). As in several cases morphological characters could not be used to identify the hybrids, hence using the mitochondrial and nuclear molecular markers (e.g., COI, *cytb*, Rag II markers and AFLP technology) have been suggested and established (Liang, Guo, Luo, Li, & Zou, 2016). These methods can be rapidly implemented and effectively determine the hybrid individuals (Liang et al., 2016).

In this study, we describe two hybrids from Tigris tributaries of the Persian Gulf basin that show intermediate characters of *Luciobarbus barbulus* X *L. kersin* and *L. barbulus* X *L. xanthopterus*. Also, molecular data of *Luciobarbus* specimens, including two hybrids are provided.

Materials and Methods

A total of 29 specimens of *Luciobarbus* were collected from two sites in the Khuzestan Province, Iran: Marun Dam (30°43'16.6"N 50°23'30.3"E) (12 individuals) and Hor al-Azim Wetland (31°19'44.9"N 47°43'30.9"E) (17 individuals), during January 2017

(Figure 1, Table 1). After anaesthesia, fishes were fixed in 5% formaldehyde and stored in 70% ethanol. In total, 27 morphometric and 12 meristic characteristics were measured and position of barbels was noticed. Measurements were made using a digital caliper and recorded to 0.1 mm. All measurements are 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 last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are noted as "1½". The morphometric characters were expressed as percentages of standard length and head length. For each metric and meristic feature, the mean and standard deviation (S.D.) were calculated.

A total of 33 sequences of cytochrome oxidase subunit I of two genera, *Luciobarbus* and *Barbus* including 20 from GeneBank and 11 samples (including hybrid morphotypes) used for this study were subjected to the molecular analysis. After

anaesthesia, the right pectoral fin from right side of fish was separated and fix in 96% ethanol. Total genomic DNA was then using phenol/chloroform procedures (Perdices, Carmona, Fernández-Delgado, & Doadrio, 2001). The standard vertebrate DNA barcode region of the COI was amplified using the primers FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3'), FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'), (Ivanova, Zemlak, Hanner, & Hebert, 2007). The PCR products were purified and sequenced in Microgen Company, South Korea. The new sequences were deposited in GenBank under accession numbers.

The sequences were edited and aligned by BioEdit version 7.0.0 software (Hall, 1999). Intergenetic distances were calculated by using Mega 6 software and Kimura 2-parameter distance method. After alignment, the new sequences with additional 19 species of *Luciobarbus* which obtained from GenBank were used to estimate phylogenetic relationships. Bayesian inference (BI) was conducted to estimate phylogenetic relationships using MrBayes 3.1.2 (Galtier, Gouy, & Gautier, 1996) and using



Figure 1. Sampling sites in the Tigris tributaries of the Persian Gulf basin.

Table 1. Number of collected specimens from two sites in the Tigris River drainages of Persian Gulf.

Species	Hor al-Azim Wetland	Marun Dam
<i>L. barbulus</i>	7	6
<i>L. xanthopterus</i>	5	6
<i>L. kersin</i>	3	-
<i>L. barbulus</i> * <i>L. kersin</i>	1	-
<i>L. barbulus</i> * <i>L. xanthopterus</i> .	1	-

Markov-chain Monte Carlo tree searches for 10 million generations with a sampling frequency of 1000. We generated maximum likelihood phylogenetic trees with 10000 bootstrap replicates in RaxML software 7.2.5 (Stamatakis, 2006) under the GTR+G+I model of nucleotide substitution, with CAT approximation of rate heterogeneity and fast bootstrap to explore species phylogenetic affinities. *Barbus lacerta* was used as appropriate outgroup to root the constructed phylogenetic tree.

Results

Description of Hybrid between *L. barbulus* and *L. kersin*

Morphometric and meristic characters of the putative hybrid are given in Table 2. The general morphology of this hybrid individual looks like *L. kersin* (Figure 2) including: deep body, maximum body depth in front of dorsal-fin origin and longer than head length (maximum body depth 130% of HL vs. 111-130% HL in *L. kersin* and 79-107% HL in *L. barbulus*); small head; last unbranched dorsal fin ray short but very strong (vs. strong but long in *L. barbulus*); outer margin of anal fin straight (vs. mostly round in *L. barbulus*).

Some of the hybrid body characteristics are similar to *L. barbulus* including: rostral barbels pass

nostril (vs. not passing nostril in *L. kersin*), maxillary barbels pass the end of the eye (vs. not reaching to middle of the eyes in *L. kersin*); median lobe on lower lip very prominent (vs. not in *L. kersin*); lateral line scales 60 (47-60 in *L. barbulus* (Coad, 2017) vs. 49-58 in *L. kersin*).

General Description

Large body, 328 mm SL. Body deep and compressed laterally, green olive at dorsal part to silver at ventral area. Head small (smaller than maximum body depth). Mouth inferior with median lobe on the lower lip. Two pairs of barbels, rostral barbels pass nostril, maxillary barbels pass posterior part of eye. Dorsal-fin origin at same level of pelvic-fin origin vertically, last unbranched dorsal-fin ray very thick, serration almost to its distal part, dorsal fin orange. Anal-fin origin at vertical of anterior of middle between dorsal- and caudal-fin origins, outer margin of anal fin straight, anal-fin origin shortly behind anus, anal fin orange. Pectoral fin not reaching to pelvic fin. Pelvic fin short and not reaching to anus. Pelvic axillary lobe present. Caudal fin is forked.

Dorsal fin with 4 unbranched and 8½ branched rays. Anal fin with 4 unbranched and 5½ branched rays. Pectoral-fin rays 16. Pelvic-fin rays 10. Lateral line scales 60, scales around caudal peduncle 24, 12 scales above (between dorsal-fin origin and lateral

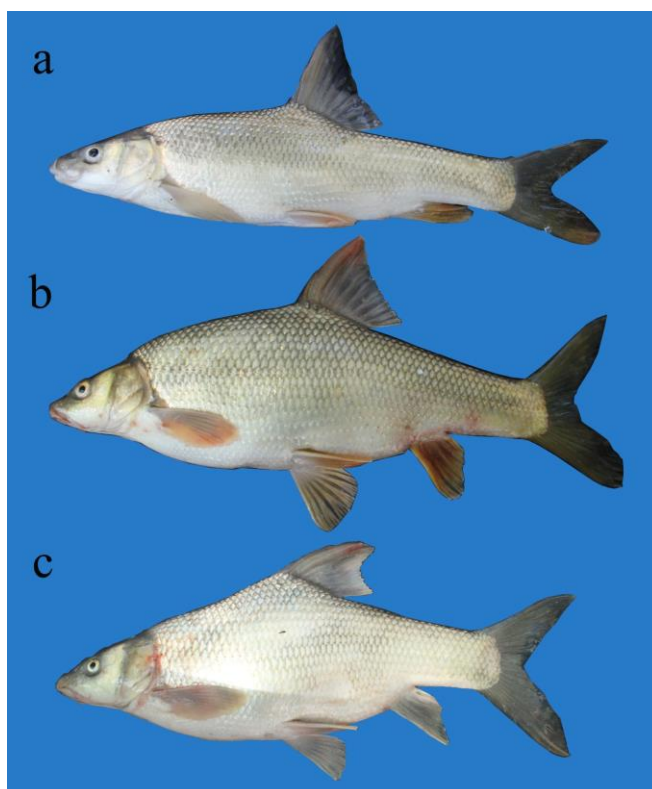


Figure 2. Putative hybrid and parents. a, *Luciobarbus barbulus* ZM-CBSU G1158, 214 mm SL; b, putative hybrid between *L. barbulus* and *L. kersin*, ZM-CBSU G1151, 328 mm SL; c, *L. kersin* ZM-CBSU G1150, 306 mm SL; all from Iran: Khuzestan prov.: Hor al-Azim wetland, Persian Gulf basin.

Table 2. Morphometric and meristic characteristics of putative hybrid between *L. barbulus* and *L. kersin*.

	<i>L. barbulus</i>				<i>L. kersin</i> hybrid			<i>L. kersin</i>		
	Min	Max	Mean	Std. Deviation		Min	Max	Mean	Std. Deviation	
Unbranched dorsal-fin ray	3	4	3.9	0.28	4	4	4	4.0	0.00	
Branched dorsal-fin ray	7	8	7.7	0.48	8	8	8	8.0	0.00	
Pectoral fin-ray	16	18	16.6	0.67	16	16	17	16.3	0.58	
Pelvic fin-ray	9	10	9.4	0.51	10	9	10	9.3	0.58	
Unbranched anal-fin ray	3	4	3.9	0.28	4	4	4	4.0	0.00	
Branched anal fin-ray	5	5	5.0	0.00	5	5	5	5.0	0.00	
Lateral line scales	50	59	55.2	3.00	60	54	57	55.0	1.73	
Lateral line scale without pore	0	2	1.0	0.58	0	1	1	1.0	0.00	
Caudal peduncle scale	22	26	23.8	1.24	24	22	25	23.7	1.53	
Scale above lateral line	10	12	10.8	0.60	12	11	11	11.0	0.00	
Scale below lateral line	6	8	7.1	0.49	9	7	8	7.7	0.58	
Pre-dorsal scale	22	29	25.9	2.06	28	23	27	25.0	2.00	
Gill rakers	15	21	18.6	2.14	18	16	18	17.3	1.15	
In percent of Standard length										
Total length	117.1	127.6	122.7	3.49	124.4	118.7	126.8	121.4	4.69	
Head length	26.2	29.7	27.3	1.03	25.8	22.8	29.3	25.3	3.49	
Snout length	9.7	13.1	10.9	1.01	9.3	11.8	11.8	11.8	1.00	
Post orbital distance	11.8	14.8	13.4	0.83	12.8	15.3	15.3	15.3	0.80	
Inter orbital distance	9.7	11.4	10.3	0.46	10.8	12.0	12.0	12.0	0.70	
Pre-dorsal fin distance	51.0	57.5	54.3	2.06	56.2	59.1	59.1	59.1	1.90	
Post-dorsal fin distance	50.2	63.7	56.9	4.35	55.4	64.6	64.6	64.6	3.35	
Dorsal-fin base length	22.2	29.7	25.4	2.12	26.3	25.0	27.1	26.3	1.11	
Dorsal-fin depth	12.5	14.2	13.3	0.59	14.7	13.0	15.7	14.3	1.91	
Anal-fin base length	16.4	19.8	18.0	1.27	16.9	18.0	18.0	18.0	1.00	
Anal-fin depth	6.3	8.3	7.3	0.65	8.6	8.4	8.4	8.4	0.55	
Pre-anal length	74.0	79.3	76.4	1.72	76.2	73.5	75.5	74.5	0.99	
Pectoral-fin length	16.7	21.4	19.2	1.19	18.5	21.7	21.7	21.7	1.11	
Pelvic-fin length	15.9	18.5	17.1	0.77	16.8	18.8	18.8	18.8	0.78	
Depth of caudal peduncle	10.3	12.2	11.0	0.58	12.1	10.6	14.1	11.8	1.94	
Body depth at dorsal-fin origin	21.7	30.6	25.6	2.66	33.7	25.5	38.0	30.1	6.88	
Distance between pectoral and anal-fin origins	47.5	58.5	52.1	2.71	53.7	53.7	53.7	53.7	2.05	
Distance between pectoral and pelvic-fin origins	24.4	31.5	26.9	1.87	30.0	29.1	29.1	29.1	1.80	
Distance between pelvic and anal-fin origins	22.2	29.3	25.7	1.81	26.9	26.7	26.7	26.7	1.67	
In percent of head length										
Head depth	49.9	55.6	52.6	1.93	49.2	47.2	47.2	47.2	1.85	
Snout length	36.2	49.3	40.0	3.61	36.2	40.3	40.3	40.3	3.00	
Post-orbital distance	44.3	53.8	49.2	2.78	49.6	52.2	52.2	52.2	2.04	
Inter-orbital distance	34.9	39.9	37.9	1.53	41.9	40.9	40.9	40.9	1.03	
Eye diameter	13.3	21.6	15.6	2.53	15.1	12.3	14.6	13.3	1.19	
Body depth at dorsal-fin origin	79.0	107.3	93.9	10.23	130.6	111.5	129.5	117.8	10.15	
Length of caudal fin	76.7	99.4	90.6	6.18	96.3	98.2	98.2	98.2	5.50	
Mouth width	20.0	32.9	24.6	3.68	21.8	23.0	23.0	23.0	3.33	

line) and 9 scales below (between pelvic-fin origin and lateral line), 28 scales in front of dorsal-fin origin. Number of total gill rakers on first gill arch 18.

Description of Hybrid between *L. barbulus* and *L. xanthopterus*

The general appearance is given in Figure 3. Morphometric and meristic characters of the putative hybrid are given in Table 3. The general appearance is somewhat between *L. barbulus* and *L. kersin*. The characters that are similar to *L. barbulus* are: prominent middle lobe on the lower lip (vs. not prominent in *L. xanthopterus*). Rostral barbel reaches to the beginning of eye (vs. not passing nostril (Coad, 2017) in *L. xanthopterus*), maxillary barbel passes eye

[(vs. not passing middle of eye in *L. xanthopterus* (Coad, 2017))]. Gill rakers on first arch 20 [(vs. 7-13 in *L. xanthopterus* (Coad, 2017))].

The characteristics that are similar to *L. xanthopterus*: very long but not strong last unbranched dorsal-fin ray (vs. moderate size and strong last unbranched dorsal-fin ray in *L. barbulus*). Outer margin of anal fin convex (vs. straight in *L. barbulus*).

General Description

Body large; 294 mm SL. Body compressed laterally, green olive at dorsal part to silver at ventral area. Head large, bigger than maximum body depth (HL 80% of maximum body depth), inferior mouth

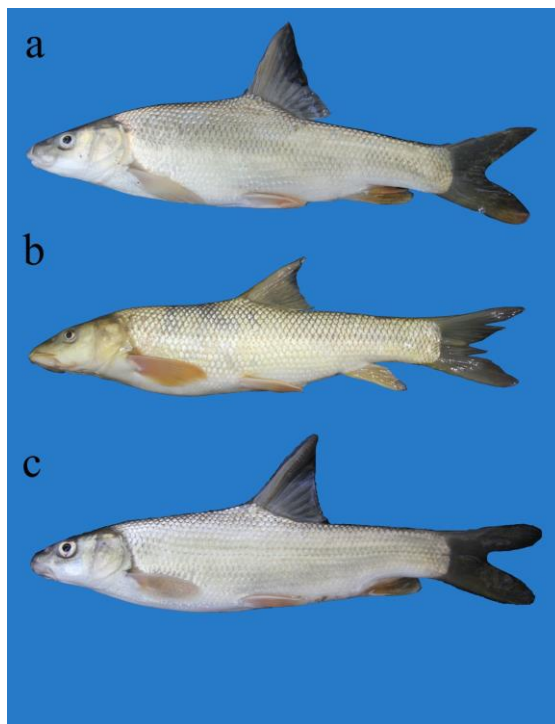


Figure 3. Putative hybrid and parents. a. *L. barbulus* ZM-CBSU G1158, 214 mm SL; b, putative hybrid between *L. barbulus* and *L. xanthopterus* ZM-CBSU G1169, 294 mm SL; c. *L. xanthopterus* ZM-CBSU G1160, 191 mm SL. All from Iran: Khuzestan prov.: Hor al-Azim wetland.

with a median lobe on lower lip. Two pairs of barbels, rostral barbels reach the eyes, maxillary barbel passing posterior margin of eye. Dorsal-fin origin almost at vertical of pelvic fin origin, last unbranched dorsal-fin ray long but not strong, serration almost to its distal part, dorsal fin orange. Anal-fin origin almost at vertical of middle between dorsal- and caudal-fin origins, outer margin of anal fin convex, anal-fin origin shortly behind anus, anal fin orange. Pectoral fin not reaching to pelvic fin. Pelvic fin short and not reaching to anus. Pelvic axillary lobe present. Caudal fin fork.

Dorsal fin with 4 unbranched and $8\frac{1}{2}$ branched rays. Anal fin with 4 unbranched and $5\frac{1}{2}$ branched rays. Pectoral-fin rays 17. Pelvic-fin rays 9. Lateral line scales 54, scales around caudal peduncle 23, 10 scales above lateral line (between dorsal-fin origin and lateral line), 7 scales below lateral line (between pelvic-fin origin and lateral line), and 26 scales in front of dorsal-fin origin. 20 gill rakers on first gill arch.

The canonical discriminant analyses for morphometric and meristic characters were separately performed (Figures 4 and 5). The obtained results revealed distinctiveness of putative hybrid and parents.

Molecular Results

We generated Bayesian and Maximum likelihood for COI barcode region of *Luciobarbus*

species (Figure 6). *Barbus lacerta* was considered as sister group. Based on the obtained tree topology, the studied *Luciobarbus* specimens were clustered in two main clades: I) include *Luciobarbus mursa* from the Caspian Sea basin; and clade II) all other *Luciobarbus* species. In clade II, *L. capito* is sister to *L. esocinus* group (*L. pectoralis*, *L. barbulus*, *L. esocinus*, *L. xanthopterus* and *L. kersin*). In the same group, *L. pectoralis* (Heckel's Orontes barbel/ Levantine barbel) from the Orontes River basin is sister to all the *Luciobarbus* species from the Tigris River drainage of the Persian Gulf basin (the Tigris *Luciobarbus* group). Within the Tigris *Luciobarbus* group all individuals of *L. barbulus* + putative hybrid) made a monophyletic group. This putative hybrid (MC1814) was considered as *L. kersin* in the morphological appearance. Within the Tigris *Luciobarbus* group, *L. kersin* which is morphologically quite different from the other species, is sister to a morphologically closely related group including *L. esocinus*, *L. xanthopterus* and putative hybrid between *L. barbulus* and *L. xanthopterus* (MC1831).

The genetic distances are given in Table 4. The closeness of *Luciobarbus barbulus*, *L. esocinus*, *L. kersin* and *L. xanthopterus* is demonstrated in Figure 6.

Discussions

Till date, five species of *Luciobarbus* have been reported from the Tigris River tributaries of the

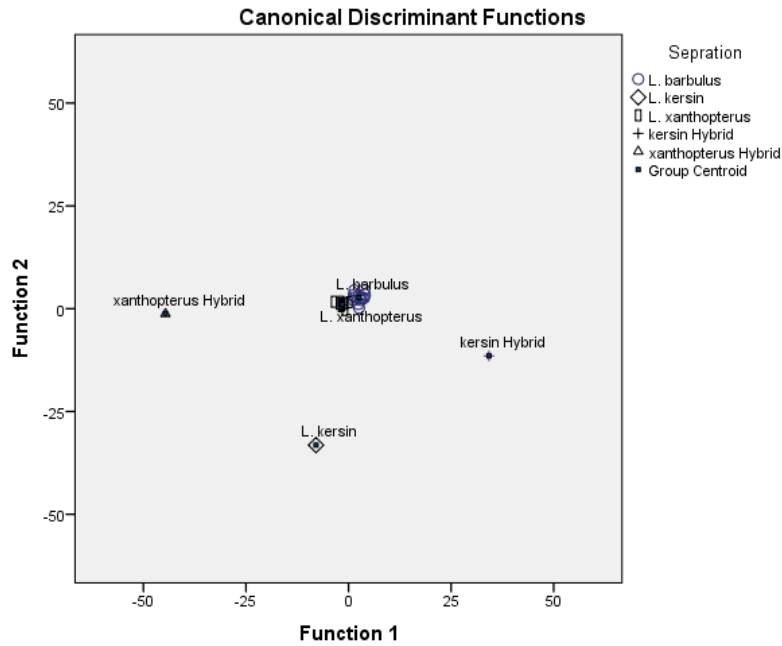


Figure 4. Canonical discriminate function scores of the morphometric characteristics the studied *Luciobarbus* species and hybrids.

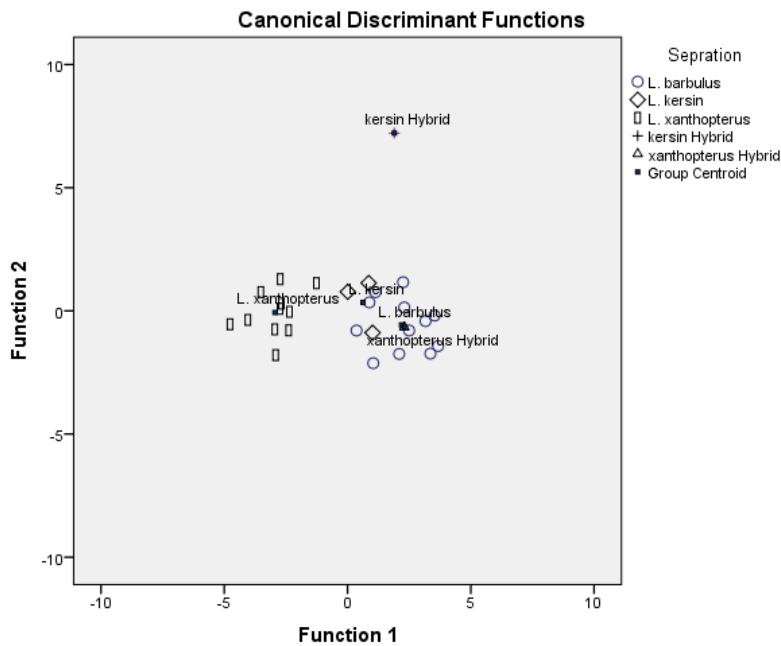


Figure 5. Canonical Discriminate Function scores of the meristic characteristics in the studied *Luciobarbus* species and hybrids.

Persian Gulf basin (Esmaeili, Coad, Gholamifard, Nazari, & Teimory, 2010; Esmaeili, Mehraban, Abbasi, Keivany & Coad, 2017). Three of them (*L. barbulus*, *L. kersin* and *L. xanthopterus*) are sympatric species and are found in the Alvand, Karun and Marun Rivers and Hor al-Azim and Shadegan wetlands. Hybridization among members of this genus has already been reported (see Almodóvar et al., 2008; Gante et al., 2015). Their spawning season in the Tigris River tributaries is around April and May (Al-Hassan, Al-Saboonchi, & Binayan, 1986; Al-

Rudainy, 2008). The overlapping in spawning season, external fertilization and also sympatricity (Esmaeili, Coad, Gholamifard, Nazari, & Teimory, 2010) are factors that can increase the hybridization chance (see also Economidis & Sinis, 1988).

Our studies showed intermediate characteristics between putative hybrids and their parents. In the putative hybrid of *L. barbulus* and *L. kersin*, the general characteristics and morphometric measurements are similar to one parent especially paternal characteristics (*L. kersin*), although most of

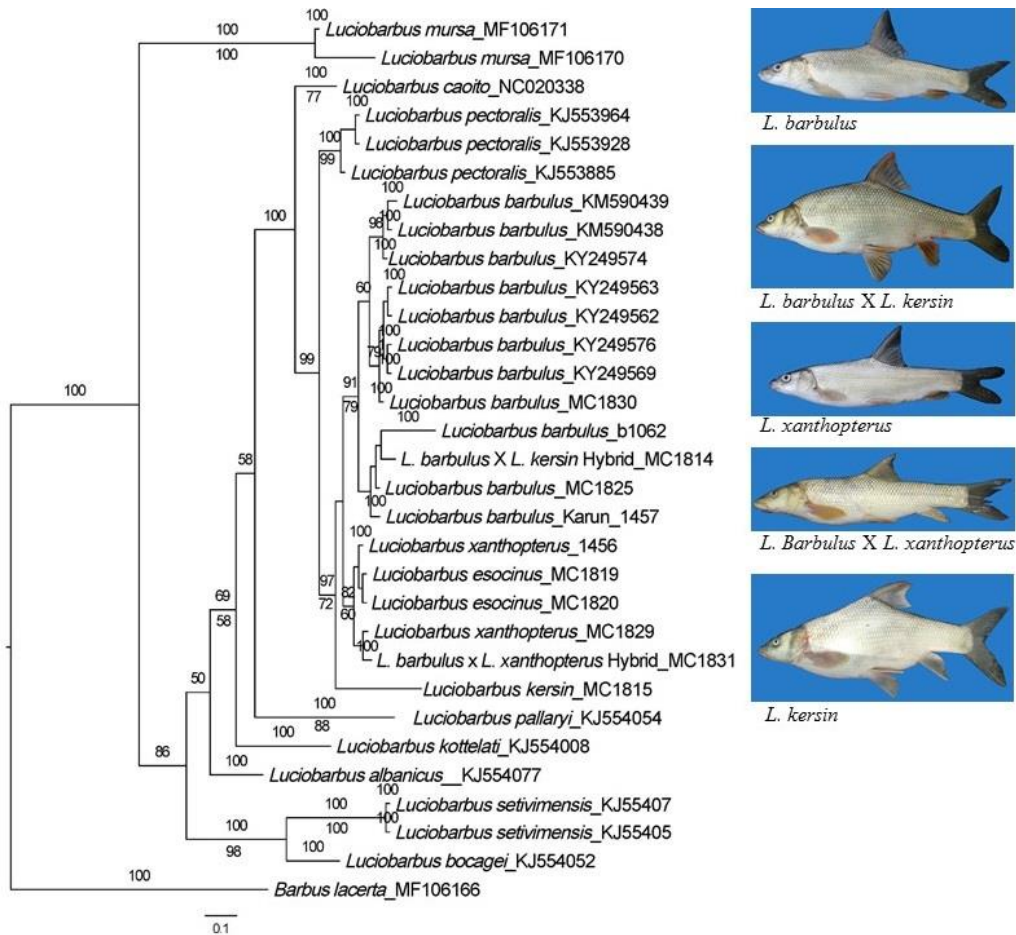


Figure 6. Phylogenetic tree of COI barcodes data from genus *Luciobarbus* based on Bayesian analysis. Values on branches indicate the indexes of support >50% for the Bayesian and ML bootstrap values. *Barbus lacerta* was used as an out-group in the analysis.

Table 4. Estimates of the evolutionary divergence over sequence pairs between species found in the COI barcode region of the *Barbus* genus complex group.

	<i>B. lacerta</i>	<i>L. mursa</i>	<i>L. capito</i>	<i>L. pectoralis</i>	<i>L. barbulus</i>	<i>L. xanthopterus</i>	<i>L. kersin</i> * <i>L. barbulus</i>	<i>L. kersin</i>	<i>L. esocinus</i>	<i>L. barbulus</i> * <i>L. xanthopterus</i>
<i>B. lacerta</i>										
<i>L. mursa</i>	11.03									
<i>L. capito</i>	9.72	9.75								
<i>L. pectoralis</i>	8.49	9.59	2.48							
<i>L. barbulus</i>	9.44	9.97	3.07	1.79						
<i>L. xanthopterus</i>	8.85	9.62	2.51	1.44	1.01					
<i>L. kersin</i>	9.28	9.73	2.60	1.72	0.71	1.01				
* <i>L. barbulus</i>	10.37	10.15	4.35	3.63	3.28	3.08	3.37			
<i>L. kersin</i>	8.85	9.51	2.60	1.35	0.91	0.09	1.10	2.98		
<i>L. esocinus</i>	8.85	9.51	2.60	1.35	0.91	0.09	1.10	2.98	0.36	
<i>L. barbulus</i>	8.85	9.74	2.22	1.35	1.83	0.27	0.73	2.98	0.36	
* <i>L. xanthopterus</i>										

the meristic characteristics are similar to maternal characteristics (*L. barbulus*). The other putative hybrid between *L. barbulus* and *L. xanthopterus* show the general appearance for *L. barbulus* (the putative father) and some meristic characteristics were similar to *L. xanthopterus* (the putative mother). Other authors reported similar results (Poly, 1997; Gante et al., 2015). It is important to point out here that the differences in lateral line scales as these meristic

characters are independent from the different trophic adaptations, and that this consilient but independent evidence strongly favors hybridization rather than intraspecific variation as an explanation for morphological intermediacy (see Vreven et al., 2016).

The low number of the captured hybrid specimens will suggest that hybridization among these species might be an accidental and natural event because there are several prezygotic and postzygotic

isolating mechanisms that prevent natural hybridization (Masoudi et al., 2016). In contrast, artificial hybridization by human, breaks these barriers and increases the chance of hybridization (Bartley, Rana, & Immink, 2000; Adah, Onyia, & Obande, 2014).

Molecular studies provided here showed that phylogenetic tree topology is similar to the other previous studies (Levin et al., 2012; Young et al., 2016). The place of putative hybrid *L. kersin* and *L. barbulus* in the tree shows that the maternal ancestor is *L. barbulus*, although the morphological characteristics and general appearance are more similar to paternal ancestor, *L. kersin*. This fact is true about the putative hybrid between *L. barbulus* and *L. xanthopterus*. Genetic distance between these species is very low and this shows the closeness of them and gene flow between them recently (Young et al., 2016).

It is important to point out here that the differences in the meristic characters are independent of the different trophic adaptations and favors hybridization rather than intraspecific variations as seen in the genus *Labeobarbus* (see Vreven, Musschoot, Snoeks, & Schliewen, 2016).

Hybridization in these species might have two reverse impacts. In one hand, hybridization may result in the extinction of unique *Luciobarbus* populations or species through unsuccessful reproductive effort or via introgression with a more common species. In the other hand, hybridization has been considered the major source of evolutionary novelty and offers the opportunity for phenotypic and genetic innovation and variation which might promote speciation as seen in the genus *Labeobarbus* (see Vreven et al., 2016).

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