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Research Article

Review and re-description of *Sabanejewia* species in Iran (Teleostei: Cobitidae)

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Abstract: The Iranian species of the genus *Sabanejewia* are reviewed based on morphological, osteological and molecular characters of the mtDNA COI barcode region. Two species, *Sabanejewia aurata* and *Sabanejewia caspia* are recognized and diagnoses are presented for both species. They are distinguishable through body pattern, length of caudal peduncle, snout and post orbital distance, shape of scale and suborbital spine, caudal complex skeleton and also the number of fixed, diagnostic nucleotide substitutions. Here we re-describe both Iranian spined loaches.

Keywords: Cobitid loach, COI, Morphological characters, Taxonomic status.

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Introduction

Spined/sting loaches of the family Cobitidae are a group of about 20 genera with about 212 species of primary freshwater fishes inhabiting Eurasia and Morocco, including Iran (Nalbant 1963; Sawada 1982; Nelson et al. 2016; Coad 2017; Eschmeyer et al. 2018). The Iranian spined loaches, have been classified into two genera i.e. *Cobitis* and *Sabanejewia*, on the basis of pigmentation pattern, number of caudal fin-branched rays and secondary sexual characters (Coad 2017; Esmaeili et al. 2010, 2017).

The genus *Sabanejewia* Vladykov, 1929 is characterized by having body pigmentation of not disposed in four Gambetta's zones (vs. presence of four Gambetta's zones in *Cobitis*); caudal fin with 12 branched rays (vs. 14 in *Cobitis*); adult males without lamina circularis at base of first pectoral rays (vs. present in *Cobitis*) but with a conspicuous swelling on each side of body before dorsal fin. The members of this genus ranges from the Aral Sea to Italy and from Asia Minor to the Baltic (Bănărescu 1992), and contains about 10 valid species (Eschmeyer et al. 2018). However, the high morphological variability and overlapping characters between taxa have resulted in a lack of consensus regarding the taxonomy of the genus (Kottelat 1997; Ludwig et al. 2000). Krupp (1985) also does not consider *Sabanejewia* to be a distinct genus.

The validity of *Sabanejewia* as a separate genus was later confirmed especially based on molecular analysis by Grossu et al. (1971), Perdices et al. (2003, 2016), Bartoňová et al. (2008) and Buj et al. (2008). Esmaeili et al. (2010, 2014, 2017, 2018), Jouladeh-Roudbar et al. (2015) and Coad (2017) recognized three species of *Sabanejewia* from Iran: *Sabanejewia aurata* (De Filippi, 1863), *S. caspia* (Eichwald, 1838) and *S. caucasica* (Berg, 1906) but presence of *S. caucasica* was under question. Here, we review the taxonomic status of the Iranian *Sabanejewia* species based on morphology, osteology and molecular characters of the mtDNA COI barcode region.

Materials and Methods

After anesthesia by 1% clove solution, fishes were fixed in 5% formaldehyde and were stored in 70% or directly fixed in 96% ethanol and deposited in the Zoological Museum, Collection of Biology Department, Shiraz University (ZM-CBSU). Measurements were made with dial caliper and recorded to 0.1mm. All measurements were made point to point, never by projections. Methods for counts and measurements follow Kottelat & Freyhof (2007). Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins were counted as "1½". Digital X-ray images were taken of the left side of all fish using a Faxitron Bioptics (LLC-Vision NDT version 2.2.5, 45k.v. and 30 sec) housed in the Zoological State Collection, Munich. The terminology of skeletal elements follows Sawada (1982).

Abbreviations used: SL, standard length; HL, head length; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz.

DNA extraction and PCR. Genomic DNA was extracted using the Salt method (Bruford et al. 1992). The standard vertebrate DNA barcode region of the COI (cytochrome c oxidase subunit 1) were amplified using primer pairs named FishF1 (5'TCAACCAACCACAAAGACATTGGCAC3') and FishR1 (5'TAGACTTCTGGGTGGCCAAAGA ATCA3') (Ward et al. 2005). Purification and sequencing of the PCR products were conducted at Macrogen (South Korea) or Daneshbod (Shiraz, Iran) Laboratories with the aforementioned primer pairs.

Molecular data analyses. We used 35 sequences from our prevoius studies and the NCBI GenBank and an additional 5 sequences in this study. Data processing and sequence assembly was done in BioEdit 7.2.5 (Hall 1999) and MEGA6 (Tamura et al. 2013) was used to create a DNA sequence alignment using ClustalW algorithm. 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 given with their respective accession numbers. The most appropriate sequence evolution model for the given data was determined with Modeltest (Posada & Crandall 1998) as implemented in the MEGA6 software, treating gaps and missing data with the partial deletion option under 95% site coverage cutoff. The model with the lowest AIC (Akaike Information Criterion) scores is considered to best describe the substitution pattern. To explore species phylogenetic affinities, according to Modeltest, we generated maximum likelihood phylogenetic trees with 10,000 bootstrap replicates in RaxML software 7.2.5 (Stamatakis 2006) under the GTR+G+I model of nucleotide substitution, with fast bootstrap and also Bayesian phylogenetic analysis (BI), using the Markov Chain Monte Carlo method (MCMC), with 6,000,000 generations under the most generalising model (GTR+G+I) using MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001). Screening for diagnostic nucleotide substitutions was performed manually from the sequence alignment. Sequence divergence values between species were calculated using Kimura two Parameter (K2P) distance model implemented in in MEGA6 (Tamura et al. 2013). As an appropriate outgroup to root the constructed phylogenetic hypothesis, we included the distantly related *Oxynoemacheilus parvinae* KX980092.

Results

We compared 5 COI barcode sequences of *Sabanejewia* species from Iran with 35 sequences from our previous studies (see Mousavi-Sabet et al. 2011; Joulade-Roudbar et al. 2017; Eagderi et al. 2017) and the NCBI GenBank (*Sabanejewia baltica*: KM287070, KR477108, KR477107; *S. larvata*: KJ554832, KJ554664, KJ554774; *S. balcanica*: KJ554702, KJ554693, KR477262, KR477260,

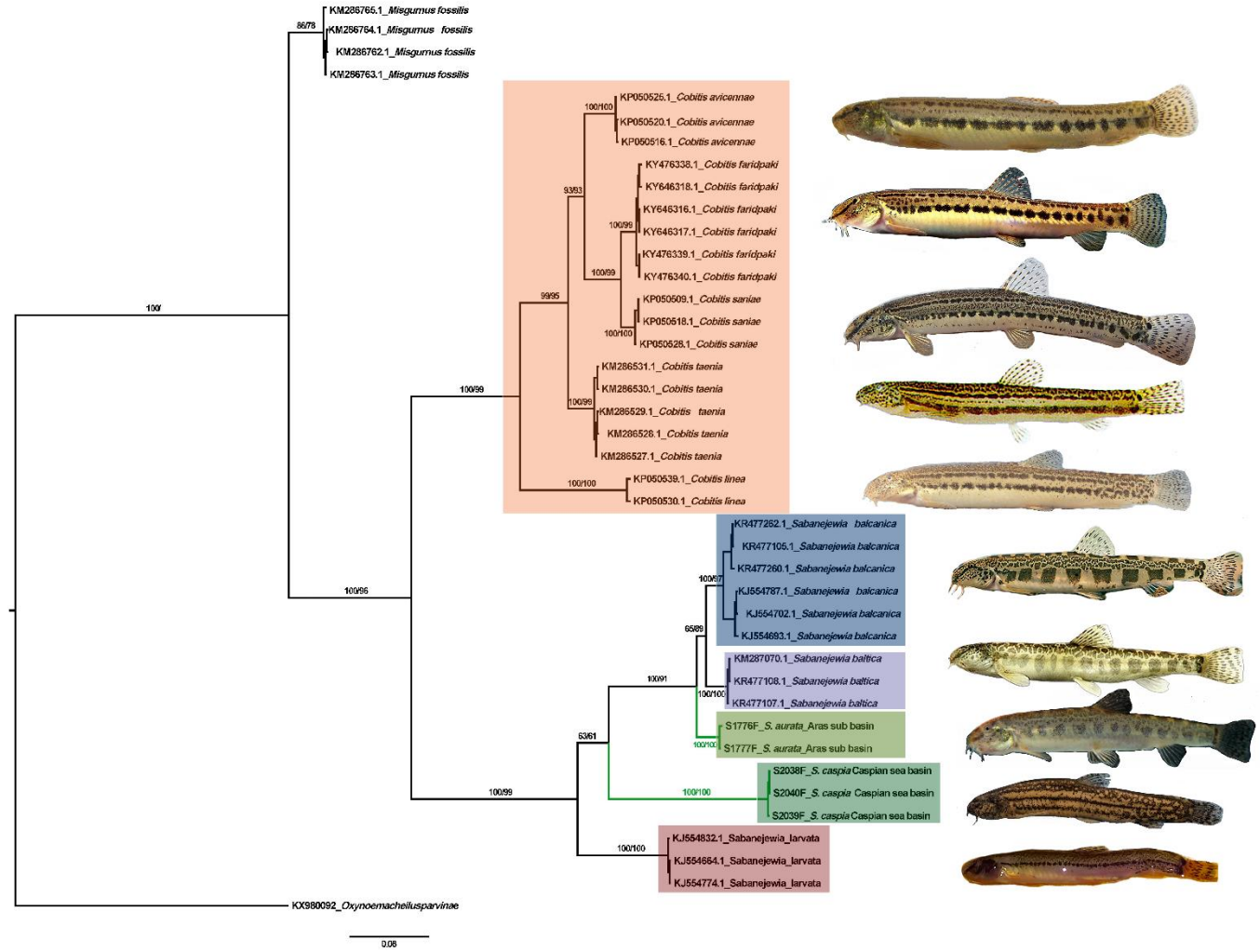

Fig.1. Maximum Likelihood and Bayesian phylogeny reconstructed based on 639 bp of COI 5' end. The values beside the branches before and after a slash are BI posterior and ML bootstrap probability values, respectively.

KR477105, KJ554787 and *Misgurnus fossilis*: KM286765, KM286764, KM286763, KM286762). Both the ML and BI phylogenetic trees were mostly similar in their topology, hence here only the BI tree including the posterior probability values from the Maximum Likelihood phylogram is presented. Based on the tree topology, the sequenced Iranian *Sabanejewia* species are split in two groups, *S. aurata* and *S. caspia* and they also form a monophyletic group beside the other species of *Sabanejewia* (Fig. 1). *Sabanejewia caspia* is sister to a lineage comprising *S. aurata* + *S. baltica* and *S. balcanica*. *Sabanejewia larvata* is sister to all the other studied *Sabanejewia* species. Table 1 lists the diagnostic nucleotide substitutions and Table 2 lists the average estimates of the evolutionary divergence in the 639 base pairs long mtDNA COI barcode region between the *Sabanejewia* species studied.

Sabanejewia aurata (De Filippi, 1863)

(Figs. 2-7)

Cobitis aurata De Filippi [F.] 1863:391 (type locality is possibly Sarcham-e Sofla (37°07'N, 47°54'E) in the Qezel Owzan River (a tributary of Sefid River drainage of the Caspian Sea basin in Iran). Lectotype: MZUT674, designated by

Table 1. Diagnostic nucleotide substitutions found in mtDNA COI of *Sabanejewia* studied species (*S. aurata*, n=2; *S. balcanica*, n=6; *S. baltica*, n=3; *S. caspia*, n=3; *S. larvata*, n=3).

Nucleotide position relative to *Oryzias latipes* complete mitochondrial genome (AP004421)																				
Nucleotide position	5550	5559	5565	5577	5592	5595	5604	5607	5673	5679	5700	5709	5712	5718	5739	5742	5745	5760	5763	5766
S. aurata	**T**	C	**C**	C	C	C	**C**	C	T	C	T	A	G	T	T	A	A	T	A	A
S. balcanica	C	C	T	C	C	C	T	C	T	C	T	A	G	T	T	A	A	T	**G**	**G**
S. baltica	C	C	T	C	C	C	T	C	T	**T**	T	A	G	T	T	A	A	T	A	A
S. caspia	C	C	T	**T**	**T**	C	T	C	T	C	T	**G**	G	T	**C**	**G**	**G**	**C**	A	A
S. larvata	C	**T**	T	C	C	**T**	T	**T**	**C**	C	**A**	A	**A**	**A**	T	A	A	T	A	**C**

Nucleotide position	5767	5772	5775	5782	5784	5790	5808	5820	5838	5844	5847	5851	5856	5862	5871	5874	5880	5883	5886	5898
S. aurata	C	C	C	C	G	A	T	C	T	C	A	G	C	C	A	C	C	A	C	C
S. balcanica	C	C	C	C	G	A	T	C	T	C	A	G	C	C	T/A	C	C	**G**	C	C
S. baltica	C	C	C	C	G	A	T	C	T	C	A	**T**	C	C	A	C	C	A	C	C
S. caspia	**T**	**A**	C	**T**	**A**	A	T	C	**C**	C	A	G	C	C	A	**G**	C	A	C	**T**
S. larvata	C	C	**A**	C	**T**	**G**	**C**	**A**	T	**T**	**C**	G	**T**	**A**	**G**	C	**T**	A	**T**	C

Nucleotide position	5902	5904	5910	5913	5919	5937	5940	5949	5952	5955	5967	5973	5979	5982	5988	5994	6003	6012	6021	6030
S. aurata	C	A	**G**	C	C	A	C	T	C	A	A	T	C	C	A	A	G	A	T	T
S. balcanica	C	A	A	C	C	A	C	T	C	A	A	T	C	C	A	A	G	A	T	T
S. baltica	C	**G**	A	C	C	C	C	T	C	**G**	A	T	C	C	A	A	G	A	T	T
S. caspia	C	A	A	C	**T**	C	C	**C**	C	A	**G**	**C**	**T**	**T**	A	A	G	A	T	**G**
S. larvata	**T**	A	A	**T**	C	**T**	**T**	T	**T**	A	A	T	C	C	**G**	**G**	**A**	**G**	**A**	**C**

Nucleotide position	6033	6039	6043	6054	6057	6058	6075	6082	6090	6096	6099	6102	6105	6108	6120	6123	6126	6132
S. aurata	A	T	C	A	C	C	T	C	C	T	A	T	A	C	A	G	A	A
S. balcanica	A	T	C	A	C	C	T	C	C	T	A	T	A	C	A	G	A	A
S. baltica	A	T	C	A	C	**T**	T	C	C	T	**G**	T	A	C	A	G	A	A
S. caspia	A	**C**	C	A	**T**	C	**A**	**T**	C	T	A	T	**T**	**A**	**G**	G	**G**	A
S. larvata	**T**	T	**T**	**T**	C	C	T	C	**T**	**C**	A	**C**	A	C	A	**A**	A	**G**

Tortonese, 1961: 188.

Cobitis hohenackeri Kessler, 1877: 177 (type locality: Azerbaidjan: Kura River, Caspian Sea basin [Berg, 1949: 894]; syntypes: ZISP 2854 [5].

Material examined: ZM-CBSU H2410, 30, 33-72mm SL; Iran: East Azarbayjan prov.: on the way from Miyaneh to Zanjan, Aydaghmoush River, a tributary of Qezel Owzan River, Sefid River drainage, Caspian Sea basin, 37°25'05"N 47°47'12"E; M. Ebrahimi, A. Teimori, A. Gholamhosseini and A. Gholamifard, 04 October 2007.

Material used in the molecular genetic analysis: ZM-CBSU M1776, M1777; Iran: Ardabil prov.: Nir, Balikhlochay River, at Yamchi Dam, entering to Gharasu River, a tributary of Aras River drainage, Caspian Sea basin, 38°04'19"N 48°09'31"E (GenBank accession number: MK370723, MK370724).

Diagnosis: *Sabanejewia aurata* is distinguished from its congener species in Iran, *S. caspia* by having the large dark spots along the flank, long caudal peduncle (18–21 vs. 13-16 %SL) and long snout (40-46 vs. 33-39 %HL), short post orbital distance (46-52 vs. 53-60 %HL), rounded scales, with large centric focal zone, and straight or very slightly curved main branch of the suborbital spine. Among *Sabanejewia* species studied for molecular characters, it is characterized by four fixed diagnostic nucleotide

Table 2. Estimates of evolutionary divergence (%) over sequence pairs between species found in the COI barcode region of cobitid studied species.

	S. aurata	*S. balcanica*	*S. baltica*	*S. caspia*	*S. larvata*	*C. avicinae*	*C. faridpaki*	*C. linea*	*C. saniae*	*C. taenia*	*M. fossilis*
S. aurata											
S. balcanica	4										
S. baltica	3	4									
S. caspia	15	16	16								
S. larvata	13	14	13	13							
C. avicinae	19	19	19	20	17						
C. faridpaki	20	20	21	22	18	6					
C. linea	20	21	20	20	20	13	12				
C. saniae	21	20	21	23	19	6	2	12			
C. taenia	18	19	20	20	18	5	6	11	6		
M. fossilis	18	18	19	18	19	18	17	17	19	17	

Fig.2. *Sabanejewia aurata*, a, ZM-CBSU H2412 (Female), 66mm SL; b, ZM-CBSU H2415 (Male), 62mm SL; c, ZM-CBSU 2423 (Female), 56mm SL; Iran: Aydaghmoush River, Caspian Sea basin.

substitutions in the mtDNA COI barcode region studied (Table 1) and a K2P nearest-neighbour distance of 3% to *S. baltica* (Table 2).

Description: For general appearance see Figures 2-5; morphometric data are provided in Table 3. Body elongate and laterally compressed. Greatest body depth at or slightly in front of dorsal-fin origin, decreasing towards caudal-fin base. Head profile slightly convex. Snout slightly blunt. Eyes situated in the middle or slightly closer to the tip of snout than to the posterior margin of the opercle. Caudal peduncle slender, compressed laterally, 2.0-2.5 (mean 2.2) times longer than deep. A small, usually triangular axillary lobe at base of pelvic fin, fully attached to body or absent. Pectoral fin shorter than head length and reaching approximately 54-68% of

Fig.3. *Sabanejewia aurata*, a, ZM-CBSU H2412 (Female), 66mm SL; b, ZM-CBSU H2415 (Male), 62mm SL; c, ZM-CBSU 2423 (Female), 56mm SL; Iran: Aydaghmoush River, Caspian Sea basin.

Fig.4. *Sabanejewia aurata*, a, ZM-CBSU H2412 (Female), 66mm SL; b, ZM-CBSU H2415 (Male), 62mm SL; c, ZM-CBSU 2423 (Female), 56mm SL; Iran: Aydaghmoush River, Caspian Sea basin.

distance from pectoral-fin origin to pelvic-fin origin. Dorsal-fin origin almost equidistant from base of caudal fin and tip of snout. Pelvic fin short, not reaching anus. Pelvic-fin origin below first or second branched dorsal-fin ray. Anal-fin origin not reaching vertical of tip of last dorsal-fin ray. Anus about one eye diameter in front of anal-fin origin. Anal fin not reaching caudal-fin base. Margin of dorsal fin straight or slightly concave. Caudal fin truncate or slightly emarginated. Largest known specimen 72.2mm SL.

Dorsal fin with 6½ branched rays. Anal fin with 5½ branched rays. Caudal fin with 6+6 branched rays. Pectoral fin with 8 (11) or 9 (9) branched rays and pelvic fin with 6 (11) or 7 (9) branched rays. Body completely covered by embedded scales. Scales rounded, with large centric focal zone (Fig. 6a). The lateral line very short, not exceeding the length of pectorals. Anterior nostril opening at end of a low, pointed and flap-like tube. Tube of anterior nostril slightly overlapping posterior nostril when folded back. Suborbital spine bicuspid, reaching slightly behind posterior margin of eye. The main branch of the suborbital spine straight or very slightly curved and 2 to 2.5 times longer than the other branch (Fig. 6b). Mouth small and arched. Lips moderately thick. The upper lip is strongly furrowed. The lower lip is fleshy, folded and interrupted at middle. The mental lobes are well developed (Fig. 4).

Inner rostral barbel reaching to base of maxillary barbel, outer one reaching to vertical of anterior eye margin or slightly beyond. Maxillary barbel reaching vertical of middle eye or in some individuals slightly beyond. There is a lateral distension of the body in front of the dorsal fin in mature males (Fig. 3b).

Table 3. Morphometric data of *Sabanejewia aurata*, (ZM-CBSU H2410, n=20) and *Sabanejewia caspia*, (ZM-CBSU H2282, n=20).

	S. aurata				***S. caspia***			
	min	max	mean	SD	min	max	mean	SD
Standard length (mm)	33	72.2	58.8		28.0	51.2	43.1	
In percent of standard length								
Head length	20	21.9	20.8	0.6	19.5	22.4	20.3	0.9
Body depth at dorsal-fin origin	13.8	16.4	15.3	0.7	14.8	18.3	16.9	0.7
Body width at dorsal-fin origin	6.5	8.7	7.5	0.5	7.35	9.86	8.8	0.6
Predorsal length	47.8	50.5	49.4	0.8	48.8	51.5	49.9	0.9
Postdorsal length	41	44.2	42.5	0.8	41.4	46.0	43.0	1.2
Prepelvic length	47.3	50.1	48.8	0.7	49.2	53.6	50.8	1.1
Preanal length	72.3	76.4	73.8	1.0	75.9	78.7	77.6	0.7
Distance between pectoral and pelvic-fin origins	24.8	29.2	27.1	1.0	26.8	31.6	29.1	1.3
Distance between pelvic and anal-fin origins	23.2	26.7	24.6	0.8	24.4	28.1	26.6	1.0
Depth of caudal peduncle	7.7	9.8	8.7	0.5	8.57	13.5	10.9	1.0
Length of caudal peduncle	18.1	20.9	19.0	0.6	13.3	16.2	14.3	0.8
Dorsal-fin depth	16.8	19.6	17.9	0.7	14.2	20.0	17.1	1.3
Pectoral fin length	14.8	17.2	15.9	0.6	13.1	18.1	15.3	1.5
Pelvic fin length	12.7	14.8	13.3	0.5	10.7	13.7	12.2	0.7
In percent of head length								
Head depth at nape	58.2	65.2	60.9	2.3	63.3	78.3	70.8	3.4
Head depth at eye	52.7	59.0	55.8	2.1	52.9	67.4	60.0	2.8
Snout length	40.1	46.4	42.9	1.5	33.0	38.7	37.2	1.8
Eye diameter	11.6	16.2	14.5	1.2	6.87	13.1	10.0	1.3
Postorbital distance	46	52.5	48.7	1.8	52.6	60.3	57.1	1.9
Maximum head width	32.2	47.3	40.3	3.9	35.1	50.0	43.4	3.6
Interorbital width	12.4	18.0	15.8	1.2	14.9	23.5	19.6	2.1

Fig.5. *Sabanejewia aurata*, about 50mm SL; Iran: Balikhlochay River, Caspian Sea basin.

Osteological characteristics: The dorsal and anal fins bear 3 unbranched rays. The total number of vertebrae is 45. The number of pre-caudal and caudal vertebrae 29 and 16, respectively. The caudal skeleton consists of five hypurals and unpaired epural, parhypural and pleurstyle bones. The pleurstyle is fused to the last centrum. The epural is broadened ventrally. The hypural-1 reaches to the ventral edge of the terminal centrum and connected to the parahypural. The 5th hypural is triangular in shape and not connected to the terminal centrum (Fig. 7).

Coloration: Based on preserved material: Body colour yellowish or bright orange-brown. On back, before and after the dorsal fin there are 3-5 and 4-6 square-like brown or dark gray blotches respectively. Lateral pigmentation is based on 10-14, large dark brown spots, generally square-like in shape. Head and its sides covered by dark brown small speckles. From the anterior rim of the orbit to the tip of snout,

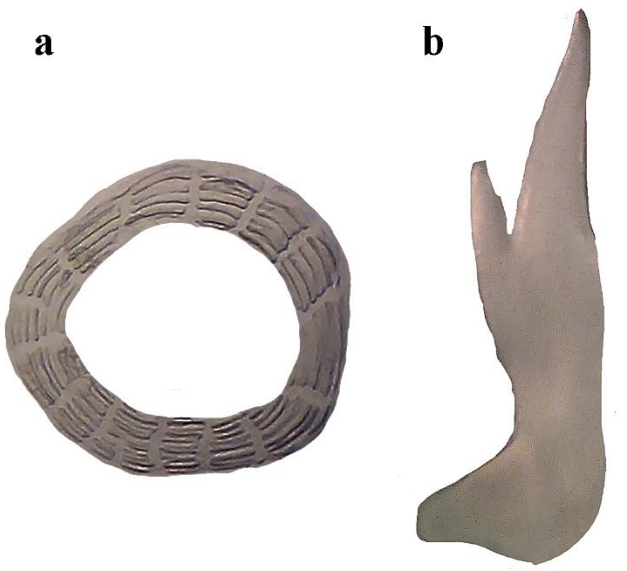

Fig.6. *Sabanejewia aurata*, ZM-CBSU H2430, 58mm SL; scale (a. left), suborbital spine (b. right).

there is a blackish stripe. At the caudal base there are always two dark-brown distinct spots. All fins are translucent but the caudal and dorsal have irregular rows of small brownish or grayish dots.

Distribution and Habitat: *Sabanejewia aurata* is found in the basins of the Baltic, Aegean, Black and Caspian seas and in the Tedzhen and Murgab Rivers of Afghanistan and Turkmenistan according to the most authors (Aliev et al. 1988; Nalbant & Bianco 1998). However, according to Kottelat (1997), this species is restricted to Iran and possibly adjacent waters. In Iran, it is found in the Aras [Balikhlochay (Fig. 8)] and Sefid River drainages, Caspian Sea basin. Balikhlochay is a perennial river that drains to the Aras river drainage. At the sampling site (Yamchi Dam), the maximum width is about 40m, with clean water, moderate to fast current and with little aquatic vegetation. The mean values of chemo-physical analysis of Sefid river during Feb. to Oct. 2013 includes NO_3^- 0.471ppm, NO_2^- 0.007 ppm, H_2PO_4 0.03ppm, total nitrogen 0.884ppm, dissolved oxygen 9.52ppm, electro-conductivity 1410.0µs/cm, pH 8.13 and water temperature 18.5°C. Macrobenthic studies of the Sefid River substrate revealed presence of Chironomidae (43.5%), Tubificidae (37.76%) and

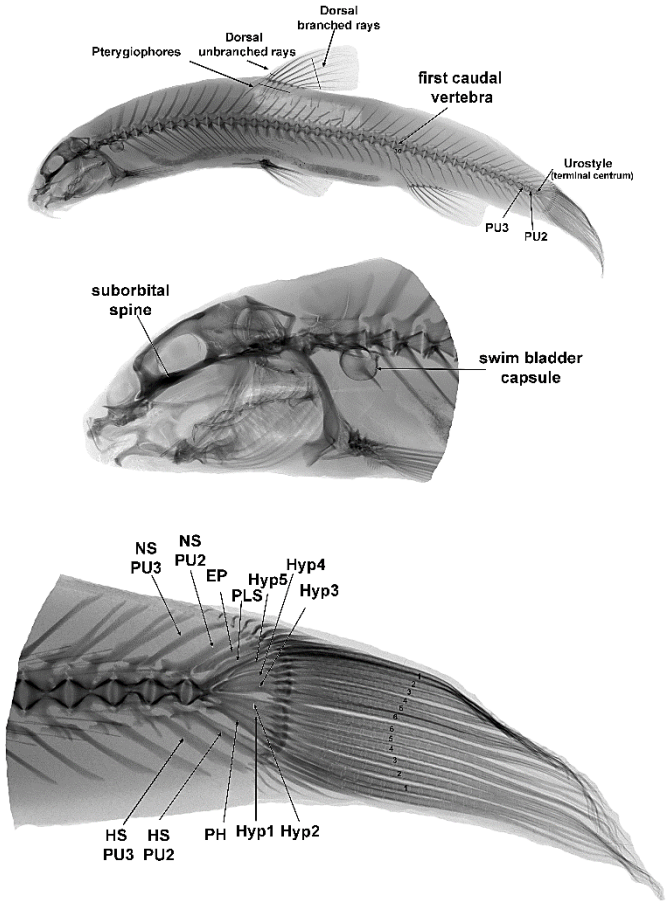

Fig.7. The skeleton of *Sabanejewia aurata* (X-ray images), ZM-CBSU MO215, 60mm SL; Iran: Balikhlochay River, Caspian Sea basin. (PU: preural; NS PU: neural spine of preural; HS PU: haemal spine of preural; Hyp: hypural; PH: parahypural; EP: epural; PLS: pleurstyle).

Sphaeriidae (19.19%) belonging to dipters (insects), oligochaete worms and bivalve mollusks, respectively.

Sabanejewia caspia (Eichwald, 1838)

(Figs. 9-14)

Cobitis caspia Eichwald, 1838: 133 (type locality: Azerbaidjan: Lenkoran [Lankaran, 38°45'N 48°51'E], Caspian Sea basin). No types known.

Material examined: ZM-CBSU H2282, 30, 28-51mm SL; Iran: Gilan prov.: Rasht city, Anzali wetland, Caspian Sea basin, 37°29'13"N 49°21'2"E; K. Abbasi, June 2017.

Material used in the molecular genetic analysis: ZM-

Fig.8. Natural habitat of *Sabanejewia aurata*, Iran: Yamchi, Balikhlochay River.

Fig.9. *Sabanejewia caspia*, a, ZM-CBSU H2284 (Female), 51mm SL; b, ZM-CBSU H2286 (Female), 48mm SL; c, ZM-CBSU 2285 (Male), 47.9mm SL; Iran: Anzali wetland, Caspian Sea basin.

CBSU M2038, M2039, M2040; Iran: Gilan prov.: Rasht city, Anzali wetland, Caspian Sea basin, 37°29'13"N 49°21'2"E (GenBank accession number: MK370720, MK370721, MK370722).

Diagnosis. *Sabanejewia caspia* is distinguished from *S. aurata* by having no large dark spots along the flank, short caudal peduncle (13-16 vs. 18–21 %SL), short snout (33-39 vs. 40-46 %HL), long post orbital

Fig.10. *Sabanejewia caspia*, a, ZM-CBSU H2284 (Female), 51mm SL; b, ZM-CBSU H2286 (Female), 48mm SL; c, ZM-CBSU 2285 (Male), 47.9mm SL; Iran: Anzali wetland, Caspian Sea basin.

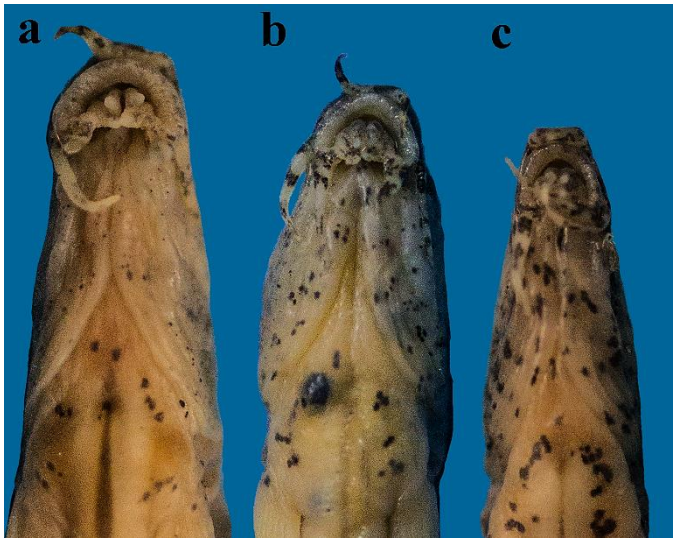

Fig.11. *Sabanejewia aurata*, a, ZM-CBSU H2284 (Female), 51mm SL; b, ZM-CBSU H2286 (Female), 48mm SL; c, ZM-CBSU 2285 (Male), 47.9mm SL; Iran: Anzali wetland, Caspian Sea basin.

distance (53-60 vs. 46-52 %HL), elliptical scales with large slightly centric focal zone and curved main branch of the suborbital spine. Among *Sabanejewia* species studied for molecular characters, it is characterized by 29 fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region studied (Table 1) and a K2P nearest-neighbour distance of 13% to *S. larvata* (Table 2).

Description: For general appearance see Figures 9-12; morphometric data are provided in Table 3. Body moderately elongate and laterally compressed. Greatest body depth at or slightly in front of dorsal-fin origin, decreasing towards caudal-fin base. Head profile slightly convex. Snout slightly blunt. Eyes small and situated closer to the tip of snout than to the posterior margin of the opercule. Caudal peduncle short and high, compressed laterally, 1.0-1.5 (mean 1.3) times longer than deep. A small, usually triangular axillary lobe at base of pelvic fin, fully attached to body or absent. Pectoral fin shorter than head length and reaching approximately 42-65% of distance from pectoral-fin origin to pelvic-fin origin. Dorsal-fin origin slightly closer to the tip of snout than to base of caudal fin. Pelvic fin short, not reaching anus. Pelvic-fin origin below first or second branched dorsal-fin ray. Anal-fin origin not reaching vertical of tip of last dorsal-fin ray. Anus about one eye diameter in front of anal-fin origin. Anal fin not reaching caudal-fin base. Margin of dorsal fin straight or slightly concave. Caudal fin truncate. Largest known specimen 51.2mm SL.

Dorsal fin with 6½ branched rays. Anal fin with 5½ branched rays. Caudal fin with 6+6 branched

Fig.12. *Sabanejewia caspia*, about 50mm SL; Iran: Anzali wetland, Caspian Sea basin.

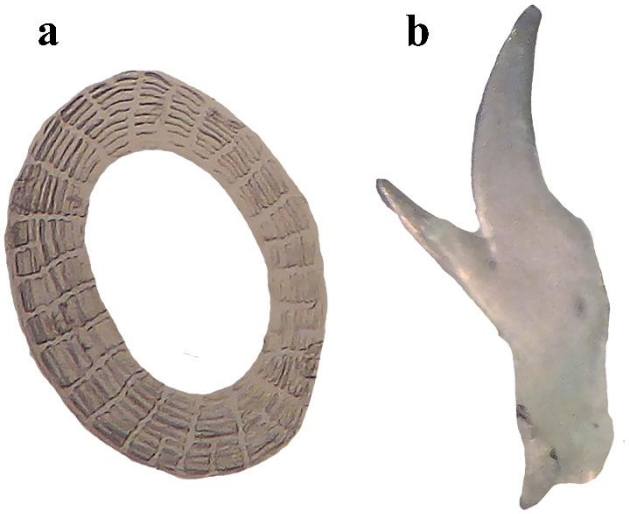

Fig.13. *Sabanejewia caspia*, ZM-CBSU H2302, 46mm SL; scale (a. left), suborbital spine (b. right).

rays. Pectoral fin with 8 (14) or 9 (6) branched rays and pelvic fin with 5 (9) or 6 (11) branched rays. Body completely covered by embedded scales. Scales elliptical, with large slightly centric focal zone (Fig. 13a). The lateral line very short, not exceeding the length of pectorals. Anterior nostril opening at end of a low, pointed and flap-like tube. Tube of anterior nostril slightly overlapping posterior nostril when folded back. Suborbital spine bicuspid, reaching slightly posterior margin of eye. The main branch of the suborbital spine is curved and is about

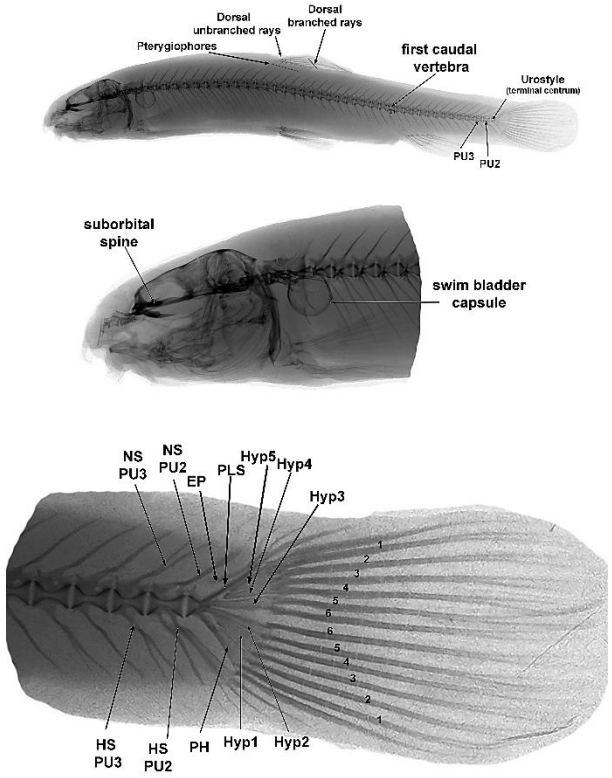

Fig.14. The skeleton of *Sabanejewia caspia* (X-ray images), ZM-CBSU H2282, 45mm SL; Iran: Anzali wetland, Caspian Sea basin. (PU: preural; NS PU: neural spine of preural; HS PU: haemal spine of preural; Hyp: hypural; PH: parahypural; EP: epural; PLS: pleurstyle).

2 times longer than the other branch (Fig. 13b).

Fig.15. Natural habitat of *Sabanejewia caspia*, Iran: Anzali wetland.

Fig.16. *Sabanejewia caucasica*, 55mm SL; Russia: Samur. Photo by A.M. Naseka.

Mouth small and arched. Lips thin. The upper lip furrowed. The lower lip interrupted at middle. The mental lobes short with two small lobules. (Fig. 11). Inner rostral barbel reaching to base of maxillary barbel, outer one reaching to vertical of anterior eye margin or slightly beyond. Maxillary barbel reaching vertical of middle eye or in some individuals slightly beyond. There is a lateral distension of the body in front of the dorsal fin in mature males (Fig. 10c, 12b).

Osteological characteristics. The dorsal and anal fins bear 3 unbranched rays. The total number of vertebrae is 44. The number of pre-caudal and caudal vertebrae 30 and 14, respectively. The caudal skeleton consists of five hypurals and unpaired epural, parhypural and pleurostyle bones. The pleurstyle is fused to the last centrum. The epural is a long, narrow and free bone. The hypural-1 almost reaches to the ventral edge of the terminal centrum and not connected to the parahypural. The 5th hypural is triangular in shape and not connected to the terminal centrum (Fig. 14).

Coloration. Based on preserved material: Body colour yellowish or bright orange-brown. Marbled pigmentation and a narrow continuous dark brown streak along flank not forming a midlateral row of dark blotches. Head and its sides covered by dark brown small speckles. From the anterior rim of the orbit to the tip of snout there is a blackish stripe. At the caudal base there are always two dark-brown distinct spots. All fins are translucent but the caudal,

dorsal and anal have irregular rows of small brownish or grayish dots.

Distribution and Habitat. *Sabanejewia caspia* is principally found in the southern Caspian Sea basin. In Iran, it is found in the Anzali wetland (Fig. 15), Sefid River and Amirkolayeh Wetland (Abbasi et al. 1999; Abbasi 2017), Caspian Sea basin. Anzali wetland is located west of the Sefid River delta and drains to Caspian Sea basin. The mean values of chemo-physical analysis of Anzali Wetland during 2016-2017 includes NO_3^- 0.083ppm, NO_2^- 0.004ppm, H_2PO_4 0.102ppm, NH_4^+ 0.416ppm, dissolved oxygen 8.2ppm, electro-conductivity 1798.5µs/cm, pH 7.44 and water temperature 18.18°C.

A study of stomach contents of 12 specimens of *S. caspia* in the Anzali Wetland showed presence of detritus, zooplankton and benthic animals, which detritus was dominant and *Daphnia* sp. (cladoceran), *Cyclops* sp. (cyclopoida), tubificid (oligochaete worms) and chironomid (insects) constituted 23.39, 8.77, 13.45 and 54.39% of total number, respectively.

Remark. Up to now, the Iranian spined loaches of the genus *Sabanejewia* have been classified into three species, *S. aurata*, *S. caspia* and *S. caucasica* (Esmaeili et al. 2010, 2014, 2017, 2018; Jouladeh-Roudbar et al. 2015; Coad 2017) but presence of *S. caucasica* (Fig. 16) in Iran was under question. It is morphologically similar to *S. caspia* than to *S. aurata* and is distinguished from *S. caspia* by having marbled pigmentation along flank not forming a streak (vs. a narrow continuous dark brown streak along flank in *S. caspia*); dark blotches on dorsum often fused (vs. no large dorsal blotches in *S. caspia*); branches of suborbital spine the same size (vs. the anterior one much shorter than posterior in *S. caspia*) (Kottelat & Freyhof 2007). We also failed to find any spined loach in our materials as *S. caucasica*, so our data (morphologically and genetically) confirm presence of two *Sabanejewia* species in the Iranian drainage of the Caspian Sea basin.

The phylogenetic relationships recovered for the genus *Sabanejewia* provide support for the monophyly of five main evolutionary mtDNA lineages: *Sabanejewia aurata, S. baltica, S. balcanica, S. caspia* and *S. larvata*. The Italian loach, *S. larvata* is sister to all other studied species. This highly divergent clade, representing the species *S. larvata* is strongly supported and is the most basal group of the genus and exhibits high mtDNA divergences from the rest of the mtDNA lineages (range 13-14%) showing that *S. larvata* is not phylogenetically closely related to any other *Sabanejewia* mtDNA lineage and does not confirm the sister relationships of *S. larvata* and *S. caspia* previously suggested (Perdices et al. 2003). The second clade represents the *S. caspia* lineage as a basal group (morphologically characterized by having no large dark spots along the flank) + *S. aurata, S. baltica, S. balcanica* (having large dark spots along the flank). The genetic differentiation of these five major lineages and their evolutionary distinctiveness seems to agree with their morphological separation that some authors have previously noted (Economidis & Nalbant 1996; Witkowski 1994; Perdices et al. 2003). Several authors considered most of the European *Sabanejewia* taxa as a subspecies of *S. aurata* (Banarescu et al. 1972; Economidis & Nalbant 1996), but our data agreeing with Perdices et al. (2003) supporting the monophyly of *S. aurata*.

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مقاله پژوهشی

بازبینی و توصیف مجدد گونه‌های جنس *Sabanejewia* در ایران (ماهیان استخوانی عالی: لوچ ماهیان خاردار آب‌شیرین)

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چکیده: گونه‌های جنس *Sabanejewia* بر اساس ویژگی‌های ریخت‌شناسی، استخوان‌شناسی و نشانگر مولکولی ناحیه بارکدینگ (ژن COI) ژنوم میتوکندریایی مورد بازبینی قرار گرفت. دو گونه *Sabanejewia aurata* و *Sabanejewia caspia* شناسایی شدند و ویژگی‌های تشخیصی هر دو گونه ارائه شده است. این دو گونه بر اساس الگوی رنگی، طول ساقه دمی، فاصله جلو و عقب چشم، شکل فلس و خار زیر چشم، اسکلت ساقه دمی و همچنین تعداد جابجائی‌های نوکلئوتیدهای تشخیصی قابل تشخیص می‌باشند. در این مطالعه هر دوگونه مذکور مورد بازبینی و توصیف مجدد قرار گرفته است.

کلمات‌کلیدی: لوچ‌ماهیان خاردار آب‌شیرین، سیتوکروم اکسیداز ۱، ویژگی‌های ریخت‌شناسی، وضعیت آرایه‌شناختی.