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Article

Oxynoemacheilus freyhofi, a new nemacheilid species (Teleostei, Nemacheilidae) from the Tigris basin, Iran

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Abstract

A new species of Nemacheilidae, *Oxynoemacheilus freyhofi* sp. nov. is described from the Roudbar River, a tributary of the Bakhtiyari River, Karoun River drainage, Iran. The species differs from its congeners in the combination of the following characters: dorsal fin with 10½ branched rays; forked caudal fin with equal size of lobes; slender and compressed caudal peduncle and complete lateral line; very small or absent median incision in upper lip; absence of suborbital groove in male. *Oxynoemacheilus freyhofi* is also distinguished from its congeners by a K2P distance of 4.5% to *O. argyrogramma* and 12.9% to *O. bergianus.*

Keywords: Freshwater fish, Taxonomy, Morphology, COI, Loach.
Zoobank: urn:lsid:zoobank.org:pub:B9CC32FB-1251-4518-9F89-EA013F203070
urn:lsid:zoobank.org:act:3A899D53-95FE-44C2-A624-249AA8A77946

Introduction

Stone loaches, family Nemacheilidae, are small fishes occurring in fresh waters of Asia and its islands, Europe, and northeast Africa (Coad 2015). They inhabit a variety of water bodies from turbulent mountain streams to salty rivers in dry lowlands (Mafakheri et al. 2015a, b, 2016). Nemacheilidae has about 48 genera and more than 661 species (Nelson et al. 2016; Eschmeyer and Fong 2011; Freyhof et al. 2015), with more expected to be described (Coad 2015; Kamangar et al. 2014). This family has a great diversity in Iranian inland waters (Coad 1995, 2006; Jouladh-Roudbar et al. 2015) with about 44 reported species that about 25 of them are endemic to Iran indicating their importance (Esmaeili et al. 2010; Kamangar et al. 2014; Esmaeili et al. 2014; Freyhof et al. 2014, 2015; Jouladh-Roudbar et al. 2015).

Stoumboudi et al. (2006) and Prokofiev (2009) placed most Nemacheilid loaches from Eastern Europe and the Middle East in the genus *Oxynoemacheilus* (Freyhof et al. 2011). The members of this genus is distinguished from the other genus of the family Nemacheilidae by a caudal fin emarginate or truncate if caudal fin forked, the body and caudal peduncle cylindrical, and usually 7-9½ branched dorsal-fin rays (Freyhof et al. 2011). The genus *Oxynoemacheilus* has 11 reported species from Iranian inland waters, including *O. angorae, O. bergianus*, *O. brandtii*, *O. frenatus, O. kiabii*, *O. longipinnis, O. persa* and *O. tongiorgii*, as well as three recently described species from the Choman River drainage of the Tigris basin i.e. *O. kurdistanicus*, *O. chomanicus* and *O. zagrosensis* (Kamangar et al. 2014). In the present paper, we describe an additional species of the genus *Oxynoemacheilus* collected from the Roudbar River, a tributary of the Bakhtiyari River (Karoun River drainage, Lorestan Province, Iran) in 2015 that demonstrated some morphological and molecular features compared to other members of this genus.

Methods

Morphological examinations: 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”. Simple rays of dorsal and anal fins are not counted as they are deeply embedded.

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Measurements were taken using an electronic caliper and rounded to the nearest 0.1 mm. The data are presented as standard length and head length. Type specimens are deposited in the Ichtyological Museum of Natural Resources Faculty, University of Tehran (IMNRF-UT).

DNA extraction and PCR: DNA was extracted from muscle tissue at base of the dorsal fin using a Genomic DNA Purification Kit (#K0512; Thermo Scientific Corporation, Lithuania) following the manufacturer's protocol. The COI gene was amplified using primers FishF1-(5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1-(5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'), designed by (Ward et al. 2005). Polymerase chain reaction (PCR) conditions were as follows: a 50 µl final reaction volume containing 5 µl of 10X Taq polymerase buffer, 1 µl of (50 mM) $MgCl_2$,1 µl of (10 mM) deoxynucleotide triphosphate (dNTP), 1 µl (10 µm) of each primer, 1 µl of Taq polymerase (5 $U\mu l^{-1}$), 7 µl of total DNA and 33 µl of H_2O. Amplification cycles were as follows: denaturation for 10 min at 94°C; 30 cycles at 94°C for 1 min, 58.5°C for 1 min, 72°C for 1 min and a final extension for 5 min at 72°C. PCR products were purified using purification Kit (Expin Combo GP – mini; Macrogen incorporation, Korea). The PCR products were sequenced using Sanger method by a robotic ABI-3130xl sequencer using manufacturer's protocol. The forward and revers primer was used to single strand sequencing.

Molecular data analysis: The sequences were compared to published *Oxynoemacheilus* sequences using (BLASTn) basic local alignment search tool (Altschul et al. 1990). All sequence data were aligned using MEGA6 software (Tamura et al. 2013). To unify the length of the sequences, the common 650 bp length segments were selected and used for phylogenetic analysis. Modeltest (Posada and Crandall 1998), implemented in the MEGA 6 software (Tamura et al. 2011) was used to determine the most appropriate sequence evolution model for the given data, treating gaps and missing data with the partial deletion option under 95 % site coverage cut-off. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern (Nei and Kumar 2000; Posada and Crandall 2001). Bayesian analyses of nucleotide sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because over parametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck and Ranala 2004). Each Bayesian analysis comprised two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies <0.01. Estimates of evolutionary divergence over sequence pairs between species were conducted in Mega6 (Tamura et al. 2013). Analyses were conducted using the Kimura 2-parameter model (Kimura 1980). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. As appropriate outgroup to root the constructed phylogenetic hypothesis, *Paracobitis molavii* (accession number: KJ23516, KJ23517), was included.

Abbreviations used: SL, standard length; HL, lateral head length; K2P, Kimura 2-parameter; IMNRFI-UT, Ichtyological Museum of Natural Resources Faculty, University of Tehran; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz.

Results

Oxynoemacheilus freyhofi, sp. nov.

(Figs. 1-7)

Holotype: IMNRF-UT-1036-1, male, 60.3 mm SL; Iran: Lorstan prov.: Roudbar River, tributary of Bakhtiyari River, near Kazem Abad village, 33°08'17.8"N 49°40'43.9"E, S. Eagderi & A. Jouladeh-Roudbar, 19 August 2015.

Figure 1. *Oxynoemacheilus freyhofi* sp. nov., IMNRF-UT- 1036-1, holotype, 55 mm SL; Iran: Lorestan prov.: Roudbar River.

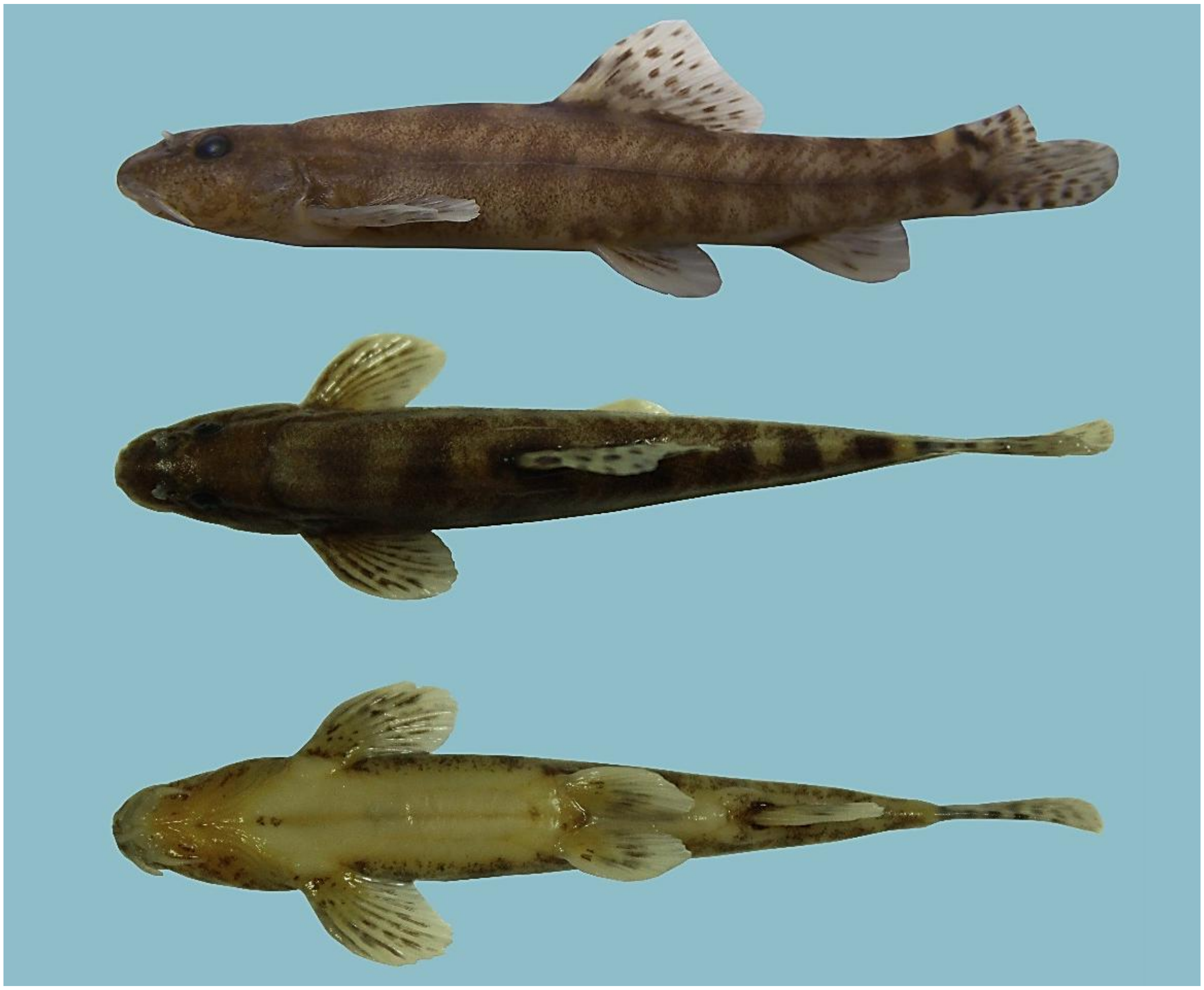

Figure 2. *Oxynoemacheilus freyhofi*, IMNRF-UT- 1036-1, holotype, 60.3 mm SL; Iran: Roudbar River River.

Paratypes: IMNRF-UT-1036, 3, 44-45 mm SL; Iran: Lorstan prov.: Roudbar River, tributary of Bakhtiyari River, near Kazem Abad village, 33°08'17.8"N 49°40'43.9"E, S. Eagderi & A. Jouladeh-Roudbar, 19 August 2015. IMNRF-UT-2036, 7, 43-44 mm SL; Iran: Lorstan prov.: Roudbar River, tributary of Bakhtiyari River, near Dehmirza gholi village, 33°07'05.8"N 49°40'03.4"E, S. Eagderi & A. Jouladeh Roudbar, 19 August 2015.

Diagnosis: *Oxynoemacheilus freyhofi* sp. nov. is distinguished from the other species of *Oxynoemacheilus* in Iran by a combination of characters, none of them unique. Dorsal fin with 10½ branched rays; forked caudal fin

Table 1. Morphometric data of *Oxynoemacheilus freyhofi* (holotype, IMNRF-UT-1036-1; paratypes, IMNRF-1036, 3 specimens, IMNRF-UT-2036, 7 specimens).

		Paratypes		
	Holotype	**Range**	**Mean**	**SD**
Standard length (mm)	60.3	51.4-55.6		
In percent of standard length (SL)				
Body depth maximal	17.2	17.3-17.9	17.6	0.3
Caudal peduncle depth	9.4	9.6-10	9.8	0.2
Predorsal length	49.9	50.4-51.9	51.1	0.7
Postdorsal length	49.1	51.3-52.4	52.0	0.7
Prepelvic length	54.5	51.7-54.9	52.8	1.7
Preanal length	75.5	74-74.4	74.1	0.3
Caudal peduncle length	15.9	17.2-18	17.5	0.4
Dorsal-fin base length	17.4	16.6-19.7	18.0	1.6
Dorsal-fin depth	15.8	19.5-21.1	20.3	0.8
Anal-fin base length	8.5	6.6-7.6	7.1	0.5
Anal-fin depth	16.2	15.1-16.3	15.7	0.6
Pectoral fin length	18.7	22.8-22.9	22.9	0.0
Pelvic fin length	15.9	17-19	17.8	1.1
Pectoral - pelvic-fin origin distance	32.4	29.3-31.7	30.5	1.2
Pelvic - anal-fin origin distance	21.3	19.9-22.4	21.5	1.4
Caudal-fin length	18.1	19.8-20.7	20.3	0.5
Body width	13.7	13.6-14	13.8	0.2
Caudal peduncle width	2.4	2.5-2.8	2.6	0.1
Head length	21.0	21-22.5	21.8	0.8
In percent of Head length (HL)				
Snout length	45.7	42-44.9	43.9	1.6
Eye horizontal diameter	20.2	18.9-21.4	20.0	1.3
Postorbital distance	40.2	42.2-49.1	44.8	3.8
Head depth at nape	64.8	60.6-70	65.2	4.7
Head width	67.0	59.4-66.5	63.1	3.6
Inter orbital	30.2	29.7-31.3	30.3	0.9
Inter nasal	18.2	18.5-21.7	19.8	1.7
Mouth width	26.0	24.4-30.9	27.9	3.3
Inner rostral barble	19.8	22.6-25.2	23.8	1.3
Outer rostral barble	33.5	31.9-33.4	32.7	0.8
Maxillary barble	30.2	30.3-31.1	30.8	0.4

with equal size of lobes; slender and compressed caudal peduncle and complete lateral line. very small or absent median incision in upper lip, absence of suborbital groove in male. *Oxynoemacheilus freyhofi* is also distinguished from its congeners by a K2P distance of 4.5% to *O. argyrogramma* and 12.9% to *O. bergianus.*

Description: See Figures 1-7 for general appearance and Table 1 for morphometric data of holotype and 10 paratypes. Medium sized, elongate, laterally compressed body with short head. Body deepest at front of dorsal-fin origin; depth moderately decreasing towards caudal-fin base; a notch at junction of head to trunk; highest body width at middle between pectoral- and pelvic-fin bases. Section of head roundish; flattened on ventral surface; snout length about equal postorbital length; eyes diameter moderate, in dorsolateral position. nostrils separated by a narrow space; anterior nostril opening pointed and flap-like tube, overlapping posterior nostril when folded back; no suborbital flap or groove. Mouth small, strongly arched; lips moderately thick, with poorly marked furrows; very small or absent median incision in upper lip; deep median interruption in lower lip; processus dentiformis small and pointed. Inner rostral barbel reaching beyond base of maxillary barbel; outer barbel not reaching to vertical of anterior eye margin; maxillary barbel reaching vertical of middle eye or in some individuals vertical of posterior eye margin. Caudal peduncle compressed laterally, 1.5-1.8 (mean 1.7) times

Figure 3. *Oxynoemacheilus freyhofi*, paratypes; Iran: Roudbar River; a, IMNRF-UT-1036-2, 45 mm SL; b, IMNRF-UT-1036-3, 43 mm SL; c, IMNRF-UT-1036-4, 42 mm SL.

longer than depth; pectoral fin reaching approximately 60-80 %pectoral-fin to pelvic-fin origins' distance; small, usually roundish axillary lobe at base of pelvic fin, fully attached to body; very small or absent in some individuals; pelvic fin insertion under third ray of dorsal-fin, not reaching anal-fin origin; pelvic fin reaching to a point about 1.5-2.0 eye diameter in front of anus; anal fin origin about one eye diameter behind anus. Margin of dorsal fin straight or slightly concave; margin of anal fin convex; caudal fin forked with equal lobes. Dorsal-fin branched rays 10½; Anal-fin branched rays 6½; caudal-fin branched rays 9+9; pectoral-fin branched rays 9-10; pelvic fin branched rays 8. Scales minute, cycloid, embedded covers whole body. Lateral line straight, on the mid-flank, ends just anterior to caudal fin base. Largest known specimen 60.3 mm SL.

Coloration: Body pale-brown in life and yellowish in preserved specimens. Head and cheeks dark-brown, fawn in below; 7-10 dark-brown bars regularly or irregularly shaped and set as wide. Bars usually meeting their homologues on the back and appear an irregular large blotches. Usually two, rarely three pale bars in front of dorsal-fin origin; one at dorsal fin origin; one at posterior dorsal-fin base; four or five bars on caudal peduncle; bars indistinct or a mottled colour pattern in juveniles smaller than 35 mm SL. two small, often faded black spot at base of caudal peduncle usually confluent. Pelvic- and pectoral fins hyaline with few dark-brown or black spots on rays.

Molecular results: We are able to generate 6 new COI genes of *O. freyhofi* (n=3) and *O. bergianus* (n=3). Additional 71 sequences of 33 species have been downloaded from Genbank (Table 2). Tables 3 and 4 list the diagnostic nucleotide substitutions and estimates of the average evolutionary divergence found in the mtDNA

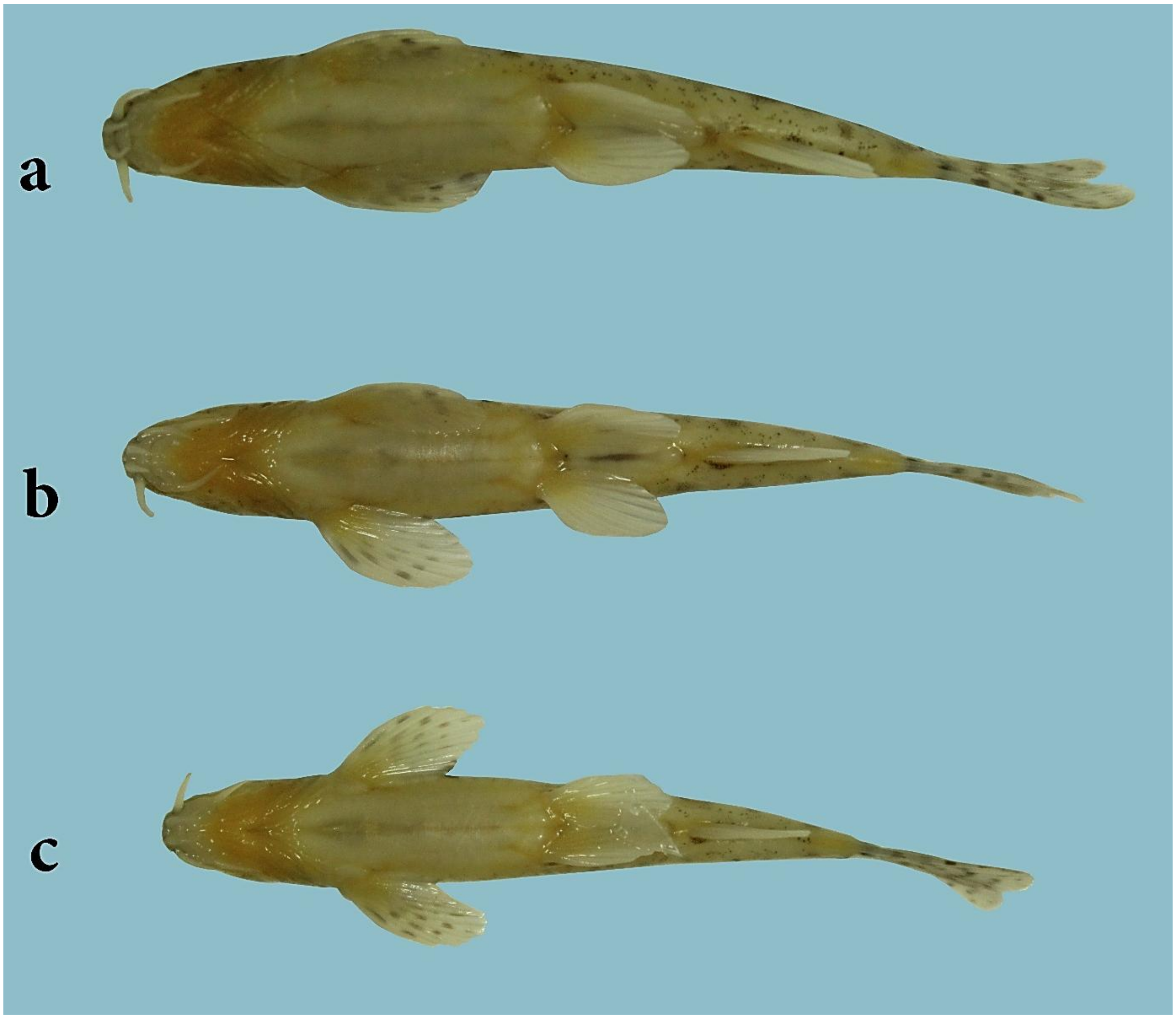

Figure 4. *Oxynoemacheilus freyhofi*, paratypes; Iran: Roudbar River; a, IMNRF-UT-1036-2, 45 mm SL; b, IMNRF-UT-1036-3, 43 mm SL; c, IMNRF-UT-1036-4, 42 mm SL.

COI barcode region, which show between 4.12% and 16.56% K2P sequence divergence in their COI gene. Two phylogenetic approaches i.e. Bayesian Inference (BI) and Maximum Likelihood (ML), gave the same tree topologies and therefore that of maximum likelihood was presented (Fig. 8). Two methods produced trees with 5 major lineages supported by high posterior probability and bootstrap values (Fig. 8): (I) *O. germanicus*, *O. mesudae*, *O. cinicus*, *O. anatolicus*, *O. mediterraneus*, *O. theophilii*, *O. phoxinoides* and *O. angorae*. (II) *O. bergianus*, *O. samanticus*, *O. samavicus*, *O. seyhanensis*, *O. eregliensis*, *O. atili*, *O. hamwii* and *O. evreni*. (III) *O. namiri*, *O. leontinae*, *O. panthera*, *O. galilaeus*, *O. Kiabii*, *O. frenatus*, *O. insignis*, *O. persa*, *O. freyhofi*, *O. argyrogramma*, *O. zagrosensis*, *O. ercisianus*, *O. tongiorgii* and *O. cyri*, (IV) *O. pindus* and *O. bureschi,* and (VI) *O. brandtii* and *O. merga*.

Etymology: The new species is named after Jörg Freyhof, a well-known ichthyologist for his valuable contribution to the knowledge of freshwater fishes of Iran.

Distribution: *Oxynoemacheilus freyhofi* is known from Roudbar River, a tributary of Bakhtiyari River. It is also widespread in the Sezar and Dez rivers which drainage to the Persian Gulf.

Habitat: At the type locality, the Roudbar River has 7-11 m width, with substrate consisting of coarse gravel and boulders, good riparian vegetation and almost fast-flowing and turbid waters. The physicochemical parameters at the sampling site were: dissolved oxygen, 7.01 mg L^{-1}, total dissolved solids, 548 mg L^{-1}, conductivity, 292

Figure 5. *Oxynoemacheilus freyhofi*, paratypes; Iran: Roudbar River; a, IMNRF-UT-1036-2, 45 mm SL; b, IMNRF-UT-1036-3, 43 mm SL; c, IMNRF-UT-1036-4, 42 mm SL.

µm cm^{-1}, pH=7.43 and water temperature 18.9°C.

Remarks: *Oxynoemacheilus freyhofi* is distinguished from *O. araxensis* by a deeply emarginated or forked caudal fin (vs. almost straight), absence of suborbital groove in males (vs. present).

Oxynoemacheilus freyhofi is distinguished from *O. bergianus* by having more branched dorsal-fin rays (10½ vs. 8-9½), caudal peduncle depth (9.8 vs. 7.5 %SL), dorsal-fin base length (18.0 vs. 15.5 %SL), pelvic-fin length (17.8 vs. 15.0 %SL), more caudal peduncle depth/length (56.6 vs. 38.5), longer snout (43.9 vs. 39.8 %HL), longer interorbital (30.3 vs. 26.9 %HL), outer rostral and maxillary barbles (respectively 32.7, 30.8 vs. 29.2, 24.8 %HL), absence of suborbital groove in males (vs. present), poorly marked or no furrows (vs. marked and developed furrows) and forked caudal fin (vs. slightly emarginate or truncate).

Oxynoemacheilus freyhofi is distinguished from *O. brandtii* by having more branched dorsal-fin rays (10½ vs. 8-9½), longer dorsal-fin length (18.0 vs. 15.6 %SL), smaller pectoral - pelvic-fin distance (30.5 vs. 33.0 %SL), larger eye horizantal diameter (20.0 vs. 17.6%HL), caudal peduncle depth/ length (56.6 vs. 46.4), caudal peduncle depth/ body depth maximal (55.7 vs. 47.2), absence of suborbital groove in males (vs. present), very small or absent median incision in upper lip (vs. marked incision in upper lip), poorly marked or no furrows (vs. marked and developed furrows).

Oxynoemacheilus freyhofi is distinguished from *O. chomanicus* by having more branched dorsal-fin rays (10½ vs. 8½-9½), elongate and slender body (vs. stout and deep-bodied), dorsal-, pectoral- and pelvic-fin base lengths (respectively 18.0, 22.9, 17.8 vs. 15.3, 16.4, 12.2 %SL), body depth (17.6 vs. 21.4 %SL), caudal peduncle depth (9.8 vs. 13.3 %SL), longer snout (43.9 vs. 39.1 %HL), eye horizontal diameter (20.0 vs. 16.9 %HL), interorbital distance (30.3 vs. 27.0 %HL), outer and maxillary barbles (respectively 32.7, 30.8 vs. 25.9, 27.1 %HL), shorter

Table 2. List of species used for molecular analysis for COI and GenBank Accession Number. *Paracobitis molavii* was considered as outgroup.

No.	Accession No.	Species	No.	Accession No.	Species
1	KJ553916	*O. anatolicus*	40	KP342071	*O. kiabii*
2	KJ553947	*O. anatolicus*	41	KP342070	*O. kiabii*
3	KJ553966	*O. angorae*	42	KJ553816	*O. leontinae*
4	KU928279	*O. angorae*	43	KJ553697	*O. leontinae*
5	KJ553824	*O. angorae*	44	KJ553660	*O. leontinae*
6	KU928280	*O. argyrogramma*	45	KJ553948	*O. mediterraneus*
7	KJ553726	*O. atili*	46	KJ553711	*O. mediterraneus*
8	KX946209	*O. bergianus* 1	47	KU928285	*O. merga*
9	KX946210	*O. bergianus* 2	48	KJ553883	*O. mesudae*
10	KX946211	*O. bergianus* 3	49	KJ553765	*O. namiri*
11	KU928285	*O. brandtii*	50	KJ553891	*O. namiri*
12	KU928281	*O. brandtii*	51	KJ553813	*O. namiri*
13	KJ553997	*O. bureschi*	52	KJ554027	*O. panthera*
14	KJ554035	*O. bureschi*	53	KP050538	*O. persa*
15	KJ553898	*O. bureschi*	54	KP050531	*O. persa*
16	KJ553905	*O. cinicus*	55	KP050529	*O. persa*
17	KJ553921	*O. cinicus*	56	KJ553825	*O. phoxinoides*
18	KJ723498	*O. cyri*	57	KJ553804	*O. phoxinoides*
19	KU928282	*O. cyri*	58	KJ553820	*O. phoxinoides*
20	KJ723513	*O. cyri*	59	KJ554021	*O. pindus*
21	KU928283	*O. ercisianus*	60	KJ554005	*O. pindus*
22	KJ553815	*O. eregliensis*	61	KJ553899	*O. pindus*
23	KJ554068	*O. eregliensis*	62	KJ553908	*O. samanticus*
24	KJ554051	*O. eregliensis*	63	KJ553807	*O. samanticus*
25	KJ553678	*O. evreni*	64	KJ553992	*O. seyhanensis*
26	KJ553954	*O. evreni*	65	KJ553980	*O. seyhanensis*
27	KU928284	*O. frenatus*	66	KJ554003	*O. seyhanensis*
28	KX946206	*O. freyhofi* 1	67	KJ553724	*O. simavicus*
29	KX946207	*O. freyhofi* 2	68	KJ553970	*O. simavicus*
30	KX946208	*O. freyhofi* 3	69	KJ554038	*O. theophilii*
31	KJ553821	*O. galilaeus*	70	KJ553850	*O. theophilii*
32	KJ553768	*O. galilaeus*	71	KJ553798	*O. theophilii*
33	KJ553856	*O. germencicus*	72	KP050536	*O. tongiorgii*
34	KJ553793	*O. germencicus*	73	KP050534	*O. tongiorgii*
35	KJ553999	*O. hamwii*	74	KP050535	*O. tongiorgii*
36	KJ554024	*O. hamwii*	75	KU928286	*O. zagrosensis*
37	KJ553963	*O. hamwii*	76	KJ723517	*Paracobitis molavii*
38	KJ553801	*O. insignis*	77	KJ723516	*Paracobitis molavii*
39	KP342072	*O. kiabii*			

head depth (65.2 vs. 71.6 %HL), smaller caudal peduncle depth/ length (56.6 vs. 82.6), smaller caudal peduncle depth/ body depth maximal (55.7 vs. 62.1), a deeply emarginated or forked caudal fin (vs. truncate).

Oxynoemacheilus freyhofi is distinguished from *O. frenatus* by having more branched dorsal-fin rays (10½ vs. 8-9½), smaller body depth (17.6 vs. 19.0 %SL), caudal peduncle depth (9.8 vs. 11.2 %SL), longer pre- and post-dorsal lengths (respectively 51.1, 52.0 vs. 48.4, 50.9 %SL), shorter prepelvic length (52.8 vs. 55.1 %SL), longer dorsal fin base (18.0 vs. 16.5 %SL), snout length (43.9 vs. 41.0 %HL), inner, outer, maxillary barbles (respectively 23.8, 32.7, 30.8 vs. 21.3, 30.1, 27.7 %HL), caudal peduncle depth/ length (56.6 vs. 66.7), smaller caudal peduncle depth/ body depth maximal (55.7 vs. 59.4), processus dentiformis narrow and pointed process (vs. well-developed).

Oxynoemacheilus freyhofi is distinguished from *O. lenkoranensis* by having more branched dorsal-fin rays (10½ vs. 8-9).

Oxynoemacheilus freyhofi is distinguished from *O. kiabii* by having more branched dorsal-fin rays (10½ vs.

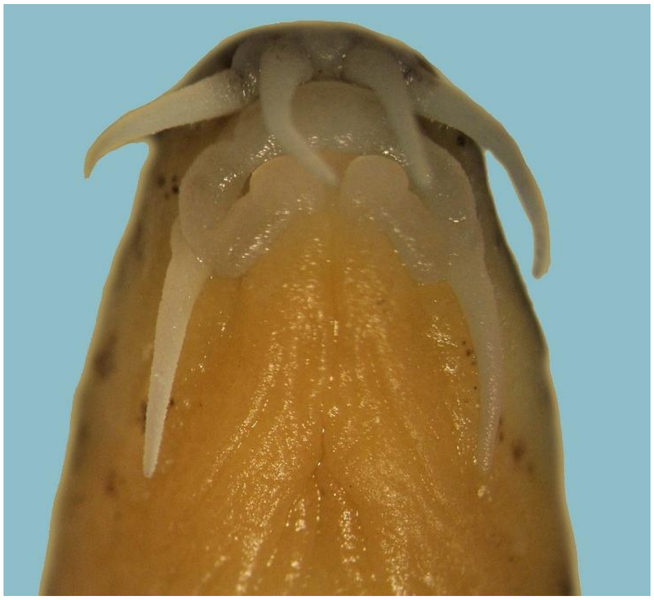

Figure 6. *Oxynoemacheilus freyhofi* sp. nov., IMNRF-UT-1036-1, holotype, 60.3 mm SL; Iran: Lorestan prov.: Roudbar River.

Figure 7. *Oxynoemacheilus freyhofi* sp. nov., IMNRF-UT-1036-1, holotype, 60.3 mm SL; Iran: Lorestan prov.: Roudbar River.

Table 3. Estimates of the average evolutionary divergence between *Oxynoemacheilus freyhofi* and other species of this genus, expressed as number of base substitutions per site. All positions with less than 95% site coverage were eliminated before analysis, leading to a total of 650 nucleotide positions.

No.	Species	1	2	3	4	5	6	7	8	9
1	*O. angorae*									
2	*O. argyrogramma*	12.74								
3	*O. bergianus*	8.07	14.49							
4	*O. brandtii*	13.31	12.96	12.62						
5	*O. frenatus*	11.86	8.86	11.68	14.86					
6	*O. kiabii*	12.09	10.26	12.49	16.56	6.75				
7	*O. freyhofi*	12.18	4.50	12.96	12.68	7.24	8.14			
8	*O. persa*	12.88	4.88	12.76	13.17	8.42	9.58	2.73		
9	*O. tongiorgii*	12.51	11.31	11.51	14.08	10.46	9.29	9.12	9.73	
10	*O. zagrosensis*	13.14	4.12	14.12	13.87	8.84	9.58	3.95	4.50	10.83

9½ rarely 10½), elongate and slender body (vs. stout and deep-bodied), smaller body depth (17.6 vs. 21.7 %SL), caudal peduncle depth (9.8 vs. 15.4 %SL), prepelvic length (52.8 vs. 58.7 %SL), preanal length, (74.1 vs. 78.6 %SL), longer caudal peduncle (17.5 vs. 14.5 %SL), dorsal-fin base length (18.0 vs. 14.7 %SL), longer snout (43.9 vs. 36.4 %HL), eye horizontal diameter (20.0 vs. 17.0 %HL), shorter head depth (65.2 vs. 75.7 %HL), interorbital length (30.3 vs. 35.1 %HL), longer outer rostral barble (32.7 vs. 26.0 %HL), smaller caudal peduncle depth/ length (56.6 vs. 105.3), smaller caudal peduncle depth/ body depth maximal (55.7 vs. 70.9), very small or absent median incision in upper lip (vs. marked incision in upper lip), complete lateral line (vs. incomplete lateral line).

Oxynoemacheilus freyhofi is distinguished from *O. kurdistanicus* by having longer snout (43.9 vs. 39.5 %HL), postorbital length (44.8 vs. 41.2 %HL), head width (63.1 vs. 59.7 %HL), outer and maxillary barble lengths (respectively 32.7, 30.8 vs. 18.6, 17.9 %HL), larger caudal peduncle depth/ length (56.6 vs. 49.2), very small or absent median incision in upper lip (vs. marked incision in upper lip).

Oxynoemacheilus freyhofi is distinguished from *O. persa* by having more branched dorsal-fin rays (10½ vs. 8-9½), longer predorsal (51.1 vs. 48.4 %SL), prepelvic length (52.8 vs. 49.4 %SL), dorsal-fin base length (18.0 vs. 14.7 %SL), body width (13.8, vs. 11.5 %SL), longer snout (43.9 vs. 40.5 %HL), interorbital length (30.3, vs.

Table 4. Diagnostic nucleotide substitutions found in mtDNA COI barcode region of *Oxynoemacheilus* species. Nucleotide position relative to *Oxynoemacheilus freyhofi* (KX52724) in mtDNA COI barcode region.

	27	30	69	72	81	84	88	90	114	117	123	159	165	174	177	180	186	192	195	198	204	210	213	219	228	234	240	249	261	262	264	267	279	286	291	294	297	303	306	309	315	318
O. freyhofi	T	G	A	G	C	A	C	G	T	T	A	G	C	T	C	A	G	A	C	A	T	C	A	A	C	G	A	A	T	C	G	T	C	T	G	A	C	G	G	C	C	A
O. kiabii	T	G	A	G	C	A	C	A	C	T	A	G	C	T	T	G	C	A	T	A	T	T	G	T	C	A	G	A	T	T	G	A	C	T	G	A	C	A	G	T	C	G
O. tongiorgii	T	G	A	G	T	A	C	A	T	T	C	G	C	T	T	G	G	A	T	A	A	T	G	G	C	G	G	G	C	T	A	C	C	T	G	A	C	A	G	T	C	A
O. persa	T	G	A	A	C	A	C	A	T	T	A	G	C	T	C	A	G	A	C	A	T	C	A	A	C	G	A	G	T	C	G	T	C	T	A	A	C	A	G	C	C	A
O. angorae	C	A	G	C	C	G	C	C	T	A	T	A	T	C	C	G	A	G	C	G	C	T	G	C	C	G	G	G	C	T	G	C	G	T	G	A	G	A	A	T	C	G
O. frenatus	C	G	A	G	C	A	C	A	C	T	A	G	C	C	T	G	G	A	C	A	T	T	G	T	C	A	A	G	T	T	G	G	C	T	G	A	C	A	A	C	T	A
O. zagrosensis	T	G	A	G	C	A	T	A	T	T	A	G	C	T	C	A	G	A	C	A	T	T	A	A	C	G	A	G	T	C	A	T	C	T	A	A	C	G	G	C	C	A
O. brandtii	T	A	G	G	A	G	C	C	T	A	T	A	C	C	C	G	T	A	T	G	A	C	G	A	T	G	A	G	C	C	G	C	G	C	G	G	A	G	G	C	C	A
O. argyrogramma	T	G	A	G	C	A	T	A	T	T	A	G	C	T	C	A	A	A	C	G	T	C	A	A	C	G	A	A	T	C	A	T	C	T	A	G	C	A	G	C	C	A
O. bergianus	T	A	G	A	A	G	C	C	T	A	T	A	T	T	T	G	G	G	C	A	T	T	G	C	T	A	G	G	C	T	G	C	C	C	G	A	A	A	A	T	T	G

	321	327	330	336	342	357	369	381	384	387	393	399	402	403	414	420	429	474	477	483	496	498	504	507	513	519	522	528	529	531	532	534	537	540	555	561	564	570	573	579	582	588
O. freyhofi	G	A	A	T	C	G	G	C	A	C	C	A	C	C	T	C	G	T	C	A	C	A	A	G	C	G	C	G	C	A	C	T	C	A	A	T	A	C	A	A	A	C
O. kiabii	G	A	A	C	C	A	A	T	A	C	C	A	C	C	C	C	G	C	C	A	T	A	A	A	C	A	T	A	C	A	C	T	T	A	G	T	G	C	G	A	G	T
O. tongiorgii	G	G	A	C	C	G	A	T	A	T	C	A	C	T	T	A	G	T	T	A	C	G	A	A	C	G	C	A	C	G	C	T	C	G	G	C	A	C	A	G	T	T
O. persa	A	A	A	T	C	G	G	C	A	C	C	T	C	C	T	C	G	T	C	A	C	A	G	A	C	G	C	G	C	A	C	T	C	G	A	T	A	C	G	G	A	C
O. angorae	G	G	A	C	T	A	G	C	A	T	T	C	T	C	C	T	A	T	C	G	C	G	A	A	T	A	T	G	T	A	T	A	T	C	G	T	A	T	A	G	G	T
O. frenatus	G	A	A	C	A	A	G	C	G	C	C	A	C	C	C	T	G	T	T	G	T	A	A	G	C	G	C	A	C	A	C	T	T	T	G	T	A	C	G	A	A	C
O. zagrosensis	G	A	G	C	C	G	G	C	A	C	C	A	C	C	G	C	A	T	T	A	C	A	G	G	C	A	C	G	C	A	C	T	C	A	A	T	G	T	G	A	A	C
O. brandtii	A	G	A	C	T	G	G	C	A	T	C	T	C	T	A	T	G	C	T	A	C	G	G	G	C	A	C	G	T	A	C	A	C	G	G	C	C	C	G	A	A	A
O. argyrogramma	G	A	G	C	C	G	G	C	A	C	C	C	C	C	T	T	G	T	T	G	C	A	G	G	T	A	C	G	C	A	C	T	C	A	A	T	G	C	G	G	A	C
O. bergianus	G	G	A	C	C	A	A	C	G	T	T	T	T	T	C	T	G	T	T	G	C	G	C	A	C	A	C	G	T	G	T	A	C	G	G	T	A	C	G	A	A	T

25.6 %SL), mouth width (27.9, vs. 20.3 %HL), larger caudal peduncle depth/ length (56.6 vs. 46.6), very small or absent median incision in upper lip (vs. marked incision in upper lip), lips and barbles without tubercles and marked furrows (vs. lips and barbles have small tubercles and marked furrows), processus dentiformis narrow and pointed process (vs. well-developed).

Oxynoemacheilus freyhofi is distinguished from *O. tongiorgii*, by having more branched dorsal-fin rays (10½ vs. 8-9½), elongate body (vs. deep), thin caudal peduncle (vs. deep), deeply emarginated or forked caudal fin (vs. truncate), a slenderer caudal peduncle (vs. deep), complete lateral line (vs. incomplete lateral line).

Oxynoemacheilus freyhofi is distinguished from *O. zagrosensis* by having more branched dorsal-fin rays (10½ vs. 8½-9½), elongate and slender body (vs. stout and deep-bodied), larger dorsal-, pectoral-, and pelvic-fin base lengths (respectively 18.0, 22.9, 17.8 vs. 14.5, 13.5, 18.0 %SL), shorter body depth (17.6 vs. 20.1 %SL), caudal peduncle depth (9.8 vs. 13.7 %SL), longer snout (43.9 vs. 37.2 %HL), eye horizontal diameter (20.0 vs. 17.6 %HL), interorbital distance (30.3 vs. 22.3%HL), outer and maxillary barbell lengths (respectively 32.8, 30.8 vs. 25.8, 25.7 %HL), shorter head depth (65.2 vs. 72.7 %HL), smaller caudal peduncle depth/ length (56.0 vs. 77.4), smaller caudal peduncle depth/ body depth maximal (55.7 vs. 68.1), a deeply emarginated or forked caudal fin (vs. truncate).

Oxynoemacheilus freyhofi is distinguished from *O. longipinnis* by having more branched dorsal-fin rays (10½ vs. 8-9½), longer maximum body depth, (17.6 vs. 14.0 %SL), pectoral-pelvic-fin origin distance (30.5 vs. 27.1 %SL), shorter head length (21.8 vs. 26.2 %SL), caudal peduncle length (17.5 vs. 20.0 %SL), head depth at nape (65.2 vs. 51.0 %HL), eye horizontal diameter (20.0 vs12.9 %HL), inter orbital distance (30.3, vs. 24.6 %HL), larger caudal peduncle depth/ length (56.6 vs. 38.8), very small or absent median incision in upper lip (vs. marked incision in upper lip).

Comparative Material: *Oxynoemacheilus angorae*: IMNRF-UT-1038, 5, 64-77 mm SL; Iran: Ardebil prov.: Near Meshkin shahr, at Ghadirlo village, Qara Su River, Caspian Sea basin, 38°34'48.5"N 47°53'12.2"E, 5 July 2016,

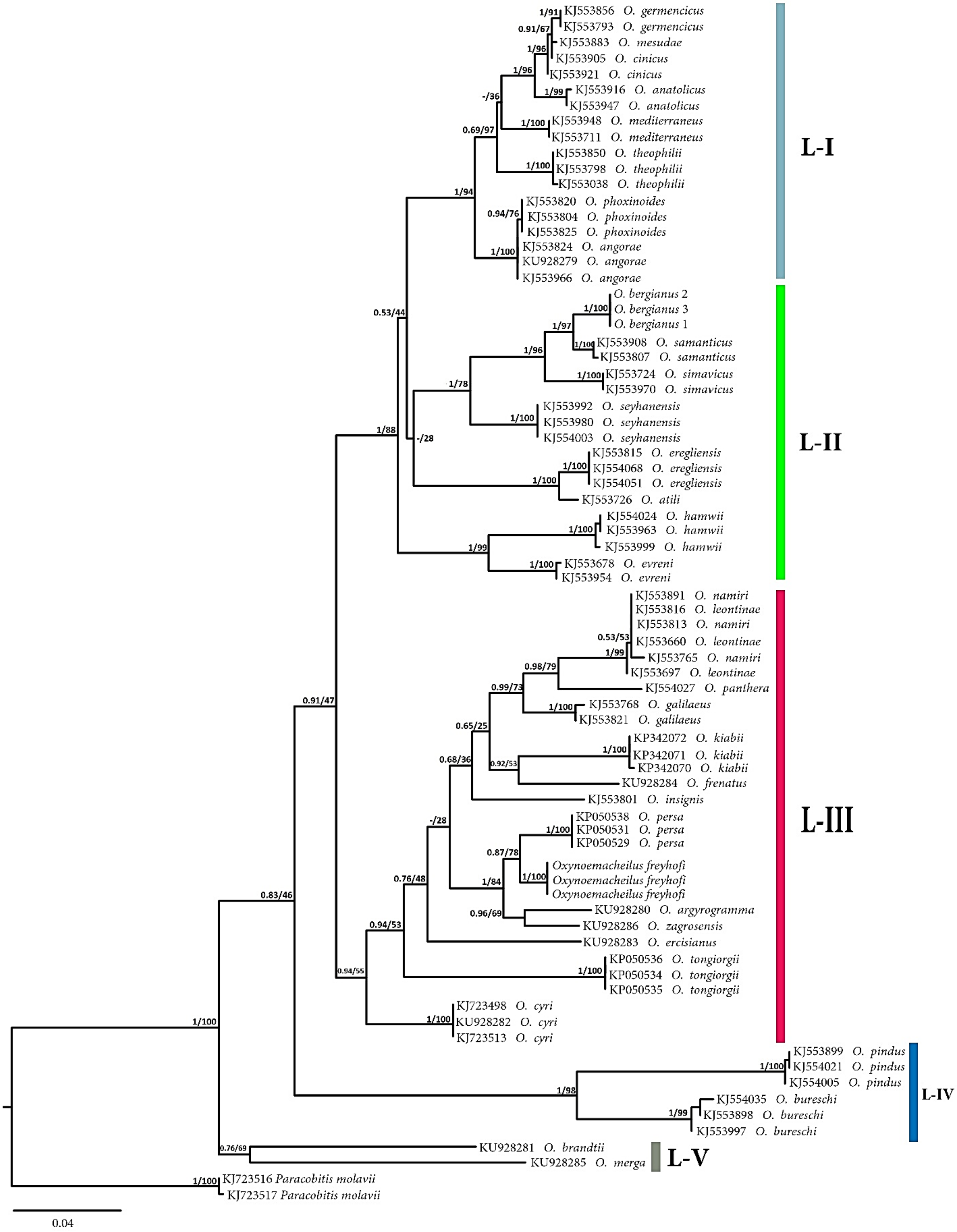

Figure 8. Maximum likelihood consensus tree inferred from COI data. Bayesian posterior probabilities followed by Maximum likelihood bootstrap values are listed above the nodes.

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Oxynoemacheilus bergianus: IMNRF-UT-1035, 11, 41-70 mm SL, Iran: Markazi prov.: Saveh-Hamedan road at Khalife kandy village, Mazlaghan Chay River, Namak basin, 35°09'43.2"N 49°36'44.2"E, 15 May 2016, A. Jouladeh-Roudbar, A. Rahmani, M. A. Jahazi & R. Rahbar-zare. - IMNRF-UT-1040, 4, 37-45 mm SL, Iran: Gilan prov.: near Gisum town, Sefid-rud River, Caspian Sea basin, 37°14'00.0"N 49°51'00.0"E, 20 Jun 2014, S. Eagderi.

Oxynoemacheilus brandtii: IMNRF-UT-1030, 20, 51-73 mm SL, Iran: Ardebil prov.: Near Meshkin shahr at Ghadirlo village, Qara Su River, Caspian Sea basin, 38°34'48.5"N 47°53'12.2"E, 5 July 2016, S. Eagderi, & A. Jouladeh-Roudbar.

Oxynoemacheilus chomanicus: IMNRF-UT-1028, 4, 51-73 mm SL, Iran: Kurdistan prov.: Near Bane, at Boein Sofla village, Bioein River, Tigris basin, 35°56'31"N 45°56'36"E, 5 July 2016,S. Eagderi, H. Pourbagher, V. Etemad, P. Jalili, T. Hosseinpour & A. Jouladeh-Roudbar. - IMNRF-UT-1041, 1, 71 mm SL, Iran: Kurdistan prov.: Near Bane, Sabadloo village, Choman River, Tigris basin, 35°56'31"N 45°56'36"E, 5 July 2016, S. Eagderi, H. Pourbagher, P. Jalili, T. Hosseinpour & A. Jouladeh-Roudbar. - IMNRF-UT-1044, 3, 55-73 mm SL, Iran: Kurdistan prov.: Near Bane at Sabadloo village, Choman River, Tigris basin, 36°01'25"N 45°56'55"E, 5 July 2016, S. Eagderi, H. Pourbagher, P. Jalili, T. Hosseinpour & A. Jouladeh-Roudbar.

Oxynoemacheilus frenatus: IMNRF-UT-1034, 13, 31-63 mm SL, Iran: Kermanshah prov.: Near Direh, at Goleyn village, Goleyn River, Tigris basin, 34°16'09"N 45°56'29"E, 25 July 2016, S. Eagderi & A. Jouladeh-Roudbar.

Oxynoemacheilus kiabii: IMNRF-UT-1025, 15, 50-70 mm SL, Iran: Kermanshah prov.: Near Songhor, at Hossein Abad village, Dinevar River, Tigris basin, 34°33'51.8"N 47°25'43.1"E, 15 Sep 2016, T. Hosseinpour, A. Soleymani & A. Jouladeh-Roudbar.

Oxynoemacheilus kurdistanicus: IMNRF-UT-1042, 4, 34-49 mm SL, Iran: Kurdistan prov.: Near Bane, at Bariso village, Bariso River, Tigris basin, 36°00'22.6"N 45°53'54.4"E, 5 July 2016,S. Eagderi, H. Pourbagher, P. Jalili, T. Hosseinpour & A. Jouladeh-Roudbar.

Oxynoemacheilus persa: IMNRF-UT-1033, 21, 34-49 mm SL, Iran: Fars prov.: Near Shiraz at Chame-Sohrab Khani village, Kor River, Kor basin, 30°28'03.6"N 52°05'59.4"E, 1 Aug 2014, S. Eagderi & H. Mossavi-Sabet.

Oxynoemacheilus zagrosensis: IMNRF-UT-1028, 8, 40-55 mm SL, Iran: Kurdistan prov.: Near Bane at Boein Sofla village, Bioein River, Tigris basin, 35°56'31"N 45°56'36"E, 5 July 2016, S. Eagderi, H. Pourbagher, P. Jalili, T. Hosseinpour & A. Jouladeh-Roudbar.

Oxynoemacheilus tongiorgii: ZM-CBSU H1844, 8, 31-58 mm SL; Iran: Fars prov.: Ghadamgah spring at Dorudzan, Kor basin, 30°14'19.65"N 52°22'23.3"E. M. Masoudi, G. Sayyadzade & H.R. Esmaeili.

Oxynoemacheilus longipinnis: ZM-CBSU H1168, 20, 28-48 mm SL; Iran: Ilam prov.: Meymeh River at Zarab, 33°08'25.6"N 46°55'42.6"E; H.R. Esmaeili, M. Masoudi, 12 Sep. 2012. - ZM-CBSU H1968, 5, 42-50 mm SL; Iran: Ilam prov.: Godarkhosh River, Tigris River tributary; S. Vatandoust.

Material used in the molecular genetic analysis: *Oxynoemacheilus bergianus*: Fin clip. Iran: Gilan prov.: near Gisum town, Sefid-rud River, Caspian Sea basin, 37°14'00.0"N 49°51'00.0"E, 20 Jun 2014, S. Eagderi. (GenBank accession numbers: KX946209, KX946210, KX946211).

Oxynoemacheilus freyhofi: Fin clip; Iran: Roudbar River, tributary of Bakhtiyari River, near Kazem Abad village, 33°08'17.8"N 49°40'43.9"E, S. Eagderi & A. Jouladeh-Roudbar, 19 August 2015 (GenBank accession numbers: KX946206, KX946207, KX946208).

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