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Re-description and molecular systematics of *Paraschistura delvarii* (Teleostei: Nemacheilidae)

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Abstract. *Paraschistura delvarii* Mousavi-Sabet & Eagderi, 2015 was originally described based on six specimens collected from the Mond River drainage of the Persian Gulf basin. However, based on examination of types and newly collected materials, it is clear that *P. delvarii* was mis-described. Here we re-describe *P. delvarii* based on correct morphological characters and molecular data set and provide its distinguishing characters with the sympatric loaches, *P. naumanni*, *P. nielseni* and *Oxynoemacheilus persa*.

Key words: Nemacheilid loach, COI, Persian Gulf basin, Sympatricity, mis-description.

Introduction

Nemacheilid loaches are the largest group within loaches senso lato that inhabit diverse freshwater habitats of Eurasia and the northernmost parts of Africa (Kottelat 1998, 2001, 2004, Kottelat & Lim 1993, Tan 2006, Kottelat & Freyhof 2007). In Iran they are distributed in almost all drainage basins and some of them are found in sympatry (Esmaeili et al. 2010, 2014, 2017, Freyhof et al. 2014, 2015) as seen in the Mond River, Persis sub-basin of the Persian Gulf. In some localities of the Mond River drainage there are four sympatric nemacheilid species including *Paraschistura delvarii*, *P. naumanni*, *P. nielseni* and *Oxynoemacheilus persa*. Researchers have problems in description of loaches due to their small size and interspecies similarity in meristic data despite intraspecies dissimilarity in color patterns. So the systematic position of some genera and species of the loaches is still unsettled and many taxa are artificial assemblages (Kottelat 2004). Here we present some molecular and morphological evidences for mis-description of the newly described species *P. delvarii* based on a pre-approved and more improved molecular character and re-describe it with a taxonomic review of other loaches in the Mond River drainage.

Material and Methods

After anesthesia by 1% clove solution, fishes were fixed in 5% formaldehyde and later stored in 70% or directly fixed in 96% ethanol and were deposited in the Zoological Museum, Collection of Biology Department, Shiraz University (ZM-CBSU). Measurements were made with dial calipers and recorded to 0.1 mm. 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".

Abbreviations used: SL, standard length; HL, head length; GUIC, Collection of the Ichthyology Museum, Department of Fisheries Sciences, Faculty of Natural Resources, the University of Guilan, Guilan province, Iran; NMW, Naturhistorisches Museum, Wien; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz; VMFC, Vatandoust and Mousavi-Sabet Fish Collection, Tehran.

DNA extraction and PCR: Genomic DNA was extracted using Macherey & 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'CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5'TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC) and FR1d_t1 (5'CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA) (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'CAGGAAACAGCTATGAC).

Molecular data analysis: We used 60 sequences from Freyhof et al. (2015) and Sayyadzadeh et al. (2016) and an additional 15 sequences in this study. Data processing and sequence assembly was done in BioEdit (Hall 1999) and the ClustalW algorithm (Higgins and Sharp 1988) was used to create a DNA sequence alignment. Modeltest (Posada & Crandall 1998), implemented in the MEGA 7 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 cutoff. 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 CAT approximation of rate heterogeneity and fast bootstrap to explore species phylogenetic affinities. Bayesian analyses of nucleotide sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because overparametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck & 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.

Bayesian inference of phylogeny was conducted for 6,000,000 generations. Seven hundred bootstrap replicates were used as ML branch support values. The posterior probabilities equal/higher than 0.95 and bootstrap supports equal/higher than 70% were considered as strong support values (Toussaint et al. 2015). MEGA 7 was also used to compute intra-clade and inter-clade K2P genetic distances. As appropriate out-group to root the constructed phylogenetic hypothesis we included the *Cobitis avicennae* KP050525.

Results

We compared 15 COI barcode sequences of loaches from the Mond River drainage with 60 sequences from our last studies (mentioned above). 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 (Fig. 1).

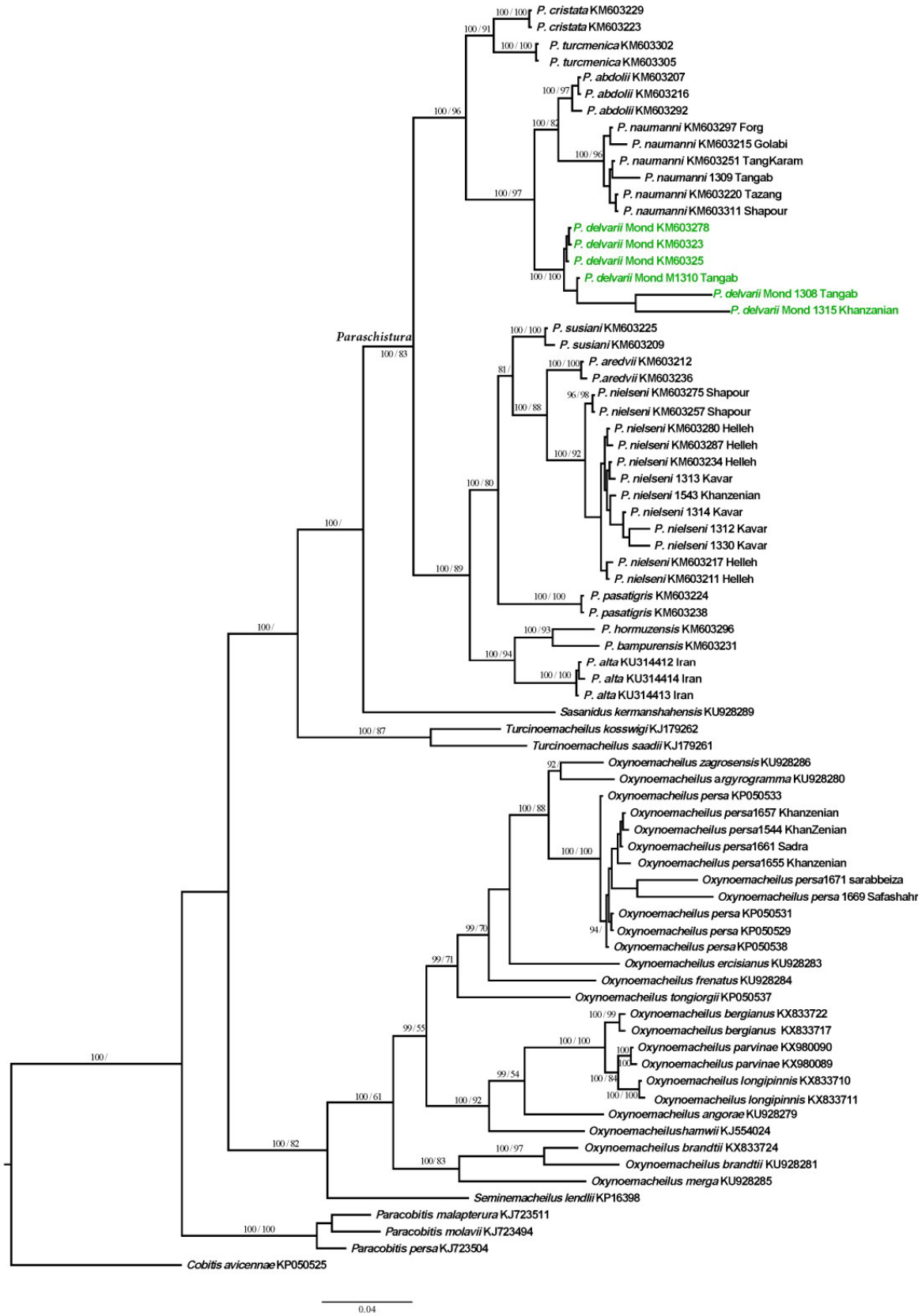

Figure 1. Maximum Likelihood and Bayesian phylogeny reconstructed based on 617 bp of COI 5′ end. The values beside the branches before and after a slash are BI posterior and ML bootstrap probability values, respectively.

Table 1. Diagnostic nucleotide substitutions found in mtDNA COI of loach species in the Mond River drainage (*O. persa*, n=12; *P. delvarii*, n=6; *P. naumanni*, n=6; *P. nielseni*, n=13).

Nucleotide position relative to *Oryzias latipes* complete mitochondrial genome (AP004421)																				
Nucleotide position	5571	5572	5575	5578	5581	5616	5628	5637	5643	5652	5664	5667	5679	5682	5691	5700	5706	5709	5712	5724
O. persa	C	A	A	G	G	T	A	T	C	C	G	A	T	C	G	C	C	T	G	A
P. delvarii	T	C	T	T	T	C	T	C	T	T	A	G	C	A	G	C	A	A	G	C
P. naumanni	T	C	C	G	T	C	T	C	T	T	A	G	C	G	G	C	A	A	A	C
P. nielseni	T	C	C	G	T	C	T	C	T	T	A	G	C	G	A	T	G	A	G	C
Nucleotide position	5736	5739	5748	5751	5754	5766	5772	5778	5781	5785	5787	5790	5796	5802	5805	5811	5814	5817	5820	5829
O. persa	C	G	T	C	G	T	T	C	T	C	T	C	A	C	C	G	C	G	C	G
P. delvarii	T	A	C	C	A	C	G	T	T	T	A	C	C	T	T	G	G	A	T	G
P. naumanni	T	A	C	C	A	C	A	T	T	C	A	C	C	T	T	G	A	G	T	G
P. nielseni	T	A	C	T	A	C	A	T	C	C	A	T	C	G	T	A	G	G	T	A
Nucleotide position	5835	5847	5850	5853	5860	5862	5865	5868	5871	5874	5887	5895	5904	5910	5925	5929	5931	5958	5961	5967
O. persa	A	C	A	G	T	G	C	C	A	G	C	C	T	G	C	C	T	A	T	G
P. delvarii	G	T	A	A	C	G	T	T	G	G	T	T	G	G	A	T	G	C	T	A
P. naumanni	G	T	A	A	C	G	C	T	A	A	T	T	A	G	A	T	G	C	C	A
P. nielseni	G	T	G	A	C	A	C	T	A	T	T	T	A	A	A	T	A	T	T	A

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

	O. persa	*P. delvarii*	*P. naumanni*	*P. nielseni*
O. persa				
P. delvarii	20			
P. naumanni	17	7		
P. nielseni	18	13	11	

Table 1 lists the diagnostic nucleotide substitutions and Table 2 lists the average estimates of the evolutionary divergence in the 617 base pairs long mtDNA COI barcode region between the loach species from the Mond River drainage.

Key to Nemacheilid loach fishes of the Mond River drainage:

1a- No prominent black spot at base of unbranched dorsal fin-rays and sometimes on first and second branched rays; a dark brown blotch or saddle present at dorsal-fin origin, extending on dorsal fin; lateral line complete; 8 ½ branched dorsal fin rays ..... *O. persa*

1b- Prominent black spot at base of unbranched dorsal fin-rays and sometimes on first and second branched rays; lateral line incomplete; 6 ½ -7 ½ branched dorsal fin rays 2

2a- Male with suborbital groove ..... *P. nielseni*

2b- Male without suborbital groove 3

3a- Distance between anus and the end tip of pelvic fin 7.2-10.5% SL, no distinct bars on the flank especially in small specimens ..... *P. delvarii*

3b- Distance between anus and the end tip of pelvic fin 4.2-7.1% SL, distinct bars on the flank. ..... *P. naumanni*

Re-description of *Paraschistura delvarii*

Mousavi-Sabet & Eagderi, 2015 (Figs 2-7)

Material examined: VMFC PSD1-H: (Holotype), 38.0 mm SL; Iran: Fars prov.: upstream of Mond River, Mond River drainage, the Persian Gulf basin, 29°40'22" N, 52°08'57" E, 13 August 2013, H. Mousavi-Sabet & S. Eagderi. —VMFC PSD1-P1 to VMFC PSD1-P5: 5 specimens, 27.2 – 42.1 mm SL; same data as holotype. —GUIC PSD1-P6 and GUIC PSD1-P7, 2 specimens, 31.1 – 35.2 mm SL; same data as holotype. —ZM-CBSU J3304, 9, 36.9-50.7 mm SL; Iran: Fars prov.: Firuzabad city, near Tangab dam, Qareh Aqaj River, a tributary of Mond River drainage, Persis Basin, 28°57'48.11''N 52°33'15.54''E; G. Sayyadzadeh, A. Khajeh Panah, E. Izadi and A. Danesh Nia, 08 Oct 2014. —ZM-CBSU H2038,1, 38.2 mm SL; Iran: Fars prov.: Shiraz, Khanzenian, Qareh Aqaj River, Mond River drainage, 29°41'13.0"N, 52°02'58.6"E; G. Sayyadzadeh, H. Darvish Nia, 24 July 2014.

Material used in the molecular genetic analysis: ZM-CBSU M1308, M1310; Iran: Fars prov.: Firuzabad, near Tangab dam, Qareh Aqaj River, Mond River drainage, 28°57'48.11''N 52°33'15.54''E (GenBank accession number: KY808477, KY808478). —ZM-CBSU M1315; Iran: Fars prov.: Shiraz, Khanzenian, Qareh Aqaj River, Mond River drainage, 29°41'13.0"N, 52°02'58.6"E (GenBank accession number: KY808479).

Diagnosis: *Paraschistura delvarii* is distinguished from all other species of *Paraschistura* in Iran except *P. naumanni* by having the pelvic-fin origin situated below or slightly in front of the vertical of the dorsal-fin origin (vs. behind dorsal-fin origin in other species). *Paraschistura delvarii* is distinguished from *P. naumanni* by having: distance between anus and the end tip of pelvic fin 7.2-10.5% SL (vs. 4.2-7.1% SL) and no distinct bar on the flank especially in small specimens (vs. presence). It is distinguished from *P. abdolii*, *P. cristata*, *P. kessleri* and *P. turcmenica* by having the body fully covered by scales (vs. scales absent on the back and on the flank in front of the dorsal-fin origin in *P. abdolii* and *P. cristata* and scales completely absent in *P. kessleri* and *P. turcmenica*). *Paraschistura delvarii* is distinguished from *P. alta* by having a deeply forked caudal fin (vs. emarginated) and from *P. cristata* by the absence of a long dorsal adipose crest (vs. presence) and an incomplete lateral line (vs. complete). *Paraschistura delvarii* is distinguished from *P. bampurensis* and *P. hormuzensis* by the absence of a suborbital flap in males (vs. presence) and from *P. nielseni* by the absence of a suborbital groove in males (vs. presence).

Among loach fishes studied for molecular characters in the Mond River drainage, *P. delvarii* is characterized by 9 fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region studied (Table 1) and a K2P nearest-

Figure 2. *Paraschistura delvarii*, ZM-CBSU J33012, 45.4 mm SL; Iran: Qareh Aqaj, Mond basin.

Figure 3. *Paraschistura delvarii*, ZM-CBSU J33012, 45.4 mm SL; head (above) and caudal (below) regions; Iran: Qareh Aqaj, Mond basin.

Figure 4. *Paraschistura delvarii*, a, ZM-CBSU J3304, 50.7 mm SL; b, ZM-CBSU J33011, 47.8 mm SL; c, ZM-CBSU J33010, 37 mm SL; Iran: Qareh Aqaj River, Mond basin.

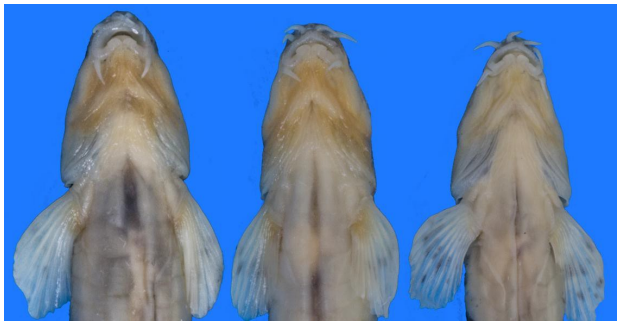

Figure 5. *Paraschistura delvarii*, head region, a, ZM-CBSU J3304, 50.7 mm SL; b, ZM-CBSU J33011, 47.8 mm SL; c, ZM-CBSU J33010, 37 mm SL; Iran: Qareh Aqaj River, Mond basin.

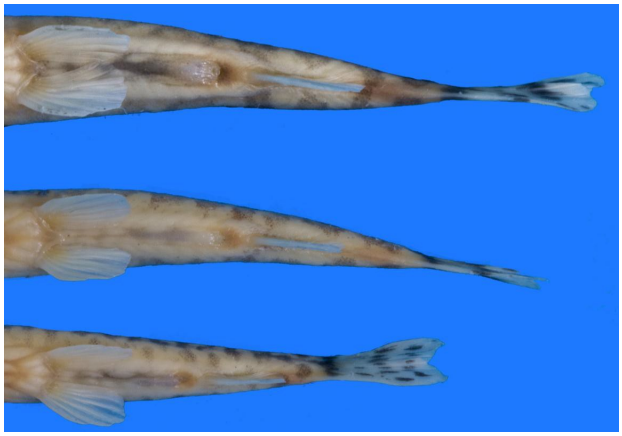

Figure 6. *Paraschistura delvarii*, caudal region, a, ZM-CBSU J3304, 50.7 mm SL; b, ZM- CBSU J33011, 47.8 mm SL; c, ZM-CBSU J33010, 37 mm SL; Iran: Qareh Aqaj River, Mond basin.

Figure 7. *Paraschistura delvarii*, ZM-CBSU H2038, 38.2 mm SL; Iran: Qareh Aqaj River at Khanzenian, Mond basin.

neighbour distance of 7% to *P. naumanni* (Table 2).

Description: For general appearance see Figures 2-7; morphometric data are provided in Table 3. Small sized, moderately elongate species with short head. Body deepest at or slightly in front of dorsal-fin origin, depth moderately decreasing towards caudal-fin base. No hump at nape. Greatest body width at pectoral-fin base. Section of head roundish, flattened on ventral surface. Caudal peduncle compressed laterally, 1.2-1.9 (mean 1.5) times longer than deep. Pectoral fin reaching approximately 43-55 % of distance from pectoral-fin origin to pelvic-fin origin. Pelvic axillary lobe ovoid, fully attached to body or absent. Pelvic-fin origin below or slightly in front of vertical of dorsal-fin origin. Pelvic fin reaching to a point about 2.5-3.0 eye diameter

Table 3. Morphometric data of *Paraschistura delvarii* ZM-CBSU J3304, n=9).

	Min	Max	Mean	SD
Standard length (mm)	36.8	50.6	52.4	
In percent of standard length				
Head length	21.2	23.3	22.2	0.7
Body depth at dorsal-fin origin	13.1	16.4	14.5	1.1
Body width at dorsal-fin origin	9.9	13.0	11.2	1.0
Predorsal length	52.2	55.6	54.5	1.2
Postdorsal length	35.3	40.4	37.2	1.5
Prepelvic length	52.6	55.8	54.2	1.3
Preanal length	73.0	81.7	79.7	2.6
Distance between pectoral and pelvic-fin origins	30.2	33.1	31.4	1.0
Distance between pelvic and anal-fin origins	23.4	26.9	25.4	1.2
Depth of caudal peduncle	8.7	11.2	10.2	0.7
Length of caudal peduncle	13.7	19.1	15.6	1.7
Dorsal-fin depth	11.3	17.8	14.4	1.7
Pectoral fin length	13.3	16.7	15.4	1.0
Pelvic fin length	12.6	14.7	13.6	0.6
In percent of head length				
Head depth at nape	51.6	63.0	56.2	3.6
Head depth at eye	44.3	53.5	49.5	2.9
Snout length	37.3	41.5	39.0	1.7
Eye diameter	14.1	16.3	15.1	0.8
Postorbital distance	46.9	51.1	49.5	1.4
Maximum head width	59.6	70.0	64.7	3.3
Interorbital width	29.1	32.8	31.1	1.3

in front of anus. Anal-fin origin about one eye diameter behind anus. Anal-fin origin at vertical or behind middle between dorsal- and caudal-fin origins. Very short and shallow dorsal and ventral adipose keels on caudal peduncle in some individuals. Margin of dorsal fin straight or slightly convex. Caudal fin emarginated. Largest known specimen 51 mm SL. Dorsal fin with 6-7 ½ branched rays. Anal fin with 5 ½ branched rays. Caudal fin with 15-16 branched rays. Pectoral fin with 10-11 and pelvic fin with 6-7 branched rays. Back and flank covered by embedded scales especially in post-dorsal part and few in pre-dorsal. Lateral line incomplete, reaching to a point slightly in front of dorsal-fin origin or below dorsal-fin base. Anterior nostril opening at the end of a low, pointed and flap-like tube. Nostrils separated by a narrow space, anterior nostril slightly overlapping (or not in some individuals) posterior nostril when folded backwards. No suborbital flap or groove. Mouth small, strongly arched (Figs 3, 5). Lips moderately thick, with many deep furrows. A median interruption in lower lip. Upper lip with median incision. Barbels short, inner rostral barbel not reaching to base of maxillary barbel; outer one reaching to base of maxillary barbel. Maxillary barbel reaching vertical of anterior part or middle eye.

Coloration: Body is cream yellow with dark to pale brown marbled pattern on flank and back. There are a series of connected blotches on flank, most obvious as bars post-dorsally. Post-dorsal with 3-7 dark-brown bars irregularly shaped and set (indistinct bars in small individuals) as wide as or wider than interspaces. An obvious black spot at the base of unbranched and first branched dorsal-fin rays. An obvious black bar at caudal-fin base. Upper part of head, opercula and snout covered by dark-brown small blotches, cheeks pale with few dark-brown dots on top. Dorsal and caudal fins with black spots and stripes on rays, pectoral fin with few dark-brown or black spots, anal and pelvic fins hyaline.

Distribution: *Paraschistura delvarii* is known from the Qareh Aqaj River, a tributary of the Mond River drainage, Persis sub-basin which drains into the Persian Gulf.

***Paraschistura naumanni* Freyhof, Sayyadzadeh, Esmaeili, Geiger 2015** (Figs 8-9)

Material examined: See Freyhof et al. (2015).

Material used in the molecular genetic analysis: ZM-CBSU M1309; Iran: Fars prov.: Firuzabad, near Tangab dam, Qareh Aqaj River, Mond River drainage, 28°57'48.11''N 52°33'15.54''E (GenBank accession number: KY808480).

Figure 8. *Paraschistura naumanni*, ZM-CBSU J2941, holotype, 35 mm SL; Iran: Golabi spring.

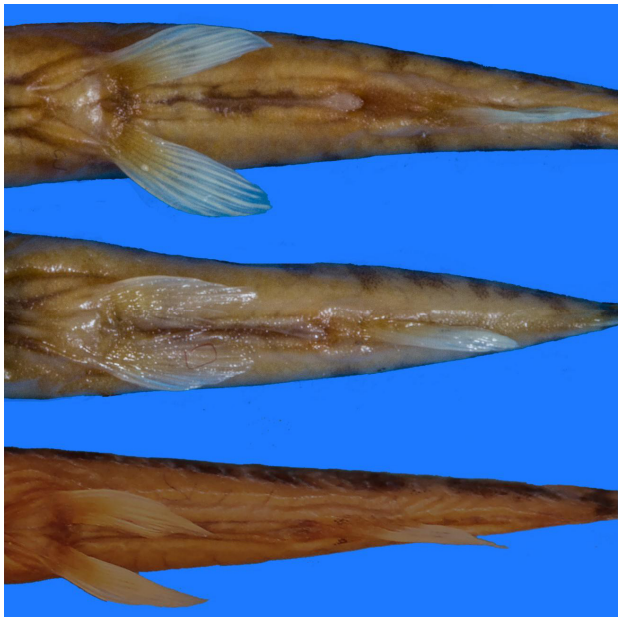

Figure 9. *Paraschistura naumanni*, paratypes; caudal region, Golabi spring; a, ZM-CBSU J2948, 48 mm SL; b, ZM-CBSU J2944, 38 mm SL; c, ZM-CBSU J2941, 35 mm SL.

Diagnosis: *Paraschistura naumanni* is distinguished from the other loach species in the Mond River drainage except *P. delvarii* by having the pelvic-fin origin situated below or slightly in front of the vertical of the dorsal-fin origin (vs. behind dorsal-fin origin). *Paraschistura naumanni* is distinguished from *P. delvarii* by having: distance between anus

and the end tip of pelvic fin 4.2-7.1% SL (vs. 7.2-10.5% SL) and having distinct bars on the flank (vs. absence). It is distinguished from *P. nielseni* and *O. persa* by the absence of a suborbital groove in males (vs. presence).

Among loach fishes studied for molecular characters in the Mond River drainage, *P. naumanni* is characterized by four fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region studied (Table 1) and a K2P nearest-neighbour distance of 7% to *P. delvarii* (Table 2).

***Paraschistura nielseni* (Nalbant & Bianco, 1998)** (Fig. 10)

Material examined: ZM-CBSU H2039, 12, 23-46.8 mm SL; Iran: Fars prov.: Shiraz, Khanzenian, Qareh Aqaj River, Mond River drainage, 29°41′13.0″N, 52°02′58.6″E; G. Sayyadzadeh, H. Darvish Nia, 24 July 2014. —ZM-CBSU H2073, 15, 34.5-53.9 mm SL; Iran: Fars prov.: Shiraz, Kavar, Qareh Aqaj River, Mond River drainage, 29°10'55.10"N 52°41'32.80"E; G. Sayyadzadeh, H. Darvish Nia, M. Masoudi, 23 July 2014.

Figure 10. *Paraschistura nielseni*, a, ZM-CBSU H2039,43.5 mm SL; b, ZM-CBSU H2040,35.7 mm SL Iran: Qareh Aqaj River at Khanzenian, Mond basin.

Material used in the molecular genetic analysis: —ZM-CBSU M1543; Iran: Fars prov.: Shiraz, Khan zenian, Qareh Aqaj River, Mond River drainage, 29°41′13.0″N, 52°02′58.6″E (GenBank accession number: KY808481). —ZM-CBSU M1312, M1313, M1314, M1330; Iran: Fars prov.: Kavar, Qareh Aqaj River, Mond River drainage, 29°10'55.10"N 52°41'32.80"E (GenBank accession number: KY808482, KY808483, KY808484, KY808485).

Diagnosis: *Paraschistura nielseni* is distinguished from the other loach species in the Mond River drainage except *O. persa* by having a suborbital groove in males (vs. absence). It is distinguished from *O. persa* by having a prominent black spot at base of unbranched dorsal-fin rays and sometimes on first and second branched rays (vs. absence) ; lateral line incomplete (vs. complete) and 6½ -7½ branched dorsal fin rays (vs. 8½). For distinguishing from the other congeners see Freyhof et al. (2015).

Among loach fishes studied for molecular characters in the Mond River drainage, *P. nielseni* is characterized by 16 fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region studied (Table 1) and a K2P nearest-neighbour distance of 11% to *P. naumanni* (Table 2).

Remarks: In a review of the genus *Paraschistura* in Iran, It was mentioned that there is an undescribed species in the Mond River drainage without giving a description (Freyhof et al. 2015), so Mousavi-Sabet & Eagderi (2016) referred to this molecular data and described it as *P. delvarii*. But we could not match our data with the description of *P. delvarii*; for example, they gave the presence of sub-orbital groove in male (vs. absence in our materials). We checked all of the type specimens in Gilan University, none of them have a sub-orbital groove. It seems that Mousavi-Sabet & Eigderi (2016) confused *P. delvarii* with *P. nielseni* or even with *O. persa* as all of them are sympatric. Our data shown that these three loach species, with *P. naumanni*, are found in the same localities in the Mond River (Figs 13-14).

***Oxynoemacheilus persa* (Heckel, 1848)** (Figs 11-12)

Cobitis Persa Heckel, 1847

Orthrias farsicus Nalbant and Bianco, 1998

Material examined: NMW-48567 (Holotype), 47.6 mm SL; Iran: Fars prov.: Springs at Persepolis. —ZM-CBSU H2051, 22, 27.5-53.4 mm SL; Iran: Fars prov.: Shiraz, Khanzenian, Qareh Aqaj River, Mond River drainage, 29°41′13.0″N, 52°02′58.6″E; G. Sayyadzadeh, H. Darvish Nia, 24 July 2014. —ZM-CBSU H2088, 2, 51.8-52.5 mm SL; Iran: Fars prov.: Shiraz, Kavar, Qareh Aqaj River, Mond River drainage, 29°10'55.10"N 52°41'32.80"E; G. Sayyadzadeh, H. Darvish Nia, M. Masoudi, 23 July 2014. —ZM-CBSU H1852, 17, 34-56mm SL; Fars prov.: Ghadamgah spring at Dorudzan, Kor basin, 30°14'19.65"N 52°22'23.3"E. —ZM-CBSU H1869, 98, 24-71mm SL; Iran: Fars prov.: Archin Qant at Safashahr, Kor basin, 30°36'16.9"N 52°56'40.1"E.

Figure 11. *Oxynoemacheilus persa*, ZM-CBSU H2051, 53.4 mm SL; Iran: Qareh Aqaj River at Khanzenian, Mond basin.

Figure 12. *Cobitis persa* NMW-48567 Holotype.

Material used in the molecular genetic analysis: ZM-CBSU M1661; Iran: Fars province: Shiraz, Sadra, Lake Maharlu basin, 29°44'47.80"N, 52°26'48.47"E (GenBank accession number: KY808486). —ZM-CBSU M1544, M1655, M1657; Iran: Fars province: Shiraz, Khanzenian, Qareh Aqaj River, Mond River drainage, 29°41′13.0″N, 52°02′58.6″E (GenBank

Figure 13. Qareh Aqaj River at Kavar, a tributary of Mond River drainage.

Figure 14. Qareh Aqaj River at Khanzenian, a tributary of Mond River drainage.

accession number: KY808487, KY808488, KY808489). —ZM-CBSU M1669; Iran: Fars province: Safashahr, Archin Qanat, Kor River basin, 30°36'16.9"N, 52°56'40.1"E (GenBank accession number: KY808490). —ZM-CBSU M1671; Iran: Fars province: Beiza, Sarab spring, Kor River basin, 29°55'28.95"N, 52°26'42.77"E (GenBank accession number: KY808491).

Diagnosis: *Oxynoemacheilus persa* belongs to a group of *Oxynoemacheilus* having a suborbital groove in males and elongated body. It is distinguished from the other loach species in the Mond River drainage by no prominent black spot at base of unbranched dorsal fin-rays and sometimes on first and second branched rays (vs. presence), lateral line complete (vs. incomplete) and 8½ branched dorsal fin rays (vs. 6½ -7½). It is also diagnosed from the other loach species in the Mond River drainage by 40 fixed (Table 1) diagnostic nucleotide substitutions in the mtDNA COI barcode region, and a K2P nearest-neighbour distance of 17% to *P. naumanni* (Table 2).

Remarks: According to Heckel (1847) the type locality of *Cobitis Persa* is "Quellen um Persepolis". Kähsbauer (1964) reports a syntype of this species in the Naturhistorisches Museum Wien under NMW 48567 (Fig. 12). This specimen is in poor condition and not readily comparable to fresh material (Coad 2017).

Oxynoemacheilus farsicus (Nalbant & Bianco 1998) is the other loach species which has been described from the Kor River basin, but Freyhof et al. (2011) couldn't find any differences between it and *O. persa*, so they considered it as a synonym of *O. persa*. We also didn't find any diagnostic character to distinguish populations oxynoemacheilid fishes from the Kor River, Lake Maharlu and Mond River basins (see Figure 1, *O. persa* clade). Therefore, we treat *O. farsicus* as a synonym of *O. persa*.

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