

Ponticola patimari sp. nov. (Gobiiformes: Gobiidae) from the southern Caspian Sea basin, Iran

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Abstract

Ponticola patimari, new species, is described from the Kheirud River, southern Caspian Sea basin, Iran. It is distinguished from its congeners in Iran by having the following features: D1 VI, D2 I / 15½–18½, A I / 11½–13½; mottled bars on flanks, small scattered spots on pectoral-fin base, cycloid scales covering upper part of opercle, longer least depth of caudal peduncle, longer ventral disc, reaching corner of lip to middle of orbit, longer interorbital width / eye diameter, pelvic fin reaching to anus or even extending beyond anus, two dark brown spots under orbital and 3 fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region. *Ponticola patimari* sp. nov. belongs to *P. syrman* group and it is separated by a minimum K2P distance of 3.0 and 3.4% from *P. iranicus* and *P. syrman*, respectively.

Keywords: Freshwater fish, Taxonomy, Cytochrome oxidase I, Caspian Sea.

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Introduction

The freshwater fish species of the southern Caspian Sea basin comprise 119 species, which Gobiiformes with 38 species form the second most divers order (Esmaili et al. 2014, 2018). This order contains the family Gobiidae with 11 genera in the southern Caspian Sea basin and the genus *Ponticola* is one of the most diverse group with five species, including *P. cyrius*, *P. geobelli*, *P. gorlap*, *P. iranicus* and *P. syrman* (Neilson and Stepien 2009; Esmaili et al. 2018). However, Neilson and Stepien (2009) included *Chasar bathybius* in the genus *Ponticola* based on their molecular analysis, but as they did not include any material of this species nor any discussion of the nominal genus, we do not include it in the genus *Ponticola* based on our unpublished molecular data. The genus *Ponticola* includes 16 species characterized by having well-developed anterior pelvic membrane with acute lateral lobes, dentary with several large conical teeth on its rear part, maxillary with expanded posterior end, and some other craniological features (see Vasil'eva et al. 1993; Miller and Vasil'eva 2003).

Based on Vasil'eva et al. (2015), the members of the genus *Ponticola* have high diversity in the southern Caspian Sea. Therefore, they described *P. iranicus* from this region based morphological and karyological features, also Nikmehr et al. (2018) verified the molecular validity of this species based on COI gene, as a sister species of *P. syrman* with 3.0% K2P genetic distance. During an ichthyological study on the members of the genus *Ponticola* in the southern Caspian Sea basin (Nikmehr et al. 2020), we collected samples of gobies from the Kheirud, Chalous, and Tonekabon rivers that based on morphological and molecular (mtDNA COI gene) data showed differences with other known *Ponticola* species from Iran. Therefore, this study is aimed to describe a new goby species of the genus *Ponticola* from the southern Caspian Sea basin based on our new materials but also considering the data from Iljin (1956), Pinchuk (1976, 1991), Bauchot (1991), Ahnelt and Holčík (1996), Eschmeyer (1996), Ragimov (1998) and Vasil'eva et al. (2015).

Material and Methods

Morphological examinations: The goby specimens were collected using electrofishing device and a small boat

trawler and three specimens per location fixed into 96% ethanol for molecular works and others fixed into 10% buffered formalin, after anesthetizing in 1% clove oil solution. All measurements were made point to point using caliper with precision of 0.1 mm. Methods for counts and measurements followed Armbruster (2012). The last two branched rays articulating on a single pterygiophore in the second dorsal (D2) and anal (A) fins are noted as “1½”. For lateral line system, we used terminology from Miller (1986). For comparative morphological analysis, we calculated mean values and standard deviations for the sample included the holotype and paratypes on all morphometric characters.

DNA extraction and PCR: DNA was extracted from the collected pectoral-fin tissues using a modified phenol-chloroform method (Sambrook et al. 1989). The COI gene was amplified using primers FishF1 - (5' – TCAAC CAACCACAAAGACATTGGCAC - 3') and FishR1 - (5'-TAGACTTCTGGGTGGCCAAAGAATCA - 3') (Ward et al. 2005). Polymerase chain reaction (PCR) conditions were as follows: a 50 µl final reaction volume containing 25 µl of Taq 2X Mastermix red, 1 µl (10 µm) of each primer, 5 µl of total DNA and 18 µl of H₂O. 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 (Bioneer, Inc, Daejeon, Korea). The PCR products were sequenced using Sanger method by a robotic ABI-3130xl sequencer by Macrogen, Inc, Daejeon, Korea. The forward and reverse primers were used to single strand sequencing.

Molecular data analysis: The sequences were compared to published *Ponticola* sequences using (BLASTn) basic local alignment search tool (Altschul et al. 1990). The retrieved sequences of the other members of the genus *Ponticola* from GenBank database (NCBI) following a blast search are shown in Table 1. COI sequences were aligned together with GenBank sequences with CLUSTAL W using default parameters (Larkin et al. 2007). We determined the best-fit model of molecular evolution for the gene dataset using the Bayesian information criterion (BIC) in IQTREE 1.6.0 (Kalyaanamoorthy et al. 2017). The model with the lowest BIC scores (Bayesian Information Criterion) was considered to best describe the substitution pattern (Nei and Kumar 2000; Posada and Crandall 2001). For phylogenetic reconstructions, the datasets were analysed by Bayesian Inference (BI) using MrBayes 3.1.2 (Ronquist et al. 2012) and maximum likelihood (ML) method in IQTREE 1.6.0 (Hoang 2018). MrBayes was run with 6 substitution types (nst = 6) and considered gamma-distributed rate variation across sites and a proportion of invariable sites (GTR) for the COI datasets. For BI, we ran four simultaneous Monte Carlo Markov Chains for 10000000 generations, sample frequency every 1000 generations, chain temperature 0.2. Log-likelihood stability was attained after 10000 generations, and we excluded the first 1000 trees as burn-in. The remaining trees were used to compute a 50% majority rule consensus tree. For ML analyses, we conducted heuristic searches (1000 runs) under TIM2+F+I+G4 model.

The reconstructed ML-based hypothesis of the mitochondrial relationships was used as input to infer putative species boundaries (molecular species delimitation approach) using Poisson Tree Processes (PTP) model introduced by Zhang et al. (2013) and Kapli et al. (2017), respectively, conducted in <http://mptp.h-its.org/#/tree> (accessed July 7 2020). The aim is to find a group delimitation that maximizes the likelihood of the partition of branch lengths. A uniform evolutionary rate (lambda) is used. Uncorrected pairwise genetic distances (p-distances) were investigated based on Kimura two-parameter (K2P) distances (Tamura et al. 2013). As outgroups, *Neogobius melanostoma* (accession numbers: FJ526801) and *Benthophilus abdurahmanovi* (accession numbers: FJ526832) were retrieved from GenBank.

Abbreviations used: TL, total length; SL, standard length; HL, lateral head length; K2P, Kimura 2-parameter. Collection codes: IMNRF-UT, Ichthyological Museum of Natural Resources Faculty, University of Tehran.

Table 1. List of species used for molecular analysis COI and GenBank Accession Number.

species	Drainage	Country	Published by	Genbank Acc. No.
<i>P. ratan</i>	Sea of Azov	Ukraine	Neilso and Stepien, 2009	FJ526848
<i>P. syrman</i>	Danube River, Vilkovce,	Ukraine	Neilso and Stepien, 2009	FJ526829
<i>P. syrman</i>	Danube River, Vilkovce,	Ukraine	Neilso and Stepien, 2009	FJ526830
<i>P. cephalargoides</i>	Dniester River Estuary	Ukraine	Neilso and Stepien, 2009	FJ526813
<i>P. cyrius</i>	Ptsa River	Georgia	Neilso and Stepien, 2009	FJ526845
<i>P. rhodioni</i>	Vostochnyy Dagomys River	Russia	Neilso and Stepien, 2009	FJ526842
<i>P. constructor</i>	Khobi River	Georgia	Neilso and Stepien, 2009	FJ526846
<i>P. kessleri</i>	Dniester River	Georgia	Neilso and Stepien, 2009	FJ526823
<i>P. eurycephalus</i>	Cape Langeron, Odessa Bay	Ukraine	Neilso and Stepien, 2009	FJ526814
<i>P. gorlap</i>	Karpovska Reservoir, Iliovka	Russia	Neilso and Stepien, 2009	FJ526819
<i>P. gorlap</i>	Caspian Sea	Azerbaijan	Neilso and Stepien, 2009	FJ526817
<i>P. gorlap</i>	Caspian Sea	Azerbaijan	Neilso and Stepien, 2009	FJ526818

Table 2. Estimates of evolutionary divergence over sequence pairs between *Ponticola* species.

Species	N	1	2	3	4	5	6	7	8	9	10	11
<i>P. ratan</i>	1											
<i>P. syrman</i>	2	9.8										
<i>P. iranicus</i>	3	10.2	2.9									
<i>P. patimari</i> sp. nov.	4	9.8	3.4	3.0								
<i>P. cyrius</i>	5	9.6	8.8	9.6	9.3							
<i>P. cephalargoides</i>	6	9.6	8.1	8.8	8.6	3.6						
<i>P. constructor</i>	7	9.8	8.3	9.0	8.4	4.9	3.2					
<i>P. platyrostris</i>	8	9.6	8.1	8.8	8.2	4.4	3.6	1.0				
<i>P. rhodioni</i>	9	9.8	7.9	8.7	8.1	4.4	3.7	1.4	1.4			
<i>P. gorlap</i>	10	10.0	7.5	8.8	8.2	6.7	6.7	6.9	6.7	6.4		
<i>P. eurycephalus</i>	11	12.3	8.7	9.4	10.1	7.2	7.2	7.9	7.7	7.4	4.9	
<i>P. kessleri</i>	12	11.9	8.3	9.8	9.7	7.2	6.8	7.6	7.4	7.0	4.5	1.3

Results

We were able to generate the COI sequence of four *Ponticola patimari* sp. nov. individuals from the Kheirud, Chalous and Tonekabon rivers, and one *P. gorlap* from Siah River. In addition, we used the COI sequence of *P. iranicus* from Nikmehr et al. (2018) for the Sefid River (selected from the same batch of the paratypes collected with Vasil'eva et al. (2015)). The Bayesian and ML analyses yielded similar topologies with well-supported nodes (Fig. 1). Two methods produced trees with 12 species supported by high posterior probability and bootstrap values (Fig. 1): *P. cephalargoides*, *P. cyrius*, *P. constructor*, *P. eurycephalus*, *P. gorlap*, *P. kessleri*, *P. platyrostris*, *P. ratan*, *P. syrman*, *P. rhodioni*, *P. patimari* sp. nov. and *P. iranicus*. *Ponticola patimari* sp. nov. appears related to *P. syrman* group and it is separated by a minimum K2P distance of 3.0 and 3.4% from *P. iranicus* and *P. syrman* (two other members of this group), respectively (Table 2). The PTP model-based species delimitation approaches using the ML topology detected 13 entities (P = 0.001, Null-model score: 96.750792, best score for single coalescent rate: 104.072120.) (Fig. 1). *Ponticola patimari* sp. nov. from Kheirud River is also distinguished from its congeners by having 3 fixed diagnostic nucleotide substitutions in the mtDNA COI barcode region.



Figure 1. Maximum likelihood estimation of the phylogenetic relationships based on the mitochondrial COI barcode region. Nucleotide positions with less than 95% site coverage were eliminated. Values at nodes correspond to BI posterior probability/ML bootstrap (Solid bars right to the specimen labels indicate species delimitation results from PTP except outgroups).

Ponticola patimari, new species

(Table 3, Figs. 2-4)

Holotype: IMNRF-UT-1088, male, 79.1 mm TL, 63.5 mm SL, Iran: Mazandaran prov.: Caspian Sea basin, Kheirud River at Najar-deh Village, 36°36'46"N; 51°34'03"E.

Paratypes: IMNRF-UT-1087, 1 female, 7 males, 48.8-79.1 mm TL, 38.1-76.7 SL, same data as holotype.

Additional materials: IMNRF-UT-1086, 8 females, 42.6-76.7 mm TL, 39.8-6.0 SL, Iran: Mazandaran prov.: Caspian Sea basin, Chalous River at Chalous, 36°38'48"N, 51°24'43"E. – IMNRF-UT-1089, 8 females, 3 males., 47.8-69.2 mm TL, 37.4-58.0 SL, Iran: Mazandaran prov.: Caspian Sea basin, Tonekabon River, 36°44'02"N, 50°50'42"E.

Diagnosis: A species of genus *Ponticola*; D1 VI, D2 I / 15½–18½, A I / 11½–13½; nape scaled completely, scales cycloid, cycloid scales covering upper part of opercle; lateral line system with sub-orbital row *d* continuous; predorsal area uniform; first dorsal fin with 2-3 oblique dark-brown stripe some brown spots on base of pectoral-fin. Two dark brown spots under orbital. Brown hypens between genipores (δ , τ , θ , ρ , β).



Figure 2. *Ponticola patimari*, sp. nov. holotype; IMNRF-UT-1088, male, 79.1 mm TL, Iran: Kheirud River.

Description of holotype: D1 VI, D2 I / 16½, A I / 11½; l.l. 50. Body laterally compressed, gradually tapering from head to tail; caudal peduncle relatively elongated, minimum body depth almost 1.4 times in caudal peduncle; first and second dorsal fins almost touching, second dorsal fin having uniform height; head relatively high and short, its length 1.3 times in SL, its width slightly larger than depth; cheeks slightly swollen; nape scaled completely, scales cycloid; cycloid scales cover upper part of opercle; jaws slightly protruding; upper lip slightly swollen, with sharpened end at end of mouth gap; pelvic disc elongated, its length about 0.4 ventro-anal, sometimes reaching anus; anterior pelvic membrane with well-developed pointed lateral lobes, their length about 3.8 times in membrane length; pectoral fins reaching base of second dorsal unbranched ray and sometimes reaching anus; caudal fin rounded.

Coloration of preserved holotype yellowish brown flanks molted brown bands; irregular dark brown spots on yellowish brown background; predorsal area uniform, with numerous small dark brown tiny spots; clear line of dark brown yellowish longitudinal stripes along midline of flanks; ventral, pectoral and anal fins yellowish-grey; light edging present along outer margin of second dorsal, ventral, anal, and caudal fins; second dorsal-fin with some brown strips blotches; three blotches scattered on first dorsal-fine strips; short dark brown spots on upper part of pectoral fin base. Brown hyphens between genipores (δ , τ , θ , ρ , β). Two dark grey spots under orbital. Small scales. Morphometric characters presented in Table 2.

Table 3. Morphometric characters (ranges, means, and standard deviation) of type specimens of *Ponticola patimari* sp. nov.

Character	Holotype + Paratype (n= 9)		
	Holotype	Paratypes	
		Range	Mean ± SD
total body length	79.1	42.6-79.1	59.7±12.43
standard body length	63.5	39-64.0	50.1±9.24
number of anal fin branched rays	11.5	11.5-13.5	12.0±0.72
number of the second dorsal fin branched rays	16.5	15.5-18.5	16.6±0.78
In percent of SL			
maximum body depth	21.2	18.2-24.0	20.8±1.6
least depth of caudal peduncle	11.3	10.5-11.8	11.3±0.5
predorsal distance	34.3	26.3-37.9	32.7±2.7
length of the second dorsal fin base	33.0	30.6-38.3	35.6±2.2
height of the second dorsal fin	16.3	8.6-16.3	12.3±2.3
preanal distance	54.0	52.1-61.0	55.7±2.4
length of anal fin base	18.2	18.2-28.8	25.1±2.8
length of pectoral fin	21.2	18.2-29.4	23.0±3.0
length of ventral disc	21.2	20.2-26.0	22.2±1.6
caudal peduncle length	16.6	15.6-26.1	20.0±3.1
width of caudal peduncle at the anal fin	5.6	3.4 -10	7.15±1.8
minimum width of caudal peduncle	3.3	2.1-6.8	3.8±1.3
head length	31.0	25.2-31.0	28.1±2.0
In percent of HL			
horizontal diameter of eye	25.8	15.5-39.4	23.1±6.1
preorbital distance	30.9	23.9-49.0	33.0±6.1
postorbital distance	52.7	45.0-61.4	54.3±4.3
interorbital distance	31.4	20.5-42.7	32.4±6.2
width of upper lip	42.1	38.1-57.2	46.5±4.6
head depth at nape	52.7	45.4-92.9	55.1±7.1
head width	68.5	67.6-81.2	74.9±5.0

Extended description based on all type specimens: Overall meristic characteristics: D1 VI, D2 I / 15½–18½ (16.6), A I / 11½–13½; l.l. 43-51; Specimens of Kheirud River with 16½ branched dorsal rays (66%) and 11½ branched anal rays (66%); in Chalous River, specimens with 16½ dorsal rays (83%) and 11½ anal rays were predominant (50%); in Tonekabon River, majority of specimens have 15½ dorsal rays (75.0%) and 11½ anal rays (62.0%). Majority of specimens from Chalous River with 49–53 scales in lateral series and in Tonekabon River 44–56 scales.

Examined specimens with relatively short body, laterally compressed tapering toward caudal peduncle; minimum body depth 1.5–1.10 times in caudal peduncle length; head relatively deep and short, its length 3.7–3.9 times in SL; head width slightly larger than depth; interorbital distance short, lower jaw slightly protruded, upper and low jaws same length; upper lip slightly swollen; pelvic disc sometimes reaching anus or even extending beyond anus in smaller specimens. In Chalous River, largest specimen a female with 64.0 mm SL 76.7 mm TL; in Kheirud River, largest one a female with 63.5 mm SL, and 79.1 mm TL; in Tonekabon River, largest one a male with 58.0 mm SL and 68.4 mm TL.

Coloration: Examined specimens after fixation yellowish brown some molted bands on glanks; fins grey with many tiny spots on pectoral, pelvic-disc and pelvic fin; belly covered by scattered black spots; predorsal area uniform, brown; upper part of pectoral fin base with some brown spots; 2-3 clear oblique brown stripe on first and second dorsal-fins. Two dark brown spots under orbital. Brown hypens between genipores (δ , τ , θ , ρ , β).

Head lateral-line system: *Ponticola patimari* sp. nov. is almost similar to other species in the genus *Ponticola* (Fig. 4): infers orbital neuromast organs composed of six transverse rows, four (1-4) before and two (5s, 6s) above hyomandibular row *b*, two (C₁-C₂) under PN and lacking row *a*. Row 6 consist of several anterior



Figure 3. *Ponticola patimari*, sp. nov. IMNRF-UT-1086-2, male, 69.6 mm TL; Iran: Chalous River.

oculoscapular pore α . Rows $5i$ and $6i$ separated, with row $5i$ well behind anterior and hyomandibular row b and row $6i$ short of row b , sub-orbital longitudinal row d consist of two parts; anterior $d1$ oblique, following border of upper lip and reaching below anterior origin of $d2$, and posterior longitudinal row $d2$. Anterior and posterior oculoscapular canals and preopercular canals present with pores σ , λ , κ , α , β , ρ , θ , τ , and δ , respectively. Anterior oculoscapular pore ρ and posterior oculoscapular pore θ well-separated.

Etymology: The species is dedicated to Dr. Rahman Patimar for his long and outstanding contributions in biological studies of Iranian fishes.

Distribution: The new species is found in rivers of the southern Caspian Sea basin, including Kheirud, Chalous and Tonekabon rivers.

Habitat: *Ponticola patimari* sp. nov. inhabits rivers with rapid-normal current and bottom covered by larger stones.

Comparative remarks. *Ponticola patimari* sp. nov. is distinguished from *P. gorlap* by having mottled bars on flanks (vs. irregular mottled colour pattern of cheeks, opercula, and flanks), small spots on pectoral-fin bases (vs. dark black blotch on pectoral-fin base), possessing 2-3 dark blotches on anterior part of first dorsal-fin (vs.



Figure 4. Diagram of head lateral-line sensory system in *Ponticola patimari* sp. nov.; pores are designated by Greek letters, rows of neuromasts (genipores/ sensory papillae) by Latin letters and Arabic numerals; AN, anterior nostril, PN, posterior nostril.

absent), longer maximum body depth (18.2-24.0 vs. 15.0-18.1 % SL), longer least depth of caudal peduncle (10.5-11.8 vs. 7.8-9.4% SL) and longer ventral disc (20.2-26 vs. 17.0-20.0 % SL).

Ponticola patimari sp. nov. differs from *P. syrman* by having corner of lip reaching middle of orbit (vs. reaching end of orbit or passing it), less branched rays in second dorsal-fin (15½–18½ vs. 14½–15½), longer least depth of caudal peduncle (10.5-11.8 vs. 9.3-10.4 % SL), longer ventral disc (20.2-26.0 vs. 19.0-20.0% SL) and shorter preanal distance (52.1-61.0 vs. 61.3-64.4 % SL).

Ponticola patimari sp. nov. is distinguished from *P. cyrius* by having a convex shaped membrane of pelvic disc (vs. concaved shaped), brown molted bars on predorsal area (vs. marbled coloration of predorsal area), reaching corner of lip to middle of orbit (vs. not reaching to orbit), longer least depth of caudal peduncle (10.5-11.8 vs. 9.4-10.0 % SL) and shorter head width (67.6-81.2 vs. 81.4-100.0 % SL).

Ponticola patimari sp. nov. differs from *P. geobelii* by having longer interorbital width/eye diameter (1.1-1.3 vs. 0.4-0.6 % SL); more spines in first dorsal fin (5 vs. 5-7 usually 6), pelvic fin reaching to anus or even extending beyond anus (vs. almost reaches anal fin or extends beyond anal fin origin), upper lip width/lateral preorbital width (1-1.1 vs. 0.4-0.67 % SL), ration of caudal peduncle depth to its length (half vs. almost equals).

Ponticola patimari sp. nov. is distinguished from *P. iranicus* by having less branched dorsal-fin rays (15½–18½ vs. 15½–16½), longer least depth of caudal peduncle (10.5-11.8 vs. 8.9-10.0 % SL), small scale (vs. large scale), six transverse rows (vs. seven transverse rows) of cephalic lateral line, pectoral fine reaching to second dorsal fin (vs. pectoral fine not reaching to second dorsal fin).

Comparative materials: *Ponticola cyrius*: IMNRF-UT-1092, 47.4-84.6 mm SL, 58.5-102.9 mm TL, 13, Turkey: Caspian Sea basin, Kura River, 41.17°56'61"N; 42.94°91'79"E.

Ponticola gorlap: IMNRF-UT-1091, 68.1-112.8 mm SL, 80.6-134.2 mm TL, 10, Iran: Caspian Sea basin, Siah River, Mazandaran prov.: Ghaem Shahr, 36°26'35"N; 52°53'47"E.

Ponticola iranicus: IMNRF-UT-1090, 35.6-86.1 mm SL, 47.8-101.2 mm TL, 10, Iran: Caspian Sea basin, Sefid River, Guilan prov.: Rostam Abad, 36°53'29"N; 48°30'48"E.

Ponticola syrman: IMNRF-UT-1093, 55.7-71.6 mm SL, 69.3-85.4 mm TL, 4, Iran: Caspian Sea basin, Golestan Prov.: Gharesoo Village. 36°49'37"N; 54°02'34"E.

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