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Three new species of *Turcinoemacheilus* from Iran and Turkey (Teleostei: Nemacheilidae)

Hamid Reza Esmaeili*, Golnaz Sayyadzadeh*, Müfit Özulug, Matthias Geiger*** and Jörg Freyhof*****

Three new species of *Turcinoemacheilus* are described from Iran and Turkey raising the number of species in this genus to six, five of them in the Middle East. *Turcinoemacheilus bahaii*, new species, from the Zayandeh River in Iran is distinguished by having the anus situated in a posterior position and a dark brown blotch on each side of the anal-fin base in both sexes. *Turcinoemacheilus minimus*, new species, from the upper Euphrates drainage in Turkey is distinguished by having the anus situated in an anterior position, a slender and long caudal peduncle and a very small maximum size (up to 38 mm SL). *Turcinoemacheilus saadii*, new species, from the Karoun drainage in Iran is distinguished by having the anus situated in an anterior position and an unique colour pattern of large dark brown saddles. In the Middle East, all *Turcinoemacheilus* species are well distinguished by molecular characters and show between 5.3 and 8.5 % K2P sequence divergence in their COI barcode region.

Introduction

Since the description of the Zagros dwarf loach *Turcinoemacheilus kosswigi* from headwaters of the Great Zab River in Turkey (Bănărescu & Nalbant, 1964), this genus was so rarely recognized that we learned only recently about its distribution. Breil & Bohlen (2001) published the first records of *Turcinoemacheilus* from the Euphrates drainage (now referred to *T. minimus*), Golzarianpour et al. (2009) first reported the genus from Iran (now referred to *T. hafezi*), Golzarianpour et al. (2013) recorded *T. kosswigi* from Sirvan, Great and Little Zab rivers in Iraq and described *T. hafezi* from Iran. This scarcity of records is surprising, since loaches of the genus *Turcinoemacheilus* are now known to be very widespread, often common, and easy to distinguish from all other nemacheilids in the Middle East by the pelvic fin origin situated in front of the dorsal fin origin and an anterior position of the anus, a particular colour pattern and a very slender body (Bănărescu & Nalbant, 1964; Freyhof et al., 2011; Golzarianpour et al., 2013). While *Turcinoemacheilus* was a mono-

* Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran. E-mail: esmaeili@susc.ac.ir, golnaz_sayad@yahoo.com
** Istanbul University, Science Faculty, Department of Biology, 34134 Vezneciler, İstanbul, Turkey. E-mail: mozulu@istanbul.edu.tr
*** Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Adenauerallee 160, 53113 Bonn, Germany. E-mail: joerg.freyhof@idiv.de

typic genus for 47 years, two new species have been described in the last years (Conway et al., 2011; Golzarianpour et al., 2013). There were already indications that more undescribed species might exist in the Middle East. Breil & Bohlen (2001) observed that the examined fishes from the Tigris drainage were relatively large, but those from the Euphrates were much smaller. Golzarianpour et al. (2013) found two sympatric species of *Turcinoemacheilus* in the Karoun drainage, one with a more anterior and one with a more posterior position of the anus. They did not identify the species with the more anterior position of the anus. Finally, fishes superficially similar to *T. hafezi* were discovered in the Iranian endorheic Zayandeh drainage. In this study, the morphological characters as well as the colour patterns of all these populations is analysed, and we also sequenced their COI barcode region. These data together supported the view that five species of *Turcinoemacheilus* are present in the Middle East, three of them still undescribed. We describe these three new *Turcinoemacheilus* based on combined findings from morphology and molecular genetic characters.

Material and methods

After anaesthesia, all fishes were fixed in 5 % formaldehyde and stored in 70 % ethanol. Measurements were made with dial calliper and recorded to 0.1 mm. All measurements are made point to point, never by projections. Methods for counts and measurements follow Kottelat & Freyhof (2007). Standard length (SL) is measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle is measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are noted as "1½". The holotype is included in the calculation of means and SD.

Abbreviations used: SL, standard length; HL, lateral head length; K2P, Kimura 2-parameter; CMK, Collection of Maurice Kottelat, Cornol; FSJF, Fischsammlung J. Freyhof, Berlin; IUSHM, Istanbul University, Science Faculty, Hydrobiology Museum, İstanbul; KU, University of Kansas Natural History Museum and Biodiversity Research Center, Lawrence; ZFMK, Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Bonn; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz; and ZMH, Zoologisches Museum, Hamburg.

DNA extraction and PCR. Genomic DNA was extracted using Machery & 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. Data processing and sequence assembly was done in Geneious (Biomatters, 2013) and the Muscle algorithm (Edgar, 2004) was used to create a DNA sequence alignment. Modeltest (Posada & Crandall, 1998), implemented in the MEGA 5 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. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern. We generated neighbor-joining (Saitou & Nei, 1987), maximum parsimony (Swofford, 2002; with PAUP4b) and maximum likelihood phylogenetic trees with 1000 bootstrap replicates to explore species phylogenetic affinities. Screening for diagnostic nucleotide substitutions was performed manually from the resulting sequence alignment.

Results

Maximum Likelihood based estimation of the phylogenetic relationships based on the mitochondrial COI barcode region place the sequenced fishes into five groups (Fig. 1), which show between 5.3 and 8.5 % K2P sequence divergence in their COI barcode region. Two of these groups correspond to the described species *T. kosswigi* and *T. hafezi*. The other three groups correspond to the new species described below. A map with all records of *Turcinoemacheilus* species in the Middle East is shown in Figure 2.

Key to species of *Turcinoemacheilus* in the Middle East

1 – Anus situated behind middle between pelvic-fin and anal-fin origins. 2
– Anus situated at or in front of middle between pelvic-fin and anal-fin origins. 3

2 – An elongated, irregularly shaped dark blotch on sides of anal-fin base; anal-fin origin situated at vertical of tip of dorsal fin when adpressed to body. .....*T. bahaii*
– No dark blotch on side of anal-fin base; anal-fin origin situated behind vertical of tip of dorsal fin when adpressed to body. .....*T. hafezi*

3 – Lateral stripe or row of blotches absent along lateral midline, 7–9 distinct dark saddles on body. .....*T. saadii*
– Prominent row of dark brown blotches along lateral midline, usually fused into a lateral stripe. 4

4 – Standard length up to at least 53 mm; caudal peduncle depth 1.9–2.2 times in its length. .....*T. kosswigi*
– Standard length up to at least 38 mm; caudal peduncle depth 2.6–3.2 times in its length. .....*T. minimus*

Turcinoemacheilus bahaii, new species
(Figs. 3–5)

Holotype. ZM-CBSU 7193B, 52.2 mm SL; Iran: Esfahan prov.: Zayandeh River between Azadegan and Qalee Shahrokh, 32°40'54" N 50°27'47" E; H. R. Esmaeili, A. Gholamifard, G. Sayyadzadeh, B. Parsi, R. Zamanian Nejad, S. Mirghiasi & M. Ghasemeian, 16 Aug 2011.

Paratypes. ZM-CBSU 7192B, 1, 38.6 mm SL; ZM-CBSU 7194B–7208B, 15, 24.1–53.9 mm SL; same data as holotype. – ZM-CBSU 7209B–7221B, 13, 26.2–45.1 mm SL; FSJF 3449, 4, 28.9–33.8 mm SL; Iran: Esfahan prov.: Zayandeh River at Eskandari village, 32°49'26" N 50°25'47" E; H. R. Esmaeili, A. Gholamifard, G. Sayyadzadeh, B. Parsi, R. Zamanian Nejad, S. Mirghiasi & M. Ghasemeian, 16 Aug 2011.

Additional material (non types). Material used in the molecular genetic analysis: ZM-CBSU T550F, T551F; GenBank accession numbers: KJ179246, KJ179247; same data as holotype.

Diagnosis. *Turcinoemacheilus bahaii* is the only species of *Turcinoemacheilus* having a dark brown blotch on each side of the anal-fin base (Fig. 6) (vs. absent) and it is the only species in which the anal-fin origin is situated at a vertical of the tip of the dorsal fin when adpressed to the body (vs. anal-fin origin clearly behind vertical of tip of dorsal fin). It is further distinguished from all other species of *Turcinoemacheilus* in the Middle East except *T. hafezi* by the more posterior position of the anus (distance from anus to anal-fin origin 0.1–0.4 times in distance from pelvic-fin to anal-fin origins). Compared to all its four congeners in the Middle East, *T. bahaii* is characterized by five fixed nucleotide substitutions in the mtDNA COI barcode region studied (Table 1).

Description. See Figures 3–5 for general appearance and Table 2 for morphometric data of holotype and 29 paratypes. Small, very slender and roundish bodied species with short head. Body deepest at about pelvic-fin base, depth decreasing towards caudal-fin base. No hump at nape. Greatest body width at pectoral-fin base or at middle between pectoral- and pelvic-fin bases, body almost equally wide until dorsal-fin origin. Section of head roundish, flattened on ventral surface. Caudal peduncle compressed laterally, 1.8–2.5

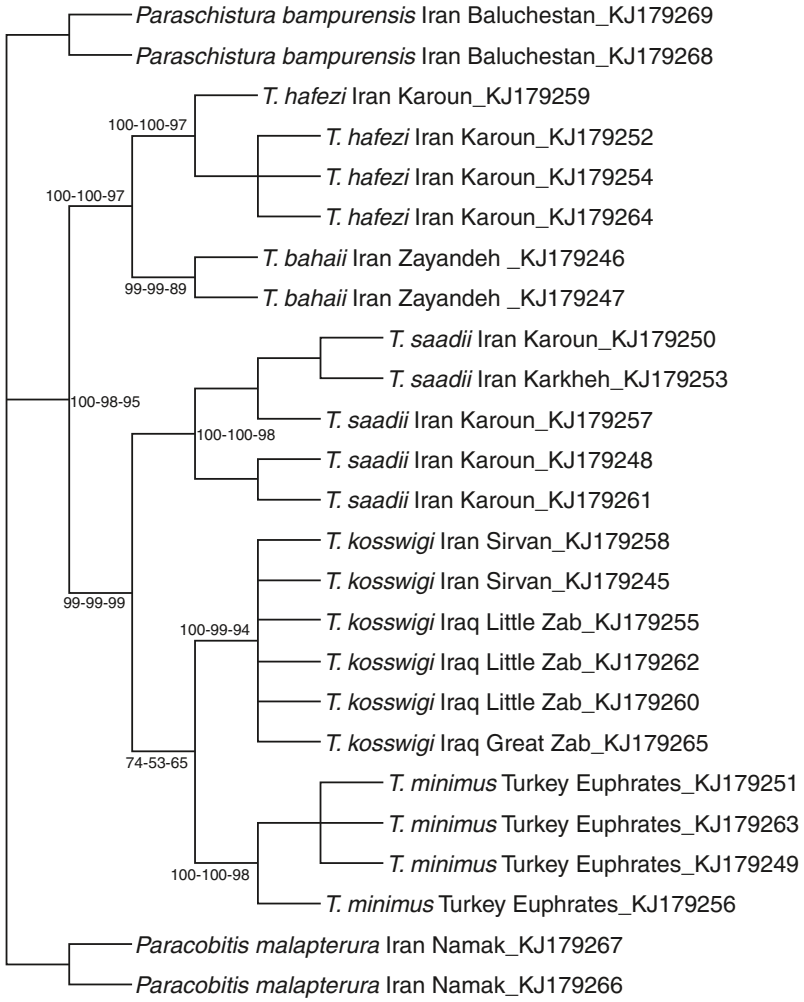

Fig. 1. Maximum Likelihood (ML) estimation of the phylogenetic relationships based on the mitochondrial COI barcode region. Nucleotide positions with less than 95 % site coverage were eliminated before analysis. Numbers of major nodes indicate bootstrap values from the Neighbour joining-, Maximum Parsimony-, and Maximum Likelihood method from 1000 pseudo-replicates. Branch lengths are not drawn to scale.

Table 1. List of the diagnostic nucleotide substitutions for the Middle-East species of *Turcinoemacheilus* found in the mtDNA COI barcode region. Nucleotide position is given with reference to the complete mitochondrial genome of *Oryzias latipes* (GenBank accession number AP004421). The * denotes and amino acid change from isoleucine to valine.

codon position	5529 3	5553 3	5593 3	5601 3	5673 3	5707 1	5715 3	5736 3	5766 3	5775 3	5793 3	5844 3	5850 3	5862 3	5874 3	5901 3	5904 3	5910 3	5919 3	5955 9	5980 1	6000 3	6012 3	6027 3	6042 3	6051 3	6066 3	6069 3
T. hafezi (n=4)	G	T	C	C	C	C	T	C	C	C	A	G	A	A	T	A	A	G	T	A	A	A	G	C	T	A	C	A
T. bahaii (n=2)	G	T	C	C	C	C	T	C	C	C	A	G	A	A	T	A	**G**	G	**C**	**G**	A	A	**A**	C	**C**	A	C	A
T. kosswigi (n=6)	G	T	C	C	C	C	T	C	C	A	A	G	A	A	T	A	A	C	A	A	A	C	G	C	T	A	C	C
T. minimus (n=4)	**A**	T	C	C	C	C	T	**T**	**T**	**T**	A	G	A	**C**	T	A	A	C	T	A	**G***	**T**	G	C	T	**G**	**T**	**G**
T. saadii (n=5)	G	**C**	**T**	**T**	**T**	**T**	**C**	C	C	C	**G**	**A**	**G**	A	**C**	**G**	A	**A**	A	A	A	C	G	**T**	T	A	C	A

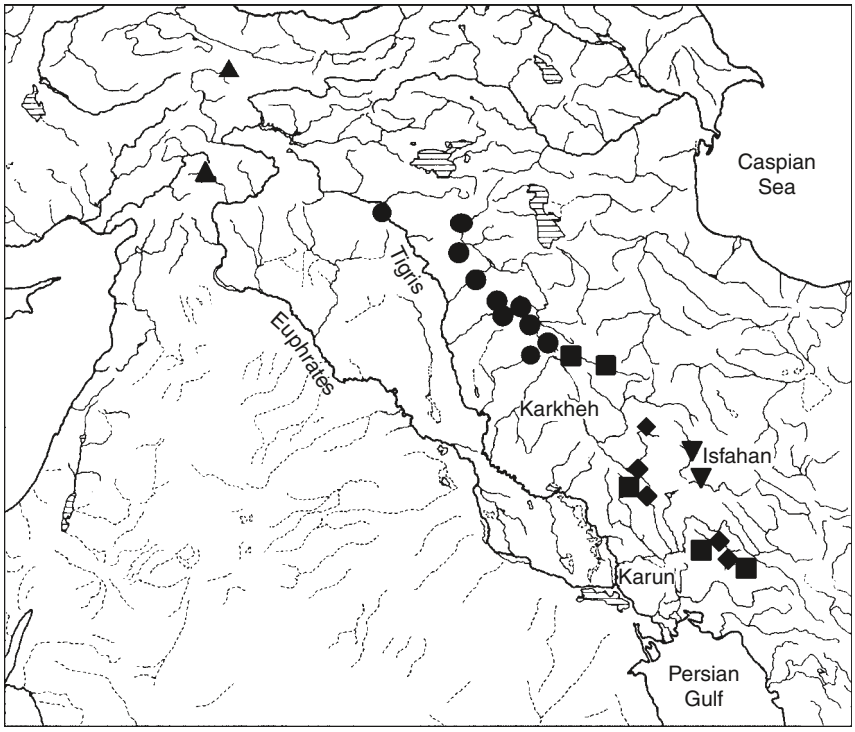

Fig. 2. Records of *Turcinoemacheilus* species in the Middle East. ▲, *T. minimus*; ●, *T. kosswigi*; ■, *T. saadii*; ◆, *T. hafezi*; ▼, *T. bahaii*.

(mean 2.2) times longer than deep. Pectoral fin reaching approximately 50 % of distance from pectoral-fin origin to pelvic-fin origin. Pelvic axillary lobe present, fully attached to body. Pelvic-fin origin distinctly in front of dorsal-fin origin. Pelvic fin reaching slightly beyond or not reaching to anus. Distance from anus to anal-fin origin 0.1–0.4 times in distance from pelvic-fin to anal-fin origins. Anal-fin origin at vertical of tip of dorsal fin when adpressed to body. Anal fin reaching beyond middle of caudal peduncle. No adipose crest on caudal peduncle. Margin of dorsal fin straight. Caudal fin emarginated. Largest known specimen 53.9 mm SL.

Dorsal fin with 7½ branched rays. Anal fin with 5½ branched rays. Caudal fin with 8+8 branched rays. Pectoral fin with 8–10 (usually 8) and pelvic fin with 6 branched rays. Body without scales. Lateral line incomplete, with 12–33 pores, not reaching to dorsal-fin origin. Anterior nostril opening on anterior side of a low, pointed and flap-like tube. Nostrils separate, posterior tip of anterior nostril not reaching posterior nostril when folded backwards. No suborbital flap or groove in males. Mouth small, slightly arched (Fig. 7a). Lips moderately thick. A median interruption in lower lip. Upper lip without median incision. Processus dentiformis small and blunt. No median notch in lower jaw. Barbels short, inner rostral barbel not reaching base of maxillary barbel; outer one reaching slightly beyond base of maxillary barbel, not reaching vertical of anterior margin of eye. Maxillary barbel reaching vertical of middle of eye. No external sexual dimorphism observed.

Coloration. In alcohol, body pale yellow with pale or dark brown mottled colour pattern. Irregular blotches on body forming irregularly set and shaped bars in some individuals or blotches more prominent along lateral midline. Flank below a line between pectoral- and pelvic-fin bases without pigmentation. An irregularly shaped, dark brown or black bar at posterior extremity of caudal peduncle, usually most prominent at the middle. In front of this bar, a whitish or yellowish irregular quadratic or triangular patch. Cheeks and ventral surface of head

Fig. 3. *Turcinoemacheilus bahaii*, ZM-CBSU 7193B, holotype, 52.2 mm SL; Iran: Zayandeh River.

Fig. 4. *Turcinoemacheilus bahaii*, Iran: Zayandeh River; paratypes; **a,** ZM-CBSU 7194B, 49.1 mm SL; **b,** ZM-CBSU 7192B, 41.7 mm SL; and **c,** ZM-CBSU 7195B, 38.6 mm SL.

Fig. 5. *Turcinoemacheilus bahaii*, ZM-CBSU 7198b, paratype, 44.1 mm SL; Iran: Zayandeh River.

cream or pale yellow, head above cheeks with many small brown spots and blotches sometimes fused into a marbled pattern or plain brown. An elongated, faint, brown blotch on each side of anal-fin base (Fig. 6). Dorsal and caudal fins hyaline, with elongated spots on rays, forming a mottled pattern of 1–2 dark vertical rows approximately in middle of ray length in dorsal fin and 1–2 vertical rows in caudal fin. Last unbranched dorsal-fin ray black at base, black at about middle of ray and hyaline on posterior part. Anal-, pelvic- and pectoral fins hyaline.

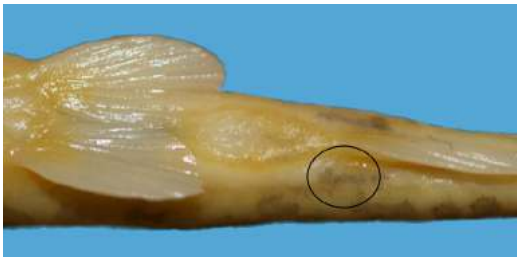

Fig. 6. *Turcinoemacheilus bahaii*, ZM-CBSU 7194B, 49.1 mm SL; diagnostic dark blotch on sides of anal-fin origin.

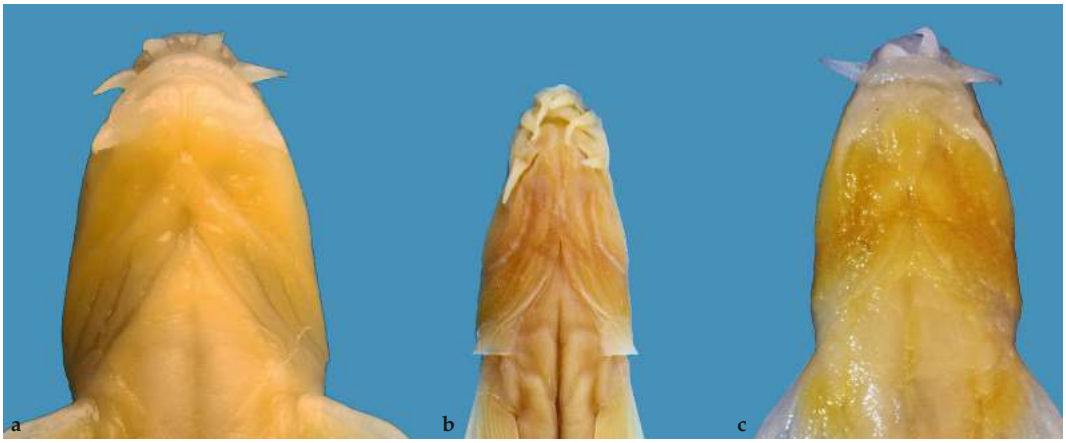

Fig. 7. Ventral view of head of species of *Turcinoemacheilus*. **a,** *T. bahaii*, ZM-CBSU 7197b, paratype, 53.6 mm SL; **b,** *T. minimus*, IUSHM 2013-1050, holotype, 35.2 mm SL; and **c,** *T. saadii*, ZM-CBSU 7170b, paratype, 46.8 mm SL.

Fig. 8. Zayandeh River between Azadegan and Qalee Shahrokh, Iran; type locality of *Turcinoemacheilus bahaii*.

In life, body creamy whitish with very faint, pale brown colour patterns.

Distribution and habitat. *Turcinoemacheilus bahaii* is known from the Zayandeh River which is an endorheic river in Central Iran flowing from the Zagros Mountains to the Gavkhoni wetlands. At the type locality (Fig. 8) the river is about 20 m wide, the substrate consists of coarse gravel and boulders and the water is fast-running.

Etymology. The species is named for Bahā' al-Dīn Muḥammad ibn Ḥusayn al-'Āmilī (also known as Shaykh-i Bahā'ī, Persian: شیخ بهایی) a scholar, philosopher, architect, mathematician, astronomer and poet in 16th century Iran.

Remarks. The phylogenetic tree reconstruction (Fig. 1) suggests that *T. bahaii* is closely related to *T. hafezi* from the Karoun and Dez drainages, both flowing to the deltaic area of the Arvand River or Shatt al Arab, which is the lowermost part of the joint Euphrates and Tigris drainages. Both species are superficially similar in body shape and colour pattern, and are separated by 5.3 % K2P distance in the studied COI gene region, which is proposed as a strong indicator that two species are involved (see Herbert et al., 2003). Beside molecular characters, *T. bahaii* is distinguished from *T. hafezi* by having a dark brown blotch on the sides of the anal-fin base (Fig. 6) (vs. absence). It is distinguished from *T. hafezi*, *T. kosswigi* and *T. saadii* by the anal fin situated in a more anterior position, at a vertical of the tip of dorsal fin when adpressed to the body (vs. anal-fin origin clearly behind vertical of tip of dorsal fin). *Turcinoemacheilus bahaii* is further distinguished from *T. kosswigi*, *T. minimus* and *T. saadii* by the more posterior position of the anus, which is behind the middle between the pelvic-fin origin and the anal-fin origin (vs. at the middle or in front of the middle). *Turcinoemacheilus bahaii* is distinguished from *T. himalaya* from Nepal by a completely scaleless body (vs. small cycloid scales present on the posterior half of the body).

Table 2. Morphometric data of *Turcinoemacheilus bahaii* (holotype ZM-CBSU 7193B, paratypes, ZM-CBSU 7192B–7208B, ZM-CBSU 7209B–7221B, n = 29).

	holotype	min	max	mean	SD
Standard length (mm)	52.2	24.1	53.9		
In percent of standard length					
Head length	20.4	20.4	25.2	23.0	1.3
Body depth at dorsal-fin origin	12.3	11.5	16.2	13.5	1.2
Prepectoral length	17.8	17.8	24.7	21.6	1.7
Predorsal length	53.9	36.4	57.5	54.4	3.6
Postdorsal length	35.0	30.1	36.6	34.2	1.4
Preanal length	72.8	66.7	74.2	71.1	1.2
Prepelvic length	50.5	49.8	55.4	52.8	1.4
Distance between pectoral and pelvic-fin origins	31.8	29.0	33.9	31.4	1.2
Distance between pelvic and anal-fin origins	21.4	15.5	22.2	19.0	1.7
Distance between vent and anal-fin origin	6.7	2.4	7.9	4.2	1.3
Depth of caudal peduncle	8.8	8.0	10.6	9.0	0.5
Length of caudal peduncle	20.7	17.5	21.6	19.4	1.1
Dorsal-fin depth	14.6	13.9	18.7	16.3	1.4
Anal-fin base length	7.5	5.3	8.1	6.9	0.7
Pectoral-fin length	14.9	14.0	17.7	16.0	1.0
Pelvic-fin length	12.2	11.2	14.0	12.0	0.8
In percent of head length					
Head depth at eye	46	42	53	47	2.8
Snout length	43	31	44	39	2.8
Eye diameter	13	11	17	14	1.5
Postorbital distance	53	42	55	48	3.0
Maximum head width	63	55	64	61	2.7
Interorbital width	25	21	32	28	3.1

Turcinoemacheilus minimus, new species
(Figs. 9–11)

Holotype. IUSHM 2013-1050, 35.2 mm SL; Turkey: Adıyaman prov.: Upper Göksu, 5 km northeast of Gölbaşı, 37°50.22' N 37°41.09' E; M. Özuluğ & J. Freyhof, 18 June 2008.

Paratypes. IUSHM 2013-1051, 5, 32.1–35.3 mm SL; FSJF 2454, 4, 33–38 mm SL; same data as holotype.

Additional material (non types). CMK 16824, 3, 25.7–30.3 mm SL; Turkey: Erzincan prov.: Karasu near Mercan, 39°45' N 40°14' E; M. Breil, 17 June 1999. – Material used in the molecular genetic analysis: FSJF DNA-968; GenBank accession numbers: KJ179249, KJ179251, KJ179256, KJ179263; same data as holotype.

Diagnosis. *Turcinoemacheilus minimus* is distinguished from the other species of *Turcinoemacheilus* in the Middle East by a combination of characters, none of them unique. *Turcinoemacheilus minimus* is superficially similar to *T. kosswigi* and both share the same colour pattern. They are distinguished by the more slender and longer caudal peduncle in *T. minimus* (caudal peduncle length 19.5–21.0 % SL vs. 17.7–19.0 in *T. kosswigi*). *Turcinoemacheilus minimus* is distinguished from *T. hafezi* and *T. bahaii* by the more anterior position of the anus, which is in the middle (or in front) of the distance between the pelvic-fin and the anal-fin origins (vs. behind the middle in *T. hafezi* and *T. bahaii*).

Turcinoemacheilus minimus is also distinguished by its very small size, up to 38 mm SL observed (vs. larger in all other species). Relative to its four congeners in the Middle East, *T. minimus* is characterized by ten fixed nucleotide substitutions in the mtDNA COI barcode region studied (Table 1), one of them non-silent and thus leading to an amino-acid change from isoleucine to valine.

Description. See Figures 9–11 for general appearance and Table 3 for morphometric data of holotype and 5 paratypes. Small, very slender and roundish bodied species with short head. Body deepest at about pelvic-fin base, depth decreasing towards caudal-fin base. No hump at nape. Great-

Table 3. Morphometric data of *Turcinoemacheilus minimus* (holotype IUSHM 2013-1050; paratypes IUSHM 2013–1051; n = 6).

	holotype	min	max	mean	SD
Standard length (mm)	35.2	32.1	35.3		
In percent of standard length					
Head length	20.5	18.1	21.3	19.6	1.1
Body depth at dorsal-fin origin	9.8	9.8	11.6	10.9	0.5
Prepectoral length	20.4	19.0	21.4	20.2	0.7
Predorsal length	58.0	56.8	59.5	58.1	0.8
Postdorsal length	34.8	34.7	36.9	35.8	0.9
Preanal length	73.5	70.4	73.6	72.8	1.2
Prepelvic length	54.2	49.7	54.2	51.9	1.4
Distance between pectoral and pelvic-fin origins	32.0	29.2	32.9	31.1	1.4
Distance between pelvic and anal-fin origins	20.7	20.7	23.3	21.9	1.0
Distance between vent and anal-fin origin	11.5	11.5	15.2	12.8	1.2
Depth of caudal peduncle	7.0	6.3	7.0	6.8	0.3
Length of caudal peduncle	20.1	17.1	21.0	19.7	1.3
Dorsal-fin depth	14.3	12.8	17.0	14.3	1.3
Anal-fin base length	7.4	6.3	9.4	7.6	1.1
Pectoral-fin length	16.7	14.9	16.7	15.7	0.6
Pelvic-fin length	12.8	12.0	13.9	13.0	0.6
In percent of head length					
Head depth at eye	35	35	42	38	2.4
Snout length	39	38	45	42	2.9
Eye diameter	16	16	21	18	1.5
Postorbital distance	47	45	50	48	1.5
Maximum head width	48	49	57	53	2.7
Interorbital width	28	25	29	27	1.4

Fig. 9. *Turcinoemacheilus minimus*, IUSHM 2013-1050, holotype, 35.2 mm SL; Turkey: Göksu River.

Fig. 10. *Turcinoemacheilus minimus*, Turkey: Göksu River: paratypes, IUSHM 2013-1051; **a,** 35.3 mm SL; **b,** 35.0 mm SL; and **c,** 33.3 mm SL.

Fig. 11. *Turcinoemacheilus minimus*, collected with IUSHM 2013-1051, about 35 mm SL; Turkey: Göksu River.

Fig. 12. Upper Göksu River northeast of Gölbaşı, Turkey; type locality of *Turcinoemacheilus minimus*.

est body width at pectoral-fin base or at middle between pectoral- and pelvic-fin bases, body almost equally wide until dorsal fin-origin. Section of head roundish, flattened on ventral surface. Caudal peduncle compressed laterally, 2.6–3.2 (mean 2.9) times longer than deep. Pelvic axillary lobe present, its tip not attached to body. Pelvic-fin origin distinctly in front of dorsal-fin origin. Pectoral fin reaching approximately 50 % of distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin reaching beyond anus. Distance from anus to anal-fin origin 0.5–0.6 times in distance from pelvic-fin to anal-fin origins. Anal-fin origin behind vertical of tip of dorsal fin when adpressed to body. Anal fin not reaching to middle of caudal peduncle. No adipose crest on caudal peduncle. Margin of dorsal fin straight. Caudal fin slightly emarginated. Largest known specimen 38 mm SL.

Dorsal fin with 6–7½ (usually 6½) branched rays. Anal fin with 5½ branched rays. Caudal fin with 8+8 or 8+7 branched rays. Pectoral fin with 8–9 and pelvic fin with 7 branched rays. Body without scales. Lateral line incomplete, with 15–20 pores, reaching beyond tip of pectoral fin but not reaching to dorsal-fin origin. Anterior nostril opening on anterior side of a low, blunt and flap-like tube. Nostrils closely together, posterior tip of anterior nostril reaching into posterior nostril when folded backwards. No suborbital flap or groove in males. Mouth small, slightly arched (Fig. 7b). Lips moderately thick. A median interruption in lower lip. Upper lip without median incision. Processus dentiformis small and blunt. No median notch in lower jaw. Barbels short, inner rostral barbel reaching base of outer rostral barbel; outer one reaching to base of maxillary barbel. Maxillary barbel reaching vertical of anterior part of eye. No external sexual dimorphism observed.

Coloration. In alcohol and life, body pale cream yellow. A row of large irregular, brown, longitudinally elongated blotches along lateral midline, often fused into a prominent irregular lateral stripe. Large, brown saddles on back, connected to lateral blotches along whole body. On predorsal back, saddles sometimes dissociated into a marbled pattern, reaching downwards to stripe. Flank below lateral stripe without pigmentation.

An irregularly shaped, dark brown or black bar at caudal-fin base. In front of this bar, a whitish or yellowish triangular patch on upper and lower caudal peduncle. Cheeks and ventral surface of head cream or pale yellow, head above cheeks plain brown. Dorsal and caudal fins hyaline, with elongated spots on rays, forming 1–2 dark vertical rows, approximately in middle of ray length in dorsal fin and 1–2 vertical rows in caudal fin. Last unbranched dorsal-fin ray hyaline at base, black at anterior half and hyaline on posterior half. Anal-, pelvic- and pectoral fins hyaline.

Distribution and habitat. *Turcinoemacheilus minimus* is known from two localities in the upper Euphrates drainage in Turkey. The Göksu at the type locality of the species (Fig. 12) is about 30 m wide, the substrate consists of coarse gravel and boulders and the water is fast-running.

Etymology. The species is named for its small size. An adjective.

Remarks. The phylogenetic tree reconstruction suggests that *T. minimus* is most closely related to *T. kosswigi* from the Tigris drainage. It had initially been identified as *T. kosswigi* by Breil & Bohlen (2001). The most striking character of *T. minimus*, the very small maximum size (up to 38 mm SL vs. 53 in *T. kosswigi*), was not considered as a difference by Breil & Bohlen (2001), as only five individuals of *T. kosswigi* were known then. When MÖ & JF collected this species again in the Euphrates, the small fishes were considered as juveniles. When fresh materials of *T. kosswigi* became available from the Iraqi Tigris drainage, no such small juveniles were found despite the fact that both samplings were conducted in June and the same fishing device, with a 150 mm anode ring that caught small juvenile cyprinids without problems. When molecular data became available, it turned out that both species are well distinguished. Based on the complete COI barcode fragment mean smallest K2P distance of *T. minimus* is 5.9 % to *T. kosswigi*, its closest relative. While there is discussion about *a priori* thresholds and their use in species delimitation and recognition (e. g. Meier et al., 2006; Srivathsan & Meier, 2012), a 5.9 % distance between two populations is strongly indicative that two species are involved (see Herbert et al., 2003).

Interestingly, the fishes described by Breil & Bohlen (2001) (now CMK 16824) were kept about one year in captivity before they were preserved, and they did not grow during this time (J. Bohlen, pers. comm.). This observation and the absence of larger fishes in our samples suggest that the *Turcinoemacheilus* from the upper Euphrates is a dwarf species. Taking this information and the molecular data together, we do not consider the available material from the upper Euphrates as juveniles and we treat their small size as a diagnostic character distinguishing it from *T. kosswigi*. Beside its small size and molecular characters, *T. minimus* is superficially very similar to *T. kosswigi* and both share the same colour pattern. They are distinguished by several morphometric characters, but as no individuals of similar sizes were available, it cannot be totally excluded that these differences are the result of allometric growth. The most obvious difference is the more slender and longer caudal peduncle in *T. minimus*.

Turcinoemacheilus minimus is distinguished from *T. hafezi* and *T. bahaii* by the more anterior position of the anus, which is in the middle (or in front) of the distance between the pelvic-fin and the anal-fin origins (distance from anus to anal-fin origin 0.5–0.6 times in distance from pelvic-fin to anal-fin origins) while it is behind the middle (0.2–0.4 times) in *T. hafezi* and *T. bahaii*.

Turcinoemacheilus minimus is distinguished from *T. himalaya* by a completely scaleless body (vs. presence of small cycloid scales on the posterior half of body).

Turcinoemacheilus saadii, new species
(Figs. 13–15)

Holotype. ZM-CBSU 7169B, 47.1 mm SL; Iran: Fars prov.: stream Tang-e-Tizab, a tributary to Bashar River which drains to the Karoun, 30°23' 12" N 51°46'50" E; H. R. Esmaeili, G. Sayyadzadeh, M. Masoudi, S. H. Aminaghai, 5 Sep 2012.

Paratypes. ZM-CBSU 7166b-7175B, 9, 44.6–63.6 mm SL; ZM-CBSU 7165B, 1, 45.4 mm SL; same data as holotype.

Additional material (non types). FSJF 1512, 10, 41.4–52.6 mm SL; Iran: Kohrang river at Kaj village, 32°03'16.97" N 50°34'46" E. — FSJF 3232, 2, 30–44 mm

SL; Iran: Kermanshah prov.: Sepidbarg river west of Javanrud, 34°48'21"N 46°27'29"E. – Material used in the molecular genetic analysis: FSJF DNA-1995; Iran: Kermanshah prov.: Sepidbarg river west of Javanrud, 34°48'21"N 46°27'29"E, GenBank accession numbers: KJ179250, KJ179257. – ZM-CBSU T540, T542; same data as holotype, GenBank accession numbers: KJ179248, KJ179261. – ZM-CBSU T524; Iran: Gamasiab, Karkheh, Tigris drainage, 34°23'16"N 47°41'14"E, GenBank accession number: KJ179253.

Diagnosis. *Turcinoemacheilus saadii* is distinguished from the other species of *Turcinoemacheilus* in the Middle East by its unique colour pattern organized in 7–9 distinct dark saddles never forming a lateral stripe (vs. mottled colour pattern or presence of a lateral stripe). It is further distinguished by the combination of a deeply emarginated caudal fin and the distance from anus to anal-fin origin 0.4–0.5 times in the distance from pelvic-fin to anal-fin origins. Relative to its four congeners in the Middle East, *T. saadii* is characterized by 13 fixed nucleotide substitutions in the mtDNA COI barcode region studied (Table 1).

Description. See Figures 13–15 for general appearance and Table 4 for morphometric data of holotype and 10 paratypes. Small, very slender and roundish bodied species with short head. Body deepest at about pelvic-fin base, depth decreasing towards caudal-fin base. No hump at nape. Greatest body width at pectoral-fin base or at middle between pectoral- and pelvic-fin bases, body almost equally wide until dorsal fin-origin. Section of head roundish, flattened on ventral surface. Caudal peduncle compressed laterally, 2.4–3.2 (mean 2.7) times longer than deep. Pelvic axillary lobe present, not attached to body. Pelvic-fin origin distinctly in front of dorsal-fin origin. Pectoral fin reaching approximately 40 % of distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin reaching to anus. Distance from anus to anal-fin origin 0.4–0.5 times in distance from pelvic-fin to anal-fin origins. Anal-fin origin behind vertical of tip of dorsal fin when adpressed to body. Anal fin not reaching to middle of caudal peduncle. No adipose crest on caudal peduncle. Margin of dorsal fin straight or convex. Caudal

Table 4. Morphometric data of *Turcinoemacheilus saadii* (holotype ZM-CBSU 7169B; paratypes ZM-CBSU 7166b–7175B, ZM-CBSU 7165B; n = 11).

	holotype	min	max	mean	SD
Standard length (mm)	47.1	44.6	56.4		
In percent of standard length					
Head length	19.4	17.3	20.0	19.0	0.8
Body depth at dorsal-fin origin	13.3	11.8	13.4	12.5	0.6
Prepectoral length	18.4	16.9	19.6	18.0	0.9
Predorsal length	53.5	51.6	54.7	52.7	1.0
Postdorsal length	39.6	36.5	39.6	37.9	1.0
Preanal length	73.3	70.4	74.4	72.9	1.0
Prepelvic length	48.6	48.1	51.3	49.8	1.0
Distance between pectoral and pelvic-fin origins	32.2	30.9	33.8	32.5	0.9
Distance between pelvic and anal-fin origins	23.2	20.9	24.3	22.6	1.0
Distance between vent and anal-fin origin	8.6	8.6	12.1	9.8	1.1
Depth of caudal peduncle	7.9	6.8	8.1	7.6	0.4
Length of caudal peduncle	21.1	17.8	21.9	20.6	1.2
Dorsal-fin depth	15.5	12.5	15.5	14.4	1.0
Anal-fin base length	6.7	5.9	7.2	6.5	0.5
Pectoral-fin length	15.3	13.3	16.0	14.9	0.8
Pelvic-fin length	13.8	11.7	13.8	12.4	0.7
In percent of head length					
Head depth at eye	43	37	49	43	2.9
Snout length	44	38	45	42	1.9
Eye diameter	11	11	16	13	1.4
Postorbital distance	44	40	48	44	2.1
Maximum head width	57	57	66	60	3.0
Interorbital width	25	22	28	26	2.1

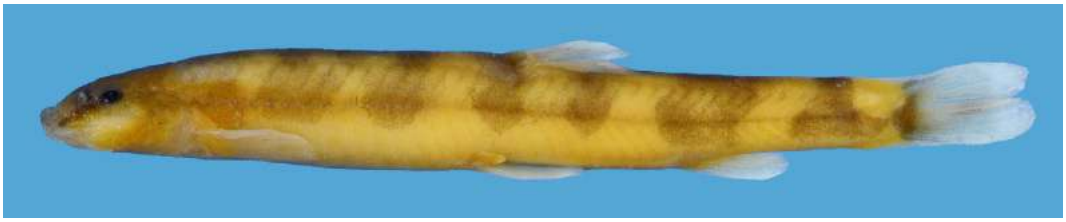

Fig. 13. *Tucinoemacheilus saadii*, ZM-CBSU7169B, holotype, 47.1 mm SL; Iran: stream Tang-e-Tizab.

Fig. 14. *Turcinoemacheilus saadii*, Iran: stream Tang-e-Tizab; paratypes; **a,** ZM-CBSU 7168B, 56.4 mm SL; **b,** ZM-CBSU 7166B, 49.5 mm SL; **c,** ZM-CBSU 7171B, 47.1 mm SL.

Fig. 15. *Turcinoemacheilus saadii*, collected with ZM-CBSU7169B, about 50 mm SL; Iran: stream Tang-e-Tizab.

fin deeply emarginated. Largest known specimen 63.6 mm SL.

Dorsal fin with 7½ branched rays. Anal fin with 4½–5½ (usually 5½) branched rays. Caudal fin with 8 + 8 branched rays. Pectoral fin with 7–8 and pelvic fin with 5–6 (usually 5) branched rays. Body without scales. Lateral line incomplete, with 18–24 pores, not reaching to dorsal-fin origin. Anterior nostril opening on anterior side of a low, pointed and flap-like tube. Anterior nostril open-

Fig. 16. Stream Tang-e-Tizab, a tributary to Bashar River, Iran; type locality of *Turcinoemacheilus saadii*.

ing on anterior side of a low, blunt and flap-like tube. Nostrils close together, posterior tip of anterior nostril reaching into posterior nostril when folded backward. No suborbital flap or groove in males. Mouth small, slightly arched (Fig. 7c). Lips moderately thick. A median interruption in lower lip. Upper lip without median incision. Processus dentiformis small and blunt. No median notch in lower jaw. Barbels short, inner rostral barbel not reaching base of maxillary barbel; outer one reaching slightly beyond base of maxillary barbel, not reaching vertical of anterior margin of eye. Maxillary barbel reaching vertical of middle of eye. No external sexual dimorphism observed.

Coloration. In alcohol and in life, body pale cream yellow. Seven to nine wide, irregularly shaped dark brown saddles on body; three to four in front of dorsal fin, sometimes dissociated or partly dissociated into two narrow bars on flank. One wide saddle starting in front of and reaching below dorsal-fin origin and one saddle at posterior part of dorsal-fin base reaching behind dorsal-fin base. Two or three saddles on caudal peduncle. Predorsal saddles reaching slightly below lateral midline, saddles behind dorsal-fin base reaching to ventral body. Some saddles, usually those on predorsal flank, Y-shaped in few individuals. Saddles usually wider on back than on flank and wider on lateral midline than below and above lateral midline. Saddles always disconnected from each other, never fused to form a lateral stripe. A very faint inner axial streak visible in preserved specimens. A dark brown or black bar at posterior extremity of caudal peduncle. In front of this bar, a whitish or yellowish blotch on upper caudal peduncle. Cheeks and ventral surface of head cream or pale yellow, head above cheeks dark brown without spots or blotches. Fins hyaline.

Distribution and habitat. *Turcinoemacheilus saadii* is known from the Bashar River which flows to the Karoun and from the Gamasiab River which flows to Karkheh. At the type locality, the stream Tang-e-Tizab (Fig. 16) is about 20 m wide, the substrate consists of coarse gravel and boulders and the water is fast-running.

Etymology. The species is named for Abū-Muhammad Muslih al-Dīn bin Abdallāh Shīrāzī, Saadii Shirazi (Persian: عبدالله شیرازی ابومحمد مصلح الدین بن) better known by his pen-name as Sa'dī (Persian: سعدی) or, simply, Saadi, one of the major Persian poets of the medieval period.

Remarks. The phylogenetic tree reconstruction (Fig. 1) suggests that *T. saadii* is the sister species of the pair *T. kosswigi* and *T. minimus*. It is separated from *T. kosswigi* by 8.5 % and from sympatric *T. hafezi* by 18.2 % K2P COI sequence divergence, which is considered as a strong indicator that these fishes represent different species (see Herbert et al., 2003). *Turcinoemacheilus saadii* is further distinguished from *T. kosswigi* and *T. minimus* by colour pattern (7–9 distinct saddles vs. lateral stripe made by a series of blotches connected to dorsal blotches). In *T. saadii*, the caudal fin is always deeply emarginated and the central rays of the caudal fin are 60–80 % of the length of the lower caudal-fin lobe. In *T. kosswigi*, the caudal fin is only slightly emarginated in most individuals and the central rays of the caudal fin are 80–95 % of the length of the lower caudal-fin lobe. However, in some individuals of *T. kosswigi*, the caudal fin is more deeply emarginate and the ratio is down to 70–80 % and largely overlapping with *T. saadii*.

Turcinoemacheilus saadii occurs in sympatry with *T. hafezi*. It is distinguished from this species by the more anterior position of the anus, which is in front of the middle or in the middle between the pelvic-fin origin and the anal-fin origin (distance from anus to anal-fin origin 0.4–0.5 times in distance from pelvic-fin to anal-fin origins) while it is behind the middle (0.2–0.4 times) in *T. hafezi*. *Turcinoemacheilus saadii* is further distinguished by a free distal part of the axillary pelvic lobe (vs. completely attached to body), a narrower caudal peduncle (depth 2.4–3.2 times in its length vs. 1.9–2.4), anterior and posterior nostrils very closely together (vs. separated), tip of anterior nostril reaching into posterior nostril when folded down (vs. not) and differences in colour patters (7–9 distinct saddles vs. lateral stripe present, disconnected from dorsal blotches or mottles colour pattern in *T. hafezi*).

Turcinoemacheilus saadii is distinguished from *T. himalaya* by a completely scaleless body (vs. small cycloid scales present on the posterior half of the body).

Comparative material. *Turcinoemacheilus hafezi*: all from Iran: FSJF 3222, 33, 24–46 mm SL; Yasouj prov.: Beshar (Bashar) river at Doruhan, 30°51'07"N 51°20'31"E. – FSJF 3002, 3, 32.4–45.8 mm SL; Sezar river, a tributary of Dez river, 33°28'N 49°03'E. – ZFMK 48841, holotype, 41.0 mm SL; ZFMK 48842–48848, paratypes, 7, 34.1–66.7 mm SL; FSJF 1649, 10, 34.4–54.7 mm SL; Chaharmahal and Bakhtiari prov.: stream Shalamzar 10 km after Joneghan in the direction to Ardal, a tributary of Ab-Kohrang River, 32°05'22"N 50°39'58"E. Material used in the molecular genetic analysis: FSJF DNA-1985; Yasouj prov.: Bashar river at Doruhan, 30°51'07"N 51°20'31"E (GenBank accession numbers: KJ179254, KJ179259). – ZM-CBSU T497, ZM-CBSU T539; Fars prov.: stream Tang-e-Tizab, a tributary to Bashar river which drains to the Karoun, 30°23'12"N 51°46'50"E (GenBank accession numbers: KJ179252, KJ179264).

T. himalaya: paratypes, all from Nepal: KU 40557, 2, 38.0, 59.3 mm SL; Indrawati river at Melamchi township, 27°49'42"N 85°34'37"E. – KU 40568, 3, 39.0–51.4 mm SL; Melamchi river, 3 miles upstream from confluence with Indrawatir river, 27°57'22"N 85°32'27"E. – KU 40280, 4, 44.9–49.7 mm SL; Gaundi river at Gumti, 28°03'54"N 83°33'36"E. – KU 40281, 2, 36.4, 38.8 mm SL; Seti river at Khairenitar, 28°02'00"N 84°04'00"E.

T. kosswigi: ZMH H1884, holotype, 53 mm SL; ZMH H1885, 5 paratypes, 41–52 mm SL; Turkey: Hakkari prov.: Kapozik Kadun. – FSJF 3345, 7, 41.3–50.8 mm SL; Iraq: stream north-west of Saburawa, a tributary of Tabin river, a tributary to Little Zab, 35°50'01"N 45°06'16"E. – FSJF 3350, 7, 40.9–50.2 mm SL; Iraq: Kuna Massi stream in Sevanja, a tributary to Little Zab, 35°47.35'N 45°24.18'E. – FSJF 3358, 1, 52.3 mm SL; Iraq: stream Zalm at Khurmal, a tributary to Sirvan, 35°18.38'N 45°58.26'E. – FSJF 3370, 4, 40.1–45.9 mm SL; Iraq: stream Suraw near Suraw village, a tributary to Little Zab, 35°45.76'N 45°59.09'E. – FSJF 3377, 3, 41.5–46.1 mm SL; Iraq: Chami Rean river near Ziraran, a tributary to Great Zab, 36°56.60'N 44°11.72'E. Material used in the molecular genetic analysis: FSJF DNA-2214; Iraq: stream Suraw near Suraw village, a tributary to Little Zab, 35°45.76'N 45°59.09'E (GenBank accession number: KJ179260). – FSJF DNA-2222; Iraq: stream north-west of Saburawa, a tributary of Tabin river, a tributary to Little Zab, 35°50'01"N 45°06'16"E (GenBank accession number: KJ179255). – FSJF DNA-2226; Iraq: Chami Rean river near Ziraran, a tributary to Great Zab, 36°56.60'N 44°11.72'E (GenBank accession number: KJ179265). – FSJF DNA-2232; Iraq: Kuna Massi stream in Sevanja, a tributary to Little Zab, 35°47.35'N 45°24.18'E (GenBank accession number: KJ179262). – ZM-CBSU T507, ZM-CBSU T509; Iran: Sirvan river, 35°06'43"N 46°15'24"E (GenBank accession numbers: KJ179258, KJ179245).

Paracobitis malapterura: material used in the molecular genetic analysis: FSJF DNA-1992; Iran: Albroz prov.: Kordan river near Karaj, 35°57'11"N 50°50'15"E (GenBank accession numbers: KJ179266, KJ179267).

Paraschistura bampurensis: Material used in the molecular genetic analysis: FSJF DNA-2001; Iran: Sistan and Baluchestan prov.: Karvander river at bridge north of Karevander, 27°51'18" N 60°46'03" E (GenBank accession numbers: KJ179268, KJ179269).

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