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Garra roseae, a new species from the Makran region in southern Iran (Teleostei: Cyprinidae)

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Abstract

Garra roseae, new species, is described from the stream Tang-e-Sarhe in the Iranian Makran region. It is distinguished from its congeners in the Middle East by lacking barbels, having a small mental disc, 42–58 total scales along the lateral line, 24–30 scales along the predorsal midline, and 20–24 circumpeduncular scales. It is further characterised by having five diagnostic nucleotide substitutions and a minimum K2P distance of 5.39% to *G. rossica* and 5.49% to *G. nudiventris* in the mtDNA COI barcode region. *Garra phryne* from eastern Iran is considered to be a synonym of *G. nudiventris*.

Key words: Freshwater fish, Taxonomy, Cytochrome oxidase I, Middle East

Introduction

Labeonine cyprinids of the genus *Garra* are widespread in the Middle East, South- and East Asia and tropical Africa (Menon 1964). In the Middle East, the genus *Garra* is well separated in two species groups (Hamidan *et al.* 2014): the *Garra rufa* group and the *G. variabilis* group. In the *G. variabilis* group, Hamidan *et al.* (2014) recognised four species, *G. variabilis*, *G. rossica*, *G. kemali* and *G. klatti*. Esmaeili *et al.* (2016) also recognised *G. nudiventris* as a valid species in this group. Interestingly, the *G. variabilis* species group has been only known from the Middle East and Central Asia until now and further research is needed to reveal its relationships with the Oriental *Garra* species.

The Makran region is geographically at the border between the Palearctic and Oriental biogeographic realms. The Oriental starts here and reaches then through Pakistan and India to the east. The Makran region, which is shared by Iran and Pakistan, is located along the coastal region in the north of the Oman Gulf between the Cape Dschask in the Iranian Hormozgan province and the Sonmiani bay about 25 km north of Karachi. The Minab River in the west and the Sarbaz River in the east are the two most important rivers in the Iranian Makran. There are also some small streams between the two river systems, which often do not have perennial or continuous flow. The Makran has biogeographical affinity to the South Asian fauna by containing oriental species, which are not much further distributed in the Middle East such as *Cabdio morar*, *Tariqilabeo diplochilus*, *Bangana dero*, and *Glossogobius giuris*. The *Garra* populations of the Iranian Makran region was identified as *Garra persica* and *G. rossica* by Sayyadzadeh *et al.* (2015), Esmaeili *et al.* (2016) and those from the Pakistani Makran was identified as *G. rossica* and *G. gotyla* by Mirza (1972). Nebeshwar & Vishwanath (2013) re-described *G. gotyla*, which is a species with four barbels and an elevated proboscis and restrict its distribution to Sikkim in north-eastern India. These authors also pointed out that many *Garra* species had been misidentified as *G. gotyla* and this was for sure also the case by Mirza (1972).

We were able to examine *Garra* populations from the Makran region including one from the stream Tang-e-Sarhe, in southern Iran (Fig. 1). A detailed morphological study in combination with DNA barcoding routines support the hypothesis that the population represents an undescribed species, which is diagnosed below. Furthermore,

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we test, (a) if *G. rossica* and *G. nudiventris* represent two species as already suggested by Esmaeili *et al.* (2016), despite their low genetic distance and (b) if *G. phryne* is a synonym of *G. rossica* as considered by Coad (1981)

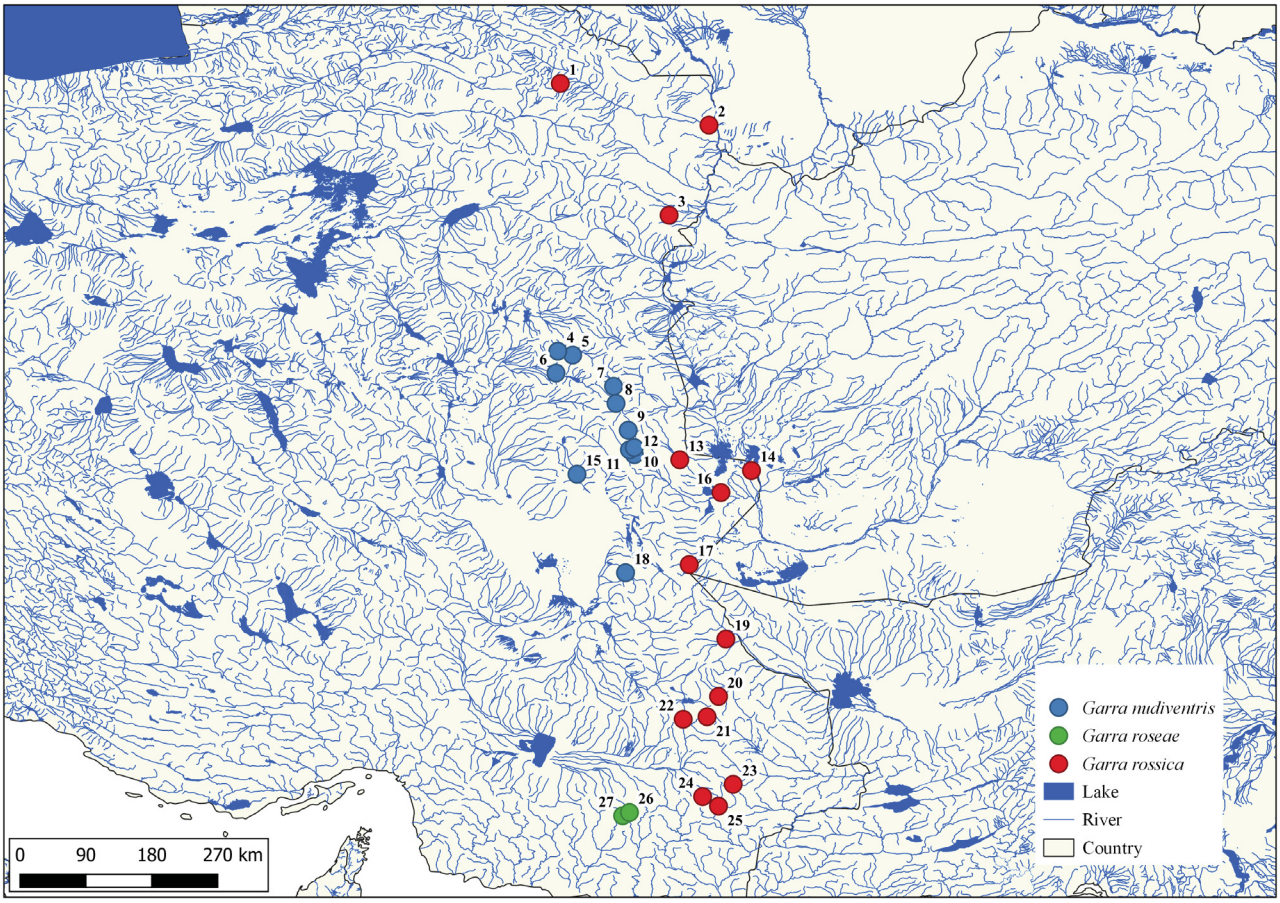

FIGURE 1. Records of the *Garra variabilis* species group in Iran. See below for the sources of records the map is based on.

Material and methods

After anaesthesia, fishes were fixed in 5% formaldehyde and stored in 70% ethanol or directly fixed in 99% ethanol. Measurements were made with a dial calliper 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). The terminology of the snout morphology and the oromandibular structures follow Stiassny & Getahun (2007) and Nebeshwar & Vishwanath (2013). Standard length (SL) was 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 counted as "1½". The holotype is included in the mean and SD calculation of morphometric characters. Scales along the lateral line are counted from the first one (the first one to touch the shoulder girdle) to the last one on the caudal-fin base (total lateral line scales). Gill rakers are counted on the outer margin of the first gill arch. *Garra kemali* and *G. klatti* are redescribed by Küçük *et al.* (2015) and Yoğurtçuoğlu *et. al.* (2018) and we base our comparison on these re-descriptions and own materials examined. Except those published by Annandale (1919), Esmaeili *et al.* (2016), and our own data points, all records of *Garra* species shown in Figure 1 were identified as *G. rossica* in the publications or databases these were taken from. As we were not able to identify these from the materials, we re-identify them based on their geographic range derived from properly identified materials.

Abbreviations used. SD, standard deviation, SL, standard length; K2P, Kimura 2-parameter. Collection codes: GUIC, Guilan University Ichthyological Collection, Sowmeh-Sara; VMFC, Vatandoust & Mousavi-Sabet Fish Collection, Tehran; FSJF, Fischsammlung J. Freyhof, Berlin.

DNA extraction and PCR: Total genomic DNA was extracted from fin tissue of the specimens, using the

commercial kit Biosprint15 for tissue and blood (Qiagen). The COI gene was amplified using primers FishF1-(5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1-(5'-TAGACTTCTGGGTGGCCAA AGAATCA-3') (Ward *et al.* 2005). PCR mixtures were prepared in 25 µ with a final concentration of 0.5 mM each primer, 0.2 mM dNTP, 1.5 mM MgCl2, and 1-unit Taq DNA polymerase (Invitrogen). Amplification cycles followed as denaturation for 2 min at 95°C; 30 cycles at 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 1 min and a final extension for 10 min at 72°C. Afterward, PCR products has been checked on 1% agarose gels, then purified by ExoSAPITTM (USB, Cleveland, USA) then sent to MACROGEN Inc (Madrid, Spain; https://dna.macrogen.com) for sequencing.

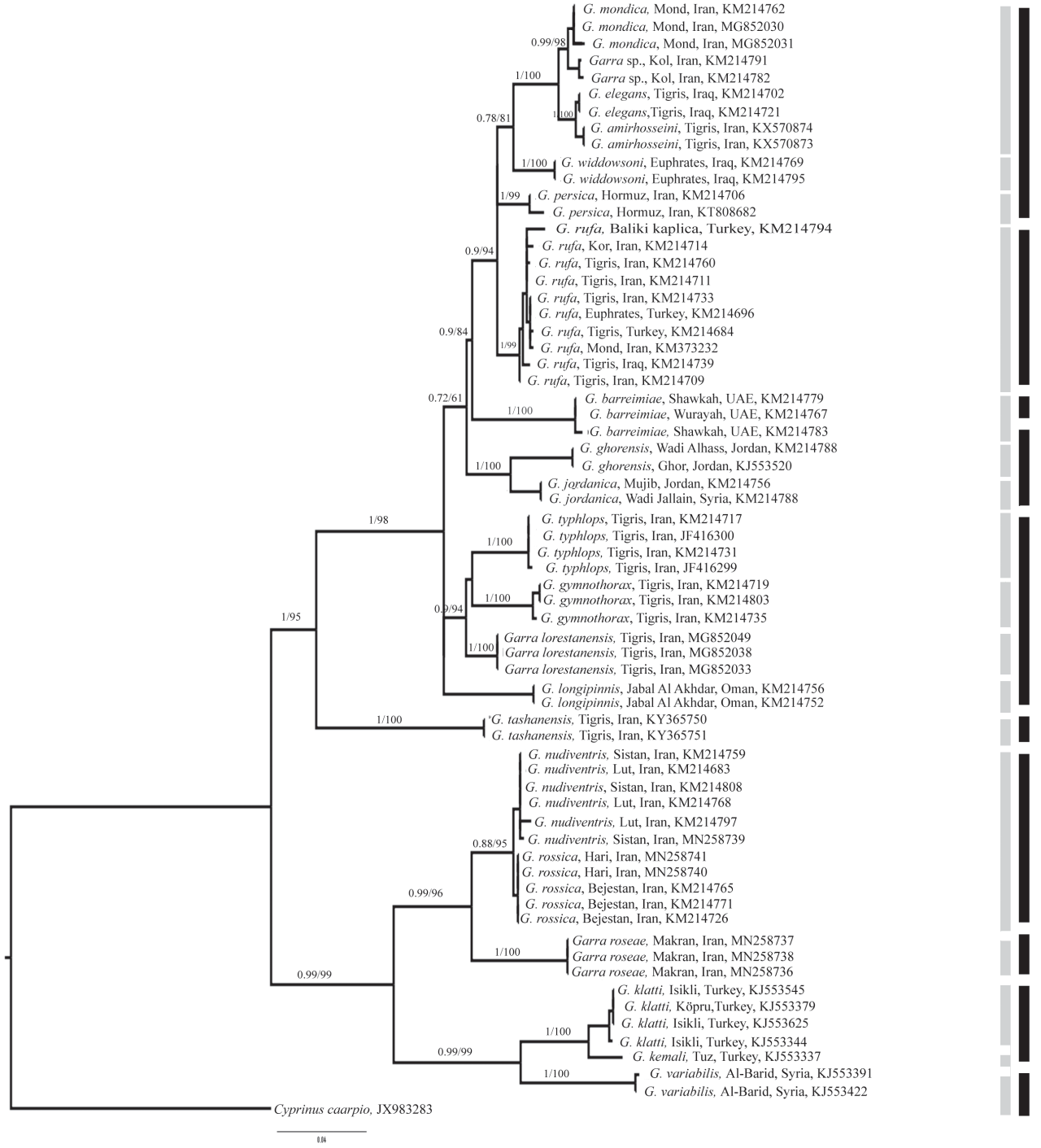

FIGURE 2. Phylogenetic tree of mitochondrial COI barcode region rendered by Bayesian inference (BI). Numbers on branches indicate posterior probability values for BI and Maximum Likelihood (ML) bootstrap. Model of rate heterogeneity: Gamma with 4 categories Gamma shape alpha: 0.1905. Black solid bars right to the specimen labels indicate species delimitation results from mPTP followed by the results of the PTP approach as gray solid bars. The tree is drawn to scale with branch lengths depicting number of substitutions per site.

TABLE 1. List of COI-sequences downloaded from NCBI Genbank with information on drainage and country of origin.

Species	Drainage	Country	Genbank	Reference
Garra amirhosseini	Tigris	Iran	KX570873	Esmaeili *et al.* 2016
Garra amirhosseini	Tigris	Iran	KX570874	Esmaeili *et al.* 2016
Garra barreimiae	Shawkah	UAE	KM214783	Hamidan *et al.* 2014
Garra barreimiae	Wurayah	UAE	KM214767	Hamidan *et al.* 2014
Garra barreimiae	Shawkah	UAE	KM214779	Hamidan *et al.* 2014
Garra elegans	Tigris	Iraq	KM214702	Hamidan *et al.* 2014
Garra elegans	Tigris	Iraq	KM214721	Behrens-Chapuis et al. 2015
Garra ghorensis	Ghor	Jordan	KJ553520	Geiger *et al.* 2014
Garra ghorensis	Wadi Alhass	Jordan	KM214788	Hamidan *et al.* 2014
Garra gymnothorax	Tigris	Iran	KM214803	Behrens-Chapuis *et al.* 2015
Garra gymnothorax	Tigris	Iran	KM214735	Behrens-Chapuis *et al.* 2015
Garra gymnothorax	Tigris	Iran	KM214719	Hamidan *et al.* 2014
Garra jordanica	Mujib	Jordan	KM214756	Hamidan *et al.* 2014
Garra jordanica	Wadi Jallain	Syria	KM214788	Hamidan *et al.* 2014
Garra kemali	Tuz	Turkey	KJ553337	Geiger *et al.* 2014
Garra klatti	Isikli	Turkey	KJ553545	Geiger *et al.* 2014
Garra klatti	Isikli	Turkey	KJ553625	Geiger *et al.* 2014
Garra klatti	Isikli	Turkey	KJ553344	Geiger *et al.* 2014
Garra klatti	Köpru	Turkey	KJ553379	Geiger *et al.* 2014
Garra longipinnis	Jabal Al Akhdar	Oman	KM214752	Hamidan *et al.* 2014
Garra longipinnis	Jabal Al Akhdar	Oman	KM214756	Hamidan *et al.* 2014
Garra lorestanensis	Tigris	Iran	MG852049	Hashemzadeh Segherloo *et al.* 2018
Garra lorestanensis	Tigris	Iran	MG852033	Hashemzadeh Segherloo *et al.* 2018
Garra lorestanensis	Tigris	Iran	MG852038	Hashemzadeh Segherloo *et al.* 2018
Garra mondica	Mond	Iran	MG852031	Hashemzadeh Segherloo et al. 2018
Garra mondica	Mond	Iran	MG852030	Hashemzadeh Segherloo *et al.* 2018
Garra mondica	Mond	Iran	KM214762	Behrens-Chapuis *et al.* 2015
Garra persica	Hormuz	Iran	KT808682	Sayyadzadeh *et al.* 2015
Garra persica	Hormuz	Iran	KM214706	Hashemzadeh Segherloo *et al.* 2017
Garra rossica	Bedjestan	Iran	KM214765	Hamidan *et al.* 2014
Garra rossica	Bedjestan	Iran	KM214771	Behrens-Chapuis *et al.* 2015
Garra rossica	Bedjestan	Iran	KM214726	Hamidan *et al.* 2014
G. nudiventris	Sistan	Iran	KM214808	Hamidan *et al.* 2014
G. nudiventris	Lut	Iran	KM214768	Hamidan *et al.* 2014
G. nudiventris	Sistan	Iran	KM214759	Hashemzadeh Segherloo *et al.* 2017
G. nudiventris	Lut	Iran	KM214683	Hamidan *et al.* 2014
G. nudiventris	Lut	Iran	KM214797	Hamidan *et al.* 2014
Garra rufa	Tigris	Iran	KM214709	Hashemzadeh Segherloo *et al.* 2017
Garra rufa	Tigris	Turkey	KM214684	Hamidan *et al.* 2014
Garra rufa	Mond	Iran	KM373232	Hashemzadeh Segherloo *et al.* 2017
Garra rufa	Tigris	Iran	KM214733	Hashemzadeh Segherloo *et al.* 2017
Garra rufa	Baliki Kaplica	Turkey	KM214794	Hamidan *et al.* 2014
Garra rufa	Euphrates	Turkey	Km214696	Hamidan *et al.* 2014
Garra rufa	Tigris	Iran	KM214760	Hashemzadeh Segherloo *et al.* 2017
Garra rufa	Tigris	Iran	KM214711	Hashemzadeh Segherloo *et al.* 2017

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TABLE 1. (Continued)

Species	Drainage	Country	Genbank	Reference
Garra rufa	Tigris	Iran	KM214739	Geiger *et al.* 2014
Garra rufa	Kor	Iran	KM214714	Hashemzadeh Segherloo *et al.* 2017
Garra tashanensis	Tigris	Iran	KY365750	Mousavi-Sabet *et al.* 2016
Garra tashanensis	Tigris	Iran	KY365751	Mousavi-Sabet *et al.* 2016
Garra typhlops	Tigris	Iran	KM214717	Hamidan *et al.* 2014
Garra typhlops	Tigris	Iran	KM214731	Hamidan *et al.* 2014
Garra typhlops	Tigris	Iran	JF416299	Hashemzadeh Segherloo *et al.* 2012
Garra typhlops	Tigris	Iran	JF416300	Hashemzadeh Segherloo *et al.* 2012
Garra variabilis	Al-Barid	Syria	KJ553391	Geiger *et al.* 2014
Garra variabilis	Al-Barid	Syria	KJ553422	Geiger *et al.* 2014
Garra widdowsoni	Euphrates	Iraq	KM214769	Hamidan *et al.* 2014
Garra widdowsoni	Euphrates	Iraq	KM214795	Hamidan *et al.* 2014
Garra sp.	Kol	Iran	KM214791	Hashemzadeh Segherloo *et al.* 2017
Garra sp.	Kol	Iran	KM214782	Behrens-Chapuis *et al.* 2015
Cyprinus carpio	Narmada	India	JX983283	Khedkar *et al.* 2014

Molecular data analysis: The sequences were compared to published *Garra* sequences recording in genebank (Table 1) [(BLASTn) basic local alignment search tool] (Altschul *et al.* 1990). Sequence data were aligned using MEGA7 software (Kumar *et al*. 2016). Sequences of COI gene were trimmed to the size of the smallest fragment, resulting in a dataset of 651 base pairs (bp). The best-fit nucleotide substitution models and partitioning scheme were calculated and selected simultaneously on Partition Finder v 2.1.1 (Lanfear *et al.* 2012) under the Bayesian Information Criterion (BIC). All parameters except topology and branch lengths were unlinked between partitions, and rate variation (prset ratepr = variable) was cited (invoked). A Bayesian inference method can examine the optimal tree topology of the combined, partitioned dataset by MrBayes v 3.1.2 (Ronquist *et al.* 2012). The model referring to the lowest BIC scores (Bayesian Information Criterion) is found as the best describe of the substitution pattern (Nei & Kumar 2000; Posada & Crandall 2001). Here, Bayesian analyses were performed using two independent runs of four Markov Monte Carlo-coupled chains of 10^7 generations each to estimate the posterior probability distribution. Topologies were sampled every 1000 generations. Phylogenetic analyses were conducted using maximum likelihood (ML) software implemented in the http://iqtree.cibiv.univie.ac.at online server (Trifinopoulos *et al.* 2016). 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 outgroup, *Cyprinus carpio* from genebank (accession number: JX983283) was used in COI Bayesian inference tree carried out in previous study (Mousavi-Sabet *et al.* 2016).

The Species Delimitation Plugin (Masters *et al.* 2011) for Geneious Pro (Biomatters 2013) was used for summarizing measures of genetic K2P distances to provide readily comparable data with other studies using this standard DNA barcoding metric. In addition, we used the reconstructed ML-based hypothesis of the mitochondrial relationships as input for a species delimitation approach using Poisson Tree Processes (PTP) and the refined multi-rate PTP (mPTP) version (Zhang *et al.* 2013, Kapli *et al.* 2017, http://mptp.h-its.org/#/tree (accessed March 25 2019). In both versions, the aim is to find a group delimitation that maximizes the likelihood of the partition of branch lengths, in PTP using a uniform evolutionary rate (lambda) and assuming different rates for each group (species) in the newer mPTP model. The null model is delimitation with all tips of the tree belonging to a single species. In PTP, a p-value test decides whether to keep the null model or reject it and use the maximum likelihood delimitation instead. Since mPTP compares models with different numbers of parameters (separate lambdas for each species), the p-value test cannot be applied and instead the Akaike Information Criterion (AIC) is used to decide, which number of groups best fits the given topology and branch lengths. In theory, this approach avoids over-splitting into too many groups (Kapli *et al.* 2017).

Results

Analysis of the COI sequence data place the included *Garra* species into the same groups found by Hamidan *et al.* (2014) and Mousavi-Sabet *et al*. (2016), adding a well-supported clade for *G. roseae* which is placed in the *G. variabilis* group. *Garra roseae* is separated by a minimum K2P distance of 5.39% to *G. rossica* and 5.49% to *G. nudiventris* in the mtDNA COI barcode region and all three individuals with DNA barcode available share five diagnostic nucleotide substitutions relative to all other included *Garra* species (Table 2).

The PTP model-based species delimitation approaches using the ML topology delivered two different estimates for the total species number present in the data: PTP detected 17 entities (p=0.001, Null-model score: 205.318054, best score for single coalescent rate: 237.101820) and mPTP 10 entities representing putative species (Null-model score: 205.318054, equal to the best score for multi coalescent rate: 231.477476). As depicted in Figure 2, the 17 detected entities using PTP much better match the morphology-based identification of the specimens and support all *a priori* identified species. The mPTP result leads to lumping of several species into one entity.

TABLE 2. Estimates of the average evolutionary divergence between *Garra* species. All positions with less than 95% site coverage were eliminated.

	G. roseae	*G. klatti*	*G. kemali*	*G. rossica*	*G. nudiventris*
G. roseae					
G. klatti	11.88				
G. kemali	12.45	2.63			
G. rossica	5.39	11.12	12.03		
G. nudiventris	5.49	10.63	11.61	0.46	
G. variabilis	12.35	7.34	7.77	11.71	11.29

Garra roseae, new species

(Figs. 3–8)

Holotype. GUIC 7847, 38 mm SL; Iran: Sistan-va-Baluchistan prov.: stream Tang-e-Sarhe near Siahangari, at km 465 on road from Zahedan to Chabahar, 26.5383, 59.9406; H. Mousavi-Sabet & M. Amouei.

Paratypes. VMFC GR-P1122, 22, 31–51 mm SL; FSJF 4071, 4, 34–37 mm SL; same data as holotype.

Material for molecular genetic analysis. VMFC DNA-GR1397, 3; same data as holotype (Genbank accession numbers: MN258736, MN258737, MN258738).

Diagnosis. *Garra roseae* is distinguished from the other species of the *Garra variabilis* group by a unique combination of characters. It is distinguished from the two Central Anatolian species *G. kemali* and *G. klatti* by having a mental disc (vs. absent) and 42–58 scales on the lateral line (vs. 35–45 in *G. kemali* and *G. klatti*). The new species is distinguished from *G. nudiventris*, *G. rossica*, and *G. variabilis* by lacking barbels (vs. one pair in *G. nudiventris* and *G. variabilis*; and one or two pairs in *G. rossica*). It is further distinguished by having the predorsal mid-line fully covered by scales (vs. naked in *G. nudiventris*), a naked breast (vs. scaled in *G. rossica*), the belly covered by scales (vs. naked in *G. nudiventris* and *G. variabilis*), 7½–8½ transverse scale rows between the lateral line and the dorsal-fin origin (vs. 5½–6½ in *G. rossica*), 6½ transverse scale rows between the lateral line and the pelvic-fin origin (vs. 4½–5½ in *G. rossica*), no axillary scale at the pelvic-fin origin (vs. present in *G. nudiventris* and *G. rossica*), and 11–13 total gill rakers on the first branchial arch (vs. 10–11 in *G. nudiventris*, 13–15 in *G. rossica*).

Description. For general appearance see Figs. 3–8; morphometric data are provided in Table 3. Small sized and elongated species with laterally compressed caudal peduncle. Dorsal head profile rising gently, slightly convex. Predorsal contour slightly convex between nape and dorsal-fin origin. Prepelvic contour convex, ventral profile more or less straight from pelvic to anal-fin origins. Body deepest at about dorsal-fin origin or about middle between nape and dorsal-fin origin, depth decreasing towards caudal-fin base. Greatest body width at pectoral-fin base, body almost equally wide until dorsal-fin origin, width decreasing towards caudal-fin base. Head moderately small, section of head roundish, flattened on ventral surface; slightly depressed, almost conical; slightly convex or flat interorbital space; height-at-nape shorter than head length; width-at-nape greater or about equal to depth-at-nape. Head length

0.9–1.1 times in body depth. Snout rounded, its length 1.2–1.6 times in postorbital length; no obvious tubercle on transverse lobe, demarcated posteriorly by a shallow transverse groove in some individuals, no transverse groove. No obvious tubercle on proboscis and lateral surface of snout. Depressed rostral surface always without tubercles; moderately separating transverse lobe from lateral surface, not clear in some specimens. No groove between transverse lobe and lateral surface. Head tubercles present only on opercula surface. Eyes relatively large, eye diameter 2.3–2.9 times in head depth at eye, 2.0–2.3 times in interorbital width. Eyes located dorso-laterally on the anterior half of head or at mid head. Barbels absent. Rostral cap well-developed, fimbriate, papillate on ventral surface. Upper lip present. Upper jaw almost or completely covered by rostral cap. Disc elliptical, shorter than wide and narrower than head width; papillae on anterior fold of same size, regularly arranged; groove between antero-median fold and central callous-pad narrow and deep, latero-posterior flap absent; surface of central callous pad without or with sparsely arranged small papillae; posterior margin of central callous pad extending vertical to anterior edge of eye. Nostrils located just anterior to eyes, round-shaped. Anterior nostril opening developed as a low, pointed and flap-like tube. Posterior nostril narrow, nostrils adjacent, posterior tip of anterior nostril reaching to posterior nostril when folded down.

TABLE 3. Morphometric data of *Garra roseae*. Holotype, GUIC 7847; paratypes, VMFC GR-P (n=22), FSJF 4071 (n=4). The holotype is included in the calculations.

	holotype	holotype and paratypes (n=26)			
		min	max	mean	SD
Standard length (mm)	38.0	31.0	51.0		
In percent of standard length					
Head length	22.6	20.4	23.3	21.7	1.3
Body depth at dorsal-fin origin	20.9	20.9	22.0	21.5	0.5
Predorsal length	51.1	48.8	51.2	50.3	0.9
Postdorsal length	37.6	36.6	39.1	37.5	1.0
Preanal length	76.2	73.4	76.5	75.3	1.4
Prepelvic length	54.9	53.0	57.0	54.7	1.5
Distance between pectoral and pelvic-fin origins	35.0	31.1	37.3	34.7	2.3
Distance between pelvic and anal-fin origins	21.2	17.2	21.8	20.3	1.8
Depth of caudal peduncle	11.9	11.7	13.0	12.4	0.5
Length of caudal peduncle	15.4	15.0	16.3	15.5	0.6
Anal-fin base length	8.5	7.9	9.3	8.8	0.6
Pectoral-fin length	16.9	13.6	16.9	15.5	1.5
Pelvic-fin length	12.4	10.7	13.1	12.0	1.0
In percent of head length					
Head depth at eye	64	58	71	63.7	2.7
Snout length	33	29	34	31.3	2.2
Eye diameter	22	22	26	24.4	1.4
Postorbital distance	44	41	46	43.9	1.8
Maximum head width	85	78	85	82.9	2.3
Interorbital width	51	50	54	51.7	1.9

Dorsal fin with 3 simple and 6½ (1), 7½ (24) or 8½ (1) branched rays, last simple ray shorter than head length; distal margin slightly concave; origin closer to caudal-fin base than to snout tip; inserted anterior to vertical of pelvic-fin origin; first branched ray longest, tip of last branched ray reaching vertical to, or slightly in front of anus when folded down. Pectoral fin with one simple and 10 (10), 11 (13) or 12 (3) branched rays. Pectoral fin reaching approximately 38–54% of distance from pectoral-fin origin to pelvic-fin origin, length shorter than head length. Pelvic fin with one simple and 7 (2) or 8 (24) branched rays. Pelvic fin not reaching anus/anal-fin base, or reaching to anus in some in individuals, origin closer to anal-fin origin than to pectoral-fin origin, inserted below third or fourth branched dorsal-fin ray. Anal fin short, with 3 simple and 5½ branched rays; first branched ray longest; distal margin straight or slightly convex; origin closer to pelvic-fin origin than to caudal-fin base. Anal fin reaching

approximately to ½ to $^{3}/_{4}$ of caudal peduncle when folded. Caudal peduncle length 1.1–1.4 times longer than deep. Caudal fin forked with 9+8 branched rays; tip of lobes rounded (or slightly pointed). Caudal fin emarginated (length of the ray in the middle of caudal fin 68–72 % of the longest branched ray in the upper lobe of the fin), with 9+8 branched rays. Total gill rakers on first branchial arch 11–13 [11(3), 12(3), 13(5)]. Lateral line complete, with 42–58 [42(3), 44(2), 45(3), 48(2), 50(3), 51(1), 53(4), 54(1), 55(3), 56(2), 58(2)] scales, which 2–3 of them were on caudal-fin base. Transverse scale rows above lateral line 7–9; between lateral line and pelvic-fin origin 6–7 and between lateral line and anal-fin origin 6. Circumpeduncular scale rows 20–24. Usually, 24–30 scales on predorsal midline between dorsal-fin origin and nape, embedded in some specimens. Scales on flank regularly arranged. Chest naked and belly scaled (scales presence from mid of pectoral fin when folded back). No axillary scale at base of pelvic fin. Largest known individual 51 mm SL.

FIGURE 3. *Garra roseae*, GUIC 7847, holotype, 38 mm SL; Iran: stream Tang-e-Sarhe.

Coloration. In preserved individuals: Background colour pale yellowish or whitish. Scales brown, grey in life, with whitish or yellowish margins. Dorsal surface of head pale yellow or brown. Flank above lateral line dark or pale brown. Abdominal edge and caudal fin origin pale yellow. Lateral head and flank anterior to dorsal-fin base pale yellow to whitish below lateral line. Cheek pale yellowish or whitish. A faint irregularly shaped, grey inner axial stripe most prominent on flank behind dorsal-fin base. Mouth, chest and abdomen yellowish. A wide, often indistinct, black or dark-brown bar at posterior-most caudal peduncle faded in most individuals, up to 2–3 scales wide. Bar reaching dorsal midline in some individuals, not reaching ventral midline. Lateral line beige, in contrast to brown colour on mid-lateral flank. A dark-brown blotch at base of unbranched dorsal-fin rays, followed by beige base of branched rays 2–3 and black or dark brown base of rays 4–7. All fins hyaline with irregularly set black spots on rays.

In life: Background colour silvery, all fins hyaline with irregular black spots. Head grey and scales on flank and back dark grey, whitish or pale grey on lower flank and belly. Iris silvery orange with dark grey spots, internal ring without spots. Dark grey dots at pectoral-fin base in some individuals.

Distribution. Until now, *G. roseae* is known from the stream Tang-e-Sarhe.

Etymology. The species is named after Rose, daughter of the first author.

Notes on habitat. *Garra roseae* was collected in a shallow stream with slow current at 1116 m altitude (Fig. 9). At the sampling site, the water was polluted from villages along the stream. The length of the stream Tang-e-Sarhe is about 70 kilometres.

Remarks. *Garra persica* is the only species of *Garra* known from the Makran region not belonging to the *G. variabilis* group. While *G. persica* is widespread in Iran and most likely also in adjacent Pakistan, it has not been found in sympatry with *G. roseae*. *Garra roseae* is distinguished from *G. persica* by lacking barbels (vs. having two pairs of barbels), having 42–58 scales along the lateral line (vs. 32–37), 20–24 circumpeduncular scales (vs. 14–16), 9+8 branched caudal-fin rays (vs. usually 8+8), a naked breast (vs. covered by scales), 7½–8½ transverse scale rows between the lateral line and the dorsal-fin origin (vs. 4½), 6½ transverse scale rows between the lateral line and the pelvic-fin origin (vs. 4½), no axillary scale at the pelvic-fin base (vs. present), and 11–13 total gill rakers on the first branchial arch (vs. 17–19).

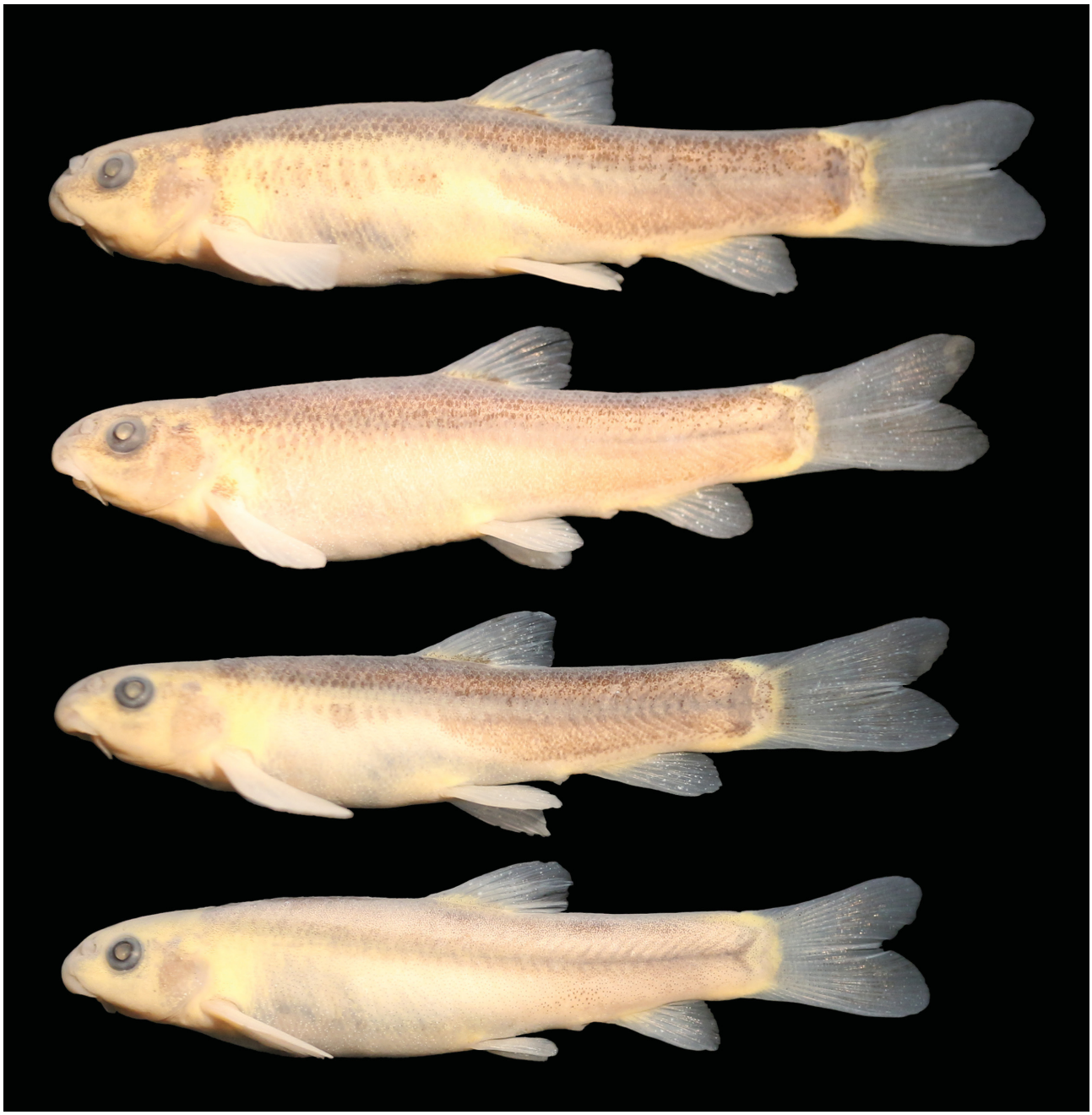

FIGURE 4. *Garra roseae*, VMFC GR-P, paratypes; from above: 36 mm SL; 35 mm SL; 34 mm SL; 34 mm SL; Iran: stream Tang-e-Sarhe.

FIGURE 5. *Garra roseae*, VMFC GR-P, paratypes; from above: 36 mm SL; 35 mm SL; 34 mm SL; 34 mm SL; Iran: stream Tang-e-Sarhe.

Within the frame of this study, we examined materials of the extirpated populations of *G. klatti* from Central Anatolia (ZMH 320: Lake *Gölcük,* ZMH 1122, Lake *Eğirdir*) and found these to indistinguishable from *Hemigrammocapoeta menderesensis* described by Küçük *et al*. (2015). Therefore, we follow Geiger *et al*. (2014) and treat *Hemigrammocapoeta menderesensis* as a synonym of *Garra klatti*.

This study also allows us to re-assess the taxonomic status of *G. nudiventris*, which was treated as a valid species different from *G. rossica* by Esmaeili *et al*. (2016). In our molecular dataset, *G. rossica* is very closely related to *G. nudiventris*, and both are characterised by a minimum K2P distance of 0.46% in the COI barcode region. Despite this low genetic distance, *G. rossica* and *G. nudiventris* are separated clearly in two distinct clades. All *G. rossica* examined by us are distinguished from all *G. nudiventris* by having the predorsal mid-line, the breast, and belly covered by scales (vs. naked; see Figs. 10–11). Therefore, we confirm the results published by Esmaeili *et al*. (2016), and consider *G. rossica* and *G. nudiventris* as two distinct taxa.

It should be noted, that *Discognathus phryne*, described by Annandale (1919) from "Nasratabad, Seistan, eastern Iran", was characterised by one pair of barbel, 36–39 scales on the lateral line, and a naked chest and back. Coad (1981) considered *D. phryne* as synonym of *G. rossica*, but our results strongly suggest that it is a synonym of *G. nudiventris,* but not of *G. rossica. Garra nudiventris* is found in the Seistan region and has a naked breast and back as described by Annandale (1919).

The *Garra* species from the Pakistani Makran region are poorly known and we have to discuss here *G. wanae*, which was originally described by Regan (1914) from the Wana Toi, tributary of the Gomal River in the upper Indus River drainage in north-western Pakistani Waziristan. Based on the description of *G. wanae* by Regan (1914), *G. roseae* is distinguished from *G. wanae* by lacking barbels (vs. two pairs of barbels) and having a naked breast (vs. covered by scales).

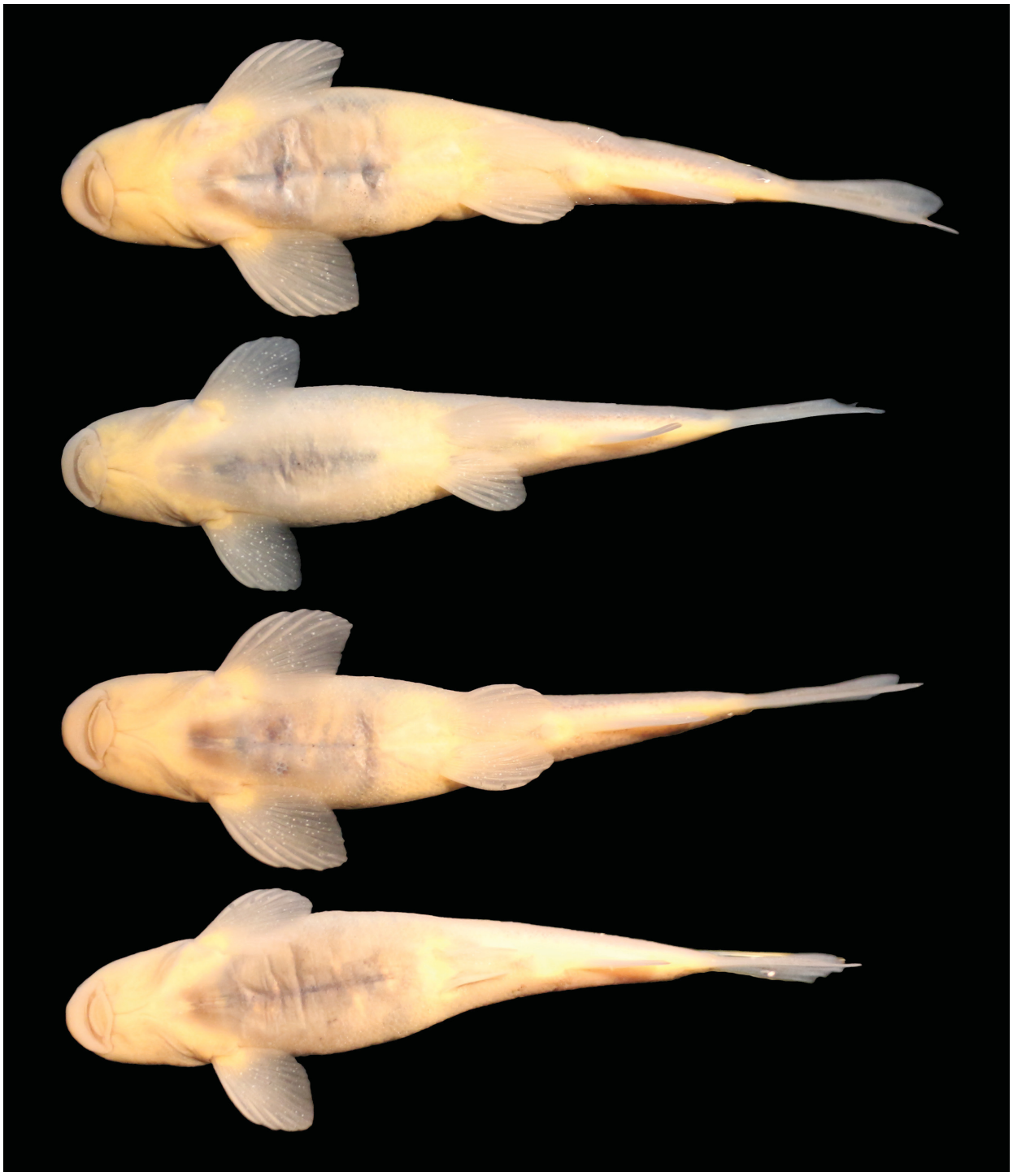

FIGURE 6. *Garra roseae*, VMFC GR-P, paratypes; from above: 36 mm SL; 35 mm SL; 34 mm SL; 34 mm SL; Iran: stream Tang-e-Sarhe.

FIGURE 7. *Garra roseae*, VMFC GR-P, paratype; 36 mm SL; Iran: stream Tang-e-Sarhe.

Comparative material

Garra kemali: FSJF 2490, 7, 36–52 mm SL; Turkey: Konya prov.: Lake Meyil between Yenikent and Esentepe, 37.9861 33.3514.—FSJF 2597, 1, 68 mm SL; Turkey: Konya prov.: Eflatun Pınarı at Sadıkhacı, 37.8252 31.6743.—FSJF 3734, 12, 49–55 mm SL; Turkey: Hirfanlı Reservoir at Geçitli, 39. 1559 33. 6327.

Garra klatti: ZMH 320, 5, 41–50 mm SL; Turkey: Isparta prov.: Lake Gölcük. C. Kosswig, 1954.—ZMH 1122, 2, 53–65 mm SL; Turkey: Isparta prov.: Lake Eğirdir. C. Kosswig, 1953.—FSJF 1873, 2, 41–54 mm SL; Turkey: Denizli prov.: spring running to Lake Isikli at Isikli, 38.3215 29.8512.—FSJF 2466, 1, 69 mm SL; Turkey: Isparta prov.: stream Aksu at Bağıllı, 37.7636 31.0336.—FSJF 2537, 21 44–66 mm SL; Turkey: Afyon prov.: stream west of Kızıllı northwest of Dinar, 38.1228 30.0954.

Garra nudiventris: VMFC GND, 18, 39–81 mm SL; Iran: Sistan-va-Baluchistan prov.: qanat near Nehbandan, 31.5642, 60.0989.

Garra persica: VMFC GPR, 11, 41–85 mm SL: Iran: Sistan-va-Baluchistan prov.: qanat Abtar, 27.2289, 60.8783.

Garra rossica: VMFC GROS, 33, 36–86 mm SL: Iran: Khorasan-e-Razavi prov.: spring Golbahar, 36.5336, 59.0892.

Garra variabilis: FSJF 2554, 8, 56–91 mm SL; Turkey: Adıyaman prov.: stream Eğri km south of Adıyaman, tributary to Atatürk reservoir, 37.7417 38.3351.—FSJF 2658, 21, 54–94 mm SL; Syria: River Orontes at Shayzar, 35.2717 36.5628.—FSJF 2737, 13, 67–105 mm SL; Syria: Nahr al Barid at Nahr al Barid, 35.3022 36.3453.—FSJF 2927, 7, 77–110 mm SL; Turkey: Kilis prov.: stream Sünnep 10 km east of Kilis, 36.7641 37.2541.—FSJF 2991, 2, 52–66 mm SL; Turkey: Gaziantep prov.: stream Merziman south of Yavuzeli, 37.2924 37.7231.

New material used in the molecular genetic analysis. *Garra nudiventris*, VMFC GND, Iran: qanat near Nehbandan, 31.5642, 60.0989. (Genbank accession number: MN258739).

Garra rossica, VMFC GROS, Iran: spring Golbahar, 36.5336, 59.0892. (Genbank accession numbers: MN258740, MN258741).

Source of records shown in Figure 1. Annandale, 1919: **18**, 29.8554 59.9798; Esmaeili *et al.* 2016: **5,** 32.8260 59.2581; **7,** 32.4 59.8166; **9,** 31.8000 60.0167; **14,** 31.2500 61.7000; **16,** 30.9500 61.2833; **21,** 27.8897 61.0933; **26,** 26.58333 60.0333; FSJF: **4,** 32.8797 59.0548; **22,** 27.855 60.767; Sayyadzadeh *et al*. 2015: **3,** 34.7361 60.5749; www.briancoad.com: **8,** 32.17 59.85; **11,** 31.53 60.03; **12,** 31.5 60.1; **15,** 31.2 59.3; **17,** 29.97 60.85; **19,** 28.95 61.35; **20,** 28.17 61.25; **23,** 26.97 61.45; **24,** 26.8 61.0; www.gbif.org: **6,** 32.58 59.03; **25,** 26.67 61.25; own data: **1,** 36.5337 59.0892; **2,** 35.9649 61.1199; **10,** 31.5642 60.0989; **13,** 31.3965 60.7205; **27,** 26.5383 59.9406.

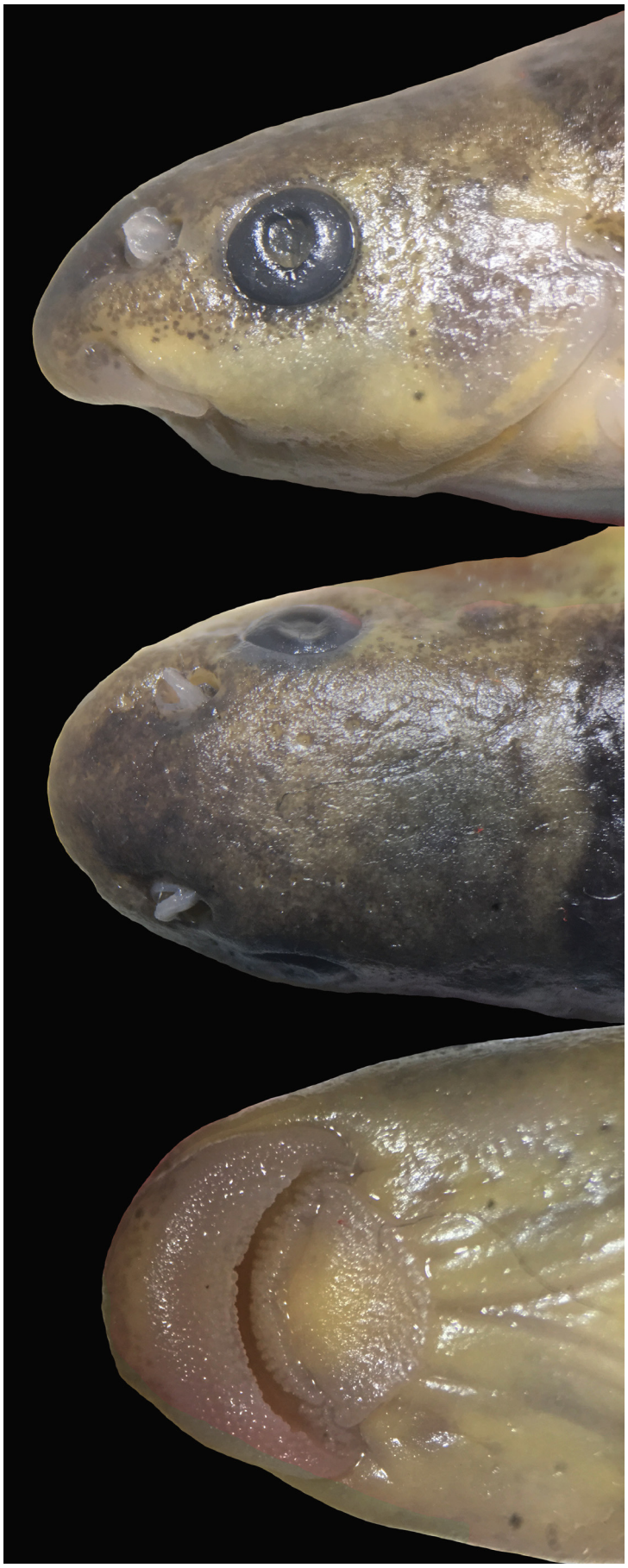

FIGURE 8. *Garra roseae*, VMFC GR-H, holotype, 38 mm SL; Iran: stream Tang-e-Sarhe.

FIGURE 9. Iran: stream Tang-e-Sarhe, type locality of *Garra roseae*.

FIGURE 10. Above: *Garra nudiventris*, VMFC GND, 81 mm SL; Iran: Qanat near Nehbandan. Below: *Garra rossica*, VMFC GROS, 86 mm SL; Iran: spring Golbahar.

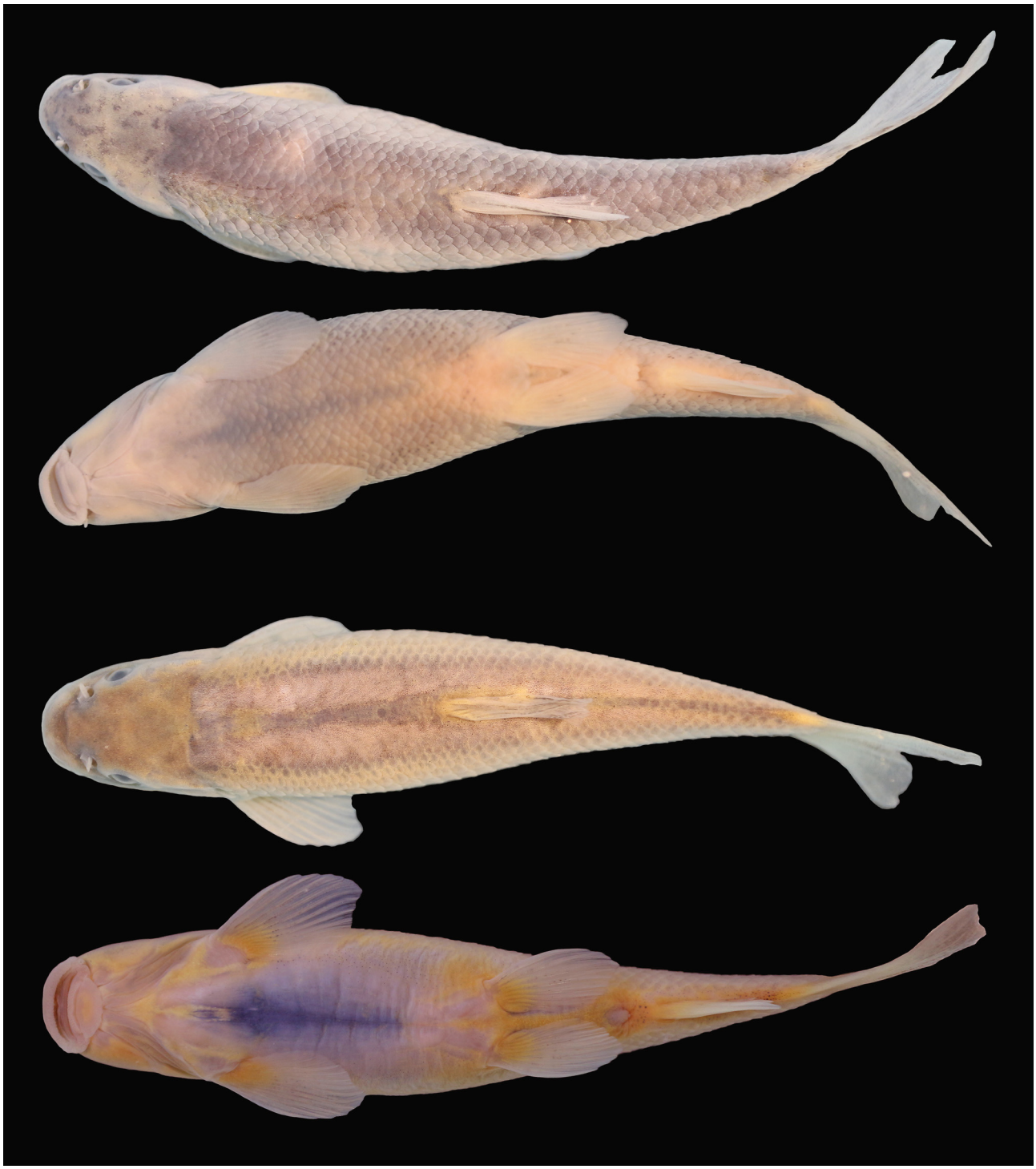

FIGURE 11. Dorsal and ventral views; above: *Garra rossica*, VMFC GROS, 86 mm SL; Iran: spring Golbahar; below: *Garra nudiventris*, VMFC GND, 81 mm SL; Iran: qanat near Nehbandan.

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