

Is *Garra rezai* (Teleostei, Cyprinidae) a species known only from two widely disjunct areas in the Tigris drainage?

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Abstract

Garra rezai was recently described from two geographically distant areas in the Tigris drainage: upper Yanarsu River (eastern Türkiye) and Bouein-Sofla Creek (Iran). In the scope of this study, we aimed to ascertain the distribution ranges of *G. rezai* and its morphologically most similar congener *G. rufa* in Türkiye by examining 1165 specimens from 73 lots, which were collected between 1957 and 2023 and currently curated in two broad fish collections. To achieve this, we focused on two important diagnostic morphological characters which distinguish these two species: scales on predorsal mid-line between dorsal-fin origin and nape, and branched dorsal-fin rays. The results revealed that *G. rufa* is a widely distributed species in the Tigris-Euphrates catchment, while *G. rezai* is regionally widespread, with populations identified in at least six different regions within the Tigris catchment. Additionally, *G. rezai* is documented for the first time in the upper Euphrates. Furthermore, we identified the drainage areas where *G. rezai* co-exists with *G. rufa*.

Key Words

Asia Minor, biodiversity, distribution, fish fauna, Mesopotamia

Introduction

Fish taxonomy, which provides a foundation for scientific research, involves the classification and naming of fish species. Taxonomy helps identify and document different species; it is also essential tasks for assessing biodiversity, tracking changes in populations and implementing effective conservation strategies (Mace 2004). Different species of *Garra* Hamilton, 1822 have gained popularity for their use in spa treatments. These species are also known as doctor fish or nibble fish. They are small freshwater fish belonging to the family Cyprinidae (Ruane et al. 2013; Aydın and Akhan 2020). Although they are not considered threatened or endangered on a global scale (Freyhof 2014), some threats such as habitat degradation and pollution might impact their populations.

Garra is one of the largest genera of the family Cyprinidae, containing approximately 150 species (Majeed et al. 2019). The species belonging to this genus are small to medium-sized fish which usually live in the bottom of fast-flowing rivers and mountain streams. However, some small-sized species of the genus *Garra* - which were previously considered under the genus *Hemigrammocapoeta* Pellegrin, 1927 - prefer vegetated and relatively more stagnant habitats. Approximately a decade ago, *Hemigrammocapoeta* was considered as a synonym of the genus *Garra* by Behrens-Chapuis et al. (2015). *Garra* is widely distributed from east, southeast, south and southwest Asia to tropical Africa (Zhang and Chen 2002; Kottelat 2020).

Garra rufa (Heckel, 1843) was traditionally considered to be distributed in Tigris, Euphrates, Orontes, Ceyhan and

Seyhan areas (Demirci et al. 2016; Ergüden 2016; Kaya et al. 2016; Bayçelebi 2020). However, after the revalidation of *Garra turcica* in Seyhan and Ceyhan (Bayçelebi et al. 2018) and the description of *Garra orontesi* Bayçelebi, Kaya, Turan & Freyhof, 2021 in Orontes (Bayçelebi et al. 2021), it was demonstrated that distribution of *G. rufa* is restricted to Persian Gulf Basin. Until 2022, only two species were known to occur in the upper Tigris-Euphrates River system. However, *Garra rezai* Mousavi-Sabet, Eagderi, Saemi-Komsari, Kaya & Freyhof, 2022 was recently described from the upper Yanarsu River (eastern Türkiye) and Bouein-Sofla Creek (Iran), drainage areas of the Tigris River (Mousavi-Sabet et al. 2022). Surprisingly, the two localities are over 450 km apart.

Another interesting point about *Garra rezai* is its similarity to its two congeneric species (*Garra variabilis* (Heckel, 1843) and *G. rufa*) distributed in the Euphrates-Tigris catchment. Although *G. rezai* is morphologically closer to *G. rufa*, it is genetically closer to *G. variabilis*, which has one pair of barbels, a small mental disc and a comparatively different body shape.

The description of *Garra rezai* raised some questions: Is this species a threatened species with a very limited range? Is *G. rezai* restricted to these two localities, or is it more widespread? Are *G. rezai* and *G. rufa* sympatric/syntopic?

Overall, the aims of this study are: i) to investigate the morphologically-based diagnostic characters of *G. rezai* and *G. rufa*, ii) to reveal the distribution of these species in Türkiye and iii) to answer the above-mentioned questions by analysing samples from two broad fish collections.

Materials and methods

In order to determine the distribution ranges of *Garra rufa* and *G. rezai* in Türkiye, 1165 specimens from 73 lots (Suppl. material 1) were examined from the Recep Tayyip Erdogan University Zoology Collection of the Faculty of Fisheries (FFR) and Collection of Ege University Faculty of Fisheries (ESFM). Material was examined in ESFM collected by electro-fishing and hand scoops between 1957 and 1997 and those in FFR collected with DC electro-fishing equipment between 2005 and 2023.

Since both collection samples were fixed in formaldehyde, it was not possible to perform a molecular study. However, considering the critical diagnostic characters (scales on predorsal mid-line between dorsal-fin origin and nape [PreDs]; branched dorsal fin rays [DFR]; total gill rakers on first branchial arch [GR]) determined by Mousavi-Sabet et al. (2022), many samples preserved in FFR and ESFM were examined.

In this sense, DFR and PreDs were counted for all specimens found in FFR and ESFM. In cases where the species could not be identified with these two critical diagnostic characters, individuals were identified by counting GR, the third critical diagnostic character. The reason why GR could not be counted in all specimens is that the

gill covers are less open and the gill arches are smaller in *Garra* spp. compared to other species, so that the gill lamella has to be removed and dissection from the upper and lower parts of the operculum opening has to be performed in order to count the gill spines. However, the collection authorities did not consent to the partial dissection of over a thousand *Garra* specimens in the FFR and ESFM that were part of the study. The counting methods were followed as described by Armbruster (2012).

Results

As a result, we determined that *Garra rufa* is still a widespread species in the Tigris-Euphrates catchment and *G. rezai* is regionally widespread, with populations in at least six different regions in the Tigris. Furthermore, *G. rezai* is recorded for the first time in the upper Euphrates. Our study indicates that *G. rufa* is considerably more widespread compared to *G. rezai*. Based on the collections examined in this study, *G. rufa* was observed in almost the entire Euphrates, except in the trout zones and throughout the Tigris, except in the Great Zap, Hezil and the eastern part of the Botan. The presence of *G. rufa* in the Menfez Stream near Hezil, as well as in the drainage areas flowing into Botan from the north (Destumi and Bitlis streams), suggests the possibility of *G. rufa* inhabiting the eastern side of Botan and Hezil. However, none of the specimens examined in the Great Zap in this study was identified as *G. rufa*. These assumptions, of course, require confirmation, especially through molecular studies.

On the other hand, it was observed that *G. rezai* is more dominant in all regions of the Tigris where these two species have sympatric distribution. However, in Kaynarca Stream, the only sympatric area in the Euphrates, *G. rufa* was dominant (Fig. 1). This may be attributed to *G. rezai*'s preference for clean and shallow streams rather than large rivers. Kaynarca Stream, dominated by *G. rufa*, is larger and more turbid compared to streams dominated by *G. rezai* in the Tigris. In summary, based on the data obtained from this study, we can assume that, in areas where these two species co-exist, *G. rezai* is dominant in clear, shallow and small streams, whereas *G. rufa* is dominant in turbid, large and relatively deep streams. Sympatric comparison of *G. rufa* and *G. rezai* in Kaynarca, Menfez, Botan and Yanarsu drainage areas are presented in Fig. 2.

Family Cyprinidae

Garra rufa (Heckel, 1843)

Fig. 3

Common names. Doctor fish.

Diagnosis. *Garra rufa* is distinguished from all the species of *Garra* in adjacent waters in having a combination of the following characters: Breast and belly covered by scales, scales embedded in skin, rarely ab-

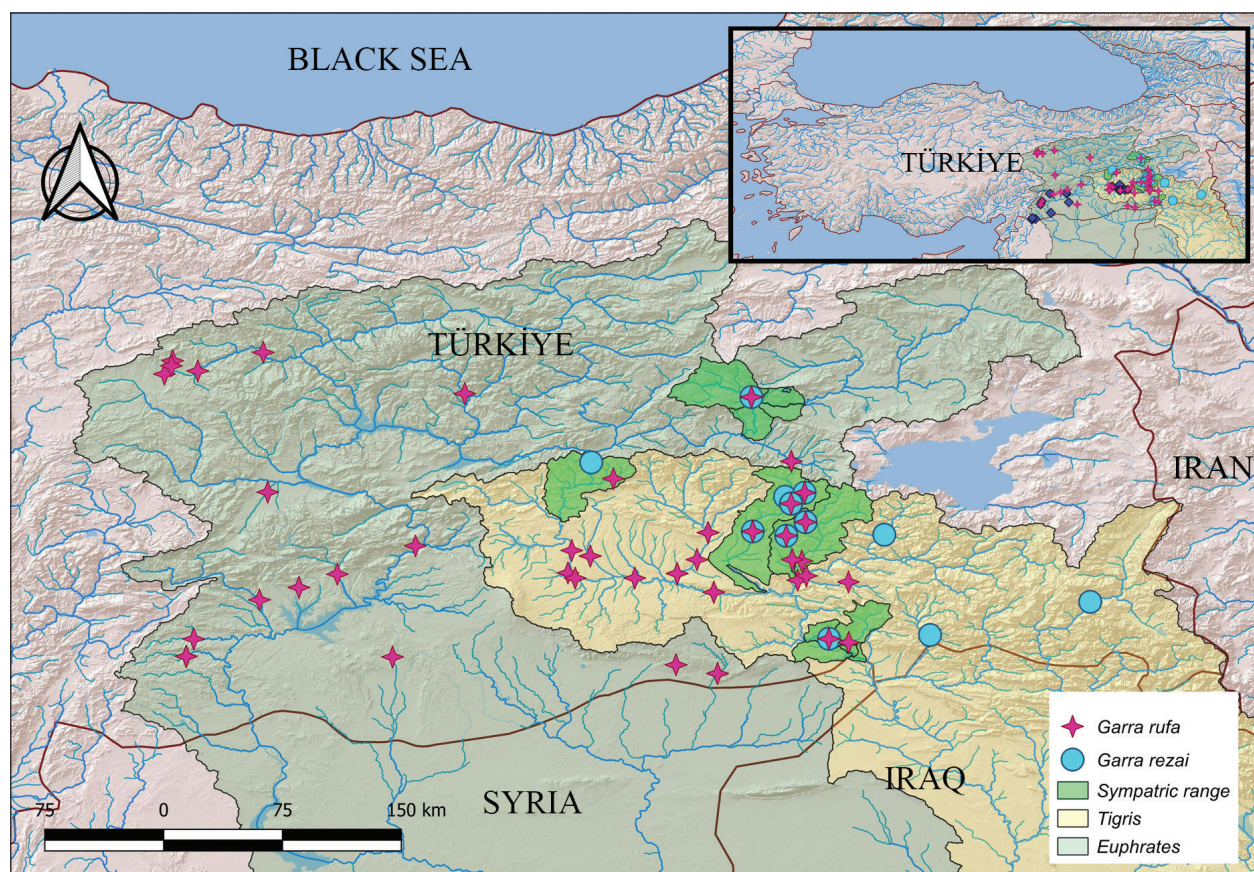


Figure 1. Distribution of the *Garra rufa* and *G. rezai* in Türkiye.

sent, mid-dorsal area in front of dorsal origin covered by (8)9–12(13–14) scales, 32–38 total lateral-line scales, usually $4\frac{1}{2}$ transverse scale rows between lateral line and dorsal origin, 11–13 circumpeduncular scales, 20–29 total gill rakers, usually $8\frac{1}{2}$ branched dorsal rays, eye fully developed.

Distribution in Türkiye. Extirpated in Qweik, does not occur in Lakes Van and Hazar. Very widespread in Euphrates. Widespread also in Tigris, but no specimens could be observed from the Great Zap, Hezil Stream and the eastern part of the Botan in FFR and EFSM.

IUCN Status. Least Concern (Freyhof 2014).

***Garra rezai* Mousavi-Sabet, Eagderi, Saemi-Komsari, Kaya & Freyhof, 2022**

Fig. 3

Common names. Tigris garra.

Diagnosis. *Garra rezai* is distinguished from all the species of *Garra* in adjacent waters in having a combination of the following characters: Breast with embedded scales, predorsal mid-line covered by (12)13–18(19–21) scales, gular disc short and wide, 35–40 total lateral-line scales, $5\frac{1}{2}$ (rarely $4\frac{1}{2}$ and $6\frac{1}{2}$) transverse scale rows between lateral line and dorsal origin, $3\frac{1}{2}$ – $4\frac{1}{2}$ transverse scale rows between lateral line and pelvic origin, 15–18 circumpeduncular scales, axillary scale at

pelvic origin large, 11–16 total gill rakers, usually $7\frac{1}{2}$ branched dorsal rays, eye fully developed.

Distribution in Türkiye. Known only from Kaynarca Stream (Murat drainage) in Euphrates. In Tigris; Yanarsu, Botan, Menfez, Hezil and Batman drainages, as well as from Aktoprak Stream, an uppermost drainage of Tigris River.

IUCN Status. Not Evaluated. The results of this study revealed that *G. rezai* is distributed in at least seven different drainage areas in Türkiye. Although *G. rezai* is known from only one locality in Iran outside Türkiye, we expected it to inhabit also other localities in Iran, Iraq and possibly Syria. In summary, this species occurs in various drainage areas and, in general, its populations appear to be in good condition; its IUCN status is suggested to be Least Concern.

Intermediate specimens

Amongst all 73 lots, we had difficulty in identifying only five samples from the Aktoprak Stream station (FFR1821, $n = 23$). A total 18 of these samples were easily recognised as *G. rezai* with 12(3), 13(3), 14(2), 15(5), 17(4), 20(1) PreDs and $7\frac{1}{2}$ (16), $8\frac{1}{2}$ (2) DFR ranges. However, five individuals (later moved to another lot with a new collection code: FFR 4062) with 9(1), 11(3), 17(1) PreDs and $7\frac{1}{2}$ (2), $8\frac{1}{2}$ (3) DFR ranges did not match either *G. rufa* or *G. rezai* and

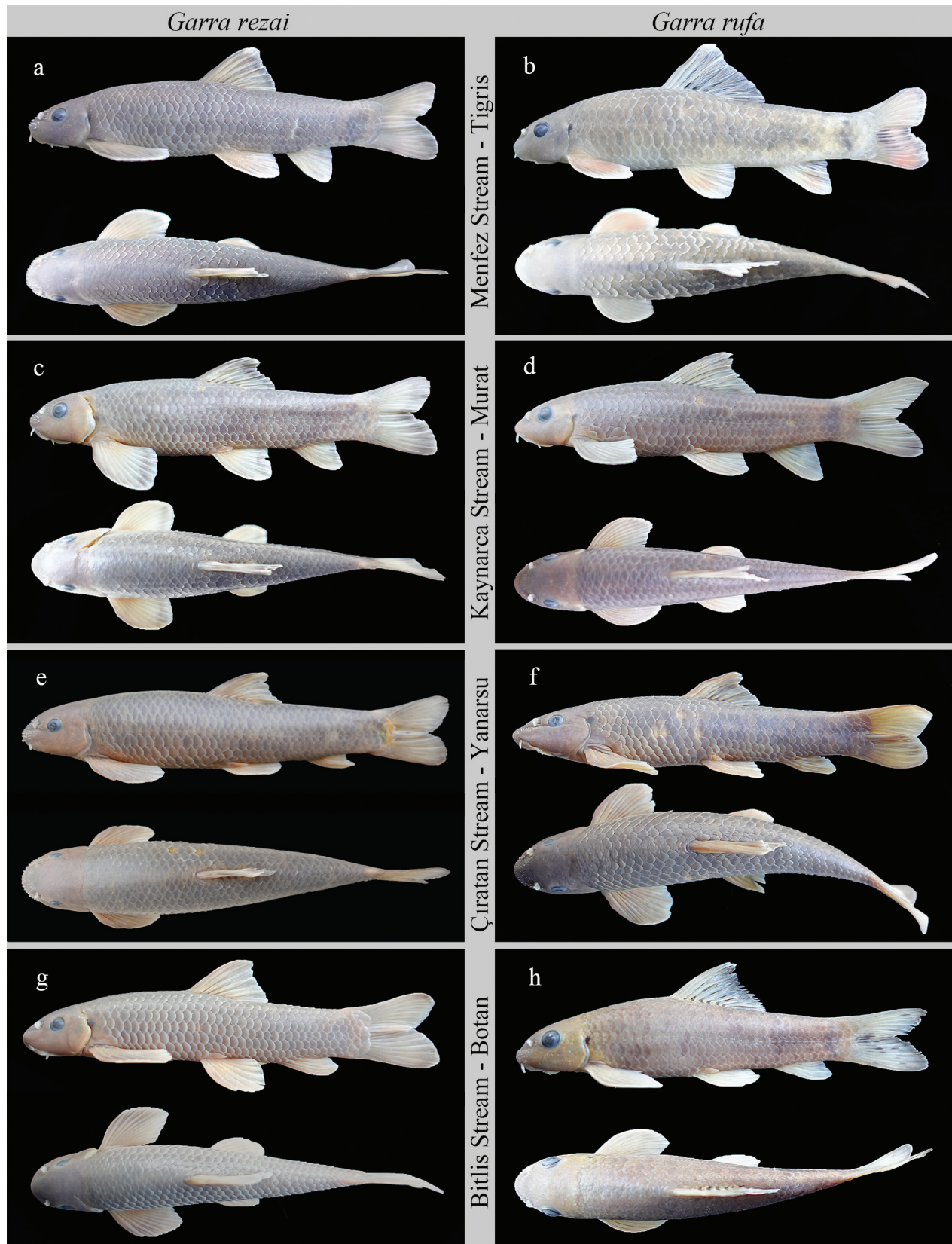


Figure 2. Sympatric comparison of *G. rufa* and *G. rezai*: Menfez Stream, Tigris drainage: (a) *G. rezai*, FFR 4044, 86 mm SL, (b) *G. rufa*, FFR 4044, 84 mm SL; Kaynarca Stream, Murat drainage, Euphrates, (c) *G. rezai*, FFR 4061, 70 mm SL, (d) *G. rufa*, FFR 1340, 69 mm SL; Çıratan Stream, Yanarsu drainage, Tigris, (e) *G. rezai*, FFR 1302, 113 mm SL, (f) *G. rufa*, FFR 4037, 125 mm SL; Bitlis Stream, Botan drainage, Tigris, (g) *G. rezai*, FFR 1274, 75 mm SL, (h) *G. rufa*, FFR 1348, 72 mm SL.



Figure 3. Upper one, *G. rufa*, not preserved, about 110 mm SL, from Merzimen Stream, Euphrates drainage: Lower one, *G. rezai*, FSJF 3824, 104 mm SL; Türkiye: Çıratan Stream, upper Yanarsu drainage, Tigris (Retrieved from Mousavi-Sabet et al. (2022)).

the values were grouped between the two species. Although GRs were analysed for control, these individuals did not match either species. The most notable example was an individual with 17 PreDs and $8\frac{1}{2}$ DFR, which was expected to be *G. rezai*; however, it moved the individual closer to *G. rufa* with 20 GR (GR range of *G. rufa* is 20–29). Therefore, here we identified these five individuals as *Garra* sp. (Fig. 4). We encourage researchers to further study these populations, especially using molecular markers.

Discussion

Mousavi-Sabet et al. (2022) distinguished *Garra rezai* from *G. rufa* by a minimum K2P distance of 14.9% in the mtDNA COI barcode region, as well as the following morphologic characters: *G. rezai* is distinguished from *G. rufa* by having 11–16 total gill rakers [GR] (vs. 20–29), 15–19 scales on predorsal mid-line between dorsal-fin origin and nape [PreDs] (vs. 11–14), $7\frac{1}{2}$ (rarely $8\frac{1}{2}$) branched dorsal-fin rays [DFR] (vs. usually $8\frac{1}{2}$, rarely $7\frac{1}{2}$ or $9\frac{1}{2}$), 15–18 circumpeduncular scales (vs. 11–13), $5\frac{1}{2}$ (rarely $4\frac{1}{2}$ and $6\frac{1}{2}$) and transverse scale rows between the lateral line and the dorsal-fin origin (vs. $4\frac{1}{2}$).

Here, we selected the most diagnostic and easily distinguishable two characters: PreDs and DFR.

Based on the two critical diagnostic characters focused on in this study, Mousavi-Sabet et al. (2022) examined 25 *G. rezai* individuals for PreDs and counted as 15–19 [15(2), 16(3), 17(10), 18(8), 19(2)], while they examined 58 individuals for DFR [$7\frac{1}{2}$ (54), $8\frac{1}{2}$ (4)]. In order to determine to what extent these characters are realistic and to reveal to which species the identified specimens belong, 1165 specimens preserved in FFR and ESFM were examined. Our morphological examination demonstrated that 1016 of these specimens belong to *G. rufa*, while 149 belong to *G. rezai* (Table 1 and Suppl. material 1). Our preliminary results are in agreement with Mousavi-Sabet et al. (2022) ranges for PreDs and DFR, even if they are partially expanded and the mode value of *G. rezai* changed. According to our comprehensive examination, PreDs range was (8)9–12(13–14) with mode 10 in 1016 *G. rufa* specimens and (12)13–18(19–21) with mode 15 in 149 *G. rezai* specimens (see Table 1 and Fig. 5 for details). Mousavi-Sabet et al. (2022) identified the PreDs range of *G. rezai* as 15–19 with a mode of 17. For DFR range of our results, we found ($7\frac{1}{2}$) $8\frac{1}{2}$ ($9\frac{1}{2}$) in 1016 *G. rufa* specimens and $7\frac{1}{2}$ ($8\frac{1}{2}$) in 149 *G. rezai* specimens (see Table 1 and Fig. 5 for details).

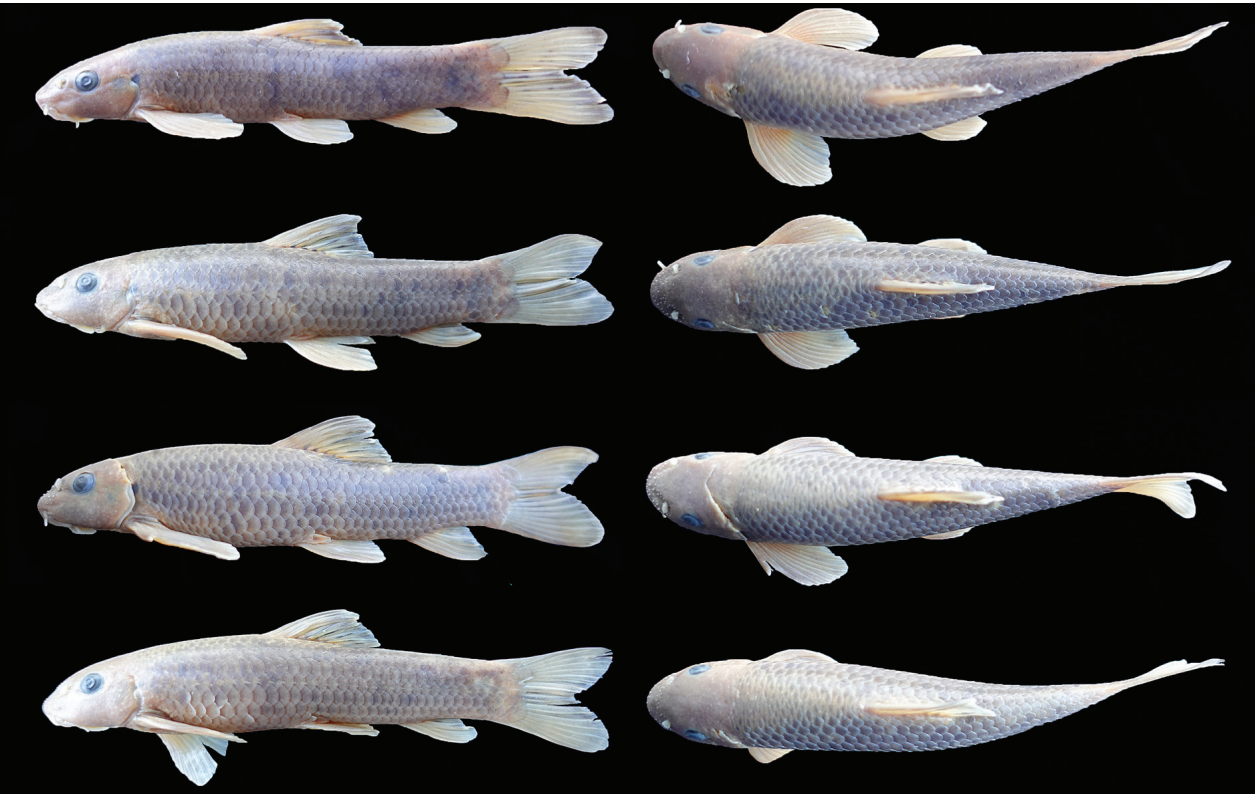


Figure 4. Some intermediate specimens: FFR 4062, Aktoprak Stream, upper Tigris drainage, from the top, 65 mm SL, 67 mm SL, 69 mm SL, 71 mm SL.

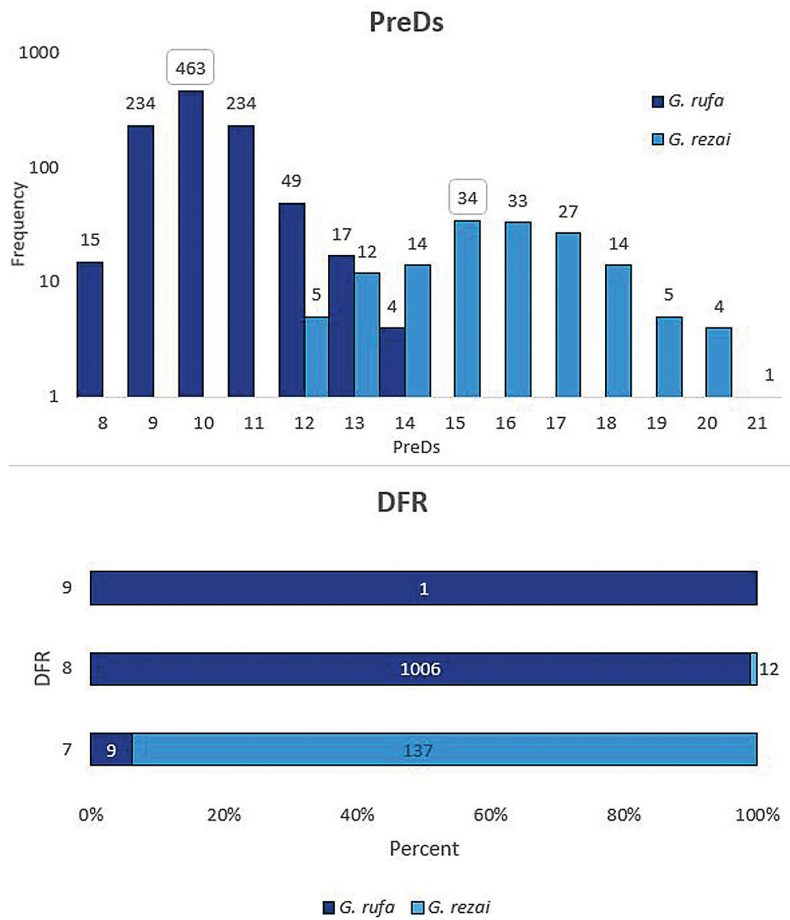


Figure 5. Frequency distributions of *G. rufa* and *G. rezai* for PreDs and DFR.

Table 1. Frequency distributions of *G. rufa* and *G. rezai* for PreDs and DFR.

	n	PreDs													
		8	9	10	11	12	13	14	15	16	17	18	19	20	21
<i>G. rufa</i>	1016	15	234	463	234	49	17	4	–	–	–	–	–	–	–
<i>G. rezai</i>	149	–	–	–	–	5	12	14	34	33	27	14	5	4	1
	n	DFR													
		7	8	9											
<i>G. rufa</i>	1016	9	1006	1											
<i>G. rezai</i>	149	137	12	–											

Our previous in-situ observations and examination of the collection specimens in FFR and ESFM revealed that there is an important relationship between *Garra rezai*, the main species of this study and the recently described *Turcinoemacheilus ekmekciae* Kaya, Yoğurtçuoğlu, Aksu, Bayçelebi & Turan, 2023, particularly concerning their distribution ranges. As mentioned in the Results section, *G. rezai* individuals were found from Kaynarca Stream (upper Murat River drainage, Euphrates), as well as from Yanarsu, Botan, Menfez, Hezil and Batman drainage areas (tributaries of the Tigris River). *T. ekmekciae* was described from Kaynarca Stream (upper Murat River drainage), as well as recorded from Yanarsu, Botan, Nerdüş and Batman drainage areas (tributaries of the Tigris River) (Kaya et al. 2024). It is obvious that both species have a very similar distribution pattern. In fact, the most interesting point in the distribution of these two species is that they were not expected to be distributed in different rivers and inhabit limited areas. Despite this, the fact that these two species - in addition to their expected distribution outside the Tigris River - also occur in the Murat River, the most important tributary of the Euphrates River, shows that there may be a strong relationship between the Murat and Tigris Rivers. In particular, the distribution of *Turcinoemacheilus ekmekciae*, which is not known to inhabit more than one different river and belongs to a genus with a generally limited distribution range and *G. rezai*, which is thought to have a regional distribution in certain areas (Fig. 1), in both the Yanarsu and Murat Rivers indicates that the connection of these two rivers naturally points to the elevation of at least part of the Muş South Mountains not being very ancient.

This study has shown that the distribution of *G. rezai* is not restricted and that many previous records of *G. rufa* (e.g. Hashemzadeh et al. (2015); Kaya et al. (2016)) may actually belong to *G. rezai*. Therefore, this study will shed light on studies to determine the distribution of this species in Iran, Iraq and Syria. We strongly encourage morphological and molecular studies to reveal the distribution of *G. rezai* and *G. rufa* in Iran, Iraq and Syria.

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Supplementary material 1

Material examined for this study with detailed collections data

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Data type: docx

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