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Morphometric Analysis of *Garra* Species collected from Ichthyotherapy Ponds in Türkiye

Türkiye’deki Balık Terapi Havuzlarından Toplanan *Garra* Türlerinin Morfometrik Analizi

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ABSTRACT

Garra rufa is commonly known as “doctor fish” and widely used for ichtyoterapy in many countries. This study aims to clarify the taxonomic identity of *Garra* specimens used in ichthyotherapy ponds in Antalya, Türkiye, a region well known for its extensive ichthyotherapy practices in hotels and spa centers, owing to its prominence as a major tourism destination. Due to increasing global demand for ichthyotherapy and the morphological similarity among *Garra* species, non-native or misidentified species may be introduced into fish spa facilities. In this study, a total of twenty-five individuals from four fish-spa centers and one reference population (*Garra rufa* from Göksu River) were examined. Twenty-two morphometric and ten meristic characters were analyzed through size-corrected comparisons and ANCOVA models. Results revealed significant morphological differences between the reference *G.rufa* and the spa fish, while the fish from commercial sources were morphologically cohesive and highly similar to each other. Comparative analysis suggests that the spa specimens are not *Garra rufa* but most likely *Garra turcica*, a species recently reported outside its native range near Manavgat, possibly due to aquaculture activities for ichthyoterapy. The findings highlight the risk of species misidentification in ichthyotherapy trade and its ecological implications. The study emphasizes the need for integrating molecular tools alongside morphological analysis to ensure accurate species identification and to inform conservation and management strategies.

Key Words
Fish spa, *Garra rufa*, ichthyotherapy, doctor fish.

ÖZ

Garra rufa genel olarak “doktor balık” olarak bilinir ve bir çok ülkede ihtiyoterapi uygulamalarında yaygın olarak kullanılmaktadır. Bu çalışma, Türkiye’nin önemli bir turizm merkezi olan Antalya ilindeki otel ve spa tesislerinde yaygın olarak bulunan balık terapisi havuzlarında kullanılan *Garra* örneklerinin taksonomik durumunu netleştirmeyi amaçlamaktadır. İhtiyoterapiye olan küresel talebin artması ve *Garra* türleri arasındaki morfolojik benzerliklere bağlı olarak, balık terapi tesislerine yerli olmayan veya yanlış tanımlanmış türler taşınabilmektedir. Çalışma kapsamında, Antalya bölgesindeki dört farklı terapi merkezi ve bir referans popülasyondan (Göksu Nehri'nden *Garra rufa*) toplam 25 birey incelenmiştir. Yirmi iki morfometrik ve on meristik karakter, boyuta göre düzeltilmiş karşılaştırmalar ve ANCOVA modelleri ile analiz edilmiştir. Sonuçlar, balık terapi merkezlerinden elde edilen bireylerin morfolojik olarak birbirlerine çok benzediğini, ancak referans *Garra rufa* popülasyonundan anlamlı farklılıklar gösterdiğini ortaya koymuştur. Karşılaştırmalı analizler, balık terapi havuzlarında kullanılan bireylerin *Garra rufa* olmadığını, büyük olasılıkla balık terapisi için yetiştiricilik faaliyetleri nedeniyle doğal yayılış alanı dışına taşındığı bilinen *Garra turcica* olduğunu göstermektedir. Bu bulgular, balık terapisi ticaretinde tür teşhisinin hatalı yapılma riskine ve bunun ekolojik sonuçlarına dikkat çekmektedir. Çalışma, türlerin doğru tanımlanmasını sağlamak ve koruma ve yönetim stratejileri geliştirilebilmek için morfolojik analizlerin moleküler verilerle desteklenmesi gerektiğini vurgulamaktadır.

Anahtar Kelimeler
Balık terapi tesisleri*, Garra rufa,* ihtiyoterapi, doktor balık.

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INTRODUCTION

Ichthyotherapy, an alternative treatment method utilizing freshwater fish as biological agents for dermatological conditions such as psoriasis and ichthyosis, has gained considerable international attention following its traditional origins in Türkiye [1,2]. The practice originated at the 'Balıklı Kaplıca' (Fish Spring) in Kangal, Sivas, where the naturally occurring thermal pools inhabited by fish have been used by the local community since the early 1900s to treat skin diseases. The therapeutic potential of these waters was first recognized when a shepherd reportedly healed his foot wounds by bathing in the spring. This led to the construction of the first public bathing facilities in 1963 [1,3].

Garra rufa, commonly known as the "doctor fish," has become the flagship species for ichthyotherapy globally, despite the fact that both *G. rufa* and *Cyprinion macrostomum* naturally inhabit the original Kangal spa [2-4]. The native distribution of the species extends across the Qweiq, Euphrates, and Tigris drainage, also occurs in the Iranian Persian Gulf basin extends from Zohreh south to Mond, including the endorheic Kor basin [5-8]. The commercial success of ichthyotherapy has resulted in its widespread adoption beyond its initial therapeutic applications, including aesthetic treatments such as fish pedicures in numerous countries, particularly in North America, Europe, and the Mediterranean region [9-12].

The increasing global demand for ichthyotherapy has led to substantial pressures on wild *G. rufa* populations, resulting in legal restrictions on the collection of these specimens from their natural habitats due to conservation concerns [10]. Consequently, commercial fish therapy facilities increasingly rely on licensed and unlicensed aquaculture operations to meet their needs. This raises questions about the origin and sustainability of the fish within the trade. As many *Garra* species are almost identical in appearance, non-*G. rufa* congeners with similar "doctor fish" characteristics are frequently used as substitutes in spa ponds. Five of the nine *Garra* species native to Türkiye are *G. orontesi*, *G. rezai*, *G. turcica*, *G. variabilis* and *G. rufa*, possess the characteristics valued in ichthyotherapy and may spread beyond their natural ranges, either intentionally or accidentally. However, visual identification by non-experts is unreliable [8,13], and commercial practices often bypass the detailed morphological or molecular analysis required for accurate species verification.

The Antalya region, a major tourism destination with numerous fish spa facilities, is a significant area for investigating the actual species composition used in commercial ichthyotherapy operations. Recent documentation of *Garra rufa* from natural waters near Manavgat, Antalya, well outside its native Seyhan and Ceyhan drainages, suggests potential anthropogenic introduction events possibly linked to aquaculture or fish therapy operations [14,15]. Such introductions raise significant ecological concerns regarding the establishment of non-native populations and their potential impacts on native aquatic communities. Furthermore, the phenotypic plasticity observed in *Garra* species under different environmental conditions, such as artificial breeding and culture systems, may complicate the accurate identification of species in commercial facilities.

The present study addresses this taxonomic uncertainty through comprehensive morphometric and meristic analysis of *Garra* specimens obtained from several fish therapy facilities in the Antalya region. By comparing therapeutic pond specimens with confirmed *G. rufa* from the Göksu River (Upper Euphrates) and evaluating morphological characteristics against diagnostic traits of potential congeners, this research aims to clarify the species identity of fish currently used in commercial ichthyotherapy operations and assess the implications for both commercial practices and conservation management.

MATERIALS and METHODS

A total of 25 live specimens of *Garra* spp. were obtained, including five individuals each from an licensed aquaculture facility supplying fish to hotels and spa centres (Station A), and from three ichthyoterapy centers (Station B near Alanya, stations C and D near Manavgat) in the Antalya region, along with five *G. rufa* from the Göksu River (upper Euphrates, 37°50'22.79"N 37°41'49.96"E) collected using backpack electrofishing (Samus MS725). The fish were over-euthanized and then fixed in 4% buffered formaldehyde before being transferred to the laboratory for morphometric and meristic examination.

We measured 22 standard morphometric characters on the left side of each specimen with a digital caliper (±0.01 mm), following Freyhof et al. [8]. These characters included body depth (BD), pre-dorsal length (PreDL), post-dorsal length (PostDL), dorsal fin height (DH), dorsal fin length (DL), caudal peduncle depth

(CPD), caudal peduncle length (CPL), pre-anal length (PreAL), pre-pelvic length (PrePL), anal fin length (AL), anal fin height (AH), distance between pectoral and pelvic fin origins (DPP), distance between pelvic and anal fin origins (DPA), pectoral fin height (PFH), pelvic fin height (PH), head length (HL), head depth at eye level (HDE), snout length (SnL), eye diameter (ED), post-orbital length (PostOL), maximum head width (MHW), and inter-orbital width (IOW). All are taken as a straight line from point to point, as shown in Figure 1, not across the body curves or as projections along the longitudinal axis. Standard length was measured from the tip of the snout to the base of the median caudal-fin rays at the end of the hypural complex. HL is the distance from the body's foremost point to the opercular membrane's posteriormost point. We also counted ten meristic characters as dorsal, pectoral, pelvic, and anal fin rays; lateral-line scales (LL); gill rakers on the first gill arch; transverse scales between lateral line and origin of dorsal fin; (LL/DF); transverse scales between lateral line and pelvic fin base (LL/PF); transverse scales between lateral line and origin of anal fin (LL/AF) and predorsal scales.

All morphometric traits were initially scaled to standard length (SL%) and examined for non-overlapping minimum-maximum ranges across stations. The traits that exhibited distinct station-specific ranges were then subjected to formal size adjustment and group comparison using ANCOVA. Prior to analysis, both morphometric traits and standard length were log-transformed (natural logarithm) to linearize allometric relationships and meet assumptions of normality and homoscedasticity. ANCOVA model was initially fitted for each morphometric character as log(Trait) ~ log(SL) × Station, where the interaction term (log(SL) × Station) tests for heterogeneity of allometric slopes among stations. When the interaction term was not significant ($p > 0.05$), indicating homogeneous allometric relationships across stations, the model was simplified to log(Trait) ~ log(SL) + Station. This reduced model allows for direct comparison of station effects while controlling for body size variation. Station A served as the reference level for all comparisons. To validate the ANCOVA results and account for potential non-normality, residuals from a size-only model [log(Trait) ~ log(SL)] were extracted and subjected to Kruskal-Wallis rank sum tests to confirm significant among-station differences. For traits showing significant station effects, allometric correction was applied using the slope coefficient (b) from the size-only model. Size-adjusted traits were calculated as Trait_adjusted = Trait / (SL^b), where b represents the allometric exponent estimated from the relationship between log-transformed trait and log-transformed standard length. Model performance was evaluated using multiple R-squared values, and all models were assessed for assumptions of linearity, homoscedasticity, and normality of residuals. The significance threshold was set at $\alpha = 0.05$ for all statistical tests. Statistical analyses were performed in R [16], using base R functions for linear modeling and analysis of variance.

RESULTS and DISCUSSION

The metric and meristic characters of the *Garra* samples collected from hotel pools in Antalya were thoroughly analyzed. The characters of fish from therapy ponds were initially compared with *Garra rufa* collected from the Göksu River to test if fish were originated from Euphrates drainage, as supposed and claimed by the fish suppliers (Table 1). Among the morphological

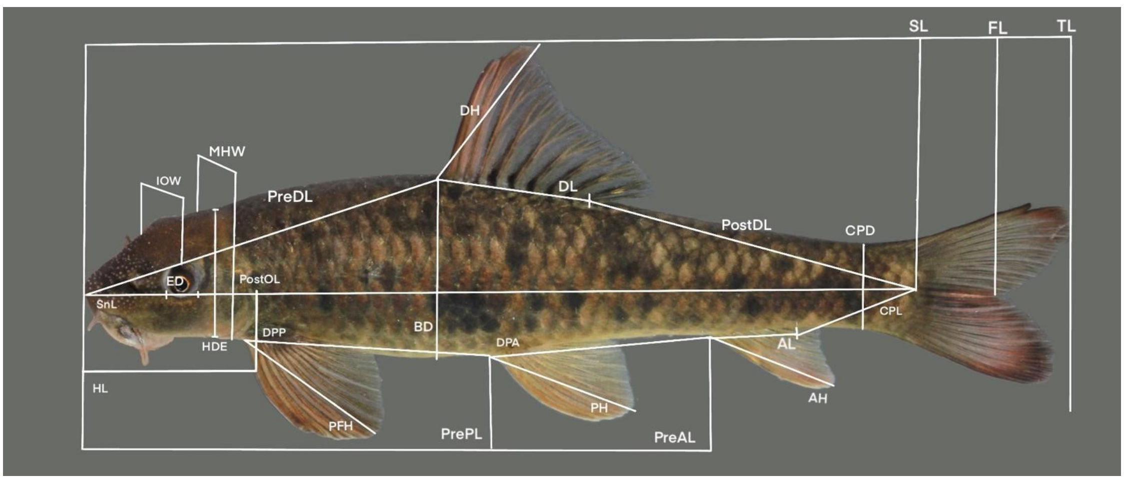

Figure 1. Morphometric measurements of *Garra* specimens.

Table 1. Standard length ratios of measurements taken from *Garra* species.

	Station A				Station B				Station C				Station D				*Garra rufa* (Göksu River)			
	Min	Max	Mean	STD	Min	Max	Mean	STD	Min	Max	Mean	STD	Min	Max	Mean	STD	Min	Max	Mean	STD
SL	28	56	40.1	11.5	33	58	39.5	7.0	33	56	40.3	7.1	35	48	40.4	4.4	56	132	92.8	32.2
In % of SL																				
BD	18.8	22.6	20.7	1.4	16.4	19.4	17.4	1.2	18.9	21.6	20.2	1.2	13.4	19.0	16.7	2.2	20.5	21.9	21.3	0.6
PreDL	43.8	49.6	46.1	2.5	45.5	49.4	47.2	1.8	45.2	47.5	46.4	1.0	45.5	50.4	47.6	2.0	44.0	47.0	45.6	1.3
PostDL	50.7	52.4	51.6	0.7	49.6	54.6	51.7	2.0	45.8	52.4	48.9	2.4	50.8	54.0	52.2	1.2	48.7	56.7	52.5	2.9
DH	21.2	22.6	22.1	0.6	20.1	23.6	21.6	1.4	20.8	24.4	22.6	1.7	22.5	24.8	23.9	0.8	21.0	24.9	23.2	1.6
DL	16.0	17.4	17.0	0.6	14.1	17.9	15.8	1.7	15.1	18.2	16.8	1.1	14.3	19.6	16.6	2.1	16.4	19.6	18.0	1.4
CPD	11.1	12.7	12.3	0.7	10.6	11.3	10.9	0.3	12.2	13.9	12.9	0.6	9.4	12.9	11.3	1.2	10.0	13.5	12.1	1.3
CPL	12.6	17.7	14.5	2.4	11.3	15.7	13.6	1.6	10.1	14.1	12.5	1.8	11.1	15.8	13.1	1.9	13.0	15.5	14.1	1.0
PreAL	72.1	76.3	74.8	2.0	75.3	80.3	77.1	1.9	72.8	77.4	75.8	1.7	75.4	79.1	76.7	1.4	73.7	79.0	75.7	2.0
PrePL	52.4	55.8	53.7	1.3	50.9	55.9	53.7	1.8	53.9	55.9	54.8	0.8	51.5	55.0	53.2	1.5	51.5	53.7	52.6	1.0
AH	17.0	19.9	18.0	1.1	14.6	19.3	16.2	1.8	16.5	20.0	18.0	1.3	18.6	20.4	19.3	0.7	16.7	20.7	18.5	1.7
AL	7.8	10.6	8.9	1.2	6.8	8.0	7.5	0.5	8.4	9.5	8.9	0.4	8.2	9.8	9.1	0.6	7.8	10.0	8.9	0.8
DPP	30.0	33.2	31.2	1.5	30.9	33.2	31.7	1.0	30.7	34.2	32.5	1.6	28.3	33.3	30.7	2.0	30.9	33.5	32.5	1.1
DPA	22.2	25.6	23.7	1.5	23.0	25.7	24.4	1.3	21.5	24.0	22.9	1.0	22.3	25.4	23.4	1.2	23.8	26.3	24.9	1.2
PFH	22.8	24.8	23.9	1.0	20.2	23.7	21.4	1.4	22.5	24.3	23.4	0.8	21.4	26.6	24.1	2.1	21.3	24.2	22.5	1.1
PH	17.3	19.9	18.8	1.1	15.6	19.3	16.9	1.5	17.8	20.1	18.8	1.0	18.2	21.0	19.7	1.1	17.5	21.1	19.2	1.5
HL	22.1	25.1	23.3	1.3	21.5	25.7	23.5	1.7	23.0	26.4	24.6	1.3	20.1	26.1	24.1	2.4	20.8	23.0	21.7	1.0
HDE	12.3	14.3	13.7	0.8	11.3	13.3	12.4	0.7	13.0	17.5	14.4	1.8	11.1	13.6	11.9	1.0	12.8	14.5	13.8	0.7
SnL	9.2	11.3	10.4	0.8	8.2	10.4	9.5	0.8	7.1	11.0	9.5	1.5	9.0	10.9	10.3	0.7	8.8	11.0	9.9	0.8
ED	5.1	6.6	5.7	0.6	5.2	6.2	5.7	0.4	4.0	5.9	5.1	0.8	4.5	5.5	5.0	0.4	4.5	6.1	5.0	0.7
PostOL	8.3	10.4	9.0	0.9	9.5	11.5	10.3	0.8	8.3	11.7	10.0	1.5	7.6	9.8	9.0	0.8	6.1	7.5	7.0	0.5
MHW	18.2	22.2	19.9	1.5	17.3	18.7	17.8	0.6	18.8	20.1	19.4	0.5	17.2	19.0	18.0	0.8	17.0	19.1	18.0	0.9
IOW	10.3	12.3	11.2	0.9	9.0	10.5	9.9	0.7	10.9	14.0	12.0	1.3	8.7	12.0	10.3	1.2	10.7	12.5	11.4	0.7

characters analyzed, nine showed at least one station with a non-overlapping size-corrected range. These are, anal fin length (AL), eye diameter (ED), head length (HL), snout length (SnL), postorbital length (PostOL), maximum head width (MHW), interorbital width (IOW), head depth at eye (HDE), and pelvic-fin height (PH) each displayed distinct, non-overlapping ranges for at least one station, suggesting clear morphological separation (Figure 2, Table 2).

The analysis performed using ANCOVA revealed a significant degree of morphometric differentiation among the four stations examined (see Table 1). The results indicated that all nine characters exhibited significant sta-

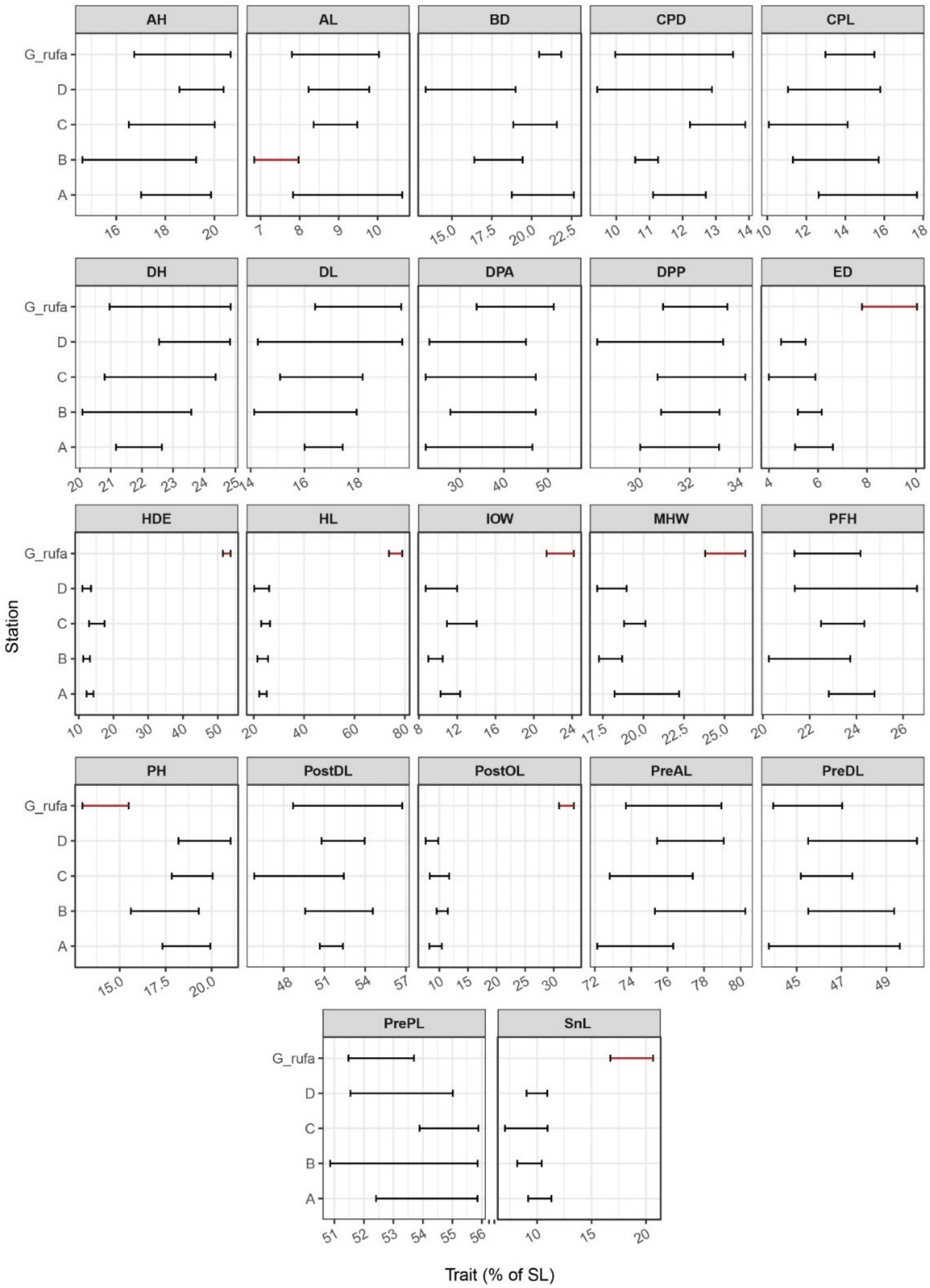

Figure 2. Minimum-maximum %SL ranges per station and morphological traits. Red marked lines indicate stations with non-overlapping (or slightly overlapping) range.

Table 2. Summary of ANCOVA Results for 9 Morphometric non-overlapping characters. NS = Not Significant; * p < 0.05, ** p < 0.01, *** p < 0.001; Station effects tested after removing interaction terms; Kruskal-Wallis tests performed on residuals from size-only models.

Character	Interaction p-value	Station Effect p-value	Allometric Slope (b)	R²	Kruskal-Wallis p-value	Station Differences
ED	0.213 (NS)	***	0.481	0.972	0.038*	StationC**, StationD***, *G_rufa****
HL	0.893 (NS)	***	0.855	0.992	0.016*	*G_rufa****
SnL	0.606 (NS)	***	0.906	0.952	0.048*	*G_rufa****
PostOL	0.066 (NS)	***	0.569	0.989	0.009**	*G_rufa****
MHW	0.950 (NS)	***	0.945	0.980	0.056 (NS)	StationB**, StationD**, *G_rufa****
IOW	0.324 (NS)	***	0.965	0.973	0.023*	StationB*, *G_rufa****
HDE	0.747 (NS)	***	0.891	0.992	0.035*	StationB*, StationD**, *G_rufa****
PH	0.592 (NS)	***	0.995	0.882	0.011*	StationB*, *G_rufa****
AL	0.332 (NS)	0.008**	0.816	0.900	0.036*	StationB**

tion effects ($p < 0.01$), with no substantial interactions between log-transformed standard length and station for any character (all $p > 0.05$). This finding suggests the homogeneity of allometric relationships across stations. The results of the Kruskal-Wallis test on size-corrected residuals revealed significant between-station differences for eight of the nine characters ($p < 0.05$). However, only MHW demonstrated marginal significance ($p = 0.056$). In the context of the obtained results, Station *G. rufa* was identified as the most morphologically distinctive, exhibiting significant disparities from the reference station (Station A) in eight of the nine characters analysed. The present station revealed highly significant differences ($p < 0.001$) in ED, HL, SL.1, PostOL, MHW, IOW, HDE, and PH, with only AL showing no significant differentiation. Station B exhibited significant differentiation in five characters (ED, MHW, IOW, PH, and AL), while Station D showed differences in three characters (ED, MHW, and HDE). Station C exhibited the least morphometric differentiation, with significant differences observed in only one character (ED), and demonstrated the greatest similarity to the reference Station A. The morphometric evidence provides substantial support for the classification of Stations A-D as geographic variants within a single species, while Station *G. rufa* is distinguished by its distinct morphologies.

A total of 10 meristic characters were examined, revealing overlapping or identical ranges across the sampling groups. One distinguishing trait was the line lateral counts, which were counted to be as 34–36 in *G. rufa* (vs. 32–34 in stations B, C and D) (Table 3). Further minor variations, for instance in the number of pelvic fin rays (7 in Station A and *G. rufa* vs. 8 in other stations), are likely due to the limited sample size.

The overall morphological analyses indicated that the fish collected from the fish therapy ponds represent a single, morphologically cohesive species, and are unlikely to be *Garra rufa* as commonly assumed. To clarify the identity of this species, we compared it with all possible congeners that share general external appearance and whose geographic distributions suggest potential overlap or misidentification. Among *Garra* species distributed in Türkiye possessing a gular disc and, the most geographically and morphologically proximate candidate is *Garra turcica*, which is native to the Seyhan and Ceyhan drainages [8,13]. Other less likely but still relevant candidates include *G. orontesi* (Orontes River), *G. variabilis* (Qweiq and upper Euphrates–Tigris basins), and *G. rezai* (Kaynarca Stream in the Murat subdrainage of the Euphrates and some tributaries of the Tigris) [17, 18]. These species can be differentiated from the fish therapy population by several diagnostic traits: *G. variabilis* has fewer gill rakers (10–15 vs. 20–23) and one pair of barbels (vs. two) [8]; *G. orontesi* has a deeper

Table 3. Meristic characters of *Garra* collected from Antalya and of *G. rufa* from Euphrates.

Group	Dorsal Fin (DF)	Pectoral Fin (PeF)	Pelvic Fin (PF)	Anal Fin (AF)	Gill rakers (GR)	Line lateral (LL)	LL/DF	LL/PF	LL/AF	Predorsal scales
Station A	7-8	11-12	7	5	20	33-35	4-5	5	4-5	12-15
Station B	8	11-12	8	4-5	20-23	32-34	4	4-5	4-5	12-15
Station C	7-8	11-12	8	4-5	20-23	32-33	4	4-5	4-5	13-14
Station D	8	11	8	4-5	16-22	32-33	4	4-5	4	12-14
Garra rufa	8	12	7	5	20-22	34-36	4-5	4-5	4-5	12-14

body (21–26% SL vs. 13–22%) and a more pointed snout (vs. blunt) [19]; and *G. rezai* differs by having more lateral line scales (35–40 vs. 32–35) and fewer gill rakers (11–16 vs. 16–23) [17]. In contrast, all these key diagnostic characters such as body depth (18–21% SL), total lateral line scales (33–37), and gill raker counts (14–24), are characteristic of *Garra turcica* [13], and the corresponding ranges observed in the fish therapy individuals (Tables 1 and 3) fully overlap with these values, which strongly supports their identification as this species. Recent records of *G. rufa* from the Ilıca Stream, west of Manavgat, Antalya [15,16], have been documented well outside the known native range of the species. Although we did not examine the specimens reported in these studies, the strong possibility that the Ilıca population originates from fish therapy facilities raises the suggestion that the individuals in question may in fact be *G. turcica*. In the absence of detailed morphological data or critical taxonomic evaluation, the accuracy of these identifications remains uncertain. Although the specimens examined from fish therapy ponds form a morphological cohesive group, some variation was observed among individuals from the four different locations (Table 1). This variability could be due to several factors, such as the collection of fish to the hotels from genetically or geographically distinct populations, or from different habitat types, the small sample sizes available for the present analysis and the influence of the artificial breeding and stocking conditions commonly used in hotels and spas. It is evident that environmental factors, including water temperature, nutrient content, stock density, and water quality, have the potential to influence the morphological traits of fish [20].

Therefore, given the morphological similarities and phenotypic flexibility observed among *Garra* species, it is important to note that species identification based solely on morphological characteristics may present certain challenges. In order to achieve more precise species identification, the utilisation of molecular tools such as COI barcoding would be of significant benefit. Integrating genetic data would not only clarify the taxonomic status of these individuals but also support more informed management, particularly as there is growing evidence that *Garra* species are being introduced into natural water bodies outside their native ranges, raising potential ecological and conservation concerns.

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