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RESEARCH ARTICLE

Intraspecific Meristic Variation in *Gambusia holbrooki* from the Middle East and its Implications for Stock Structure and Taxonomic Status

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ABSTRACT

An analysis was conducted on the variation in the counts of seven meristic traits of the eastern mosquito fish, *Gambusia holbrooki*, using samples collected from ten different sites in the Middle East. These sites are located along the Tigris, Euphrates, and Shatt al-Arab Rivers, spanning across Iraq, Iran, Türkiye, and Syria. The study revealed a northward increase in the average values of these meristic traits, with the highest recorded in Türkiye and the lowest in the southern region of Iraq. The statistical analysis using Boxplots demonstrated that the seven meristic features were distinct across different rivers, with no overlap observed. Furthermore, the meristic counts of the sexes were not substantially different ($p \geq 0.05$). Analysis of each meristic trait variable suggested that the variations between samples were likely affected by several surrounding variables, such as ambient temperature, nutrient availability, currents, and genetic isolation patterns in the studied Middle East region. On the other hand, the study discussed the potential for implementing *G. holbrooki* stock identification.

Keywords: Euphrates, Mosquitofish, Morphology, Population, Shatt al-Arab Rivers, Tigris

Introduction

Gambusia affinis and *G. holbrooki* constitute members in a genus including around forty-six [1–4] within the Cyprinodontiformes order and Poeciliidae family, known as top minnow, live-bearers. These species were plentiful in Central America, which is regarded as the hub of poeciliid diversity. [1] While the wide presence of *G. affinis* and *G. holbrooki* is a result of their ongoing translocation out of their natural distribution area for mosquito control, the remaining 20 species of *Gambusia* are each restricted to taxonomically and geographically isolated river systems in south-central United States and eastern Mexico. [5–7]

The early 1900s marked the start of introducing *Gambusia* species in various countries to combat malaria by controlling mosquito larvae. [8–10] Efforts to officially and systematically combat malaria began in the late 19th century, spurred by a growing awareness of the crucial role mosquitoes play in transmitting illnesses like yellow fever and malaria. In 1905, the mosquitofish were transported for the first time over a considerable distance, making their journey from Seabrook to Hawaii. Subsequently, interest in using

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livebearer fish as a method for controlling these diseases has increased significantly. During the 1920s and 1930s, the two species of *Gambusia* were already established in several countries around the world outside their homeland.

Gambusia holbrooki reached Iran in the 1920s[11–14] and it has been documented further in recent years,[15–18] even though it's unclear exactly when it was introduced to Iraq.[19,20] The eastern mosquitofish, *G. holbrooki*, arrived in Syria around 1929[21,22] and was transported from Spain to Italy, then moved on to Corsica, and later reached Egypt, Cyprus, Sudan, and finally Syria.[23] Beckmann,[24] in his study of Syria's freshwater fish, reported the presence of *Gambusia* exclusively in the Yarmuk River. In Türkiye, *Gambusia* species were recognized as the first exotic species to become familiar with freshwater sources.[25–27] Geldiay & Balık[28] noted that mosquitofish were adopted for biological control in the 1930s in Türkiye.

The movement of individuals within populations limits their ability to adapt and develop into separate biological entities in altered habitats. Within fish species, adequate separation can lead to demonstrable genetic and morphological isolation across populations, laying the groundwork for identifying and controlling distinct populations.[29–31] As previously noted by Heincke[32] and continued to be so by Haddon and Willis,[33] Silva,[34] Jawad et al.,[35,36] and Schroeder et al.,[37] morphological features like the shape of the body and meristic numbers may be used to identify populations. Disparity in such attributes was presumed to be exclusively hereditary in beforehand findings;[38,39] however, evidence suggests that it relies on both environmental factors and genetic inheritance.[40–42] Even with the new molecular genetic tools that identify neutral genetic changes in groups, studying physical differences between populations is still very important for separating stocks.[43,44] Meristic[45–47] and morphometric characteristics[47] are the two morphological traits most frequently used to distinguish fish stocks.[48,49].

Studies addressing the population structure of *G. holbrooki* have employed both genetic[45–47] and morphological research[50–53] in different localities worldwide. None of the previous efforts but addresses the lack of large-scale studies on populations of *G. holbrooki* in the Tigris, the Euphrates and the Shatt al-Arab river systems, and their tributaries irrigating Iran, Iraq, Türkiye and Syria, the principal rivers of the Middle East. Interestingly no available data on meristic variation among these populations is currently available, indicating a significant lack of information regarding the species' ecological and evolutionary dynamics in this geopolitically critical region. Consequently, our research intends to fill such needs.

Materials and methods

Study area

Recognized as Western Asia's longest river,[54] the Euphrates begins where two tributaries meet in southeastern Türkiye: the Murat Su and the Kara Su, converging about ten kilometers before reaching Keban. Researchers such as Daoudy[55] and Frenken[56] estimate the river's entire length, from the Murat River to its confluence with the Tigris, at roughly 3,000 km, with 1,230 km in Türkiye, 710 km in Syria, and 1,060 km in Iraq. The river's gradient varies significantly along its course: it descends 368 m over 600 km from Keban to the Turkish Syrian border, slows down upon reaching Upper Mesopotamia, dropping only 163 m through Syria and a mere 55 m between Hīt and the Shatt al-Arab Delta.[57] The Tigris River 1,750 kilometers long originates in the Taurus Mountains to the east of Türkiye 25 km southeast of Elazığ and 30 km from the Euphrates River.[58,59]

The Shatt al-Arab River is an important hydrological landmark that gradually emerged some five millennia ago in the junction of the Tigris and Euphrates Rivers close to al-Qurnah in Basrah Province.[60,61] Traversing 200 kilometers, this watercourse operates as a natural boundary between Iran and Iraq by the side of its southern path before discharging into the Arabian Gulf. Its width fluctuates significantly, narrowing to 232 m near Basrah City and widening to 800 m at its Gulf terminus.[62] The Karun River, the only tributary of Shatt al-Arab, deposits large amounts of silt from Iran's highlands, necessitating ongoing dredging to keep it navigable for commercial and maritime activities.[62]

Fish specimens

Fish were collected from four nations through which the Tigris, Euphrates, and Shatt al-Arab Rivers flow or are located. Specimens of *G. holbrooki* were gathered from ten different populations across these four countries: Iran, Iraq, Türkiye, and Syria Fig. 1 Table 1. Fish specimens from Iran and Türkiye were gathered using DC electrofishing equipment (SAMUS-725MS) in defined sample zones. In contrast, samples from other countries were randomly caught using a dip net measuring 30 cm in diameter and featuring a 2-mm mesh. This was done during the peak daily water temperatures in the mid-afternoon, after observing the active schooling of fish in shallow, vegetated regions. Captured individuals were fixed in 10% formaldehyde immediately upon arrival in 10% formaldehyde for 2 days and then transferred to 70% ethanol until processed. The collection of fish samples took place from August 1999 to January 2000, with a

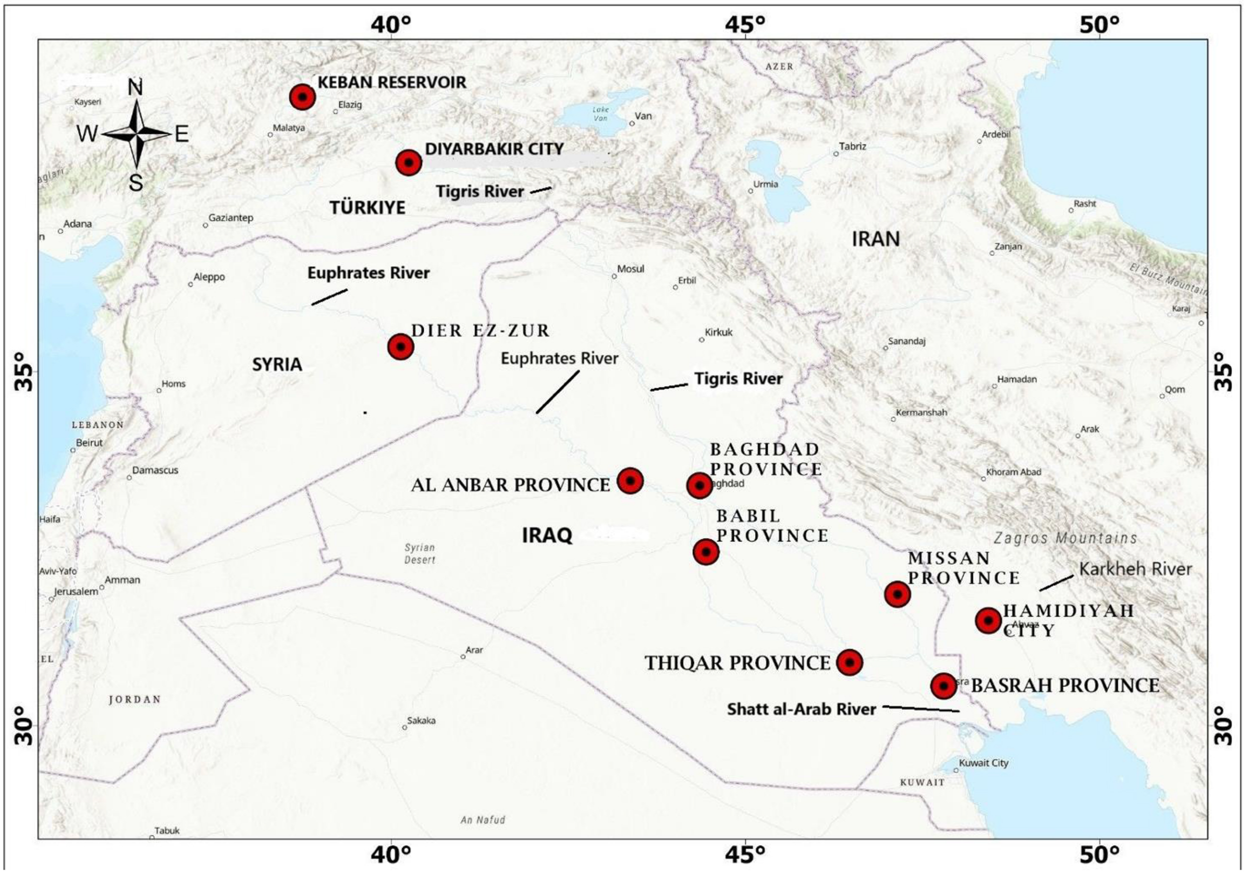

Fig. 1. Sampling locations of *Gambusia holbrooki* in the Middle East.

depth of capture ranging from 0.5 to 2.4 meters. The average total lengths and standard deviations of the sample for each location are given in Table 1.

Specimens were analyzed soon after being collected while they were still fresh. Seven meristic traits were assessed. These traits included: DFR for dorsal fin ray, AFR for anal fin ray, PFR for pectoral fin ray, CFR for caudal fin ray, GRN for the number of gills rakers on the 1st gill arch, LLS for the number of scales on the lateral line, and VN for the number of vertebrae. The meristic features were counted using the approach as described by Hubbs and Lagler.[63] The counts were all determined by the left side. Following the removal of the anterior gill arch, the number of gills rakers on the 1stgill arch was measured by light microscopy. According to Barlow,[64] size was not considered a factor because meristic traits are determined early in a fish's development and remain constant throughout its lifespan. Macroscopic analysis provided the sex.

To count the meristic traits included in this study, specimens were cleared in aqueous 1% KOH and were stained with alizarine red-S for bone, employing the method of.[65,66] Specimens were stored in 100% glycerine. Sexual variance was evaluated by one-way ANOVA tests after sex was determined microscopically. ANOVAs were also performed to compare the values of the meristic traits being studied between females and males.

Statistical analysis

Seven meristic characters were counted for 2190 specimens of *Gambusia holbrooki:* 1024 from Euphrates River; 652 from Tigris River and 302 from Shatt al-Arab River, and 312 from Karkheh River, Iran, where half of the sample consisted of females and the other half of males. The meristic characteristics were as follows: DFR: dorsal fin ray; AFR: anal fin ray; PFR: pectoral fin ray; CFR: caudal fin ray; GRN: number of gills rakers on the 1stgill arch; LLS: number of scales on the lateral line; VN: number of vertebrae. Boxplots showing the meristic data were produced based on a summary of four summary statistics: minimum, maximum, sample mean, and standard error. Given that normality was never met in any of the variables, the Mann-Whitney U test was implemented to examine gender disparities, and differences among rivers were explored by means of the

Table 1. Biodata of *Gambusia holbrooki* collected from ten localities in the middle East. F, females; M, males.

Population	Country	River	Geographic coordinates	Number of fish specimens	Fish total length range (mean ± standard deviation)
Keban Reservoir	Türkiye	Euphrates	38°52'43.78"N 38°44'44.23"E	152 (F = 76, M = 76)	F = 42–55 (48.3 ± 1.23) M = 35–50 (41.3 ± 2.32)
Dier ez-Zur	Syria	Euphrates	35°21'5.60"N 40° 7'56.01"E	148 (F = 74, M = 74)	F = 35–55 (44.2 ± 1.43) M = 32–50 (40.8 ± 1.43)
Al-Anbar Province	Iraq	Euphrates	33°27'31.54"N 43°22'21.92"E	217 (F = 108, M = 109)	F = 30–45 (37.3 ± 2.12) M = 29–35 (32.4 ± 1.43)
Babil Province	Iraq	Euphrates	32°27'14.85"N 44°26'36.81"E	287 (F = 146, M = 141)	F = 35–49 (41.9 ± 1.12) M = 32–41 (36.3 ± 1.13)
Thiqar Province	Iraq	Euphrates	30°53'35.77"N 46°28'9.57"E	220 (F = 110, M = 110_	F = 31–48 (39.2 ± 2.14) M = 30–41 (35.2 ± 1.17)
Diyarbakir Province	Türkiye	Tigris	37°57'4.24"N 40°14'41.51"E	142 (F = 71, M = 71)	F = 41–50 (45.3 ± 1.11) M = 38–43 (40.2 ± 1.16)
Baghdad Province	Iraq	Tigris	33°23'27.49"N 44°21'10.30"E	262 (F = 131, M = 131)	F = 31–42 (36.4 ± 1.21) M = 30–39 (34.1 ± 2.13)
Missan Province	Iraq	Tigris	31°51'18.12"N 47° 8'42.89"E	248 (F = 124, M = 124)	F = 30–42 (36.1 ± 1.20) M = 29–40 (43.3 ± 1.14)
Basrah Province	Iraq	Shatt al-Arab	30°33'34.45"N 47°47'53.06"E	302 (F = 151, M = 151)	F = 35–48 (41.2 ± 1.25) M = 32–43 (37.1 ± 2.14)
Hamdiyah Province	Iran	Karkheh	31°29'2.73"N 48°25'40.84"E	212 (F = 106, M = 106)	F = 32–45 (38.3 ± 1.15) M = 30–41 (35.3 ± 1.22)
Grand total				2190	

Kruskal-Wallis H test (SPSS ver.26.0). These tests are non-parametric versions of the t-test and are applied when the hypothesis of normality is not met. In order to investigate the possible existence of differences in meristic use in classifying rivers, meristic characteristics were subjected to Discriminant analysis (SPSS ver. 26.0) to calculate generalized Mahalanobis distances, discriminant functions, and the percentage of the specimens correctly classified using the functions. This was tested by means of cross-validation, that is performing repeated analyses each time dropping one subject when constructing the Discriminant function and classifying each time this individual into groups based on the function obtained. Correct classification rates are presented in percentage. Paired t-tests were used to determine if meristic counts differed between the two sides of the fish.

Results

Meristic counts did not reveal any discernible differences between sexes (*p* $\geq$ 0.05). The boxplot, along with the mean ± SD, minimum, and maximum values for meristic characteristics Fig. 2; Table 2, clearly demonstrates that the six meristic traits exhibit minimal overlap between rivers. The most distinguishing characteristics were DFR, AFR, PFR, CFR, GRN, and LLS. However, all seven traits showed significant differences among rivers Table 3. Kruskal–Wallis's tests indicated that differences among rivers were statistically significant for all variables: DFR: $H(2) = 1012.3$, $p < 0.001$; AFR: $H(2) = 915.8$, $p < 0.001$; PFR: $H(2) = 1151.5$, $p < 0.001$; CFR: $H(2) = 675.9$, $p < 0.001$; GRN: $H(2) = 616.8$, $p < 0.001$; LLS: $H(2) = 981.8$, $p < 0.001$; VN: $H(2) = 296.8$, $p < 0.00$

Given that meristic features did not significantly change across the two sides of the fish's body (paired *t*-test, *p* > 0.05), values from the left side were randomly selected for analysis.

The cross-validated discriminant analysis performed with morphometric characters and including all 2190 individuals yielded a correct classification of 89.9 % per river (Wilk's lambda = 0.068, $p < 0.001$). Rivers Tigris and Shatt al-Arab are totally discriminated with 100.0 % Table 4. The classification result was lower using the Euphrates River sample, with a rate of 78.5%, the cross-validated analysis misclassified specimens from Euphrates with those from rivers Shatt al-Arab (20.1 %) and Tigris (1.4 %) Table 2. The Mahalanobis distances could be seen in the Supplementary Table, which is available on request from the corresponding author.

Discussion

Although the population structure data for *Gambusia* fish species are more than 25 years old, it still holds value in understanding how the population may have changed over time in the study area.

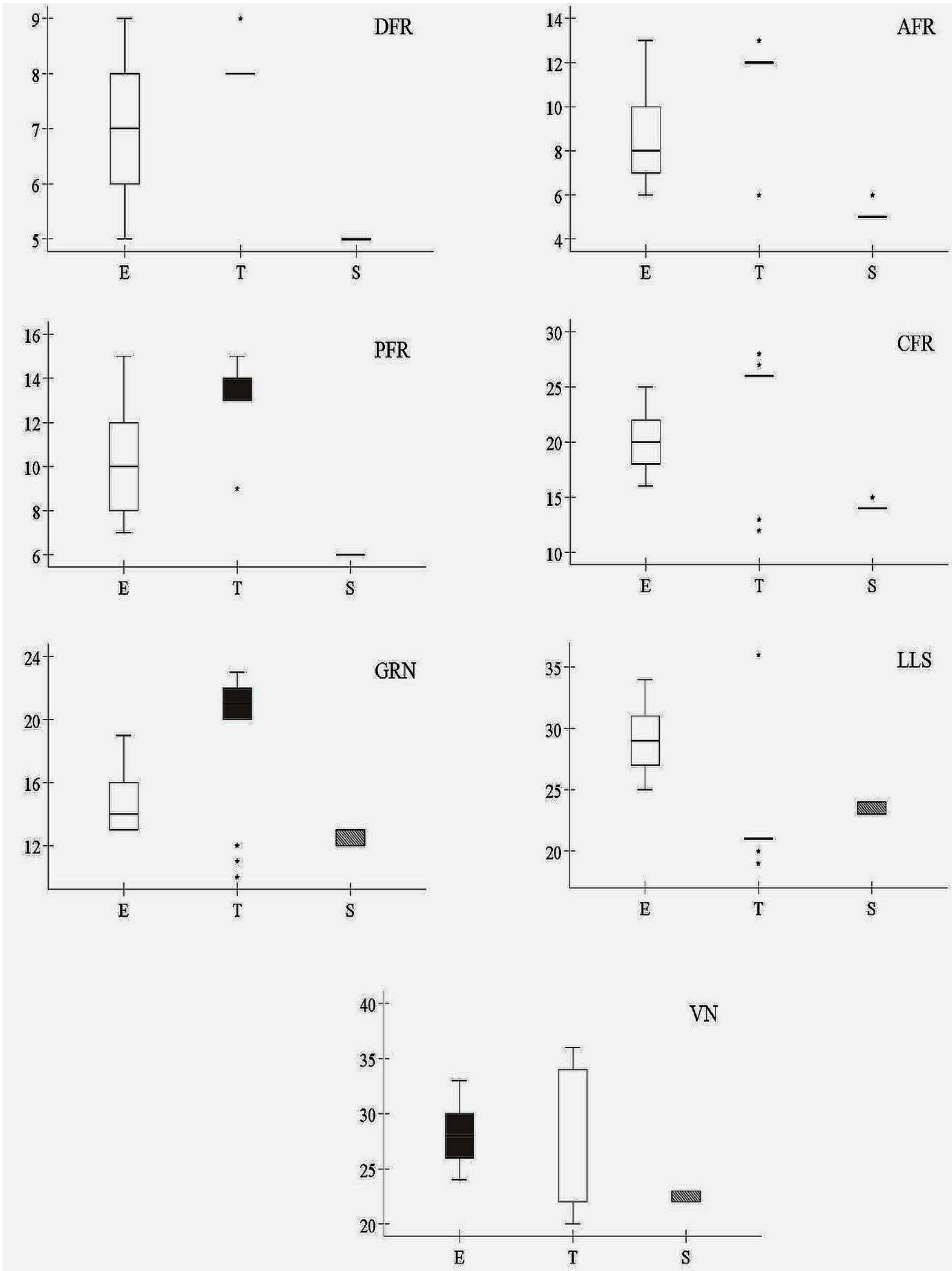

Fig. 2. Boxplot illustrating the mean ± SD, along with the minimum and maximum values of meristic characters for three rivers: Euphrates (E, white), Tigris (T, grey), and Shatt al-Arab (S, diagonals). The characters are defined as follows: DFR represents the dorsal fin ray; AFR stands for the anal fin ray; PFR denotes the pectoral fin ray; CFR is the caudal fin ray; GRN indicates the number of gill rakers on the 1st gill arch; LLS refers to the number of scales on the lateral line; VN signifies the number of vertebrae.

Table 2. Each value is presented as a range (mean ± standard deviation) for the meristic characteristics measured across ten populations of *Gambusia holbrooki* and is presented for the ten locations from which fish samples were collected. AFR represents the anal fin ray count; CFR denotes the caudal fin ray count; DFR stands for the dorsal fin ray count; GRN indicates the number of gills rakers on the 1st gill arch; PFR refers to the pectoral fin ray count; LLS is the lateral line scale count; and VN signifies the vertebrae number.

Localities	AFR	CFR	DFR	GRN	PFR	LLS	VN
Euphrates River Türkiye							
Keban Reservoir	12–13 (12.3 ± 1.765)	24–25 (24.3 ± 2.535)	9–9 (9 ± 0.001)	18–19 (18.3 ± 2.352)	14–15 (14.2 ± 1.842)	33–34 (33.2 ± 1.045)	32–33 (32.1 ± 1.105)
Syria							
Dier ez-Zur	12–13 (12.2 ± 1.310)	22–23 (22.4 ± 2.435)	8–9 (8.3 ± 1.983)	16–17 (16.2 ± 1.744)	12–13 (12.4 ± 1.234)	31–32 (31.4 ± 2.316)	30–31 (30.3 ± 2.241)
Iraq							
Al-Anbar Province	8–9 (8.4 ± 1.334)	20–21 (20.1 ± 1.785)	7–7 (7 ± 0.001)	14–15 (14.3 ± 2.035)	10–11 (10.4 ± 2.008)	29–30 (29.4 ± 1.664)	28–29 (28.3 ± 1.334)
Babil Province	7–7 (7 ± 0.001)	18–19 (18.4 ± 2.113)	6–6 (6 ± 0.001)	13–13 (13 ± 0.001)	7–7 (7 ± 0.001)	27–28 (27.3 ± 1.662)	26–27 (26.3 ± 2.554)
Thiqar Province	6–7 (6.4 ± 1.008)	16–17 (16.4 ± 1.755)	5–5 (5 ± 0.001)	14–14 (14 ± 0.001)	7–8 (7.4 ± 0.001)	25–26 (25.3 ± 0.001)	24–25 (24.3 ± 2.331)
Tigris River Türkiye							
Diyarbakir Province	13–13 (13 ± 0.001)	27–28 (27.4 ± 2.443)	8–9 (8.4 ± 1.723)	22–23 (22.2 ± 2.325)	15–15 (15 ± 0.001)	36–36 (36 ± 0.001)	35–36 (35.3 ± 1.766)
Iraq							
Baghdad Province	12–12 (12 ± 0.001)	26–26 (26 ± 0.001)	8–8 (8 ± 0.001)	20–21 (20.2 ± 1.391)	14–14 (14 ± 0.001)	21–21 (21 ± 0.001)	34–34 (34 ± 0.001)
Mysan Province	12–12 (12 ± 0.001)	26–26 (26 ± 0.001)	8–8 (8 ± 0.001)	22–22 (22 ± 0.001)	13–13 (13 ± 0.001)	21–21 (21 ± 0.001)	22–22 (22 ± 0.001)
Shatt al-Arab River							
Basrah Province	5–6 (5.4 ± 2.554)	14–15 (14.4 ± 2.035)	5–5 (5 ± 0.001)	12–13 (12.3 ± 1.335)	6–6 (6 ± 0.00)	23–24 (23.3 ± 2.635	22–23 (22.4 ± 2.555)
Iran							
Hamdiyah Province	6–6 (6 ± 0.001)	12–13 (12.4 ± 1.225)	8–8 (8 ± 0.001)	10–12 (11 ± 0.325)	9–9 (9 ± 0.001)	19–20 (19.4 ± 0.865)	20–21 (20.3 ± 1.821)

Table 3. Meristic counts are given as mean ± SD, minimum and maximum characters for *Gambusia holbrooki* in the rivers Tigris, Euphrates, and Shatt al-Arab., Euphrates, and Shatt al-Arab Rivers. Dorsal fin rays are denoted DFR, anal fin rays AFR, pectoral fin rays PFR, caudal fin rays CFR, number of gills rakers on the 1st gill arch GRN, lateral line scales LLS, and the number of vertebrae VN. *P* values of the Kruskal-Wallis test (H).

	Euphrates		Tigris		Shatt al-Arab			
Characters	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	H	p
DFR	5–9	6.80 ± 1.428	8–9	8.10 ± 0.296	5–5	5.00 ± 0.000	1012.3	< 0.01
AFR	6–13	8.38 ± 2.216	6–13	10.69 ± 2.701	5–6	5.24 ± 0.427	915.8	< 0.01
PFR	7–15	9.86 ± 2.473	9–15	12.65 ±2.184	6–6	6.00 ± 0.000	1151.5	< 0.01
CFR	16–25	19.77 ± 2.748	12–28	22.83 ± 6.174	14–15	14.22 ± 0.414	675.9	< 0.01
GRN	13–19	14.84 ± 1.797	10–23	18.93 ± 4.602	12–13	12.58 ± 0.494	616.8	< 0.01
LLS	25–34	28.78 ± 2.821	19–36	23.05 ± 5.795	23–24	23.25 ± 0.435	981.8	< 0.01
VN	24–33	27.83 ± 2.775	20–36	27.38 ± 6.648	22–23	22.66 ± 0.476	296.8	< 0.01

This is important for a couple of reasons; 1) historical historical baseline: old data provides a baseline for comparison in order for researchers to determine how population structure (e.g., age distribution, sex ratio, genetic diversity, abundance) has changed over time. - without a historical perspective, finding long-term patterns of decline, expansion, or shifts in genetic composition would be difficult; (2) discovery of changes, either of natural or anthropogenic origin, such as habitat degradation (e.g., pollution, urbanization), effects of climate change (e.g., temperature changes affecting reproductive cycles), introduction of invasive species or diseases, and human influences including water management and use of pesticides,

Table 4. Classification outcomes from the discriminant analysis of meristic characters for each of the rivers: Tigris (T), Euphrates (E), and Shatt al-Arab (S). The success rate for cross-validated predictions of river membership is 89.9%, indicating that this percentage of grouped cases was accurately classified.

	Predicted Group Membership			
Rivers	E	T	S	Total
E	78.5	1.4	20.1	100.0
T	0	100.0	0	100.0
S	0	0	100.0	100.0

that could be influencing the *Gambusia* population as a whole; 3) usage of *Gambusia* as "indicator species", as the mosquitofish are often studied for its ecosystemic functions (e.g. mosquito control) as well as for being particularly sensitive to environmental shifts. Population fluctuations could be indicative of larger-scale ecological problems.[67–69]

This is despite the fact that fish samples came from the same gene pool, which is the Euphrates-Tigris River system, but the outcome would present the impacts of different environments (pollution, habitat differences) on individuals originating from this gene pool. In this case, this should not pose a problem for comparison. Fish from the same gene pool can still be compared for two reasons: (1) geographic vs. genetic variation if the question is about geographic variation (e.g., morphological differences due to local conditions), samples from different parts of the river systems might still show meaningful patterns; and (2) alternative approaches for assessing genetic diversity, increasing sample size within the Euphrates-Tigris system (including more sites and different tributaries) could help detect subtle variations.[70–72]

Meristic features in fish, including the number of fin rays or vertebrae, are established very early, in fish cases since the larval stage. These characteristics are conditioned by environmental, habitat characteristics, particularly temperature.[66,73–75] It has been observed that lower temperatures in the early life stages result in higher vertebrae count.[75–77] Therefore, variations in meristic traits across different locations suggest that larvae experienced diverse environmental conditions, which can be interpreted as evidence of distinct spatial breeding groups.

The current study on the identification of *G. holbrooki* populations found no notable differences in the number of seven meristic traits between male and female specimens from ten populations across the Tigris, Euphrates, and Shatt al-Arab Rivers. This outcome indicates that the meristic characteristics assessed in this study exhibited similar responses in both sexes. This conclusion aligns with Arnett's[77] and Wood et al.[78] findings on *G. affinia* and *G. holbrooki*, which demonstrated that the phenotypically plastic traits of both sexes of these two *Gambusia* species respond similarly to environmental factors. Conversely, the same meristic characteristics revealed significant phenotypic differences among the samples from the rivers, Tigris, Euphrates, and Shatt al-Arab, through non-parametric analysis and meristic comparisons using the Kruskal–Wallis's test. Findings imply that the *G. holbrooki* populations in the three rivers are distinct. This divergence suggests a relationship between the variation in meristic traits and geographic distance from each other, suggesting the possibility of restricted movement between fish populations of this species in the three rivers studied. In addition, such deviations may have developed from habitat, genetic divergence, perhaps each. Even if the drivers are not identified in this study, it is clear that different groups of *G. holbrooki* may be discriminated by meristic traits. This conclusion must be contemplated throughout the designing of conservation agendas for this species.

The current findings indicated a rising pattern in the average values of all seven meristic traits studied in *G. holbrooki* populations, moving from the southern region (Basrah City, Iraq) to the northern areas (Turkish populations) alongside the Tigris and Euphrates rivers. Such an increase in the mean values of the meristic attributes can be credited to the shifts in temperature of the water, where water from the Euphrates and Tigris experienced lower temperatures, then it gradually increased southward, reaching the southern Mesopotamian plane in Basrah City, where the water temperature was higher than any localities northward. The *G. holbrooki* specimens from the Karkheh River, Iran have meristic traits mean value higher than that of Basrah City, Iraq population because this river is descending from highland in the west of Iran, where temperature is often low.[79] Alpaslan et al.[80] reported that the water temperature at Keban Dame varies between 18.6 at the surface and 13.9 °C at 30 metres depth respectively. In contrast, the Shatt al-Arab River, where the most southernmost *G. holbrooki* population was sampled, experiences water temperatures ranging from 12.0 °C during winter to 31.1 °C in summer.[81] Incubation times and meristic counts are both reduced in waters with higher temperatures.[82,83] This study also identified a relationship between latitude and higher meristic trait estimations, suggesting that *G. holbrooki* ranges in the locations studied are still impacted by Jordan's law (Jordan's Law specifies that vertebral count is positively correlated with latitude, reflecting a trend where fish and some other ectothermic species exhibit more vertebrae in colder, higher-latitude environments compared to warmer regions). Yamahira et al.[84] reported similar results for two *Menidia* species taken from the eastern and western coastlines of Florida, USA. Since the work of Tåning,[66] who demonstrated the influence of ecological variables on different meristic traits of fish, research has published reports of these influences on the meristic features of various species of fish.[73,85,86] Fish have in fact been reported to show more within- and between species morphological variation than other vertebrates.[87,88] Such modifications to the external features of fish allow them to adjust through physiological and behavioural changes to different conditions in their environment.[89]

Two possible reasons why populations of any fish species might be heterogeneous: genetic differentiation and ecological adaptability, or a combination of both. Meristic characteristics can reveal how a group responds to a specific environment.[43,90] In certain cases, deviations in the external features of the fish detected in the feral groups could be persistent and appear because of habitat flexibility.[91,92] Departure in morphological attributes anticipated from modifications in ecological issues might be preferred for stock identification, as this technique is inexpensive to perform and provides consistent findings.

Quilang et al.[93] and Alsafy et al.[94] suggest a potential connection between the total number of gills rakers on the 1stgill arch and the dietary patterns and preferences of fish. Fish that consume benthic organisms, such as those from the Shatt al-Arab River in Basrah City, southern Iraq, were observed to possess a low number of gills rakers. In contrast, fish groups from the higher parts of the Tigris and Euphrates Rivers, including areas of Syria and Türkiye,[95] exhibited a high count of gill rakers, reflecting their different feeding habits compared to those in central and southern Iraq.[96,97]

The populations of *G. holbrooki* from Baghdad City and Mysan Province exhibited similar average values for meristic traits, such as dorsal fin ray, caudal fin ray, and anal fin ray counts, as well as the number of lateral line scales. These similarities may stem from the proximity of the two locations, suggesting that the populations may share the same genetic mix. Furthermore, according to Ali et al.[98] and Olson & Speidel,[99] there are no natural obstacles in the area that might impediment fish passage between these sites. On the other hand, the three groups located along the Euphrates River within Iraq's borders have been identified as distinct based on the average values of their meristic traits. This variation might stem from the differences in the land's geomorphology that the Euphrates traverses, starting from Al-Anbar Province, moving through Babil Province, and finally reaching Thiqar Province. These geographical features create a natural barrier that limits the movement of *G. holbrooki* individuals, resulting in a restricted gene pool.[100–102]

We demonstrated the effectiveness of utilizing boxplots to represent the meristic dataset in examining the population structure of the eastern mosquito fish, *G. holbrooki*, from ten sites spanning the Shatt al-Arab, Tigris, and Euphrates Rivers, which run via Iran, Iraq, Türkiye, and Syria. Nonetheless, additional analyses are required to comprehend the links between perceived meristic disparity and disagreements in habitation, notably ecological issues that could affect the embryonic and larval phases. Additional morphological and genetic investigations on further groups of *G. holbrooki* within the Middle East are necessary to justify the persistent discrepancy in the various meristic traits detected in this study.

Taxonomic classification and environmental encounters of Gambusia holbrooki in the Middle East: steering historical uncertainty and hybridizing

The identification of *Gambusia holbrooki* (eastern mosquitofish) populations in the Middle East has been shaped by historical taxonomic challenges and evolving methodologies. Initially, *G. holbrooki* and its closely related species, *G. affinis* (western mosquitofish), were classified as subspecies under *G. affinis* until the 1990s. At that time, genetic and morphological distinctions, such as differences in dorsal fin rays (7–8 in *G. holbrooki* compared to six in *G. affinis*) and the presence of teeth on the third ray of the gonopodium in males, led to their recognition as separate species.[103] This taxonomic differentiation caused confusion in early records from the Middle East, where introductions for mosquito control often involved mixed or mislabelled groups. For instance, populations introduced to the region in the early 20th century likely came from uncertain origins, as global distribution efforts frequently combined the two species.[104] Current classification in the Middle East now relies on morphological keys, such as fin ray counts and gonopodium structure, supplemented by genetic analyses to resolve uncertainties, especially in hybrid zones or areas where both species might coexist.[103,104]

Through the analysis of meristic characters throughout the Tigris-Euphrates River system, this study confirms the taxonomical identification, and environmental interactions of *Gambusia holbrooki* in the Middle East. The result that "the seven meristic characters were distinct among rivers and there was no overlap between the boxplots" is rather good evidence for genetic isolation. This physical disjunction, which is also associated with a north-south cline (wherever the counts are higher in Türkiye and lower in southern Iraq) strongly supports the hypothesis of the adaptation of populations to local ecological variables, including local water temperature and water currents. In addition, this isolation itself, which may also drive historical uncertainty about the population structure, provides necessary conditions for divergence. Although not explicitly addressing hybridization, the section of the article where the authors discuss the future potential of their findings for "stock identification" implies the acknowledgment of discrete population units, thus laying the groundwork for the exploration of hybridization between formerly isolated groups within the species' range. To wrap up, this work goes beyond the mere documentation of *G. holbrooki* in the Middle East. It offers important empirical support for the occurrence of substantial intraspecific divergence in the Tigris-Euphrates system. The results support the view of these as unique population stocks exhibiting adaptive evolution, isolated units whose genetic integrity should be an important consideration for future taxonomic and/or conservation approaches.

Conclusion

This study demonstrated the variability in meristic characters among the identified ten populations. There is an evident northward increase in the average values of these meristic traits, with the highest in Türkiye and the lowest in south of Iraq. The study found that *G. holbrooki* populations exhibit higher meristic trait values in cooler northern regions (Türkiye) compared to warmer southern areas (Basrah, Iraq), with water temperature being a key factor. Specimens from Iran's Karkheh River also showed higher values due to cooler highland temperatures. Warmer waters, like those in the Shatt al-Arab River, correlate with reduced meristic counts and incubation times. The statistical analysis using Boxplots was efficient to show the differences between populations investigated. At each locality and for each meristic feature examined that the variations in these traits were likely influenced by the water temperature distribution pattern in the Middle East region being studied. The implementation of *G. holbrooki* stock recognition in the taxonomy of this species is emphasized.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: This project was ethically cleared by the local committee at University Unitec Institute of Technology.

Author' contributions statement

F.A.A.F. conceptualization; data curation; investigation; methodology; project administration; resources; writing an original draft; writing a review; and editing. A. L. I. statistical analyses; investigation; methodology; writing an original draft. L.A.J. conceptualization; formal analysis; investigation; methodology; project administration; supervision; validation; visualization; writing the original draft; writing a review; and editing.

Data availability

Any interested party may request access to the supporting data from the corresponding author of this study.

Journal declaration

Third author (L.A.J.) is an editor for the journal but did not participate in the peer review process other than as an author. The authors declare no other conflicts of interest.

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التباين في الصفات المعددة داخل النوع في سمك غامبوزيا هولبروكي من الشرق الأوسط وآثاره على بنية المخزون والحالة التصنيفية

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الخلاصة

تمت دراسة التباينات في سبع صفات مظهرية معدودة لسمكة الكمبوزيا هولبروكي المجمعة من عشر مناطق في الشرق الأوسط. تقع هذه المناطق ضمن أربعة أنهار هي: الفرات، ودجلة، وشط العرب، والكرخة، الموجودة في العراق وإيران وتركيا وسوريا. بينت نتائج البحث أن هناك زيادة في قيم الصفات المظهرية المدروسة من الجنوب باتجاه الشمال، حيث بلغت أعلى قيم لهذه الصفات في الأسماك المجمعة من تركيا، بينما سُجلت أقل القيم في أسماك منطقة البصرة جنوب العراق. كما أظهرت التحليلات الإحصائية عدم وجود تداخل بين قيم الصفات المدروسة، فضلاً عن عدم وجود اختلافات بين الذكور والإناث. تشير نتائج هذه الدراسة إلى أن التباينات في قيم الصفات المدروسة قد تعود إلى الاختلاف في درجات حرارة الماء بين المناطق. أخيراً، سلط البحث الضوء على التطبيقات المحتملة لدراسة تجمعات أسماك الكمبوزيا في الأبحاث التصنيفية المتعلقة بهذا النوع.

الكلمات المفتاحية: نهر الفرات، أسماك البعوض، الشكل، السكان، نهر شط العرب، نهر دجلة.