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Population Structure of the Mudskipper *Boleophthalmus dussumieri* (Valenciennes, 1837) in the Northern Persian Gulf, Iran

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ABSTRACT

Aims: This research examined the genetic structure of the Mudskipper *Boleophthalmus dussumieri* across three key intertidal zones in the north of the Persian Gulf and Oman Sea, Iran.
Materials & Methods: Genetic analysis of the mitochondrial cytochrome b gene in 28 samples (25 contemporary and 3 archived) from four regions evaluated diversity and structure using haplotype and nucleotide diversity indices, AMOVA, ΦST, and haplotype networks.
Finding: A total of 21 haplotypes were identified from 28 individuals. Overall haplotype diversity was high (Hd = 0.942), and nucleotide diversity was moderate (π = 0.013). A hierarchical analysis of molecular variance (AMOVA) revealed significant genetic structuring among the three *B. dussumieri* populations surveyed. The analysis indicated that 27.87% of the total genetic variation was partitioned among populations, while the remaining 72.16% was found within populations. The overall fixation index (Φ_{ST}) was 0.72, a statistically significant value ($p < 0.001$), confirming high and definitive genetic differentiation across the sampling region.
Conclusion: The results show a clear population-genetic pattern, marked by distinct differences between the Goban and Makran populations. In contrast, the Delvar and Khorabi populations form a single, genetically uniform cluster. These patterns align with a model of recent demographic expansion from a common ancestral source, followed by initial genetic divergence. This differentiation is influenced by current barriers to gene flow and the species' limited dispersal ability.

Keywords: Coast of Persian Gulf; Cytochrome b; Mitochondrial DNA; Phylogeography; Population Genetics.

CITATION LINKS

[1] Ansari A., Trivedi S., Saggu S., Rehman H. Mudskipper: A biological indicator for environmental m... [2] Haidari C., Khalilipour O., Aghah H., Zolgharnein H., Hosseini S.M. Survey of the Morphometric va... [3] Looi L.J., Aris A.Z., Isa N.M., Yusoff F.M., Haris H. Elemental composition and health risk asses... [4] Ghanbarifardi M., Aliabadian M., Esmaeili H.R. Shape variation of the Indian Ocean slender mudski... [5] Ghanbarifardi M., Aliabadian M., Esmaeili H.R., Polgar G. Morphological divergence in the Walton... [6] Ghanbarifardi M., Aliabadian M., Esmaeli H.R. Morphometric variation of *Periophthalmus waltoni* (T... [7] Ghanbarifardi M., Esmaeili H.R., Gholami ... [8] Muhala V., Guimarães-Costa A., Bessa-Silva A.R., Rabelo L.P., ... [9] Muhala V., Guimarães-Costa A., Macate I.E., Tembe S., Mula Y., Tóvela É., Bessa-Silva A.R., Valli... [10] Sinaei M., Mashinchian A., Fatemi M., Riazi G. Application of biomarkers in mudskipper (*Boleophth... [11] Lambeck K. Shoreline reconstructions for the Persian Gulf since the last glacial maximum. Earth P... [12] Kennett D.J., Kennett J.P. Early State Formation in Southern Mesopotamia: Sea Levels, Shorelines,... [13] Ghanbarifardi M., Zarei R. Otolith shape analysis of ... [14] Thacker C.E. Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). Mol. ... [15] Sharifiniya M., Mousavi-Sabet H., Alavi-Yeganeh M.S., Ghanbarifardi M. Molecular phylogeny and mo... [16] Liu Z.Z., Wang C.T., Ma L.B., He A.Y., Yang J.Q., Tang ... [17] Kumar S., Stecher G., Suleski M., Sanderford M., Sharma S., Tamura K. MEGA12: Molecular Evolution... [18] Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J.C., Guirao-Rico S., Librado P., Ramos-Onsins S.E., ... [19] Paradis E. Analysis of haplotype networks: The randomized minimum spanning tree method. Method. E... [20] Excoffier L., Lischer H.E. Arlequin suite ver 3.5: a new series of programs to perform population... [21] Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for t... [22] Slatkin M., Hudson R.R. Pairwise comparisons of mitochondrial DNA sequences in stable and exponen... [23] Excoffier L. Patterns of DNA sequence diversity and genetic structure after a range expansion: le... [24] Grant W.S., Bowen B.W. Shallow population histories in deep evolutionary lineages of marine fishe... [25] Fu Y.X. Statistical tests of neutrality of mutations against population growth, hitchhiking, and ... [26] Riquet F., Liautard-Haag C., Woodall L., Bierne N. Parallel pattern of differentiation at a genom... [27] Bohonak A.J. Dispersal, gene flow, and population structure. Q. Rev. Biol. 1999; 74(1): 21-45... [28] Selkoe K.A., D'Aloia C.C., Crandall E.D., Iacchei M., Liggins L., Puritz J.B., Toonen R.J. A deca... [29] Ramanadevi V., Thangaraj M., Sureshkumar A., Dubburaj J. Detection of genetic variation and gene...

Introduction

The Persian Gulf, a semi-enclosed marginal sea known for its extreme environmental conditions and dynamic geological history, provides a unique model for studying the evolution of coastal organisms. Among its diverse fauna, mudskippers (Gobiidae: Oxudercinae) are highly specialized amphibious fishes that inhabit intertidal mudflats, estuaries, and mangrove habitats [1, 2]. These zones are both biologically productive and highly vulnerable to human impacts, making resident species like *Boleophthalmus dussumieri* (Valenciennes, 1837) important for ecological and genetic research. Found along the northern Iranian coast from Khuzestan to Hormozgan Provinces, *B. dussumieri* has adapted to a semi-terrestrial lifestyle and serves as a bioindicator of coastal pollutants, including polycyclic aromatic hydrocarbons (PAHs) and heavy metals [2, 4]. Despite its ecological significance, little is known about its population genetics and phylogeography in the Persian Gulf. Previous research on regional mudskippers mainly focused on other genera, providing useful comparisons. For example, studies on *Periophthalmus waltoni* revealed noticeable morphological differences [5] and morphometric variation [6] between populations in the Persian Gulf and the Gulf of Oman. Molecular phylogenetic analyses have clarified relationships among several mudskipper species in the region [7], and otolith shape analysis has helped differentiate species within the Persian Gulf [13].

Additionally, comparisons of mitochondrial genomes revealed the unique gene arrangement of *B. dussumieri* [8], while records from outside the Persian Gulf suggest multiple intraspecific lineages, indicating hidden genetic diversity [9]. The coastal waters of the northern Persian Gulf are complex, with varying salinity, temperature, and pollutant levels, all of which can influence genetic diversity through selective pressures [4, 10]. Furthermore, the region's Pleistocene sea-level changes, which caused periodic drying and recolonization of the Persian Gulf, are believed to have left a strong demographic footprint on its marine fauna [11, 12]. Therefore, genetic studies are essential not only for assessing population health and adaptive capacity but also for reconstructing historical biogeographic patterns. This study aims to fill the existing knowledge gap by conducting the first comprehensive population genetic analysis of *B. dussumieri* along the northern Persian Gulf. We sampled individuals across three key intertidal zones representing a broad latitudinal gradient: Goban (Khuzestan Province), Delvar (Bushehr Province), and Khorabi (Hormozgan Province). Using sequence data from the mitochondrial cytochrome b gene, we specifically aimed to: (1) measure genetic diversity and haplotype distribution within and among populations; (2) evaluate population genetic structure and current gene flow; and (3) infer the demographic history of these populations to distinguish between historical colonization events and recent fragmentation. By combining these findings with established biogeographic knowledge from studies on related species [4, 5, 6, 13], this research will clarify the evolutionary forces shaping biodiversity in this ecologically important region and provide a genetic baseline for the conservation and management of *B. dussumieri* and its vulnerable intertidal habitat.

Materials & Methods

Study Samples

Sampling was conducted across three key intertidal zones along the northern Persian

Gulf, specifically targeting the Iranian coast. These included the Goban area in Khuzestan Province (48°37′54″E, 30°16′12″N), the Delvar area in Bushehr Province (51°1′32″E, 28°47′1″N), and the Khorabi area in Hormozgan Province (56°23′39″E, 27°11′1″N) (Figure 1). Specimens were collected from September to November 2017, covering roughly 830 km of coastline [34]. From muddy intertidal habitats at the three sampling sites, a total of 29 *B. dussumieri* individuals were gathered using a randomized protocol. Fish were mainly caught by hand and occasionally with fine mesh cast nets. Due to logistical challenges in these terrains, specimens were collected along with sediment and immediately placed on ice before transportation to the Marine Environment Laboratory at Khorramshahr University of Marine Science and Technology. Upon arrival, species identification was verified using the dichotomous key provided by Murdy [14]. About 2 grams of dorsal fin muscle tissue were dissected from each individual, preserved in 96% Ethanol, and stored at −20 °C until DNA extraction [15].

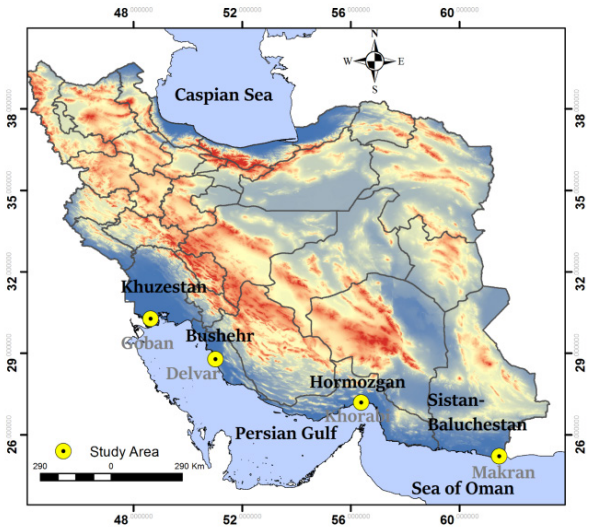

Figure 1) Map of the study area and geographic location of sampling points (i.e., Goban in Khuzestan Province, Delvar in Bushehr Province, and Khorabi in Hormozgan Province) in the Persian Gulf.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was isolated from dorsal fin tissues using a modified ammonium acetate precipitation method. Briefly, samples were homogenized in STE buffer containing 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 20% SDS, followed by protein digestion with Proteinase K. DNA was then precipitated with cold absolute ethanol, washed with 70% ethanol, air-dried, and resuspended in TE buffer. DNA quality and quantity were assessed by spectrophotometry using an Eppendorf BioPhotometer D30 and confirmed through 1% agarose gel electrophoresis stained with Safe Stain. The mitochondrial cytochrome b gene was selected for phylogenetic analysis. Primers were designed based on published sequences of related Boleophthalmus species [16] and synthesized by Sinaclon Co. PCR reactions were carried out in 25 µL volumes containing 1x Ampliqon Taq DNA Polymerase Master Mix RED, 10 µM of each primer, 3–5 ng.µL DNA template, and nuclease-free water. The thermal cycling protocol included an initial denaturation at 95 °C for 4 minutes; 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 60 seconds, and extension at 72 °C for 90 seconds; followed by a final extension at 72 °C for 10 minutes. Amplification products were verified through electrophoresis on 1% agarose gel stained with Safe Stain, visualized under UV light, and compared to a 100 bp DNA ladder. Successful PCR products were purified and sent to Royan Zistagen Company for bidirectional Sanger sequencing using an ABI 3730xl DNA Analyzer.

Alignment and Sequence Analysis

Raw sequences were edited and aligned using SeqScape v2.6 (Applied Biosystems) and MEGA 12 [17], employing the ClustalW

Table 1) Number of individuals and relative frequency for each haplotype in *B. dussumieri* populations.

Haplotype	Variable site	Goban	Delvar	Khorabi	Makran	Total
Hap1	AAGGTCTTATCCTCCCTAACACTCTACACTGTCTTCGCTCCTCCGTCTGT ATCAGCTCCCTGTCCCTCGTTTCCTTCGATGCGGTATCGGAACGA			1		1
Hap2	.....C.....A.....	1	3	3		7
Hap3	.....C....GG..A.....A.....			1		1
Hap4	.....C....GG.....			1		1
Hap5	G..AA.....C....G...A.....			1		1
Hap6	.....A.....		2			2
Hap7	.....TC.....A.....		1			1
Hap8	.....T.....A.....		1			1
Hap9	.....G...A.....		1			1
Hap10	.G.....A.....A.....		1			1
Hap11	GT...TCA.....A.....A.....		1			1
Hap12	.....A.....T..	1				1
Hap13	.G.....A.....A..T.....A.....ATG..TC...T.C	1				1
Hap14	.....C..C.....A.....A.....	1				1
Hap15	.....T.....A.....C.....	1				1
Hap16	..A.....T.....A.....A.....AA.....T.T.....C...T.C	1				1
Hap17	.....AT.....TA....AA.A.....TG...C.T.ATC	1				1
Hap18	.....C..C.....A.....TGG.....T.....A.	1				1
Hap19	.....C.....ACTCTCTACTCACT.CATAC.TCAAA.TCAC.. ATA.CTAAGCCT..AACC..T.CCA..A.T.T.TCG.A.G.AG				1	1
Hap20	.....T..TCTCTACTCACT.CATAC.TCAAA.TCAC.. ATA.CTAAGCCT..AACC..T.CCA..A.T.T.TCG.A.G.AG				1	1
Hap18	.....C.T.ATCTT.CT.ACTCTCTACTCA.TACATAC.TCAAA.TCAC.. ATA.CTAAGCCT..AACC..T.CCA..A.T.T.TCG.A.G.AG				1	1
Total		**8**	**10**	**7**	**3**	**28**

algorithm. Haplotype diversity, nucleotide diversity, and polymorphic sites were calculated with DnaSP 6 [18]. Median-joining haplotype networks were built in Network 5.0 [19]. Population genetic structure was evaluated through pairwise ΦST, Nei's genetic distance, AMOVA, and isolation-by-distance analyses using Arlequin 3.5.2.2 [20] and GenAlEx 6.5 [21]. The accession numbers 25 of the nucleotide sequences deposited in GenBank include PZ020718-PZ020742.

Findings

A total of 25 individuals were sequenced

for 488 bp of the mitochondrial cytochrome b gene from three populations. Among the 28 sampled, 21 haplotypes were identified. Haplotype 2 showed the highest frequency at 28%. Haplotype 6, the second most frequent, was also found in two individuals from the Delvar region. The distribution of haplotypes was highly structured, with most being unique to a single population. Specifically, Haplotypes 1, 2, 4, and 5 were exclusive to Khorabi; Haplotypes 6-11 were unique to Delvar; Haplotypes 12-18 were found only in Goban, and Haplotypes 19-21 were exclusive to Goban. In contrast, Haplotype 2 was the only shared haplotype, present in all three regions (Table 1). The results indicate high overall haplotype diversity. The highest genetic diversity was observed in the Goban and Makran regions (Hd = 1.00), slightly exceeding the diversity in the Delvar region (Hd = 0.911). The Khorabi region had the lowest diversity (Table 2). Nucleotide diversity across all populations was moderate (Table 2). Neutrality tests, such as Tajima's D and Fu's Fs, provide insights into population history; negative, significant values mainly suggest recent population expansion or purifying selection rather than a neutral equilibrium (Table 3).

A hierarchical analysis of molecular variance (AMOVA) revealed significant genetic structuring among the three *B. dussumieri* populations surveyed (Table 4). The analysis indicated that 27. 87% of the total genetic variation was partitioned among populations, while the remaining 72.16% was found within populations. The overall fixation index (ΦST) was 0.72, a statistically significant value ($p < 0.001$), confirming high and definitive genetic

Table 2) Sample size, Haplotype diversity (h), nucleotide diversity (π), number of haplotypes, and polymorphic sites value for the populations of *B. dussumieri* in the north of the Persian Gulf.

Population	Number of Samples	No. Haplotype	No. Polymorphic Site	Haplotype Diversity	Nucleotide Diversity
Goban (A) (Khuzestan)	8	8	28	1.00	0.021
Delvar (B) (Bushehr)	10	7	8	0.911	0.004
Khorabi(C) (Hormozgan)	7	5	9	0.857	0.007
Makran (Sistan-Baluchestan)	3	3	14	1.00	0.019
Total	**28**	**21**	**95**	**0.942**	**0.013**

Table 3) Fu's Fs and Tajima's D values for *B. dussumieri* populations in the northern Persian Gulf.

Population	Tajima's D (p-value)	Fu's F_s (p-value)
Goban (A) (Khuzestan)	-0.70 (p>0.1)	-2.03 (p>0.1)
Delvar (B) (Bushehr)	-1.07 (p>0.1)	-2.69 (p=0.05)
Khorabi(C) (Hormozgan)	0.19 (p>0.1)	-0.23 (p>0.1)
Makran (D) (Sistan-Baluchestan)	-	1.06 (p>0.1)
Total	**-1.45 (p>0.05)**	**-2.72 (p<0.01)**

differentiation across the sampling region. Pairwise population comparisons provided further resolution of this genetic structure (Table 5). At our sampling sites, the pairwise ΦST values ranged from 0.08 to 0.94. Specifically, the Goban population exhibited significant genetic differentiation from both the Delvar (ΦST = 0.125, $p < 0.01$) and Khorabi (ΦST = 0.109, $p < 0.05$) populations. In contrast, the comparison between Delvar and Khorabi showed no significant genetic differentiation (ΦST = 0.087, $p > 0.05$). This pattern was corroborated by Nei's genetic distance (Nei's GD), which was greatest between Goban and Khorabi (0.018) and between Goban and Delvar (0.16). However, it was substantially lower and non-significant between Delvar and Khorabi (0.007). An analysis of genetic variation and genetic distance between the sampled regions and the Makran population archived in the genebank revealed that the divergence between Makran and each of the three studied regions was substantially greater than that observed among the sampled regions themselves. Notably, the genetic differentiation of the Makran population in the Oman Sea was significantly higher and more robust than that of all three Persian Gulf populations. Estimates of gene flow (Nm) derived from pairwise ΦST values among sampling areas were all greater than 1, suggesting that migration partially counteracts the effects of genetic drift. The highest level of gene flow was observed between the Delvar and Khorabi populations (Nm = 5.1. 1), consistent with their lack of significant genetic differentiation. Nevertheless, relative to the Makran population, the extent of gene flow was considerably reduced.

The Median-Joining network analysis of haplotypes from different populations of *B. dussumeri* (Figure 2) reveals a characteristic genetic structure. The haplotypes from the Khorabi (blue), Goban (green), and Delvar (red) sampling areas do not show complete

Table 4) Results of hierarchical analysis of molecular variance among 3 populations of *B. dussumieri*. All individuals were divided into three groups based on the sample sites in the region; P-values were calculated using a random permutation test (10,000 permutations).

Study Factor	Percentage of Variance	Fixation Indices	P-value
Among Different Populations	27.87	Φ_{ST}=0.72	p<0.001
Within Populations	72.16		

Table 5) Pairwise ΦST values are shown below the diagonal and Nm above the diagonal in section A, and Nei's genetic distance (Nei's GD) below the diagonal in section B among *B. dussumieri* populations based on the cytochrome b gene.

	A					B			
Φ_{ST}/N_m	**Khorabi**	**Delvar**	**Goban**	**Makran**	**Nei's GD**	**Khorabi**	**Delvar**	**Goban**	**Makran**
Khorabi	-	5.1	4.0	0.04	Khorabi	-			
Delvar	0.08^{NS}	-	3.5	0.03	Delvar	0.007	-		
Goban	0.1*	0.12**	-	0.09	Goban	0.018	0.016	-	
Makran	0.92 *	0.94**	0.84*	-	Makran	0.197	0.192	0.02	-

Note: * Significant at the 0.5 level; ** Significant at the 0.01 level; ns: not significant.

genetic differentiation. However, the Makran population (the Oman Sea) exhibits complete genetic separation from all Persian Gulf samples. The size of the haplotype circles, which reflects their frequency, and the limited number of mutations between key haplotypes, as shown by the connecting lines, support this structural pattern. The overall network topology is star-like, featuring one or several central, high-frequency haplotypes separated by a small number of mutations from rare, peripheral haplotypes. This pattern typically indicates a recent population expansion where the studied populations originated from a common ancestral group. The sharing of central haplotypes between populations and the small mutational distances among them may suggest either gene flow until recent times or brief periods of minimal isolation. Overall, the results suggest that the three populations are not fully genetically distinct and share an interconnected evolutionary history. In contrast, the Makran population shows greater genetic differentiation than the three studied populations.

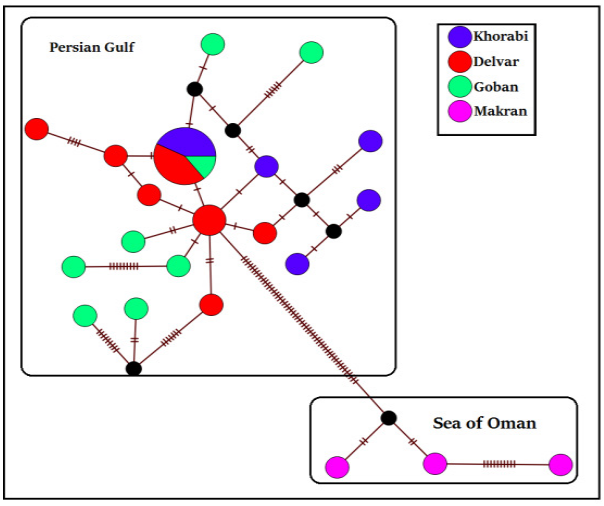

Figure 2) The Median Joining network analysis of all haplotypes of *B.dussumeri*. blue: Khorabi; red: Delvar, Green: Goban, and Pink: Makran area. Circles represent haplotypes and are sized proportionally to their frequency.

Discussion

We reconstruct the complex evolutionary history of the mudskipper *Boleophthalmus dussumieri* in the northern Persian Gulf and the Oman Sea, showing how a combination of ancient demographic shifts and modern ecological forces shapes its current genetic landscape. Mitochondrial DNA analyses reveal significant genetic diversity within populations in the Persian Gulf and the Oman Sea, identifying 21 distinct haplotypes among 28 individuals. This high haplotype diversity (Hd = 0.94), especially in the Goban and Makran regions (Hd = 1.00), surpasses values reported in many other marine fish species and suggests a large historical effective population size and rapid population growth. The distribution of these haplotypes displays clear geographic patterns, with most being unique to specific populations. Haplotypes 1, 2, 4, and 5 are exclusive to Khorabi; Haplotypes 6-11 are unique to Delvar; Haplotypes 12-18 occur only in Goban; and haplotypes 19-21 are found in Makran. This high level of population-specific haplotypes contrasts with the pattern seen in many marine species with high dispersal potential and indicates restricted current gene flow. The star-like shape of the Median-Joining network, centered around the high-frequency Haplotype 2 shared across three Persian Gulf populations, shows a typical sign of recent population expansion from a small effective size, as described by Slatkin and Hudson [22] and further explained by Excoffier [23]. This pattern is especially clear in the network, where multiple rare haplotypes connect to the central haplotype by one or a few mutational steps. The genetic evidence for expansion is also supported by the combination of high haplotype diversity and moderate nucleotide diversity (π = 0.012), a

pattern identified by Grant and Bowen [24] as indicative of rapid demographic expansion, where new mutations appear as singletons before genetic drift can significantly change their frequencies. This pattern is consistent with studies on *P. waltoni* and Scartelaos tenuis, which show that populations in more connected coastal habitats have lower genetic differentiation [4, 13].

The consistently negative Fu's Fs values across all populations, though not statistically significant at traditional levels, support the idea of population expansion, indicating an excess of rare alleles compared to expectations under neutral equilibrium conditions [25,26]. This suggests that the populations may have recently grown in size or experienced selective pressures that removed harmful alleles. These metrics together point to a dynamic evolutionary environment in which genetic diversity is maintained, and both selective and demographic processes influence the populations' genetic structure, helping ensure their long-term survival and adaptation in the changing environments of the Persian Gulf and the Oman Sea. The analysis of molecular variance highlights a key feature of marine species with limited dispersal ability: most genetic variation (72.16%) is found within populations, while a significant portion (27.87%) is among populations ($\Phi ST = 0.72$, $p < 0.001$). This pattern aligns with the species' reproductive biology; as Bohonak [27] and others, such as Selkoe et al. [28], have noted, species with demersal eggs and limited larval dispersal often exhibit this genetic pattern, in which even moderate geographic distances can create barriers to gene flow. Pairwise population comparisons reveal an asymmetrical genetic landscape that reflects the complex hydrography of the northern Persian Gulf. The genetic patterns observed in Persian Gulf populations are similar to those reported from other parts of the species' range. Muhala et al. [9] reported the first record of *B. dussumieri* in Mozambique. They identified at least two distinct lineages within the species, while Ramanadevi and Venkataraman [29] found significant genetic variation in Indian populations using RAPD markers. These consistent patterns across regions and marker systems suggest that the evolutionary forces shaping *B. dussumieri's* genetic structure in the Persian Gulf are likely active throughout its entire range. Additionally, we recognized that the extensive availability of COI sequences for *B. dussumieri* in NCBI offers a useful resource for comparison. We emphasized that future research combining COI with Cytb data would improve phylogeographic understanding and enable broader comparisons across the species' distribution.

Conclusion

This study presents the inaugural detailed assessment of the population genetic structure of *Boleophthalmus dussumieri* across three northern Persian Gulf localities. Although constrained by the analysis of a partial cytochrome b fragment (~488 bp) and a modest sample size, the results demonstrate a significant genetic differentiation of the Goban population relative to the more homogeneous genetic cluster formed by Delvar and Khorabi. In a broader comparative context, the Makran population was found to be entirely distinct. Elevated haplotype diversity, coupled with a star-like haplotype network topology, suggests a recent demographic expansion from a common ancestral source, followed by incipient divergence likely driven by

localized ecological conditions. While these findings align with broader regional studies on mudskippers, they underscore that genetic structuring within the Persian Gulf and Oman Sea is subtle and predominantly localized. Future investigations employing complete mitochondrial genomes or genome-wide markers, integrated with expanded geographic sampling, will be crucial to validate these patterns and to elucidate the relative contributions of historical versus contemporary processes in shaping the genetic architecture of *B. dussumieri*. Collectively, the evidence indicates that northern Persian Gulf and Oman Sea populations of *B. dussumieri* are in the early stages of genetic divergence, with the Makran population constituting a distinct genetic unit. Notably, in contrast to the clear phylogeographic breaks documented between the Persian Gulf and Gulf of Oman in *Periophthalmus waltoni*, the present study reveals only subtle differentiation within the Persian Gulf proper.

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Authors' Contributions: **B Moein:** Investigation, Formal analysis; **O Khalilipour:** Conceptualization, Methodology, Investigation, Formal analysis, Writing Original Draft, Visualization; **H Zolgharnein:** Methodology, Validation, Data Curation, Review & Editing.

Ethical Permission: All procedures for sampling and handling mudskipper (*Boleophthalmus dussumieri*) specimens were carried out in compliance with established ethical standards and regulations governing the use of animals in scientific research. Individuals were captured using finemesh cast nets to minimize the risk of injury, and euthanasia was performed humanely through rapid cooling on ice, a method widely accepted for poikilothermic vertebrates such as fish. Tissue samples (dorsal fin clips) were obtained only after death. Fieldwork was conducted under official collection permits granted by the Iranian Department of Environment.

Competing Interests: The authors declare that there are no competing interests, whether financial or non-financial, that could be perceived as influencing the research, analysis, or conclusions presented in this manuscript.

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Conflict of Interest: The authors declare no conflict of interest.

AI Use Declaration: To improve the clarity and readability of this work, the authors used DeepSeek V3.2 for language editing. The authors have thoroughly reviewed and revised the AI-generated suggestions and accept full responsibility for the final content.

References

1. Ansari A., Trivedi S., Saggu S., Rehman H. Mudskipper: A biological indicator for environmental monitoring and assessment of coastal waters. J. Entomol. Zool. Stud. 2014; 2(6):22-33.
2. Haidari C., Khalilipour O., Aghah H., Zolgharnein H., Hosseini S.M. Survey of the Morphometric variation on Mudskipper (*Boleophthalmus dussumieri*) in the Coastal Areas of Khuzestan. J. Anim. Environ. 2017; 9(3):285-290.
3. Looi L.J., Aris A.Z., Isa N.M., Yusoff F.M.,

Haris H. Elemental composition and health risk assessment of giant mudskipper (*Periophthalmodon schlosseri*) from the intertidal zone of the west coast of Peninsular Malaysia. Front. Mar. Sci. 2020;7(1):618284.
4. Ghanbarifardi M., Aliabadian M., Esmaeili H.R. Shape variation of the Indian Ocean slender mudskipper, *Scartelaos tenuis* (Day, 1876) from the Persian Gulf and Gulf of Makran (Gobioidei: Gobiidae). Iran.J.Fish.Sci. 2020; 19(2):612-622.
5. Ghanbarifardi M., Aliabadian M., Esmaeili H.R., Polgar G. Morphological divergence in the Walton's Mudskipper, *Periophthalmus walto*ni Koumans, 1941, from the Persian Gulf and Gulf of Oman (Gobioidei: Gobiidae). Zool. Middle. East. 2014a; 60(2):133-143.
6. Ghanbarifardi M., Aliabadian M., Esmaeli H.R. Morphometric variation of *Periophthalmus waltoni* (Teleostei: Gobiidae) in the Persian Gulf and Gulf of Oman. Iran. J. Anim. Biosyst. 2014; 10(2):137-144.
7. Ghanbarifardi M., Esmaeili H.R., Gholami Z., Aliabadian M., Reichenbacher B. Molecular phylogeny of three mudskippers (Gobiidae) from the Persian Gulf and Gulf of Oman (Makran). J. Appl. Ichthyol. 2016; 32(3):416-420.
8. Muhala V., Guimarães-Costa A., Bessa-Silva A.R., Rabelo L.P., Carneiro J., Macate I.E., Sampaio I. Comparative mitochondrial genome brings insights to slight variation in gene proportion and large intergenic spacer and phylogenetic relationship of mudskipper species. Sci. Rep. 2024; 14(1):3358
9. Muhala V., Guimarães-Costa A., Macate I.E., Tembe S., Mula Y., Tóvela É., Bessa-Silva A.R., Vallinoto M., Sampaio I. First record of mudskipper *Boleophthalmus dussumieri* (Gobiidae: Oxudercinae) on the coast of Mozambique and evidence of two putative lineages along its known distribution range. J. Fish. Biol. 2023; 102(1):281-286.
10. Sinaei M., Mashinchian A., Fatemi M., Riazi G. Application of biomarkers in mudskipper (*Boleophthalmus dussumieri*) to assess polycyclic aromatic hydrocarbons (PAHs) pollution in coastal areas of the Persian Gulf. Ecotoxicol. Environ. Saf. 2012; 84(1):311-318.
11. Lambeck K. Shoreline reconstructions for the Persian Gulf since the last glacial maximum. Earth Planet. Sci. Lett. 1996; 142(1-2): 43-57.
12. Kennett D.J., Kennett J.P. Early State Formation in Southern Mesopotamia: Sea Levels, Shorelines, and Climate Change. J. Island Coast. Archaeol. 2006; 1(1):67-99.
13. Ghanbarifardi M., Zarei R. Otolith shape analysis of three mudskipper species of the Persian Gulf. Iran. J. Fish. Sci. 2021; 20(2):333-342.
14. Thacker C.E. Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). Mol. Phylogenet. 2003; 26(3):354-368.
15. Sharifiniya M., Mousavi-Sabet H., Alavi-Yeganeh M.S., Ghanbarifardi M. Molecular phylogeny and morphological characteristics of the reef margin blenny, *Entomacrodus striatus* (Valenciennes, 1836) from the Persian Gulf of Oman (Actinopteri: Blenniiformes Blenniidae). ECOPERSIA 2022; 10(1):37-46.
16. Liu Z.Z., Wang C.T., Ma L.B., He A.Y., Yang J.Q., Tang W.Q. Complete mitochondrial genome of the mudskipper *Boleophthalmus pectinirostris* (Perciformes, Gobiidae): Repetitive sequences in the control region. Mitochondrial. DNA. 2012; 23(1):31-33.
17. Kumar S., Stecher G., Suleski M., Sanderford M., Sharma S., Tamura K. MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. Mol. Biol. Evol. 2024; 41(12):1-9.
18. Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J.C., Guirao-Rico S., Librado P., Ramos-Onsins S.E., Sánchez-Gracia A. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 2017; 34(12):3299-3302.
19. Paradis E. Analysis of haplotype networks: The randomized minimum spanning tree method. Method. Ecol. Evol. 2018; 9(5):1308-1317.
20. Excoffier L., Lischer H.E. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 2010;10(3):564-567.
21. Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinform. 2012; 28(19):2537-2539.
22. Slatkin M., Hudson R.R. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics. 1991; 129(2):555-562.
23. Excoffier L. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. Mol. Ecol. 2004; 13(4):853-864.
24. Grant W.S., Bowen B.W. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J. Hered. 1998; 89(5):415-426.
25. Fu Y.X. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics. 1997; 147(2):915-925.
26. Riquet F., Liautard-Haag C., Woodall L., Bierne N. Parallel pattern of differentiation at a genomic island shared between clinal and mosaic hybrid zones in a complex of marine snails. Mol. Ecol.

2019; 28(17):4017-4033.
27. Bohonak A.J. Dispersal, gene flow, and population structure. Q. Rev. Biol. 1999; 74(1):21-45.
28. Selkoe K.A., D'Aloia C.C., Crandall E.D., Iacchei M., Liggins L., Puritz J.B., Toonen R.J. A decade of seascape genetics: contributions to basic and applied marine connectivity. Mar. Ecol. Prog. Ser. 2016; 554(1):1-19.
29. Ramanadevi V., Thangaraj M., Sureshkumar A., Dubburaj J. Detection of genetic variation and genetic diversity in two Indian mudskipper species (*Boleophthalmus boddarti, B. dussumieri*) using RAPD marker. Not. Sci. Biol. 2013; 5(2):216-222.