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RESEARCH ARTICLE

New Record and DNA Barcoding of the Flat Needlefish (Belonidae), *Ablennes hians* (Valenciennes, 1846) from Iskenderun Bay, Eastern Mediterranean, Türkiye

Servet Ahmet Doğdu^{1,2*}, Necdet Uyğur¹, Cemal Turan², Funda Turan², Ali Uyan²

¹Iskenderun Technical University, Maritime Technology Vocational School of Higher Education, Underwater Technologies, 31200 Iskenderun, Hatay, Türkiye
²Iskenderun Technical University, Maritime Technology Vocational School of Higher Education, Underwater Technologies, 31200 Iskenderun, Hatay, Türkiye
³Iskenderun Technical University, Faculty of Marine Sciences and Technology, Molecular Ecology and Fish. Genetics Lab. 31220, Iskenderun, Hatay, Türkiye
⁴Iskenderun Technical University, Faculty of Marine Sciences and Technology, Molecular Ecology and Fish. Genetics Lab. 31220, Iskenderun, Hatay, Türkiye

<https://orcid.org/0000-0003-2939-5838>
<https://orcid.org/0000-0002-4460-1735>
<https://orcid.org/0000-0001-9584-0261>
<https://orcid.org/0000-0002-0257-6009>
<https://orcid.org/0000-0001-9308-3324>

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Abstract: This study reports the flat needlefish *Ablennes hians* on the coasts of Türkiye for only the second time. mtDNA COI gene region was used for molecular identification of the species. On 21 December 2024, fishermen caught the specimens used in the study at a depth of 24 m in Iskenderun Bay. The COI sequences obtained in this study matched *A. hians* reference sequences with 100% identity in BLAST searches of the BOLD and NCBI databases. Genetic distance results indicate that the Turkish population is extremely close to the Israel population (0.0085) this Mediterranean group is genetically distinct from the closest Red Sea (Egypt) population (0.0563) and other Indo-Pacific populations. This verified that *A. hians* may have entered the Mediterranean via the Red Sea. On the other hand, more specimens from the Mediterranean, Red Sea and Atlantic Ocean should be studied to determine the true route of the species.

Anahtar kelimeler:

Belonidae
mtDNA COI
Lessepsiyen göçü
İklim değişikliği

Türkiye'nin Doğu Akdeniz Kıyılarındaki Iskenderun Körfezi'nde Bulunan Düz İğne Balığı (Belonidae), *Ablennes hians* (Valenciennes, 1846) Türünün Yeni Kayıt ve DNA Barkodlaması

Öz: Bu çalışma Türkiye kıyılarında ikinci kez düz iğne balığı *Ablennes hians*'ın varlığını bildirmektedir. Türün moleküler tanımlanması için mtDNA COI gen bölgesi kullanılmıştır. Çalışmada kullanılan örnekler, 21 Aralık 2024 tarihinde Iskenderun Körfezi'nde 24 m derinlikte balıkçılar tarafından yakalanmıştır. Bu çalışmada elde edilen COI dizileri, BOLD ve NCBI veritabanlarında yapılan BLAST aramalarında *A. hians* referans dizileriyle %100 özdeşlik göstermiştir. Genetik uzaklık sonuçları, Türkiye popülasyonunun İsrail popülasyonuna (0,0085) son derece yakın olduğunu, bu Akdeniz grubunun, en yakın Kızıldeniz (Mısır) popülasyonundan (0,0563) ve diğer Hint-Pasifik popülasyonlarından genetik olarak ayrıştığını göstermektedir. Bu durum, *A. hians*'ın Kızıldeniz'den Akdeniz'e girmiş olabileceğini doğrulamaktadır. Ancak, türün gerçek rotasını belirlemek için Akdeniz, Kızıldeniz ve Atlantik Okyanusu'ndan daha fazla örneğin incelenmesi gerekmektedir.

Introduction

The two most important factors affecting the entry of alien species into the Mediterranean Sea are the opening of the Suez Canal and global warming (Turan et al., 2016; Langeneck et al., 2023; Deidun et al., 2024; Doğdu and Turan 2024; Sliskovic et al., 2024; Zenetos et al., 2024; Golani 2025). The combination of these two factors has led to the introduction of more than 100 exotic fish species into the Mediterranean to date (Galil et al., 2018; Turan et al., 2018; Azzurro and D'Amen 2022; Mutlu et al., 2023; Yağlıoğlu et al., 2023; Turan et al., 2024a; Khalil et al., 2025).

The family Belonidae, distributed across tropical and temperate seas worldwide, comprises 10 genera and 48

valid species (Fricke et al., 2025). This family is represented in Turkish waters by four species under three genera: *Belone belone* (Linnaeus, 1760), *Belone svetovidovi* Collette and Parin, 1970, *Tylosurus imperialis* (Lacepède, 1803), and *Ablennes hians* (Valenciennes, 1846) (Karataş et al., 2021; Turan et al., 2023; Irmak and Özden 2023; Turan et al., 2025).

Ablennes hians, commonly known as the flat needlefish, inhabits a broad range of tropical and temperate marine environments across Red Sea, Indian, Pacific and the Atlantic Oceans (Froese and Pauly 2025). The species was first reported in the Mediterranean Sea from the Israel coast (Golani 2019), and subsequently from

*Corresponding author: servet.dogdu@iste.edu.tr

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Syria (Alshawy et al., 2019), Lebanon (Crocetta et al., 2021), Malta (Deidun et al., 2021) and Türkiye (Irmak and Özden 2023). According to Irmak and Özden (2023) the specimen of *A. hians* recorded from Malta has been reported to be a misidentified specimen of *Tylosurus acus*.

DNA barcoding is an increasingly utilized tool for taxonomic identification of fish species (Landi et al., 2014). It is particularly useful for identifying cryptic species that cannot be identified morphologically (Ribeiro et al., 2012; Karahan et al., 2017; Turan et al., 2024b). Sequencing specific loci and comparing them against established reference databases (NCBI, BOLD) enable reliable species identification (Bariche et al., 2015; Turan et al., 2017; Amir et al., 2022; Bemis et al., 2023). Accurate identification of alien fish species is essential for effective ecosystem monitoring, early detection, and mitigation of potential ecological threats (Karan et al., 2019; Turan et al., 2020; Ragkousis et al., 2023).

In this study, we analyzed mtDNA *cytochrome c oxidase subunit I (COI)* gene sequences to document a new occurrence of *Ablennes hians* in Iskenderun Bay. This finding, representing the species' second record from

Turkish marine waters, contributes to understanding its growing occurrence in the Mediterranean and presents ideas on its possible pathway of entry.

Material and Methods

On December 21, 2024, five individuals of *Ablennes hians* were collected using gill nets at a depth of 24 meters in Iskenderun Bay by local fishermen (36.290870 N, 35.782803 E) (Figure 1). The collected samples were stored on crushed ice and safely transported to the laboratory while maintaining the cold chain. Tissue samples taken from the muscles were preserved in 95% ethanol and kept at -20°C until they were processed. Measurements were taken for each specimen's total length (± 0.1 mm) and body weight (± 0.01 g). All *A. hians* individuals (Figure 2) were thoroughly inspected and taxonomically identified based on diagnostic characteristics described in previous studies and identification keys, including those by Collette and Parin (1970), Alshawy et al. (2019), Golani (2019) and Irmak and Özden (2023).

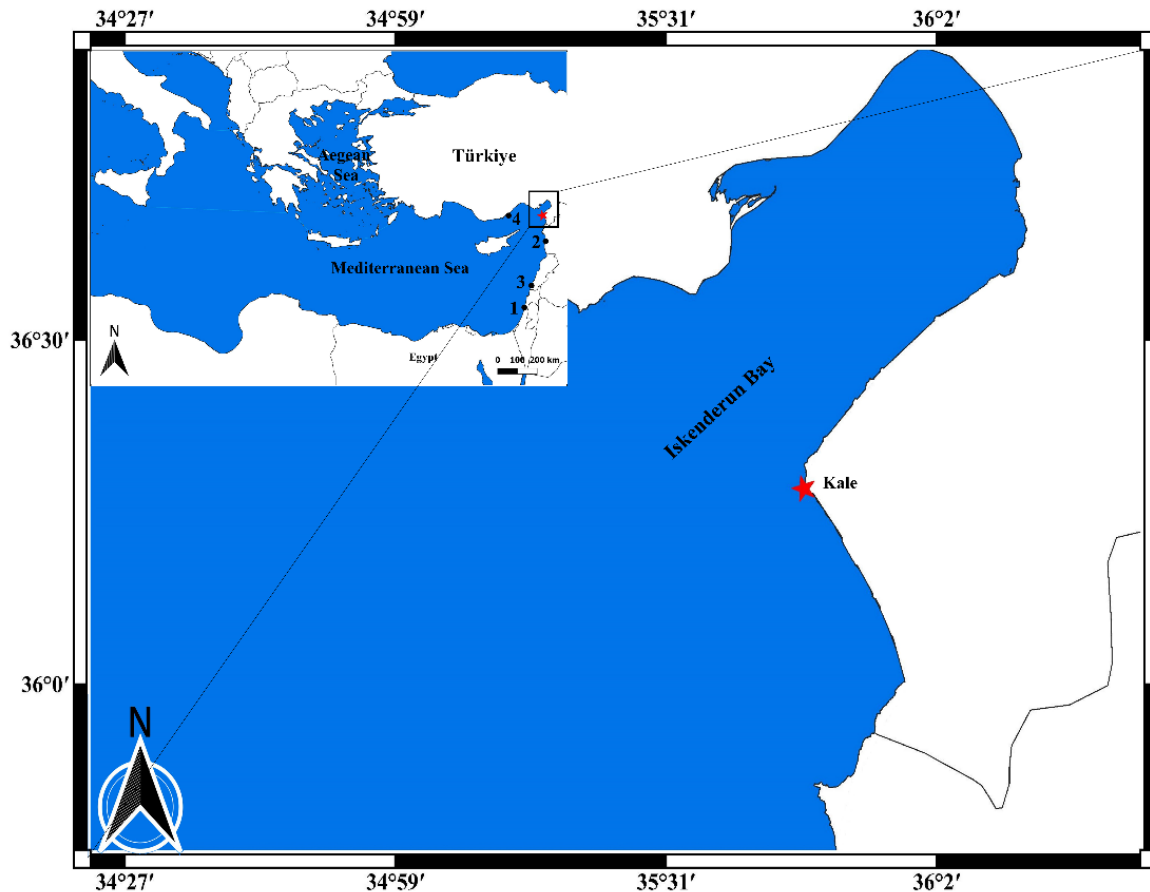


Figure 1. Sampling location (★) of *Ablennes hians* from Kale coasts in Iskenderun Bay, and its previous records in the Mediterranean Sea (●: previous records).

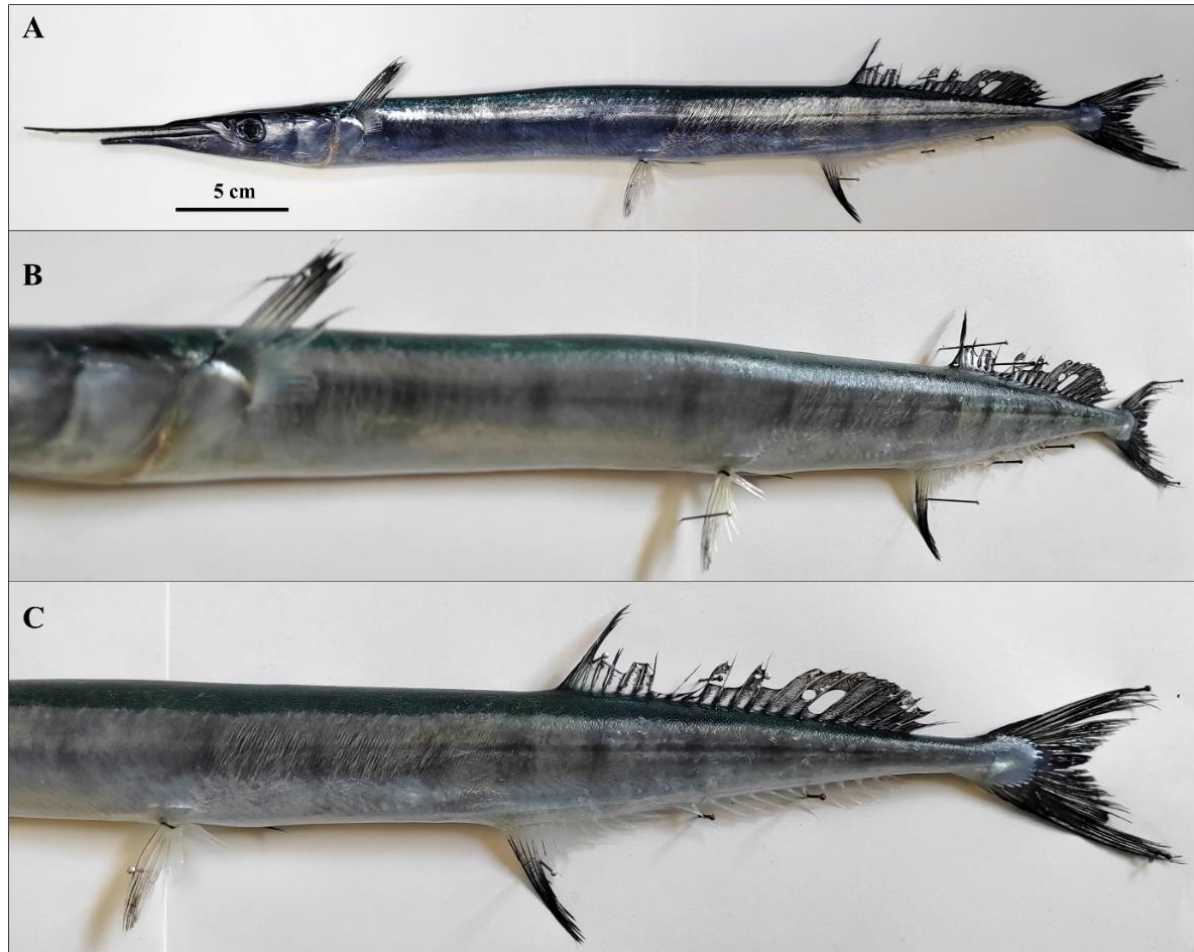


Figure 2. *Ablennes hians* (A: General view, B: Side view, C: Position of the dorsal and anal fin to the tail)

Mitochondrial DNA was isolated from muscle tissues utilizing a slightly modified version of the traditional phenol-chloroform extraction protocol. PCR amplification of the extracted mtDNA was carried out using universal primers targeting the COI gene, as described by Ward et al. (2005):

Fish_F1:

5'-TCAACCAACCACAAAGACATTGGCAC-3'

Fish_R1:

5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'

Each PCR reaction was prepared in a final volume of 50 µl, comprising 0.4 µM of both primers, 0.2 mM of dNTP mix, 1 unit of Taq DNA polymerase, and reaction buffer containing 20 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 15 mM KCl, along with 1.5 µl of template DNA. Thermal cycling was performed with 35 repetitions of the following steps: denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 75 °C

for 45 seconds, concluding with a final elongation step at 75 °C for 7 minutes. Amplification success was confirmed by running the PCR products on a 1.5% agarose gel. Sequencing of PCR products was performed via the Sanger sequencing technique (Sanger et al., 1977), utilizing a BigDye Terminator v3.1 kit on an ABI 3130 XL automated sequencer. The resulting DNA sequences were aligned and edited using the BioEdit software package (Hall et al., 2011). Analysis of nucleotide variation and genetic diversity was conducted, and phylogenetic trees were generated using MEGA 12 (Kumar et al., 2024). Pairwise genetic distances were calculated under the Kimura 2-Parameter (K2P) model (Kimura, 1980). The obtained sequences were compared against *Ablennes hians* and other Belontiidae entries available in the GenBank and BOLD repositories (Table 1).

The sequences used in the study were registered in the NCBI database with accession numbers PV124771.1-PV124775.1.

Table 1. Sequence data from databases belonging to Belonidae species used in the study

Species	Accession Numbers	Database	Country	References
<i>Ablennes hians</i>	PP422398.1	Genbank	Philippines	Unpublished
<i>A. hians</i>	OP247590.1	Genbank	China	Unpublished
<i>A. hians</i>	MN511846.1	Genbank	Pakistan	Amir et al (2022)
<i>A. hians</i>	GU674072.1	Genbank	Indonesia	Unpublished
<i>A. hians</i>	MT323766.1	Genbank	USA	Unpublished
<i>A. hians</i>	MN380039.1	Genbank	Egypt	Unpublished
<i>A. hians</i>	ABLEN001-20	BOLD System	Israel	Tadmor-Levi et al (2020)
<i>A. hians</i>	OR528792.1	Genbank	India	Toji et al., (2024)
<i>A. hians</i>	OR528791.1	Genbank	India	
<i>A. hians</i>	OR528790.1	Genbank	India	
<i>A. hians</i>	OR528789.1	Genbank	India	
<i>A. hians</i>	OR528788.1	Genbank	India	
<i>A. hians</i>	MH377855.1	Genbank	India	Unpublished
<i>A. hians</i>	MH377856.1	Genbank	India	Unpublished
<i>A. hians</i>	MH377854.1	Genbank	India	Unpublished
<i>A. hians</i>	MK340571.1	Genbank	Bangladesh	Habib et al., (2023)
<i>A. hians</i>	MK340570.1	Genbank	Bangladesh	
<i>A. hians</i>	MH235604.1	Genbank	Myanmar	Segura-Garcia and Yain Tun (2018)
<i>A. hians</i>	MH235603.1	Genbank	Myanmar	
<i>A. hians</i>	KU943251.1	Genbank	Taiwan	Chang et al., (2017)
<i>A. hians</i>	KU893033.1	Genbank	Taiwan	
<i>A. hians</i>	KU892799.1	Genbank	Taiwan	Zhang and Hanner (2011)
<i>A. hians</i>	JF952660.1	Genbank	Japan	
<i>A. hians</i>	JF952659.1	Genbank	Japan	
<i>A. hians</i>	JF492762.1	Genbank	South Africa	Steinke et al., (2011)
<i>A. hians</i>	JF492761.1	Genbank	South Africa	
<i>A. hians</i>	JF492760.1	Genbank	South Africa	
<i>A. hians</i>	JF492759.1	Genbank	South Africa	
<i>A. hians</i>	JF492758.1	Genbank	South Africa	Asgharian et al., (2011)
<i>A. hians</i>	HQ149783.1	Genbank	Iran	
<i>A. hians</i>	MK777127.1	Genbank	Vietnam	Thu et al., (2019)
<i>A. hians</i>	MK777126.1	Genbank	Vietnam	
<i>A. hians</i>	MK777125.1	Genbank	Vietnam	
<i>A. hians</i>	GBAAW70063-24	BOLD System	Philippines	Unpublished
<i>Hemiramphus far</i>	FOAE044-06	BOLD System		Unpublished

Results

The majority of alien fish species in the Mediterranean Sea are known to originate from the Red Sea, entering via the Suez Canal (Golani et al., 2021; Azzurro and D'Amen, 2022; Turan and Doğdu 2022; Golani 2025). Here, we confirm the second record of *Ablennes hians* from Turkish

waters through morphological and molecular identification using the mitochondrial *COI* gene region.

The five *Ablennes hians* specimens from İskenderun Bay measured 55.8–60.1 cm in total length. Detailed morphometric and meristic data (e.g. standard length, head length, fin-ray counts) are given in Table 2.

Morphologically, the species exhibits a slender, laterally compressed body with elongated jaws bearing small, sharp teeth; a blue-green dorsum fading to light blue flanks and a bright grey ventrum; transparent pelvic fins; and darker dorsal and anal fins with long black rays (Figure 2A).

Thirteen dark vertical bars occur along the flanks, though these are often faint in photographs owing to light reflection (Figure 2B–C). Our measurements closely match those of Irmak and Özden (2023), Alshawy et al., (2019), and Froese and Pauly (2025).

Table 2. Meristic characteristics of *A. hians* caught from Iskenderun Bay, with mean values and standard deviations ($\pm$) from previous records

Morphometric Measurements (cm)	This Study (n=5)		Alshawy et al., (2019) (n=2)	Irmak and Özden (2023) (n=1)	Froese and Pauly (2025) (n=1)
	Mean	% TL			
Total length	58 $\pm$ 1.5	100	50.0-55.3	72.7	53
Standard length	51.4 $\pm$ 1.3	88.6	44.5-50.2	-	48.1
Body depth	3.3 $\pm$ 0.1	5.7	3.4	-	3.2
Head length	13.7 $\pm$ 0.4	23.6	14.5-15.0	19	13.2
Eye diameter	1.4 $\pm$ 0.1	2.4	1.5	1.9	0.9
Interorbital distance	1.5 $\pm$ 0.1	-	-	2.39	-
Jaw length	9.3 $\pm$ 0.2	16	9.6-10.4	-	-
Dorsal Fin length	9.7 $\pm$ 0.3	16.7	9.0-9.4	-	-
Pectoral fin length	4.5 $\pm$ 0.1	7.8	3.4-3.5	5.2	-
Pelvic fin length	4.0 $\pm$ 0.1	6.9	2.7-3.0	-	-
Anal fin length	9.9 $\pm$ 0.3	17.1	9.3-9.8	-	-
Caudal fin length	6.5 $\pm$ 0.3	11.2	5.4-6.0	-	-
Pre-dorsal	40.2 $\pm$ 1.1	69.3	-	50.9	37.2
Post-dorsal	49.9 $\pm$ 1.3	86	-	-	-
Pre-anal	38.4 $\pm$ 1.0	66.1	-	50	36.6
Post-anal	48.4 $\pm$ 1.3	83.4	-	-	-
Pre-pectoral	15.4 $\pm$ 0.4	26.6	-	-	14.5
Pre-pelvic	29.7 $\pm$ 0.8	51.2	-	38.9	28.8
Weight (g)	178.94 $\pm$ 15.72	-	-	-	-
Counts					
Dorsal fin rays	24-26		25	24	23-26
Anal fin rays	26		27	26	24-28
Pectoral fin rays	15		14	13	-
Ventral fin rays	6		6	-	6

The COI gene regions from five *Ablennes hians* specimens yielded sequences 645 base pairs in length, which have been submitted to GenBank accession numbers PV124771.1-PV124775.1. There were observed 68 variable sites, 577 conservative sites, 44 parsimony-informative sites and 24 singleton sites over 645 bp DNA barcode of five *A. hians* samples. By comparing the five *A. hians* barcodes obtained from our study with sequences obtained from databases, a total of 21 haplotypes were identified in 39 sequences, and although common

haplotypes were identified between some populations, no common haplotype shared by all populations was identified. Haplotype diversity among these sequences was found to be 0.9069. Minimum spanning tree (MST) analysis indicated considerable genetic variation among the examined populations (Figure 3). At the species level, the obtained *A. hians* sequences were confirmed with 100% reliability by blast results from the Bold System and NCBI database.

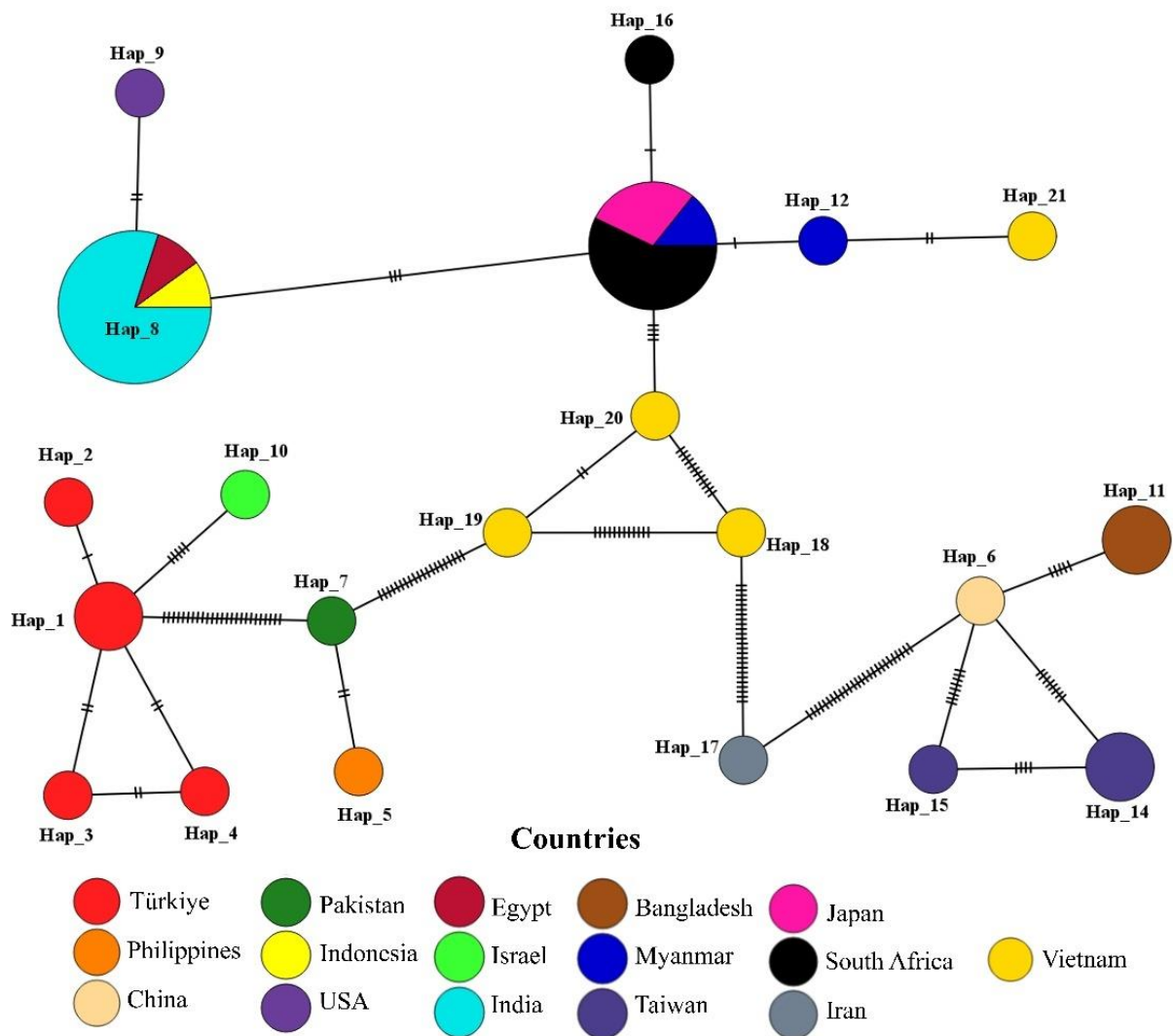


Figure 3. The minimum spanning network based on *COI* haplotypes of *Ablennes hians*, showing mutational relationships between haplotypes

Pairwise genetic distances among populations were calculated using the K2P model (Kimura, 1980), which is widely applied in DNA barcoding studies (Hebert et al., 2003; Collins et al., 2012). The obtained values are given in Table 3, showing the closest relationship between the Turkish and Israel specimens. Pairwise genetic distances revealed that the Turkish *Ablennes hians* samples were most closely related to the Israel samples (0.0085), suggesting a recent colonization event possibly via Lessepsian migration. In contrast, substantial genetic divergence was observed between Turkish and South/Southeast Asian samples (Türkiye–Bangladesh: 0.0888), indicating distinct phylogeographic lineages. These genetic distance models are consistent with the observed haplotype distribution (close relatives share the same haplotypes, while distant relatives have different haplotypes).

The *COI* gene sequences obtained in this study were subjected to maximum likelihood (ML) phylogenetic analysis together with reference sequences obtained from NCBI and BOLD (Figure 4). The evolutionary relationships were illustrated using a consensus tree generated from 1000 bootstrap replicates, following the method of Felsenstein (1985). In Figure 4, low genetic variation was observed among the individuals sampled in our study. All five Turkish samples of *Ablennes hians* barcodes form a strongly supported monophyletic clade together with the single Israel sample. Pairwise *COI* distances between the Turkish and Israel samples are exceptionally low (0.0085), whereas distances to the nearest Red Sea–Egypt population are substantially higher (0.0563). Within the Turkish samples, divergence is low, indicating that these five specimens share a very recent common ancestor.

Table 3. Pairwise genetic distances between populations (Populations: Türkiye (1), Philippines (2), China (3), Pakistan (4), Indonesia (5), USA (6), Egypt (7), Israel (8), India (9), Bangladesh (10), Myanmar (11), Taiwan (12), Japan (13), South Africa (14), Iran (15), Vietnam (16))

Pop.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.0339														
3	0.0790	0.0372													
4	0.0435	0.0035	0.0616												
5	0.0563	0.0032	0.0596	0.0396											
6	0.0600	0.0068	0.0634	0.0432	0.0033										
7	0.0563	0.0032	0.0596	0.0396	0.0000	0.0033									
8	0.0085	0.0336	0.0768	0.0487	0.0505	0.0542	0.0505								
9	0.0514	0.0000	0.0533	0.0350	0.0000	0.0032	0.0000	0.0470							
10	0.0888	0.0447	0.0084	0.0710	0.0690	0.0728	0.0690	0.0865	0.0625						
11	0.0619	0.0026	0.0653	0.0451	0.0050	0.0084	0.0050	0.0561	0.0042	0.0710					
12	0.0831	0.0399	0.0112	0.0731	0.0648	0.0686	0.0648	0.0809	0.0580	0.0129	0.0592				
13	0.0619	0.0034	0.0652	0.0450	0.0050	0.0084	0.0050	0.0560	0.0044	0.0709	0.0000	0.0591			
14	0.0619	0.0035	0.0653	0.0450	0.0050	0.0084	0.0050	0.0560	0.0045	0.0710	0.0000	0.0592	0.0000		
15	0.0789	0.0334	0.0450	0.0615	0.0558	0.0595	0.0558	0.0767	0.0492	0.0505	0.0504	0.0463	0.0503	0.0504	
16	0.0533	0.0005	0.0518	0.0317	0.0113	0.0148	0.0113	0.0574	0.0087	0.0562	0.0073	0.0544	0.0073	0.0073	0.0406

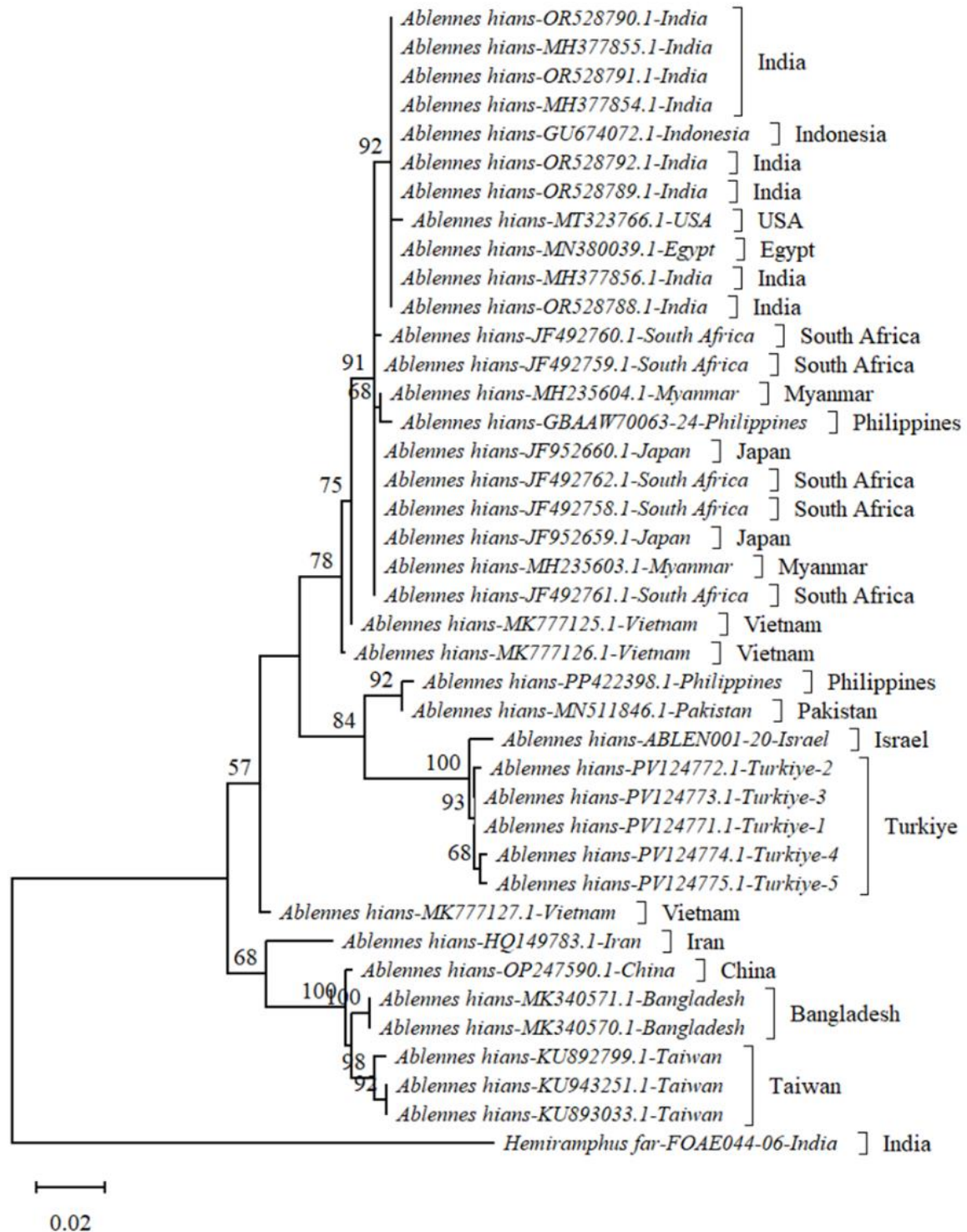


Figure 4. Phylogenetic tree generated using the Maximum-Likelihood (ML) approach based on COI region. *Hemiramphus far* was used as an outgroup in this analysis. Branches supported by bootstrap values below 50% (from 1000 replicates) were collapsed for clarity. The numbers on the branches represent the bootstrap value.

Discussion

Ablennes hians was first recorded in the Eastern Mediterranean by Golani (2019). Later, it was reported only from the Eastern Mediterranean (Alshawy et al., 2019; Irmak and Özden 2023). Tadmor-Levi et al., (2020) hypothesised that the entry route of *A. hians* into the Mediterranean may be the Strait of Gibraltar. The Israel sample (ABLEN001-20) and our sequences group in a single, fully supported clade. This perfect bootstrap support confirms that *A. hians* in Türkiye and Israel derive from a single colonization event most likely a recent Lessepsian migration through the Suez Canal. Also, pairwise COI distances between the Turkish and Israel samples are exceptionally low (0.0085), whereas distances to the nearest Red Sea–Egypt population are substantially higher (0.0563). Within the Turkish clade itself, divergence is minimal, indicating that these five specimens share a very recent common ancestor. This finding supports the possibility that *A. hians* may have entered the Mediterranean Sea from the Red Sea (Golani, 2019; Irmak and Özden 2023). However, more specimens from the Mediterranean, Red Sea and Atlantic Ocean should be studied to determine the true route of the species.

Conclusion

This study provides genetic confirmation on the presence of *Ablennes hians* in Turkish marine waters, marking the second verified record from Türkiye and the fifth from the Mediterranean Sea. The findings highlight the rapid spread of this species since its initial detection in the region in 2019. The close genetic similarity between Turkish and Israel populations and their proximity to Indo-Pacific Ocean/Red Sea specimens support the Lessepsian migration hypothesis via the Suez Canal. Further sampling and research in the Mediterranean Sea, Red Sea, and Atlantic regions are needed to fully elucidate the route and population dynamics of *A. hians*.

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Conflict of Interest

The authors declare no competing interests.

Author Contributions

Servet Ahmet Doğdu: Responsible for study design, species identification, data processing, drafting the original manuscript, overseeing the submission process, as well as contributing to the manuscript's review, editing, and visualization stages. Necdet Uyğur: Participated in sample collection and contributed to reviewing the initial manuscript draft. Cemal Turan: Involved in conceptualizing the research, identifying species, analyzing data, and reviewing the initial version of the manuscript. Funda Turan: Contributed to data

interpretation and participated in the revision of the manuscript draft. Ali Uyan: Assisted with laboratory procedures and took part in the evaluation of the original draft manuscript.

Ethics Approval

Local Ethics Committee Approval is not required as experimental animals were not used in this study. The fish samples used in this study were obtained from commercial fishermen.

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