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RESEARCH ARTICLE

The Effects of Local Aquatic Activities on Coral Cover in the Jordanian Gulf of Aqaba

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Abstract

Objective: The reef flats at the Gulf of Aqaba, Jordan, are exposed to the accelerated development associated with increased interest in recreational marine activities such as diving and snorkeling. The physical damage from net entanglement and overfishing has also affected the coral reef. This research evaluated the effects of aquatic, commercial, and land-based activities on the total coral cover in the Aqaba Marine Park (Now the Aqaba Marine Reserve) and the power station located north of the reserve.

Materials and Methods: The line transect method was used to estimate the coral cover, followed by measuring the distance of the 13 diving sites to the different landscape disturbances and applying a linear regression analysis.

Results: The results showed no significant relationship between the distance to the nearest human disturbance and the entire coral cover in the depth categories. In addition, there was no significant difference between low and high-frequency diving pressure and the mean coral cover at any depth category and no significant difference in the mean coral cover between low and high-frequency snorkeling sites. Sites with higher fishing activity had significantly lower coral cover than sites with lower fishing activity. However, there was no significant difference in the mean coral cover between sights with higher and lower fishing activity at the 21-30 m depth range.

Conclusion: The study illustrated that local marine recreational activities (diving and snorkeling) do not affect the coral cover structure up to 10 meters depths. However, considerable coral degradation was found in high fishing zones less than 10 meters deep, which correlates with illegal fishing activities. We believe illegal fishing has negatively affected the coral cover, and recreational marine activities reduced coral cover, especially in diving areas requiring shore entrance.

Keywords: Aqaba marine reserve, Coral cover, Fishing activities, Jordan, Recreational marine activities

Introduction

In the past 30 years, 60% of the world's coral reefs have vanished due to global and local anthropogenic activities (Gardner *et al*., 2003; Côté *et al*., 2005; Bruno & Selig, 2007; Carlson *et al*., 2019). The Red Sea has a biologically rich coral reef ecosystem contains thousands of fish species and other associated fauna (Golani & Bogorodsky, 2010; Fine *et al*., 2019). The reef system in the Gulf of Aqaba in Jordan consists of continuous and discontinuous fringing corals, which increase in depth as the distance from the reef crest increases (Khalaf & Kochzius, 2002a; Kotb *et al*., 2008; Al Tawaha *et al*., 2019). The Red Sea and Aqaba Gulf coral reefs are more resilient to global bleaching events but still vulnerable to local aquatic and land-based disturbances (Osman *et al*., 2017). The Red Sea and Gulf of Aqaba are popular tourist destinations, making them prime locations to study the impact of aquatic recreational activities and coastal infrastructure development on coral reef health (Gladstone *et al*., 2013). Our understanding of the anthropogenic effects on coral cover in the Red Sea and Gulf of Aqaba is a top priority, especially given that the coral reefs are suggested as a source of future translocation to assist in the global recovery of coral reef ecosystems (Osman *et al*., 2017).

The reef flats are biodiverse habitats with high primary productivity rates that support global fisheries and comprise an estimated 35% of global marine biodiversity (Barbier *et al*., 2011; Bellwood *et al.*, 2018). However, reef flats, such as those in Aqaba, Jordan, are threatened because they are adjacent to significant populations and industrial centers (Khalaf & Kochzius 2002a, 2002b; Kotb *et al*., 2008). The reefs off Aqaba are accessible for recreational and diving activities, concentrated within Jordan's limited 27-kilometer coastline (Carlson *et al*., 2019). Unsustainable large- and small-scale fishing practices can lead to a reduction of coral cover through the physical damage from net entanglement and overfishing that leads to trophic changes in the fish community (Dulvy *et al*., 2004; Wilson *et al*., 2010; Jessen *et al*., 2014; Rizzari *et al*., 2014; Ballesteros *et al*., 2018). Urban development and runoff suffocate coral polyps, which causes corals to die (Diaz & Rosenberg, 2008; Carlson *et al*., 2019).

A survey was conducted in the Aqaba Marine Park (hereafter called the Aqaba Marine Reserve), and the Power Station Center located north of the reserve (Fig. 1). The landside of the reserve is 350 meters from the coastline and extends for about seven kilometers off Aqaba. It contains around 28 active dive sites. The reef flat in Aqaba is the northernmost ecosystem in the Indo-Pacific region (Al Tawaha *et al*., 2019). This research assessed the impact of aquatic, commercial, and land-based activities on the coral cover of Aqaba, Jordan.

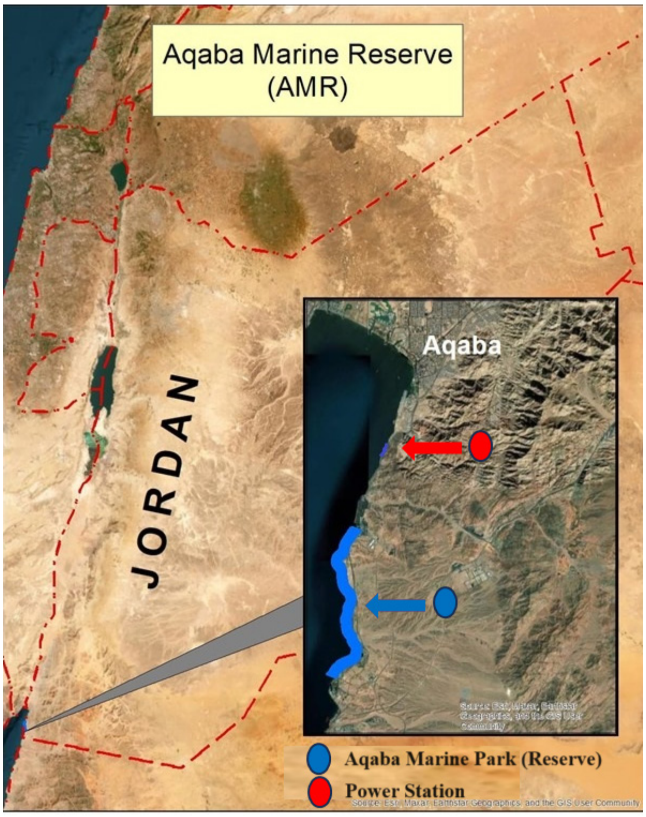

Figure 1. The Aqaba Marine Reserve and the Power Station Locations along the Gulf of Aqaba, Jordan.

Materials and Methods

The cover of the living hard and soft coral was estimated using the linear transect method (English *et al*., 1997) in May 2019, where three transects, each 20 m long and a meter on both sides, were studied at three depth zones of 1-10 m, 11-20 m, and 21-30 m at 13 diving sites approximately 500 m apart in the Aqaba Marine Reserve (Table 1), with transects performed in the Power Station Center north of the marine reserve, where data was recorded every 0.5 m using a special underwater writing board. We then measured the distance of the 13 sites to different landscape disturbances, such as distance to the nearest road, hotel, jetty, and port, using Google Earth (Table 1). There are large commercial ports at the northern and southern border of Jordan's Red Sea coast. The jetties are approximately 50 m long and are mainly used by swimmers, snorkelers, and small boats. In past surveys, we categorized the prevalence of fishing from local fishermen by examining the physical remains of entangled fishnets and/or fishing rope remnants. We ranked higher fishing density sites as those with fragments of torn fishing nets at more than one location at the dive site. In contrast, lower-density fishing sites typically had little or no fishing net remnants at any given time. Based on interviews with local dive centers, we categorized snorkel sites as low- or high-frequency. There are roughly 32 licensed diving centers in Aqaba. Most dive sites are visited from the shore. We classified high frequency sites as those used by a dive center more than twice a week. Low-frequency sites are those visited once or less a week by a dive center.

Table 1. Geographical location of the diving sites (decimal degrees) and respective distances (m) to different potential landscape disturbances.

Dive site	Latitude	Longitude	Road	Hotel	Port	Jetty
Black Rock	29.43495	34.97213	224	112	2847	93
Eel Canyon	29.41467	34.97518	288	259	3318	235
First Bay North	29.45067	34.97066	77	115	1161	406
Gorgon 1 & 2	29.41833	34.97285	213	713	3634	36
Japanese Garden	29.42695	34.97279	50	300	3722	425
King Abdullah Reef South	29.43902	34.97018	401	522	2389	577
King Abdullah Reef North	29.4433	34.96933	415	555	2010	500
Marine Science Station	29.45472	34.9725	8	570	657	874
Power Station Center	29.48806	34.98353	43	4138	1769	36
Power Station North	29.48958	34.98561	22	3756	1977	151
Power Station South	29.48283	34.98242	11	4453	1057	690
Rainbow Reef	29.4311	34.97428	115	70	3298	53
Seven Sisters	29.42264	34.97196	205	324	4056	191
Mean + SE			159.4+39	1222.1+462	2453.5+309	328.2+76
Min – Max			8 – 415	70 – 4453	657 – 4056	36 – 874

We performed linear regression analysis to determine how the coral cover percentage was affected by distance to the nearest human development (road, hotel, commercial port, and jetty). We then compared the mean coral cover at sites according to the categorically classified density of aquatic activities, such as diving, fishing, and snorkeling, using multiple ANOVAs. We analyzed the data separately for each depth class, 0-10 m, 11-20 m, and 21-30 m. We only compared the mean coral cover at sites with high and low snorkel density at the 0-10 m depth, as snorkeling rarely occurs in greater depths.

Results

Distance to human disturbance

The final linear regression models showed there was no significant relationship between the distance to the nearest human disturbance and total coral cover in any of the depth categories (0-10 m): $F(1,12)=0.013$, $p=0.99$, ii) (11-20 m): $F(1,12)=0.085$, $p=0.99$ and iii) (21-30 m): $F(1,12)=0.013$, $p=0.99$). The p-values ($p=0.99$) for the depth categories are more significant than the typical significance level, indicating that differences in the distance of human disturbance have no impact on coral cover.

Diving pressure

We found no significant difference between low and high-frequency diving density and mean coral cover at any depth category (0-10 m: $F_{1,12}=0.89$, $p=0.37$; 11-20 m: $F_{1,12}=0.83$ $p=0.38$; 21-30 m: $F_{1,12}=0.13$, $p=0.72$).

Snorkeling Sites

There was also no significant difference in the mean coral cover between low and high-frequency snorkeling sites at the 0-10m depth range ($F_{1,12}=0.38$, $p=0.55$).

Fishing Activity

Sites with higher fishing activity had significantly lower coral cover than sites with lower fishing activity at 0-10 m ($F_{1,12}=5.62$, $p=0.037$) and 11-20 m ($F_{1,12}=10.29$, $p=0.009$). However, there was no significant difference in mean coral cover between sights with higher or lower fishing activity at the 21-30 m depth range ($F_{1,12}=2.22$, $p=0.16$) (Fig. 2).

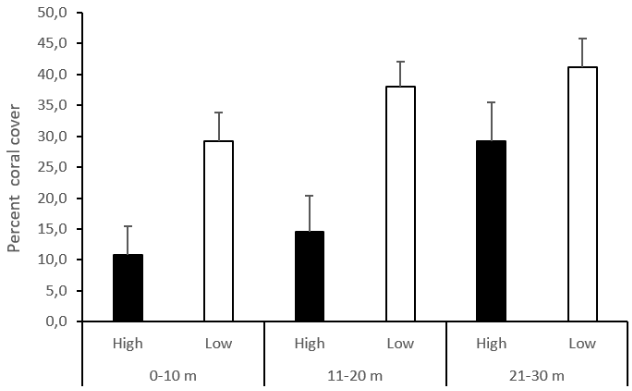

Figure 2. The mean (+ SE) percent coral cover with high and low fishing activity at three depth zones is compared.

Discussion

Our results suggest an association between illegal, small-scale fishing practices and coral cover. Study sites with high levels of fishing activity, such as the power station, as evidenced by fragments of ghost nets and fishing ropes, have reduced coral cover. The local fishermen often encircle small sections of the reef with nets, ropes, cages, nylon lines, anchors, and other angling devices that physically damage and trample corals and disrupt the ecological integrity of the coral reef (Chiappone *et al*., 2005; Abu-Hilal & Al-Najjar, 2009; Gilardi *et al*., 2010; Al Tawaha *et al*., 2019). Furthermore, even after the fishing event, the remaining ghost fishing equipment continues to damage coral, entangle fish, and reduce the penetrating light (Gunn *et al*., 2010). The effects of small-scale localized disturbance from local fishing can accumulate over time to significantly degrade coral (Edinger *et al*., 1998; Asoh *et al*., 2004; Shedrawi *et al*., 2017). The decline in coral cover as a result of damage from fishing equipment could alter the coral reef fish community by reducing the abundance of corallivorous and carnivorous fish (Khalaf & Kochzius, 2002a; 2002b) while overfishing has led to the rarity of large natural predators and commercially valuable fish species. The association between higher fishing activity and the reduced coral cover was less apparent at depths greater than twenty meters (Fig. 2). We believe that local fishermen, including the limited practices in scale by visitors and picnickers who illegally fish nets, do so at the shallower and more accessible reefs with smaller nets, which allows poachers to quickly remove their nets and leave if pursued (personal observation, OA).

Fishing activities are not allowed within 350 m of the coast to encourage catching pelagic species such as (*Thunnus albacares*), one of the primary target species for artisanal fisheries (Tesfamichael *et al*., 2016). Jordan has a small artisanal fishing fleet that provides fish to the local market (Khalaf & Kochzius, 2002a; Tesfamichael *et al*., 2012). However, fishermen can use throw nets inside the 350m to catch small planktivorous and pelagic species for bait. The legal enforcement is inconsistent as illegal fishing near the reefs was observed at sunrise at some of the survey sites in small motorboats (3-5 m length) using seine and gill nets in addition to amateur fishermen who use ropes and nylon to fish from shore in the evening and early morning hours.

We found no association between coral cover and diving and snorkeling activities. We may not have found any association with these aquatic recreations because the coral damage may have already taken place from long-term recreation in such a small area, and what remains is the reduced coral cover that can endure (Hawkins & Roberts, 1993; Tratalos & Austin, 2001; Barker & Roberts, 2004; Hasler & Ott, 2008; Lamb *et al*., 2014). Most of the entrances to the beaches and dive sites are heavily damaged (Al Tawaha *et al*., 2019), given the high levels of recreation used to create dive sites that have alleviated the diving density at natural coral reefs (Tynyakov *et al*., 2017). Most inexperienced open-water divers, discovery divers, and snorkelers visit these wreck sites. In contrast, experienced divers are more likely to visit the less frequented dive sites with higher coral cover (Lynch *et al*., 2014). In the Gulf of Aqaba, the damage that results from the irresponsible practice of amateur divers, such as touching, grabbing, or standing on coral reefs, which causes coral breakage, has been documented.

Unlike other studies, our study found no association between coral cover and landscape disturbance for several reasons (Fabricius, 2005; Carlson *et al*., 2019) One limitation of our analysis is that the study scale is relatively small as the dive sites are located close the disturbances because of current derived mass transport of contaminants (Table 1), therefore, the whole reserve is subjected to the same disturbance (Abelson *et al*., 1999; Carlson *et al*., 2019). Also, our study treats all developments equally, regardless of scale or year built. The dive sites examined are likely remnants of areas that already experienced disturbances and are degraded compared to their original state (Walker & Ormond, 1982). However, a direct relationship between the coral reef distance is significantly meaningful, and a necessary landscape disturbance is not always essential. A landscape disturbance is not always needed, not consistently substantial, and not always a crucial significant predictor of coral cover (Lirman & Fong, 2007). Establishing a comprehensive registry for diving records, including the number of divers, duration of dive, site, level of diving professionalism, and other information, is highly recommended.

An alternative explanation could be related to the flash flood events that happen in Aqaba and contribute to massive sedimentation running into the Gulf. This was discussed by several scholars who stated that the hyper-arid environment surrounding the Red Sea could mitigate local coastal disturbances, such as sedimentation and pollution from rainfall and rivers (Freiwan & Kadioğlu, 2007; Katz *et al*., 2015; Carlson *et al*., 2019). Landscape effects are often noticeably apparent after heavy rainfall carrying sediments and terrestrial contaminants run into the Gulf, negatively impacting coral health (Acevedo *et al*., 1989; Butler *et al*., 2015; Carlson *et al*., 2019).

In 2020, the Jordanian government changed the administration classification of the Aqaba Marine Park (AMP) to the Aqaba Marine Reserve (AMR) after direct orders from His Majesty King Abdulla II bin Al-Hussein to bolster its management and legal enforcement with support at the national and international levels (Eid *et al*., 2021). A management plan was developed for the reserve, and a bylaw was prepared to strengthen governance and conservation aspects. This study investigated recreational marine activities as a growing industry in the Gulf of Aqaba, Jordan, which will add an essential baseline for future research and monitoring attempts. Hopefully, this increased enforcement of no fishing in the shallow reefs and proper monitoring of recreational marine activities will contribute to the recovery of the coral reef community.

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RESEARCH ARTICLE

Historical and Contemporary Occurrence of *Odontaspis ferox* (Risso, 1810) (Lamniformes: Odontaspididae) in Turkish Seas, with New Records from the Region

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Abstract

Objective: The aim of the present article is to review historical and recent records of the critically endangered *Odontaspis ferox* from Turkish seas, and to present previously unpublished sightings.

Materials and Methods: Data of the present article was collated from the following sources: (a) observations during the visits to main fishing ports and fish markets located along Aegean and Mediterranean coasts of Türkiye; (b) screening of digital and published media reporting on the capture of large sharks in Turkish seas; (c) screening of old and contemporary ichthyological books and shark-specific publications, dealing with the fishes of Aegean Sea and eastern Mediterranean; and (d) citizen scientists' observations.

Results: Four previously unpublished records of *O. ferox* from the Turkish Mediterranean coasts are reported, and with the addition of recent sightings, the total number of documented records of the species from Türkiye has increased to eight.

Conclusion: Despite previous suspicions of the existence of *O. ferox* in Turkish seas, the present study has verified the occurrence of the species in the region. The scarcity of evidence-based observations is compatible with research findings from other parts of the Mediterranean Sea and the Turkish population of the species is currently recognized as rare. Owing to the extinction risk of *O. ferox*, it should be rapidly included in the list of species banned for fishing through official fishery legislation published by the Ministry of Agriculture and Forestry.

Keywords: Aegean Sea, Mediterranean Sea, occurrence, smalltooth, status

Introduction

The family Odontaspididae is represented by a single genus and two uncommon species in the world's oceans (small-tooth sand tiger *Odontaspis ferox* (Risso, 1810) and bigeye sand tiger *O. noronhai* (Maul, 1955)) (Ebert *et al.*, 2021; Froese & Pauly, 2023). They are characterized by stout, large heavy-bodies with bulbous conical snouts, long mouths extending behind eyes, eyes without nictitating eyelids, tearing type dentition, anal fin and second dorsal fin smaller than first dorsal fin, all three broad-based (Compagno, 2001). The bigeye sand tiger is arguably the most rarely captured extant lamniform species in the world with sporadic records from the Indian, Pacific, and Atlantic oceans (Stone & Shimada, 2019), while the small-tooth sand tiger is found circumglobally in warm-temperate and tropical seas with a very irregular and disjunctive distribution, and is the only representative of the genus in the Mediterranean Sea (Bonfil, 1995; Compagno, 2001). *Odontaspis ferox* was first described from the Nice coast (Western Mediterranean) by Risso (1810), and it is mostly associated with deep and upper slope waters along continental and insular shelves and lives on or near the bottom (Compagno, 2001). The Mediterranean population distributes mainly at depths from 10 to 250 m (Fergusson *et al*., 2008), but it is also known to inhabit depths as shallow as 1 m and as deep as 928 m elsewhere in the world (Fergusson *et al*., 2008; Francis & Lyon, 2012; Barría *et al*., 2018), and the recent findings of Higgs *et al*. (2022) support this depth range.

Despite the fact that *O. ferox* observations from the Mediterranean Sea date back to the 1800s (Hoffman & Jordan, 1892; Carus, 1893), it was mentioned to be a rare species a century ago (Desbrosses, 1930). There are relatively few reported captures globally, where Fergusson *et al*. (2008) compiled a list with nearly 160 records, 14 of which were from the Mediterranean Sea observed during the period between 1964 and 2008. The small-tooth sand tiger shark is thus regarded as a naturally rare species making *O. ferox* highly susceptible to exploitation because of its *k*-selected life strategies (slow growth, late maturation, low fecundity, long gestation periods), combined with the semi-enclosed nature of the Mediterranean Sea and existing anthropogenic stressors (Bonfil, 1995; Cavanagh & Gibson, 2007; Nuez *et al*., 2021). Populations of *O. ferox* are declining and the species has been listed by the IUCN as vulnerable on a global scale (Graham *et al*., 2016) and critically endangered at the Mediterranean Sea regional level (Pollard *et al*., 2016). It is also included in Annex II of the Specially Protected Areas and Biological Diversity Protocol (UNEP/MAP-SPA/RAC, 2018) and according to the GFCM recommendation (no. 36/2012/3), *O. ferox* cannot retained on board, transshipped, landed, transferred, stored, sold or displayed or offered for sale.

The occurrence of *O. ferox* in Turkish seas was a matter of dispute for a long time, due to the lack of stored specimens or photographic material for precise species identification (Kabasakal, 2021). Besides a couple of unverified historical records of the species (i.e., Geldiay, 1969; Fischer *et al*., 1987; Mater & Meriç, 1996), only four *O. ferox* individuals were recorded from the Turkish coast until now (Fergusson *et al*., 2008; Kabasakal & Bayrı, 2019; Kabasakal & Bilecenoğlu, 2020). In this paper, we present four additional *O. ferox* sightings from the Turkish Mediterranean coasts that would fill the knowledge gap to a great extent for this remarkably poorly known species.

Materials and Methods

Data of the present article was collated from the following sources: (a) screening of old and contemporary ichthyological books and shark-specific publications (i.e. Geldiay, 1969; Fischer *et al*., 1987; Mater & Meriç, 1996), dealing with the fishes of the Aegean Sea and the eastern Mediterranean, to extract unverified historical records of the species from the study area; (b) screening of peer-reviewed articles (Fergusson *et al*., 2008; Kabasakal & Bayrı, 2019; Kabasakal & Bilecenoğlu, 2020) to extract the evidence-based previous occurrences of *O. ferox* in Turkish seas; (c) screening of digital and published media reporting on the capture of large sharks in Turkish seas; and (d) citizen scientists' observations. The unverified historical records of *O. ferox* (data source (a)) were not included in Table1, because of their nonevidence-based nature. Only the evidence-based contemporary records (data source (b)) and unpublished additional records (data sources (c and d)) were presented in Table 1, in agreement with the protocol proposed by Kovačić *et al*. (2020). Whenever possible the following data was collated for examined specimens: total length (TL), total weight (TW), sex, date and locality of capture, type of fishing gear, post-landing remarks such as the locality where the captured *O. ferox* shipped to be displayed, auctioned and/or sold as a whole or cut into pieces. Regarding the nature of fishery-dependent opportunistic research (Jessup, 2003), information on the size and weight of the captured small-tooth sand tiger sharks were either extracted from the fishing logs or obtained after an interview with the fisherman, or collated following the data mining of digital or published media, in which the relevant data has always been provided by the fishers. Species identification follows Compagno (2001) and Ebert *et al.* (2021), and taxonomic nomenclature follows Froese & Pauly (2023). Photographs

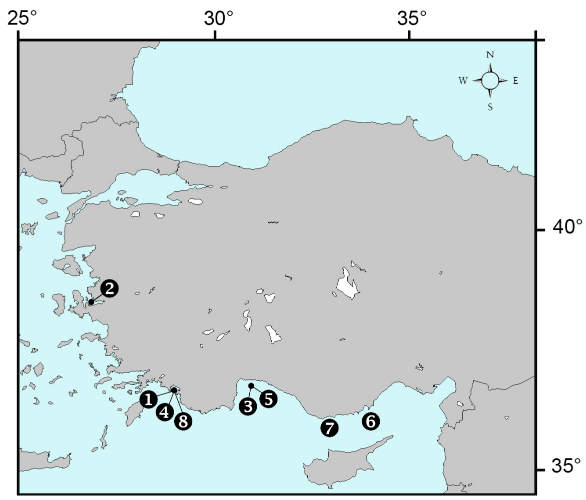

Figure 1. Map showing approximate capture localities of *Odontaspis ferox* along the Turkish coast. The numbers correspond to relevant specimens given in Table 1.

Table 1. Summary of evidence-based occurrences of *Odontaspis ferox* along the Turkish coasts in chronological order. Specimen numbers are the same as the numbers shown on the map in Figure 1. N/A: Not available

No	Year	Location	Size (cm)	Sex	Depth	Remarks	Reference
1	2002	Fethiye Bay	200	N/A	N/A	Captured in demersal trawl fishery	Fergusson *et al.* (2008)
2	2004	Urla, İzmir Bay	190	F	30	Captured in artisanal fishery, type of the fishing gear is unknown	Fergusson *et al.* (2008)
3	2009	Antalya Bay	ca. 400	N/A	N/A	Type of fishing gear is unknown; the specimen was cut to pieces and sold	Present study
4	2013	Fethiye Bay	ca. 120	F	N/A	Type of fishing gear is unknown; shipped to İstanbul Fishmarket and auctioned	Present study
5	2019	Antalya Bay	ca. 400	F	100 to 120	Captured in demersal trawl fishery	Kabasakal & Bayrı (2019)
6	2019	Taşucu, Mersin	ca. 400	F	N/A	Captured in demersal trawl fishery and sold	Kabasakal & Bilecenoğlu (2020)
7	2021	Bozyazı, Mersin	ca. 350	F	N/A	Captured in demersal trawl fishery and sold	Present study
8	2022	Fethiye Bay	272	M	N/A	Type of fishing gear is unknown; the specimen cut to pieces and sold; jaws are preserved in the personal collection of Mr. Erdi Bayrı	Present study

of the additional records of *O. ferox* were stored in the personal archive of the corresponding author and available upon request for further inspection. Capture localities of *O. ferox* in Turkish marine waters are plotted on the map (Fig. 1) and details of relevant data are presented in Table 1.

Results

Description of examined specimens (Figs. 2-5)

Small-tooth sand tiger sharks are large, bulky odontaspidids with a long bulbously conical snout, and mouth long and extending behind eyes (Figs. 2-3); tooth rows numerous and teeth moderately large with a prominent narrow cusp with two pairs of lateral cusplets (Figs. 2-5); upper anterior teeth separated from lateral teeth by 2 to 4 rows of small intermediate teeth (Fig. 5); first dorsal fin closer to pectoral-fin bases than pelvic-fin bases (Figs. 3-4); first dorsal fin noticeably larger than second dorsal fin and anal fin (Fig. 4); anal fin with strongly concave posterior margin (Fig. 3); caudal fin asymmetrical but with a strong ventral lobe (Fig. 3); color medium grey or grey-brown above, usually lighter below, sometimes with darker spots scattered on the body (Figs. 2-5). Observed descriptive characters coincide with those presented in Compagno (2001) and Ebert *et al.* (2021), thus the examined specimens (n=4) were described as *O. ferox*.

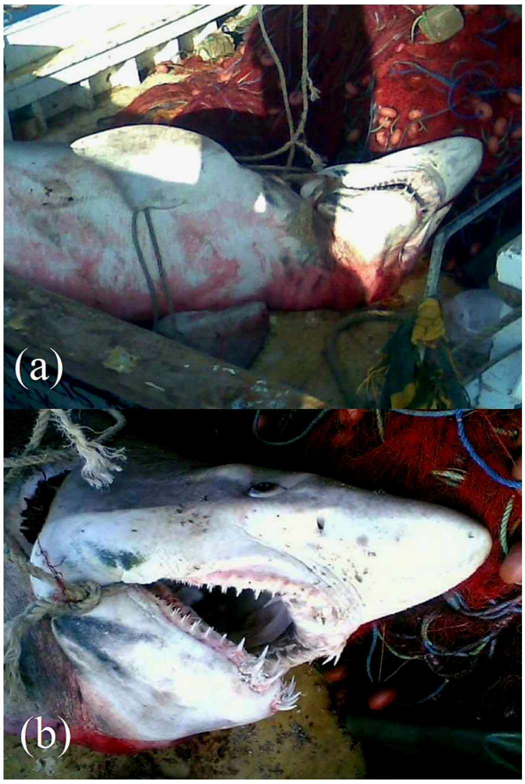

Figure 2. Specimen of *Odontaspis ferox* captured off Antalya in the summer of 2009 (record no 3 in Table 1). (a) ventral view of the specimen; and (b) ventro-lateral view of the head showing the long mouth extending behind the eyes.

Figure 3. Specimen of *Odontaspis ferox* captured off Fethiye coast in 2013 (record no 4 in Table 1). Arrow denotes the strongly concave posterior margin of the anal fin.

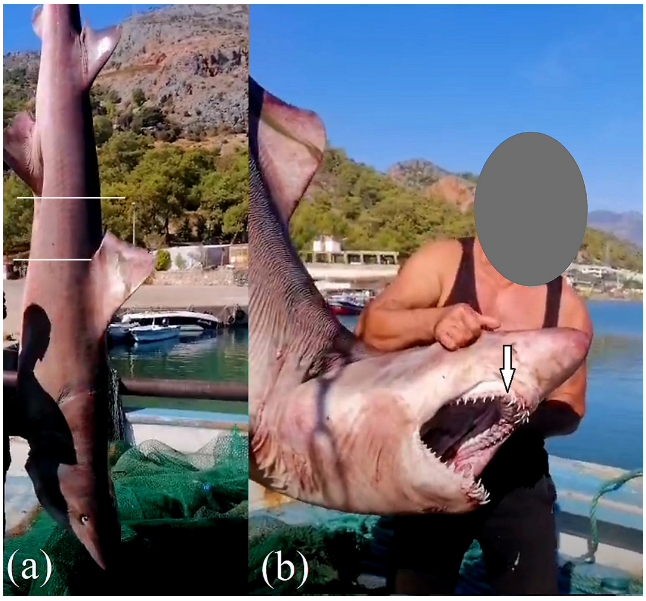

Figure 4. Specimen of *Odontaspis ferox* captured off Bozyazı on 4th of November 2021 (record no 7 in Table 1). (a) lateral lines denote that the first dorsal fin is closer to pectorals than pelvic fins; also on this panel, it is seen that the first dorsal fin is larger than the second one; and (b) arrow denotes the rows of small intermediate teeth separating upper anterior teeth.

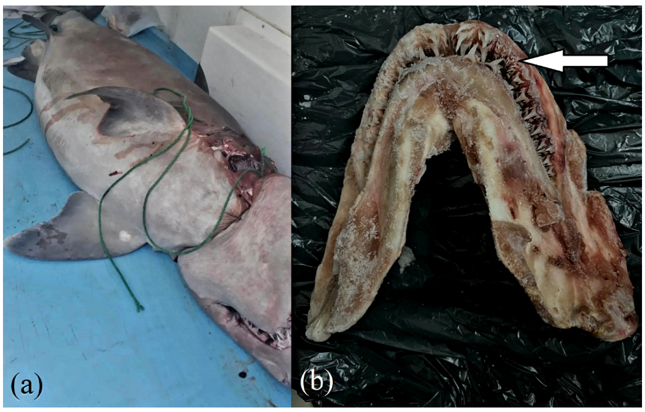

Figure 5. Specimen of *Odontaspis ferox* captured off Fethiye coast on 13th of July 2022 (record no 8 in Table 1). (a) ventro-lateral view of the specimen; and (b) arrow denotes the rows of small intermediate teeth separating upper anterior teeth.

Additional records

Available data collated from the sources, which are mentioned in the materials and methods section, revealed four additional records of *O. ferox* from the Turkish Mediterranean waters. In the summer of 2009, a specimen of *O. ferox* was captured off Antalya in commercial fisheries and sold to a hotel or a restaurant to attract customers' attention (Fig. 2). In 2013, a juvenile small-tooth sand tiger shark was captured in commercial fisheries off the Fethiye coast and shipped to the İstanbul wholeale fish market for auction (Fig. 3). On 4 November 2021, a specimen of *O. ferox* was captured in a demersal trawl fishery off the Bozyazı coast, upon landing cut to pieces and sold to a restaurant (Fig. 4). Recently, on 13 July 2022 a small-tooth sand tiger shark was captured off the Fethiye coast, which was also cut to pieces and sold (Fig. 5).

Discussion

Sharks are among the most threatened marine taxa in the Mediterranean Sea, evidently declining more severely in abundance when compared to other parts of the world (Cavanagh & Gibson, 2007; Bargnesi *et al*., 2020). Since conservation and management actions are strongly dependent on a sound knowledge of local diversity, historical and contemporary records of especially rare and threatened shark species are of great importance (Tavares *et al*., 2019), as in the present case of small-tooth sand tiger shark.

The presence of *O. ferox* along the Aegean and Mediterranean coasts of Türkiye was first mentioned by Geldiay (1969), but this research was not associated either by a stored specimen, or photographic and morphological evidence, hindering its confirmation from the region. Two additional studies also presented information on its occurrence from Türkiye (Fischer *et al*., 1987; Mater & Meriç, 1996), again without any evidence that may help with the verification of the relevant records. The occurrence of *O. ferox* in Türkiye was thus a matter of dispute for a long time until the capture of two individuals from İzmir and Fethiye Bay was presented by Fergusson *et al*. (2008). Since no further observations were available from the region in the following decade, the species was considered to be possibly extinct from Turkish coasts in the IUCN regional red list (Pollard *et al*., 2016). In a recent study, Kabasakal & Bayrı (2019) reported a specimen of *O. ferox* (TL 400 cm) caught by a bottom trawler off Antalya Bay, not only substantiating its presence in Türkiye but also proving that the species is not extinct yet.

Following the comprehensive list of Fergusson *et al*. (2008) comprising 14 captured *O. ferox* individuals from the Mediterranean Sea between 1964 and 2008, a total of 10 further evidence-based records were published at disjunct localities including Rhodes Island (Corsini-Foka, 2009), Andros Island (Damalas & Megalofonou, 2012), Malta (Vella *et al*., 2017), Cyprus (Akbora *et al*., 2019; Giovos *et al*., 2021), Tunisia (Ben Amor *et al*., 2020), Türkiye (Kabasakal & Bayrı, 2019; Kabasakal & Bilecenoğlu, 2020), eastern Aegean Sea (Moutopoulos *et al*., 2022), and Albania (Soldo *et al*., 2022), corresponding to a total of 24 records given during the last 60 years. This geographical pattern clearly reveals the widespread but patchy distribution of the species throughout the Mediterranean Sea with remarkably low density. Due to the sporadic and irregular nature of records of small-tooth sand tiger sharks from Turkish waters, we therefore consider its status of occurrence as rare, which is supported by the conclusions outlined in the most recent studies from adjacent waters, emphasizing the rarity of *O. ferox* (Damalas & Megalofonou, 2012; Akbora *et al.*, 2019; Serena *et al*., 2020; Giovos *et al.*, 2022; Moutopoulos *et al*., 2022).

Collated information from the field surveys and literature search also provided some basic information on the life history of *O. ferox* occurring in the eastern Mediterranean Sea (Table 1). The size of the small-tooth sand tiger sharks mentioned in the present study varies between approximately 120 and 400 cm, and the depth of captures ranged between 30 and 120 m. Considering the types of known fishing gears (n=8), it is seen that published or examined specimens of *O. ferox* have been captured mainly in different types of demersal fishery (Table 1). Therefore, we can suggest that a wide size range of *O. ferox* is threatened by the fishing pressure of demersal fisheries along the Turkish Mediterranean coast operated along a wide depth range of the continental shelf. Regarding the size at birth (>105 cm) of small-tooth sand tiger sharks (Ebert & Stehmann, 2013), the size of one specimen (Sp. no 4, approximately 120 cm; Table 1) coincides with the juvenile phase of the life span of *O. ferox*; however, for the moment, it is not possible to suggest whether the parturition of small-tooth sand tiger shark occurs in Turkish Aegean or Mediterranean waters, solely based on the previous occurrence of a single juvenile, and further investigation and evidence-based data is required to clarify this question.

Conclusion

Contrary to previous arguments that *O. ferox* is possibly extinct in Turkish seas (Pollard *et al*. 2019), the presence of the species has been verified by several contemporary records, of which the most recent incidental capture of this species in the mentioned region was in 2022 (Specimen no 8 in Table 1). Due to patchy occurrence and scarcity of records, the status of occurrence of *O. ferox* in Turkish seas was evaluated as "rare", which is a resident species along the Turkish Mediterranean coasts. The increasing use of smartphones and social media posts showing the large elasmobranches opened a window of opportunities to allow shark researchers to warn about the captures of uncommon sharks in remote localities. In recent years, the collaboration with citizen scientists and screening of internet media yielded several new records of sharks from Turkish waters, accompanied with a broadened understanding of the distribution ranges of rare large sharks (Kabasakal & Bilecenoğlu, 2020). Thus, in addition to traditional survey methods, this new approach, use of citizen scientists and local ecological knowledge, may allow us achieving unreported record(s), which eventually may increase our knowledge on the bioecology of the species. Since *O. ferox* is already included in Annex II of the SPA/BD Protocol covered by Recommendations GFCM/36/2012/3 and GFCM/42/2018/2, it should be rapidly included to the list of species banned for fishing through the official fishery legislation published by Ministry of Agriculture and Forestry, owing to the current threat of targeted captures and landings in commercial fisheries.

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RESEARCH ARTICLE

A new population record and habitat assessment of the endemic fish species *Pseudophoxinus battalgilae* (Teleostei: Leuciscidae) from Central Anatolia

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Abstract

Objective: *Pseudophoxinus battalgilae* is one of two species with the widest distribution of the genus in the Central and Southern Anatolia (Manavgat River) regions, the other being *Pseudophoxinus firati*. The species description was made based on samples from the Beyşehir Lake basin. Records are found from Suğla Lake (Seydişehir); the Akgöl (Ereğli) drainage canals; and the Zengen (Ereğli) and Ilgın (springs and small streams flowing into Çavuşçu Lake); the Kızılca, Akkaya, and Gümüşler reservoirs (Niğde), and the Manavgat River basin. This study attempts to identify new recorded localities of *P. battalgilae* and determines their general distribution areas. In parallel with this, an attempt has been made to evaluate the current state of the species' habitats.

Materials and Methods: *Pseudophoxinus battalgilae* specimens were caught from Gödet Stream (Karaman) with an electrical shock device, with most being released back into the habitat. The metric and meristic characters of the samples were determined. A maximum likelihood phylogenetic tree was created with closely related species and a haplotype network analysis was applied.

Results: The study records the species to have also spread to the Gödet Stream in the Central Anatolia region, thus contributing to identifying the population number and distribution area of the species. The maximum genetic distance of the *P. battalgilae* populations was determined as 0.0061 between Gödet Stream and the Aşıklar (Ereğli) canal populations. In addition to the phylogenetic analysis, the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) results confirm the Gödet Stream population to belong to the *P. battalgilae* species.

Conclusion: As a result, the evaluations in the study show a total of eight populations of the species to have been found. However, many populations of the species are expected to disappear in a short time due to drought and to domestic and agricultural pollution.

Keywords: Central Anatolia, endemic, habitat loss, biodiversity

Introduction

The first study on the phylogeny of the genus *Pseudophoxinus* stated the Anatolian species to have diversified as a result of geological isolation in the early Pliocene period 15 million years ago and the Anatolian and Eastern Mediterranean lineages to have been separated (Hrbek *et al*., 2004). Other molecular study results have found the *Pseudophoxinus* species from the upper Euphrates River and the Levant from the Jordan River north-west to the Seyhan River to form a single phylogenetic group, with the second species group being distributed in all Anatolian inland waters west of Seyhan River (Küçük *et al*., 2012; Geiger *et al*., 2014; Saç *et al*., 2019). Perea *et al*. (2010) differentiated *Pseudophoxinus* species in Anatolia as Central Anatolian species with a very complex morphological structure and as Eastern Mediterranean species with a less complex morphological structure. The genus *Pseudophoxinus* has 22 species in total in Anatolian inland waters according to the latest systematic records, with the most common species being *P. firati* (Eastern Mediterranean) and *P. battalgilae* (Central Anatolia).

This study evaluates the current status of the recorded populations of *P. battalgilae* and adds the presence of a new population in Gödet Stream, which feeds the Karaman Reservoir.

Materials and Methods

Sampling and Morphological Analysis

Pseudophoxinus battalgilae specimens were caught from Gödet Stream with an electrical shock device, with most being released back into the habitat. Some were killed by over-anaesthetization, and then fixed and stored in formaldehyde. Measurements were made with a dial caliper and recorded to an accuracy of 0.1 mm. All measurements were made point to point, never by projections. Methods for counts and measurements follow Kottelat & Freyhof's (2007) methods. Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the posterior anal-fin ray to the end of the hypural complex at the mid-height of the caudal-fin base.

Lateral line scales were counted from the anteriormost scale (the first one to touch the shoulder girdle) to the last scale at the end of the hypural complex. Scales along the lateral line were counted from the first one behind the pectoral girdle to the last one on the caudal-fin base. Gill rakers were counted on the outer margin of the anterior gill arch. The last two branched dorsal and anal-fin rays articulating on a single pterygiophore are counted as 1½. The studied materials were deposited at the Inland Fishes Collection, Isparta University of Applied Sciences, Eğirdir Fisheries Faculty (IFC-ESUF).

Care for the experimental animals was consistent with the Republic of Türkiye's animal welfare laws and guidelines, alongside the policies approved by Isparta University of Applied Sciences Local Ethics Committee for Animal Experiments (Date: 12.03.2020, No: 001).

DNA isolation, PCR and Sequence Analysis

Genomic DNA extraction was made from the fin tissue of the samples which were kept in 70% ethanol. The DNA extraction was performed using the Hibrigen DNA Purification kit in accordance with manufacturer's instructions. Polymerase chain reaction (PCR) was performed on the Biorad Thermal Cycler using the mitochondrial cytochrome c oxidase subunit I (COI) gene universal barcoding primers Fish-F1 (5'- TCA ACC AAC CAC AAA GAC ATT GG CAC -3') and Fish-R1 (5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3'; Ward *et al*., 2005). PCR was performed using the New England Biolabs Taq DNA polymerase in a 50 µl reaction volume. The reaction mixture used 100 ng DNA, 5 µl 10X PZR buffer, 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 0.25 µM each primer, and 0.25 U Taq DNA polymerase. PCR product purification and sequence analysis were performed in a commercial laboratory (Macrogen Europe Inc.). The raw data from sequence analysis were edited by visually checking the peaks with the software Bioedit 7.2.5 (Hall, 1999). Sequences were compared with the databases of the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The molecular identification analysis used 20 newly produced cytochrome c oxidase I (COI) barcodes and 6 sequences (KJ554376, KJ554454, KJ554116, KJ554089, KJ554385, KJ554428) from a previously published study (Geiger *et al*., 2014). *Alburnoides smyrnae* (GenBank number: MZ539436) was chosen as the outgroup taxa for rooting the phylogenetic tree. Haplotype network inference was constructed using a median-joining (MJ) algorithm (Bandelt *et al*., 1999) and Farris's maximum-parsimony (MP and implemented in the software Network (version 10.0; www.fluxus-engineering.com). The software MEGA X was used for the nucleotide substitution model, the maximum likelihood (ML) analysis, and the pairwise genetic distance. The nucleotide substitution model K2+ G was selected for the

ML analysis, and *p* distance was selected for determining genetic distance.

Comparative materials

IFC-ESUF 03-0962: 17 specimens, 40.0-149.8 mm SL (Konya, Türkiye: Çarşamba canal, Seydişehir)

IFC-ESUF 03-0965: 4 specimens, 43.94-49.30 mm SL (Karaman, Türkiye: Bozkır Stream)

IFC-ESUF 03-0960: 12 specimens, 71.73-123.20 mm SL (Antalya, Türkiye: Manavgat Reservoir)

IFC-ESUF 03-0961: 9 specimens, 70.38-113.45 mm SL (Antalya, Türkiye: Manavgat Reservoir)

IFC-ESUF 03-0963: 12 specimens, 49.24-88.72 mm SL (Antalya, Türkiye: Manavgat River and Manavgat Reservoir)

IFC-ESUF 03-1010: 4 specimens, 66.3-73.4 mm SL (Konya, Türkiye, Tatlıkuyu Village Ereğli)

IFC-ESUF 03-1018: 9 specimens, 29.05-51.8 mm SL (Konya, Türkiye: Aşıklar Village-Ereğli)

IFC-ESUF 03-0967: 3 specimens, 37.32-39.90 mm SL (Konya, Türkiye: Zengen Village-Ereğli)

IFC-ESUF 03-0966: 11 specimens, 33.05-82.81 mm SL (Niğde, Türkiye: Akkaya Reservoir)

IFC-ESUF 03-1025: 17 specimens, 43.30-59.28 mm SL (Niğde, Türkiye: Gümüşler Reservoir)

IFC-ESUF 03-0964: 10 specimens, 28.1-57.8 mm SL (Konya, Türkiye: Springs on the shores of Çavuscu Lake)

IFC-ESUF 03-1016: 3 specimens, 43.69-53.83 mm SL (Konya, Türkiye: Canal near Çavuşcu Lake)

IFC-ESUF 03-1027: 26 specimens, 41.43-61.16 mm SL (Konya, Türkiye: Bulasın Creek, near Ilgın)

IFC-ESUF 03-1037: 25 specimens, 74.44-91.53 mm SL (Karaman, Türkiye: Gödet Stream)

Results and Discussion

The species was first recorded in Beyşehir Lake as *Acanthorutilus maeandricus* by Kosswig (1952; *nomen nudum*). Later, a second sampling was made from the same lake in 1964 (Bogutskaya, 1997). Its scientific identification was made by Bogutskaya (1997) based on ZMH examples (Haplotype ZMH 8861, Beyşehir Lake, August-September, Collector C. Kosswig, 1964; Paratypes: ZMH 2701 (3), ZMH 6634 (3), ZMH 1080 (2) same date and same collector as holotype).

The scientific identification distinguished the species from other *Pseudophoxinus* species, with its complete lateral line, greater number of branched rays in the dorsal fin (D: III-IV, 8-9) and anal fin (A: III-IV, 8-9), significantly flattened lateral body structure, and the presence of an uninterrupted keel between the pelvic-fin and the anal fin particularly notable.

Apart from the records of Kosswig (1952; 1964) recording its type locality as Lake Beyşehir, no other scientific records of *P. battalgilae* are found in any other scientific studies conducted to date. The current study encountered no specimens in the samplings conducted in Beyşehir Lake and its surrounding spring waters-streams (Eflatunpınarı, Deliktaş spring, karst springs mixing into the lake on the western shores of the Lake Beyşehir, Sarıöz Canal, Üstünler, Soğuk and İli Stream) between 1997-2021.

Meanwhile, we encountered a few samples between 2000-2014 in the Taşağıl and Çarşamba canals flowing into the Suğla Reservoir in Beyşehir Lake basin and in some small springs around Seydişehir. However, the recently conducted field studies (2018-2021) found no *P. battalgilae* samples due to heavy pollution (sewage and agricultural fertilizer and pesticide inputs) in the above-mentioned habitats of the Taşağıl and Çarşamba canals, as well as due to seasonal drying in some springs near Seydişehir and damage caused by construction equipment.

Recently, Atalay (2005), Küçük (2007), Freyhof & Özuluğ (2010), Küçük *et al*. (2016), Bayçelebi *et al*. (2020) and Küçük *et al*. (2020) have provided information regarding the morphology and zoogeography of the species. The molecular phylogenetic analysis of the species is available in the studies conducted by Hrbek *et al*. (2004), Perea *et al*. (2010), and Geiger *et al*. (2014). The existence of eight populations has been reported so far (Atalay, 2005; Freyhof & Özuluğ, 2010; Küçük *et al*., 2016; Küçük *et al*., 2020). This study has evaluated the current status of the populations given to date and added the presence of a new population in Gödet Stream, which feeds Karaman Reservoir (Fig. 1). The coordinates for the locations of the *P. battalgilae* are given in Table 1.

The current field studies have determined *P. battalgilae* to be distributed over 5 basins: the spring waters, rivers, and lakes in Central Anatolia (3); Manavgat River in the Mediterranean basin (1), and the streams and spring waters around Ilgın (1) adjacent to Sakarya River. Evaluations regarding these inland waters and *P. battalgilae* populations are given below:

1-Beyşehir Lake basin: In the current population of this region, the species is encountered to a limited extent in the drainage canals where the spring waters flow into the Suğla Reservoir (Kuğu Park surroundings, Seydişehir) are collected. In the early 2000s, the outflow canal of Beyşehir Lake, known as Çarşamba canal, used to host a population

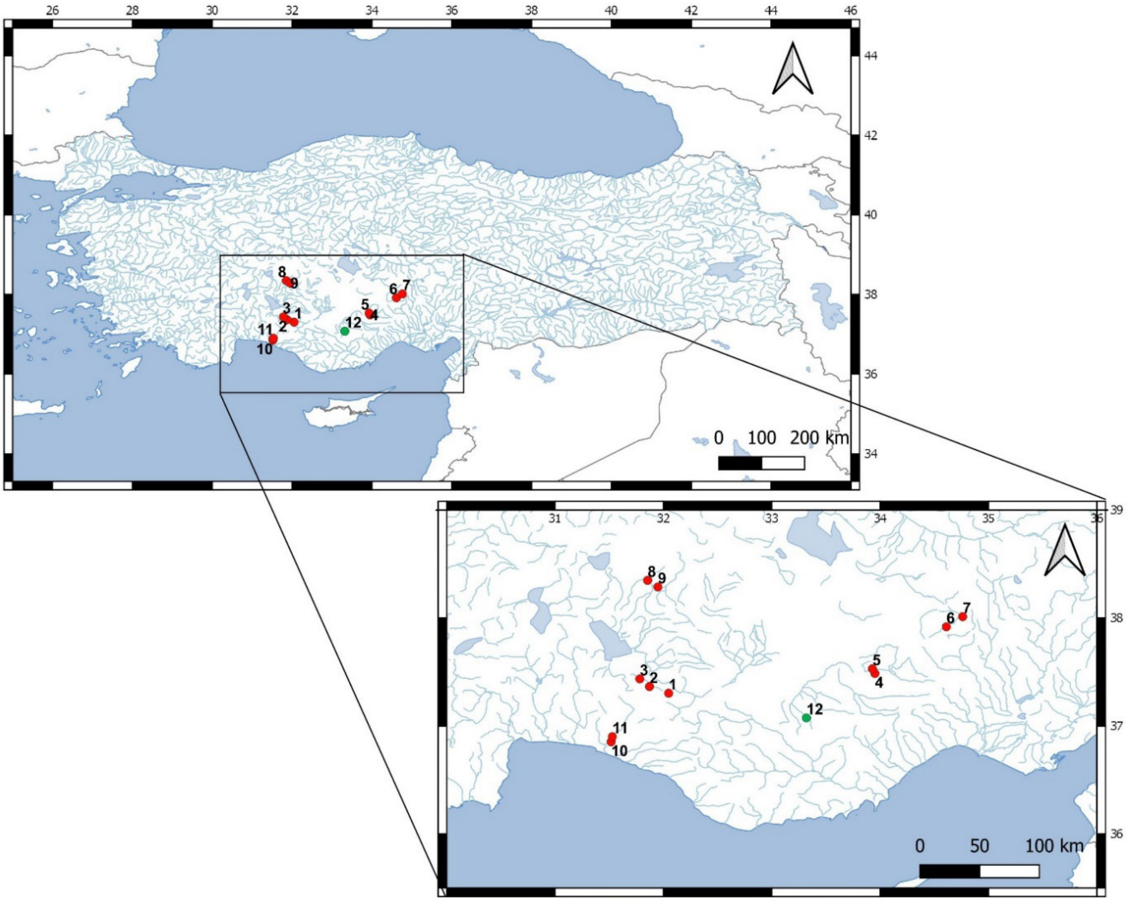

Figure 1. Distribution areas of *Pseudophoxinus battalgilae* (● = new record; ● = old records).

of this species in a relatively healthy state. However, due to intense agricultural, domestic and industrial pollution over the last 20 years, this species is no longer found in this habitat. Similarly, in Bozkır Stream, specimens were last encountered in 2005, but recent studies have failed to find any specimens due to intense domestic pollution and water drying in recent years. However, it has been reported that in 2023, a very few examples were found during fishing with very porous nets on the Yeşildağ shores of Beyşehir Lake (Dr. Vedat Yeğen personal opinion). For this reason, it is important to monitor the population of the species in Lake Beyşehir.

2-Manavgat River basin: The species forms dense populations along the coastal areas of the Manavgat and Oymapınar reservoirs (especially in the spring), as well as in the cold river waters of the river section where the Manavgat River flows into the dam lake. However, irregular flow in the dam lakes, water level instability in the lakes, and the effects of exotic species (e.g., *Carassius gibelio, Squalius anatolicus*, and *Alburnus escherichii*) are external factors pressuring their populations.

3-Akgöl (Ereğli) basin: The region is under severe drought pressure. The canals flowing from the above localities to Akgöl (near Tatlıkuyu and Aşıklar villages) have dried up over the past 8-10 years. The species has not been recently detected in the Akkaya and Gümüşler reservoirs around Niğde. In this basin, *P. battalgilae* individuals are encountered to a limited extent only in the agricultural irrigation canals near Taşağıl Village (Ereğli), which are supplied by the İvriz Reservoir. All recorded populations in the region are undergoing extinction.

4-Çavuşcu Lake Basin: The study conducted sampling from several small spring waters flowing into Çavuşcu Lake and from Bulasın Creek in the southeastern part of the lake. *Pseudophoxinus* has been known since the early 2000s to be widespread in this region. This study's samplings encountered very few *P. battalgilae* specimens in the habitats. Çavuşcu Lake (Ilgın) is reported as being isolated from Sakarya River; therefore, the fish fauna are similar in both regions (Turan & Kaya, 2019). Unlike Central Anatolia and the Manavgat River basin, the distribution of *P. battalgilae* in this lake basin is considered interesting from a zoogeographical point of view.

Table 1. Locality and coordinates for the distribution areas of *P. battalgilae.*

No	Locality	Coordinates (DMS)
1	Suğla reservoir-Seydişehir	37°18'11.55"N; 32°02'50.29"E
2	Kuğu Park-Seydişehir	37°21'53.65"N; 31°52'15.94"E
3	Gökçehüyük Pond-Seydişehir	37°26'08.63"N; 31°46'53.71"E
4	Taşağıl village- Ereğli Marshes	37°29'10.13"N; 33°57'03.91"E
5	Aşıklar village- Ereğli Marshes	37°31'44.77"N; 33°55'38.34"E
6	Akkaya reservoir- Niğde	37°55'03.83"N; 34°36'37.53"E
7	Gümüşler reservoir- Niğde	38°00'42.40"N; 34°45'37.49"E
8	Çavuşcu Lake Spring-Ilgın	38°20'50.28"N; 31°51'11.96"E
9	Bulhasan Creek-Ilgın	38°17'11.32"N; 31°56'54.56"E
10	Manavgat reservoir-Manavgat	36°51'11.53"N; 31°31'02.02"E
11	Manavgat River-Manavgat	36°54'04.04"N; 31°31'41.03"E
12	Gödet Stream- Karaman	37°04'29.91"N; 33°19'07.46"E

5-Gödet Stream (Karaman) drainage: The Gödet Stream originates as two separate streams from springs near Güldere and Paşabağı villages and goes on to forms the Gödet Reservoir near Karaman after the confluence of both streams (Fig. 2). Its approximate length is 20 km. The stream is used primarily for drinking water and agricultural irrigation. *Pseudophoxinus* specimens (Fig. 3) were caught from a location near where the stream empties into the reservoir.

Figure 3. *Pseudophoxinus battalgilae* (IFC-ESUF 03-1037: 6.73 mm SL [Karaman, Türkiye: Gödet Stream]).

The Gödet Stream specimens' lateral line have a total of between 52-64 scales, with 12-15 scale rows found between the lateral line and the origin of the dorsal fin, 4-5 (4-5.5) scale rows are found between the origin of the lateral line anal-fin, and 11-13 gill rakers are found on outer side of the first gill arch. The dorsal fin has 7 branched rays, while the anal-fin mostly has 8-9 branched rays (Tables 2, 3). The taxonomic characteristics of the Gödet Stream samples are similar to other *P. battalgilae* populations in Anatolia.

According to the results from the phylogenetic analyses, the *Pseudophoxinus* specimens from Gödet Stream clearly inhabit the same monophyletic group as the other *P. battalgilae* populations found in Türkiye (Aşıklar canal in Ereğli, Akkaya Reservoir in Niğde, Seydişehir's surroundings, Manavgat River, Tuz drainage, and Ilgın surroundings; see Figs. 4 and 5). Furthermore, *P. iconii* is a

Figure 2. A new record habitat of *Pseudophoxinus battalgilae* (Gödet Stream-Karaman).

Table 2. Frequency distribution of certain meristic characteristics of the *P. battalgilae* from the Central Anatolia and the Manavgat River Basin

	Number of rows of scales between							Number of rows of scales between					
	N	**11**	**12**	**13**	**14**	**15**	**X̄**	**4**	**$4_{1/2}$**	**5**	**$5_{1/2}$**	**6**	**X̄**
Gödet Stream	26	-	4	14	7	1	**13.2**	12	5	4	5	-	**4.5**
Ereğli basin	13	-	4	7	2	-	**12.8**	11	-	2	-	-	**4.2**
Akkaya Reservoir	5	-	2	2	1	-	**12.8**	-	-	5	-	-	**5.0**
Beyşehir basin	10	2	7	-	-	1	**12.1**	2	-	7	-	1	**4.9**
Manavgat basin	12	3	3	5	1	-	**12.0**	5	-	6	-	1	**4.7**
Ilgın basin	5	4	1	-	-	-	**11.2**	2	-	3	-	-	**4.6**
	Branched dorsal-fin rays				**Branched anal-fin rays**								
	N	**7**	**8**	**X̄**	**8**	**9**	**X̄**						
Gödet Stream	26	26	-	7.0	18	8	8.3						
Ereğli basin	13	13	-	7.0	13	-	8.0						
Akkaya Reservoir	5	5	-	7.0	5	-	8.0						
Beyşehir basin	8	7	1	7.1	8	-	8.0						
Manavgat basin	12	12	-	7.0	7	5	8.4						
Ilgın basin	5	5	-	7.0	1	4	8.8						

Table 3. Frequency distributions for the lateral line scales and gill rakers of the *P. battalgilae* from Central Anatolia and the Manavgat River basin.

	Lateral Line														
	N	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**X̄**
Gödet Stream	**25**	-	2	1	-	5	1	2	2	2	5	3	2	-	**59.7**
Ereğli basin	**13**	-	1	2	-	4	2	2	2	-	-	-	-	-	**57.4**
Akkaya Reservoir	**5**	-	-	-	-	1	1	-	-	1	1	-	1	-	**60.4**
Beyşehir basin	**10**	3	1	1	1	1	1	-	2	-	-	-	-	-	**56.0**
Manavgat basin	**13**	-	-	1	1	-	3	1	1	1	-	4	-	1	**60.2**
Ilgın basin	**5**	3	1	1	-	-	-	-	-	-	-	-	-	-	**53.6**
	Gill Rakers														
	N	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**X̄**						
Gödet Stream	**11**	4	6	1	-	-	-	-	11.7						
Ereğli basin	**13**	4	7	2	-	-	-	-	11.8						
Akkaya Reservoir	**5**	1	3	1	-	-	-	-	12.0						
Beyşehir basin	**4**	-	1	1	-	-	1	1	14.5						
Manavgat basin	**9**	-	1	7	-	1	-	-	13.1						
Ilgın basin	**5**	1	-	3	1	-	-	-	12.8						

sister species to *P. battalgilae*, with the other *Pseudophoxinus* species (e.g., *P. hitittorum*, *P. firati*) inhabiting nearby basins being in different monophyletic groups (Fig. 4).

The maximum genetic distance among the *P. battalgilae* populations was identified as 0.0061 between the specimens from Gödet Stream and Aşıklar canal specimens in the

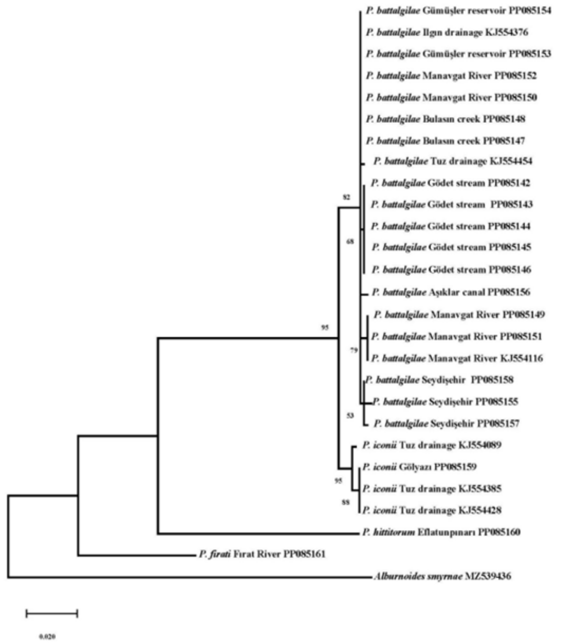

Figure 4. Maximum likelihood phylogenetic tree of *Pseudophoxinus* populations..

Figure 5. *Pseudophoxinus battalgilae* (IFC-ESUF 03-0961: 96.0 mm SL [Antalya, Türkiye: Manavgat Reservoir]).

Figure 6. *Pseudophoxinus iconii* (IFC-ESUF 03-1026: 63.8 mm SL [Konya, Türkiye: Gölyazı village]).

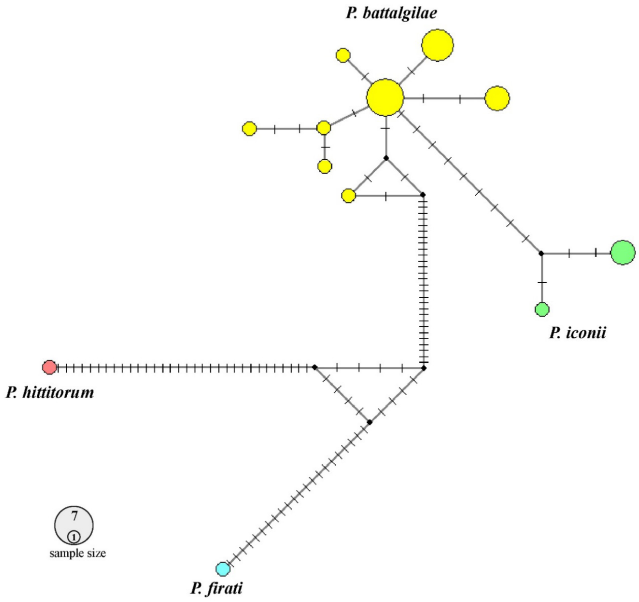

Figure 7. Haplotype network analysis for the *Pseudophoxinus* species.

Table 4. Genetic distance between *Pseudophoxinus* species/populations based on COI sequence data

		P. battalgilae								*P. iconii*	*P. firati*
		GS	**BC**	**GR**	**ID**	**TD**	**MR**	**S**	**AC**		
P. battalgilae	**GS**										
	BC	0.0015									
	GR	0.0015	0.0000								
	ID	0.0015	0.0000	0.0000							
	TD	0.0031	0.0015	0.0015	0.0015						
	MR	0.0034	0.0018	0.0018	0.0018	0.0034					
	S	0.0046	0.0031	0.0031	0.0031	0.0046	0.0043				
	AC	0.0046	0.0031	0.0031	0.0031	0.0046	0.0049	0.0061			
P. iconii		0.0180	0.0165	0.0165	0.0165	0.0181	0.0183	0.0196	0.0196		
P. firati		0.0874	0.0859	0.0859	0.0859	0.0864	0.0877	0.0890	0.0844	0.0901	
P. hittitorum		0.0997	0.0982	0.0982	0.0982	0.0988	0.0982	0.0982	0.0982	0.0985	0.0890

GS = Gödet Stream; BC = Bulasın Creek; GR = Gümüşler Reservoir; ID = Ilgın Drainage; TD = Tuz Lake Drainage; MR = Manavgat River; S = Seydişehir's surroundings; AC = Aşıklar Canal

Ereğli Marshes basin in Konya, which are geographically very close (Table 4). The minimum genetic distance between *P. battalgilae* and the most related species (*P. iconii*; see Fig. 6) was determined as 0.0165 (Table 4). Furthermore, a maximum of five nucleotide differences are found among the *P. battalgilae* populations, with the populations showing a star-like structure in the haplotype network analysis (Fig. 7). As a result, the genetic analysis has confirmed the *Pseudophoxinus* specimens sampled from Gödet Stream to be *P. battalgilae.* The NCBI BLAST analysis has also confirmed the Gödet Stream population to belong to the *P. battalgilae* species (> 99% identity).

Conclusion

As a result of the recent taxonomic studies on and revisions to the *Pseudophoxinus* genus, a total of 22 valid species (*P. handlirschi* EX) has been recorded in Anatolia (Güçlü & Küçük, 2017). While the molecular phylogeny of the genus has been partially determined (Central Anatolia, Southeastern Anatolia and Eastern Mediterranean phylogenies), its morphology exhibits a rather complex structure (Hrbek *et al*., 2004; Perea *et al*., 2010). A v ariety of species have been noted to have diversified regarding a morphology-based classification, including those with a complete or nearly complete lateral line, relatively large body size, and small scales (e.g., *P. anatolicus*, *P. handlirschi*, *P. fahrettini*), as well as species with an underdeveloped lateral organ, relatively small body size, and large scales (e.g., *P. alii*, *P. maeandri*, *P. libani*). *P. battalgilae*, which is distributed in Anatolia and has the largest population after *P. firati*, has a complete lateral line and very small scales. The most distinctive feature of the species is the presence of a partially developed keel between the ventral and anal fins and of seven or more branched rays in the anal fin. Regarding the sequence analysis based on the COI barcoding region, no significant genetic difference is understood to be present among the *P. battalgilae* populations.

As a result based on what can be understood from the evaluations in this study, a total of eight populations are present, five in the Central Anatolian basin (two in the Gümüşler and Akkaya reservoirs in Niğde, one in the soil canals that supply the Akgöl and Ereğli marshes, one in the Gödet Stream in Karaman, and one in the small springs around Seydişehir) and two in the Manavgat River basin (one in the Oymapınar and Manavgat reservoirs and one in the area surrounding Ilgın, namely the two rivers flowing into Çavuşçu Lake). These populations, especially those around Akgöl, Niğde, and Ilgın, are expected to disappear in a short time due to drought resulting mainly from groundwater withdrawal for agricultural irrigation and the decreased rainfall in recent years, as well as due to domestic and agricultural pollution.

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RESEARCH ARTICLE

Investigation of Fish Species Diversity in the Shuhada River in Badakhshan Province, Afghanistan

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Abstract

Objective: The present research was conducted on the existence and species diversity of fish in the Shuhada River, one of the Kokcha River tributaries situated in Badakhshan Province, Afghanistan. There has not been a previous study on fish species diversity; this is the first attempt to fill out this gap and identify the fish species of the river.

Materials and Methods: Fish specimens were collected from three selected sites in the Shuhada River. The study was performed twice in each season of the year 2022, by using a variety of fishing nets, like gill nets with a length of 5 m and a height of 2 m, although with meshes ranging from 3 to 3 cm knot to knot and hooks. Two samples were taken from each site in each season.

Results: Generally, 463 fish specimens were collected from the three selected zones. The Futtah was one of the selected zones that had the highest number of fish species (38.8%), followed by Yasich (34.04%), and the least number of fish species (28.07%) were collected from the Maidan zone. It found that *Salmo trutta* is the most abundant species at 52.9%, followed by *Schizothorax curvifrons* at 34.5% and *Paracobitis longicauda* at 12.5% was the least abundant species. During the cold seasons of autumn and winter, fish migrate down in the Kokcha River, and in spring and summer migrate to the upper zone in cold water.

Conclusion: During the current study, three fish species, *Salmo trutta, Schizothorax curvifrons,* and *Paracobitis longicauda*, were documented in the study area. Fish hunting and flooding are the main harmful forces causing the reduction of fish diversity in the Shuhada River.

Keywords: Shuhada River, fish species, *Salmo trutta, Schizothorax curvifrons, Paracobitis longitude*

Introduction

Fish constitute the most significant number of species and the greatest abundant group of vertebrates globally and live in vast aquatic ecosystems with varied ecological types found in various environments (Kelzang *et al*., 2021; Shendge, 2007; Yang *et al*., 2021). Fish show vast diversity in habitats, morphology, and biology (Mirza *et al*., 2018). Fish are a significant part of the biological diversity and the most essential bioindicator of the ecosystem. These aquatic living organisms have had a significant effect on human civilization (Majidi *et al*., 2023). Fish have impact on food web structure, nutrient cycle, energy dynamics, and different ecological functions in aquatic ecosystems (Wang *et al*., 2021). Knowledge of fish diversity has scientific, ecological, and economic significance, as it provides elementary guides on the diversity of different aquatic ecosystems (Mirza *et al*., 2011; Taiwo, 2023).

Afghanistan is rich in natural resources, and its stunning landscapes of deserts, mountains, open woods, forests, and rivers are home to a vast range of biological diversity. Unfortunately, recent decades of conflict, climate change, and population growth have destroyed ecosystems (UNEP, 2008). Afghanistan is mountainous and landlocked; the average altitude is 1300 m. The weather differs between the lowlands and highlands. Rivers are a vital landscape of the globe and are considered the principal factor in urban, agricultural, rural, and industrial development, as well as vital from the viewpoint of biological diversity (Majidi *et al*., 2023). 101 fish species can be found in Afghanistan, and an additional 38 species are assumed to exist in the country. Many fish species are described as being endemic in Afghanistan, especially in the genera *Schizothorax* and *Nemacheilus*. However, the classification of these genera is consequently ambiguous, so they may be mistaken for more extensively spread species (Coad & Bogutskaya, 2012; UNEP, 2017). Fish diversity in Afghanistan is poorly studied by Canadian ichthyologists relative to other fauna. Because of the civil war for a few decades, the fish diversity of Afghanistan has not been studied, and there has been no attempt to record fish species in this country (Coad, 2009; Majidi *et al*., 2023).

The Badakhshan territory is known as a biological diversity hotspot in Afghanistan because of its vast range of biodiversity and unique fish resources. Because of its geographical situation and topographical conditions, there are few studies on the diversity and distribution patterns of fish species in the study area. According to the National Environmental Protection Agency (NEPA)," the local office says that excessive and improper fishing has endangered several fish species in this aquatic ecosystem. Based on NEPA's evidence, so far nobody has been prosecuted over the crime of fish hunting which has further encouraged and contributed to the poaching of animals" (Majidi *et al*., 2023).

The Shuhada River originates from the high mountains located in the eastern part of the Shahada district, where the waters of Khoshdare, Yaghurde, Gharspan, and Korkhodare have all found the Shahada River, which flows into the Kokche River in the Bahark district of Badakhshan province, Afghanistan. The Shuhada River supports some aquatic organisms, and fish are one of the most important components of this aquatic ecosystem. Moreover, there are severe threats to the fish diversity in the Shuhada River from illegal fishing and water pollution. It is the first effort to study the Shuhada River ichthyofauna in the Badakhshan province of Afghanistan. This survey aims to identify and document fish species that inhabit the Shuhada River in Badakhshan Province, Afghanistan.

Material and Methods

Study area

This survey was performed in the Shuhada River, situated in the Shuhada District of the Badakhshan Province, Afghanistan, and lies between latitudes 36°59'59" and 36°4'16" and longitudes 70°51'15" and 71°16'7" (Fig. 1). The river originates from the high mountains located in the eastern part of the Shahada district, where the waters of Khoshdare, Yaghurde, Gharspan, and Korkhodare have all found the Shahada River, which flows into the Kokche River in the Bahark district. This river originates from springs and natural caves in the area. Its fish is famous throughout the country. Those who come for tourism in this area will benefit from this fish meat. We selected three sites (Yasich, Maidan, and Futtah) for the assistance of fish species in the Shuhada River. The Shahada River is 35 km long and 1 to 2 m deep. Its temperature fluctuates from -13 to 25°C. There are numerous fish species in this river. Its fish have short migration, in the cold seasons of autumn and winter, fish migrate down the Kokcha River. In the spring and summer, the fish migrate to the upper zone in cold water. Fish hunting and flooding are the main harmful forces causing the reduction of fish diversity in the study area. Thus, we saw more than fifty people hunting in the river during the survey (Khattak *et al*., 2015; Majidi *et al*., 2023; Muhammad *et al*., 2017).

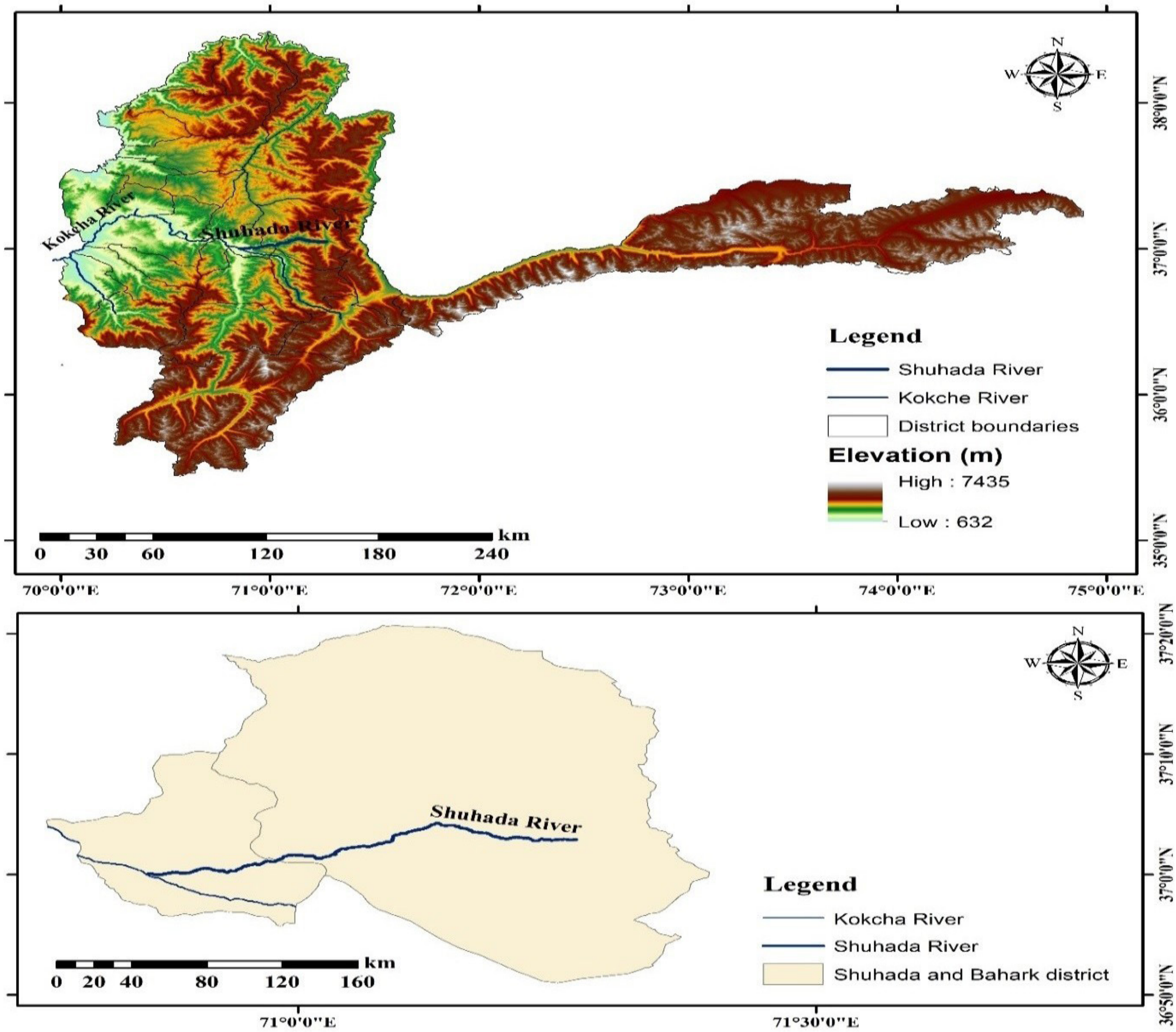

Figure 1. The location of the Shuhada River in Badakhshan Province.

Methods and analyses

The species diversity of fish in the Shuhada River was collected from three selected sites (Yasich, Maidan, and Futtah) in 2022. We obtained permission from the Wildlife Protection Department of Badakhshan province to capture fish in the study area. The study was conducted twice in each season of the year by using a variety of fishing nets, such as gill nets with a length (5 m) and height (2 m), although with meshes ranging from 3 to 3 cm knot to knot and hooks. Two samples were taken from each site in each season.

The collected specimens were stored in 10% formalin for further study in the biology laboratory of Badakhshan University (Pazira *et al*., 2016). The specimens were identified by species, genus, family, and order using taxonomic keys (FishBase, 2023; Coad, 2015). The calculation of data for Shannon (h), Dominance (D), Simpson (S), Margalef (R), Evenness (E), Brillouin index (B), Menhinick index (M) and Fisher's alpha of fish species was documented using PAST 4.03 and Ms Excel 2016 (Altaf *et al*., 2015; Dube & Kamusoko, 2013; Mirza *et al.*, 2011).

Percentage frequency of occurrence was calculated using the following formula;

$$\text{Percentage frequency} = \frac{\text{number of individual species}}{\text{total number of species}} \times 100$$

Results

The current survey is of the Shuhada River, one of the tributaries of the Kokcha River, situated in the Shuhada District of Badakhshan Province, Afghanistan. A total of 463 fish specimens were collected from the three sampling zones. The recorded three fish species were belonging to three families and three orders. The list of collected fish species is presented in Table 1 and Figures 2 – 4. These

Table 1. Fish species in the Shuhada River of Badakhshan Province.

Station No	Order	Family	Species	Local name
1	Salmoniformes	Salmonidae	*Salmo trutta*	Alahbuqa
2	Cypriniformes	Cyprinidae	*Schizothorax curvifrons*	Shirmahi
3	Cypriniformes	Nemacheilidae	*Paracobitis longicauda*	Mohidehantang

Figure 2. *Salmo trutta* (Order Salmoniformes, Family Salmonidae).

Figure 3. *Schizothorax curvifrons* (Order Actinopterygii, Family Cyprinidae).

Figure 4. *Paracobitis longicauda* (Order Cypriniformes, Family Nemacheilidae).

species were *Salmo trout* 52.9%, *Schizothorax curvifrons* 33.5%, and *Paracobitis longicauda* 12.5% (Table 2). We found that *S. trutta* was a highly abundant species, and *P. longicauda* was the least abundant species in the study region (Khattak *et al*., 2015; Majidi *et al*., 2023; Mirza *et al*., 2012).

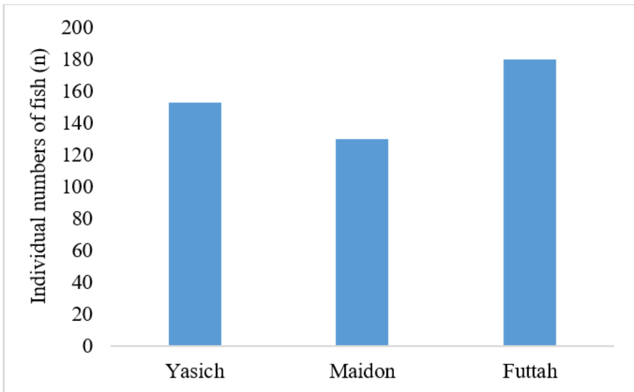

Figure 5. The abundance values (n) of fish in the sampling sites.

In the sampling areas, Futtah had the highest number of collected fish (38.8%), followed by Yasich (33.04%), and the least amount of fish (28.07%) was collected from the Maidan site (Fig. 5). All fish collected from the study site are of Asian origin (Bari *et al*., 2014), and it is known that natural flow systems are the main factors controlling riverine fish groups in aquatic ecosystems (Mirza *et al*., 2011).

Table 2. Fish numbers documented in three sites of the Shuhada River

Fish name	Yasich	Maidon	Futtah	Total collected specimens
Salmo trutta	98	79	68	245
Schizothorax curvifrons	43	36	81	160
Paracobitis longicauda	12	15	31	58
Total number of fish observed	**153**	**130**	**180**	**463**

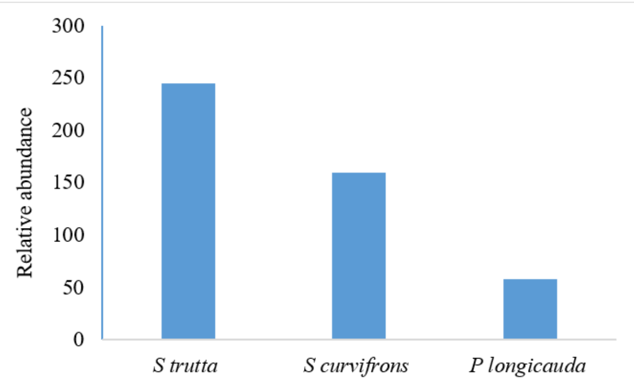

Figure 6. The relative abundance of each fish species in the study area.

In the present survey, we found that *S. trutta* is the most abundant species with 52.9% (n = 245) in all three sites, followed by *S. curvifrons* with 33.5% (n = 160), and *P. longicauda*'s abundance significantly differs in the three sampling sites with 12.5% (n = 58), which is the least abundant in the study region. In the following graph, *S. trutta* is 245 ± 4.5, *S. curvifrons* is 160 ± 3.7, and *P. longicauda* is 58 ±1.5 (Fig. 6) (Kelzang *et al*., 2021; Dube & Kamusoko, 2013).

As Altaf *et al.* (2015), in the fish species diversity indices (Table 3), the surveyed area exhibited the Shannon of the fish at Yasich 0.84, followed by Maidan 0.90, and Futtah 1.03. The dominance of the fish at the Yasich is 0.49, followed by Maidan, 0.45, and Futtah, 0.37. The Simpson index at the Yasich is 0.50, followed by Maidan, 0.54, and Futtah, 0.62. The Margalef index at Yasich is 0.39, followed by Maidan 0.41 and Futtah 0.38. Evenness index at 0.77, followed by Maidan at 0.82 and Futtah at 0.93. Brillouin index at Yasich: 0.81, followed by Maidan: 0.87, and Futtah: 1.00. Menhinick index at Yasich is 0.24, followed by Maidan 0.26 and Futtah 0.22. Fisher's alpha index at Yasich is 0.52, followed by Maidan 0.54 and Futtah 0.51. Biodiversity indices and statistically computed results indicate that the site of Futtah is marked by an abundance of fish species. According to local people, there is a lot of flooding during the spring season; it may be transferring fish, fingerlings, and eggs from the upper to the lower zone of this aquatic ecosystem (Altaf *et al*., 2015; Majidi *et al.,* 2023).

Table 3. Statistical analysis of the fish diversity in the Shuhada River

Diversity indices	Yasich	Maidan	Futtah
Numbers	3	3	3
Individuals	153	130	180
Shannon (H')	0.84	0.90	1.03
Dominance (D)	0.49	0.45	0.37
Simpson (S)	0.50	0.54	0.62
Margalef (R)	0.39	0.41	0.38
Evenness (E)	0.77	0.82	0.93
Brillouin index (B)	0.81	0.87	1.00
Menhinick index (M)	0.24	0.26	0.22
Fisher's alpha	0.52	0.54	0.51

Majidi *et al.* (2023) in a survey, on fish diversity in the Kokcha River used a range of fishing tackle, e.g., dragnets, hooks, and gill nets with the same length (5 m) and height (2 m) with meshes varying from 3 to 3 cm, knot to knot. Altaf *et al.* (2015) in a survey, on the diversity of fish in the Chenab River used gill nets with the same length (100 m to 20 m) and height (1.6 m), but with a mesh size of 1.5 inches.

During the current survey, we documented two main threats to Ichthyofauna in the Shuhada River: hunting and floods. According to the study area's locality, the fish population in this aquatic ecosystem is declining due to overfishing (Fig. 7). The Shuhada River has been heavily affected in recent decades by various types of human activities, such as agricultural fertilizers and dumping of household waste, which have severely affected its fauna.

Similar research has been done in some rivers of Afghanistan and other countries. The current outcome corresponds with a survey that reported four fish species from the Kokcha River of Badakhshan province, Afghanistan (Majidi *et al*., 2023). Another study reported 1190 fish belonging to Cypriniformes, Salmoniformes, and Cichliformes from the Kabul River of Afghanistan. However,

Figure 7. The hunted fish in the study region.

Afghanistan's aquatic habitats are less suitable for and geographically remote from many of the more widespread Asian Siluriformes, resulting in a comparatively limited diversity of catfishes. Cyprinids can live in cold water and tolerate low oxygen levels. As a result, they are frequently found to be more prevalent in freshwater ecosystems in most of Asia when combined with historical events (Kelzang *et al*., 2021). Furthermore, Coad (1981) mentioned that coldwater fish stocks in the upper zone of the Kabul River basin are dominated by various cyprinid snow trutta (Schizothoracini) and Cobitidae. Afghanistan's rivers and streams contain Oriental and Palaearctic species, northern and southern species, and high and low-altitude-adapted fish species. The fauna is dominated by Cyprinidae (56.9%), Cobitidae (24.5%), and, to a lesser extent, Siluriformes (11.8%). In another research, Coad & Bogutskaya (2012) reported Cyprinidae from the northwestern region of Afghanistan and the northeastern region of Iran. Other corresponding reports are Khattak *et al.*, 2015, Mirza *et al.*, 2011, Muhammad *et al.*, 2017, and Hossain *et al.*, 2013.

Afghanistan has been endowed with natural riches and scenic beauty. The beautiful scenery of mountains, deserts, woodlands, forests, and water sustains a rich diversity of flora and fauna in a variety of environmental conditions (Majidi, 2023; UNEP, 2008). Biological diversity exhibits itself in a wide range of behaviors, species numbers, and differences in species groupings in various habitats and also in the different ecosystems that can be found in various zones of the country (Adil, 2000; Majidi *et al*., 2022). Decades of conflict and unrest, poor education, lawlessness, the wood mafia, high unemployment, general poverty, drought and other natural catastrophes, population increase, and the migration of displaced or returning peoples have all had significant impacts on the environment and wildlife in Afghanistan (Saidajan, 2012).

The current research is the first time to document fish species in the Shuhada River of Badakhshan province. Shuhada District has enough water and an ideal environment for fish aquaculture. Aquatic species are a smaller proportion of the Badakshan Province diet since fish producers are unable to produce enough fish to meet client demand. Fish hunting is currently outlawed across Badakhshan province, and as a result, the region's wildlife status has improved. There have been no previous reports on the Shuhada River's fish diversity. Therefore, these findings are of great importance for future studies on fish species diversity in Afghanistan.

Conclusion

The current investigation focused on assessing the fish species diversity in the Shuhada River one of the Kokcha River tributaries located in the Badakhshan Province of Afghanistan. The present research was conducted in 2022, and three fish species belonging to three different orders and families were recorded. *Salmo trutta* was found to be the most abundant species, accounting for 52.9% of the total fish population across all three sampling sites followed by *Schizothorax curvifrons*, making up 33.5% of the population, while *Paracobitis longicauda* was the least abundant species, comprising only 12.5% of the fish population. The study also highlighted hunting and flooding as significant threats to the fish population in this aquatic habitat. Generally, this study provides valuable insights into the fish species composition and the potential challenges faced by the ichthyofauna in the Shuhada River.

Ethics Committee Approval: We obtained permission from the Wildlife Protection Department of Badakhshan province to capture fish in the study area.

Peer-review: Externally peer-reviewed.

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RESEARCH ARTICLE

New Locality Records of *Testudo graeca* (L., 1758) in the Eastern Black Sea Region of Türkiye

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Abstract

Objective: The literature does not clearly specify the locations inhabited by the spur-thighed tortoise (*Testudo graeca*) from the eastern Black Sea coast of Türkiye. This study thus aimes to reveal new locality records for *T. graeca* in Trabzon province.

Materials and Methods: Two adult male specimens were caught from the Darıca and Konaklar neighborhoods in the respective Akçaabat and Ortahisar districts. Some of the morphological characteristics of these specimens have been recorded using a digital caliper. After taking morphometric measurements, the tortoises were returned to their habitat. No anesthetic procedure was performed on the turtles.

Results: Both the Darıca and Konaklar specimens have five vertebral scutes, 11 pairs of marginal scutes, and four pairs of costal scutes on their carapace. In addition, both specimens were seen to have one undivided supracaudal scute and one nuchal scute on their carapace. The Darıca specimen has a straight carapace length (SCL) of 208.19 mm, and plastron length (PL) of 188.08 mm while the Konaklar specimen has an SCL of 216.33 mm and a PL of 196.28 mm.

Conclusion: The study compared its specimens' the pholidosis and morphometric characteristics and color-pattern features with those of specimens reported in the literature. The morphological features of the Darıca and Konaklar specimens are similar to those for the samples of *Testudo graeca ibera* in the literature. The study's findings concluded that the samples of Darıca and Konaklar belong to the *T. g. ibera* subspecies.

Keywords: Pholidosis, spur-thighed tortoise, Trabzon, distribution

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Introduction

The spur-thighed tortoise, or *Testudo graeca* (L., 1758) is listed as vulnerable (VU) on the International Union for Conservation of Nature (IUCN) Red List (Van Dijk *et al.*, 2004). It has two sub-special clades one being the western subspecies clade native to northern Africa and southwestern Europe, including Morocco, Algeria, Libya, Tunisia, and Spain (introduced since historic times on the Balearic Islands of Spain and western Sardinia, Italy; Escoriza *et al.*, 2023). The other is the eastern subspecies clade native to the Balkans and Southwestern Asia including Greece, Bulgaria, North Macedonia, Romania, Kosovo, Türkiye, Serbia, Russia, Georgia, Azerbaijan, Armenia, Iraq, Iran, Syria, Jordan, Lebanon, Israel, and Palestine (Türkozan *et al.*, 2023).

According to morphological traits and molecular data, five subspecies clades are currently recognized among the eastern clade of the species: the Armenian tortoise (Araxes tortoise), or *T. g. armeniaca*, found in Armenia, Azerbaijan, Iran, Russia (Dagestan), and Türkiye; the Zagros tortoise (Buxton's tortoise), or *T. g. buxtoni* found in Iran, Iraq, and Türkiye; the Anatolian tortoise (Greek tortoise/Asia Minor tortoise), or *T. g. ibera* found in Bulgaria, Greece, Georgia, North Macedonia, Kosovo, Romania, Serbia, Türkiye, and Russia (Krasnodar); the Levantine tortoise (Mesopotamian tortoise), or *T. g. terrestris* found in Jordan, Lebanon, Israel, Palestine, Syria, and Türkiye; and the Kerman tortoise (Iranian tortoise), or *T. g. zarudnyi* found in Iran (Türkozan *et al.*, 2023). Similarly, five subspecies are recognized in the western clade of the spur-thighed tortoise: *T. g. cyrenaica* in northeastern Libya; *T. g. graeca* in southwestern Morocco; *T. g. marokkensis* in the northern and central Atlantic plain of Morocco; *T. g. nabeulensis* in Tunisia, extreme northeastern Algeria, northwestern Libya, and introduced in Sardinia; and *T. g. whitei* in northeastern Morocco, western Algeria, peninsular Spain, and introduced in Mallorca and Formentera (previously referred to as *T. g. graeca*; Escoriza *et al.*, 2023).

According to Türkozan *et al.* (2018), four mitochondrial clades (i.e., *armeniaca*, *buxtoni*, *ibera*, and *terrestris*) represent the *T. graeca* species complex in Türkiye. Those authors suggested that the *ibera* mtDNA clade distributes from west to east, while the *terrestris* mtDNA reaches the Taurus range across Türkiye (except for one locality in which *terrestris* and *ibera* are syntopic). The *terrestris* clade is in close contact with the *buxtoni* mtDNA clade along the Anatolian Diagonal (a significant barrier) in the east (Gür, 2016). The *buxtoni* mtDNA clade is distributed in the Zagros Mountain forest-steppe, an ecoregion among the Irano-Anatolian hotspots. The other mtDNA clade, *armeniaca*, is only found in the lowlands of the Araxes Valley in Türkiye. However, syntopic occurrences of *terrestris* and *ibera* (Türkozan *et al.*, 2018), *armeniaca* and *ibera* (Mashkaryan *et al.*, 2013), and *buxtoni* and *armeniaca* (Javanbakht *et al.*, 2017) have combined extensive gene flows among these clades (Mashkaryan *et al.*, 2013; Mikulíček *et al.*, 2013), which suggests the presence of parapatric speciation distributions and hybrid zones.

The species is found in all regions of Türkiye except the eastern Black Sea region (Başoğlu & Baran, 1977; Baran & Atatür, 1998; Baran *et al.*, 2021). Only one record (Lortet, 1887) exist indicating the occurrence of the species in Trabzon province from the eastern Black Sea region. However, Lortet (1887) provided no details about the locality or localities where the species was found.

The present study provides two new locality records for *T. graeca* in the Trabzon province of Türkiye and a comparison of some morphological characteristics of the specimens caught in Trabzon with those of other specimens reported in the literature.

Material and Methods

During a field survey on June 20, 2022, a male specimen of *T. graeca* was observed in the Darıca neighborhood of Akçaabat district in Trabzon province (41°2'342''N, 39°31'234''E, 209 m a.s.l.; Fig. 1). The individual was caught by hand in its natural habitat (Fig. 2).

On June 9, 2023, another male individual of *T. graeca* was found in the Konaklar neighborhood of Ortahisar district in Trabzon province (40°58'736''N, 39°46'430''E, 278 m a.s.l.; Fig. 1). The individual was also caught by hand in its natural habitat (Fig. 2). The two individuals from Darıca and Konaklar were photographed, measured, and released back into their natural habitat.

Body measurements were taken with a digital caliper (accuracy ± 0.01 mm). Morphometric measurements were taken in the same way as Türkozan *et al.* (2005, 2010, 2018) with straight carapace length (SCL) being measured from the outermost projection of the cervical scale to the outermost projection of the posteriors marginals; median carapace width (CW) being measured at the center of the carapace; maximum carapace width (MCW) being measured at the posterior marginals 7-9; carapace height (CH) being the vertical measurement between the most dorsal point of the carapace and the most ventral point of the plastron; and plastron length (PL) being measured from the outermost projection of the gulars to the posterior end of the anals.

The number of scutes on the carapace and plastron were counted. The study also noted the appearance of the

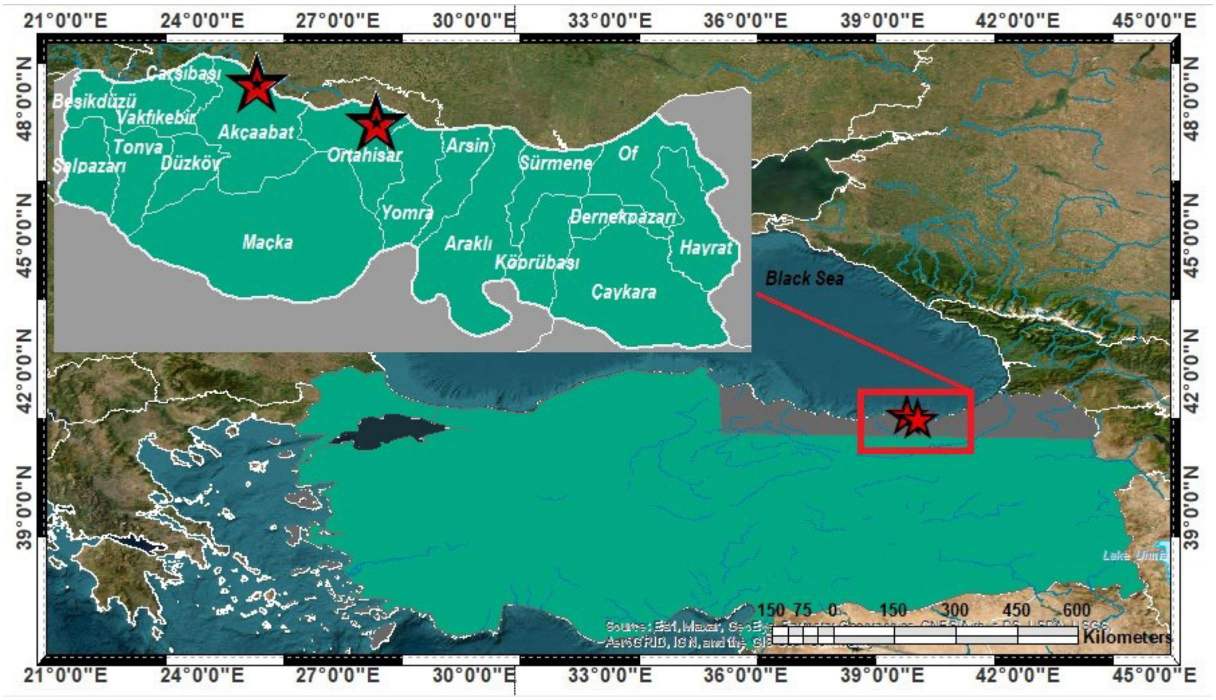

Figure 1. Map of the distribution areas of *Testudo graeca* in Türkiye. Green represents the locations of populations known in the literature. Grey indicates areas where locality records have not been previously reported for *T. graeca*. The red stars show the new localities found in the current study.

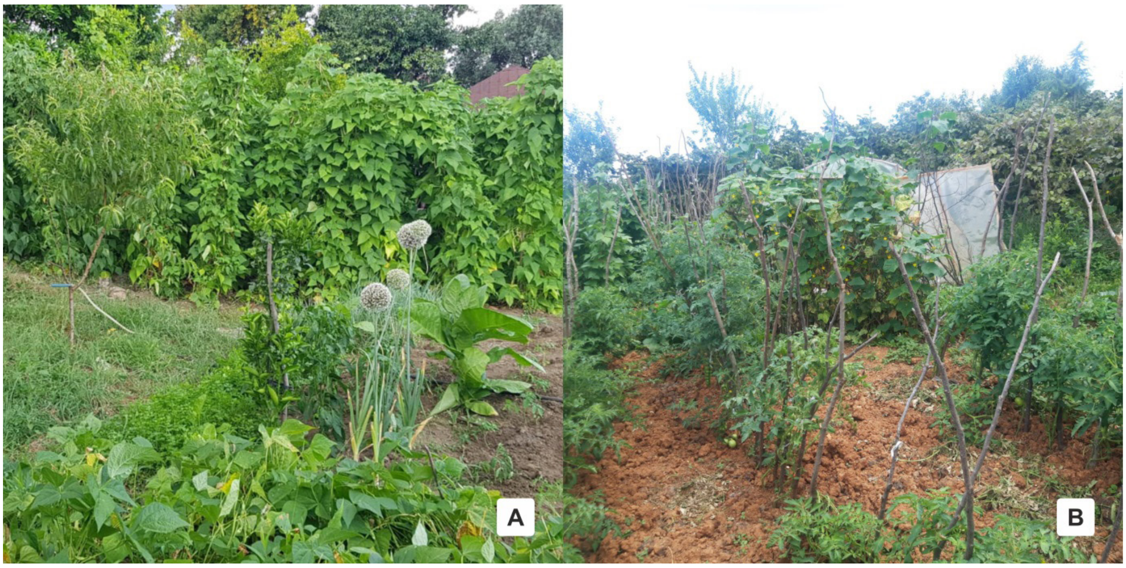

Figure 2. (A) Habitat of *Testudo graeca* in the Darıca neighborhood of Akçaabat district in Trabzon province. (B) Habitat of *T. graeca* in the Konaklar neighborhood of Ortahisar district in Trabzon province.

carapace, plastron and plaques, as well as certian features indicated Türkozan *et al.*'s. (2023) study.

Results

The habitat in the Darıca neighborhood consists of a small field with vegetables such as beans, spring onions, lettuce, and surrounding fruit trees. The sympatric reptiles are *Dolichophis caspius* (Gmelin, 1789), *Natrix natrix* (L., 1758), *Darevskia rudis* (Bedriaga, 1886), and *Anguis colchica* (Nordmann, 1840).

The habitat in the Konaklar neighborhood consists of a small field with vegetables such as tomatoes, beans, and hazelnut trees. The sympatric reptiles are *Zamenis longissimus* (Laurenti, 1768), *Darevskia rudis* (Bedriaga, 1886), and *Anguis colchica* (Nordmann, 1840).

Pholidolial characteristics: The specimens from Darıca and Konaklar each have five vertebral scutes, 11 pairs of marginal scutes, and four pairs of costal scutes on their carapace. In addition, one undivided supracaudal scute and one nuchal were seen on the carapaces of each sample. The plastron for both specimens consists of six pairs of scutes.

Morphometric measurements: For the Darıca specimen, the SCL measures 208.19 mm and the PL measures 188.08, while the Konaklar specimen's SCL measure 216.33 mm and its PL 196.28 mm. Comparisons of the morphometric measurements of the Darıca and Konaklar specimens of *T. graeca* to those in the studies of Türkozan *et al.* (2005, 2010) are given in Table 1.

Color-pattern: The Darıca specimen's carapace has a dark color. Dark blue coloration occurs on the head and the costal and vertebral scutes. In addition, this specimen has black spots on the marginal, costal, and vertebral scutes (Fig. 3). The Konaklar specimen's carapace also has a dark color and dark blue coloration on the head (especially on the posterior) and the costal and vertebral scutes. This specimen also has tiny black spots on the marginal, costal, and vertebral scutes (Fig. 3). Both the Darıca and Konaklar specimens's plastron have a light background pattern and include elongated black spots parallel to the longitudinal axis, which form two nearly continuous bands.

Discussion

The presence of *Testudo graeca* on the eastern Black Sea coast of Türkiye was first mentioned by Lortet (1887) without giving any locality name, simply stating it to be present in the province of Trabzon. In addition, Türkozan *et al.* (2023) showed Trabzon province to be in the species' distribution map and explained that this species had either been introduced there or some situation had likely occurred such as an individual trade, translocated specimens or historically relict populations. The present study provides two new locality records (Darıca neighborhood in Akçaabat district and Konaklar neighborhood in Ortahisar district) for *T. graeca* in the Trabzon province of Türkiye. The study has concluded that Trabzon falls within the natural distribution area for this species.

The study compared the pholidolial characteristics and morphometric measurements of the Darıca and Konaklar specimens to those of other Turkish specimens used in the studies of Türkozan *et al.* (2005, 2010, 2023). The morphometric measurements (i.e., SCL, CW, MCW, CH, and PL) of the Darıca and Konaklar specimens have been found to be similar to the *Testudo graeca ibera* measured specimens measured Türkozan *et al*.'s (2005, 2010) studies. In accordance with the geographical distribution of the subspecies of *T. graeca* in Türkiye, this study has also compared the Trabzon specimens with the specimens of the *T. g. ibera* subspecies clades in the literature. The numbers of scutes the Darıca and Konaklar specimens have on their carapace and plastron have been found to be similar to the data shown in Türkozan *et al.* (2023). In addition, Türkozan *et al.* (2023) stated the plastron of *T. g. ibera* to consist of six pairs of scutes and *T. g. ibera* to have wide abdominals, moderately sized femorals and gulars, and relatively narrow humerals. Similar characteristics were seen in the plastrons of the specimens found in Darıca and Konaklar.

This study provides two locality records for the species with only one adult individual beeing seen per locality.

Table 1. Comparison of some of the morphometric measurements of the Darıca and Konaklar specimens of *Testudo graeca* with those for the *Testudo graeca ibera* presented in Türkozan *et al.* (2005, 2010). Lengths are measured in millimeters (mm). For other abbreviations, see the text. Note: Türkozan *et al.*'s (2005) study standardized the CW, MCW, CH, and PL characteristics for maximum carapace length.

	This Study (Darıca specimen)	**This Study (Konaklar Specimen)**	**Türkozan *et al.* (2005)**		**Türkozan *et al.* (2010) (Different Regions of Anatolia)**
Character	**1 ♂**	**1 ♂**	**Mean values of 7 ♂♂ from Agean Region**	**Mean values of 6 ♂♂ from Central Anatolia**	**Mean values of 257 ♂♂**
SCL	208.19	216.33	186.71	211.00	183.5
CW	158.24	166.26	-	-	131.70
MCW	166.18	174.38	-	-	140.30
CH	112.12	120.46	-	-	87.60
PL	188.08	196.28	-	-	160.30

Figure 3. (A) The male *Testudo graeca* specimen found in Darıca. (B) The male *T. graeca* specimen found in Konaklar.

According to the IUCN Red List, the species is in the vulnerable (VU) category and the number of individuals belonging to the species is decreasing. Field observations that will reveal the existence of individuals of this species in other districts of Trabzon and other provinces in the Eastern Black Sea Region should be maintained.

Although the results for the specimens from the Darıca and Konaklar populations are similar to those for the specimens of *T. g. ibera* in the current literature, the number of specimens in this study is very low. Based on the study's morphological findings, these two specimens have been concluded to belong to *T. g. ibera*. However, this conclusion is not based on molecular data.

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DESCRIPTION

Turkish Journal of Bioscience and Collections is an international, scientific, open-access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of the Center for Research and Practice in Natural Riches in Istanbul University and it is published biannually in February and August. Turkish Journal of Bioscience and Collections was published first time in August 2017. The journal has been published two issues in a volume since 2018. The publication languages of the journal are English and Turkish.

AIM AND SCOPE

Turkish Journal of Bioscience and Collections aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of bioscience. The journal publishes original research, review articles, short communications, and obituary that are prepared in accordance with the ethical guidelines in all fields of biology and life sciences.

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