

Range extension of *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) in the northeastern Mediterranean, İskenderun Bay, Turkey

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Abstract: This paper reports the range extension of *Kyphosus vaigiensis* in the northeastern (NE) Mediterranean. On 18 November, 2018, a single specimen of *K. vaigiensis* was caught in İskenderun Bay (36°45.6' N, 35°40.8' E) by a recreational fisherman and reported through a social media application. The specimen was identified using morphological and molecular methods. Its occurrence and distribution in the NE Mediterranean were investigated based on interviews with fishers.

Key words: Brassy chub, fishers' ecological knowledge, Levant Basin, first record, DNA barcoding

Sea chubs (Kyphosidae, *Kyphosus*) currently consist of 12 species (Knudsen and Clements, 2013, 2016, 2019) occurring in tropical and subtropical regions of the Atlantic, Indian, and Pacific oceans (Sakai and Nakabo, 1995, 2014; Knudsen and Clements, 2013). They generally inhabit shallow waters of less than 10 m deep in various habitats like reefs, seagrass meadows, and sandy bottoms (Nelson, 2006). Moreover, juveniles are commonly found in nearby floating algae or below flotsam (Knudsen and Clements, 2013). Sea chubs are strictly herbivorous, mainly feeding on macroalgae (Knudsen and Clements, 2013). Although they are considered popular game fish, they have low economic importance because of their characteristic bad odor and taste (Bae et al., 2008).

Kyphosus vaigiensis is a circumtropical species distributed along the Indian, Atlantic, and Pacific oceans. Its presence in the central and western Mediterranean (Azzurro et al., 2013a; Brutto, 2017), and Red Sea (Knudsen and Clements, 2013), has been known for a long time. It was first recorded in Spain, western Mediterranean in 1998 (Azzurro et al., 2013a), and additional occurrences were reported thereafter (Orsi-Relini et al., 2010; Ligas et al., 2011; Peña-Rivas and Azzurro, 2013; Mannino et al., 2015; Vella et al., 2016). Recently, its occurrence has been documented in the eastern Mediterranean. A single specimen was caught along the Israeli coast for the first time (Goren et al., 2016). Gerovasileiou et al. (2017) reported a range extension from the southern coast of Cyprus. These occurrences seem temporally scattered throughout

the Mediterranean, indicating a clear movement pattern from west to east or in the opposite direction. Herein, we reported a further range extension of *K. vaigiensis* from the northern coast of the Levant Basin, in İskenderun Bay, and investigated its establishment status based on the fishers' ecological knowledge approach.

A single specimen of *K. vaigiensis* was observed on 18 November, 2018, in İskenderun Bay (36°45.6' N, 35°40.8' E), northeastern Mediterranean (Figure 1). A recreational fisherman caught the specimen at approximately 4 m deep, near the sandy-rocky bottom, at dawn, using a jig hook, and reported it through a social media application. After making contact, the specimen was transported to the fish laboratory under cold storage conditions. After the meristic characters were counted, the specimen was photographed. The morphometric characters were measured to the nearest 0.1 mm on the digital image using ImageJ 1.52 software (Schindelin et al., 2012). Next, fin tissue was plucked out for DNA analysis. The specimen was preserved in 4% neutralized formaldehyde and kept in the collection of the Fisheries Faculty, Çukurova University, under catalog number CUKV001-18. Identification of the specimen was carried out on the basis of the meristic and morphometric characters reported by Sakai and Nakabo (1995, 2014) and Knudsen and Clements (2013). In order to evaluate its establishment status on Turkish coasts, its photographs were shown to 30 local amateur and professional fishermen operating around the Karataş and Yumurtalık fishery ports, near the location where the specimen was observed.

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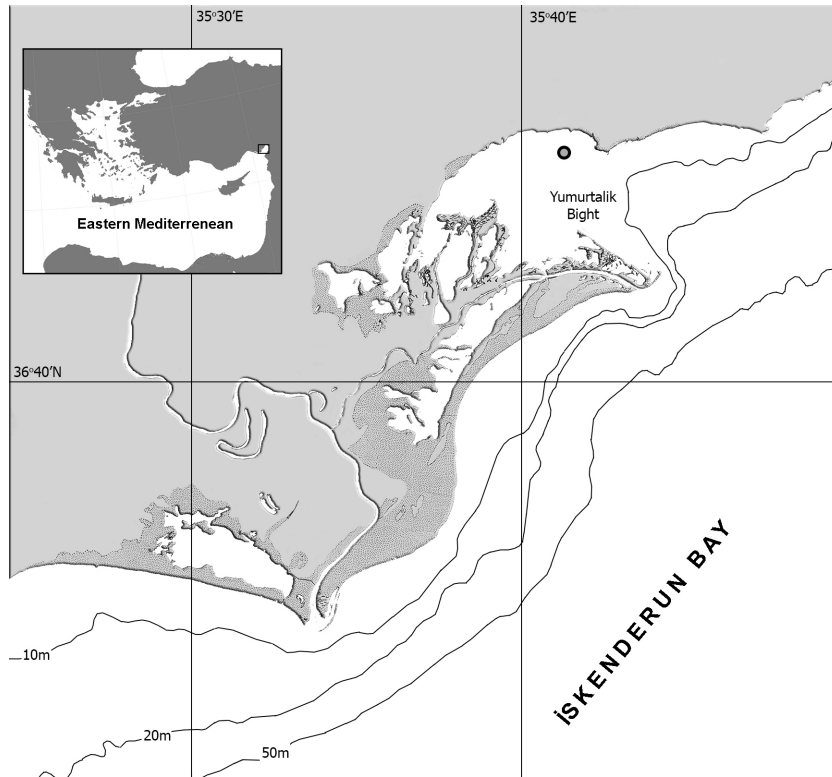


Figure 1. Map of the İskenderun Bay showing the location where the *Kyphosus vaigiensis* specimen was caught.

Validation of the morphological identification was carried out by amplifying and analyzing a region of the mitochondrial cytochrome c oxidase subunit I (COI) gene. For this purpose, genomic DNA was isolated from 20 mg of fish fin tissue using the Qiagen DNeasy blood and tissue kit (Qiagen N.V., Dusseldorf, Germany). The targeted barcoding gene region was amplified using a primer pair fish F1 (TCAACCAACCACAAAGACATTGGCAC) - fish R1 (TAGACTTCTGGGTGGCCA AAGAATCA) (Ward et al., 2005) and DreamTaq DNA polymerase (Thermo Fisher Scientific Baltics, UAB, Vilnius, Lithuania). A 50- μ L polymerase chain reaction (PCR) mixture was prepared that included 2 μ L isolated genomic DNA (29 ng/ μ L), 5 μ L 10X buffer (containing 25 mM Mg²⁺), 1 μ L 10 mM dNTP mix, 1.4 μ L of each primer (10 pmol/ μ L), 0.25 μ L Taq DNA polymerase (5 U/ μ L), and 37.95 μ L nuclease-free water. The thermal cycling program was performed using a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA) for the PCR using the following conditions: 2-min initial denaturation at 95 °C, 35 cycles of 30-s denaturation at 95 °C, 30-s annealing at 58 °C, 1-min extension at 72 °C, followed by 7-min final extension at 72 °C. After attaining the expected band in the gel electrophoresis, the PCR product was purified using NucleoSpin® gel and the PCR clean-up kit (Macherey Nagel, Düren, Germany) and sequenced using the same primers.

Moreover, the obtained sequence was subjected to a phylogenetic analysis to determine its genetic similarity and distance from other known species. For this purpose, the nucleotide sequences of the COI genes of related species, which had more than 97% similarity with the obtained sequence, were aligned using ClustalW (Thompson et al., 1994) followed by the formation of a phylogenetic tree, which was constructed using the neighbor-joining method (Saitou and Nei, 1987). All of the ambiguous positions were removed for each sequence pair (pair-wise deletion option) and distances between the sequences were computed using the Kimura 2-parameter method (Kimura, 1980). All of the phylogenetic analyses were conducted using Mega X software (Kumar et al., 2018).

K. vaigiensis can be distinguished from other species of the genus by the presence of scales in the interorbital region, golden horizontal scale rows along the body, the length and number of soft rays in the dorsal and anal fins, and the number of gill rakers on the upper and lower limbs of the first gill arch (Knudsen and Clements, 2013).

The morphological characteristics of the specimen were as follows: the total length was 531.1 mm and total weight was 2270 g. The body was elongated and moderately compressed, with a small terminal mouth full of incisiform teeth. The head was small and gently convex in front of the

eyes. Scales were present in the interorbital region. The color was silver-gray laterally with a bluish-shine dorsally. There were a number of yellow-golden longitudinal stripes along the body. The head had 2 yellow-golden stripes, one below and the other behind the eyes. The proximal part of the pectoral fins and outer pelvic fins was bright silver. The distal half of the pectoral fins and inner pelvic fins was slightly darker. The dorsal and anal fins were in dark brown color and not elevated. The margin of the soft rayed portion had darker bands (Figure 2). The meristic and morphometric characters of the specimen are presented in Table 1.

After sequencing of the specimen's targeted barcode region, 676 bp of the COI gene was obtained by assembling bidirectional (forward and reverse) sequences. In order to compare the data with other known sequences, the assembled sequence was uploaded to both the Barcode of Life Data (BOLD) system identification system and the National Center for Biotechnology Information nucleotide basic local alignment search tool (BLAST). According to the BOLD identification and BLAST results, the assembled nucleotide sequence of the species showed



Figure 2. *Kyphosus vaigiensis*, 531.1 mm total length from İskenderun Bay (northeastern Mediterranean): on the right, the first gill arch (photograph by C.E. Ozyurt).

100% similarity with that of *K. vaigiensis* samples mainly from the Mediterranean Sea and Indo-Pacific region (Table 2, Figure 3). In the BOLD database, the specimen was assigned the following the barcode index number: BOLD: AAC3456, process id: CUKV001-18.

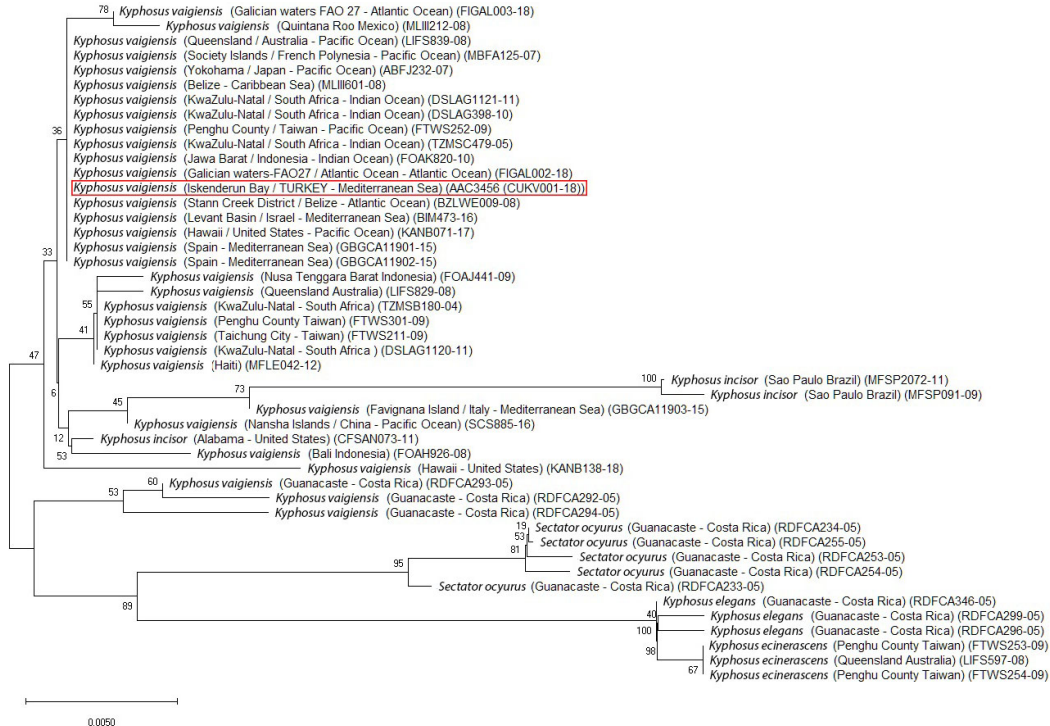
Based on the molecular analysis, the specimen that we obtained matched the specimen reported from the Israeli coast by Goren et al. (2016), for the genetic identity of the mtDNA cytochrome oxidase 1 (100%), and numerous

Table 1. Morphometric and meristic characteristics of the specimen of *Kyphosus vaigiensis* from İskenderun Bay, northeastern Mediterranean (36.76°N, 35.68°E).

Metrics	Length (mm)	% in SL	Meristic	Count
Total length	531.1	115.82	Dorsal fin spines	11
Fork length	493.0	107.53	Dorsal fin rays	13
Standard length (SL)	458.5	100.00	Anal fin spines	3
Head length	103.8	22.64	Anal fin rays	13
Body depth	191.8	41.82	Pectoral fin rays	19
Body width	7.6	1.66	Ventral fin spines	1
Caudal peduncle depth	45.7	9.96	Ventral fin rays	5
Caudal peduncle width	8.1	1.77	Gill rakers on upper limb of first gill arch	8
Snout length	23.9	5.22	Gill rakers on lower limb of first gill arch	19
Interorbital length	5.4	1.18	Incisor shaped teeth on upper jaw	34
Eye diameter	19.0	4.15	Incisor shaped teeth on lower jaw	37
Upper jaw length	13.4	2.93		
Preanal length	259.6	56.62		
Dorsal fin base length	240.6	52.47		
Soft dorsal fin base Length	149.4	32.59		
Spinous dorsal fin base Length	116.5	25.42		
Pectora fin length	89.8	19.59		
Anal fin base length	124.6	27.18		
Pelvic fin length	74.3	16.20		
Body depth at opercle	154.7	33.73		

Table 2. BOLD accession numbers and sampling locations of the *Kyphosus vaigiensis* specimens, which revealed 100% similarity to our specimen (marked in bold).

BOLD Accession number	Sampling locality	Region
CUKV001-18	İskenderun Bay, Turkey	Mediterranean Sea
DSLAG398-10	KwaZulu-Natal, South Africa	Indian Ocean
DSLAG1121-11	KwaZulu-Natal, South Africa	Indian Ocean
MLIII601-08	Belize	Caribbean Sea
ABFJ232-07	Yokohama, Japan	Pacific Ocean
MBFA125-07	Society Islands, French Polynesia	Pacific Ocean
LIFS839-08	Queensland, Australia	Pacific Ocean
BZLWE009-08	Stann Creek District, Belize	Atlantic Ocean
FTWS252-09	Penghu County, Taiwan	Pacific Ocean
GBGCA11903-15	Favignana Island, Italy	Mediterranean Sea
GBGCA11902-15	Spain	Mediterranean Sea
GBGCA11901-15	Spain	Mediterranean Sea
TZMSC479-05	KwaZulu-Natal, South Africa	Indian Ocean
FOAK820-10	Jawa Barat, Indonesia	Indian Ocean
BIM473-16	Levant Basin, Israel	Mediterranean Sea
SCS885-16	Nansha Islands, China	Pacific Ocean
KANB071-17	Hawaii, United States	Pacific Ocean
FIGAL002-18	Galician waters-FAO27, Atlantic Ocean	Atlantic Ocean

**Figure 3.** The neighbor-joining phylogenetic tree for our specimen and the 45 related specimens in the BOLD database. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. In the phylogram, each species is represented by its name, sampling location, and barcode index number, respectively. The obtained sequences are enclosed in a red box for clarity.

specimens mainly from the Mediterranean Sea and Indo-Pacific region (Table 2, Figure 3). Although this could be considered evidence of Suez Canal intrusion, specimens from the Caribbean Sea and Galician coast also revealed a high match to the obtained sample. Therefore, as Goren et al. (2016) stated, Gibraltar could also be considered as another possible migration route.

Fishermen are often the very first to observe changes in marine fish and this knowledge can provide useful information to monitor alien species (Azzurro, 2011, 2013b). The specimen presented herein was recognized by a recreational fisherman and reported to fishery scientists through a social media application. Although *K. vaigiensis* has broadened its distribution and can be considered to be commonly occurring in the northeastern Mediterranean, its establishment was also investigated by interviewing the fishermen. However, none of the fishermen interviewed had previously encountered any

Kyphosid species in that part of the Mediterranean. Nevertheless, subsequent records from Israel (Goren et al., 2016), Cyprus (Gerovasileiou et al., 2017), and Turkey can now be considered as a preliminary indication of this Kyphosid species broadening its distribution and potentially establishing a population in that part of the Mediterranean.

In conclusion, the case presented herein was the result of a collaborative effort between fishermen and scientists, and it provided further support of the importance of such participatory approaches in the monitoring and management of alien species, especially in the eastern Mediterranean, which is a biological invasion hotspot.

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