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A new alien fish in the Mediterranean Sea; *Oxyurichtys keiensis* (Smith, 1938) (Gobiiformes: Gobiidae)

Uğur ÖZDEN, Dilruba SEYHAN ÖZTÜRK, Erhan IRMAK, Adnan Çağlar ORUÇ and Semih ENGİN

İzmir Katip Celebi University, Faculty of Fisheries, Havaalanı Sosesi Cd. No:33/2, 35620 Ciğli, Izmir, Turkey

Corresponding author: Uğur ÖZDEN; ugurozden.ks@gmail.com

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Abstract

An Indian Ocean goby, *Oxyurichtys keiensis* (Smith, 1938), is recorded from the northern Levantine coast of Turkey. The identification of *Oxyurichtys keiensis* was carried out using morphological characters and DNA barcoding data. This species is distinguished from only lessepsian congeneric, in the Mediterranean Sea, *Oxyurichtys petersii,* by sensory papilla pattern, scales in lateral series and tongue shape. In this study, the presence of the 13rd alien gobiid species of the Mediterranean Sea, which tends to create established populations far from its native distribution is presented.

Keywords: Kei goby; cryptobenthic fish; *Oxyurichtys;* Mediterranean Sea; Gobiidae; first record.

Introduction

The entry of an alien species to a new ecosystem is considered a major threat to the biodiversity, structure and function of the ecosystem, and this entry may result from anthropogenic factors or the species expanding its native range (Pajuelo *et al*., 2016; Çınar *et al*., 2021).

Gobiid fish are the species-richest fish taxon among exotic fish in the Mediterranean Sea (Kovačić & Patzner, 2011). A total of 12 exotic gobiid species are known in the Mediterranean Sea (Engin *et al*., 2018; Goren & Stern, 2021). The genus *Oxyurichthys* contains large and distinctive gobies that are widespread in the tropical Indo-west Pacific in a range of estuarine and coastal marine habitats. The genus has only one representative, *Oxyurichthys petersi* (Klunzinger, 1871), present as an alien species in the Mediterranean, and it is common in the shallow soft-bottom regions all over the eastern Mediterranean from Israel to the Southern Aegean Sea (Ben-Tuvia, 1983; Goren *et al*., 2009; Akyol *et al*., 2006).

The aim of this paper is to report the establishment of a resident population of *Oxyurichthys keiensis* in the Mediterranean Sea.

Material and Methods

Samples were detected for the first time in *Upogebia pusilla* (Petagna, 1792) packages, which is sold as live baits for angling. Afterward, surveys were carried out in the potential habitats of *O. keisensis* with the help of the fishermen. Five specimens were collected with hand net at 0-2 m depth in the mouth of the creek (36.65333°N, 29.11528°E) in Fethiye Bay (Fig. 1) in November 2018. Specimens were fixed in 4% formalin solution for morphological analysis and pectoral fin clips were fixed in 96% ethanol for genetic analyses. The collected specimens were deposited in the Fish Collection Centre of İzmir Katip Çelebi University (IKC PIS 1254-1258) (Fig. 2A). Morphological measurements and counts followed Pezold & Larson (2015).

Total genomic DNA was isolated using with the PureLink Genomic DNA mini kit (Invitrogen; www.invitrogen.com). Universal primers described in Ward *et al*. (2005) were used amplify the COI gene. The PCR thermal profile consisted of an initial step of 2 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C, followed in turn by 10 min at 72°C. Sequences were aligned using CLUSTAL W (Thompson *et al*., 1994) and manually edited in BIOEDIT 7.2.5. (Hall, 1999). *Buenia jeffreysii* (Günther, 1867) (GenBank accession number KM077819) was used to root the tree. The best-fit model of nucleotide substitution was calculated by the Akaike and Bayesian Information Criteria (AIC and BIC) approaches. The model with the lowest BIC and AIC scores is considered to describe the substitution pattern the best. Maximum likelihood (ML) analysis was performed with the software package MEGA v.7.0 (Kumar *et al*., 2016), using the Hasegawa- Kishino (HKY) model which was chosen after running the 'Model Se-

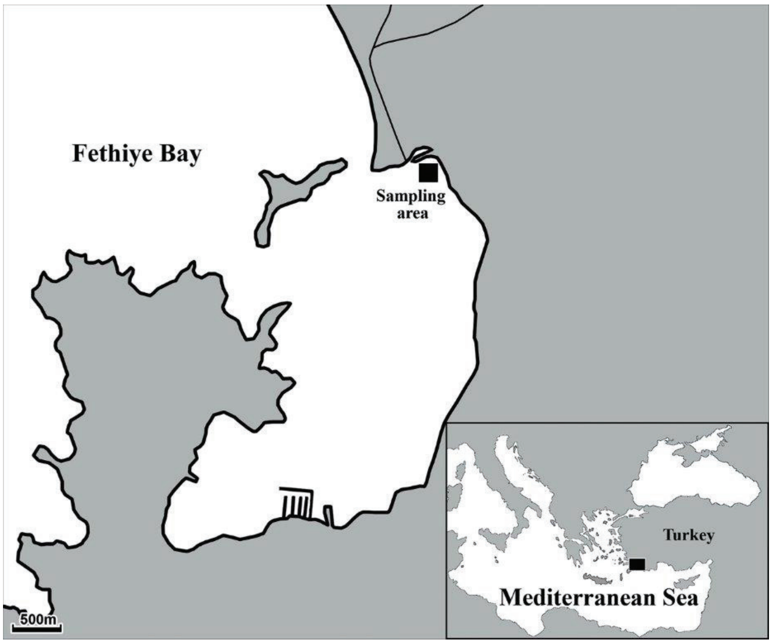

Fig. 1: Sampling area of *Oxyurichthys keiensis.*

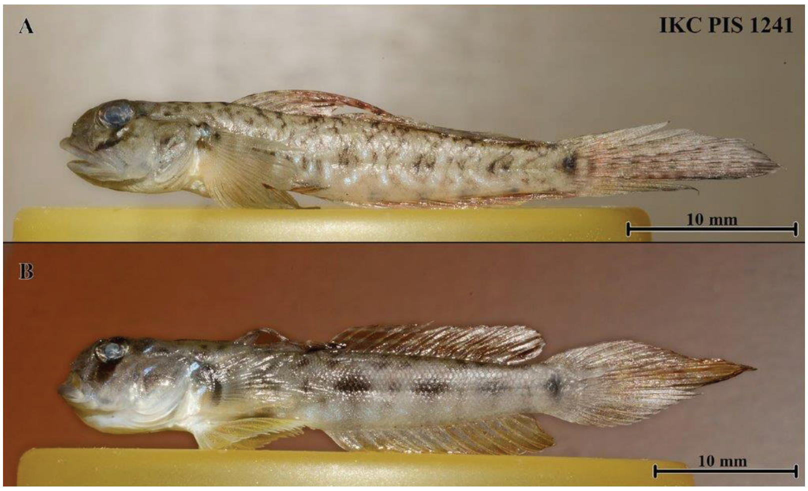

Fig. 2: *Oxyurichthys keiensis* (A); *Oxyurichtys petersii* (B) (Photo credit: Semih Engin).

lection' tool in MEGA. A bootstrap test with 1000 replicates was performed to verify the robustness of the tree. Sequence divergence values within and between species were calculated using the uncorrected p distances.

Aligned sequences were compared with existing data and submitted to GenBank with the accession numbers MZ558389-MZ558391.

Results

Diagnosis

Oxyurichthys differs from the closely related gobionelline genera by having paired anterior interorbital pores, two pairs of nasal canal pores, a single row of upper-jaw teeth with a partial row anteriorly in some large specimens), a transverse sensory papillae pattern, no flaps on the shoulder girdle, a tongue largely fused to the floor of the mouth with a free tip, a lower jaw that curves slightly upwards and having no preopercular canal pores (Pezold & Larson, 2015). The genus *Oxyurichthys* differs from all other native and alien Mediterranean gobies by the presence of an additional head canal pore σ^1 on the snout (Kovačić, 2020). The specimens were assigned to *Oxyurichthys keiensis* and distinguished from its congeners by having the following features: upper-jaw teeth on two to three rows; fleshy tongue truncate to bifurcate, not rounded; no dark spot, cornification or tentacle on the dorsoposterior surface of the eye; anterior nares not darkly pigmented; no membranous crest on the nape; 27-34 lateral scales (in this study, 29-30); spines of the first dorsal fin elongate in males, the third and fourth spines of the appressed fin reaching beyond the second dorsal fin to the near caudal-fin base; dorsal fins VI, I+11 and anal fin, I+12.

Ecology

Oxyurichthys keiensis was found only on muddy ground between 1-2 m depths. Juvenile individuals of *Gobius niger* Linnaeus, 1758 were also sampled at the sampling location. Besides, the other non-indigenous gobies, *O. petersii* and *Vanderhorstia mertensi* Klausewitz, 1974 were observed in the sampling area.

Description

A total of 5 specimens with total length 30.1-44.1 mm were collected. Morphometric and meristic characters are given in Table 1. Body elongated, mouth front position wide and slightly oblique. Head moderately long. Eyes lateral, high on head. Caudal peduncle compressed. Pelvic fin rounded to oval, not reaching anus in females, reaching anus or anal-fin origin in males. Caudal fin lanceolate, no scales on head, nape, pectoral base, or prepelvic region. Anterior oculoscapular canal present onto snout and diverging interorbital canals before posterior pore κ (pores λ double) (Fig. 3). Pore σ^1 present and extending to anterior nostril. Row *a* with three transverse poliferations. Four transverse well organized suborbital rows of sensory papillae. *c1* begining below eye and reaching row *d*. *c2*, *c3, c4* between row *b* and row *d*. Longitudinal row *b* and row *d* extending to preoperculum.No preopercular canal present.

Coloration of freshly preserved material

Body green to brown, (Fig. 2A), pectoral fins transparent, other fins including pelvic fins with reddish hue, an oblique V-shaped dark blotch on the suborbital area from the eye to the upper lip and a bright oblique bluish stripe inside this V shape, lips greenish, upper lip with bright bluish stripe. Body with lateral sides covered with irregular bright bluish blotches, 4 doubled small black blotches on the lateral midline and a triangle dark blotch on the caudal peduncle. A dark and a bright bluish blotch on the pectoral fin base. The dorsal side covered with irregular small dark blotches, and the caudal fin with 9 dark narrow bars.

Genetics analyses

A total of 561 bp for COI mitochondrial gene fragments were obtained (GenBank accession numbers**:** MZ558389-MZ558391). The mean intraspecific and interspecific uncorrected p-distances for *Oxyurichthys* species is 0.6 %±0.002 S.D. and 13.3 %±0.03 S.D. respectively. The intraspecific uncorrected p-distances of *O. keiensis* species is observed as % 0.8 ±0.006 S.D. The maximum genetic distance was observed between *O. cornutus* McCulloch & Waite, 1918 and *O. notonema* (Weber, 1909) (17%) while the minimum was observed between *O. cornutus* and *O. ophthalmonema* (Bleeker, 1856) (4.2%).

Discussion

Oxyurichthys keiensis is known from the South-Western Indian Ocean, including Southeastern Africa, the Seychelles and Madagascar (Pezold & Larson, 2015). This species is distinguished from the Lessepsian congeneric *O. petersii*, which has an established population in the Mediterranean Sea, by its sensory papillae pattern, scales in lateral series and tongue shape, coloration pattern (Fig. 2A and 2B) and genetics (Fig. 4).

Table 1. Morphometric and meristic characters of *Oxyurichthys keiensis*.

	Oxyurichthys keiensis
n	5
TL (mm)	30.01-44.18 (34.8 ± 6.1)
SL (mm)	22.46-32.86 (26.3 ± 4.3)
HL (mm)	6.2-9.36 (7.5 ± 1.3)
% of SL	
Head Length	27.25-30.16 (28.8 ± 1.1)
Body Depth at Anal-fin Origin	14.78-16.4 (15.03 ± 0.6)
Caudal peduncle Length	11.29-14.82 (13.06 ± 1.5)
Caudal Peduncle Depth	8.01-9.07 (8.42 ± 0.5)
Pelvic Fin Length	21.85-26.37 (23.52 ± 2.1)
% of HL	
Depth at Posterior Preopercular Margin	52.84-61.29 (56.58 ± 3.1)
Width at Posterior Preopercular Margin	45.13-54.06 (49.48 ± 3.7)
Eye Diameter	20.83-26.45 (23.47 ± 2.1)
PreOrbital	21.53-29.91 (24.29 ± 3.4)
InterOrbital	4.21-7.48 (5.56 ± 1.5)
Meristic Characters	
First dorsal fin spine	VI
Second dorsal fin rays	I-11
Anal fin rays	I-12
Pectoral fin rays	20
Scales in lateral line	29-30

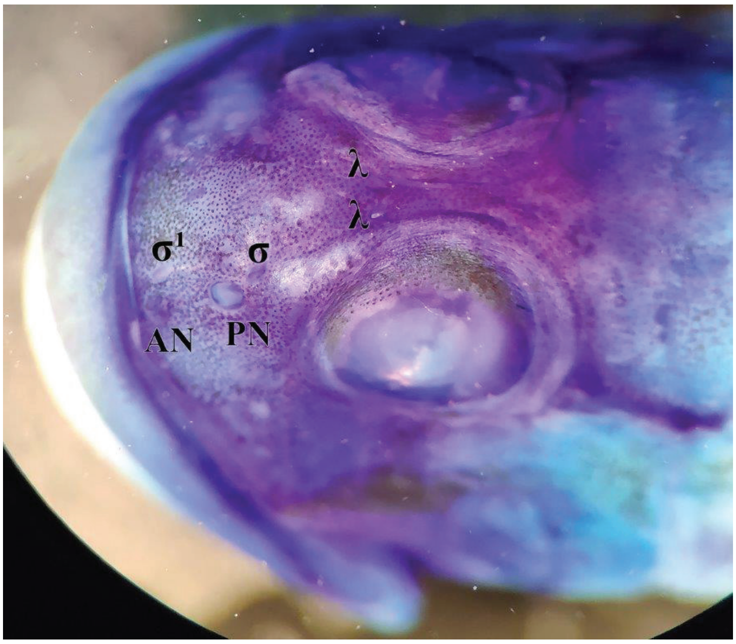

Fig. 3: Head anterior oculoscapular canal pores of *Oxyurichthys keiensis.*

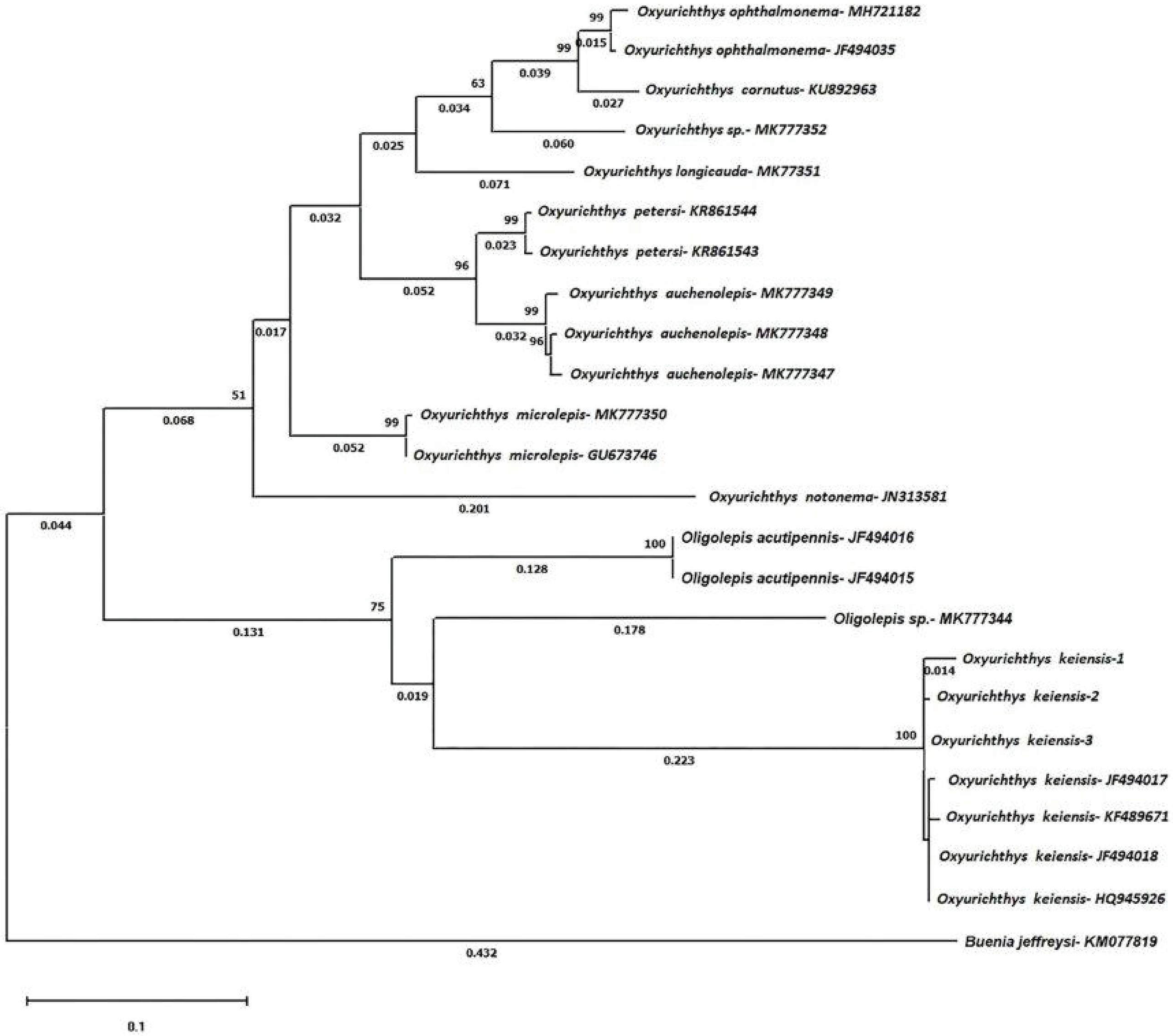

Fig. 4: ML tree constructed from COI sequences based on HKY model with *Buenia jeffreysii* (KM077819) as outgroup. Numbers at nodes are for ML bootstrap percentages (≥50%). The tree is drawn to scale, with branch lengths (under the branches) measured in the number of substitutions per site.

There are two possibilities to explain the entry of this species into the Mediterranean Sea. Shipping activities associated with this species' high ecological tolerance, if the native range is really restricted as we presume, is one of these possibilities. An example of this pathway is the West Pacific goby *Tridentiger trigonocephalus* (Gill, 1859), that has established populations in the north of the Black Sea and in the Mediterranean (Boltachev *et al*., 2007). The second possibility is the expanse of the native range of the species through the Suez Canal. *O. keiensis* has not been reported from the Red Sea. However, its morphologic similarities to and misidentification with the other gobiid may have resulted in overlooking this species in this area. Moreover, trawling, dredging and other conventional methods are often insufficient to sample and detect such small and cryptobenthic fish (Kovačić, 2008).

The generated sequences of *O. keiensis* were grouped with other references labeled as *Oligolepis keiensis* (Smith, 1938) sequences that were obtained from Genbank (Fig. 4). Hoese (1986) regarded this species as in the genus *Oligolepis.* However, in recent years, the taxonomic status of *O. keiensis* has been clarified by Pezold & Larson (2015) with consistent morphological characters, and this species was assigned to the genus *Oxyurichthys*. Pezold & Larson (2015) indicated that *O. keiensis* could be most easily confused with *Oligolepis acutipennis* (Valenciennes, 1837), but it differs from that species in terms of having no preopercular canal pores and having prominent transverse rows of sensory papillae, while those rows in *Oligolepis acutipennis* are reduced or lacking, such that only the horizontal rows are developed. In this study, although our morphological observations completely matched with Pezold & Larson (2015), the sequences were clustered with other *Oligolepis* species instead of *Oxyurichthys* (Fig. 4). Nevertheless, it should be noted that neither the sample sizes nor the conducted genetic analyses were enough to the clarify taxonomic statuses of these species. Further genetic analyses and more samples belonging to these two genera are needed to see a better resolution.

We conducted only one survey for this species in the sampling area which indicate an established and abundant population.

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