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Rapid Communication

First records of the fish *Abudefduf sexfasciatus* (Lacepède, 1801) and *Acanthurus sohal* (Forsskål, 1775) in the Mediterranean Sea

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Abstract

To date, the Mediterranean Sea has been subjected to numerous non-indigenous species' introductions raising the attention of scientists, managers, and media. Several introduction pathways contribute to these introduction, including Lessepsian migration via the Suez Canal, accounting for approximately 100 fish species, and intentional or non-intentional aquarium releases, accounting for at least 18 species introductions. In the context of the citizen science project of iSea "Is it alien to you?… Share it", several citizens are engaged and regularly report observations of alien, rare or unknown marine species. The project aims to monitor the establishment and expansion of alien species in Greece. In this study, we present the first records of two popular high-valued aquarium species, the scissortail sergeant, *Abudefduf sexfasciatus* and the sohal surgeonfish, *Acanthurus sohal,* in along the Mediterranean coastline of Greece. The aggressive behaviour of the two species when in captivity, and the absence of records from areas close to the Suez Canal suggest that both observations are the result of aquarium intentional releases, rather than a Lessepsian migration.

Key words: Scissortail sergeant, Sohal surgeonfish, Eastern Mediterranean Sea, Greece, Aegean Sea, citizen science

Introduction

The potential impacts associated with the introduction of marine Non-Indigenous Species (NIS) to new areas are well-studied and recognised. Although only a small fraction of marine NIS can survive outside of their native range (Mack et al. 2000), successful invaders can negatively affect ecosystems and economy through an enormous range of individual and cumulative impacts. NIS can displace native species, change community structure and food webs and alter fundamental biological processes, such as nutrient cycling and sedimentation (Molnar et al. 2008). To date, NIS have damaged economies by diminishing fisheries and aquaculture operations, fouling ships' hulls and clogging intake pipes, and others are noxious, poisonous, or venomous and thus pose a direct threat to human health (Ruiz et al. 1997; Galil et al. 2015).

The Mediterranean Sea is one of the most affected areas by NIS worldwide (Katsanevakis et al. 2013) as a result of the intense shipping activity, high connectivity with corridors and other ecoregions, and the multiple, cumulative, and increasing anthropogenic stressors that currently exist (Coll et al. 2012). Fish invasions in the Mediterranean have dramatically increased in the last two decades, with more than 40% of the total number of fish species introductions being recorded after 2001 (Galil et al. 2017; Zenetos et al. 2017). The list of Mediterranean NIS is increasingly expanding (see Galil et al. 2017; Zenetos et al. 2017), particularly due to the influx of Lessepsian migration (i.e., Red Sea species entering the Mediterranean through the Suez Canal). Most of the NIS introduced from the Red Sea have established thriving populations along Eastern Mediterranean coastlines (Galil et al. 2017). As a result, the Eastern basin currently includes some of the major hotspots of alien species impacts (Katsanevakis et al. 2016). Direct impacts of fish species introductions include the outcompetition of native herbivorous fish *Sparisoma cretense* (Linnaeus, 1758) and *Sarpa salpa* (Linnaeus, 1758) by rabbitfishes *Siganus luridus* (Rüppell, 1829) and *S. rivulatus* (Forsskål & Niebuhr, 1775) (Papaconstantinou 1987; Bariche et al. 2004), the displacement of the native red goatfish *Mullus barbatus* (Linnaeus, 1758) by *Upeneus molluccensis* (Bleeker, 1855) (Galil 2007), and the socioeconomic severe effects associated with the introduction of the poisonous puffer *Lagocephalus sceleratus* (Gmelin, 1789) (Kalogirou et al. 2013). Similar concerns exist for other recent fish introductions, whose environmental and socioeconomic effects have not been studied yet, but which appear to be expanding very rapidly such as the lionfish *Pterois miles* (Bennett, 1828) (see Kletou et al. 2016), the goatfish *Parupeneus forskalii* (Fourmanoir & Guézé, 1976), and the pufferfish *Torquigener flavimaculosus* (Hardy & Randall, 1983) (pers. obs.).

In this article, we report the first records of the scissortail sergeant *Abudefduf sexfasciatus* (Lacepède, 1801) and the sohal surgeonfish *Acanthurus sohal* (Forsskål, 1775) in the Mediterranean Sea. *Abudefduf sexfasciatus* is a reef-associated damselfish, distributed in the Indo-Pacific and the Red Sea (Allen 1991), inhabiting inshore and offshore coral or rocky reefs (Allen and Erdmann 2012) where it feeds on zooplankton and algae (Lieske and Myers 1994). It is the third species of the genus *Abudefduf* that has been recorded in the Mediterranean after *A. saxatilis* (Linnaeus, 1758), and *A. vaigiensis* Quoy & Gaimard, 1825) (Azzurro et al. 2013; Tsadok et al. 2015). *Acanthurus sohal* is an endemic species of the Arabian Peninsula, distributed from the Red Sea to the Arabian Gulf. It can reach up to 40 cm TL, is commonly found in seaward edges of reefs exposed to surge, and is known for its aggressive and territorial behavior (Lieske and Myers 1994). Both *A. sexfasciatus* and *A. sohal* are very popular among the aquarium enthusiasts, reaching high prices in the market.

Methods

Citizen science

In 2016, an online data repository was established by iSea, in which citizen scientists could easily upload photographic material along with information on specimen size (length and/or weight), depth, number of specimens, exact location, date and type of observation (freediving, underwater photography, shore-base fishing, boat-based fishing, spearfishing). A Google Form and a Group on Facebook were established to facilitate these reports. The project's Facebook group currently numbers ~3000 members, with 200 of those actively engaged on a daily basis. The vast majority of the participants are recreational fishers, followed by scuba divers, naturalists and professional fishermen. Further, several experts on marine alien species are part of the group community offering their expertise for the identification of the specimens reported.

Table 1. Morphometric measurements taken from *A. sohal* specimen.

Total Length	37.51 cm
Fork Length	29.82 cm
Standard Length	26.55 cm
Total Weight	429 gr
Head Length	5.3 cm
Pre-anal length	9.6 cm
Pre-pectoral length	6.2 cm
Pre-pelvic length	7.8 cm
Body Depth	12.2 cm

Specimens

On 13th of August 2017 a spear fisherman from Kalymnos Island, Dodecanese, caught an individual *A. sohal* in Liani Punda (36.934607N; 26.987879E; Figure 1) at a depth of 12 m inside a cave where a specimen of *Epinephelus marginatus* (Lowe, 1834) was caught from the same fisherman a few seconds before. The fishermen captured some photographs and froze the *A. sohal* specimen. A few days later, the specimen was reported via pictures on the iSea Facebook group, and identified by the experts' group based on the unique shape and coloration pattern of the species. Later the specimen was sent to iSea headquarters where morphometric measurements were taken (Figure 2; Table 1).

On 21th of August 2017, an individual of *A. sexfasciatus* of approximately 7–8 cm total length was video recorded in Sounio, Greece (37.677306N; 24.055472E; Figure 1) at 2 m depth by a spear fisherman. The video was posted on the 24th of August 2017 on the iSea Facebook group. The species was identified by the experts' group of the project based on the dark longitudinal stripe on both lobes of the caudal fin, which represents a specific taxonomic character (Figure 2). Additional information on the location, the approximate size and the type of observation, was further collected after personal communication with the fisherman.

Visual and genetic identifications

Experts associated with the iSea Facebook group identified the samples using the posted pictures. In

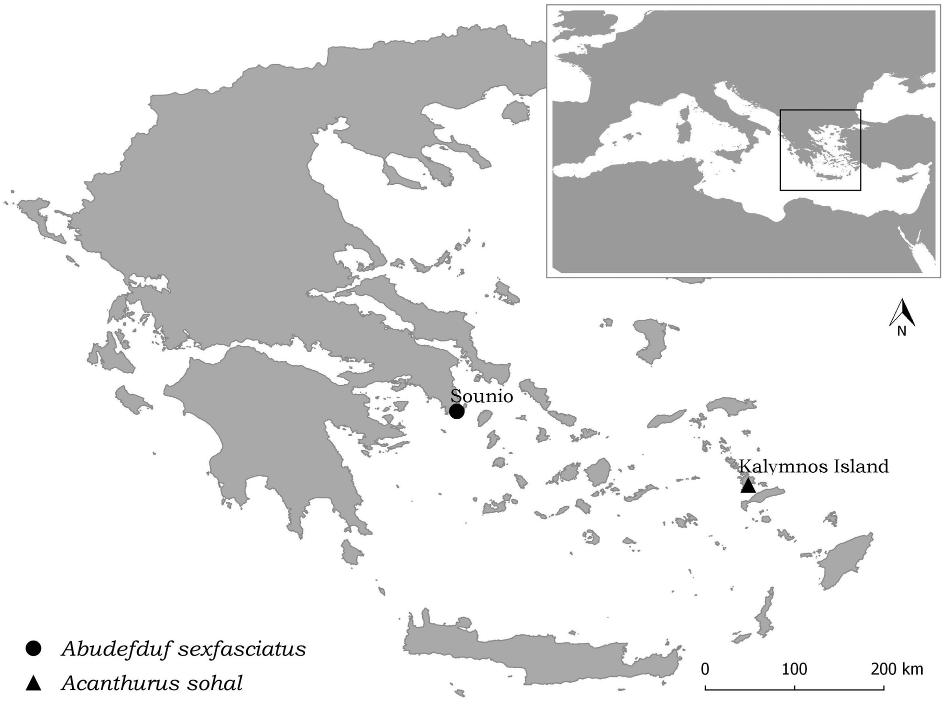

Figure 1. Locations of *Acanthurus sohal* and *Abudefduf sexfasciatus* individuals found in waters surrounding Kalymnos Island and Sounio, Greece, respectively, in 2017.

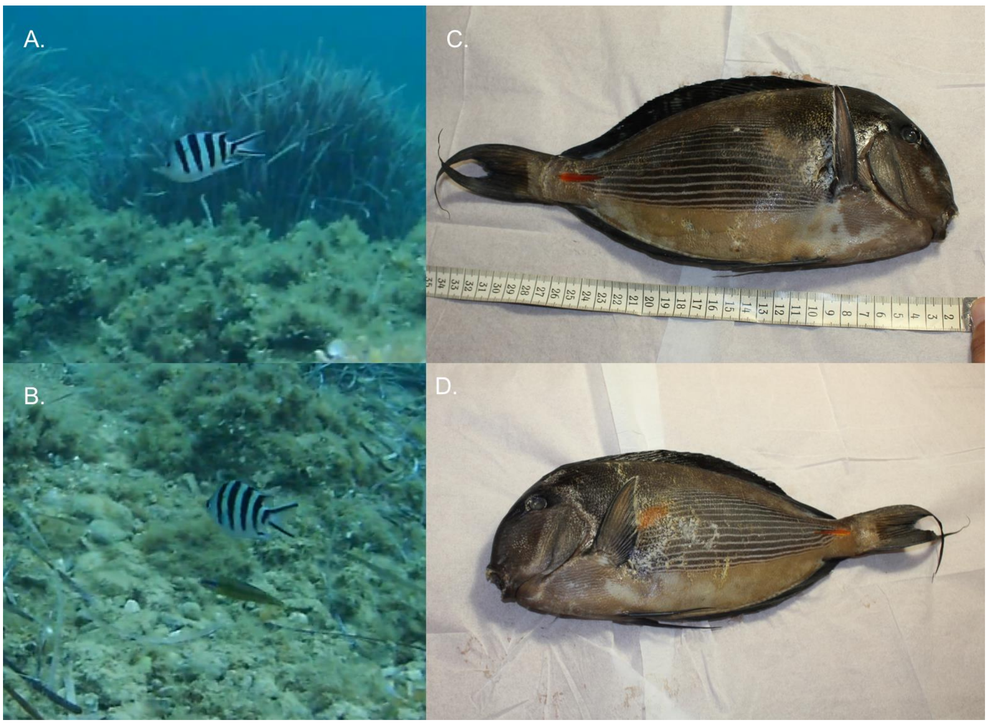

Figure 2. A, B: *Abudefduf sexfasciatus* specimen from Sounio, Greece in 2017; C, D: *Acanthurus sohal* specimen found in Kalymnos Island, Greece in 2017. Photos A. and B. provided by Iraklis Tsonos and photos C and D by Ioannis Giovos.

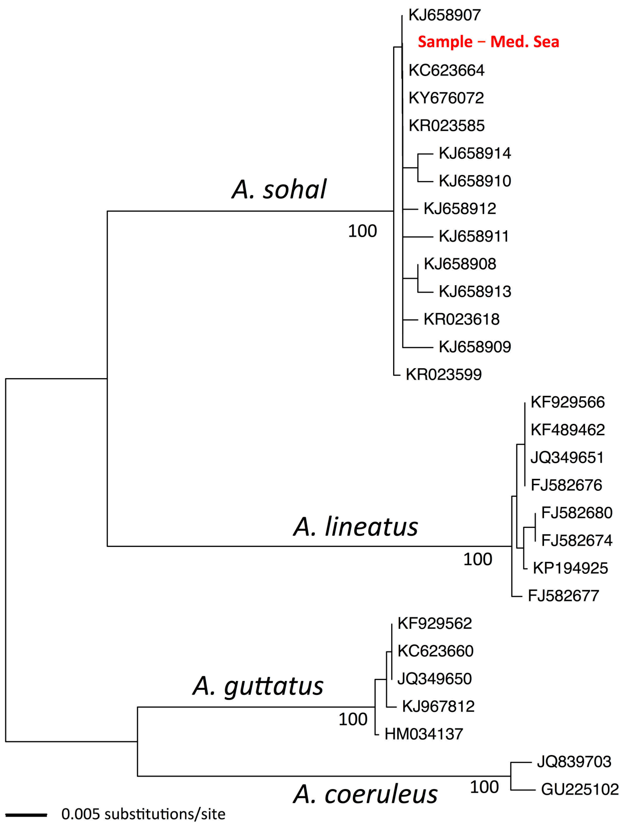

Figure 3. Phylogenetic relationships of *Acanthurus* sequences. The Neighbour-Joining method was used to identify the phylogenetic relationships of the putative *A. sohal* sample (in red, noted as sample – Med Sea) and sequences obtained from GenBank. Tree labels correspond to Genbank Accession numbers. Numbers by the nodes correspond to bootstrap support, and species names of GenBank sequences are presented above the corresponding branches.

addition, a genetic barcode was used on both samples to definitively identify the species. DNA was extracted from the sample identified as *Acanthurus sohal*. The mitochondrial barcode gene CO1 (Cytochrome oxydase 1) was sequenced following published protocols (Bariche et al. 2015). Briefly, the amplification of COI used fish specific primers VF2T1 and VR1dT1 (Ward et al. 2005). PCR amplified fragments were sequenced in both directions using the same primers, and then compared with available sequences in GenBank. Potential relationships among sequences were examined using a Neighbour-Joining (NJ) approach generated in R (R Core Team 2013) using the *ape* package (Paradis et al. 2004). The Kimura-2 parameter model was used to estimate genetic distances, and two close relatives, *Acanthurus coeruleus* (Bloch & Schneider, 1801) and *A. guttatus* (Forster, 1801) were used as outgroups.

Results

Visual identification

A group of experts visually identified the first (13th August 2017) sample as *A. sohal*, and the second sample (20th August 2017) as *A. sexfasciatus*. In addition, measurements of the *A. sohal*, sample were taken as the specimen recovered (Table 1).

Genetic identification

The amplification of the Mediterranean sample collected on 13th August 2017 and suspected to be *Acanthurus sohal* resulted in a CO1 sequence of 653 base pairs (GenBank Accession number MG310258). Genetic identification based on the barcode agreed with the visual identifications. Genetic distance between the Mediterranean sample and *Acanthurus sohal* sequences from Genbank was consistent with within-species range (0.36%). In fact, three sequences available in GenBank were identical to our sample. The average genetic distance between the Mediterranean sample and its closest relative, *A. lineatus* was 8.86%, a value within the range of expected inter-specific values. Grouping of the sample with available sequences of *A. sohal* was well supported (bootstrap values of 100%), and separation of this group from close relatives (*Acanthurus lineatus*) resulted in reciprocally monophyletic groups (Figure 3).

Discussion

The field of citizen science is flourishing, and the contribution of citizens in documenting, understanding, and responding to species introductions has been widely acknowledged by scientists, policy-makers, and conservationists (Thiel et al. 2014; Scyphers et al. 2015). Although some scientists remain skeptical about the reliability of citizens to detect and adequately characterize ecological changes (Brandon et al. 2003; Bhattacharjee 2005), engaging citizens in the collection of data can have definite benefits which include increasing social and environmental awareness, improved collection of large amount of data, and coverage of large areas with reduced cost. The citizen science project of "iSea" was launched in May 2016 with the aim to record information on the occurrence, distribution and expansion of marine alien species in Greece and Cyprus. To date, the project has gathered a vast amount of information regarding the presence of non-indigenous species (NIS) in Greece, including several new records, helping to further elucidate the current state of the country's marine bioinvasions. Specifically, the project database currently numbers more than 800 NIS observations from four Mediterranean countries (Greece, Cyprus, Albania and Turkey). Similar initiatives are also available from other Mediterranean areas in the form of Facebook groups, e.g. "Mediterranean Marine Life" (see Bariche and Azzurro 2016; Langeneck et al. 2017) and "Oddfish", or web based platforms such as seawatchers.org (see Azzurro et al. 2013).

Through the iSea citizen science project, the present study documents the first records of *Abudefduf sexfasciatus* and *Acanthurus. sohal* in the Mediterranean Sea. We think that both ballast water and Lessepsian migration vectors can be excluded as possible introduction pathways for these species. Indeed, larval duration is too short for ballast transport and, to our best knowledge, there are no records of these two species in areas closer to the Suez Canal. Moreover, both *A. sexfasciatus* and *A. sohal* are visually conspicuous fishes and would not have gone undetected in the area. To us, the most likely vector is aquarium release. Both species are highly commercialized and popular species in the aquarium industry, but are also very demanding pets given their aggressive behaviour (Lieske and Myers 1994). An *A. sohal* individual was observed in Florida USA in 2003, once again far away from its native range, and the authors directly linked the introduction to an aquarium release or escape (Semmens et al. 2004).

Due to the strict environmental requirements of these two species (primarily temperature), the probability of establishing permanent populations in the Mediterranean Sea seems unlikely. However, their likely release from aquaria raise concerns about the level of awareness and invasive species education of aquarium-holders in Greece. The aquarium trade is one of the most significant pathways of introduction of NIS, responsible for some 18 species introductions in the Mediterranean Sea (Zenetos et al. 2016), including five Acanturids (Langeneck et al. 2012; Langeneck et al. 2015; Weitzmann et al. 2015; Evans et al. 2017; Marcelli et al. 2017). Thus, it is important that policy-makers, conservationists, scientists and pet-shop owners take action in order to establish good practices and educate aquarium users.

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