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# The Indo-Pacific Sergeant *Abudefduf vaigiensis* (Quoy & Gaimard, 1825) (Perciformes: Pomacentridae) in Libya, South-Central Mediterranean Sea

David Osca <sup>1</sup>, Valentina Tanduo <sup>1</sup>, Francesco Tiralongo <sup>2,3</sup>, Ioannis Giovos <sup>4</sup>, Sara A. A. Almabruk <sup>5</sup>, Fabio Crocetta <sup>1,\*</sup>  and Jamila Rizgalla <sup>6</sup>

<sup>1</sup> Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, I-80121 Napoli, Italy; david.osca@szn.it (D.O.); valentina.tanduo@libero.it (V.T.)

<sup>2</sup> Ente Fauna Marina Mediterranean, Scientific Organization for Research and Conservation of Marine Biodiversity, I-96012 Avola, Italy; francesco.tiralongo@unict.it

<sup>3</sup> Department of Biological, Geological and Environmental Science, University of Catania, I-95131 Catania, Italy

<sup>4</sup> iSea, Environmental Organization for the Preservation of the Aquatic Ecosystems, GR-55438 Thessaloniki, Greece; ioannis.giovos@isea.com.gr

<sup>5</sup> Zoology Department, Faculty of Science, Omar Al-Mokhtar University, P.O. Box 919 El Bayda, Libya; sara.almabruk@omu.edu.ly

<sup>6</sup> Department of Aquaculture, Faculty of Agriculture, University of Tripoli, P.O. Box 13275 Tripoli, Libya; jamilarizgalla@gmail.com

\* Correspondence: fabio.crocetta@szn.it

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**Abstract:** The Indo-Pacific Sergeant *Abudefduf vaigiensis* (Quoy & Gaimard, 1825) (Chordata: Pisces: Actinopterygii: Perciformes: Pomacentridae) is first recorded in the south-central Mediterranean Sea (Libya), based on the external morphology and the barcoding of a fragment of the *cytochrome c oxidase subunit I* gene. Present sightings from field surveys and social media include juveniles, sub-adults, and a single adult specimen, suggesting that the species is now established in Libyan waters. No certainties occur regarding timing and possible pathway of arrival of this species in the area, and it may have simply gone undetected for years. The joint effort of field studies and citizen science projects in collaboration with international organizations continues shedding light on bioinvasions in Libya, with valuable outcomes for the Mediterranean marine biology as a whole.

**Keywords:** coastal monitoring; citizen science; alien species; bioinvasions; damselfishes

## 1. Introduction

Biological pollution is a common phenomenon worldwide. Although marine invasions have been widely documented all over the world [1], they are very relevant in the Mediterranean Sea, where native species are often even outnumbered by congeneric or confamilial taxa [2]. The family Pomacentridae Bonaparte, 1831 in the Mediterranean Sea, as an example, includes a single native species, *Chromis chromis* (Linnaeus, 1758), and seven additional alien or cryptogenic taxa, namely *Abudefduf hoefleri* (Steindachner, 1881), *Abudefduf saxatilis* (Linnaeus, 1758), *Abudefduf sexfasciatus* (Lacepède, 1801), *Abudefduf vaigiensis* (Quoy & Gaimard, 1825), *Chrysiptera cyanea* (Quoy & Gaimard, 1825), *Chrysiptera hemicyanea* (Weber, 1913), and *Stegastes variabilis* (Castelnau, 1855) (Table 1, Figure 1).

**Table 1.** Records of alien and cryptogenic taxa belonging to the family Pomacentridae Bonaparte, 1831 from the Mediterranean Sea, with origin, country and locality with coordinates, year of first sighting, number of specimens (N), environment, method of observation, and references (R) as listed in literature (see also Figure 1). Abbreviations used: AO—Eastern and Western Atlantic Ocean; EA—Eastern Atlantic; IP—Indo-Pacific; IWP—Indo-West Pacific; WA—Western Atlantic.

Taxon	Origin	Country and Locality	Year	N	Environment	Method	R
<i>Abudefduf hoefleri</i> (Steindachner, 1881)	EA	Malta, Delimara 35.8352° N, 14.5626° E	2014	1	shallow coastal waters	sampled	[3]
	AO	Spain, Tarragona 41.1310° N, 1.3704° E	2009	1	2 m	observed	[4]
Malta, Valletta Grand Harbour 35.5337° N, 14.3052° E		2013	6-7	close to the cruise-ship passenger terminal	observed	[5]	
Israel, Sdot-Yam 32.2932° N, 34.5315° E		2013	10	shallow rocky reef 1.5–2 m	sampled	[6]	
Malta, Valletta Grand Harbour 35.8982° N, 14.5206° E		2013–2014	3	-	observed	[7]	
Turkey, Çandarlı Bay 38.5144° N, 26.5251° E		2016	1	1–3 m	observed	[8]	
<i>Abudefduf sexfasciatus</i> (Lacepède, 1801)	IP	Greece, Sounio 37.6773° N, 24.0554° E	2017	1	2 m	observed	[9]
	IP	Italy, Gulf of Naples 40.4754° N, 14.1230° E	1957	1	100 m from seashore	sampled	[10]
Israel, Rosh Ha'Nikra 33.0885° N, 35.1053° E		1997	1	littoral platform 1–2 m	sampled	[11]	
Italy, Gulf of Genoa 44.1510° N, 9.2541° E		1998	1	vertical rocky wall 2 m	observed	[12]	
Malta, Valletta Grand Harbour 35.8982° N, 14.5206° E		2013	2	-	sampled	[7]	
Malta, Valletta Grand Harbour and nearby 35.9029° N, 14.5205° E		2015	6	-	sampled	[7]	
Lebanon ~34.0085° N, 35.0211° E		≤2015	1	-	sampled	[13]	
<i>Chrysiptera cyanea</i> (Quoy & Gaimard, 1825)	IWP	Slovenia, Portoroz 45.3048° N, 13.3459° E	2013	1	salt storehouse	observed	[14]

Table 1. Cont.

Taxon	Origin	Country and Locality	Year	N	Environment	Method	R
<i>Chrysiptera hemicyanea</i> (Weber, 1913)	IWP	Malta, Marsaxmett Harbour 35.5445° N, 14.3011° E	2017	1	large rocks and boulders 2 m	observed	[15]
<i>Stegastes variabilis</i> (Castelnaud, 1855)	WA	Malta, Senglea 35.8910° N, 14.5142° E	2013	1	waterfront 2 m	sampled	[16]

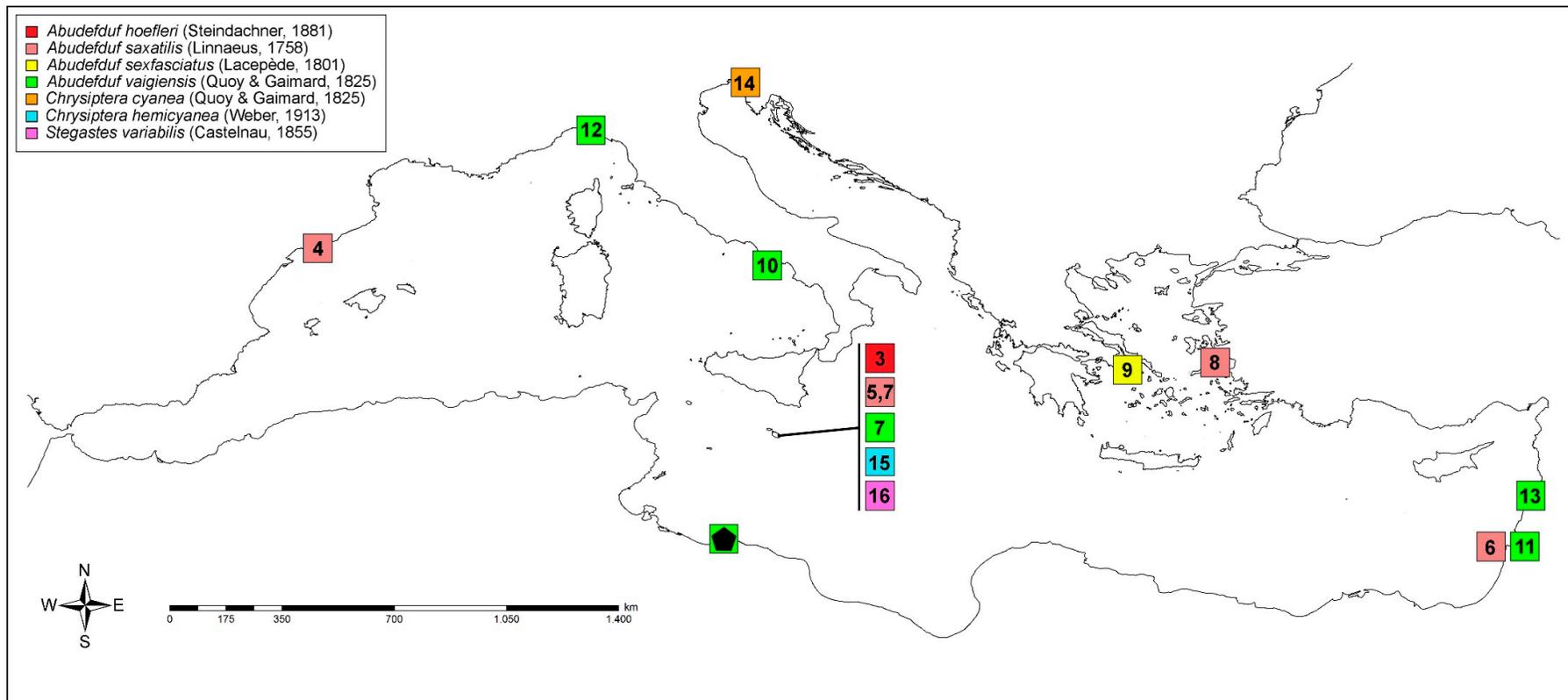


Figure 1. Sightings of alien and cryptogenic taxa belonging to the family Pomacentridae Bonaparte, 1831 from the Mediterranean Sea. Numbers refer to literature records (see also Table 1). Black pentagon refers to present record.

The majority of these species are known from the Mediterranean basin based on single records, some of which were obtained through social media and citizen scientist projects [4,9]. However, the Sergeant-major (*A. saxatilis*) and the Indo-Pacific Sergeant (*A. vaigiensis*) were recorded from larger parts of the Mediterranean Sea for decades, with *A. vaigiensis* being first recorded in Italy more than 60 years ago [4–7,10–13]. In addition, despite putative distinct colour-pattern features should allow their easy visual identification (according to the literature, in *A. saxatilis* the fifth vertical dark bar extends without gap on the posterior margin of the dorsal fin, whereas in *A. vaigiensis* it has a gap between it and the dorsal fin; in *A. saxatilis*, two black spots are present on the caudal peduncle, whereas they are absent in *A. vaigiensis* [6,7,17,18]), the local distribution and state of these two species still remain ambiguous due to misidentifications between the two congeners, with some researchers even doubting the presence of consistent meristic or morphometric differences (Gerald R. Allen, Western Australian Museum, Perth, Australia; pers. comm. [5]). In this light, recent confirmed records from the Mediterranean Sea only relied on barcoding of selected specimens [6,7,13].

The introduction pathway in the Mediterranean Sea of both *A. saxatilis* and *A. vaigiensis* also remains unclear, an uncertainty which is partly due to the patchy distribution with long distances between the observation points (Table 1 and Figure 1). Several pathways were proposed for *A. saxatilis*, including aquarium release, ship transport, or even natural range expansion through the Gibraltar Strait [4,5,19], and the same holds for *A. vaigiensis*, whose proposed pathways included aquarium release, ship transport, or Lessepsian invasion through the Suez Canal [11,13,19–21].

The aim of the present paper is to first report the presence of *A. vaigiensis* in south-central Mediterranean Sea (Libya) based on external morphology and barcoding of a fragment of the *cytochrome c oxidase subunit I* (COI) gene. In addition, we here highlight the additional observation of an *Abudefduf* specimen matching the morphological characters of *A. saxatilis*, thus suggesting that also the occurrence of *A. saxatilis* in Libya may be possible.

## 2. Materials and Methods

The data reported in the present paper fall within the framework of an ongoing project that aims at monitoring the marine biodiversity in Libya by integrating information gained from field surveys and social media (citizen science projects and mining Facebook™ posts).

Field surveys were conducted in two stages, from 14th June to 7th November 2018 and from 5th May to 29th October 2019, in an area of approximately 90 hectares with a 2.2 km long coastline in a natural shallow water bay, situated 12 km west of Tripoli Harbour and known as “Regatta” or “Magribi Arabic Touristic Village”. Along this coastline, there is a shallow water natural bay (32.8539° N, 13.0543° E) formed between two islands subjected to tidal movements. The natural bay consists of sandy and rocky substrates. Snorkel surveys were undertaken, weather permitting, at several locations within the bay and along the island margins, and consisted of a consecutive series of 2–6-h-long snorkel dives up to 2 m depth. Pictures and short pieces of footage were taken in situ using Tough TG-3 and TG-4 Olympus underwater cameras. A single *Abudefduf* specimen was taken with the help of a hand net, and then placed in 90–96% ethanol for preservation and further study.

Six Facebook™ groups with an interest in both recreational and professional fishing were monitored daily for recent postings from January 2018 to September 2019. Furthermore, relevant posts prior to these dates and extending back to 2012 were also screened. Owners of relevant posts were contacted to obtain information on posted material, and permission was sought from the owners of the Facebook™ post.

Genomic DNA of the single *Abudefduf* specimen sampled was extracted from the caudal fin by using the NucleoSpin® Tissue (Macherey-Nagel) kit, following the manufacturer’s protocol. Partial sequence of the COI gene was amplified using the primers developed by Baldwin et al. [22]: forward - FISH-BCL: 5′-TCAACYAATCAYAAAGATATYGGCAC-3′; reverse - FISH-BCH: 5′-TAAACTTCAGGGTGACCAAAAATCA-3′. The polymerase chain reaction (PCR) was conducted in 25 µL volume reaction, containing 2.5 µL of Roche buffer (10×), 2.5 µL (2 mM) of dNTPack Mixture

(Roche), 2 µL each of forward and reverse primers, 0.25 µL (5 U/µL) of Roche Taq DNA polymerase, 1 µL of DNA (15 ng/µL) and sterilized distilled water up to 25 µL. Amplification was performed with an initial denaturation for 5 min at 95 °C, followed by 39 cycles of 1 min at 95 °C, 1 min annealing at 47 °C and 1 min at 72 °C, with a final extension of 5 min at 72 °C. The successful PCR product was purified and Sanger sequenced through an automated DNA sequencer (ABI PRISM 3730) at the Molecular Biology and Sequencing Service of SZN (Naples, Italy). Forward and reverse sequences obtained were assembled using Sequencher v. 5.0.1 (GeneCodes Co.) and compared with reference sequences from the entire NCBI nucleotide (NT) database using BLASTn [23] as to confirm the morphological identification and further verify the diagnostic nucleotide positions reported by Tsadok et al. [6].

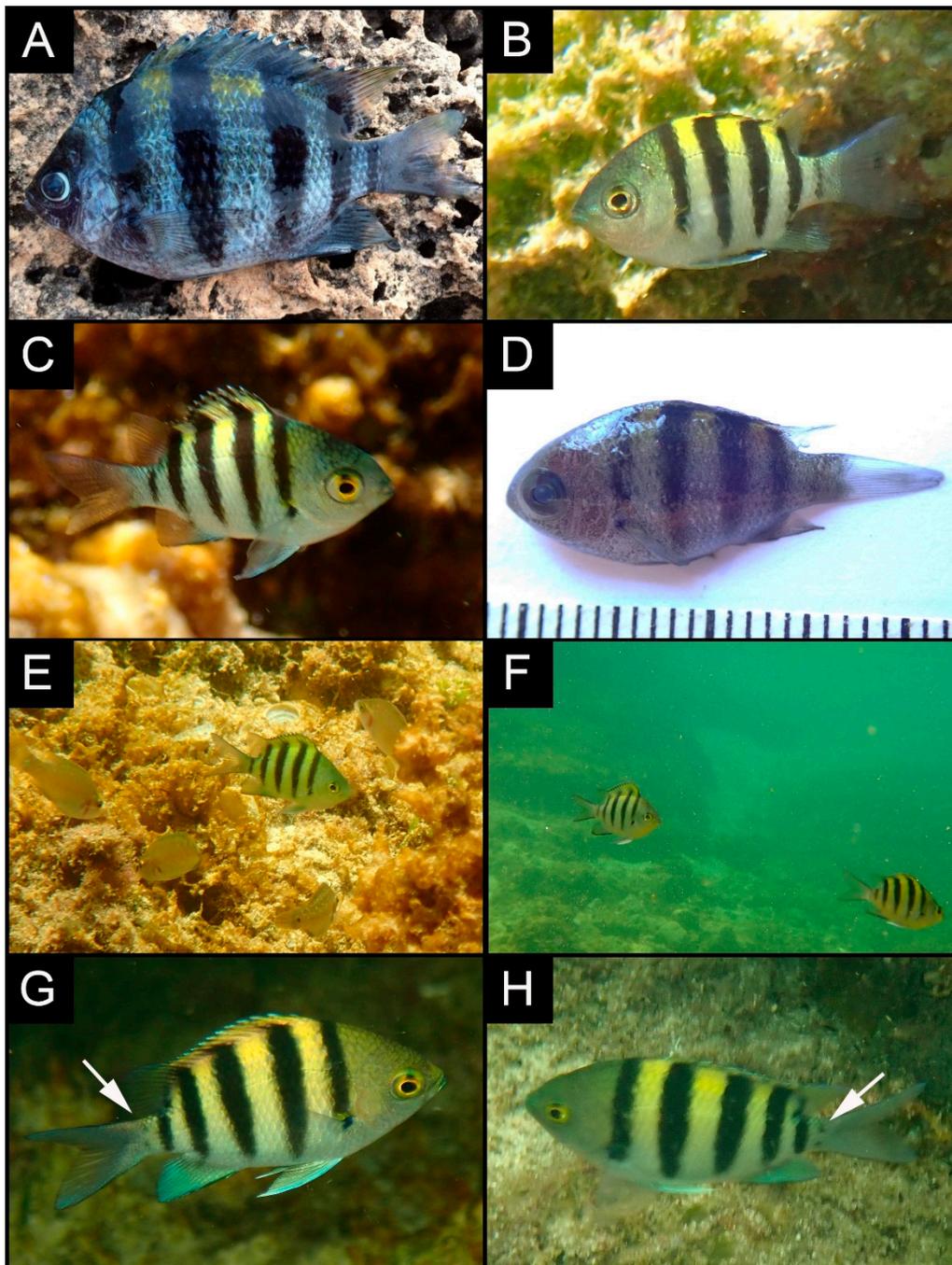
### 3. Results

The field surveys held in Regatta yielded 6 different records, for a total of 9 specimens belonging to the genus *Abudefduf* (Table 2, Figure 2). However, some of these records may be based on the same individuals; in fact, from a cautious point of view, the records reported comprise at least 4 different specimens. The majority of the sightings (6) regarded specimens with an oval-shaped body, silvery-white belly in color with a yellow part around the base of the dorsal fin and five vertical conspicuous black bars, four on the trunk and the fifth located on the caudal peduncle, thus coinciding with morphological characters of *A. vaigiensis*. Among specimens showing such characters, a single juvenile individual (TL ~2.2 cm) was sampled on the 25th August 2019. All remaining individuals were only photographed, and these comprise at least a pair of larger individuals (TL ~5 cm) observed on the 18th September 2019. On the other hand, among the observed fishes, an individual showing morphological traits characteristic of *A. saxatilis* (and in particular two black spots on the caudal peduncle) was seen in three different days during October 2019 (Table 2, Figure 2).

Mining of Facebook™ posts backdated the presence of the genus *Abudefduf* in Libya to as early as the 10th July 2018, when a single adult specimen (TL ~10 cm) of *A. vaigiensis* was sampled at shallow depths in Janzur, a locality 5 km west of Regatta, by a professional fisherman with a trammel net (Table 2, Figure 2).

**Table 2.** Records of specimens belonging to the genus *Abudefduf* Forsskål, 1775 from Libya, with locality, coordinates, date, method of observation, morphological identification, number of specimens (N), GenBank accession number, and figures (see Figure 2).

Locality	Coordinates	Date	Method	Morphological Identification	N	GenBank	Figure
Janzur	32.5028° N, 13.0106° E	10.07.2018	Facebook	<i>A. vaigiensis</i>	1	-	Figure 2A
Regatta	32.8539° N, 13.0543° E	7.09.2018	field survey	<i>A. vaigiensis</i>	1	-	Figure 2B
Regatta	32.8539° N, 13.0543° E	25.08.2019	field survey	<i>A. vaigiensis</i>	1	MN852855	Figure 2C–D
Regatta	32.8539° N, 13.0543° E	18.09.2019	field survey	<i>A. vaigiensis</i>	2	-	Figure 2E–F
Regatta	32.8539° N, 13.0543° E	1.10.2019	field survey	<i>A. cf. saxatilis</i>	1	-	Figure 2G
Regatta	32.8539° N, 13.0543° E	19.10.2019	field survey	<i>A. cf. saxatilis</i> and <i>A. vaigiensis</i>	2	-	-
Regatta	32.8539° N, 13.0543° E	29.10.2019	field survey	<i>A. cf. saxatilis</i> and <i>A. vaigiensis</i>	2	-	Figure 2H



**Figure 2.** Specimens belonging to the genus *Abudefduf* Forsskål, 1775 reported in the present study from Libya. (A–F) *Abudefduf vaigiensis*. (G,H). *Abudefduf cf. saxatilis*. White arrows highlighting the two black spots on the caudal peduncle.

A 663 bp partial sequence of the COI gene was obtained. BLASTn queries generated sequences with a 100% identical score to *A. vaigiensis*, although high similarities (until 99.85%) were also obtained with *A. sexfasciatus*. Our analysis on the diagnostic nucleotide positions revealed: i) two sequences identical to our (AP006016 with an unknown sampling locality, and MF123716 from the Gulf of Aqaba, Red Sea) [24,25]; ii) four sequences (MF123711, MF123713, MF123717, KF186627) from the Gulf of Aqaba and generally the Red Sea with a difference in position 346 [6,25]; iii) one sequence (FJ237571) from India with a difference in position 2 [26]; iv) two sequences (MF123715 and MF123714) from the

Gulf of Aqaba with differences in positions 328 and 346 [25]; and v) one sequence (MF123712) from the Gulf of Aqaba with six differences in positions 58, 81, 105, 322, 346, and 484 [25].

#### 4. Discussion

Molecular results obtained here are in agreement with data reported by Bertrand et al. [27], who noticed that an *A. vaigiensis* lineage was found to be nested within *A. sexfasciatus* in the mitochondrial gene tree, whereas both species were only found to be distinct based on nuclear information. Alternatively, another possibility is that some misidentifications may be at the basis of the sequences deposited in GenBank. However, *A. vaigiensis* and *A. sexfasciatus* clearly differ with regards morphology and color pattern, which definitively excludes *A. sexfasciatus* and confirms that the sequenced specimen from Libya belongs to *A. vaigiensis*. In addition, the 25 diagnostic positions reported by Tsadok et al. [6] to differentiate between *A. vaigiensis* and *A. saxatilis* are lowered here to 22 due to the presence of three *A. saxatilis* diagnostic nucleotide positions (105, 322, and 484) in the MF123712 sequence of *A. vaigiensis*.

To date, records of *A. vaigiensis* and of *A. saxatilis* were still unknown from the Mediterranean coastline of Africa, despite *A. vaigiensis* being first found in the central Mediterranean more than half a century ago [10], whereas *A. saxatilis* has become a commonly encountered taxon along the easternmost coastline of the Mediterranean Sea [6]. The present record of *A. vaigiensis* therefore fills a gap in the distribution of this taxon in the newly invaded area, and suggests that the species is now established in Libyan waters. With respect to its congeneric species *A. saxatilis*, records based only on morphology were published in Spain [4] and Malta [5,7]. If these records are true, then the sighting of an Atlantic species in the easternmost Mediterranean Sea [6] could be due to the presence of populations even in the western and central Mediterranean. In this light, its potential occurrence in Libya may also be possible; however, further field work and molecular analysis is needed to confirm that.

The new records reported here immediately raise the question of the origin of these specimens. Indeed, the most plausible hypothesis is that these fishes may have recently spread from nearby detected or yet-undetected populations. However, another plausible hypothesis is also that these and additional central Mediterranean populations may have simply gone undetected since years. In this light, the finding of a larger individual showing morphological features associated with reproductive activity, as well as the discovery of juveniles in two consecutive years, also strongly suggests the presence of this fish in the area well before the dates recorded here, and the possible presence of an undetected population in the area. Alternatively, these specimens may have been locally introduced through ship transport, and the findings in Janzur and Regatta may be the result of different intrusion attempts.

Alien invasion in the Mediterranean Sea is an increasing phenomenon, with reports of fishes held from various countries in the recent years (e.g., [28–32]). Libya, due to its geographic position, situated in the south-central Mediterranean Sea and possessing a coastline of ~1770 km, would constitute a key country to study the spreading of alien, cryptogenic, and newcomer species from the western Atlantic to the eastern Mediterranean and from the Red Sea to the western Mediterranean. However, it still lacks intensive systematic efforts for monitoring invasive marine species [33], a situation which is amplified by the wide political unrest in the region. Despite that, the number of alien species recorded in recent years is increasing thanks to field surveys, citizen-science projects, and international collaborations [33–44]. Therefore, if these joint efforts continue, the outcomes obtained will be valuable and useful for the Mediterranean marine biology as a whole.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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