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RESEARCH ARTICLE

The first records of Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy & Gaimard, 1825) and further notes on the occurrence of sergeant major *A. saxatilis* (Linnaeus, 1758) in Malta: expanding populations of an invasive genus in the Mediterranean Sea

Adriana Vella*, Sandra Agius Darmanin, Noel Vella

Conservation Biology Research Group, Department of Biology, University of Malta, Msida MSD2080, MALTA

***Corresponding author:** adriana.vella@um.edu.mt

Abstract

Morphometric, meristic analyses and genetic barcoding of *Abudefduf* spp. specimens collected from Maltese waters have led to the confirmation of the Indo-Pacific sergeant, *A. vaigiensis* (Quoy and Gaimard, 1825), in the central Mediterranean Sea. Though *A. vaigiensis* was first visually spotted in April 2013, the first specimen was caught in September 2013 from the Valletta Grand Harbour. Additional specimens were subsequently collected, with some showing morphological signs of reproductive activity indicating the establishment of a growing population.During the same study, observations on the occurrence of the sergeant major, *A. saxatilis* (Linnaeus, 1758) were also noted revealing that both species are co-existing in the same area and are expanding their geographical range along the Maltese coast. These findings direct toward sustained research and monitoring efforts on these new invasive species which may rapidly influence local coastal communities.

Introduction

The ongoing invasion of marine species into the Mediterranean Sea, particularly evident by the increasing number of reported alien fish species (Coll *et al.* 2010; Golani 2010; Zenetos *et al.* 2010, 2012; Kalogirou *et al.* 2012; Katsanevakis *et al.* 2014) encourages long-term monitoring and research to be undertaken side by side with sea-user and stakeholder involvement. The latter are often seeking scientific advice on the presence, identification and possible impacts of new species observed or caught in local waters so collaboration with fishermen of every sector from full-time, part-time to sport-fishermen promotes useful data-gathering relevant to understanding the changes in fish catches and fish

communities. At the same time fishermen contribute their experience and effort to marine biodiversity research programmes by working in collaboration with researchers to obtain the information necessary for better management of resources (Vella 2009; Zenetos *et al.* 2009; Kalogirou *et al.* 2012; Azzurro *et al.* 2013). Such collaboration has led to better monitoring for alien species, such as for the genus *Abudefduf* in Maltese waters.

The family of damselfish, Pomacentridae, of which the genus *Abudefduf* is a member, is diverse and circumglobal with over 385 species primarily inhabiting tropical and temperate near-shore, shallow waters (Allen and Woods 1980; Allen 1991; Bessa *et al.* 2007; Feitosa *et al.* 2012; Litsios *et al.* 2012; Cowman *et al.* 2013; Froese and Pauly 2015). Only one member of this family, *Chromis chromis* (Linnaeus 1758), is native to the Mediterranean Sea (Dulčić 2005; Froese and Pauly 2015). However other species of this family are spreading as aliens in the Mediterranean Sea (Coll *et al.* 2010; Golani *et al.* 2014). The sapphire devil, *Chrysiptera cyanea* (Quoy and Gaimard 1825) recorded in Italy (Lipej *et al.* 2014) and the cocoa damselfish, *Stegastes variabilis* (Castelnau 1855) recorded in Malta (Vella 2014 a,b; Vella *et al.* 2015a) are such examples. Pomacentridae species of the genus *Abudefduf* (Linnaeus, 1758) were first recorded in the Mediterranean in 1957 where a young individual, identified as *A. saxatilis vaigiensis*, was caught from the Gulf of Naples in Italy (Tardent 1959). With more recent records, both the Indo-Pacific sergeant *A. vaigiensis* (Quoy and Gaimard 1825) and the sergeant major *A. saxatilis* (Linnaeus, 1758) are described below.

A. vaigiensis has a wide geographic distribution ranging from the Central Pacific, to the eastern coast of Africa including the Red Sea. In the past years the distribution of this species has been expanding easterly with the colonization of the Hawaiian Islands (Severns and Fiene-Severns 1993; Randall 2007; Coleman *et al.* 2014), and westerly into the Mediterranean Sea. The first isolated record for this species in the Mediterranean Sea dates back to 1957 from the Gulf of Naples in Italy (Tardent 1959). This was followed by another western Mediterranean record from the Gulf of Genoa in the Ligurian Sea (Vacchi and Chiantore 2000). Considering only these two records for this species in Italy, Occhipinti-Ambrogi *et al.* (2011) referred to *A. vaigiensis* as a non-established alien species along the Italian coast which has been transported via shipping. However, the scenario in the eastern Mediterranean is different, where possible migration through the Suez Canal to the Levantine Basin has led to the identification of the species in 1997 along the north coast of Israel (Goren and Galil 1998) with a later report of its establishment (Golani *et al.* 2014). In 2015 it was reported in Lebanon (Bariche *et al.* 2015).

The sister species, *A. saxatilis*, was first reported in the Mediterranean in 2009 in Spain through visual reports (Azzurro *et al.* 2013), followed by more recent records from both Malta (Vella 2014a,b; Deidun and Castriota 2014) and Israel

(Tsadok *et al.* 2015) where visual records and specimens were respectively collected in 2013.

*A. vaigiensi*s and *A. saxatilis* have very similar morphological characteristics. On visual inspection of photos or footage, *A. saxatilis* is distinguished by the continuous extension of the fifth dark vertical bar on the posterior margin of the dorsal fin (*A. vaigiensis* has a gap between the bar and the extension) and the occurrence of two black spots on the tail peduncle (absent in *A. vaigiensis*) (de Beaufort 1940; Allen 1991; Randall 1996; Azzurro *et al.* 2013; Froese and Pauly 2015; Tsadok *et al.* 2015). To address the possible confusion between these two very similar congeners, Tsadok *et al.* (2015) used genetic analyses and found that all the specimens sampled from Israel were identified as *A. saxatilis,* therefore shedding some doubt on the possible presence of *A. vaigiensis* in the Mediterranean Sea. However, a more recent study by Bariche *et al.* (2015) genetically barcoded a specimen of *A. vaigiensis* from Lebanon, confirming the occurrence of this species in the Levantine Basin. This study presents data assessing the presence of both of these *Abudefduf* species in Maltese waters since 2013.

Materials and Methods

On 9 September 2013, during the research by the CBRG-UoM in collaboration with Maltese fishermen, an *A. vaigiensis* specimen (Figure 2) of total body length 117.0 mm was captured inside the Valletta Grand Harbour (GPS: 35.898291N, 14.520632E). Three other specimens were subsequently collected on 7 and 23 June 2015 and on 7 September 2015 (GPS: 35.902975N, 14.520526E; GPS: 35.888615N, 14.517597E; GPS: 35.867721N, 14.575003E, respectively) (Figure 1). In total, three specimens of *A. vaigiensis* were collected from the Valletta Grand Harbour (Figures 2 to 4) and another (Figure 5) was collected from a couple of kilometers away (Figure 1). Once the fish were captured the whole specimens were kept for scientific analyses. The diagnostic features that were used in the morphological identification of the specimens according to de Beaufort (1940), Allen (1991), Randall (2007), Froese and Pauly (2015) were analysed under a dissecting microscope.

In addition to the specimens collected, scientific surveys led to visual records of *Abudefduf* spp. that were recorded through photography and videography (dates and locations for these records presented in Figure 1). During these surveys both *A. vaigiensis* and *A. saxatilis* were morphologically identified and distinguished according to de Beaufort (1940), Allen (1991), Randall (1996, 2007), Azzurro *et al.* (2013), Froese and Pauly (2015).

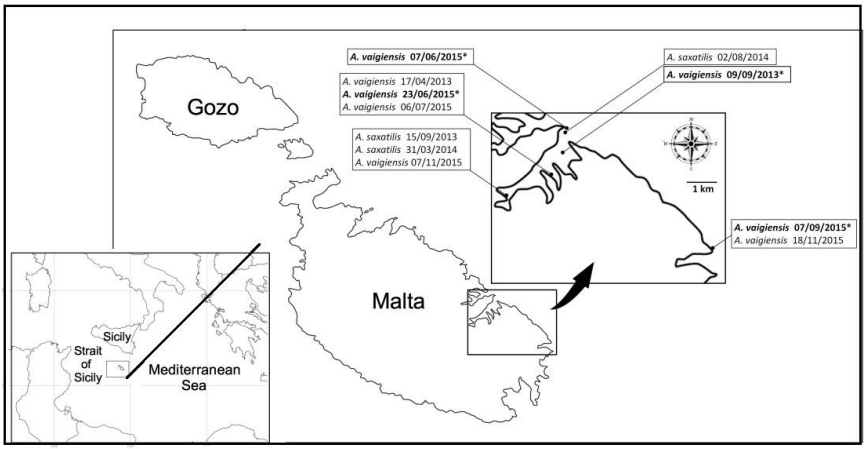

Figure 1. A map of Malta showing the locations and the dates when *A. vaigiensis* and *A. saxatilis* specimens were encountered during ongoing surveys around the Maltese coast. (*****indicates specimens collected refer to Figures 2 to 5)

Genetic analyses:
Tissue samples were collected from the four specimens of *A. vaigiensis* and preserved in non-denatured 95% ethanol for long-term storage. The specimens were subsequently deposited in the ichthyological collection of the Conservation Biology Research Group Laboratory at the University of Malta with reference code numbers:
CBRG/F.130909/AV001; CBRG/F.150607/AV001; CBRG/F.150623/AV001 and CBRG/F.150907/AV001.

Genomic DNA was extracted from muscle tissue and analysed for cytochrome c oxidase I gene (COI); NADH dehydrogenase subunit 3 and its flanking tRNA genes (nd3); cytochrome b (cytb); and 12S to 16S ribosomal RNA genes (12S-16S) as described by Vella *et al.* (2015a), while ATP synthase subunit 6 and its flanking protein coding genes (ATPase) was amplified using L8331 and H9236 as described in Quenouille *et al.* (2004). Blastn was used to compare the current genetic data with that available in genomic databases to confirm the species' identity.

The sequences obtained were deposited in GenBank accession numbers KT283589-91, KU363020 (COI); KT283592-94, KU363021 (ATPase); KT283595-97, KU363022 (nd3); KT283598-600, KU363023 (cytb); and KT283601-03, KU363024 (12S-16S) for CBRG/F.130909/AV001; CBRG/F.150607/AV001; CBRG/F.150623/AV001; and CBRG/F.150907/AV001 respectively.

COI gene sequences for different *Abudefduf* spp. available in GenBank and BOLD, allowed for phylogenetic analyses of specimens sampled locally. The

sequences were aligned using Geneious v6.1.6 (www.geneious.com, Kearse *et al.* 2012). A 550 bp sequence representing the smallest homologous COI sequence was used and genetic divergences were calculated using the Kimura 2-parameter distance model (Kimura 1980), while MEGA v5.2.1 (Tamura *et al.* 2011) was used to construct a phylogenetic tree using Maximum Likelihood.

2a. *Abudefduf vaigiensis* Specimen 1 just after being caught on September 9th 2013 Valletta Grand Harbour, Malta

2b. *Abudefduf vaigiensis* Specimen 1: Reference number: CBRG/F.130909/AV001

3a. *Abudefduf vaigiensis* Specimen 2 just after being caught on June 7th 2015 at Valletta Bridge, Malta

3b. *Abudefduf vaigiensis* Specimen 2: Reference number: CBRG/F.150607/AV001

4a. *Abudefduf vaigiensis* Specimen 3 just after being caught on June 23rd 2015 at Senglea waterfront, Malta showing the intense blue coloration associated with reproductively active individuals.

4b. *Abudefduf vaigiensis* Specimen 3 Reference Number: CBRG/F.150623/AV001

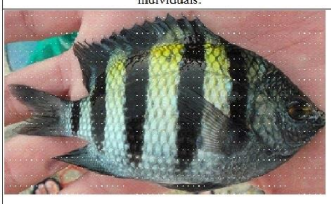

Figure 5a. *Abudefduf vaigiensis* Specimen 4 just after being caught on September 7th 2015 at Zonqor point, Malta.

Figure 5b. *Abudefduf vaigiensis* Specimen 4: Reference Number: CBRG/F.150907/AV001

Figures 2 to 5 (a&b). Photographs of the *Abudefduf vaigiensis* specimens a) immediately after being caught in Malta between 2013 and 2015 and b) with scale and reference number

Figure 6. *Abudefduf saxatilis* observed in Maltese waters since September 2013 first reported by Vella (2014 a&b, CBRG-UoM).

Results

Morphometrics:
The morphology and meristics of the specimens identified as *A. vaigiensis* are presented in Table 1 and Figures 2-5 present the photographs of the specimens a) immediately after being caught and b) subsequently with a scale during analyses. All specimens had five prominent vertical black bars (Figures 2-5), while the body had a white to light bluish colouration with a yellow top in the first, second and fourth specimens, while the third specimen exhibited dark bluish colouration. The colouration of each specimen was more pronounced just after being caught (Figures 2-5). The specimens had XIII+12 to 14 dorsal fin rays, II+11 to 13 anal fin rays and I+4 to 5 ventral fin rays (Table 1). All features agree with those reported by de Beaufort (1940), Allen (1991), Randall (1996, 2007), Froese and Pauly (2015), with the largest specimen exceeding the maximum length quoted in Froese and Pauly (2015).

Apart from *A. vaigiensis*, the authors also briefly reported the presence of *A. saxatilis* (Figure 6) in Maltese waters which was first recorded in September 2013 and mentioned by Vella (2014a,b). The latter species was subsequently reported from visual detection by Deidun and Castriota (2014) in Malta. The current study identified *A. saxatilis* through visual encounters, photographs and videography of various individuals between September 2013 and 2015 confirming an established population (Figure 1).

Genetic analyses:
A total of 3888 bp were sequenced covering a total of 23.3% of *A. vaigiensis* mtDNA. The sequence lengths obtained were 616 bp, 833 bp, 408 bp, 765 bp and 1266 bp for COI, ATPase, nd3, cytb and 12S-16S respectively, and each was run via BLASTn to identify sequence matches. All the genes confirmed the genus with identity matches ranging from 87.9% to 100% at COI; 84.1% to 99.8% at ATPase; 87.0% to 100% at nd3; 87.0% to 100% at cytb; and 99.9% at 12S-16S.

Table 1. Measurements and meristics for *Abudefduf vaigiensis* specimens caught in Malta

Parameter	**Specimen 1**		**Specimen 2**		**Specimen 3**		**Specimen 4**	
Mass (g)	31.25		63.39		225.88		35.30	
Total length (cm)	11.70		14.50		22.00		11.20	
Fork length (cm)	10.10	86.3%TL	12.40	85.5%TL	19.90	90.5%TL	9.80	87.5%TL
Standard length (cm)	9.10	77.7%TL	11.00	75.9%TL	16.90	76.8%TL	8.90	79.5%TL
Maximum body depth (cm)	5.10	56.0%SL	6.55	59.6%SL	9.27	54.9%SL	5.08	57.1%SL
Length of dorsal fin base (cm)	5.20	57.1%SL	6.73	61.2%SL	9.76	57.8%SL	4.84	54.4%SL
Pectoral fin base (cm)	0.97	10.7%SL	1.46	13.3%SL	1.59	9.4%SL	0.87	9.8%SL
Anal fin base (cm)	1.40	15.4%SL	3.90	35.5%SL	3.74	22.1%SL	1.90	21.4%SL
Pre-pelvic length (cm)	3.70	40.7%SL	4.10	37.3%SL	5.90	34.9%SL	2.94	33.0%SL
Pre-anal length (cm)	6.60	72.5%SL	7.30	66.4%SL	10.50	62.1%SL	5.44	61.1%SL
Head length (cm)	2.20	24.2%SL	3.50	31.8%SL	4.95	29.3%SL	2.40	27.0%SL
Pre-orbital length (cm)	1.02	11.2%SL	1.42	12.9%SL	1.49	8.8%SL	0.97	10.9%SL
Eye diameter (cm)	0.80	8.80%SL	1.45	13.2%SL	1.30	7.7%SL	0.73	8.2%SL
Counts								
Dorsal fin spines	13		13		13		13	
Dorsal fin soft rays	12		12		14		12	
Ventral fin spines	1		1		1		1	
Ventral fin soft rays	4		4		5		4	
Anal fin spines	2		2		2		2	
Anal fin soft rays	11		11		13		11	
Pectoral fin soft rays	16		16		18		16	
Lateral line scales	21		22		22		22	
Gillrakers on first arch	28		25		25		25	

At species level, all the specimens had high identity matches (99.8-100%) with other *A. vaigiensis* sequences at all the genes deposited at GenBank. The COI sequences of the Maltese specimens were also compared with another *Abudefduf* sequence from the Mediterranean which was identified as *A. saxatilis* (Tsadok *et al.* 2015). The sequences of these two species differed by 4.6% (Figure 7), genetically identifying the presence of *A. vaigiensis* species in Maltese waters. The local research results merged with that by Bariche *et al.* (2015) genetically confirm *A. vaigiensis* presence in the Mediterranean Sea, with the local study revealing the establishment of a growing population.

The specimens of *A. vaigiensis* analysed in the current study exhibited different haplotypes (Figure 7), suggesting different maternal origins. When considering all the 3888 bp, a total of 19 positions (0.5%) exhibited genetic differences, with the maximum difference between specimens being 11 bp. All the genetic differences led to no changes in the amino acid sequences. The different maternal DNA indicates possible multiple releases of the species through transport in ballast water or aquaria (Vacchi and Chiantore 2000).

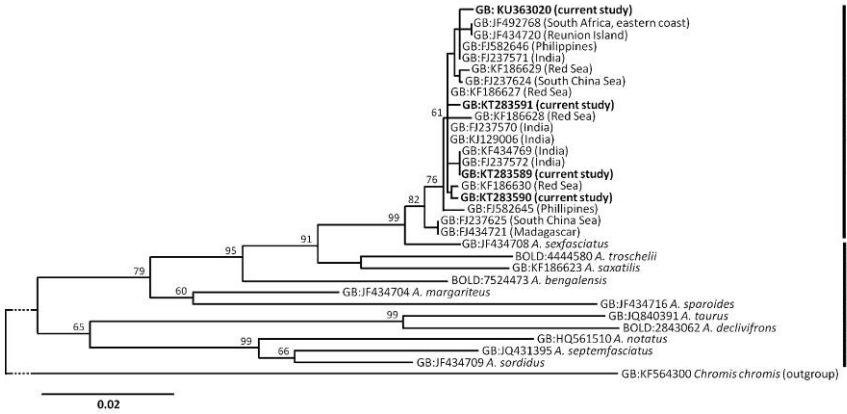

Figure 7. Maximum Likelihood tree of a homologous 550 bp sequence of the COI gene from *Abudefduf* spp. using K2P distances and 1500 bootstraps. The numbers indicate the accession numbers used in the tree construction

Discussion

Visual morphological identification, photographs and footage collected during field surveys were corroborated by molecular techniques and have led to the genetic confirmation of *A. vaigiensis* in Maltese waters. Though different reports, based on morphological identifications, indicated two *Abudefduf* species in the Mediterranean (Golani *et al.* 2014; Vella 2014a,b), some doubt was shed on the possible presence of *A. vaigiensis* (Tsadok *et al.* 2015; Evans *et al.* 2015) since Tsadok *et al.* (2015) carried out genetic analyses on specimens of *Abudefduf* from Sdot-Yam, Israel and concluded that the specimens were all *A. saxatilis*.

The results presented here, however, confirm the presence of an expanding population of *A. vaigiensis* in the Central Mediterranean Sea through this first genetic and morphological study of this species from Maltese waters. This study also reports the occurrence of *A. saxatilis* in the same area at least since 2013 Vella (2014a,b) (Figure 1). As species of this genus have very similar morphological characteristics, one cannot exclude the presence of various *Abudefduf* species in the Mediterranean Sea without detailed examinations.

The introduction of tropical species into the Mediterranean may be possible through various means (Coll *et al.* 2010; Zenetos *et al.* 2010, 2012; Kalogirou *et al.* 2012; Katsanevakis *et al.* 2014). *Abudefduf* spp. have been reported to have colonized new areas by rafting, as juvenile *Abudefduf* spp. have often been observed among marine debris (Gooding and Magnuson 1967; Hunter and Mitchell 1967; Jokiel 1990; Mundy 2005; Nishida *et al.* 2008; Carlton and Eldredge 2009; Coleman *et al.* 2014). This vector is considered responsible for transporting alien species across vast distances (Bryan *et al.* 2012; Williams *et al.* 2013). Members of the Family Pomacentridae are also reported to shelter under large ships (Occhipinti-Ambrogi *et al.* 2011) therefore may travel with these vessels in their ballast waters (Vacchi and Chiantiore 2000; Wonham *et al.* 2000; Galil 2006; Azzurro *et al.* 2013; Vella *et al.* 2015a). Additionally aquaria on cruise liners and the aquarium industry of ornamental species around the Mediterranean are also possible means facilitating alien species introductions (Calado 2006; Papavlasopoulou *et al.* 2014; Katsanevakis *et al.* 2014; Vella *et al.* 2015a,b). Moreover the aquaculture industry, such as that operating a short distance from the sampling sites, may be promoting and sustaining these alien species as studies in Indonesia have shown that *A. vaigiensis* feed on food pellets that pass through the aquaculture cages (Sudirman *et al.* 2009).

From observations presented here *A. saxatilis* and *A. vaigiensis* were both initially recorded in the Valletta Grand Harbour, but since the first visual records in 2013 (Vella 2014a,b) the populations of these two species have been expanding both in numbers and range. Specimens of the former have been recorded just on the outside of the Valletta Grand Harbour, while the latter has recently been recorded 6 km away from the harbour (Figure 1). The dark bluish coloration of the largest *A. vaigiensis* specimen, caught on 23 June 2015 from Maltese waters, may be associated with *Abudefduf* species' reproductive behaviour (Prappas *et al.* 1991; Froese and Pauly 2015).

Apart from the pressure on local biodiversity, the co-occurrence of the tropical Atlantic migrant *A. saxatilis* and the tropical Indo-Pacific lessepsian migrant *A. vaigiensis* in the same harbour marks the first record of sympatric occurrence for these two species. The lack of geographic barriers between these very closely related species may provide fertile grounds for cross-breeding and hybridization among different *Abudefduf* species breaking the barriers between species as has been reported elsewhere (Coleman *et al.* 2014). These different alien fish species presence needs to be followed to assess the evolution of both local and alien fish populations and communities with respective effects on coastal biodiversity and ecosystem functioning (UNEP-MAP RAC/SPA 2010).

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