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Morphological and genetic barcoding study confirming the first Stegastes variabilis (Castelnau, 1855) report in the Mediterranean Sea

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Abstract

This paper presents morphometric and genetic barcoding analyses of the first record of the Cocoa Damselfish, *Stegastes variabilis* in the Mediterranean Sea. A single specimen was captured from Senglea waterfront, Malta (Central Mediterranean) on the 15th of September 2013. The species is non-indigenous in the Mediterranean, as it is native to the tropical Western Atlantic. Apart from undertaking identification through meristics and morphometric measurements of the specimen, genetic analyses of the 3410 bp mtDNA genes were carried out to confirm the species' identity. The latter was useful given that the genus *Stegastes* is known to be composed of morphologically very similar species, with variable colour patterns.

Keywords: *Stegastes variabilis*, alien species, genetic barcoding, fish morphometrics, Mediterranean Sea, Malta

Introduction

Pomacentridae is an ecologically important species-rich and highly abundant Family of reef fishes (Brunt & Davies, 1994) composed of more than 350 species that primarily inhabit tropical and temperate near-shore, shallow waters around the world (Allen & Woods, 1980; Bessa *et al.*, 2007; Cooper, *et al.*, 2009; Feitosa *et al.*, 2012; Litsios, *et al.*, 2012; Froese & Pauly, 2015), with a preference for hard rocky or reef substrates (Bessa *et al.*, 2007). Some species reach a maximum length of 35 centimetres, although most species are usually less than 15 centimetres (Carpenter, 2002; Froese & Pauly, 2015).

Damselfish species in the Mediterranean are represented by the native *Chromis chromis* (Linnaeus, 1758) which is distributed from the eastern Atlantic along the coast of Portugal to the Gulf of Guinea and the Mediterranean Sea (Dulčić, 2005; Froese & Pauly, 2015).

Introduced Pomacentridae species of the genus *Abudefduf* (Linnaeus, 1758) (Goren & Galil 1998; Azzurro *et al.*, 2013; Tsadok *et al.* 2015) have been reported in the Mediterranean in 1957 from the Gulf Naples in Italy (Tardent, 1959), where a young individual was identified as *Abudefduf saxatilis vaigiensis.* Western Mediterranean records followed by another from the Gulf of Genoa in the Ligurian Sea (Vacchi & Chiantore 2000). However there have also been reports of species of this genus entering the Mediterranean Sea through the Suez Canal with a first recorded in Israel in 1998 (Golani *et al.*, 2012), while other reports of this genus have been recorded more recently along the coast of Tarragona in Spain (Azzurro *et al.*, 2013) and in Malta by the authors in 2013 (Vella, 2014 a & b), followed by Deidun & Castriota (2014). These local reports were in turn listed in a review by Evans *et al*. (2015). Another Pomacentridae that has been recorded in the Mediterranean, *Chrysiptera cyanea* (Quoy & Gaimard, 1825), was collected from the Gulf of Trieste, North Adriatic Sea (Lipej *et al.*, 2014).

Methods

On the 15th of September, 2013 during ongoing research by the Conservation Biology Research Group of the University of Malta (CBRG-UoM) in collaboration with Maltese sport fishermen, a single individual (Fig. 1) of total body length 113.4 mm was captured during a shore sport-fishing competition along the Senglea waterfront (GPS: 35.891074N, 14.514230E), Malta. It was caught using a reel rod with paternoster rig set-up using a 0.16 mm line and two hooks size 10, and *Alitta virens* (M. Sars, 1835) as bait placed at a depth of 1 to 2 metres, around 0.5 to 1 metres from the seabed. Upon capture the whole specimen was kept for scientific analyses. The diagnostic features that were used in the morphological identification of the specimen were analysed under a 20X magnification dissecting microscope. All length measurements were taken to the nearest 0.1 mm using electronic calipers and mass was recorded to the nearest 0.1 g.

A small piece of muscle tissue was stored in 96% ethanol, later digested with Proteinase K and the total DNA was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer). PCR amplifications was carried out for the complete sequence of cytochrome b (cytb) using GluDG-L14724, CB3H-15560 and CB6ThrH-15930 primers (Martin & Palumbi, 1993) and NADH dehydro-

genase subunit 3 (nd3) using nd3-F270 and nd3-R750 primers (Cooper *et al.*, 2009). The partial sequence of cytochrome c oxidase I gene (COI) using FishF2 and FishR2 primers (Ward *et al.,* 2005) and 12S to 16S ribosomal RNA genes (12S-16S) using 12SA and 16SA primers (Palumbi, 1996) were also amplified following the protocols of their respective sources. PCR products were sequenced using Applied Biosystems 3730xl. The sequences obtained were deposited in Genbank with accession numbers: KT002410 (cytb); KT002411 (nd3); KT002412 (COI); and KT002413 (12S to 16S). These sequences were compared to other sequences available in GenBank using BLASTn.

Results

Morphological analyses

The fish specimen's morphology, meristics (Table 1) and colour (Fig. 1), matched with the descriptions of the species *Stegastes variabilis* given by Allen & Woods (1980), Allen (1991), McEachran & Fechhelm (2010) and Froese & Pauly (2015). The specimen had XII+15 dorsal finrays, II+12 anal finrays and 1+5 ventral finrays. The body had a dark yellow to brownish colouration (Figure 1 and Table 1) similar to one recorded by Souza *et al.* (2011).

This specimen lacked the large black spot on the rayed section of the dorsal fin, possibly because the occurrence of this spot is mostly visible in juveniles (Allen, 1991). Moreover Souza *et al.*, (2011) has noted that *S. variabilis* exhibits a wide range of colouration and did not exclude the possibility of an undescribed species. For this purpose, a molecular genetics approach was considered to confirm the species' identification.

Genetic analyses

A total of 3410bp were sequenced, 543 bp, 1140 bp, 351 bp and 1376 bp obtained from COI, cytb, nd3 and 12S-16S respectively, and each were run via blastn to identify sequence matches. The cytb and nd3 gene sequences confirmed the genus, as the closest identity matches (78.1% to 92.6% and 80.0% to 98.0% respectively) were with those of the genus *Stegastes*. The 543 bp of COI sequence resulted in 100% identity match to *S. variabilis* [gb|JQ842713; JQ842714; JQ841972 (Weigt *et al.*, 2012)]. Additionally, the 308 bp portion of the 12S rRNA gene sequence also gave a 100% identity match to *S. variabilis* [gb|AF285938 (Tang, 2001)], confirming the species identification of the specimen. The latter forms part of the CBRG-UoM marine specimen and tissues collection with the reference number: CBRG/F.130915/SV001.

Discussion

These results provide relevant scientific data related to the first record of *S. variabilis* in the Maltese waters and the Mediterranean Sea. These results also confirm that this first documented occurrence of *S. variabilis* in the Mediterranean Sea reported by Vella (2014 a & 2014 b) and subsequently listed in Evans *et al.*'s (2015) review as '*questionable*' is in fact certain and confirmed as shown by the results presented here.

Most of the alien fishes recorded in the Mediterranean are considered to be Lessepsian migrants (Golani, 2010) which entered through the Suez Canal, followed by adaptation in the Levantine Sea before spreading westerly throughout the Mediterranean Sea (Psomadakis *et al.*, 2009; Jribi & Bradai, 2012; Souissi *et al.*, 2014; Evans *et al.* 2015). As *S. variabilis* has never been recorded in any other region of the Mediterranean Sea its occurrence in the Southern Central Mediterranean indicates that this specimen may have not been spotted before due to its close resemblance to the Mediterranean damselfish, *C. chromis*. Moreover, the natural habitat of *S. variabilis* consists of inshore and offshore algal and rocky reefs, usually at 0 to 30 metres (Allen, 1991; McEachran & Fechhelm, 2010), a habitat similar to that occupied by *C. chromis*. These two factors make it difficult for the occurrence of this alien species to be detected.

This alien species could have been carried from its natural habitat range (Western Atlantic) by vessel transportation, such as in ship ballast water tanks (Galil, 2006) given that the specimen was found in a port which is regularly visited by large yachts, cruise-liners and other commercial ships. Another possibility is that the specimen was transported for the aquarium trade given that this species is also reported as an ornamental species both outside and within the Mediterranean (Monteiro-Neto *et al.,* 2003; Papavlasopoulou *et al.,* 2014), similarly to the above mentioned *C. cyanea* (Lipej *et al.*, 2014).

The occurrence of *S. variabilis* in the Mediterranean Sea must be closely traced, as the damselfish of the genus

Fig. 1: The individual *Stegastes variabilis* caught from Senglea Waterfront, Malta on the 15th of September 2013 (image by S. Agius Darmanin, CBRG-UoM).

Table 1. Measurements and meristics for *Stegastes variabilis* specimen caught in Malta.

Measurements	mm	Proportion %
Total length (TL)	113.4	
Fork length (FL)	105.9	93.39 TL
Standard length (SL)	83.0	73.19 TL
Maximum body depth (BD)	46.7	41.18 TL
Length of dorsal fin base	56.7	68.31 SL
Pectoral fin base	4.5	5.42 SL
Anal fin base	21.1	25.42 SL
Pre-pelvic length	31.6	38.07 SL
Pre-anal length	61.6	74.21 SL
Head length (HL)	28.2	24.87 TL
Pre-orbital length	7.3	25.89 HL
Eye diameter	6.9	6.08 TL
Counts		
Dorsal fin spines	12	
Dorsal fin soft rays	15	
Ventral fin spines	1	
Ventral fin soft rays	5	
Anal fin spines	2	
Anal fin soft rays	12	

Stegastes are considered to be among the most aggressive and highly territorial benthic feeders (Letourneur *et al.*, 1997; Santangelo *et al.*, 2002; Osório *et al.*, 2006; Hutchings *et al.*, 2008; Steele *et al.*, 2010), and show to have significant impact on coral community structure and algal assemblages (Gobler *et al.*, 2006), increasing the algal biomass and productivity of reef systems (Hinds & Ballantine, 1987; Ferreira *et al.*, 1998; Ceccarelli, 2007), and thus have a potential of altering the ecosystems which they inhabit.

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