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The first record of the African Sergeant, *Abudefduf hoefleri* (Perciformes: Pomacentridae), in the Mediterranean Sea

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Abstract

Background: Alien fish species are increasing in the Mediterranean Sea, urging closer monitoring of coastal habitats and biodiversity towards achieving timely assessment and management. Among the alien fish species found in Maltese waters there are members of the *Abudefduf* genus.

Methods: Research work undertaken with the cooperation of fishermen allows for additional monitoring effort to record the presence of alien species and their possible impacts. One such alien fish specimen collected from Maltese waters in January 2014 was studied through morphological, meristic and genetic analyses. Mitochondrial DNA barcoding analyses were undertaken to confirm the species' identity given that the specimen found belonged to the genus *Abudefduf* which is composed of a number of congeners that have similar morphological characters.

Results and conclusion: This research led to the identification of the first record of the African Sergeant, *Abudefduf hoefleri* (Steindachner 1881) (Perciformes: Pomacentridae), in the Mediterranean Sea. This alien tropical species is native of the East Atlantic and may aggressively compete with native Mediterranean reef species for territories and resources.

Keywords: African sergeant, *Abudefduf hoefleri*, Alien, Morphometrics, mtDNA barcoding, Mediterranean Sea, Malta

Background

The Pomacentridae is a highly diverse family of reef fishes that primarily inhabit tropical and temperate shallow waters around the world (Allen & Woods 1980; Allen 1991; Bessa et al., 2007; Cooper et al., 2009; Feitosa et al., 2012; Litsios et al. 2012; Froese and Pauly 2013). Till a few years ago, the only representative of this Family in the Mediterranean Sea was the native *Chromis chromis* (Linnaeus 1758) which is distributed from the eastern Atlantic along the coast of Portugal to the Gulf of Guinea and throughout the Mediterranean Sea (Dulčić 2005; Froese & Pauly 2013). However, in the past two decades, alien species have been on the increase in the Mediterranean Sea (Coll et al., 2010; Golani et al., 2014), including species that are not natural migrants from neighbouring waters (Vacchi et al., 2010; Lipej et al., 2014; Vella et al., 2015 and 2015). Recent records of non-native Pomacentridae members in the Mediterranean include *Chrysiptera cyanea* (Quoy and Gaimard 1825) that was collected from the Gulf of Trieste, North Adriatic Sea (Lipej et al., 2014) and *Stegastes variabilis* (Castelnau 1855) that was recently caught from Maltese waters (Vella 2014a and 2014b; Vella et al., 2015a), in the central Mediterranean Sea.

The genus *Abudefduf* (Linnaeus, 1758) was first recorded in the Mediterranean in 1957 (Tardent, 1959), with several more recent records of *A. vaigiensis* (Goren & Galil, 1998; Vacchi & Chiantore, 2000; Golani et al., 2014; Vella 2014a and 2014b), and *A. saxatilis* (Azzurro et al., 2013; Vella 2014a and 2014b; Deidun & Castriota 2014; Tsadok et al., 2015) at various coastal locations around the Mediterranean. However, there have been no records of the African sergeant, *A. hoefleri* in this region. This fish is native to the tropical Eastern Atlantic region, ranging from Senegal to Benin, including Ilheu das Rolas, Sao Tome and Cape Verde (Loris & Rucabado 1990; Edwards et al., 2001; Cooper et al., 2009; Cowman & Bellwood, 2013), with its most northern records being the Canary Islands (Cooper et al., 2009; Triay-Portella et al., 2015).

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Results and discussion

Morphometrics

The specimen caught weighed 94.98 g and had a total body length of 174.0 mm. This length is close to the maximum size recorded for this species (Froese & Pauly, 2013). Its appearance, morphology and meristics presented in Tables 1 and 2 and Figs. 1 and 2 matched the descriptions of *A. hoefleri* given by Allen (1991), Edwards et al. (2001), and Froese & Pauly (2013). This specimen had XIII + 14 dorsal finrays, II + 13 anal finrays, I + 5 ventral fin rays and 18 pectoral finrays. The body had a dark bluish colouration with four darker blue strips which were mostly pronounced just after being caught (Fig. 1).

Genetic analyses

A total of 3992 bp were sequenced from the *A. hoefleri* specimen's mtDNA. The sequence lengths obtained were 606 bp, 833 bp, 458 bp, 829 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 high identity matches. At species level, the corresponding sequences available in GenBank are limited to one specimen collected from the Canary Islands, studied by Cooper et al. (2009). Therefore, it was not possible to compare all the currently studied genes with other species specific genetic data. Both the current study and that by Cooper et al. (2009) analysed part of the 12S rRNA gene and nd3. When the latter genes were compared against each other, 352 bp of the 12S rRNA gene gave 100 % identity match while 408 bp of nd3 gave a 99.8 % identity match with FJ616290 and FJ616506 respectively Cooper et al. (2009), thus confirming the species identity. Additionally, phylogenetic analyses of the genus have placed the currently studied *A. hoefleri* specimen on a distinct branch, with the closest congeners being *A. troschelii* and *A. saxatilis* (Fig. 3).

Table 1 Measurements for the *Abudefduf hoefleri* specimen caught in Malta

Parameter	*A. hoefleri* Specimen	*Proportion %*
Mass (g)	94.98	
Total length (mm)	174.0	
Fork length (mm)	145.3	83.5 % TL
Standard length (mm)	127.3	73.1 % TL
Maximum body depth (mm)	69.1	54.3 % SL
Length of dorsal fin base (mm)	74.3	58.4 % SL
Pectoral fin base (mm)	13.0	10.2 % SL
Anal fin base (mm)	32.2	25.3 % SL
Pre-pelvic length (mm)	39.6	31.1 % SL
Pre-anal length (mm)	78.5	61.7 % SL
Pre-pectoral length (mm)	31.4	24.7 % SL
Head length (mm)	35.7	28.0 % SL
Pre-orbital length (mm)	10.3	8.1 % SL
Eye diameter (mm)	10.4	8.2 % SL
Depth of caudal peduncle (mm)	19.6	15.4 % SL

Table 2 Meristics for the Abudefduf hoefleri specimen caught in Malta

Parameters measured for *A. hoefleri* specimen	Counts
Dorsal fin spines	XIII
Dorsal fin soft rays	14
Ventral fin spines	I
Ventral fin soft rays	5
Anal fin spines	II
Anal fin soft rays	13
Pectoral fin soft rays	18
Gill rakers	25
Lateral line scales	20

Discussion

Unlike most of the alien fish species noted in the Mediterranean which have entered through the Suez Canal (Occhipinti-Ambrogi et al., 2011; Kalogirou et al. 2012; Golani et al., 2010; Golani et al., 2014; Vella et al., 2015b), this new alien species has probably entered through the Strait of Gibraltar, given that its typical range is of tropical eastern Atlantic origin (Loris & Rucabado 1990; Edwards et al., 2001; Cowman & Bellwood, 2013). The lack of records of *A. hoefleri* in other regions of the Mediterranean Sea might indicate that this species is not a natural migrant but has been introduced through shipping activity, especially given that the occurrence of this species has been noted to coincide with the presence of port activities and oil platforms in the Canary Islands (Triay-Portella et al., 2015). At Mediterranean level, shipping activity is one of the main vectors introducing alien marine

Fig. 1 *Abudefduf hoefleri* just after it was caught showing its intense blue coloration

Fig. 2 *Abudefduf hoefleri* a few hours after being caught

species in the region (Galil, 2006; Katsanevakis et al., 2014). The record presented here occurred in a location very close to harbours, transhipment hubs and off-shore bunkering sites in the Malta. Moreover, the specimen was caught from the semi-enclosed embayment of the *Ħofra ż-Żgħira* (Fig. 4), an area that receives thermal effluent discharges from a nearby power station. On exit, this effluent has a temperature higher than the ambient temperature, causing the water at the *Ħofra ż-Żgħira* to have temperatures up to 8 °C warmer than surrounding ambient temperatures (Enemalta, 2011). The warmer environmental conditions at this site may have helped this tropical species survive. Nonetheless one cannot exclude the possibility that this species might be a very recent natural migrant which has not been yet recorded in other stepping stone locations and is expanding its natural home range, possibly colonizing regions that are undergoing gradual warming. The latter being reported as facilitating immigration and the survival of exotic species in the Mediterranean Sea (Occhipinti-Ambrogi & Galil, 2010).

Conclusion

Ongoing monitoring may verify the spread or loss of this non-indigenous species in these waters. The already at risk Mediterranean fish species (Coll et al. 2010) are being faced with increasing threats from alien species (Coll et al., 2015). As a member of the genus *Abudefduf*, this new alien can be aggressively competitive towards other fishes, therefore like other alien species its occurrence can have detrimental effects to coastal fishes communities with potential negative repercussions on marine conservation of local species.

Methods

On the 4th of January 2014 a specimen of *A. hoefleri* (Figs. 1 and 2) was caught in shallow coastal waters close to Delimara, Malta [GPS: 35.835260 N, 14.562635E] (Fig. 4) during sport-fishing being monitored by researchers from the Conservation Biology Research Group of the University of Malta. 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 according to Allen (1991), Edwards et al. (2001), and Froese & Pauly (2013). All length measurements were taken to the nearest 0.1 mm using electronic calipers and mass was recorded to the nearest 0.01 g.

Tissue samples were collected from this specimen and preserved in 95 % ethanol. The specimen was subsequently deposited in the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta with reference code number CBRG/F.140104/AH001. For genetic analyses, the tissue was digested with Proteinase K and the total DNA was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer). PCR amplifications were carried out for: cytochrome c oxidase I gene (COI) using FishF2 and FishR2 primers (Ward et al., 2005); ATP synthase subunit 6 and its flanking protein coding genes (ATPase) using L8331 (Meyer 1993) and H9236 primers (Quenouille et al., 2004); NADH dehydrogenase subunit 3 and its flanking tRNA genes (nd3) using nd3-F270 and nd3-R750 primers (Cooper et al., 2009); cytochrome b (cytb) using GluDG-L14724 and CB3H-15560 primers (Martin and Palumbi 1993); and 12S to 16S ribosomal RNA genes (12S-16S) using 12SA and 16SA primers (Palumbi 1996). The amplification for each primer set was performed using an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s,

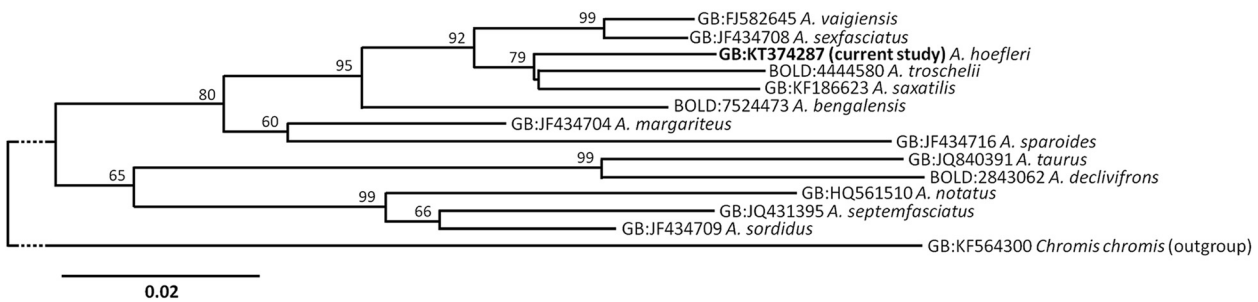

Fig. 3 Maximum Likelihood tree of a homologous 549 bp sequence of the COI gene from *Abudefduf* spp. using K2P distances and 1500 bootstraps. The numbers indicate the accession numbers used in the tree construction

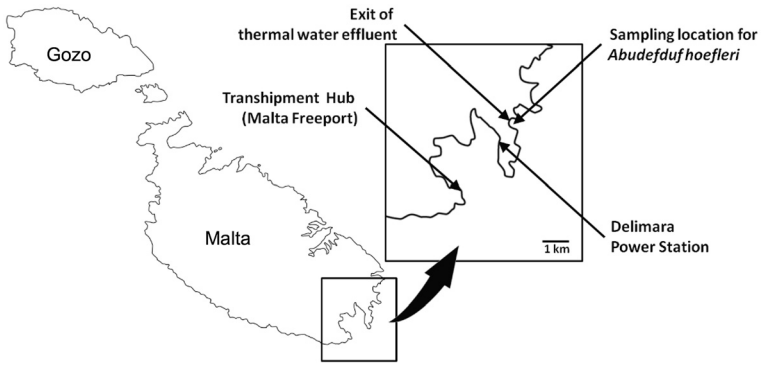

Fig. 4 Map showing the location from where the *Abudefduf hoefleri* specimen was caught

annealing at 52 °C for 45 s and extension at 72 °C for 40 s and a final extension at 72 °C for 10 min, except 12S-16S where an annealing temperature of 48 °C was used. The PCR products were purified and sequenced via ABI3730XL sequencer using both the forward and reverse primers. The sequences, at both nucleotide and amino acid level, were analyzed using Geneious v6.1.6 (http://www.geneious.com, Kearse et al., 2012). The sequences obtained were deposited in GenBank accession numbers KT374287-91 for COI, ATPase, nd3, cytb and 12S-16S respectively. These sequences were compared to other sequences available in genomic databases using BLASTn.

Various COI gene sequences for different *Abudefduf* species available in GenBank and BOLD, allowed for phylogenetic analyses of this specimen. The sequences were aligned using Geneious v6.1.6 (http://www.geneious.com, Kearse et al., 2012). A 549 bp sequence representing the smallest homologous COI sequence was selected and genetic divergences were calculated using the Kimura 2-parameter distance model (Kimura 1980), while the phylogenetic tree was constructed using 1500 bootstraps with Maximum Likelihood utilizing MEGA v5.2.1 Tamura et al. (2011).

Availability of supporting data

The genetic sequence data supporting the results of this article are available in GenBank, as accession numbers KT374287-91 for COI, ATPase, nd3, cytb and 12S-16S respectively.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AV has contributed to all aspects of the research work presented here including the conception and design of the molecular genetics research, analyses and interpretation of both genetic and morphological data and was involved in finalizing the manuscript. NV contributed to molecular genetic research, analyses and interpretation. SAD contributed to the morphological research work of the specimen collected. All three authors were involved in the drafting of the manuscript and in giving approval for the version for publication.

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