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Research Article

On the occurrence and identification of *Abudefduf saxatilis* (Linnaeus, 1758) in the easternmost Mediterranean Sea

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Abstract

Specimens of *Abudefduf* sp. collected in the Levantine region were previously assumed to be the Lessepsian-immigrant Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy and Gaimard, 1825). Using phylogenetic analysis of mitochondrial genes cytochrome c oxidase I (COI) and 16S rRNA probed in 10 individuals sampled in the Mediterranean Israel and on 10 individuals sampled in the Red Sea, we established that Atlantic-origin sergeant major *Abudefduf saxatilis* (Linnaeus, 1758) now occurs along the eastern Mediterranean in shallow coastal rocky habitats. This study showed that misidentifications resulting from phenotypic plasticity and conservative morphological analysis may lead to wrong conclusions regarding the biogeography of studied species. In this respect, molecular tools are necessary to rule between the Lessepsian and Atlantic origin of non-indigenous cryptic species.

Key words: sergeant major, Indo-Pacific sergeant, Mediterranean, non-indigenous species, phylogeny

Introduction

Numerous non-indigenous species have recently colonized the Mediterranean Sea and have noticeably affected the local ecosystems. These colonization events have, however, allowed the scientific community to study the processes of arrival, naturalization, and geographic spread on easily accessible spatial and temporal scales (Galil 2000, 2007, 2009; Golani et al. 2007; Zenetos et al. 2005). Fish comprise a significant portion of the Mediterranean marine xenobiota and approximately 70% of the non-indigenous fish species are of Indo – Pacific origin (CIESM 2014). The passage of Red Sea species through the Suez Canal is the major route of introduction of Indo – Pacific species into the Mediterranean Sea (Por 1978). These species, often referred as 'Lessepsian migrants' (Por 1978), are widely distributed along the eastern coast of the Mediterranean, an area that is characterized by warm oligotrophic conditions, often resembling the Lessepsian's original habitat (Galil and Goren 1994; Galil 2000, 2009; Mavruk and Avsar 2008).

The *Abudefduf* (Pomacentridae) genus is represented by tropical and subtropical warm-water fishes that typically occur on coral reefs (Allen 1991). In the past, the Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy and Gaimard, 1825) has often been confused with an Atlantic species, the sergeant major *Abudefduf saxatilis* (Linnaeus, 1758) (Allen 1991). *A. saxatilis* is a common Atlantic representative of the genus, abundant on Caribbean reefs and in the tropical coastal waters of western Africa to Angola (Randall 1996). The closely-related *A. vaigiensis* populates the Indo - Pacific region (Allen 1991). Tardent (1959) provided the first observation of *Abudefduf* from the Mediterranean Sea (Gulf of Naples) and identified it as *A. vaigiensis*. Later on, other *Abudefduf* individuals were reported from both the Levantine basin (Goren and Galil 1998) and the Western Mediterranean (Vacchi and Chiantore 2000). All these individuals were identified morphologically as *A. vaigiensis* with traversing through the Suez Canal considered the most likely route of arrival. As well, this species was considered to be one of the fastest-spreading Lessepsian

species (Zenetos et al. 2010). Yet, the occurrence of *A. saxatilis* from Western and Central Mediterranean was recently reported (Azzurro et al. 2013; Deidun and Castriota 2014). These authors based their identification on two distinctive morphological characteristics of *A. saxatilis*: the extension of the fifth vertical bar from the posterior margin of dorsal and anal fin; and the presence of two black spots on the caudal peduncle (following Beaufort 1940; Allen 1991).

During last three years (2011–2013), individuals from the *Abudefduf genus* established large populations in the shallow rocky habitats of the Israeli coast from South to North and were observed in large schools. Here we provide new observations of *Abudefduf* sp. in the easternmost Mediterranean Sea combined with identification of these specimens using molecular techniques.

Methods

Specimens of *Abudefduf* sp. were sampled from both Mediterranean and Red Sea locations. In the Mediterranean Sea, we collected 10 *Abudefduf* specimens at Sdot-Yam, Israel, on a shallow (1.5-2 m) rocky reef (32°29'32.66"N; 34°53'15.00"E) by angling with bait. In the Red Sea, we collected other 10 specimens of *Abudefduf*, from two different locations off the city of Eilat (29°30'06.65"N; 34°55'03.98"E and 29°29'49.33"N; 34°54'36.84"E). Fish from the Mediterranean and the Red Sea were collected on 10 January 2013 and 15 January 2013, respectively. Immediately after capture, the individuals were photographed and preserved in 76% ethanol and stored at -20°C. Voucher specimens HURedSea1 and HUMed1 were kept in the University of Haifa, Israel.

Total genomic DNA was extracted from each preserved specimen using the Wizard SV Genomic DNA Purification System kit (Promega) according to manufacturer's instructions. The template DNA was amplified using GoTaq Green Master Mix (Promega) with T100 thermal cycler (Bio-Rad). Partial sequence (544 bp) of the mitochondrial cytochrome c oxidase I (COI) gene was amplified using the FishBCL and FishBCH primers (Baldwin et al. 2009). A 480 bp partial mitochondrial 16S rRNA gene fragment was amplified using a primer pair 16SL and16SH (Kocher et al. 1989). The PCR conditions were as follows: denaturation at 94°C for 20 seconds, annealing at 50°C for 30 seconds, and extension at 72^{0}C for 40 seconds for 35 cycles. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega) and subsequently cloned in the pGEM-T Easy Vector (Promega) using Escherichia coli JM109 (Promega) as the host. The sequencing was performed by HyLabs (Israel) using the T7 and SP6 primers. The sequences were submitted to the GeneBank with accession numbers KF186623-KF186630 and KF925346-KF925347.

The COI and 16S rRNA genes datasets used to reconstruct the *Abudefduf phylogenetic* relationships contained 69 and 25 sequences, respectively. The sequence of the green chromis *Chromis viridis* (Cuvier, 1830) (JF434903) was chosen as an outgroup. Alignment was performed with CLUSTALX (Thompson et al. 1997) using default parameter settings and manually refined with BIOEDIT (Hall 1999). The best probabilistic model-of-sequence-evolution was determined with the program MEGA5.1 (Tamura et al. 2011), using the Akaike information criterion (Posada and Crandall 1998). Bayesian analyses using the Markov-chain Monte-Carlo technique were performed by MrBayes v3.1.2 (Ronquist et al. 2011). We used the HKY+G+I model for the COI analysis, HKY+G for 16S rRNA gene analysis (Hasegawa 1985) and initiated an analysis from a random starting tree, four Markov chains sampled every 1000 generations, and were terminated after two million generations once the average standard deviation fell below 0.01. The graphical view of the phylogenetic tree made using MEGA5.1 (Tamura et al. 2011).

Results

The molecular analysis clearly distinguished between Mediterranean and Red Sea specimens. There were between 0 and 2 variations in the 480 bp 16S rRNA sequence between the Mediterranean *Abudefduf* and Genebank *A. saxatilis* sequences. There were 7 fixed differences between Mediterranean *Abudefduf* and *A. vaigiensis* 16S rRNA gene sequences. The *Eilat Abudefduf* 16S rRNA gene sequences were identical to Indo-Pacific *A. vaigiensis* (Figure 1a). Overall, 25 fixed differences between the *A. saxatilis* and *A. vaigiensis* 608 bp COI gene markers were established, yielding a clear diagnosis for both the Red Sea and the Mediterranean specimens (Figure 1b). The Red Sea species belong to Indo- Pacific *A. vaigiensis* group, while the eastern Mediterranean specimens were identified as the Atlantic *A. saxatilis*.

All the sampled specimens from the Mediterranean Sea presented two black spots on the caudal peduncle (Figure 2a). This feature was absent

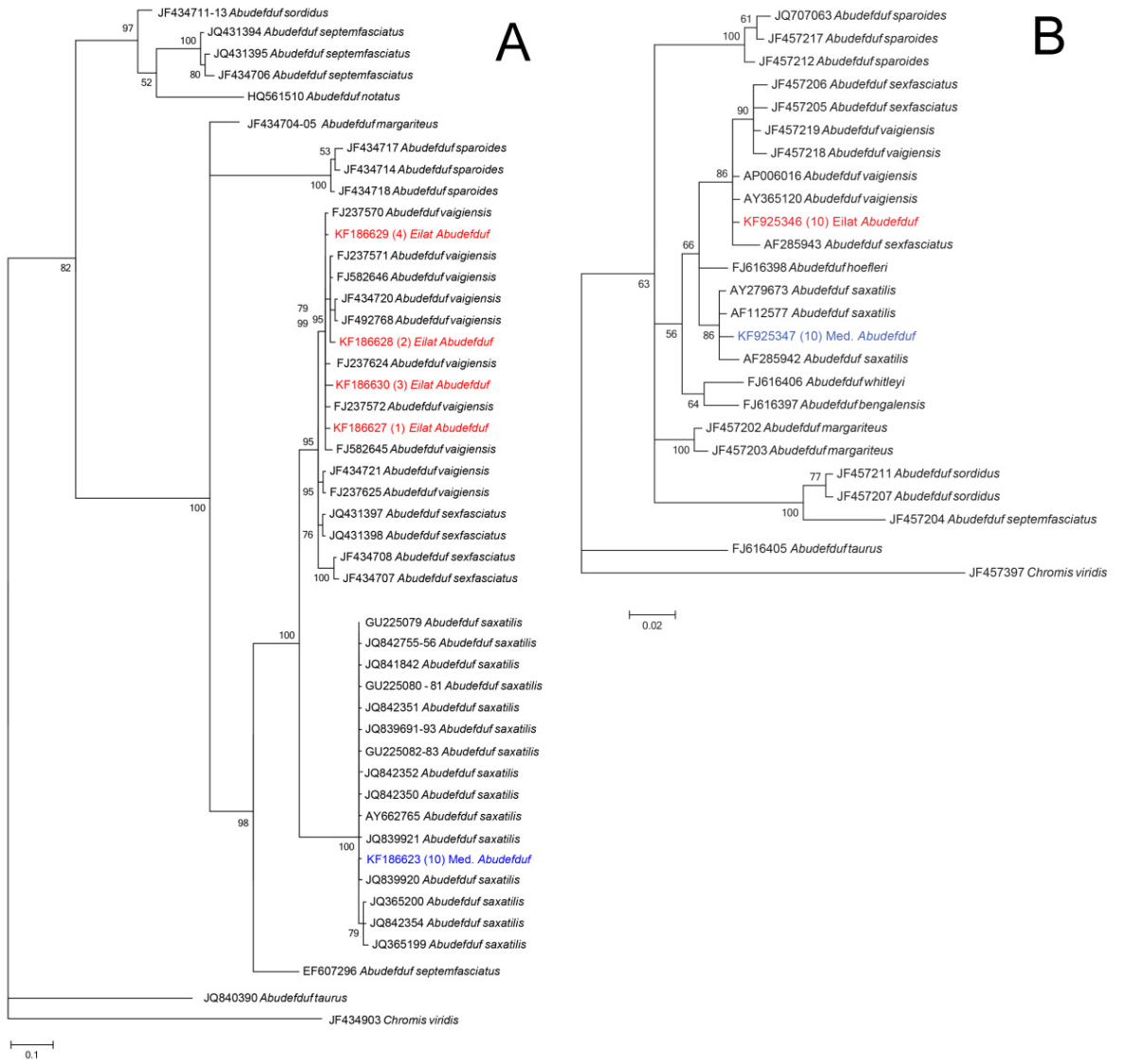

Figure 1. Bayesian analysis of *Abudefduf* 16S rRNA gene (A) and COI gene (B) sequences. At each node, numbers indicate the posterior probabilities (%) recovered by Bayesian analysis using MrBayes. Scale bar represents the number of substitutions per site. Genebank accession numbers are provided for each sequence. Red Sea and Mediterranean *Abudefduf* specimens` branches are marked with red and blue, respectively. The number within the parentheses indicates the number of specimens with respective gene phylotype.

from the Red Sea specimens (Figure 2b). The extension of the fifth vertical bar from the posterior margin of dorsal and anal fin was present in Mediterranean *A. saxatilis*, although to a lesser extent this feature was also observed in young Red Sea specimens (Figure 2b). In Mediterranean *A. saxatilis* specimens the fifth bar extended continuously to the dorsal fin while in the Red Sea *A. vaigiensis* specimens there was a gap between the bar and the extension.

Discussion

Using molecular tools, we have identified *Abudefduf* specimens collected in the eastern Mediterranean as *A. saxatilis* and *Abudefduf* specimens collected from the Red Sea as *A. vaigiensis*. Interestingly, the Red Sea *Abudefduf* sp. was first identified as *A. saxatilis* (Fishelson 1970), and since then the reports regarding the identity

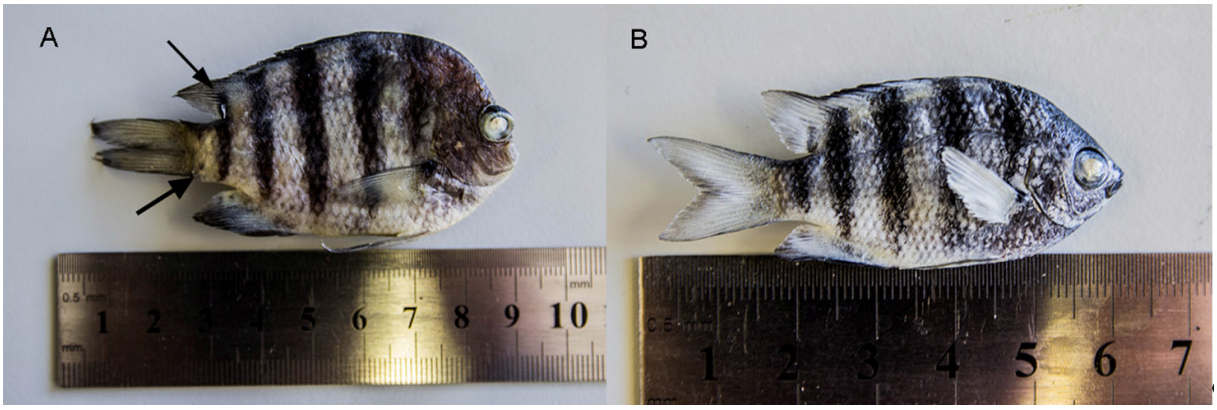

Figure 2. Images of preserved *Abudefduf* specimens. a) Mediterranean specimen *A. saxatilis* voucher HUMed1 (Genebank sequences KF925347 and KF186623), the arrows point at two black spots on the caudal peduncle and the extension of the fifth vertical bar from the posterior margin of dorsal fin. b) Red Sea specimen *A. vaigiensis* voucher HURedSea1 (Genebank sequences KF925346 and KF186627). Photographs by Dr. Maxim Rubin-Bloom.

of specimens collected in the Gulf of Aqaba have been confusing (Goulet 1995; Khalaf and Kochzius 2002). Fish species identification based on morphological description is often hindered by cryptic morphology (e.g. Griffiths et al. 2010; Puckridge et al. 2013; Thomas et al. 2014). Further morphological studies in association with molecular analyses are needed to clarify the stability of the morphological characters used so far for their identification (Azzurro et al. 2013). *A. vaigiensis* has naturally invaded the Hawaiian Islands, where it interbreeds with the local *Abudefduf abdominalis*, establishing a hybrid population (Maruska and Peyton 2007). We cannot exclude the possibility that interbreeding between *Abudefduf* species might also occur in the Mediterranean Sea. In such case, the speciation of *A. saxatilis* and *A. vaigiensis* has shifted to sympatric after being allopatric for last 4 million years (Quenouille et al. 2011).

The presence of *A. vaigiensis* in the western Mediterranean Sea was previously used as an example of the quickly spreading Lessepsian migration (Zenetos et al. 2010). Our findings open the possibility that previous morphological identification of *A. vaigiensis* in the Mediterranean (Goren and Galil 1998; Tardent 1959; Vacchi and Chiantore 2000) could actually be misidentification of the Atlantic *A. saxatilis*. Further genetic analysis of the Mediterranean *Abudefduf* is needed to clarify this point and to assess if *A. vaigiensis* does really occur in the Mediterranean Sea.

Conclusions

Non-indigenous species in the Mediterranean are mostly classified based on morphological traits that, in the case of cryptic species, may lead to erroneous conclusions. In contrast, the molecular approach can provide a near-unequivocal tool for species identification together with further information on population structure and biogeography. The case of *A. saxatilis* in the easternmost Mediterranean Sea reinforces the importance of genetic analysis for the study of Lessepsian bioinvasions (e.g. Bernardi et al. 2010). In the future, the establishment of key species molecular barcoding can help much the study and monitoring of biological invasions in the Mediterranean Sea and elsewhere.

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