First record of *Lutjanus fulviflamma* (Osteichthyes: Lutjanidae) in the Mediterranean Sea

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Abstract

This paper presents the first record of Dory snapper, *Lutjanus fulviflamma* in the Mediterranean Sea. The specimen was caught from Valletta Waterfront, Malta (Central Mediterranean) on the 15th of December 2013. The species was identified through morphometric and meristic characters. Genetic analyses of the mitochondrial DNA sequences (COI, CO2, ND4 and Cytb) verified the species' identity, while phylogenetic analyses revealed that the specimen was of East African origin possibly finding its way into the Mediterranean Sea as a Lessepsian migrant through the Suez Canal due to the ongoing Erythrean invasion.

Keywords: *Lutjanus fulviflamma*, alien, genetics, Mediterranean, Malta

Introduction

The Dory snapper, *Lutjanus fulviflamma* (Forsskål 1775), also known as the blackspot napper, is a widely distributed fish species in the Indo-Pacific Ocean. Its distribution ranges from the Islands of Samoa to Australian coast, along the Southern Asian coast, to the Eastern African coast, up to the Red Sea (Allen 1985; Smith and Heemstra 1986; Froese and Pauly 2015). This carnivorous fish, which reaches a maximum total length of 350mm (Allen 1985; Kamukuru et al. 2005; Nanami et al. 2013) is able to form large schools (Froese and Pauly 2015) and is a commercially valuable species in the Indian Ocean (Allen 1985).

The genus *Lutjanus* is not native to the Mediterranean Sea, however there have been few casual reports of *L. argentinamaculatus* in 1977 (Mouneimne 1979) and *L. jocu* in 2005 (Vacchi et al. 2010). The former is an Indo-Pacific species, possibly a Lessepsian migrant for the Eastern Mediterranean (Zenetos et al. 2005; Oral 2010). On the other hand, *L. jocu* is a tropical western Atlantic Ocean migrant that has been recorded once in the Ligurian Sea (Vacchi et al. 2010).
Material and Methods

On the 15th of December, 2013, a single specimen of the Dory snapper was caught within the Pinto Wharf, Valletta Grand Harbour, Malta (Figure 1; 35°53’19.1’’N 14°30’27.8’’E). The specimen was photographed and measured (Figure 2). The diagnostic features that were used in the morphological identification of the specimen followed those by Allen (1985), Smith and Heemstra (1986) and Froese and Pauly (FishBase 2013-2015). A small muscle tissue sample was then taken from this specimen and preserved in 95% ethanol for long-term storage prior to genetic analyses (specimen collection reference number: CBRG/F.131215/LF001).

Figure 1. Map showing location where the specimen of Lutjanus fulviflamma was caught (indicated as a black spot).

Later 30 mg of the muscle tissue sample was digested with Proteinase K and the total genomic DNA was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer Inc.). PCR amplifications was carried out for the partial sequence of cytochrome c oxidase I gene (COI) using FishF1 and FishR1 primers (Ward et al. 2005), the partial sequence of tRNA-Asp and the complete cytochrome c oxidase II genes (CO2) using COII-forward and COII-reverse primers (Guo et al. 2007). The partial NADH dehydrogenase subunit 4, complete tRNA-His, tRNA-Ser and the partial tRNA-Leu (ND4) using ND4 and Leu-Scyliorhinus primers (Naylor et al. 2005); and partial cytochrome b (Cytb) using GluDGL14724 and CB3H-15560 primers (Martin and Palumbi 1993) were also amplified. The amplification for each primer set was performed using an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 52°C for 45 seconds and extension at 72°C for 40 seconds for 35 cycles. The sequences obtained were deposited in
GenBank accession numbers KT283585 (COI); KT283586 (CO2); KT283588 (ND4); and KT283587 (Cytb). These sequences were compared to other sequences available in GenBank using BLASTn.

Phylogenetic analyses of COI gene sequences for different *L. fulviflamma* specimens of known geographic origins available in GenBank and BOLD, allowed for the determination of the possible origin of the specimen collected in Malta. The sequences were aligned using Geneious v6.1.6 (www.geneious.com, Kearse *et al.* 2012). A 571 bp sequence representing the smallest homologous COI sequence was selected and genetic divergences were calculated using the Kimura 2-parameter distance model (Kimura 1980), with Maximum Likelihood utilizing MEGA v5.2.1 (Tamura 2011).

**Figure 2.** Photograph of the specimen of *Lutjanus fulviflamma* caught from Pinto Wharf, Valletta, Malta.

**Results**

*Morphological analyses*

The specimen caught weighed 105.4 grams and had a total body length of 181 mm (Table 1). This length being close to the size at which this species reaches sexual maturity (Allen 1985; Kaunda-Arara *et al.* 1997). Its appearance, morphology and meristics presented (Table 1 and Figure 2) matched the descriptions of *L. fulviflamma* as reported by Allen (1985), Smith and Heemstra (1986) and Froese and Pauly (2013). This specimen had X+14 dorsal fin rays, I+5 ventral fin rays, III+8 anal fin rays and 15 pectoral fin rays, together with a predominant black spot on the lateral line at the alignment of the anterior part of the soft rays of the dorsal fin. The dorsal and upper sides of the body were green-brown, changing to light yellow on its lower sides becoming white on the belly. The pectoral, pelvic and anal fins were yellow, while the slightly forked caudal fin changed colour from green-brown towards the peduncle to yellow towards the edge. The caudal fin had orange colouration at the tip of both forks (Figure 2).
Table 1. Measurements and meristics for *Lutjanus fulviflamma* specimen caught in Malta

<table>
<thead>
<tr>
<th>Measurements</th>
<th>mm</th>
<th>Proportion %</th>
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<tbody>
<tr>
<td>Total length (TL)</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>Fork length (FL)</td>
<td>173</td>
<td>95.5% TL</td>
</tr>
<tr>
<td>Standard length (SL)</td>
<td>151</td>
<td>83.4% TL</td>
</tr>
<tr>
<td>Maximum body depth (BD)</td>
<td>57</td>
<td>37.7% SL</td>
</tr>
<tr>
<td>Length of dorsal fin base</td>
<td>75</td>
<td>49.7% SL</td>
</tr>
<tr>
<td>Length of pectoral fin base</td>
<td>9</td>
<td>6.0% SL</td>
</tr>
<tr>
<td>Length of anal fin base</td>
<td>23</td>
<td>15.2% SL</td>
</tr>
<tr>
<td>Pre-pelvic length</td>
<td>58</td>
<td>38.4% SL</td>
</tr>
<tr>
<td>Pre-anal length</td>
<td>102</td>
<td>67.5% SL</td>
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<tr>
<td>Pre-pectoral length</td>
<td>48</td>
<td>31.8% SL</td>
</tr>
<tr>
<td>Head length (HL)</td>
<td>53</td>
<td>35.1% SL</td>
</tr>
<tr>
<td>Pre-orbital length</td>
<td>18</td>
<td>11.9% SL</td>
</tr>
<tr>
<td>Eye diameter</td>
<td>12</td>
<td>7.9% SL</td>
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</table>

<table>
<thead>
<tr>
<th>Counts</th>
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<tbody>
<tr>
<td>Dorsal fin spines</td>
<td>10</td>
</tr>
<tr>
<td>Dorsal fin soft rays</td>
<td>14</td>
</tr>
<tr>
<td>Ventral fin spines</td>
<td>1</td>
</tr>
<tr>
<td>Ventral fin soft rays</td>
<td>5</td>
</tr>
<tr>
<td>Anal fin spines</td>
<td>3</td>
</tr>
<tr>
<td>Anal fin soft rays</td>
<td>8</td>
</tr>
<tr>
<td>Pectoral fin soft rays</td>
<td>16</td>
</tr>
<tr>
<td>Lateral Line scales</td>
<td>49</td>
</tr>
</tbody>
</table>

**Genetic analyses**

A total of 3045bp were sequenced, 631 bp, 760 bp, 854 bp and 800 bp obtained from COI, CO2, ND4 and Cytb respectively, and each were run via BLASTn to identify sequence matches. All the genes confirmed the genus with identity matches ranging from 99.7% to 87.6% for COI; 98.8% to 88.6% for CO2; 92.3% to 86.8% for ND4 and 97.4% to 86.6% for Cytb. At species level the specimen gave highest identity match, of 99.7%, with *L. fulviflamma* (GB|JQ639261) for the COI gene, therefore confirming its morphological identification. In order to analyse the origin of the specimen collected from Maltese waters the COI gene was used in phylogenetic and phylogeographic analyses (Figure 3).

The *L. fulviflamma* 571 bp long COI sequences analysed exhibited a total of 23 different haplotypes, all of which translated to the same amino acid sequence. Phylogenetic analyses of the haplotypes (Figure 3) have yielded two main clusters. One clade representing specimens from Seychelles, Madagascar, Iran, East Africa and the Eastern coast of South Africa, while the other clade represents specimens collected from Malaysia, South China, Philippines, Indonesia and Taiwan. These two clades indicate clear subdivision in the *L. fulviflamma* taxon. The specimen collected from the Maltese Islands was classified within the first clade (Figure 3), showing closest matches with haplotypes of East African origin. When the specimen's COI sequence was shortened to 571 bp, a 100% match was noted to haplotype JQ639261.
Figure 3. Maximum Likelihood tree of 571 bp of the COI gene sequences of *Lutjanus fulviflamma*, using K2P distances. The numbers indicate the accession numbers used in the tree construction with their respective place of origin.

The genetic divergence noted between the two clades using the COI gene was greater than 1.2% (7 to 11 basepair differences). This divergence explains why BLASTn analyses of the Maltese specimen gave hits below 99% identity for CO2 and Cytb genes, as the genetic data available in GenBank for these two genes was obtained from *L. fulviflamma* specimens that were not of East African origin and therefore falling into clade 2.

**Discussion**

This study provides the scientific evidence obtained from the morphological, meristics and genetic analyses for the first recorded specimen of *L. fulviflamma* in the Mediterranean Sea. These results confirm the first documented occurrence of this species in Malta as briefly reported by (Vella 2014 a; Vella 2014 b) but subsequently listed in (Evans *et al.* 2015) review as 'questionable'. The results presented here confirm the identity of the specimen morphologically, genetically and also reveals the phylogeographic origin of this specimen. Genetic data indicated that the specimen collected from Maltese waters was found to be closely related to the East African clade of the *L. fulviflamma* species. Therefore this specimen is of Western Indo-Pacific origin and is possibly a Lessepsian migrant, like most alien fish recorded in the
Mediterranean Sea (Galil 2007; Psomadakis et al. 2009; Jribi and Bradai 2012; Evans et al. 2015). This species has not been previously recorded in any other region of the Mediterranean Sea and its occurrence in the Southern Central Mediterranean region may also be due to human transportation from its natural Indo-Pacific habitat. One possible mode of transportation is via ballast water tanks (Galil 2006) as this specimen of *L. fulvifamma* was collected from a harbour which is regularly visited by large commercial ships. Another possibility is that the specimen was an aquarium release from marine vessels visiting the Grand Harbour given that members of the genus *Lutjanus* are exported as ornamental fish both in the Indo-Pacific (Jayalal and Ramachandran 2012; Ekaratne 2000) and Atlantic Ocean (Monteiro-Neto et al. 2003).

The occurrence of *L. fulviflamma* in the Maltese waters has to be closely monitored. This carnivorous species (Nanami et al. 2013) is able to form shoals and may impose new treats to the native fish species found in these waters.

The genetic differences between the two clades found in this study together with their tight link to geographical distribution indicate that this species' population structure and phylogeography deserve further investigation, encompassing the whole distribution range of the species.

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**References**


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