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# Variola louti (Perciformes Epinephelidae) in the Mediterranean Sea: Incidental introduction or aquarium release?

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**On the presence of the yellow-edged lyretail grouper,  
Variola louti (Perciformes: Epinephelidae), in the  
Mediterranean: Lessepsian immigrant or aquarium release?**

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Subject Area:	Fisheries Ecology, Fisheries Resources, Genetics
Abstract:	Lessepsian immigrants are altering the composition and functioning of Eastern Mediterranean ecosystems. Here, we report the first confirmed and second published record of the yellow-edged lyretail grouper, <i>Variola louti</i> (Forsskål, 1775) in the Mediterranean Sea and Cyprus, supported by morphological and genetic analysis. Phylogenetic analyses revealed that none of the samples from the Red Sea or the Indian Ocean (Mozambique, South Africa and India), clustered with our samples; indicating that aquarium release is the most possible pathway.

1 **On the presence of the yellow-edged lyretail grouper, *Variola louti* (Perciformes: Epinephelidae), in**  
2 **the Mediterranean: Lessepsian immigrant or aquarium release?**

3 Short title: *Variola louti* in Cyprus and Mediterranean

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11 **Introduction**

12 Lessepsian immigrants (i.e. Red Sea species entering the Mediterranean through the Suez Canal) have  
13 greatly altered the composition and functioning of many Eastern Mediterranean shelf ecosystems and are  
14 expected to continue doing so, as new species arrive and establish self-sustaining populations in the region  
15 (Michailidis et al., 2019). Some of these species can potentially become invasive and through a series of  
16 mechanisms substantially change the community structure, cause the loss of native genotypes, modify  
17 habitats, affect food web properties and ecosystem processes, impede the provision of ecosystem services,  
18 impact human health, and cause substantial economic losses (Katsanevakis et al., 2014).

19 Here, we report the first confirmed record of the yellow-edged lyretail grouper, *Variola louti* (Forsskål,  
20 1775) in the Mediterranean Sea and Cyprus. *Variola louti* is an Indo-Pacific reef-associated grouper of  
21 commercial importance in its native range, and a popular species in the aquarium trade. It was first reported  
22 in Cyprus approximately a year ago based on an underwater observation, and its introduction was attributed  
23 to an aquarium release (Kousteni et al., 2019). In this report, the species was identified using both  
24 morphological characteristics and genetic analysis. The phylogenetic relationship with available sequences  
25 from specimens analysed in other regions was further examined and discussed.

26 **Materials and Methods**

27 On 29 August 2019, a recreational spearfisher caught a *V. louti* individual at the north-western tip of Cyprus,  
28 near Pomos village (32.55 E 35.17 N). The fish was caught around noon on a rocky bottom by 15 m depth,  
29 approximately 90 km ocean distance from the area of its first Mediterranean sighting a year ago (Kousteni  
30 et al., 2019) (Figure 1).

31 The specimen was morphologically examined for confirmation of its identity. DNA was extracted and the  
32 mitochondrial barcode gene COI (Cytochrome oxidase 1) was sequenced following published protocols  
33 (Bariche et al., 2015). Briefly, the amplification of COI used fish specific primers VF2T1 and VR1dT1

34 (Ivanova et al., 2007). PCR amplified fragments were sequenced in both directions using the primers used  
35 for the amplification, and then compared with available sequences in GenBank. Phylogenetic  
36 reconstructions were based on the Neighbour-Joining method generated in R (RCoreTeam, 2016) with the  
37 use of the ape package (Paradis, Claude, & Strimmer, 2004). Genetic distances were based on the Kimura  
38 2 parameter method. The maximum likelihood (ML) method was also used as a second phylogenetic  
39 reconstruction approach, as implemented in GARLI (Zwickl, 2006). To estimate support for the nodes,  
40 1000 bootstrap replicates were performed and we retained only the values supporting the nodes accounting  
41 for more than 50% of the bootstrap replicates.

## 42 **Results**

### 43 *Morphology*

44 The study specimen was a sexually immature individual of 2145 g wet weight. It had an oblong body, with  
45 the maximum body depth and head length 3 and 2.7 times in standard length respectively. The dorsal head  
46 profile and the interorbital area were convex. Both jaws had a pair of large canines at the front, while the  
47 lower jaw also had two large canines at the midsides. Palatines and vomer were also toothed. The caudal  
48 fin was lunate with the upper and lower lobes produced, about twice the length of middle rays, and the  
49 pelvic fins extended past the anus. The body and median fins were brown to orange-red with numerous  
50 small round or elongate spots of lavender and pink, while caudal, dorsal, anal, and pectoral fins had a broad  
51 yellow rear margin. All morphological characteristics and morphometric and meristic measurements (Table  
52 1) are in agreement with *V. louti*, as described in the literature (Heemstra, Randall, Carpenter, & Niem,  
53 2001).

### 54 *Genetic analysis*

55 The PCR amplification and sequencing of the cytochrome oxidase 1 resulted in a 658 bp fragment  
56 (GenBank accession number MN475883). A BLAST comparison of this sequence with available sequences  
57 in GenBank placed it in a cluster of 23 sequences, all identified as *V. louti*. Seven of those sequences were  
58 identical to the one obtained for our sample. These seven sequences belonged to samples collected in  
59 Australia, Indonesia, Philippines, and China. Two *V. louti* sequences available in GenBank were obtained  
60 from samples collected in the Red Sea. One sequence from Egypt has a 99.69% similarity with our sequence  
61 (MH707293, two mismatches), and one sequence from Eilat, Israel, has a 97.7% similarity with our  
62 sequence (MF124078, 15 mismatches).

63 Phylogenetic analyses were performed by comparing our sequence to *V. louti* sequences extracted from  
64 GenBank, using four *V. albimarginata* (the only other *Variola* species) sequences as outgroups. Maximum  
65 likelihood and Neighbor-Joining methods resulted in identical tree topologies, therefore only the NJ tree is  
66 shown here (Figure 2). As indicated above from the BLAST results, our sequence clustered with *Variola*  
67 *louti* samples, and was very well separated from the *V. marginata* sequences. When all available sequences

68 of *V. marginata* were used, and additional grouper species were used as outgroups, results remained  
69 unchanged (not shown).

70 No samples from the Red Sea or the Indian Ocean (Mozambique, South Africa and India), clustered with  
71 our samples, however, resolution at the level of this genetic marker is relatively low due to low mutation  
72 rate in CO1, therefore such a geographic association is difficult to rule out.

### 73 **Discussion**

74 Morphology and genetic results with both BLAST and phylogenetic analyses unambiguously identify this  
75 specimen as the yellow-edged lyretail grouper, *Variola louti*. While only four samples from GenBank are  
76 from the Indian Ocean (two from the Red Sea and two from Mozambique), none of them cluster with our  
77 sample (a result that would be consistent with a Lessepsian immigrant). In contrast, results tentatively  
78 indicate an aquarium release, since most aquarium trade fish are imported from other regions (e.g. Indonesia  
79 and Philippines). The presence of a single large individual in Cyprus, which could in fact be the same  
80 specimen previously observed in Cyprus (although that individual was recorded as being of a larger size  
81 >70 cm TL) (Kousteni et al., 2019), is also consistent with this hypothesis. Yet, further work, sampling and  
82 observations, as well as the engagement of citizen scientists, are necessary to conclusively settle this  
83 question.

### 84 **Acknowledgments**

85 The authors are grateful to freediving spearfishers Giorgos Panagi, Ilias Panagi, Dimitris Koyionis, and  
86 Andreas Georgiou for willingly providing the specimen for examination along with details on its capture.

### 87 **Conflict of Interest**

88 None.

### 89 **Data Availability Statement**

90 The data that support the findings of this study are openly available in "GenBank" at  
91 <https://www.ncbi.nlm.nih.gov/genbank/>, accession number MN475883.

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115 *sequence datasets under the maximum likelihood criterion*.

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125 **Table 1.** Morphometric and meristic measurements of the examined *Variola louti* specimen.

<b>Morphometric measurements</b>	<b>Absolute value (mm)</b>
Total length	565
Fork length	499
Standard length	436
Preanal length	278
Predorsal length	144
Prepelvic length	141
Prepectoral length	144
Maximum body depth	143
Caudal peduncle depth	59
Head length	161
Preorbital length	47
Eye diameter	16
<b>Meristic measurements</b>	<b># number</b>
Dorsal fin	IX+14
Anal fin	III+8
Pectoral fin	16
Lateral line scales	74
Gill rakers (upper + lower)	10+18 (including rudimentary)

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154 **Figure 1.** (A) The location of the capture, indicated with a red circle, and of the previous Mediterranean  
155 record indicated with a black circle. (B) Specimen analysed in this study.

156 **Figure 2.** Phylogenetic reconstruction of *Variola* groupers based on the cytochrome oxidase marker. Tree  
157 topology is based on the Neighbour-Joining, NJ, method (identical to Maximum Likelihood, ML,  
158 topology), numbers on nodes are bootstrap values derived from 1000 replicates (only numbers above 50%  
159 are shown). First number is for NJ, second number for ML. Mediterranean sample is from Cyprus and is  
160 in red. All other sequences are from GenBank and are in black. Their sample origin is indicated after their  
161 accession number.

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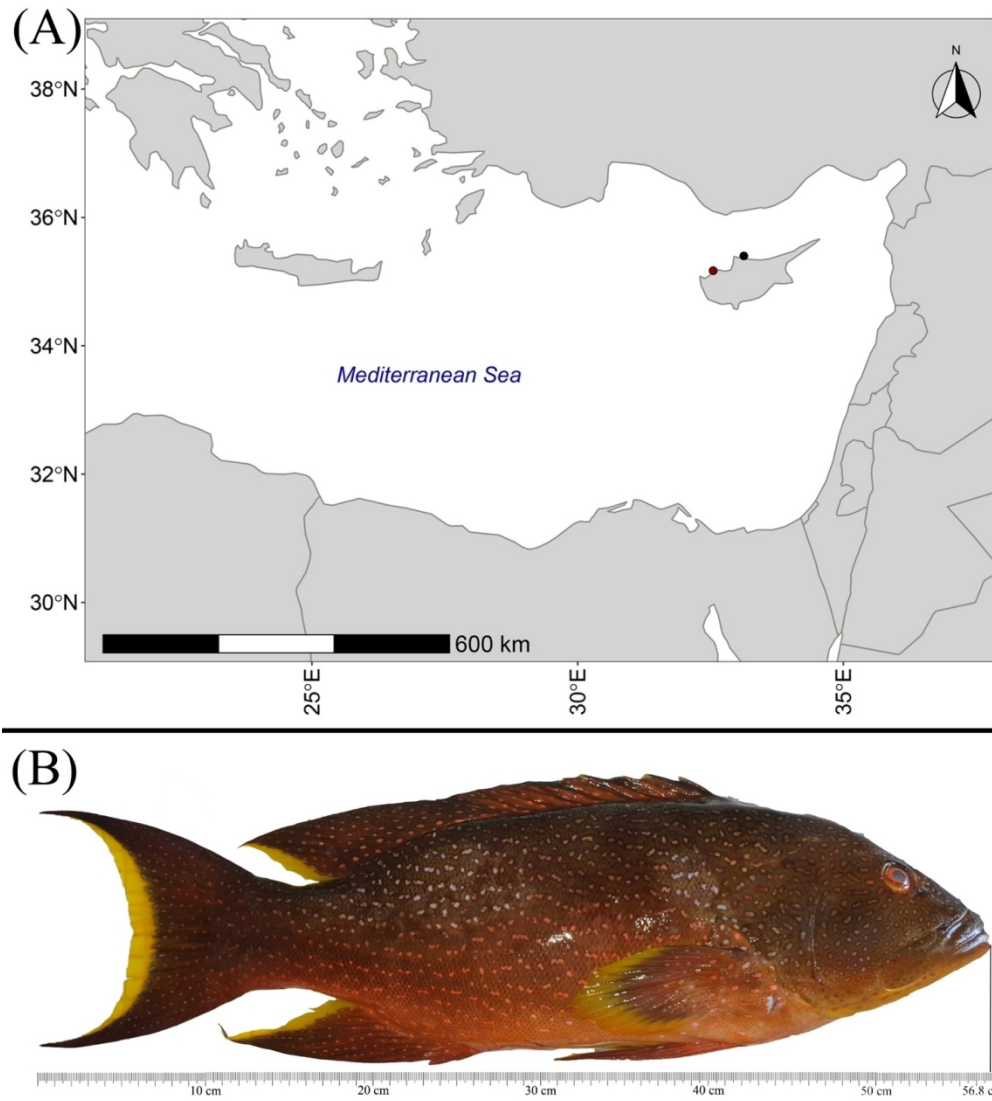


Figure 1. (A) The location of the capture, indicated with a red circle, and of the previous Mediterranean record indicated with a black circle. (B) Specimen analysed in this study.

169x197mm (220 x 220 DPI)

