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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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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, Variola louti (Forsskål, 1775) in the Mediterranean Sea and Cyprus, supported by morphological and 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.

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

Short title: *Variola louti* in Cyprus and Mediterranean

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Introduction

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

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

Materials and Methods

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

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

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

Results

Morphology

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

Genetic analysis

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

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

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

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

Discussion

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

Acknowledgments

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Conflict of Interest

None.

Data Availability Statement

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

References

Bariche, M., Torres, M., Smith, C., Sayar, N., Azzurro, E., Baker, R., & Bernardi, G. (2015). Red Sea fishes in the Mediterranean Sea: a preliminary investigation of a biological invasion using DNA barcoding. *Journal of Biogeography, 42*(12), 2363-2373.

Heemstra, P., Randall, J., Carpenter, K., & Niem, V. (2001). FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 4. Bony fishes part 2 (Mugilidae to Carangidae).

Ivanova, N. V., Zemplak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, *7*, 544-548. 10.1111/j.1471-8286.2007.01748.x.

Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppakoski, E., Cinar, M.E., Ozturk, B., . . . Cardoso, A.C. (2014). Impacts of marine invasive alien species on ecosystem services and biodiversity: a pan-European review. *Aquatic Invasions 9*, 391-423.

Kousteni, V., Bakiu, R., Benhmida, A., Crocetta, F., Di Martino, V., Dogrammatzi, A., . . . Gokoglu, M. (2019). New Mediterranean Biodiversity Records (April, 2019). *20*(1), 230-247. doi:doi: 10.12681/mms.19609

Michailidis, N., Corrales, X., Karachle, P. K., Chartosia, N., Katsanevakis, S., & Sfenthourakis, S. (2019). Modelling the role of alien species and fisheries in an Eastern Mediterranean insular shelf ecosystem. *Ocean & Coastal Management, 175*, 152-171.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics, 20*(2), 289-290.

RCoreTeam. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *URL* http://www.*R-project. org*.

Zwickl, D. J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.*

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

Morphometric measurements	**Absolute value (mm)**
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
Meristic measurements	**# number**
Dorsal fin	IX+14
Anal fin	III+8
Pectoral fin	16
Lateral line scales	74
Gill rakers (upper + lower)	10+18 (including rudimentary)

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.

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

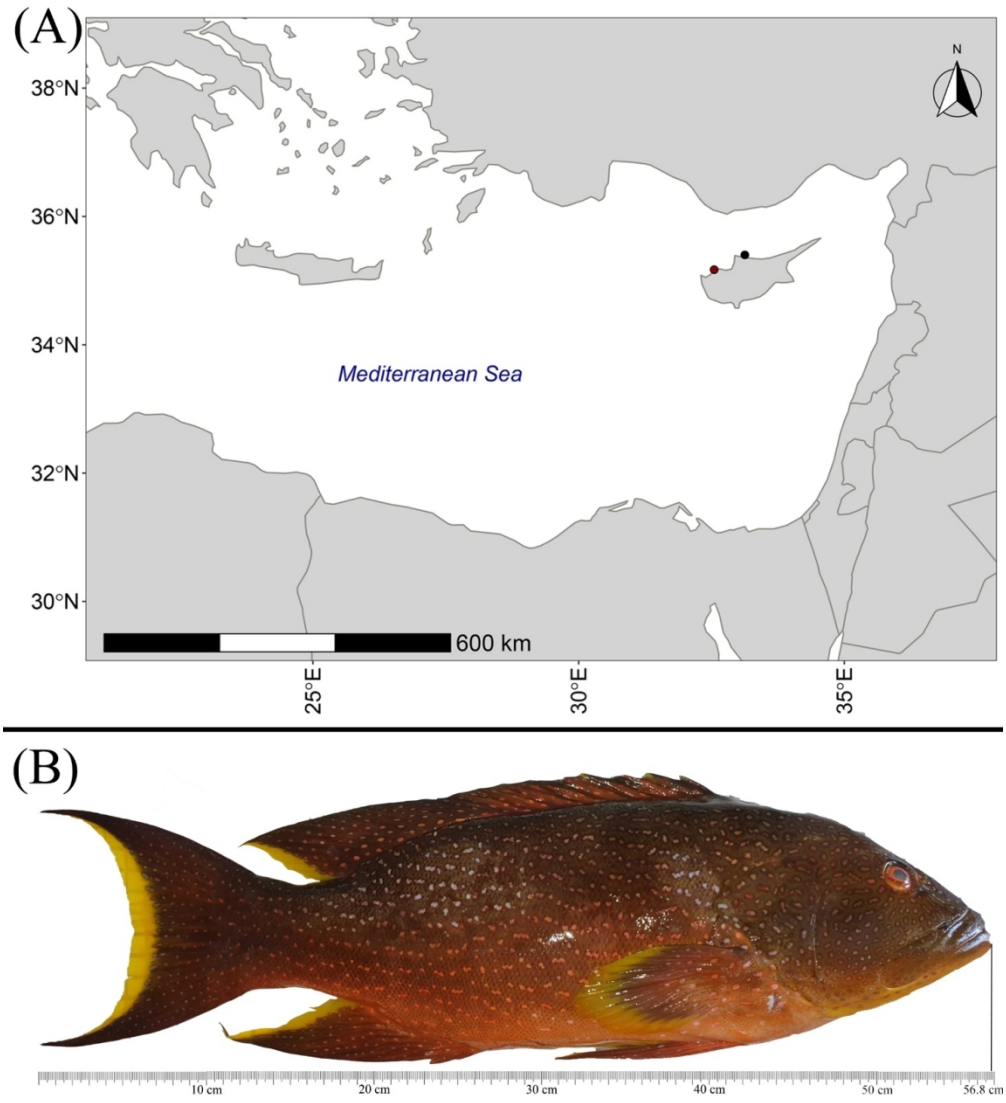
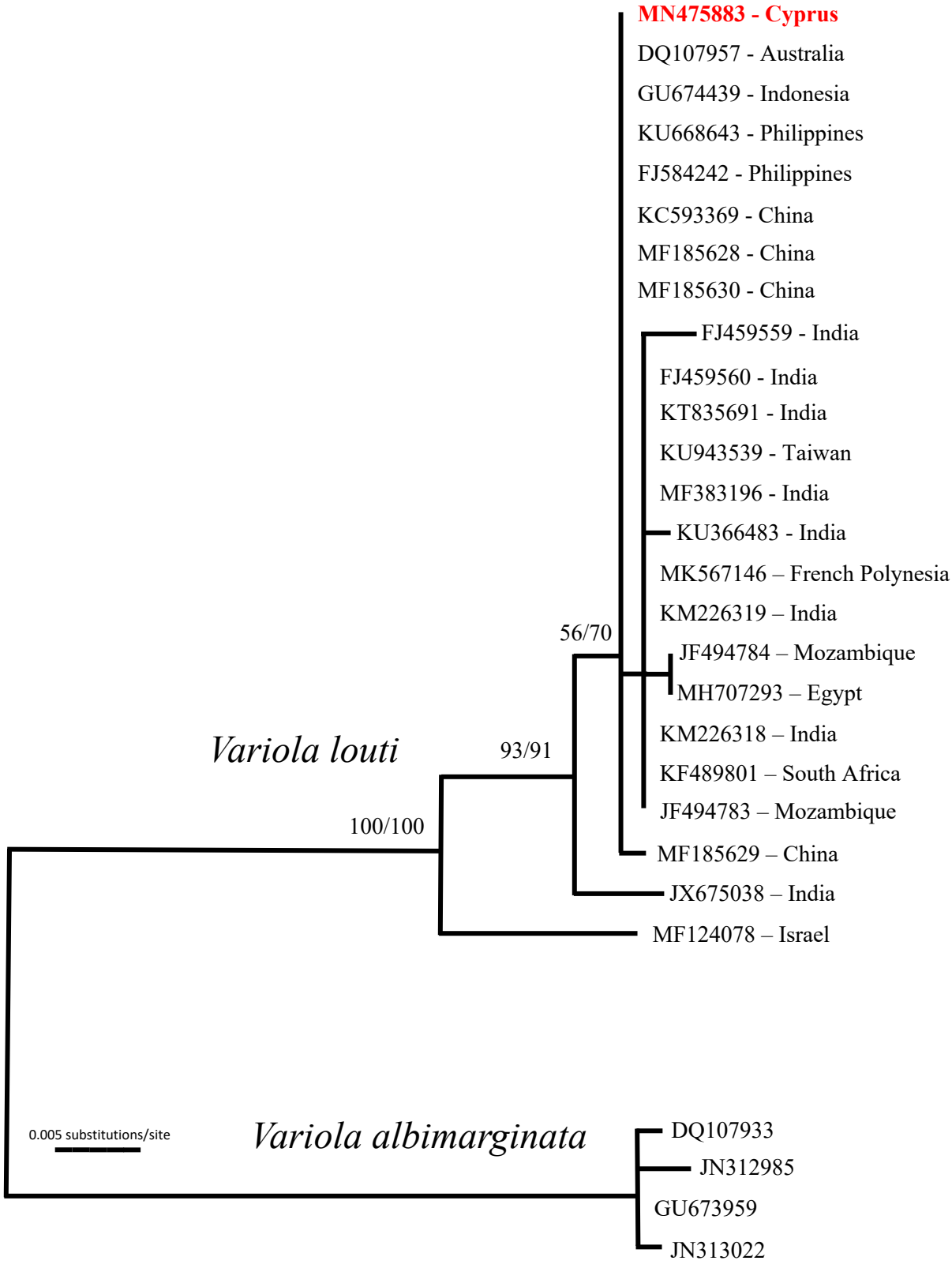


Figure 1. (A) The location of the capture, indicated with a red circle, and of the previous Mediterranean record indicated with a black circle. (B) Specimen analysed in this study.

169x197mm (220 x 220 DPI)


MN475883 - Cyprus
DQ107957 - Australia
GU674439 - Indonesia
KU668643 - Philippines
FJ584242 - Philippines
KC593369 - China
MF185628 - China
MF185630 - China
FJ459559 - India
FJ459560 - India
KT835691 - India
KU943539 - Taiwan
MF383196 - India
KU366483 - India
MK567146 – French Polynesia
KM226319 – India
56/70
JF494784 – Mozambique
MH707293 – Egypt
Variola louti
93/91
KM226318 – India
KF489801 – South Africa
JF494783 – Mozambique
100/100
MF185629 – China
JX675038 – India
MF124078 – Israel
0.005 substitutions/site
Variola albimarginata
DQ107933
JN312985
GU673959
JN313022