

## CORRECTED PROOF

## Rapid Communication

## The first record of the marbled spinefoot, *Siganus rivulatus* Forsskål & Niebuhr, 1775 and further records of the dusky spinefoot, *Siganus luridus* (Rüppell, 1829) from Malta

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## OPEN ACCESS

### Abstract

Here we report the first record of the Marbeled spinefoot, *Siganus rivulatus* Forsskål and Niebuhr, 1775 (Acanthuriformes: Siganidae), collected in July 2022 from Maltese coastal waters. The specimen was identified through morphological characters and confirmed genetically through DNA barcoding, given that the family Siganidae is composed of several species belonging to only one genus with similar and overlapping morphological characters. Previous to this report, the closest record of *S. rivulatus* was off the island of Sicily. Thus, the presence of *S. rivulatus* in Maltese waters suggests that this species is expanding its distribution within the Mediterranean Sea. Additionally, this study compares this central Mediterranean record of *S. rivulatus* with genetic data of the Dusky spinefoot, *Siganus luridus* (Rüppell, 1829), a previously established species. Individual records of *Siganus* (rabbitfishes) are important to note, as they represent the first warning signs of a possible invasion. Previous work has shown that these species drastically alter the ecosystems of the Mediterranean region through their herbivorous diet and grazing behaviour. Therefore, we need to closely monitor for more individuals and establish mitigation measures against established and expanding populations of these two species.

**Key words:** first record, Lessepsian migrant, Siganidae, DNA barcoding, Mediterranean Sea

### Introduction

The marine teleost family Siganidae is composed of a single extant genus, *Siganus* Forsskål, 1775, and encompasses 29 valid species of rabbitfish (Ahyong et al. 2022; Froese and Pauly 2022). In the Mediterranean Sea this genus is represented by two non-indigenous invasive species, the Dusky spinefoot, *Siganus luridus* (Rüppel, 1829) and the Marbled spinefoot, *Siganus rivulatus* Forsskål and Niebuhr, 1775 (Ben Rais Lasram et al. 2008; Zenetos and Galanidi 2020). These two species have an Indo-Pacific origin and are

both considered Lessepsian migrants. *Siganus luridus* has been reported in Syria in 1930 (Zenetos and Galanidi 2020) and Israel in 1955 (Ben-Tuvia 1964; Golani et al. 2021), while *S. rivulatus* has been first reported off the coast of Israel in 1924 (Steinitz 1927; Ben-Tuvia 1964). Since their first records, they have formed established populations in the Eastern Mediterranean and have been expanding their range westerly (Ben Rais Lasram et al. 2008), with *S. luridus* reaching the French Mediterranean coast by 2008 (Daniel et al. 2009). *Siganus rivulatus* reached Tunisia by 1974 (Ktari and Ktari 1974) and more recently was recorded in Sicily and the Pelagie Islands, both in the central Mediterranean (Karachle et al. 2016; Stamouli et al. 2017). These two species are of great concern to the Mediterranean Sea, as established populations of these intensive grazers alter the composition of algal assemblages, often times reducing them to barren reefs. Thus the expansion and increase in abundance of populations of the rabbitfishes are a threat to the native biodiversity and the overall ecosystem function (Sala et al. 2011; Giakoumi 2014).

In addition to these two established species, another three species of *Siganus* have been reported from the Mediterranean Sea, including the Streaked spinefoot, *Siganus javus* (Linnaeus, 1766) collected in 2009 (Ibrahim et al. 2010), the Streamlined rabbitfish, *Siganus argenteus* (Quoy & Gaimard, 1825) collected in 2015 (Abdelghani et al. 2021), and the Mottled spinefoot, *Siganus fuscescens* (Houttuyn, 1782) collected in 2020 (Azzurro and Tiralongo 2020). Until now, these three species had been considered casual records as there is no evidence that they have formed any established population in the Mediterranean Sea (Zenetos and Galanidi 2020; Golani et al. 2021).

The possible occurrence of *Siganus* species in Maltese waters has been documented in local guidebooks (Lanfranco 1993) and scuba divers' observations (Castriota and Andaloro 2008), with the first authenticated record dating back to 2002. A specimen of *S. luridus* was collected from Wied iż-Żurrieq and analysed morphologically by one of us (AV) (Ragkousis et al. 2023). All records of siganids in Maltese waters have always been *S. luridus* (Schembri et al. 2012; Borg et al. 2023). However, here, we report a rabbitfish specimen that was collected in 2022 and differed in appearance from the already known *S. luridus*. Species of the family Siganidae have very similar meristic counts and so species identification relies strongly on colour patterns (Woodland and Anderson 2014). To avoid any possible misidentifications during the current study we utilized both morphological and genetic characters to accurately identify the specimens collected down to the species level. Recognising that there has been an increasing number of records of *S. luridus* in the central Mediterranean (Schembri et al. 2012; Azzurro et al. 2017), we investigated this and other previously collected specimens from Maltese waters in detail.



**Figure 1.** The first recorded *Siganus rivulatus* specimen caught from Malta in July 2022. Photograph by AV and YS.



**Figure 2.** A map showing the location from where the first records of *Siganus luridus* (blue) and *Siganus rivulatus* (red) were collected.

## Materials and methods

On 23 July 2022, a specimen of *S. rivulatus* (Figure 1) was caught from Delimara, Malta (GPS: 35.822; 14.565; Figure 2) by a Maltese fisherman using trammel nets. The specimen was transferred to the authors, photographed and morphologically analysed following Fischer and Bianchi (1984), and Golani et al. (2021). All body length measurements were taken to the nearest 0.1 mm using electronic callipers. Later a tissue biopsy was taken and preserved in 100% ethanol, and the specimen was subsequently deposited in the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta (CBRG-UM) as voucher specimen SMM009. As part of long-term on-going research around Malta, the CBRG-UM also has a collection of *S. luridus* that were used for this study, including tissue biopsies from the first confirmed Maltese record of *S. luridus* that was analysed by AV in 2002 (Supplementary material Figure S1).

Total genomic DNA was extracted from *S. rivulatus* ( $n = 1$ ) and *S. luridus* ( $n = 16$ ) (Table S1) using the GF-1 Tissue DNA Extraction Kit (Vivantis,

Malaysia). PCR amplifications were carried out for cytochrome *c* oxidase I gene (COI), 16S rRNA (16S) and 12S rRNA gene (12S) using primers sets and amplification protocols as described in Vella et al. (2016a, b). PCR products were sequenced using both the forward and reverse primers via ABI3730XL. The quality check, editing, and assembly of complementary sequences were carried out using Geneious R10 (<https://www.geneious.com>, Kearse et al. 2012). The genetic data generated during this study were deposited into GenBank (Table S1).

The final data set for all three genes was compared with the NCBI GenBank database via Blastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The COI sequences were also compared to BOLD data using the Species Level Barcode Records Identification Engine (<http://www.boldsystems.org>; Ratnasingham and Hebert 2007) to identify the closest match and the Barcode Index Number associated with the data produced in this study. The COI gene sequences from GenBank allowed for phylogenetic analyses of these specimens and for comparative analyses of the currently-analysed specimens with other Siganidae species. Sequences chosen were aligned using Geneious R10 (<http://www.geneious.com>; Kearse et al. 2012), via MUSCLE (Edgar 2004). The model of best fit as identified by jModel (Posada 2008) was used for constructing a phylogenetic tree using a Maximum Likelihood with 1500 bootstraps through MEGA X (Kumar et al. 2018). The phylogenetic tree allowed for better visualization of species delimitation using DNA barcodes.

## Results

The *S. rivulatus* specimen collected during this study weighed 195 g and had a total length of 240.0 mm (Table 1). The specimen had a compressed, oval body covered with very small cycloid scales. The head was slightly concaved above the orbit. When fresh, the dorsal area of the specimen was olive-green with some darker brown areas, grading to a paler grey colour ventrally. The lower two-thirds of the specimen's sides were characterized by golden-yellow undulating transverse stripes, which lost most of their colouration upon freezing. The caudal fin was moderately forked. The dorsal fin had 13 hard spines and 10 soft rays and was preceded by a forward-projecting procumbent spine, while the pelvic fin was composed of two spines separated by three soft rays (I+3+I), both characteristics typical of *Siganus* species. The overall appearance, morphology and meristic counts (Table 1 and Figure 1) matched the descriptions of *S. rivulatus* (Fischer and Bianchi 1984; Golani et al. 2021).

The meristic counts of the analysed *S. rivulatus* overlapped with those of the analysed *S. luridus* (Table 1), however the two differed in the shape of the caudal fin as in *S. luridus* it was truncated rather than forked. In *S. rivulatus* the dorsal ray margin and the anal fin margin were less rounded

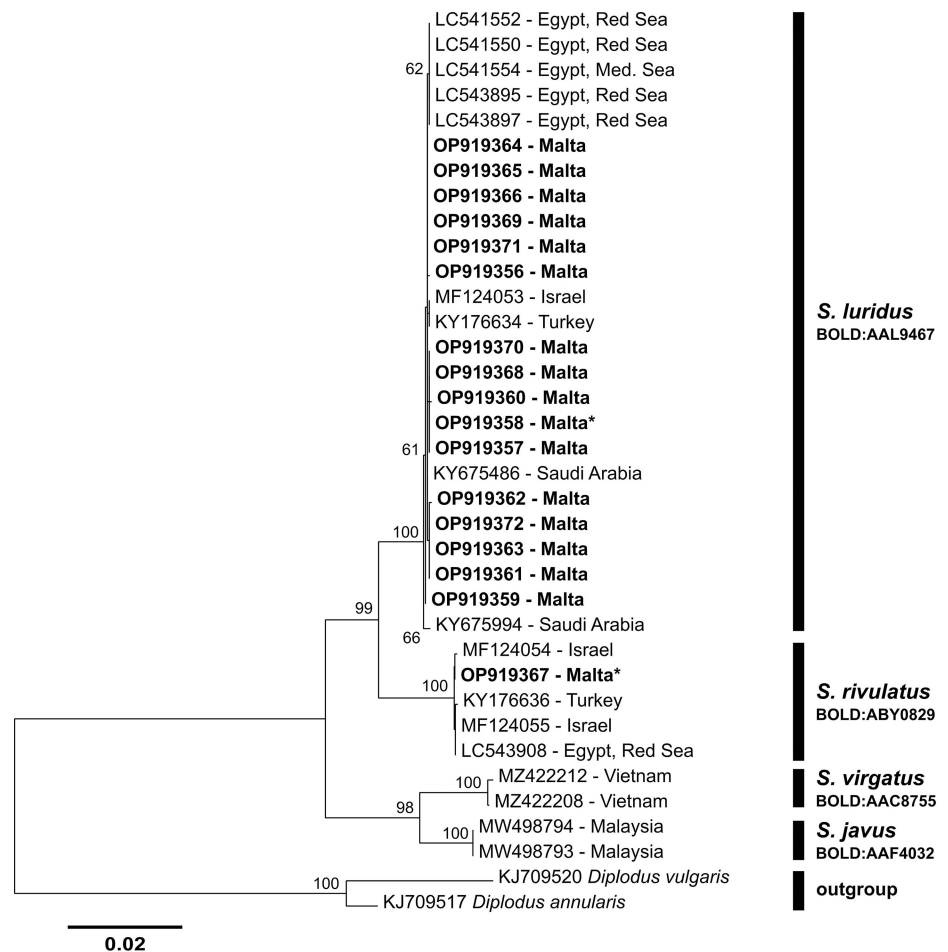
**Table 1.** Meristic counts and body measurements of one specimen of *Siganus rivulatus* and 21 specimens of *Siganus luridus* collected from Malta between 2002 and 2022. Abbreviations: SL = Standard Length; SD = Standard Deviation.

	<i>Siganus rivulatus</i> (n = 1)		<i>Siganus luridus</i> (n = 21)	
				mean ± SD
<b>Meristic counts:</b>				
Procumbent spine	I		I	
Dorsal fin hard spines	XIII		XIII	
Dorsal fin soft rays	10		10–11	10.1 ± 0.2
Pectoral fin	16		14–17	15.9 ± 0.9
Pelvic fin	I+3+I		I+3+I	
Anal hard spines	VII		VII	
Anal fin soft rays	9		8–9	8.9 ± 0.2
Caudal fin	20		16–20	18.2 ± 1.1
<b>Measurements<sup>1</sup>:</b>				
Total length	240.0		154.5–279.0	
Fork length	226.2		n/a	
Standard length	203.3		126.2–231.0	
Maximum body depth	71.0	34.9% (SL)	55.0–104.1	44.2% ± 1.8% (SL)
Length of dorsal fin base	144.2	70.9% (SL)	85.4–202.0	71.2% ± 2.3% (SL)
Pectoral fin base	21.6	10.6% (SL)	20.2–38.3	16.9% ± 1.1% (SL)
Anal fin base	82.5	40.6% (SL)	50.3–96.0	39.2% ± 1.5% (SL)
Pre-pelvic length	50.2	24.7% (SL)	29.5–68.5	23.7% ± 1.8% (SL)
Pre-pectoral length	38.7	19.0% (SL)	22.0–44.2	19.1% ± 1.5% (SL)
Pre-anal length	101.1	49.7% (SL)	31.1–115.2	49.5% ± 2.3% (SL)
Caudal peduncle depth	10.1	5.0% (SL)	8.7–21.6	8.2% ± 1.3% (SL)
Head length	39.8	19.6% (SL)	27.6–50.8	21.2% ± 1.3% (SL)
Pre-orbital length	15.5	7.6% (SL)	10.0–27.3	6.8% ± 1.1% (SL)
Eye diameter	9.7	4.8% (SL)	8.6–14.0	6.1% ± 0.5% (SL)
Inter-orbital distance	13.0	6.4% (SL)	11.4–20.4	8.9% ± 0.7% (SL)
Weight	195		55.0–460.0	296.4 ± 102.8

than those of *S. rivulatus*, while the head in *S. rivulatus* was moderately concave. The specimens of *S. luridus* were darker in colour than *S. rivulatus* and lacked the golden-yellow stripes over their body.

The morphological identification of both species was supported by genetic analyses. After trimming and processing the data, the sequence lengths used for this study were 614 bp, 304 bp and 489 bp for COI, 12S and 16S respectively. The BOLD Species Level Barcode Records Identification Engine placed the *S. rivulatus* within BOLD:ABY0829, while *S. luridus* specimens were placed within BOLD:AAL9467, with both species matching conspecifics by at least 99.3% (Figure 3). Using Blastn, COI produced a similar outcome, with matches confirming species identification. As for the 12S, *S. rivulatus* matched 100% with MW376910 while the *S. luridus* sequences identified in this study matched by 99.7% to 100% with MW376909. The 16S sequences identified in this study completely matched with the GenBank data for each respective species. This work provided the genetic resources required to correctly identify *S. rivulatus* and *S. luridus*, especially given that prior to this study there was little publicly accessible data for the 16S and even less so for the 12S.

When compared against each other, the two species genetically differed by 7.5% on the COI gene, 2.3% on the 12S gene and 0.8% on the 16S gene.



**Figure 3.** The phylogenetic relationships between specimens analysed as part of this manuscript (noted in bold; \* indicate the first Maltese records) and other *Siganus* species using COI data. The values at the nodes indicate bootstrap values > 60. The numbers on the right are the GenBank accession numbers, sampling locations and BOLD BINs.

The *S. luridus* specimens genetically analysed here produced seven COI haplotypes leading to a haplotype diversity of 0.842 (SD  $\pm$  0.059). These haplotypes shared 99.0% identical nucleotides, with a mean pairwise percentage identity of 99.8% and a maximum p-distance of 0.7%. For the 12S and 16S genes, *S. luridus* produced only two haplotypes per gene, and in each case the difference between haplotypes was of 1 bp, with the less common haplotype appearing only one time.

## Discussion

The current study provides the first record of *S. rivulatus* in Maltese waters, based on both morphological and genetic identification. This individual likely represents a natural range expansion from nearby populations. It has been recently recorded from a number of neighbouring central Mediterranean islands, including Sicily (Karachle et al. 2016) and the Pelagie Islands (Stamouli et al. 2017), and well-established populations exist in both Libyan and Tunisian waters (Ktari and Ktari 1974; Shakman et al. 2008). Additionally, this record comes from the same location where the first records of other

alien teleost species, *Abudefduf hoefleri* and *Kyphosus vaigiensis*, were noted in Malta (Vella et al. 2016a, c). This area is prone to heavy marine traffic, as it occurs close to Marsaxlokk Harbour (Figure 2) which has a major international transshipment facility (Malta Freeport Terminal 2023). Given that *S. rivulatus* can survive three weeks in ballast water (Wonham et al. 2000), we cannot exclude the possibility that marine activities might have facilitated its spread as well.

The specimen of *S. rivulatus* collected during this study was larger than the length at which the species matures (Soykan et al. 2020) and close to the maximum length recorded in most Mediterranean studies (Bilecenoglu and Kaya 2002; Shakman et al. 2008; Soykan et al. 2020). Similarly, a number of the *S. luridus* specimens collected during this study were large enough to be considered mature, as they match maturity sizes for individuals collected from the central Mediterranean (Azzurro et al. 2007). Genetic analyses have shown that *S. luridus* exhibits a large haplotype diversity ( $0.842 \text{ SD} \pm 0.059$ ), corroborating other studies that found similarly high levels of genetic variation in other areas of the Mediterranean (Hassan et al. 2003; Azzurro et al. 2006). This may indicate that the colonization around Malta involved the migration of several individuals from neighbouring areas.

In the Mediterranean, both *S. rivulatus* and *S. luridus* have modified their diet from their Red Sea counterparts to adapt to new algal resources (Bariche 2006). Unlike the Red Sea, the Mediterranean Sea hosts few native herbivorous fish species, namely *Sarpa salpa* and *Sparisoma cretense*, thus *Siganus* species find little competition in the region (Verges et al. 2014). This, together with increasing sea temperatures, is creating the right conditions for the establishment and expansion of rabbitfish populations (Marras et al. 2015; D'Amen and Azzurro 2020). They are becoming the most abundant herbivorous fish in most of the invaded areas, and their feeding behaviour has led to overgrazing and alteration of native ecosystems (Sala et al. 2011; Giakoumi 2014; Verges et al. 2014).

Since the first record of *S. luridus* in 2002, fishermen and divers have reported an increasing presence of this species in Maltese waters (*pers. comm.*). In this regard, citizen science projects can be a powerful tool in the monitoring of the range expansion and abundance of invasive alien fish (Tiralongo et al. 2020). Consequently, the current new record of *S. rivulatus* calls for careful monitoring of these invasive species using effective tools to aid timely management in accordance with Descriptor 2 of the European Marine Strategy Framework Directive and other relevant European and regional legislation on invasive alien species.

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## Authors’ contribution

AV: conceptualization, data collection, first draft preparation and review of the manuscript; NV: data collection, first draft preparation and review of the manuscript; YS, CMM: data collection and review of the manuscript; CM, IP, DT and FT: review of the manuscript.

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### Supplementary material

The following supplementary material is available for this article:

**Figure S1.** An image of the first record of *Siganus luridus* collected from Malta on 10<sup>th</sup> April, 2002.

**Table S1.** A list of specimens, collected from Malta, whose tissue biopsies were genetically analysed in this study, including the corresponding GenBank Accession Numbers and notes.