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# First Record of the Silverspot Squirrelfish *Sargocentron caudimaculatum* (Rüppell, 1838) in Mediterranean Waters

*Raouia Ghanem*[1], *Wafa Rjiba Bahri*[1], *Amani Chaffai*[1], *Jeanne Zaouali*[2], *Bilel Hassen*[3], *Sahar Karray*[3], *Monia El Bour*[3], *Jamila Ben Souissi*[1,2*] *and Ernesto Azzurro*[4,5]

[1] Biodiversity, Biotechnology and Climate Change Laboratory (LR11ES09), Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia, [2] National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia, [3] National Institute of Marine Sciences and Technologies, Tunis, Tunisia, [4] Institute for Marine Biological Resources and Biotechnology (IRBIM)-National Research Council (CNR), Ancona, Italy, [5] Stazione Zoologica Anton Dohrn Naples (SZN), Napoli, Italy

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**Edited by:**
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***Correspondence:**
*Jamila Ben Souissi*
*jbensouissi@yahoo.com*

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On 14th October 2021, a single specimen of *Sargocentron caudimaculatum* was captured in the locality of Cape Bon, Eastern Tunisia, by a local fisher using gillnets at 60 m depth, on rocky bottom. This observation represents the first record of this species in the Mediterranean Sea. General information on the squirrelfishes and the importance of molecular tools for the proper identification of morphologically challenging non-indigenous organisms were raised and discussed.

**Keywords: *Sargocentron caudimaculatum*, Tunisian coasts, climate change, non-indigenous species, citizen, DNA barcoding**

## INTRODUCTION

The squirrelfishes and soldierfishes (Holocentridae) are reef associated species, widely distributed from tropical to warm temperate waters of Indian, Pacific and Atlantic Oceans. Based on swimbladder characteristics, they are separated in two valid subfamilies: Holocentrinae and Myripristinae (Nelson, 1955). The Holocentrinae subfamily includes only three genera: *Holocentrus* Scopoli, 1777, *Neoniphon* Castelnau, 1875 and *Sargocentron* Fowler, 1904. The genus *Sargocentron* includes 33 species (Froese and Pauly, 2022). Eight species, out of the 85 valid species listed in the Holocentridae family (Eschmeyer and Fong, 2017), naturally occur in the Red Sea (Golani and Bogorodsky, 2010), whilst no native species occur in the Mediterranean Sea. The first documented occurrence of squirrelfishes in the Mediterranean Sea was made by Haas and Steinitz (1947) from Palestine, who reported *Holocentrus ruber* (Forsskål, 1775), which was later transferred to the genus *Sargocentron*. The Redcoat *Sargocentron rubrum* (Forsskål, 1775) is one of the first Red Sea species that entered the Mediterranean Sea *via* the Suez Canal (Golani and Ben-Tuvia, 1985) and it is recognized today as one of the most successful invaders of the Basin (Azzurro et al., 2014; Golani et al., 2021). In recent times, the species expanded geographically (Golani et al., 2021) reaching the coasts of Tunisia in 2013 (Ounifi-Ben Amor et al., 2016; Bradai et al., 2019).

Until recent times, *S. rubrum* was considered to be the only representative of the squirrelfish family in the Mediterranean. However, Deef (2021a) reported the occurrence of two squirrelfishes, *Sargocentron spinosissimum* (Temminck & Schlegel, 1843) and *S. tiereoides* (Bleeker, 1853) from the Egyptian Mediterranean coast.

The silverspot squirrelfish *Sargocentron caudimaculatum* (Rüppell, 1838), is a reef associated species, native to the Indian and Pacific Oceans from East Africa to Japan and northern Australia and as far east as the Marshall Islands. It is usually found at depths between 2 and 40 m (Randall and Heemstra, 1984) and to the best of our knowledge it has been never reported from Mediterranean waters.

## MATERIALS AND METHODS

### Study Area and Sampling Processing

On 14th October 2021, a single specimen of *Sargocentron caudimaculatum* was captured in the locality of Cape Bon, Eastern Tunisia, (37°02'57"N and 10°54'18"E) by a local fisher using gillnets at 60 m depth, on rocky bottom. Immediately after capture, specimen was provided by fishermen to researchers. The same individual was later photographed (**Figure 1**), measured (to the nearest millimeter) weighted (to the nearest gram) and frozen for subsequent study. Meristic and morphometric analyses were performed in the laboratory on the defrosted individual following the taxonomic keys provided by Randall and Heemstra (1984); Randall (1998) and Randall and Greenfield (1999). Finally, the collected specimen was stored in ethanol 80% and deposited to the Ichthyological collection of INAT, under the accession number: INAT-HOL-SA- cau01.

### DNA Extraction, Amplification of Mitochondrial COI, and Sequencing

DNA was isolated from fin samples using the Pure Link™ Genomic DNA Mini Kit (Thermo Fisher Scientific) following the manufacturer's protocol, under sterile conditions. The concentration of the isolated DNA was measured in Nano Drop™ spectrophotometer to evaluate its quality and quantity. A 658 bp long fragment from the 5′ region of the COI gene was PCR-amplified using the primer pair as recommended by Ward et al. (2005):

FishF2 5 ′TCGACTAATCATAAAGATATCGGCAC3′ and FishR2 5 ′ACTTCAGGGTGACCGAAGAATCAGAA3.

The PCR amplification of each sample was conducted in a 25 µl volume, including 13.25 µl ultrapure water, 2.5 µl of 10x PCR buffer, 2 µl $MgCl_2$ (25 mM), 1 µl each primer (10 mM), 1 µl (10 mM) of total dNTPs Mix, 0.3 µl of 5 U/µl Taq DNA polymerase (HOT FIREPol® DNA Polymerase), and 4 µl DNA template (ca. 10–100 ng).

The PCR amplification was carried out in a Bio-Rad iCycler Thermocycler where the thermocycling profile was customized as follows: an initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 50 s, primer annealing at 50°C for 2 min, extension for 72°C for 90 s, and a final extension at 72°C for 6 min. Amplification success was checked. Sequencing in one direction (forward) was performed and newly obtained sequence was uploaded in the BLASTn suite to verify whether they meet the threshold value of ≥97% for both the percent identity and query coverage. Two phylogenetic trees were constructed using neighbor-joining (NJ) (**Figure 2A**) and fast minimal evolution methods (**Figure 2B**). The sequences of the high-fidelity amplicon were submitted to the Gen Bank, assisted by the Barcode Submission Tool with detailed source information and feature annotation.

## RESULTS

The specimen under consideration (**Figure 1**) measured 214 mm total length (TL), weighed 187 g and it is described as follows: Head slightly convex and very large eyes; a long preopercular spine; head and body red; edges of scales silver; spinous portion of dorsal fin whitish dappled with light red except for spines and adjacent edges and triangular outer part of each membrane which are bright red. Dorsal fin with XI spines and 14 soft rays, deeply notched between

**FIGURE 1** | The silverspot squirrelfish *Sargocentron caudimaculatum* caught out of the Cape Bon locality.

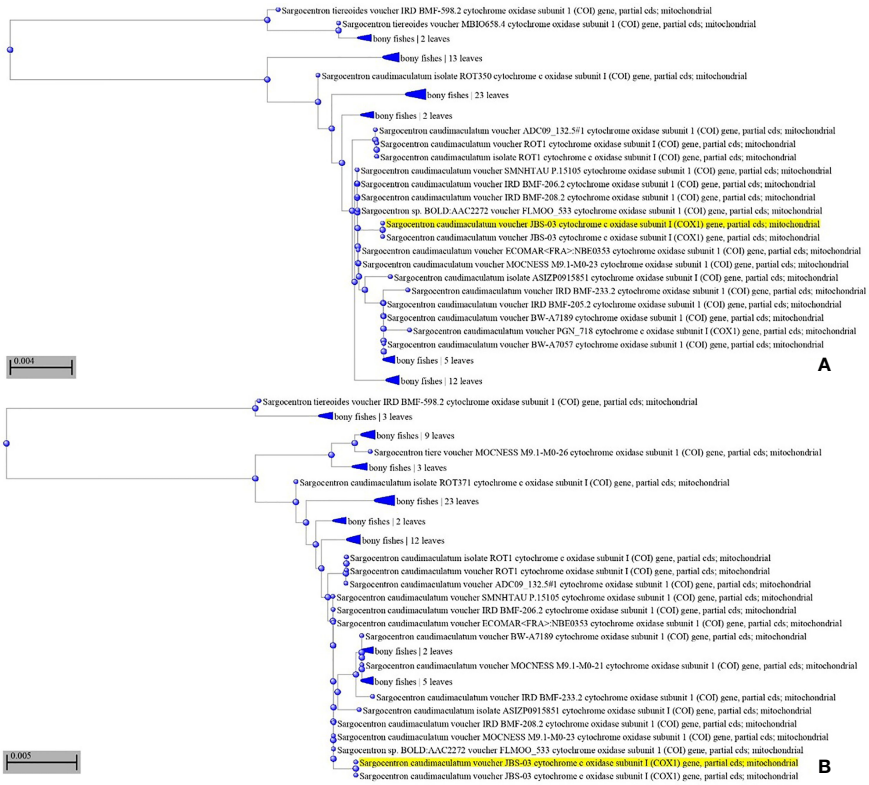


**FIGURE 2** | Phylogenetic tree created with **(A)**, the neighbor joining method (NJ), **(B)**, The fast minimum evolution method by using Blast tree viewer to show the relationships between aligned cytochrome c oxidase subunit I (COI) gene partial sequence of *S. caudimaculatum* with comparison to Genbank records.

spinous and soft portions; last dorsal-fin spine shortest, anal fin with IV spines and 10 soft rays; pelvic fins with I spine and 7 soft rays; oblique rows of scales on cheek 5; anterior end of nasal bone with 2 short diverging spines; preopercular spine long, subequal to orbit diameter, uppermost of 2 large spines posteriorly on opercle the longest; caudal fin forked. Meristic and morphometric characters are reported in **Table 1**.

Genetic sequences: The alignment of 100 sequences of cytochrome c oxidase subunit I (COI) gene and the resulting phylogenetic tree supported by a total of 644 nucleotide informative positions, revealed that the sequences of the Cape Bon specimen were identical to the sequences of *Sargocentron caudimaculatum* present in GenBank (collected in the in the Northeastern of the Red Sea (Gulf of Aqaba; MF124029) (**Figure 2**), with a high bootstrap support (97%).

The new sequences of *S. caudimaculatum* were deposited in GenBank and the accession number obtained (COI gene: OM265164).

## DISCUSSION

Morphological traits, color and both morphometric and meristic characters are in accordance with previous descriptions of *Sargocentron caudimaculatum* (Randall and Heemstra, 1984; Randall, 1998; Randall and Greenfield, 1999). Further molecular identification using DNA barcoding confirmed the taxonomic identity of the species. Considering that *S. caudimaculatum* naturally occurs in the Red Sea (Golani and Bogorodsky, 2010), the species could be likely added to the list of Lessepsian immigrants (Golani et al., 2021), even if the long distance between the Suez Canal exit and the record location (> 2000 Km) could also suggest a possible introduction through ship transport. In addition, the specimen was captured near Sidi Daoud harbor which could be a further evidence to support a ship born introduction. This latter is indeed an increasingly effective vector for the introduction of exotic species in the Mediterranean Sea (Zenetos and Galanidi, 2020).

The high number of nominal species, their partial descriptions, loss of holotypes, and synonymies (Schmitz and Wainwright, 2011), as well as the similarity in color pattern and external morphology of squirrelfishes makes the identification of these ones particularly challenging (Deidun et al., 2016; Deef, 2021b). This taxonomic uncertainty also applies to *S. rubrum*, indeed recent barcoding analyses carried out on Lebanese specimens (Bariche et al., 2015), highlighted different sequences from any currently genetically barcoded *Sargocentron* species (see Keskin and Atar, 2013 for the Mediterranean Sea) and a close match with *Sargocentrum*

**TABLE 1** | Morphometric and meristic characters of *Sargocentron caudimaculatum* caught out of the Cape Bon locality.

| Morphometric characters | In mm | In % of SL |
|---|---|---|
| Total length (TL) | 214 | – |
| Standard length (SL) | 172 | – |
| Predorsal length | 62.9 | 36.56 |
| Preanal length | 118.5 | 68.89 |
| Pre-anal fin length | 132.67 | 77.13 |
| Pre-pectoral fin length | 58.88 | 34.23 |
| Pre-pelvic fin length | 69.73 | 40.54 |
| Head length (HL) | 61.09 | 35.51 |
| Body depth | 68.44 | 39.79 |
| Body width | 30.29 | 17.61 |
| Pectoral fin length | 36.31 | 21.11 |
| Pelvic fin length | 40.89 | 23.77 |
| 1st dorsal fin spine | 12.75 | 7.41 |
| 2nd dorsal fin spine | 21.29 | 12.37 |
| 3rd dorsal fin spine | 24.03 | 13.97 |
| 4th dorsal fin spine | 25.33 | 14.72 |
| 5th dorsal fin spine | 25 | 14.53 |
| 6th dorsal fin spine | 22.15 | 12.87 |
| 7th dorsal fin spine | 19.44 | 11.3 |
| 8th dorsal fin spine | 17.36 | 10.09 |
| 9th dorsal fin spine | 13.85 | 8.05 |
| 10th dorsal fin spine | 11.11 | 6.45 |
| 11th dorsal fin spine | 6.89 | 4 |
| 1st dorsal fin ray | 20.81 | 12.09 |
| Length dorsal fin base | 97.42 | 56.63 |
| 2nd anal fin spine | 10.5 | 6.10 |
| 3rd anal fin spine | 26.97 | 15.68 |
| 4th anal fin spine | 39.76 | 23.11 |
| 1st anal fin ray | 33.99 | 19.76 |
| Length of last anal ray | 9.56 | 5.55 |
| Length of anal fin base | 25.22 | 14.66 |
| Length of caudal peduncle | 37.98 | 22.08 |
| Depth of caudal peduncle | 17.39 | 10.11 |
| Width of caudal peduncle | 3.28 | 1.90 |
| Snout length | 9.8 | 5.69 |
| Eye diameter | 11.49 | 6.68 |
| Interorbital width | 6.76 | 3.93 |
| Upper jaw length | 15.48 | 9 |
| **Meristic characters (counts)** | | |
| Dorsal fin spines | XI | – |
| Dorsal fin rays | 14 | – |
| Pectoral fin rays | 14 | – |
| Pelvic fin spines | 1 | – |
| Pelvic fin rays | 7 | – |
| Anal fin spines | IV | – |
| Anal fin rays | 10 | – |
| Pored lateral-line scales | 43 | – |
| Oblique rows of scales on cheek | 5 | – |
| Scales rows above lateral-line to base of middle dorsal-fin spines | 2.5 | – |
| Gill rakers | 8 | – |

*seychellense* (Smith & Smith, 1963). These results led to hypothesize the occurrence of a species complex for *S. rubrum* and to raise the possibility that unnoticed species of *Sargocentron* had entered the Mediterranean from the Red Sea (Bariche et al., 2015; Deidun et al., 2016; Vella et al., 2016). Hence we stress the importance of molecular tools for the proper identification of morphologically challenging non indigenous organisms, in addition to the precious collaboration with local communities, especially fishers, which always amplify our potential to detect these new arrivals.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, OM265164.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because It's a new record of non-indigenous fish species (not protected).

## AUTHOR CONTRIBUTIONS

RG performed the data analyses and wrote the first draft of manuscript. All the authors contributed to manuscript revision, read and approved the submitted version.

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