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First record of the Gracile lizardfish *Saurida gracilis* (Quoy & Gaimard, 1824) in Mediterranean waters

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Abstract

In January 2021 during a scientific survey to assess the economic impacts of a Mass Mortality Event, a single individual of the Gracile lizardfish *Saurida gracilis* (Quoy & Gaimard, 1824) was caught out of Korbous (northern Tunisia). This observation, confirmed by genetic analysis, provides evidence on the occurrence of a new non-indigenous species in Mediterranean waters. The relevance of this finding for the current monitoring strategies implemented at the regional level are briefly raised and discussed.

Keywords: *Saurida gracilis*; Tunisia; Mediterranean Sea; Lessepsian migration; non-indigenous species.

Introduction

Mediterranean waters are increasingly populated by non-indigenous species (NIS), with new taxa regularly reported in the scientific literature. According to the most recent revisions (Zenetos *et al.*, 2020), 70 new species were added to the inventory of established taxa during the period 2017-2019 and, in the last two decades, many exotic fishes have expanded their geographical distribution, primarily into the central Mediterranean and the Adriatic seas but also into the westernmost sectors of the Mediterranean basin (Golani *et al.*, 2021).

Here we document the occurrence of a new non-indigenous lizardfish for the Mediterranean Sea. Lizardfishes belonging to the family Synodontidae, currently include four valid genera and 83 valid species (Eschmeyer, 2021). Fishbase (Froese & Pauly, 2021) reports 22 valid species of the lizardfish genus *Saurida* Valenciennes 1850. These species are widely distributed along the Indo-West Pacific continental shelves, five of which (*S. gracilis* (Quoy & Gaimard, 1824), *S. longimanus* Norman, 1939, *S. lessepsianus* Russell, Golani & Tikochinski, 2015, *S. tumbil* (Bloch, 1795), *S. golanii* Russell, 2011) occur in the Red Sea and one (*S. lessepsianus* Russell, Golani & Tikochinski, 2015) historically invaded the Mediterranean Sea as a result of Lessepsian migration (Ben Souissi *et al.*, 2005;

Bogorodsky *et al.*, 2014; Russell *et al.*, 2015).

Species belonging to the genus *Saurida* can be morphologically very similar, leading to possible misidentifications (Mohanchander *et al.*, 2019; Jawad *et al.*, 2021) but recent work has better delineated their taxonomy, with several new species described in the past few decades (e.g., Shindo & Yamada, 1972; Waples, 1981; Inoue & Nakabo, 2006; Russell, 2015; Russell *et al.*, 2015; Ye & Kim, 2018).

The Gracile lizardfish, *Saurida gracilis* is a small demersal fish, widely distributed in the Indo-Pacific Ocean (Froese & Pauly, 2021), from the Hawaiian Islands to East Africa and the Red Sea (Golani & Bogorodsky, 2010; Bogorodsky *et al.*, 2014; Akel & Karachle, 2017; Golani & Fricke, 2018). Chabanaud (1934a, b), reported the occurrence of *S. gracilis* from the Suez Canal, but these records should probably be attributed to a misidentification of *Saurida lessepsianus* (Russell *et al.*, 2015). The Gracile lizardfish primarily occurs inshore, usually between 2 - 60 m (Allen *et al.*, 2013) over sand and silty bottoms. It is commonly observed in shallow lagoons, reef flats, and sheltered seaward reefs where it feeds on fishes and occasionally on crustaceans (Froese & Pauly, 2021). To the best of our knowledge, *S. gracilis* has been never reported from Mediterranean waters.

Material and Methods

The specimen under consideration was captured on 13 January 2021, during a field trip carried out by the National Agronomic Institute of Tunisia (INAT). This survey was initially conducted to investigate the economic impacts of a Mass Mortality Event, which occurred between 12 and 14 January 2021 in the area between Port aux Princes and Korbous, in northern Tunisia (Garrabou *et al.*, 2019a) and involved several commercial fish species and invertebrates (INAT unpublished data).

Field sampling was performed over randomly allocated linear transects (of 150 m × 20 m), by collecting, counting, and weighing all the species stranded on the beach or picked up by snorkelling among dense marine vegetation in very shallow waters. During these surveys, one single individual of the Gracile lizardfish was captured by hand by one of the authors (FK), whilst snorkelling along the coast of Korbous (36.813889 N, 10.565 E). It was found at a very shallow depth (50 cm) still alive among a dense *Posidonia oceanica* meadow.

Immediately after capture, the collected individual was photographed (Fig. 1a), measured to the nearest millimetre, weighed to the nearest gram, and then stored frozen for subsequent analyses. Morphological analyses were made on the defrosted specimen with the help of a stereomicroscope. Standard length (SL) and preanal length were measured with a steel ruler; all other measurements were made with a dial caliper to the nearest 0.1 mm. X-rays were used to count the vertebrae. Taxonomic identification followed Waples *et al.* (1981), Fischer & Bianchi (1984), Cressey & Waples (1984), and Inoue & Nakabo (2006). Finally, the collected specimen was stored in ethanol 80% and deposited in the ichthyological collection of INAT, under the accession number INAT-SYN-SA-gra01.

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' ACTTCAGGGTGACCGAAGAAT-CAGAA3.

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 MgCl₂ (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 customised 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. The amplicons were further identified through electrophoresis in a 1% agarose gel with working solution TAE under voltage 100V. 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. The sequences of the high-fidelity amplicons were submitted to the Gen Bank, assisted by the Barcode Submission Tool with detailed source information and feature annotation.

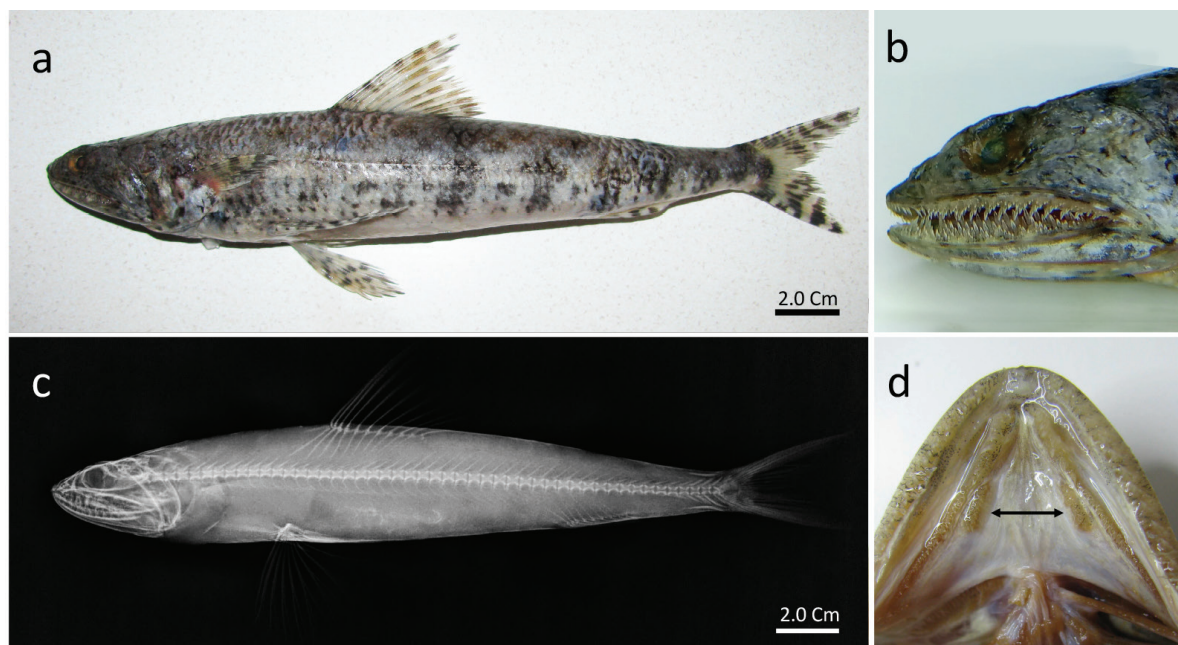


Fig. 1: The Gracile lizardfish *Saurida gracilis* caught along the coast of Korbous. a) Fresh specimen photographed immediately after capture; b) Detail of the head, showing the rows of villiform teeth extending out of the lip; c) X rays of the same specimen; d) Detail of vomer showing the inner vomerine teeth (arrow).

Results

The Korbous individual (Fig. 1a) measured 242 mm total length (TL), weighed 117.3 g and is described as follows.

Description: body elongate and cylindrical, somewhat depressed on head and slightly compressed on caudal peduncle; snout somewhat elliptical when viewed from above; pectoral axillary scale short and broad. Several rows of villiform teeth visible in both jaws even when mouth is closed (Fig. 1b), extending outward onto the lips beyond the normal row of large canine. Two series of teeth on palate, with teeth of the inner palatine series irregularly disposed in three or more rows (Fig.1d). No visible vomerine tooth between the two series of outer palatine teeth.

Dorsal fin rays 10; Pectoral fin rays 13, longest ray 12% or more of SL and extending as far as 1st to 3rd

pre-dorsal scale rows; pelvic fin rays subequal in length; lateral line scales 46 – 52; pre-dorsal scales 3; pelvic fin rays 9; lateral line scales 49; vertebrae 49 (Fig.1c); pectoral fin with 13 rays and extending posteriorly to past pelvic insertion up to three scale rows before dorsal origin (Table. 1). Pectoral axillary scale short and broad.

Colour: large dark splotches mottling the grey background of body. Dorsal, pelvic, anal, and caudal fins mottled with dark spots. Eye circular, covered with fleshy adipose eyelid anteriorly and posteriorly. Interorbital region broad, slightly concave. Peritoneal chromatophores concentrated in a narrow band on the upper part of the peritoneum, forming a dark coloured stripe, right above the kidney.

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 612 nucleotide informative positions, revealed that the se-

Table. 1. Morphometric and meristic characters of *Saurida gracilis* caught out of Korbous (total weight 117.3 g).

Morphometric characters	mm	% of SL
Total length (TL)	242	-
Standard length (SL)	240.5	-
Predorsal length	86.35	35.9
Preadipose length	171	71.1
Preanal length	151.5	62.99
Pre-anal fin length	157.5	65.49
Pre-pectoral fin length	51.44	21.39
Pre-pelvic fin length	71.8	29.85
Head length (HL)	46.58	19.37
Body depth	30.19	12.55
Body width	28.49	11.85
Interpelvic width	8.99	3.74
Pectoral fin length	22.84	9.5
Pelvic fin length	36.65	15.24
Length of 2 nd dorsal ray	35.49	14.76
Length of last dorsal ray	12.56	5.22
Length dorsal fin base	28.56	11.88
Length of 2 nd anal ray	21.31	8.86
Length of last anal ray	11.18	4.65
Length of anal fin base	18.16	7.55
Length of caudal peduncle	12.32	5.12
Depth of caudal peduncle	7.23	3.01
Width of caudal peduncle	6.96	2.89
Snout length	11.52	4.79
Snout width	13.81	5.74
Eye diameter	7.09	2.95
Interorbital width	9.6	3.99
Post orbital length	26.83	11.16
Upper jaw length	32.92	13.69

Continued

Table 1 continued

Morphometric characters	mm	% of SL
Meristic characters (counts)		
Dorsal fin rays	10	
Pectoral fin rays	13	
Pelvic fin rays	9	
Anal fin rays	9	
Pored lateral-line scales	49	
Transverse scales above lateral-line	5	
Transverse scales below lateral-line	6	
Pre-dorsal scales	18	
Pre-adipose scales	16	
Post-adipose scales	10	
No. of vertebrae	49	
No. of rows of palatine teeth	3 or more	
No. of vomerine teeth	0	
No. of teeth rows across tongue	3	

quences of the Korbous specimen were identical to the sequences of *Saurida gracilis* collected in the northern Red Sea and available in GenBank (MN560893), with a high bootstrap support (99%). The new sequences of *S. gracilis* collected in Korbous, were deposited in GenBank with the (COI gene) accession number OK188994.

Discussion

The Gracile lizardfish *S. gracilis* was primarily identified on the basis of morphological differences that separate this species from other members of the genus, including the most similar Clouded lizardfish *S. nebulosa* Valenciennes, 1850 and Orange mouth lizardfish *S. flamma* Waples, 1982. Meristic counts of the Korbous specimen agree with those reported by Waples (1981) for *S. gracilis* and morphological characters, such as the inner palatine teeth arranged in three or more irregular rows, the pigmentation pattern of the peritoneum, the number of pre-dorsal scales, and the shape and length of the axillary scale, which clearly distinguish this species from other congeners (Waples 1981; Cressey & Waples, 1984). Further molecular identification using DNA barcoding confirmed the taxonomic identity of the species *S. gracilis*.

Considering that *S. gracilis* naturally occurs in the Red Sea (Golani & Bogorodsky, 2010; Golani & Fricke, 2018), its possible introduction via the Suez Canal is here proposed as the most likely pathway. Nevertheless, due to the large distance (more than 2,000 km) between the putative introduction point (the exit of the Suez Canal at Port Said) and the capture location in Korbous, other possible vectors, primarily ship transport, cannot be excluded (Zenetos & Galanidi, 2020). Regardless of the possible means of introduction, the finding of a new non-indigenous species in the Mediterranean Sea reinforces the need of conducting regular monitoring activities (Garrabou *et*

al., 2019b; Tsiamis *et al.*, 2019; Gücü *et al.*, 2021), especially in light of the current pace of species introductions (Zenetos & Galanidi, 2020). Accurate and comprehensive information on NIS occurrence and distribution are today required to fulfil the needs of both the Integrated Monitoring and Assessment Programme (IMAP) and related Assessment Criteria, adopted by the Contracting Parties to the Barcelona Convention (IMAP, 2017) under the guiding principles of the Ecosystem Approach (EcAp) and by the Marine Strategy Framework Directive (MSFD) (EU, 2017). At both the European (MSFD) and Barcelona Convention (IMAP) levels, NIS are treated as a distinct descriptor (D2) or Ecological Objective (EO2), respectively. A desired outcome is an empowered and coordinated monitoring system implemented by the different Mediterranean countries.

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