

## Rapid Communication

## Range expansion of a restricted lessepsian: westbound expansion breakthrough of *Lagocephalus spadiceus* (Richardson, 1844) (Actinopterygii: Tetraodontidae)

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### Abstract

The half-smooth golden puffer fish (*Lagocephalus spadiceus*) is a member of the Tetraodontidae family and one of the earliest lessepsian fishes, originally reported in the Mediterranean during the early 1920s. Since then, the species has extended its distribution from the Egyptian coasts to the Sea of Marmara yet remaining confined near the shore or to regions adjacent to the coastline. In May 2016, a single specimen of *L. spadiceus* was fished in South Crete. This is the first time the species has been recorded in the island and the first verified indication that the species is expanding its range westwards over open-sea areas since its entrance in the basin. Thorough morphological and biological description as well as DNA verification of the captured specimen is provided. This record, coupled with the recent first record in the Sea of Marmara, may be an indication of a change in the expansion pattern of the species or of its population status.

**Key words:** alien marine fishes, lessepsian migration, pufferfish, Eastern Mediterranean, bioinvasions, Crete

### Introduction

The half-smooth golden puffer fish (*Lagocephalus spadiceus* Richardson, 1844) is a member of the Tetraodontidae family which includes 184 species distributed in tropical and temperate seas and fresh waters around the world (Matsuura 2015). It is a demersal shallow water fish of Indo-Pacific origin, found over various types of substrata but mainly sandy and in depths ranging from 3 to 200 m but most frequently in depths less than 50 m (Matsuura et al. 2014; Tuncer et al. 2008). In the places of its natural distribution it is harvested for human consumption and in parts of its range it has likely

undergone population declines due to overfishing (Matsuura et al. 2014). Yet, despite its commercial value, information on its biology and ecology is quite scarce (Tortonese 1986; Tuney 2016). Targeted research on the species is limited and in most of the cases the species is examined along, or compared, with other *Lagocephalus* species.

*Lagocephalus spadiceus* is one of the oldest lessepsian fishes and the first of the four *Lagocephalus* species that have entered the Mediterranean via the Suez (*Lagocephalus suezensis*, *Lagocephalus sceleratus* and *Lagocephalus guentheri*, being the other three). It was originally reported in the early 1920s by Sanzo (1930) from a specimen caught in

the Dodecanese islands. Since then, the species was not reported again in any Mediterranean area until the 50s when there were three new records: in Iskenderun, Turkey (Kosswig 1950), NW of Samos, Greece (Ananiadis 1952) and in the Israeli coasts (Ben-Tuvia 1953). Thereafter, reports of the species along the Levantine coastal areas either in professional fishing or in research cruises progressively increased, yet, the majority of the reported cases originated from a few localities, mainly from Iskenderun and Mersin in Turkey. In 2008, there was another record of this species from the Sea of Marmara (Tuncer et al. 2008) which is the outermost north expansion verified for this species. In May 2016, a single specimen of *L. spadiceus* was fished in South Crete. This is the first time the species was recorded in the island and the first verified indication that the species is spreading westwards, crossing over open-sea areas, since its entrance in the basin.

In this paper, we present a thorough morphological and to the best possible extent biological description of our specimen, providing all the information that can be extracted from the examination of a single specimen. An important aspect of the Tetraodontidae family is the ambiguity about the taxonomy of its members. Notably, much confusion occurs on the status of several species of the genus *Lagocephalus* (*L. spadiceus* included), since classification at the species level has not been comprehensively reviewed (Matsuura et al. 2011; Matsuura 2015). For overcoming any identification ambiguities and validate the specimen's taxonomic status, DNA sequencing has also been conducted. Due to the small number of descriptions provided in the literature for *L. spadiceus* and the variability of the characters used as diagnostics, DNA sequencing seems as the most reliable technique for verifying the taxonomic identity of specimens of this genus.

The description of our specimen from South Crete adds to our knowledge on the biology and morphology of the species, and provides further insight into its range expansion, which, coupled with the recent record of northward expansion (Tuncer et al. 2008), may indicate an alteration of its population status.

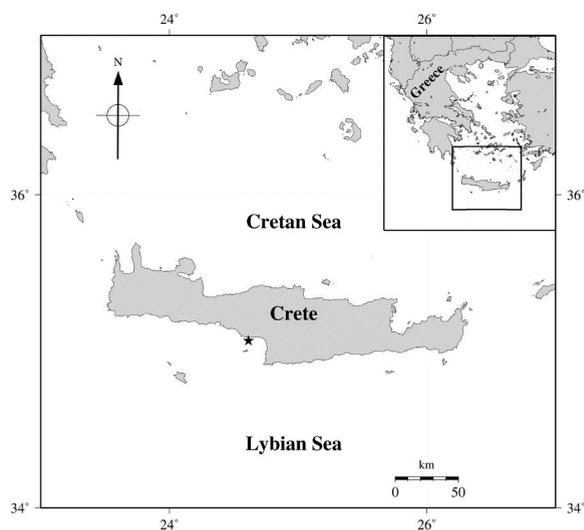
## Material and methods

### Morphology examination

The specimen (Figure 1) was caught on May 27, 2016 in South Crete, at 60 m depth (Figure 2). It was caught in the trammel nets of the professional fishing boat "Tolmiros" and it was the only specimen of this species in the particular catch. The fish was brought to the laboratory the same day, where it was identified.



**Figure 1.** The *Lagocephalus spadiceus* specimen caught in South Crete. Photo by S. Kiparissis



**Figure 2.** Capture site (denoted by the star) of the *Lagocephalus spadiceus* specimen caught in South Crete at 60 m depth.

All body measurements and counting of its meristics were conducted while the fish was fresh. All measurements were taken on the left side of the body and represent the direct distances between points, in accordance to Hubbs and Lagler (1958). Apart from the overall length measurements, which were recorded to the nearest mm, all other measurements were recorded to the nearest 0.1 mm with digital callipers. Weight measurements were recorded to the nearest 0.01 g. Upon completing the external morphology examination, the fish was dissected and stomach distension and perivisceral fat deposits were recorded before viscera were removed to examine the stomach and intestine contents. The degree of stomach distension and perivisceral fat deposits were ranked according to the subjective indices of Lebedev (1946) and Prozorovskaya (1952) respectively. Gonads

were examined for sex determination, and their maturity stage was ranked according to Nikolsky (1963). Finally, after extracting tissue for DNA analysis, the specimen was preserved in 70° alcohol. The voucher was deposited in the Natural History Museum of Crete, accredited with the collection number: NHMC80.1.100.1.

#### DNA isolation and barcoding

Total genomic DNA was extracted from muscle tissue using the NucleoSpin® Tissue (Macherey-Nagel), according to the manufacturer's instructions. The DNA barcoding of the analyzed sample was based on the PCR amplification of four loci sequences mtDNA markers (*cytb*, *12S rRNA*, *16S rRNA* and *mtCOI*), using the universal primers L14841/H15149 (Kocher et al. 1989), 12SAL/12SBH and 16SarL/16SbrH (Palumbi et al. 1991) and FishF1/FishR2 (Ward et al. 2005), respectively. Briefly, 3 min of denaturation (95 °C) followed by 35 PCR cycles of 1 min at 94 °C, 1 min at 54 °C (for the *cytb*, the *12S rRNA* and the *16S rRNA* gene) or 56 °C for *mtCOI* and 1 min at 72 °C, with a final extension at 72 °C for 5 min. PCR reactions were carried out in 20 µl volume of reaction mixture, which included final concentrations of 1x reaction buffer (Kapa), 1 mM MgCl<sub>2</sub>, 0.5 mM dNTPs, 0.5 mM of each primer, 0.5U µl of Taq (Kapa) and 1 µl of template DNA (~ 30–50 ng). The PCR products were purified by a PEG (Polyethylene glycol)-NaCl method (Sambrook et al. 1989). Both strands were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (PE Applied Biosystems) in a 3500 Genetic Analyzer (Applied Biosystems). All marker gene sequences generated in this study were manually edited with Geneious 7.1.2. and have been deposited in the GenBank under accession numbers KY130420–KY130423.

## Results

#### Morphological and biological description of the specimen

The diagnostic characters of our specimen were in agreement with those described for the Tetraodontidae family and the *Lagocephalus* genus by Tortonese (1986) and Matsuura (2001). Its meristics were in accordance to those described by Golani et al. (2002) for this species, except for the number of pectoral fin rays, which, in our specimen were 16 compared to the range of 17–18 described by these authors. The coloration of the specimen was dark green-olive on the back, silvery on the sides with a golden sheen and white on the abdomen. There were dark bands on

**Table 1.** Description of the meristic characters of the *Lagocephalus spadiceus* specimen caught south of Crete.

Meristic parameters	Counts
Dorsal Fin rays	13
Anal Fin rays	11
Pectoral Fin rays	16
Caudal Fin rays	13

the back similar to those described by Farrag et al. (2016) for *Lagocephalus guentheri*. There were two patches of spinules, one on the back and one on the belly, with the latter ending in the mid distance of the edge of the pectoral fin and the anus. The spinulose area on the back extends from the origin of the inter-orbital space anteriorly, to shortly before the outer margin of the pectoral fins posteriorly, ending in a conical shape with a pointy tip. The coloration of the caudal fin was dark olive to brown with a lighter hue at the origin of the fin, with the posterior half of both lobes darker and both ending in white tips. The lower lobe had a whitish tint (Figure 1). The specimen lacked pelvic fins and spines in all fins, as do all members of the Tetraodontidae family (Tortonese 1986).

The total length of the specimen was 202 mm and weighed 159.31 g (eviscerated weight: 127.08 g). The meristics and the values of its morphometric parameters are summarized in Table 1 and Table 2, correspondingly, where body and head measurements are presented both as direct measurements and as percentages of the standard length (SL) and head length (HL), respectively.

Inspection of the gonads of the specimen showed that it was a mature female, with its light orange ovaries occupying about half of the abdominal cavity (degree 3 on Nikolsky's scale). The stomach of the specimen was half distended (degree 3 on Lebedev's subjective stomach-distension index), while macroscopically there was no detectable fat deposition in the abdominal cavity and on the viscera (degree 0 on Prodorovskaya's fat-deposition grading scale). The stomach contents were in the form of a shapeless mass, with almost all ingested food digested and with only a few parts of the foraged organisms still recognizable. The digested remains that still retained a recognizable shape were identified as fish fin apparatuses and as parts of ophiuroid species.

#### DNA sequence alignment and comparisons

The query length of the amplified product was 329 bp, 427 bp, 593 bp and 704 bp for the *cytb*, the *12S rRNA*, the *16S rRNA* and the *mtCOI* gene, respectively. Based on the BLAST analysis of the datasets of the

**Table 2.** Morphometric data of the *Lagocephalus spadiceus* specimen caught south of Crete. Body and head measurements are also presented as percentages of total length and head length respectively.

Morphometric Parameters	Size (mm)	%SL	%HL
Total Length (TL)	202		
Fork Length (FL)	193		
Standard Length (SL)	172		
Head Length (HL)	51.2	0.30	
Head Depth at the end of the gill slit	56.2		1.10
Head Depth at the middle of orbit	49.7		0.97
Head Width (HW) at gill slit	37.0		0.72
Head Width (HW) at orbits	24.3		0.47
Orbit Diameter (horizontal)	14.7		0.29
Orbit Diameter (vertical)	12.6		0.25
Eye Diameter	11.2		0.22
Inter-Orbital space	26.1		0.51
Pre-Orbital Length	22.6		0.44
Post-Orbital Length	22.7		0.44
Body Width at dorsal fin origin (BW <sub>d</sub> )	31.7	0.18	
Body Depth (BD) measured at the dorsal fin origin	38.1	0.22	
Pre-Dorsal Length	109.4	0.64	
Pre-Anal Length	112.2	0.65	
Caudal Peduncle Depth	9.5	0.06	
Caudal Peduncle Length (end of anal base to end of SL)	47.4	0.28	
Dorsal Fin Base Length	18.1	0.11	
Dorsal Fin Length (posterior part of fin base to edge of lobe)	32.8	0.19	
Pectoral Fin Base Length	16.7	0.10	
Pectoral Fin Length (from dorsal insertion point to end of longest ray)	29.3	0.17	
Anal Fin Base Length	13.3	0.08	
Anal Fin Length	29.2	0.17	
Caudal Fin Height (vertically extended)	42.6	0.25	

four loci, it seems that the unidentified sample is assigned as the species *Lagocephalus spadiceus*. In more details, the *cytb* sequence showed the highest similarity (98.5% and 97.6%) with two *L. spadiceus* specimens (KM667972 and KF781159, respectively), the *12S rRNA* sequence was identical with two *L. spadiceus* specimens (KM667972 and KF781140 respectively), and the highest similarity for the *16S rRNA* gene was 99.7% and 99.3%, with two *L. spadiceus* specimens (KM667972 and KT718804), respectively. Finally, the *mtCOI* sequence was identical with two *L. spadiceus* specimens (KM538373 and KM538370, with coverage 663 bp/704 bp both of them) and most closely related with another *L. spadiceus* specimen (KM667972, with coverage 697 bp/704 bp). The remarkable morphological similarity of *L. spadiceus* and *L. guentheri* (also stressed by the overlapping of their meristics described in the present work), raises justified concern. In our case, K2P distances for the *Lagocephalus* genus sequences found in GenBank for the *cytb* gene suggested distinct separation of these two species (13.5% distance of our specimen to *L. guentheri*). However, the authors consider that further and more elaborate comparisons of genetic material of more specimens are necessary in order to validate the existence of two

separate species, provided the identifications have been correct, a difficult task considering their morphological resemblance.

## Discussion

There is a general agreement in the literature regarding the ranges of the meristics of *L. spadiceus*. The counts in our specimen agreed with these descriptions (e.g. Ben-Tuvia 1953; Golani et al. 2002), the only exception being the number of pectoral fin rays, which were 16 in our specimen compared to the range of 17–18 given by other authors. Yet, a recent record from West India (Suvarna Devi 2016) has also delivered a count of 16 pectoral fin rays and these two exceptions indicate a wider range of the number of pectoral rays that has been described so far for the species, and one that overlaps with the corresponding range described for *L. guentheri* (Matsuura et al. 2011), a species very similar to *L. spadiceus* which recently was reported in the eastern Mediterranean (Farrag et al. 2016; Akyol and Aydin 2016). Another notable similarity of our specimen with *L. guentheri* is the dark band coloration pattern on the back, which was similar to the pattern Farrag et al. (2016) and Matsuura et al.

(2011) described for *L. guentheri*. These similarities, added to those already described in the literature, render *L. spadiceus* and *L. guentheri* morphologically very similar, hence the difficulty in distinguishing these two species and the substantive possibility of misidentifications of the specimens caught in the Mediterranean, as has also been suggested by Akyol and Aydin (2016) and Farrag et al. (2016). So, even though the taxonomy of these two species has been described (Matsuura et al. 2011), it is the authors' opinion that due to their close similarity, the identified specimens of both species captured in the basin, should be examined (or perhaps reexamined) more attentively. DNA sequencing will eventually provide the necessary validation. In their recent publication, Zenetos et al. (2017) have synonymized *L. spadiceus* with *L. guentheri*. However, up to date evidence supports the opinion of two discrete species (Matsuura and Satoh 2017) and no nomenclatural changes have yet been established.

The coloration of our specimen's caudal fin partly differed from that described by Matsuura et al. (2011) (dark yellow with the ventral one-third white and with no white tips at the endings), a coloration description used by Farrag et al. (2016) as a diagnostic character. From our specimen, we understand that there is a certain variation in the coloration of the caudal fin and in that respect it may not constitute a strong taxonomic character. Further descriptions from other fresh specimens, genetically verified, will be useful in order to understand the extent of the coloration variability of this fin and to determine its gravity as a diagnostic character. Regarding the diet of *L. spadiceus*, Naik and Jalihal (1998) have provided a detailed analysis, describing fish, crustaceans, molluscs and algae as the groups that comprise the diet spectrum of this species. The detection of ophiurids in the stomach of our specimen provides evidence that it forages on an even broader spectrum of organisms, a feature that can be advantageous to a species that colonizes a new environment.

*Lagocephalus spadiceus* is one of the earliest lessepsian fishes. It passed through the Suez, decades before the salinity reduction of the Bitter Lakes facilitated the passage of the erythrean species through the Canal (Ben-Tuvia 1978; Bianchi and Morri 2003), and before the global warming effect and the operation of the Aswan dam altered the Mediterranean environment in their favor (Bianchi and Morri 2003). The rapidity with which *L. spadiceus* overcame the considerable salinity barriers (the high salinity of the Bitter Lakes and the low salinity of the Nile estuary) and its tolerance to the low winter Mediterranean Sea temperatures indicates a very resilient species and, thus, a strong candidate for rapid colonization.

So, it would be only logical to expect the species to thrive after its entrance into the Mediterranean, if not all around the basin, at least in the south-eastern areas where the environmental conditions are closer to those of the areas of its origin. Paradoxically, even though the species spread swiftly after entering the basin, moving east of the Nile Estuary to the north along the Levantine coastline and to the Aegean Sea, it did not establish any foothold populations along its migration route for many decades. Even today, at least ninety three years after its first detection, the only Mediterranean area known to hold a population of considerable size is the Iskenderun Bay and Mersin (SW Turkey) along with the neighboring areas (Başusta et al. 2013; Gökçe et al. 2016). In all the other areas of its distribution, even those closer to the Suez Canal, the species is rare or not abundant (Bilecenoglu 2010). Moreover, in all these decades of its presence in the basin and all along its distribution range from the Egyptian coasts to the Sea of Marmaras, the species has remained confined to near shore areas and has migrated only to regions adjacent to the coastline, such as the Dodecanese islands and Cyprus. The above suggests that other parameters (biological or environmental) hindered its expansion. These parameters (or some of them) seem to have been lifted, since the species recently recommenced its expansion progress as the two late records reveal, the first from the Sea of Marmara in the north (Tuncer et al. 2008) and the second from the island of Crete in the west (the present record). Considering this species' expansion history, these records constitute expansion breakthroughs, with the latter constituting the first true westward migration since it was advanced via open-sea areas, an unprecedented feature in its migration pattern as it has been unfolded so far.

Up to date, there have been another three reports that denote a westbound expansion of the species' distribution, describing the species present in the coast of Tunisia (Charfi-Cheikhrouha 2004) in the Sallum Gulf, north-west coastline of Egypt (El-Haweet et al. 2011) and in the Ionian Sea (Lefkadiou et al. 2010). However, while the report from Tunisia is an obvious misidentification, which has also been challenged by Enajjar et al. (2015), (the described meristics and the spinule distribution do not accord with that of the species), the reference from the Sallum Gulf is not supported with any specimen description. The report from the Ionian is also another case of misidentification as we have been reassured (Lefkadiou, personal communication). Thus the present record in Crete is the first verified evidence of a westbound expansion of this species in the Mediterranean.

Since there is limited knowledge of the biology and ecology of this species, and no knowledge of the species' mode of adaptation in the Mediterranean whatsoever, we cannot make any safe assumptions or speculations as to which parameters have been altered to permit this new dispersion recommencement to areas beyond its distribution boundaries. Yet, since all evidence suggests the progressive change of the Mediterranean environment, resulting to the progressive tropicalization of the basin (Bianchi and Morri 2003), we could ascribe these last findings to the generation of more favorable conditions for the species, at least in the eastern part of the basin, permitting its expansion to areas formerly inaccessible due to unfavorable environment. In any case, these two recent events of range expansion (to the north and west) may portend a new era for this species' abundance and expansion in the East Mediterranean regions.

The geographical distribution patterns of the non-indigenous species (NIS) are important background information for understanding and describing the dynamics of their dispersion. This type of information is not sufficient for many of these species, a situation exacerbated by the NIS increasing influx rates during the last decades (Bilecenoglu 2010). Additionally, frequent misidentifications (examples for the *Lagocephalus* genus in Farrag et al. 2016), only obfuscate the general picture. In that respect, the accuracy and validity of the distribution data are fundamental prerequisites and a more scrupulous and painstaking effort must be embraced in order to accurately identify and describe NIS from captured or deposited specimens. To that end, genetic analysis proves a valuable tool.

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