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FIRST SUBSTANTIATED RECORD OF *RAJA ASTERIAS* DELAROCHE, 1809 (ELASMOBRANCHII: RAJIFORMES: RAJIDAE) IN THE GULF OF CÁDIZ, NORTH-EASTERN ATLANTIC

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Abstract. The Mediterranean starry ray, *Raja asterias*, considered endemic in the Mediterranean, has recently been reported by other authors from Atlantic fisheries in southern Portugal and northern Morocco. There has been, however, no substantiated record of the species outside the Mediterranean. This fact and the easiness of confusion of *R. asterias* with other species have probably prevented those records from being taken into account in the recent catalogue of North Atlantic elasmobranchs species, in the assessment of the population status of this species, and in recent annotated global checklists of chondrichthyans. In our study, a specimen of *R. asterias* was collected from the Gulf of Cádiz and its species identity was assessed based on morphological and molecular methods. The presently reported finding constitutes a substantiated record of *R. asterias* from the Atlantic. Based on this record, previously reported catches of the Mediterranean starry ray in the Atlantic should be confirmed, and if so, these populations should be included in future assessments of this vulnerable species and in the description of its geographic distribution.

Keywords: Mediterranean starry ray, Atlantic record, morphological description, molecular evidences

The Mediterranean starry ray, *Raja asterias* Delaroche, 1809, was described based on a specimen of a fish species that was commonly found in the fish market of Barcelona, in the north-eastern coast of Spain. It is a benthic species that predominantly lives in the continental shelf, from very shallow waters near the shore down to 200 m depth (Serena 2005), although it has been reported from 343 m depth in the Ionian Sea (Mytilineou et al. 2005). The maximum size of the species is 94 cm total length, although the majority of specimens have a total length of 76 cm or less (Weigmann 2016). The Mediterranean starry ray is present in the whole Mediterranean, it less commonly occurs in the eastern part, and is absent in the Black Sea (Serena 2005), but in general, it shows a decline in terms of abundance (Abella and Serena 2005, Coll et al. 2013, Navarro et al. 2013). In the western and central Mediterranean the species can be locally more abundant, occurring as a by-catch in the landings from the Catalan Sea, the Gulf of Lions, the Ligurian-, Tyrrhenian-, and Adriatic seas, and off the coasts of Tunisia, Algeria, and Morocco (Barone et al. 2007, Hemida et al. 2007, Romanelli et al. 2007, Coll et al. 2013, Navarro et al. 2013, Farrugio 2015, Serena et al. 2015, Ferrà et al. 2016, Fatimetou and Younes 2016).

The IUCN Red List assessed *R. asterias* as a near threatened species (Serena et al. 2015). In that assessment, regarding the geographic range, it was pointed out that this species "can probably be considered endemic in the Mediterranean Sea", although it "may also occur in the Atlantic, near the southern coast of Portugal (Serena 2005)". The extended distribution of *R. asterias* to the adjacent areas of the Atlantic had already been suggested before by Stehmann and Bürkel (1984), who reported its possible distribution in southern Portugal although considered it as questionable, and by Fischer et al. (1987). More recently, the species has not been included in the

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FAO species catalogue of sharks, batoids and chimaeras of the North Atlantic, which includes the Gulf of Cádiz (Ebert and Stehmann 2013), and was reported as endemic to the Mediterranean Sea in an annotated global checklist of chondrichthyans (Weigmann 2016).

However, the species appears in works from the North Atlantic coast of Morocco (Serghini et al. 2008, Tai et al. 2010), and the southern coast of Portugal (Coelho et al. 2005). Tai et al. (2010) reported *R. asterias* as the most important skate species exploited in the northern area off the Moroccan Atlantic coast, whereas Coelho et al. (2005) ranked the species among the most important elasmobranchs from trammel net catches in southern Portugal. None of those works presented morphological or molecular evidences, or even a picture, of the species in order to evidence the identification.

The absence of a substantiated record confirming the presence of *R. asterias* with morphological and/or molecular evidences has probably prevented that those records were taken into account in recent works dealing with the geographical distribution of this species (Ebert and Stehmann 2013, Serena et al. 2015, Weigmann 2016). Hence, in this work we used morphological characteristics and molecular techniques to report the presence of *R. asterias* from the Gulf of Cádiz, North-eastern Atlantic.

An individual of *R. asterias* was collected during the bottom trawl survey ARSA0316. The survey was carried out on board the R/V *Miguel Oliver* in the Gulf of Cádiz (Fig. 1) from 21 February to 6 March 2016. The fishing gear used during the survey was the otter bottom trawl Baca 44/60 (ICES 2010) with a codend mesh size of 20 mm. The individual was caught on 22 February from a 42 m depth sampling station located at 36º10′47″N and 06º12′03″W (Fig. 1). Morphometric measurements and external observations were recorded for the specimen. The individual was fixed and preserved in 4% formalin and is kept in the Marine Fauna Collection* based at the Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, where it is labelled as CFM-IEOMA6087. Genomic DNA was extracted from muscle tissue sample following the protocol proposed by Terrasa et al. (2009). The PCR (polymerase chain reaction) and the universal primers L14724 and H15175 (Ivanova et al. 2007) were used to amplify the cytochrome c oxidase subunit I (COI) fragment. The PCR thermal profile used during the PCR cycles at 96°C for 5 min; then 35 cycles at 94°C for 60 s, 56°C for 60 s and 72°C for 60 s, followed with a final extension at 72°C for 10 min. The PCR product was purified using the commercial kit QIAquick PCR Purification Kit (QIAGEN). Both heavy and light strands were sequenced on an automated ABI 3130 sequencer using ABI Prism Terminator BigDye TM Cycle Sequencing Reaction Kit (Applied Biosystems). Sequence was edited and aligned using BioEdit v. 7.0.5.2 (Hall 1999). The DNA sequence obtained was deposited in the GenBank database** and, BOLD database*** (Barcode of Life Data Systems) (Ratnasingham and Hebert 2007).

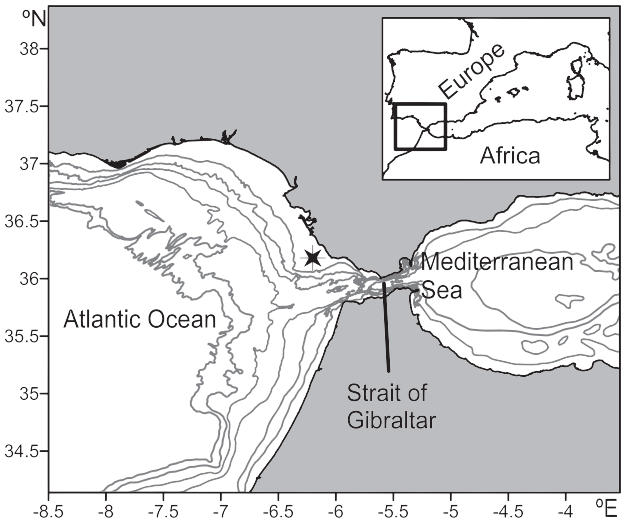

Fig. 1. Map showing the sampling station (black star) where the studied individual of *Raja asterias* (CFM-IEOMA6087) was collected. The 50, 100, 200,500, 800 and 1000 m isobaths are shown

In order to compare our sample with other *Raja* species from the Mediterranean and North-eastern Atlantic, we downloaded from the GenBank the COI sequences of the phylogenetically and morphologically closest species present in the area (Ball et al. 2016). The maximum likelihood (ML) method was used to infer phylogenetic relation between taxa, using the Hasegawa-Kishino-Yano (1985) methodology with invariant sites (HKY+I) as substitution model, selected by AIC test implemented in jModelTest v.2.1.7 (Darriba et al. 2012). The phylogenetic tree was assessed by bootstrap procedure (1000 replicates) using Mega v.6 (Tamura et al. 2013). This software was also used to estimate the genetic distance (*p*-distance) and identity percentage between DNA sequences.

The *R. asterias* individual from the Gulf of Cádiz was a female of 61.5 cm total length and weighing 1504.3 g. It was capable to reproduce and was found in a pre-spawning maturity stage, with presence of eggs but not capsules. It belongs to the genus *Raja* Linnaeus, 1758, which includes small to large skates, with a snout very short and broad, and a tail moderately long and slender (Last et al. 2016). The morphometric and meristic measures of the specimen are summarized in Table 1. The collected specimen was identified as a *R. asterias* based on the following traits: snout short and obtuse; disc wider than long with anterior margin slightly sinuous; upper surface mainly prickly; regular median row of thorns (76) from nape to first dorsal fin, and one parallel row of thorns on each side of tail (19–21 thorns); dorsal fins prickly with length of the base longer than distance between them; 3 preorbital and 2 postorbital thorns; 42 tooth rows in upper jaw; underside surface of disc white

* *http://www.ma.ieo.es/cfm/.*
** *http://www.ncbi.nlm.nih.gov/genbank/.*
*** *http://www.boldsystems.org.*

Table 1
Morphometric (in cm) and meristic characters of *Raja asterias* CFM-IEOMA6087 caught in the Gulf of Cádiz (North eastern Atlantic)

Character	Value		
	[cm]	No.	[TL%]
Total length	61.5		
Disc width	41.7		67.8
Disc length	32.5		52.8
Snout tip to max disc width	27.3		44.4
Preorbital snout length	8.0		13.0
Orbit diameter	2.0		3.3
Orbit + spiracle length	2.7		4.3
Spiracle length	0.7		1.1
Interorbital width	3.1		5.0
Distance between spiracles	4.1		6.7
Precaudal length	32.8		53.3
Tail length to first dorsal fin	17.3		28.1
Tail length to second dorsal fin	21.9		35.6
Tail length	31.0		50.4
Preoral length	6.4		10.5
Prenarial length	5.0		8.0
Mouth width	5.3		8.7
Internarial distance	2.3		3.8
Distance between first gill slit	9.0		14.6
Distance between fifth gill slit	5.6		9.0
Tail width at axil of pelvic fins	2.8		4.5
Tail height at axil of pelvic fins	1.6		2.5
First dorsal fin base	4.1		6.7
First dorsal fin height	1.8		2.9
Second dorsal fin base	4.0		6.6
Second dorsal fin height	1.5		2.5
First dorsal fin to caudal fin tip	6.5		10.5
Second dorsal fin to caudal fin tip	2.1		3.4
Thorns in median row (nape to D1)		76	
Thorns in lateral row of tail (left side)		19	
Thorns in lateral row of tail (right side)		21	
Tooth rows upper jaw		42	
Tooth rows lower jaw		46	

No. = number; Percentages of morphometric characters [TL%] are relative to the total length; nape to D1 = from nape to first dorsal fin.

and smooth, except for prickly snout; upper surface grey, covered more or less uniformly by small black dots; and presence of white-yellow spots surrounded by small black dots (Fig. 2). These characters were coincident with those described for *R. asterias* in Stehmann and Bürkel (1984), Serena et al. (2010), and Last et al. (2016) except for the number of thorns in the median row which in our specimen was larger than the range given in those works (50–70).

A total of 591 base pairs of the COI mitochondrial DNA fragment were sequenced (GenBank ID: KY474344). The nucleotide frequencies were T = 28.6, C = 30.29, A = 24.03, and G = 17.09. The comparison of COI sequences revealed low genetic distance (*p* distance = 0.001/0.005) and a high percentage of identity (99.9/99.45) (Table 2) between the individual studied and *R. asterias* individuals from Algeria and Italy, supporting the morphological identification. The studied individual showed the greatest genetic distance and lowest percentage of identity when compared with *Raja brachyura* Lafont, 1871, followed by *Raja montagui* Fowler, 1910, and *Raja clavata* Linnaeus, 1758 (Table 2). Likewise, the phylogenetic tree included the studied individual into *R. asterias* cluster, which is supported by a high bootstrap probability (*p* = 100%, Fig. 3).

The presently reported study constitutes the first substantiated record of *R. asterias* from the Gulf of Cádiz, North-eastern Atlantic. Although the species appears in several works in the Atlantic (Coelho et al. 2005, Serghini et al. 2008, Tai et al. 2010), these works have not been taken into account in a recent assessment of the species (Serena et al. 2015), an elasmobranch catalogue that includes the Gulf of Cádiz (Ebert and Stehmann 2013), and a recent annotated global checklist of chondrichthyans (Weigmann 2016). The absence of a substantiated record of the species in the Atlantic and also the reported easiness of confusion of *R. asterias* with *R. brachyura* (see Serena et al. 2015), may be the reasons for which the mentioned works from the Atlantic have not been taken into account.

The phylogenetic and genetic distance of the individual studied showed a closer genetic relation with Algerian than with Italian individuals, indicating that due to the geographical proximity, the populations in the Atlantic and those in the western Mediterranean could be the same or be in contact through the Strait of Gibraltar. Further work should be done to confirm this hypothesis**.**

Assuming the easiness of confusion of *R. asterias* with other species, its presence in the Atlantic should be verified in the areas where it has been previously reported. If confirmed, and catches of the Mediterranean starry ray in the Atlantic are so important as reported in Coelho et al. (2005) and Tai et al. (2010), the species should no longer be considered endemic to the Mediterranean. Moreover, the populations in the Atlantic should be taken into account in future assessments of the state of this vulnerable species and in the description of its geographic distribution.

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Fig. 2. Dorsal view of *Raja asterias* (CFM-IEOMA6087) collected from the Gulf of Cádiz

Table 2

Mean value of genetic distances (*p* distance, lower triangular matrix comparisons) and percentage of identity (upper triangular matrix comparisons) for cytochrome c oxidase subunit I (COI) fragment from *Raja* species, below and above the diagonal, respectively

Species	*R. asterias* GC	*R. asterias* AL	*R. asterias* IT	*R. clavata* IT, PO	*R. brachyura* IT, PO	*R. montagui* IT, PO
R. asterias GC		99.94	99.45	95.24	93.23	93.47
R. asterias AL	0.001		99.5	95.18	93.17	93.41
R. asterias IT	0.0055	0.005		94.69	92.68	93.3
R. clavata IT, PO	0.0474	0.048	0.053		95.41	95.24
R. brachyura IT, PO	0.0677	0.068	0.073	0.046		94.87
R. montagui IT, PO	0.0663	0.067	0.068	0.047	0.052	

GC = Gulf Cádiz, AL = Algeria, IT = Italy, PO = Portugal; Comparison of *Raja asterias* are according to sampling localities. The GenBank accession numbers of individuals used in the comparison are those indicated in Fig. 3.

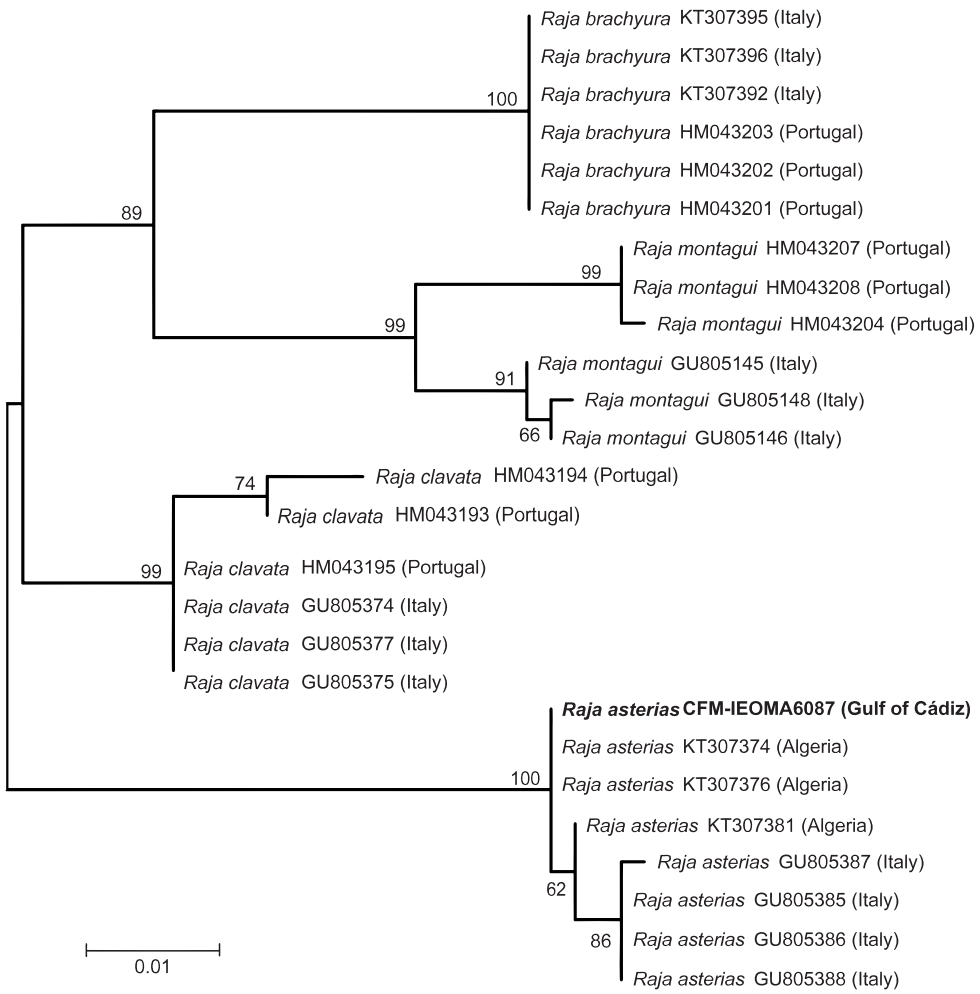

Fig. 3. Maximum likelihood tree based on the cytochrome c oxidase subunit I (COI) fragment of *Raja* species from Mediterranean and North-eastern Atlantic; The GenBank accession numbers are indicated next to species name and the sampling localities are shown between brackets; The GenBank accession number of the individual studied from the Gulf of Cádiz (CFM-IEOMA6087) is KY474344

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