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New morphological and molecular evidence confirm the presence of the Norwegian skate Dipturus nidarosiensis (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin

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Abstract

The present study confirms the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea, by means of morphological traits and molecular markers providing the first record of this species in the Alboran Sea. Cannas *et al*. (2010) reported *D. nidarosiensis* for the first time in the Mediterranean from specimens captured in the central western basin, but Ebert & Stehmann (2013) and Stehmann *et al*. (2015) considered these records "likely refer to the smaller morphotype, *Dipturus* sp.", a species not yet described. Eight specimens of the *Dipturus* genus (Rajiformes: Rajidae) were caught off the Alboran Island (western Mediterranean) in 2012, 2013 and 2016, between 620 and 819 m depth. These specimens showed morphometric diagnostic features corresponding to those of Norwegian skates from the Northeast Atlantic and the central western Mediterranean Sea. Moreover, the Alboran individuals were genetically compared to Northeast Atlantic specimens available in GenBank by means of two mitochondrial DNA fragments: cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cytb*). Analyses showed that the Northeast Atlantic Norwegian skate specimens and the Alboran Sea ones were genetically similar and shared haplotypes, corroborating the identification of the Alboran individuals as *Dipturus nidarosiensis*. However, they were different from other *Dipturus* species distributed throughout the Northeast Atlantic and the Mediterranean Sea. Our results confirm the occurrence of this deep-sea large skate species in the Mediterranean, although the IUCN Red List of Threatened Species does not consider it possible (Stehmann *et al*., 2015).

Keywords: Alboran Sea, deep-sea skate, *Dipturus nidarosiensis,* DNA barcoding, western Mediterranean.

Introduction

Skates (Rajiformes: Rajidae) are considered especially vulnerable to the impact of fishing activities because of their k-selected life-history strategy, characterized by slow growth, late attainment of sexual maturity, long life spans and low fecundity (Stevens *et al*., 2000). The Rajidae is a highly diverse family of elasmobranch fishes, including more than 285 species (Weigmann, 2016). Within this family, the genus *Dipturus* (Rafinesque , 1810) is among the most diverse, comprising at least 29 species (Ebert & Compagno, 2007). This genus includes some of the largest know skates, such as the critically endangered common skate, *Dipturus batis* (Dulvy *et al*., 2006), which was recently reported to be a species-complex in which two species were confounded: the blue skate *D*. cf. *flossada* (Risso , 1826) and the flapper skate *D*. cf. *intermedia* (Parnell , 1937) (Griffiths *et al*., 2010; Iglésias *et al*., 2010). In the Northeast Atlantic Ocean, besides the two aforementioned species, the presence of the spear-nose skate *D*. *oxyrinchus* (Linnaeus , 1758) and the Norwegian skate *D*. *nidarosiensis* (Storm, 1881) is also known, both suggested to represent species complexes similar to that reported for *D*. *batis* (Ebert & Stehmann, 2013).

The Norwegian skate occurs mainly off central and southern Norway, off Iceland, Scotland and Ireland (Ebert & Stehmann, 2013). Recent records include the Bay of Biscay (Rodríguez-Cabello *et al*., 2013) and the central western Mediterranean (Sardinia; Cannas *et al*., 2010). However, Ebert & Stehmann (2013) considered the records of 14 individuals from the Mediterranean (Cannas *et al*., 2010) "likely refer to the smaller morphotype, *Dipturus* sp.", a species not yet described. This previous study did not take into account the fact that several fish and non-fish species distributed in both the Atlantic and Mediterranean exhibited a smaller morphotype and probably genetic divergence in the latter basin, even if the population genetic boundaries are not geographically and hydrogeographically homogeneous (Patarnello *et al.,* 2007). The inference assumed by Ebert & Stehmann (2013) has been included in the latest assessment of this species by the IUCN Red List of Threatened Species (Stehmann *et al*., 2015), where the Mediterranean records are not taken into account; even though additional records were reported also in the central western basin by Follesa *et al*. (2012). Moreover, a very recent study has documented *D. nidarosiensis* in the Rajidae fauna of the Ionian Sea also in the central western Mediterranean (Cariani *et al.,* 2017).

The Norwegian skate is mainly distributed on the outer continental shelf and slope bottoms, from around 200 m to more than 1,000 m depth (Ebert & Stehmann, 2013). Although this species is not targeted by commercial fishing, it is a by-catch of the deep-water trawl and long-line fisheries in which it is occasionally caught (ICES, 2015). *D. nidarosiensis* is currently catalogued as "near threatened" in the Northeast Atlantic by the IUCN Red List of Threatened Species (Stehmann *et al*., 2015). Therefore, an improvement in knowledge regarding the distribution of this species is of significant importance for conservation purposes. The present study provides new morphological and molecular evidence that confirms the presence of *D. nidarosiensis* in the Mediterranean and enlarges its distribution range from Sardinia to the Alboran Sea, next to the Northeast Atlantic where the species is well known.

Materials and Methods

Samples

During the MEDITS bottom trawl surveys (see Bertrand *et al*., 2002 for specification on sampling scheme and gear used) in 2012, 2013, and 2016, eight specimens of *Dipturus* sp. were collected from hauls caught off the Alboran Island (Fig. 1) at depths ranging from 620 to 819 m. All the specimens were caught from three sampling stations (Fig. 1). Additionally, two specimens of *D. oxyrinchus* were collected during the MEDITS survey in 2013 off the Balearic Islands. A small piece of muscular tissue of each specimen was preserved in absolute ethanol for molecular analyses.

Morphology

Morphometric measurements and external observations were recorded for all *Dipturus* sp. specimens according to Stehmann (1995) and Cannas *et al*. (2010). All measurements are indicated in Table 1. All individuals are preserved in the Marine Fauna Collection (http://www.ma.ieo.es/cfm/) based at the Centro Oceanográfico de Málaga (Instituto Español de Oceanografía). Catalogue IDs for each sample (CFM-IEOMA samples) are indicated in Table 2.

Molecular analyses

Genomic DNA was extracted from tissue samples following the protocol proposed by Terrasa *et al*. (2009). Polymerase chain reaction (PCR) and the universal primers FF2d, FR1d and L14724, H15175 (Ivanova *et*

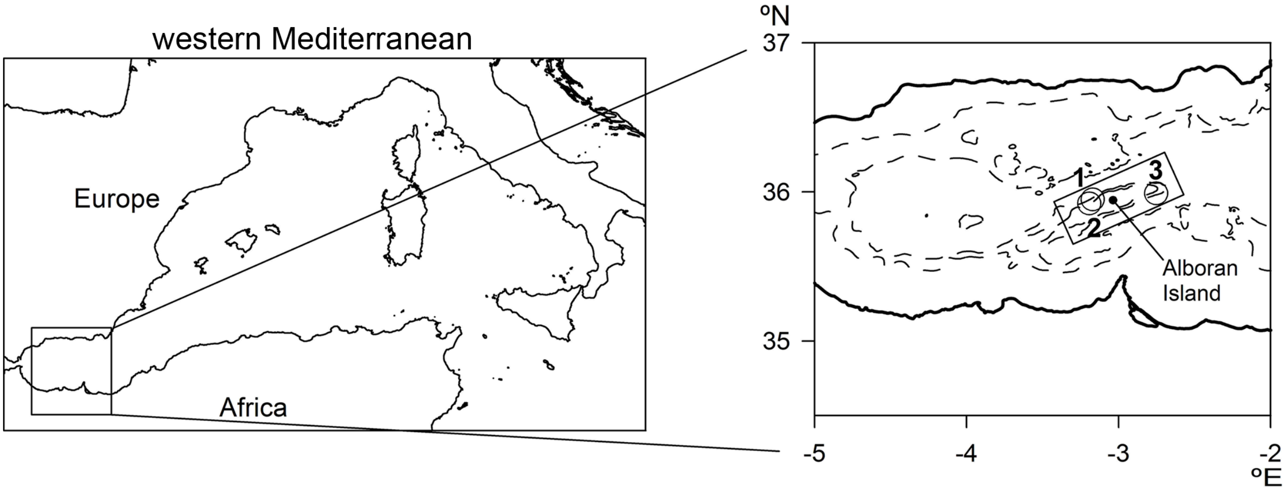

Fig. 1: Map of the study area (Alboran Sea, western Mediterranean), indicating the sampling stations (circles) where the samples were caught: (1) CFM-IEOMA 3570, CFM-IEOMA 3472, CFM-IEOMA 6040, CFM-IEOMA 6041, CFM-IEOMA 6042, CFM-IEOMA 6043; (2) CFM-IEOMA 6044, (3) CFM-IEOMA 6045. Isobaths correspond to 600 and 1,000 m depth.

Table 1. Measurements (cm) and percentage of total length (%) of CFM-IEOMA samples caught in the Alboran Sea (western Mediterranean). Sex of each specimen (M, male; F, female) is indicated in brackets.

Measurements	CFM-IEOMA 3472 (M)		CFM-IEOMA 3570 (M)		CFM-IEOMA 6040 (M)		CFM-IEOMA 6041 (M)		CFM-IEOMA 6042 (F)		CFM-IEOMA 6043 (M)		CFM-IEOMA 6044 (M)		CFM-IEOMA 6045 (F)	
	cm	**%**	**cm**	**%**	**cm**	**%**	**cm**	**%**	**cm**	**%**	**cm**	**%**	**cm**	**%**	**cm**	**%**
Total Length	34.2		32.1		24.7		27		25.6		27.9		72.5		24.7	
Disc width	24.9	72.81	22.9	71.34	17.5	70.85	19.1	70.74	18.4	71.88	19.3	69.18	51.6	71.17	17.3	70.04
Disc length	19.7	57.60	18.2	56.70	13.3	53.85	14.9	55.19	14.4	56.25	15.5	55.56	43	59.31	13	52.63
Snout tip to max disc width	16.5	48.25	15.2	47.35	12.9	52.23	13	48.15	12.9	50.39	13.4	48.03	39.8	54.90	12.8	51.82
Preorbital snout length	7.6	22.22	6.4	19.94	4.7	19.03	5.7	21.11	5.3	20.70	5.8	20.79	16.1	22.21	4.7	19.03
Eyeball length (left)	0.7	2.05	0.8	2.49	0.7	2.83	0.8	2.96	0.7	2.73	0.8	2.87	2	2.76	0.8	3.24
Orbit diameter	1.2	3.51	1	3.12	1	4.05	1.1	4.07	1	3.91	1.2	4.30	2.2	3.03	1	4.05
Orbit + spiracle length	1.6	4.68	1.4	4.36	1.2	4.86	1.4	5.19	1.3	5.08	1.5	5.38	3.1	4.28	1.1	4.45
Spiracle length	0.6	1.75	0.6	1.87	0.6	2.43	0.4	1.48	0.6	2.34	0.5	1.79	1.2	1.66	0.4	1.62
Interorbital width	1.3	3.80	1.1	3.43	1.1	4.45	1.2	4.44	1.2	4.69	1.2	4.30	3.2	4.41	1.1	4.45
Distance between spiracles	2.1	6.14	1.9	5.92	2.1	8.50	1.9	7.04	1.9	7.42	2.1	7.53	4.5	6.21	2.2	8.91
Precaudal length	17.2	50.29	15.2	47.35	12	48.58	13	48.15	12.2	47.66	13.2	47.31	37.2	51.31	11.4	46.15
Tail length to first dorsal fin	8.3	24.27	8.7	27.10	6.7	27.13	7.5	27.78	6.8	26.56	7.5	26.88	19.7	27.17	7.2	29.15
Tail length to second dorsal fin	11.4	33.33	11.5	35.83	8.8	35.63	9.7	35.93	9.3	36.33	9.4	33.69	25.2	34.76	9.2	37.25
Tail length	15.7	45.91	14.8	46.11	13.1	53.04	13.2	48.89	12.9	50.39	13.6	48.75	35.5	48.97	12.2	49.39
Preoral length	7.9	23.10	7.2	22.43	5.1	20.65	6.1	22.59	5.6	21.88	6.1	21.86	17.5	24.14	5.1	20.65
Prenarial length	6.9	20.18	6.1	19.00	4.3	17.41	5.3	19.63	5	19.53	5.5	19.71	16.5	22.76	5.4	21.86
Mouth width	3	8.77	2.5	7.79	2	8.10	2.2	8.15	2.1	8.20	2.3	8.24	5.4	7.45	2	8.10
Internarial distance	3.3	9.65	3.2	9.97	2.1	8.50	2.6	9.63	2.3	8.98	2.5	8.96	6.5	8.97	2.2	8.91
Width of the first gill slits	0.5	1.46	0.3	0.93	0.5	2.02	0.5	1.85	0.5	1.95	0.5	1.79	1.1	1.52	0.5	2.02
Width of the fifth gill slits	0.35	1.02	0.2	0.62	0.4	1.62	0.4	1.48	0.4	1.56	0.3	1.08	0.9	1.24	0.4	1.62
Distance between first gill slit	5	14.62	4	12.46	3.4	13.77	3.5	12.96	3.3	12.89	3.6	12.90	9.3	12.83	3.3	13.36
Distance between fifth gill slit	3.7	10.82	3.5	10.90	2.4	9.72	2.4	8.89	2.2	8.59	2.5	8.96	6.3	8.69	2.3	9.31
Tail width at axil of pelvic fins	0.7	2.05	0.6	1.87	0.5	2.02	0.5	1.85	0.5	1.95	0.6	2.15	1.6	2.21	0.5	2.02
Tail height at axil of pelvic fins	0.75	2.19	0.7	2.18	0.65	2.63	0.65	2.41	0.64	2.50	0.76	2.72	1.5	2.07	0.6	2.43
First dorsal fin base	1.9	5.56	1.8	5.61	1.5	6.07	1.8	6.67	1.5	5.86	1.5	5.38	4.3	5.93	1.5	6.07
First dorsal fin height	0.8	2.34	0.9	2.80	0.6	2.43	0.6	2.22	0.7	2.73	0.8	2.87	2.2	3.03	0.6	2.43
Second dorsal fin base	2.3	6.73	1.8	5.61	1.5	6.07	1.8	6.67	14.3	55.86	1.5	5.38	4.8	6.62	1.6	6.48
Second dorsal fin height	1	2.92	0.9	2.80	0.65	2.63	0.65	2.41	0.64	2.50	0.8	2.87	2	2.76	0.7	2.83
First dorsal fin to caudal fin tip	5.5	16.08	4.5	14.02	5.1	20.65	4.5	16.67	4.4	17.19	4.7	16.85	11.5	15.86	4.5	18.22
Second dorsal fin to caudal fin tip	3.5	10.23	2.5	7.79	2.7	10.93	2.4	8.89	2.3	8.98	2.6	9.32	5.1	7.03	2.5	10.12
Interdorsal distance	0.7	2.05	0.8	2.49	0.8	3.24	0.5	1.85	0.7	2.73	0.8	2.87	1.7	2.34	0.6	2.43
Teeth in upper jaw	43		39		43		43		43		43		44		43	

Table 2. GenBank and BOLD accession numbers for CFM-IEOMA samples and *Dipturus oxyrhinchus* specimens of *COI* and *Cytb* sequences.

Species	Catalogue ID	Year collected	*COI* GenBank no	*COI* BOLD no	*Cytb* GenBank no
Dipturus nidarosiensis	CFM-IEOMA 3472	2012	KX783036	AAEES003-16	KX463276
	CFM-IEOMA 3570	2013	KX783035	AAEES004-16	KX463275
	CFM-IEOMA 6040	2016	KX783034	AAEES005-16	KX686016
	CFM-IEOMA 6041	2016	KX783033	AAEES006-16	KX686017
	CFM-IEOMA 6042	2016	KX783032	AAEES007-16	KX686018
	CFM-IEOMA 6043	2016	KX783031	AAEES008-16	KX686019
	CFM-IEOMA 6044	2016	KX783030	AAEES009-16	KX686020
	CFM-IEOMA 6045	2016	KX783029	AAEES010-16	KX686021
Dipturus oxyrhinchus	166	2013	KU761956	AAEES001-16	KX463277
	176	2013	KU761957	AAEES002-16	KX463278

al., 2007; Palumbi, 1996) were used to amplify two mitochondrial fragments: cytochrome c oxidase subunit I (*COI*) and the cytochrome b (*Cytb*). The thermal profile for PCR consisted of 96°C for 5 min; then 35 cycles at 94°C for 60 s, 56/52°C for 60 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min. PCR products were 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). Sequences were edited and aligned within BioEdit v. 7.0.5.2 (Hall, 1999). The DNA sequences obtained for both mitochondrial fragments were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) and, additionally, the COI fragments were recorded in the Barcode of Life Data (BOLD) database, http://www.boldsystems.org; Ratnasingham & Hebert, 2007).

In order to compare our *Dipturus* samples with other *Dipturus* species in the Northeast Atlantic, we downloaded from GenBank the *COI* and *Cytb* sequences provided by Griffiths *et al*. (2010), Rodríguez-Cabello *et al*. (2013) and Lynghammar *et al*. (2014) (GenBank ID's reported below).

Genetic distances (*p*-distance), identity percentage, and number of base differences between pair of DNA sequences were calculated with MEGA v.6 (Tamura *et al.,* 2013) and Geneious v. 7.1.3 software (Biomatters Ltd., www.geneious.com). The average values of three previous genetic indices between our study samples and GenBank sequences were compared. Additionally, the haplotype network was made for both mitochondrial DNA fragments by statistical parsimony in TCS v.1.21 (Clement *et al*., 2000) and manually edited in Illustrator CS5 (Adobe Systems Inc., CA, USA). Phylogenetic reconstruction was estimated using the substitution model selected by AIC implemented in jModelTest v.2.1.7 (Darriba *et al*., 2012): Hasegawa-Kishino-Yano (1985) model with invariant sites (HKY+I) and Tamura 3-parameter (Tamura, 1992) for *COI* and *Cytb* (*Leucoraja naevus* as outgroup), respectively. Trees were made with MEGA v.6 using the maximum likelihood (ML) assessed by non-parametric bootstrapping (1,000 replicates).

Results

External features

Morphometric measures of all specimens are summarized in Table 1. The *Dipturus* sp. specimens (CFM-IEOMA samples) caught off the Alboran Island ranged from 24.7 to 72.5 cm and from 17.3 to 51.6 cm in total length and disc width, respectively (Table 1). In all, 6 males and 2 females were captured, all of which were immature, with males presenting soft claspers shorter than the length of the pelvic fins, and females with barely visible ovaries and undistinguishable ovarian follicles.

All specimens showed the main traits characterizing the genus *Dipturus*, including a long and pointed snout (internarial width less than 70% prenarial snout length), anterior margins deeply concave and thorns on disc usually absent (Ebert & Stehmann, 2013; Fig. 2). All individuals were identified as *Dipturus nidarosiensis* due to the combination of the following diagnostic features: thorns on disc absent, 39-43 tooth rows in the upper jaw, interdorsal thorns absent (which is common in juvenile specimens), dorsal side uniformly grey-brownish without dots and ventral side almost completely black, covered by a firm layer of black mucus. However, the specimens showed irregularly scattered tail spines, which were fewer (10-12) than those described for adult specimens of *D. nidarosiensis* in the Atlantic (40-50; Ebert & Stehmann, 2013) and the central western Mediterranean (30-41; Cannas *et al*., 2010), but similar to the 9-11 thorns reported by the latter authors for juvenile specimens.

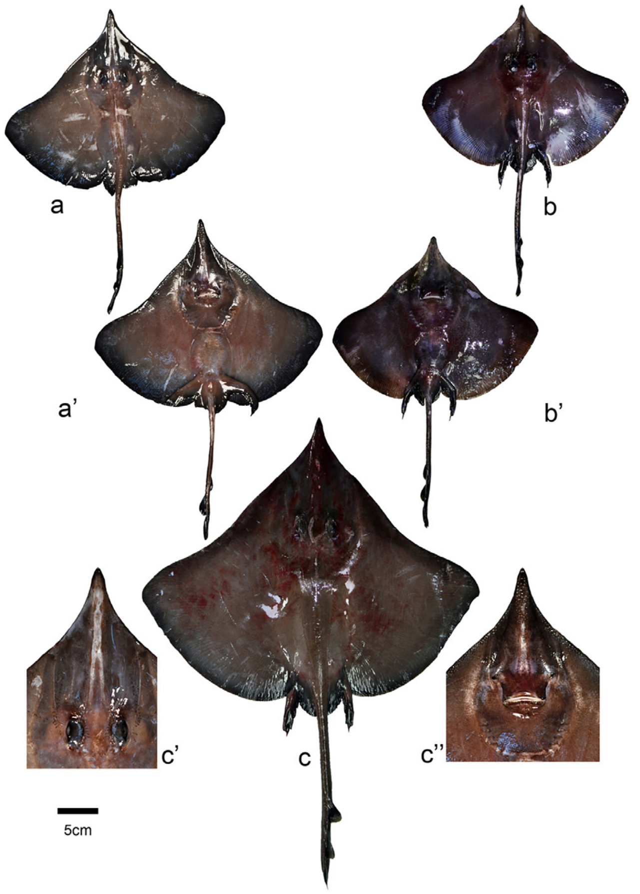

Fig. 2: Pictures of *Dipturus nidarosiensis* specimens caught in the Alboran Sea (western Mediterranean). Dorsal (a, b) and ventral (a', b') view of male (CFM-IEOMA 3472: a, a') and female (CFM-IEOMA 6042: b, b') specimens. Dorsal view of largest specimen (CFM-IEOMA 6044: c) with close view to the side (c', c'').

Genetics

All specimens were sequenced for *COI* and *Cytb* mitochondrial fragments, producing 591 and 378 bp, respectively. The GenBank and BOLD accession number for *D. oxyrinchus* and CFM-IEOMA samples are summarized in Table 2.

The haplotype network (Fig. 3) showed that CFM-IEOMA samples shared haplotypes of both mitochondrial fragments with samples of Northeast Atlantic *D. nidarosiensis*. The low values of genetic distance, reduced numbers of base differences, and the high percentage of identity for *COI* (0.002; 0.9 bp and 99.85, respectively, Table 3) and *Cytb* (0.002; 0.8 bp and 99.80, respectively, Table 3), between the CFM-IEOMA samples and *D. nidarosiensis* from the Northeast Atlantic and the Bay of Biscay supported the morphological identification of the CFM-IEOMA samples as *D. nidarosiensis*. Meanwhile, larger values for genetic distance and lower percentage identity were recorded when comparing the CFM-IEOMA samples and the other *Dipturus* species: *D. oxyrinchus*

Table 3. Average genetic distances and percentage of identity for *COI* and *Cytb* fragments from *Dipturus* species below and above the diagonal, respectively. Average numbers of base differences for both fragments are also indicated in brackets. Dn: *Dipturus nidarosiensis*; Do: *Dipturus oxyrhinchus*; Df: *Dipturus* cf. *flossada*; Di: *Dipturus* cf. *intermedia*; ss: study samples; Gb: GenBank samples.

Dipturus samples	CFM-IEOMA samples		Dn Gb		Do ss		Do Gb		Df Gb	Di Gb
	COI	*Cytb*	*COI*	*Cytb*	*COI*	*Cytb*	*COI*	*Cytb*	*COI*	*COI*
CFM-IEOMA samples	■	■	99.85	99.80	94.03	95.98	94.03	95.98	94.82	94.37
Dn Gb	0.002 (0.9)	0.002 (0.8)	■	■	94.02	96.03	94.02	96.03	94.77	94.36
Do ss	0.059 (35.1)	0.040 (15.1)	0.060 (35.5)	0.040 (15)	■	■	97.04	100	95.74	96.28
Do Gb	0.059 (35.1)	0.040 (15.1)	0.060 (35.5)	0.040 (15)	0.0 (0.0)	0.0 (0.0)	■	■	96.61	98.14
Df Gb	0.052 (30.7)	------	0.52 (30.7)	------	0.034 (20)	------	0.034 (20)	------	■	97.34
Di Gb	0.056 (33.1)	------	0.57 (33.5)	------	0.019 (11)	------	0.019 (11)	------	0.031 (18.3)	■

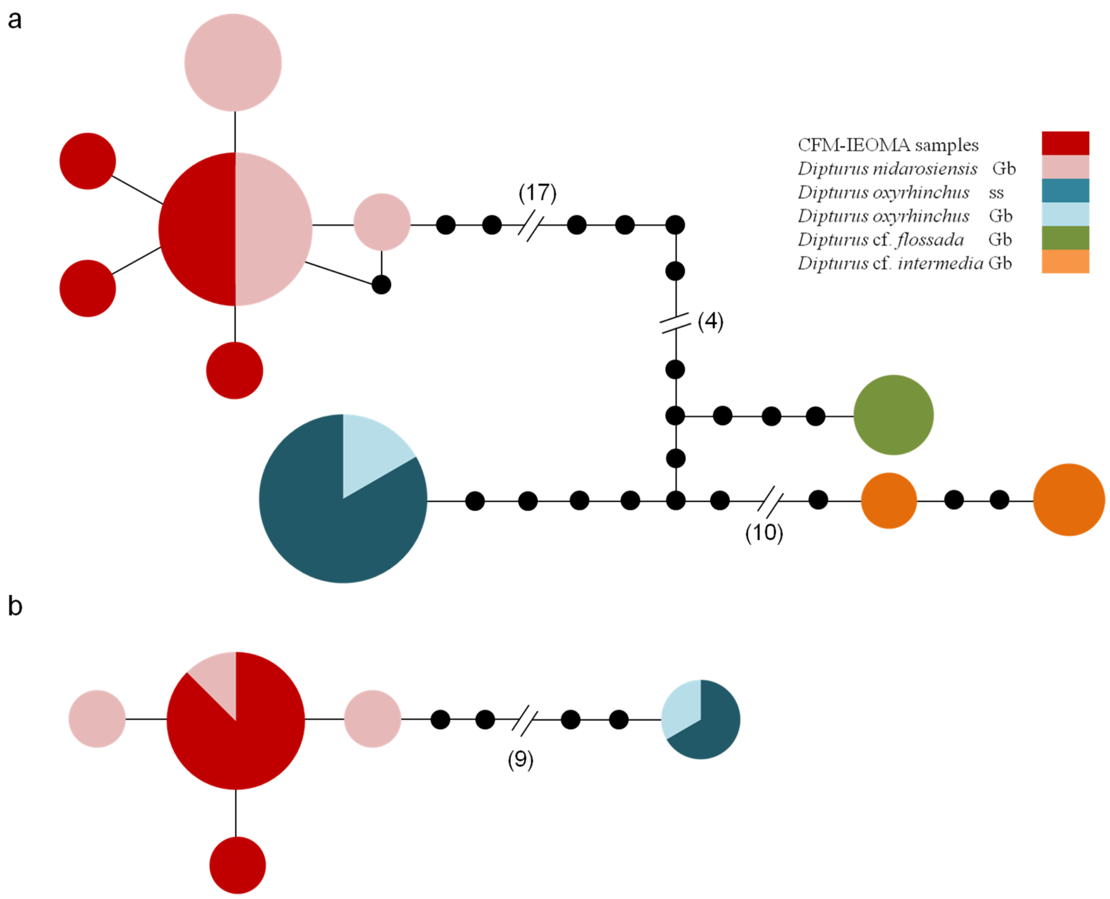

Fig. 3: Haplotype networks derived for (a) *COI* and (b) *Cytb* fragments of *Dipturus* species from the Northeast Atlantic and the Mediterranean. Sizes of circles correspond to the number of individuals displaying each haplotype. Numbers in brackets indicate the total mutational steps separating each haplotype. Black circles represent mutational steps, separated by oblique lines when necessary for the shortening of the steps connecting the largest differentiated haplotypes. ss: study samples; Gb: GenBank samples.

(0.059/94.0 and 0.040/95.9 for *COI* and *Cytb*, respectively), *D.* cf. *intermedia* (0.056/94.3 for *COI*), and *D.* cf. *flossada* (0.052/94.8 for *COI*), respectively (Table 3).

Phylogenetic reconstruction for *COI* (Fig. 4), indicated two strongly supported monophyletic clades. The first corresponds to *D. nidarosiensis* and the CFM-IEOMA samples. The second clade includes the other *Dipturus* species (*D. oxyrinchus*, *D.* cf. *intermedia* and *D.* cf. *flossada*).

Discussion

The present study uses morphological traits and molecular markers to identify 8 specimens of *Dipturus nidarosiensis* (Storm, 1881) captured in the Alboran Sea (western Mediterranean) to provide new evidence of its questioned presence in the Mediterranean Sea. These specimens had the external diagnostic features described by Storm (1881) and showed similar morphometric measurements to those present in the immature specimens of *D. nidarosiensis* collected in Sardinia. All *D. nidarosiensis* individuals from the Alboran Sea were juveniles and, similarly to the juvenile specimens reported by Cannas *et al.* (2010), showed a reduced number of median thorns on the tail. As has been recorded in other skate species, the median thorns could increase progressively as maturity approaches (e.g. *Raja eglanteria* and *Raja montagui*; Ebert & Stehmann, 2013). Little is known about juveniles of *D. nidarosiensis*, so in order to have adequate identification in the future, the characterization of these morphological changes is important.

Molecular analysis confirmed the morphological identification of the CFM-IEOMA samples as *D. nidarosiensis*. Sequence divergence in both mitochondrial fragments (*COI* and *Cytb*) indicated that the Alboran specimens were genetically similar to Northeast Atlantic specimens, and shared haplotypes with them. Phylogenetic analyses revealed that the Alboran and Northeast Atlantic specimens of *D. nidarosiensis* do form a monophyletic group that is markedly differentiated with re-

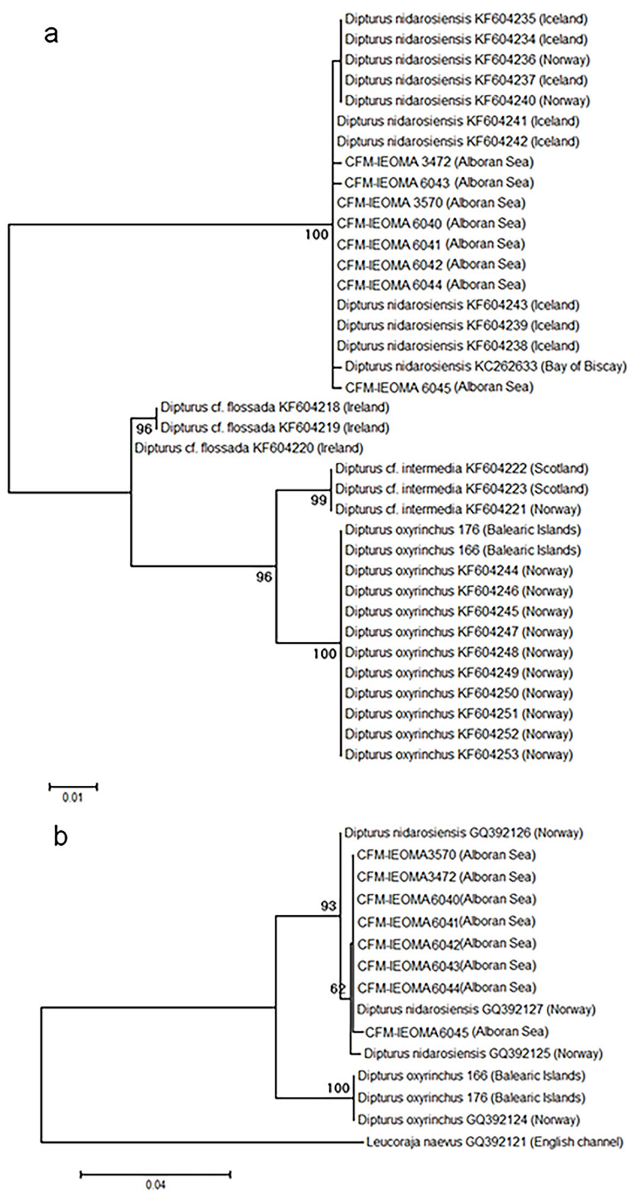

Fig. 4: Maximum likelihood tree based on *COI* (a) and *Cytb* (b) fragments of *Dipturus* species from the Northeast Atlantic and the Mediterranean. Bootstrap values and GenBank accession numbers are indicated near the nodes and the species name, respectively. Sampling localities are also shown in brackets.

spect to the other *Dipturus* species. Besides, phylogenetic analyses show a close relationship with *D. oxyrinchus* and *D.* cf. *intermedia*, similarly to the results described by Griffiths *et al*. (2010), Iglésias *et al*. (2010) and Lynghammar *et al*. (2014).

The morphometric and molecular analyses presented here, along with the comparisons with the DNA sequences of Northeast Atlantic Norwegian skates available in the GenBank, add evidence concerning the presence of this species in the Mediterranean Sea, corroborating the first record of this species in the Mediterranean made by Cannas *et al*. (2010).

The new records of the Norwegian skate, which in the Mediterranean was only known from Sardinia, suggest a wider Mediterranean distribution than previously thought. The Strait of Gibraltar and the adjacent Alboran Sea are transition zones between the Atlantic and the Mediterranean, and are influenced by the inflow of Atlantic water through the Strait and the interchange of these waters with Mediterranean waters through the Balearic channels. The Alboran Sea is characterized by complex bottom topography, where the presence of numerous submarine canyons provides a favorable environmental scenario to the enrichment of deep-water ecosystems (Millot, 1999; Baro *et al*., 2012). These conditions would be favorable to the presence of *D. nidarosiensis*, as well as to other deep-water Atlantic chondrichthyan species, such as *Galeus atlanticus*, also present in this area but unknown in other parts of the Mediterranean (Rey *et al*., 2010; Ramírez-Amaro *et al*., 2015).

The depth at which *D. nidarosiensis* has been found in the Mediterranean, from 600 to 1,420 m off Sardinia (Cannas *et al*., 2010) and from 620 to 819 m in the present work, points out that this species is one of the deepest living skates in the area. This bathymetric distribution could probably be one of the reasons for the little knowledge on the biology of this species. The background inference by Ebert & Stehmann (2013) and Stehmann *et al.* (2015) together with all the recent findings reported by Cariani *et al.* (2017) and the present study launches the challenge to resolve this still-open question by merging all Mediterranean *D. nidarosiensis* specimens (meaning those collected from Sardinia, Ionian and the Alboran Seas) in a unique specimen set and genetically comparing them against the Northeast Atlantic skate using appropriate molecular markers (e.g. microsatellites or SNPs) in order to detect intraspecific differences and assess gene flow. In addition, tagging experiments would contribute to resolve population connectivity between the Atlantic and the Mediterranean and within the Mediterranean.

The Alboran Sea has been reported as a hot spot of marine biodiversity, highlighting its ecological importance for many threatened or endangered vertebrate species (Coll *et al*., 2010; Danovaro *et al*., 2010). Despite the ecological and economic interest of the area, there is a lack of studies on Alboran deep fauna. Moreover, the deep-water trawl is not well developed in this area, and its western part and Alboran Island showed a low level of fishing exploitation mainly between depths of 500 and 800 m depth (Moranta *et al*., 2007). Current records allow an improvement of chondrichthyan biodiversity in the Alboran Sea and support the importance of this area for the biodiversity conservation of deep-sea species of these vulnerable fishes, as has been suggested in previous studies (Ramírez-Amaro *et al*., 2015).

Our results represent an important contribution to the knowledge of the distribution of *D. nidarosiensis* in the Mediterranean Sea. The confirmation of the presence of this species in the area and the new records in the most western part of the basin should finally be included in the future updates of the IUCN Red List. Moreover, special attention is recommended during the periodic activities devoted to the scientific monitoring of fisheries (e.g. European Commission's Data Collection Framework). In fact, the occurrence of *D. nidarosiensis* could potentially be even more widespread within the Mediterranean, but still unnoticed because of the possible misidentification with *D. oxyrinchus*, despite their various morphological differences, as has also been reported in the past for *D. oxyrinchus* vs *D. batis* (e.g. Dulvy *et al*., 2006).

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