

New records of chondrichthyans species caught in the Cantabrian Sea (southern Bay of Biscay)

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Seventeen chondrichthyan species were caught in the Cantabrian Sea (southern Bay of Biscay) during a multidisciplinary survey carried out in the Avilés canyon system in May 2011. This survey provided the first records of three species (Galeus murinus, Neoraja iberica, and Neoraja caerulea) in these waters, and a further record of Rajella kukujevi. To confirm the identity of these species, the cytochrome c oxidase subunit I (COI) of the specimens was sequenced. Genetic analyses revealed that the DNA sequences of the two Neoraja species were identical in all the specimens analysed. Morphometric analyses, based on 40 characters, showed 3.66% dissimilarity between the two species. The morphometric character that contributed most to this discrepancy was disc width.

Keywords: deep-water chondrichthyans, new records, Cantabrian Sea, Avilés canyon, *Rajella*, *Neoraja*, *Galeus*, morphological divergence.

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INTRODUCTION

Several new deep-water chondrichthyans have been described from the north-east Atlantic in recent years (Iglésias *et al.*, 2004; Stehmann *et al.*, 2008; Luchetti *et al.*, 2011). In some cases, records are restricted to certain areas, suggesting that species are endemic to these areas, while in other cases the limited numbers of records makes it impossible to define their distribution. Most of these species are not commercially important, and so they are discarded from commercial fisheries. However, onboard observers in certain fisheries or dedicated scientific surveys studying deep-water areas, including potential Marine Protected Areas (MPAs) have provided more information on the fauna inhabiting deep water ecosystems.

A multidisciplinary survey was carried out in the Cantabrian Sea (southern Bay of Biscay) in May 2011 within the INDEMARES project. The main objective of this project was to contribute to the protection and sustainable use of the biodiversity in Spanish seas by identifying valuable areas for the Natura 2000 network. One of the proposed areas, the Avilés Canyon System, is located 12 km offshore at 6°W longitude, and covers a depth-range from 150 m near the continental shelf to 4750 m at the abyssal plain of the Bay of Biscay (Figure 1). The Avilés Canyon System is a complex, structurally controlled system of canyons and valleys comprising three main canyons of different morphostructural character. These are, from east to west, La Gavierra canyon, El Corbiro canyon and Avilés canyon. The system also includes

two geomorphologic structures: El Agudo de Fuera rocky outcrop and El Canto Nuevo marginal shelf. The near continental shelf is generally narrow and features rocky outcrops of different relief and morphologies and a near horizontal surface corresponding to sedimentary areas with unconsolidated sediment cover or heavily eroded basement surfaces. The strong currents on the shelf are responsible for the limited sedimentary cover of the area. More information about the geomorphology of the Avilés Canyon System is found in Gomez-Ballesteros *et al.* (in press).

Avilés Canyon System is a deep-water ecosystem of great interest within a highly productive area characterized by poleward currents in winter and equatorward currents in spring/summer (Ruiz-Villarreal *et al.*, 2004; Gil, 2008), remarkable phytoplankton blooms, as observed in other canyons (Gonzalez-Quiros *et al.*, 2003), and a diverse benthic community (Louzao *et al.*, 2010). Increased primary production is associated with the shelf break and the topography of the canyon, resulting in upwelling events, especially in summer (González-Quirós *et al.*, 2003; Ruiz-Villarreal *et al.*, 2004). Important local fisheries target hake, blue whiting, anglerfish, megrim, mackerel and horse mackerel (Punzon & Gancedo, 1998; Castro *et al.*, 2011).

The objective of this study was to present data of some deep-water chondrichthyans caught for the first time in the Cantabrian Sea (southern Bay of Biscay) and discuss the similarities found between two skates of the genus *Neoraja* (Rajidae) caught in this area, *Neoraja iberica* Stehmann, Séret, Costa and Baro, 2008 and *Neoraja caerulea* (Stehmann, 1976), based on genetic and morphological results.

Molecular tools, and DNA barcoding in particular, have proven to be very useful not only for phylogenetic and phylogeographic studies but also for identifying cryptic species and

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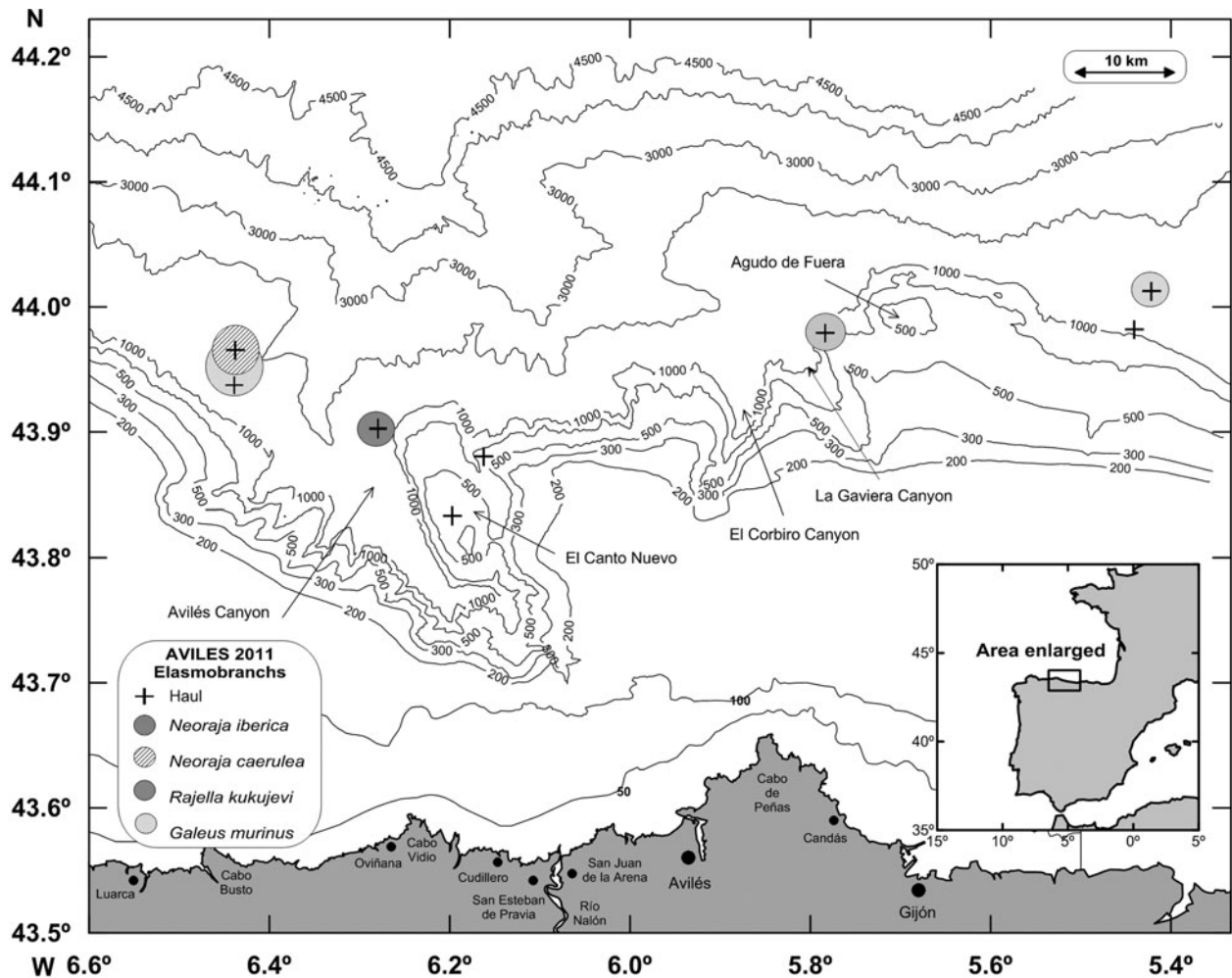


Fig. 1. The study area and the spatial distribution of the new species recorded in the Avilés Canyon System.

species complexes (Herbert *et al.*, 2003; Moura *et al.*, 2008; Ward *et al.*, 2008; Iglésias *et al.*, 2009; Serra-Pereira *et al.*, 2011; Dugdeon *et al.*, 2012; White & Last, 2012). Although these techniques are widely used they have some limitations, and therefore the integration of both genetic and morphological studies is recommended (Tinti *et al.*, 2003; White & Last, 2012), especially if reliable features to aid field identification are to be identified.

MATERIALS AND METHODS

All the chondrichthyes reported here were caught during the survey INDEMARES-AVILES 0511 using a bottom trawl net (GOG-73) with a mesh size of 10 mm and a haul duration of 30 min, which is the standard sampling gear used in the MEDITS surveys (Bertrand *et al.*, 2002). A total of seven hauls were performed in the Avilés Canyon System at depths of 400–1650 m (Figure 1). Mean depth refers to the average between the depth recorded at the start of the tow, when the gear contacts the sea floor and at the end of the tow when the gear leaves the sea floor. Depth-range refers to the minimum and maximum depth recorded in these points. Species were identified according to several texts (Compagno, 1984; Whitehead *et al.*, 1984; Quéro *et al.*, 2003; Iglésias, 2011). Morphometric measurements followed Compagno (1984) and Last *et al.* (2008). All measurements

were made point to point to the nearest mm. Values are expressed as a percentage of the total length (L_T), from the tip of the snout to the tip of the caudal fin.

Similarity between morphometric characters was calculated using the Bray–Curtis index (Clarke & Warwick, 1994) and the resulting dendrogram was obtained with the group average clustering algorithm using the PRIMER software. Species typifying similarities and dissimilarities among different clusters were determined using the SIMPER routine. The non-parametric Kolmogorov–Smirnov test at the 5% significance level was applied to determine the differences between the main morphometric measurements.

Total genomic DNA was extracted from ethanol-preserved muscle tissue using the FENOSALT method (Pérez & Presa, 2011). A 657 bp fragment of the COI locus was amplified and sequenced using the primer pair FishF2 and FishR2 (Ward *et al.*, 2005). Amplifications were carried out in a Mastercycler thermocycler gradient (Eppendorf). The PCR program consisted of 5 min at 95°C, then 35 cycles of 95°C for 30 s, 50°C for 60 s, 72°C for 1 min and a final extension of 10 min at 72°C. Each PCR reaction had a total volume of 20 µL, containing between 10 and 20 ng of purified DNA, 2 µL of 10X NH₄ Reaction Buffer (160 mM (NH₄)₂SO₄, 670 mM Tris–HCl, pH 8.8), 0.16 µL of 100 mM mix dNTPs (Bioline), 1.5 mM of MgCl₂, 15 pmol of each primer, and 1 U of BioTaq DNA polymerase (Bioline). PCR products were cleaned before the

Table 1. Total number and mean biomass of chondrichthyan species caught in the Avilés Canyon System during the INDEMARES-AVILES 0511 survey. No. hauls refers to the number of hauls in which the species was caught ($N_{\text{total}} = 7$).

Species	Author	Number		Kg/haul	Depth range (m)	Length range (cm)		No. hauls
		Male	Female			Male	Female	
<i>Centrophorus squamosus</i>	(Bonnaterre, 1788)	0	1	0.87	1051–1075	—	100	1
<i>Centroscyrnus coelelepis</i>	Barbosa du Bocage & de Brito Capello, 1864	5	12	16.66	1457–1650	72–90	72–115	1
<i>Centroselachus crepidater</i>	(Barbosa du Bocage & de Brito Capello, 1864)	1	5	0.68	1244–1650	63	28–73	2
<i>Chimaera monstrosa</i>	Linnaeus, 1758	14	32	6.53	361–1650	5–63	3–71	7
<i>Deania calcea</i>	(Lowe, 1839)	20	9	8.98	990–1650	49–89	58–100	4
<i>Deania profundorum</i>	(Smith & Radcliffe, 1912)	3	13	0.60	578–1032	31–52	27–50	4
<i>Dipturus nidaorensis</i>	(Storm, 1881)	0	3	2.17	1244–1650	—	91–108	2
<i>Etmopterus princeps</i>	Collett, 1904	27	15	5.71	1244–1650	16–64	16–71	2
<i>Etmopterus pusillus</i>	(Lowe, 1839)	2	0	0.13	1051–1075	42–44	—	1
<i>Etmopterus spinax</i>	(Linnaeus, 1758)	1	2	0.09	530–784	33	29–44	1
<i>Galeus melastomus</i>	Rafinesque, 1810	21	23	2.33	361–1075	21–66	16–71	5
<i>Galeus murinus</i>	(Collett, 1904)	1	7	0.24	1249–1650	43	14–48	2
<i>Hydrolagus mirabilis</i>	(Collett, 1904)	2	3	0.08	1457–1650	17–21	15–32	1
<i>Neoraja caerulea</i>	(Stehmann, 1976)	3	1	0.04	1457–1650	9–27	30	1
<i>Neoraja iberica</i>	Stehmann, Séret, Costa & Baro, 2008	1	1	0.02	530–784	35	30	1
<i>Rajella kukujevi</i>	(Dolganov, 1985)	1	1	0.36	1051–1075	57	70	1
<i>Scyliorhinus canicula</i>	(Linnaeus, 1758)	0	1	0.10	578–624	—	56	1
<i>Scymnodon ringens</i>	Barbosa du Bocage & de Brito Capello, 1864	1	0	0.30	530–784	70	—	1

sequencing reaction using Exo-Sap (USB) according to the manufacturer's protocol. The purified fragments were directly sequenced on the two DNA strands with the same primer pair used for PCR amplification. Sequencing was performed in an ABI Prism 3100 capilar sequencer using the BigDye Terminator Cycle Sequencing Standard (Applied Biosystems).

Identifications were made using: (i) Basic Local Alignment Search Tool (BLAST, Zhang *et al.*, 2000) available in GenBank, and; (ii) the identification engine provided in the Barcode of Life Data Systems (BOLD). Evolutionary analyses were conducted using MEGA5 (Tamura *et al.*, 2011). The phylogenetic tree was inferred using the neighbour-joining method (Saitou & Nei, 1987) and the maximum likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa-Kishino *et al.*, 1985). In both cases, the bootstrap consensus trees were inferred from 500 replicates (Felsenstein, 1985). HKY + G was the model with the lowest Bayesian information criterion (BIC), and it was selected to describe the substitution pattern using MEGA5 (Tamura *et al.*, 2011).

Sediment (grain size, organic content, redox) and infauna data were also recorded for the sampling areas using a mega box corer. The dredge sampled a 0.175 m² area and penetrated 15–20 cm into the sediment. Particle size analysis of sediments was performed by a combination of dry sieving and sedimentation techniques (Buchanan, 1984). Sediment characteristics include mean particle size (Q_{50}), sorting coefficient (S_o), weight percentage of gravel and coarse sands (>500 μm), medium and fine sands (62–500 μm), and silt (<62 μm). Organic matter (OM) in the sediment was estimated as weight loss of dried (100°C, 24 h) samples after combustion (500°C, 24 h).

RESULTS

During this survey a total of 17 chondrichthyan species were caught (Table 1). The most abundant species in terms of biomass were *Centroscyrnus coelelepis* Barbosa du Bocage & de Brito Capello, 1864, *Deania calcea* (Lowe, 1839) and

Table 2. Summary data of the chondrichthyan species recorded for the first time in the Cantabrian Sea, and sediment characteristics in each respective position. % OM, percentage of organic matter; Q_{50} , mean particle diameter; S_o , sorting coefficient; weight percentage of gravel and coarse sands (>500 μm), medium-fine sands (62–500 μm) and silt (<62 μm).

Species	Sex	No. ind.	Length (cm)	Location (Lat/Long)	Depth range (m)	% OM	Q_{50} (phi)	S_o	% >500 μm	% 62–500 μm	% <62 μm
<i>Galeus murinus</i>	Female	6	16–48	43.57°N 6.26°W	1457–1650	4.67	2.55	2.20	6.11	68.57	25.02
	Male	1	43	44.00°N 5.25°W	1244–1254	2.79	2.71	1.71	3.77	79.11	17.13
	Female	1	46	44.00°N 5.25°W	1244–1254	2.79	2.71	1.71	3.77	79.11	17.13
<i>Rajella kukujevi</i>	Male	1	58	43.54°N 6.16°W	1051–1075	6.32	3.72	2.67	1.80	56.39	41.81
	Female	1	71	43.54°N 6.16°W	1051–1075	6.32	3.72	2.67	1.80	56.39	41.81
<i>Neoraja iberica</i>	Male	1	35	43.58°N 5.46°W	530–784	3.11	2.98	1.59	2.18	81.72	16.10
	Female	1	30	43.58°N 5.46°W	530–784	3.11	2.98	1.59	2.18	81.72	16.10
<i>Neoraja caerulea</i>	Male	3	9–27	43.57°N 6.26°W	1457–1650	4.7	2.55	2.20	6.11	68.87	25.02
	Female	1	30	43.57°N 6.26°W	1457–1650	4.7	2.55	2.20	6.11	68.87	25.02

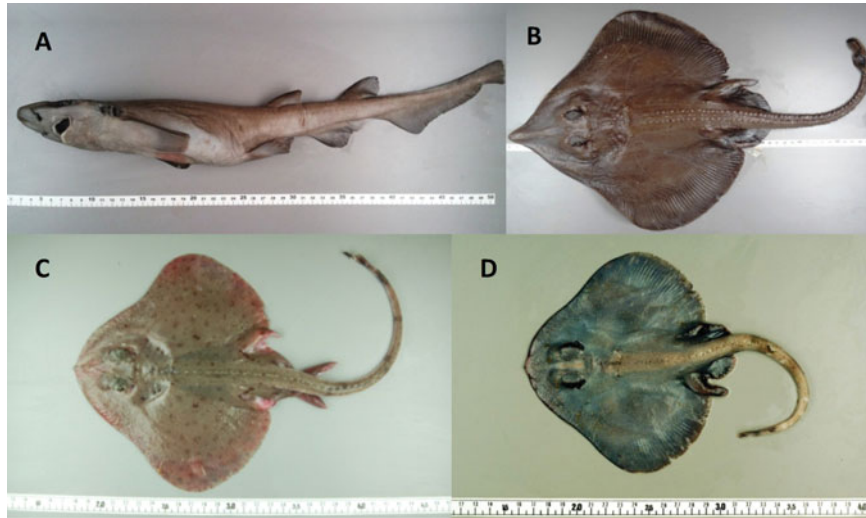


Fig. 2. New records of some deep-water chondrichthyan species caught in the Cantabrian Sea: (A) *Galeus murinus*; (B) *Rajella kukujevi*; (C) *Neoraja iberica*; (D) *Neoraja caerulea*.

Chimaera monstrosa Linnaeus, 1758 while in terms of the total number of specimens observed, *C. monstrosa*, *Galeus melastomus* (Rafinesque, 1810) and *Etmopterus princeps* Collett, 1904 were the most numerous. The following species were recorded for the first time in this area (Table 2).

Mouse catshark *Galeus murinus* (Collett, 1904) (Figure 2A): a total of eight specimens were caught in two hauls at mean depths of 1554 and 1249 m, respectively

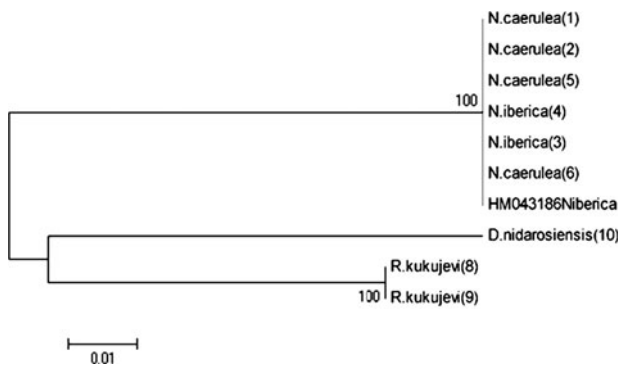


Fig. 3. Molecular phylogenetic analysis by the neighbor-joining method (Saitou & Nei, 1987). Bootstrap values (%) are shown next to the branches.

(Figure 1) on opposite sides (east/west) of the study area at a distance of 111 km from each other. In both cases the sediments comprised medium and fine sand, as the highest proportion of the sediment was between 62 and 500 μm . However, in the deepest haul, located in the west of the canyon, the percentage of organic matter and the sorting coefficient was comparatively higher (Table 2). Six female specimens (16–48 cm L_T) were caught in this haul, with two further specimens (a 43 cm male and a 46 cm female) caught on the east side of the canyon at a mean depth of 1249 m.

Mid-Atlantic skate *Rajella kukujevi* (Dolganov, 1985) (Figure 2B): two specimens (a 57.5 cm male and a 70.0 cm female) were caught at one station at 1063 m mean depth. The sediment consisted of silt and very fine sand with a high percentage of organic matter (6.32%) and a relatively large sorting coefficient, indicating that grain sizes were mixed.

Iberian pygmy skate *Neoraja iberica* Stehmann, Séret, Costa and Baro, 2008 (Figure 2C): two specimens (a 30.0 cm female and 35.2 cm male) were caught at one station (mean depth 657 m). The sea floor at this site consisted of fine sand containing 3.11% organic matter with a moderate size selection coefficient.

Table 3. Results obtained in BOLD and GenBank (BLAST) database. Ss, specimen similarity, Mi, maximum identity, and the respective GenBank Accession numbers.

Code	BOLD species	Ss (%)	BLAST (GenBank) species	Mi (%)	Accession numbers
1	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	100	KC262625
2	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	100	KC262626
3	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	100	KC262627
4	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	99	KC262628
5	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	100	KC262629
6	<i>Neoraja caerulea</i>	100	<i>Neoraja iberica</i>	100	KC262630
7	<i>Galeus murinus</i>	99.84	Carcharhiniformes sp. BOLD:AAA9550 voucher S000008	91	
8	<i>Rajella kukujevi</i>	100	Rajiformes sp. BOLD:AAA4360 voucher RA001134	100	KC262631
9	<i>Rajella kukujevi</i>	100	Rajiformes sp. BOLD:AAA4360 voucher RA001134	99	KC262632

Table 5. Morphometric measurements of the Rajidae species caught in Avilés Canyon System (Cantabrian Sea) during INDEMARES 2011 survey. Values are expressed as percentage of total length (L_T), except total length.

Species	<i>N. iberica</i>		<i>N. caerulea</i>			<i>R. kukujevi</i>	
	G4NIB1	V4NIB2	G3NCA1	G3NCA2	G3NCA3	G3RKU1	G3RKU2
Code (CA11_)	Male	Female	Male	Female	Male	Female	Male
1 – Total length (cm)	35.2	30.0	27.5	30.1	23.4	70.0	57.5
2 – Disc width	52.0	52.7	56.0	58.1	56.4	55.4	58.4
3 – Disc length	44.0	45.0	47.3	48.2	45.3	52.9	52.5
4 – Snout width	26.7	26.7	25.1	26.2	27.4	35.3	32.9
5 – Snout length (preorbital)	9.9	11.0	10.2	11.6	10.3	15.6	14.6
6 – Snout-spiracle	15.3	16.0	16.4	17.3	15.8	20.3	19.7
7 – Head length (dorsal)	17.3	17.7	18.9	19.9	18.4	22.6	21.2
8 – Orbit diameter	4.7	4.7	5.1	5.0	4.7	3.9	3.8
9 – Orbit and spiracle length	6.0	5.8	6.2	6.0	5.8	4.7	5.0
10 – Spiracle length (main aperture)	2.7	3.3	3.3	3.7	3.4	2.9	2.1
11 – Distance between orbits	3.1	3.0	2.9	3.3	3.2	3.9	3.7
12 – Distance between spiracles	6.3	6.0	7.1	7.0	7.1	7.0	6.4
13 – Snout to cloaca	40.1	41.0	41.5	43.2	40.8	50.9	48.7
14 – Cloaca to caudal-fin	60.2	58.0	57.5	56.5	58.1	49.7	51.0
15 – Snout length (pre upper jaw)	10.2	11.7	10.5	11.6	10.7	16.6	15.3
16 – Prenasal length	8.2	9.5	7.6	9.6	8.5	14.6	13.6
17 – Head length (to fifth gill)	22.7	23.0	24.0	23.9	23.1	29.1	27.1
18 – Mouth width (exposed part)	2.8	2.7	2.9	3.0	2.7	5.1	4.9
19 – Distance between nostrils	6.5	6.3	6.3	7.3	6.6	7.7	8.2
20 – Nasal length	4.5	4.3	4.4	4.7	4.7	4.6	4.3
21 – Nasal width	7.5	7.5	8.1	8.0	8.1	8.7	8.9
22 – Width of first gills	1.3	1.7	1.6	2.0	1.7	1.7	1.3
23 – Width of fifth gills	0.9	0.7	1.3	1.3	1.3	1.1	1.0
24 – Distance between first gills	12.5	12.7	13.8	13.6	12.8	13.4	14.1
25 – Distance between fifth gills	5.7	6.0	7.3	8.3	7.1	8.6	8.5
26 – Length anterior pelvic lobe	13.1	12.3	13.5	15.0	14.3	15.6	15.7
27 – Length posterior pelvic lobe	17.9	15.7	16.4	15.9	14.7	18.1	17.8
28 – Pelvic base width	8.0	8.7	10.5	9.0	8.1	10.3	9.0
29 – Tail at pelvics (width)	4.5	4.0	4.7	4.7	4.3	5.1	4.9
30 – Tail at pelvics (height)	2.8	2.8	3.6	3.3	3.0	3.3	3.0
31 – Tail at midlength (width)	2.3	2.2	2.9	2.5	2.1	2.3	2.6
32 – Tail at midlength (height)	1.6	1.5	1.8	1.8	1.5	1.5	1.6
33 – Tail at D1 (width)	1.1	1.3	1.8	2.0	1.3	2.0	1.6
34 – Tail at D1 (height)	0.9	0.9	1.1	1.3	0.9	1.1	1.3
35 – D1 base	4.5	3.7	4.9	4.0	3.4	5.9	5.6
36 – D1 height	2.0	2.3	2.2	2.3	1.7	2.9	3.3
37 – D1-Caudal	12.2	11.3	10.5	10.0	10.7	12.9	12.5
38 – D2-Caudal	7.1	7.7	6.5	7.0	6.8	7.0	7.3
39 – Caudal length	3.1	3.3	2.2	3.0	2.6	1.1	1.4
40 – D2 base	5.4	5.0	4.5	4.2	6.0	6.1	6.1
41 – D2 height	1.7	2.0	2.5	2.3	2.6	3.0	3.0
42 – Clasper (post-cloacal length)	18.0		10.5		7.5		9.9
43 – Cloaca to pelvic-clasper insertion	3.7		6.9		3.0		4.3

analysis using the non-parametric Kolmogorov–Smirnov test did not reveal any significant differences among the first ten selected characters (Table 6). However due to the low sample size ($N_1 = 2$, $N_2 = 3$) these results should not be considered decisive.

DISCUSSION

The knowledge on the biology and geographical distribution of deep-water skates in the North Atlantic is limited because several deep-water species have only been discovered relatively recently. Furthermore some records are restricted to certain areas and only a few individuals of these species

have been recorded, which is the case of *Rajella kukujevi*, *Neoraja iberica* and *Neoraja caerulea*, (Figure 5).

The mouse catshark *Galeus murinus* was originally described by Collett in 1904 as *Pristiurus murinus*. Authors later recognized *Pristiurus* as a junior synonym of *Galeus*. The first specimen, an immature female of 22 cm, was caught at a depth of 1100–1300 m, at a location 93 miles north-west of the Hebrides Islands. Since then, *Galeus murinus* has been noted to be fairly common. In recent years it has been found to be more widespread in the north-east Atlantic than previously thought (Anonymous, 2000), occurring from western Iceland, Faroe Islands, north-west Scotland, Ireland, France (two records at 46°N 10°W) and as far south as the Western Sahara (four records, one at 20°43'N 17°71'W, 280 m depth and three at 24°–25°N 6°–6.6°W,

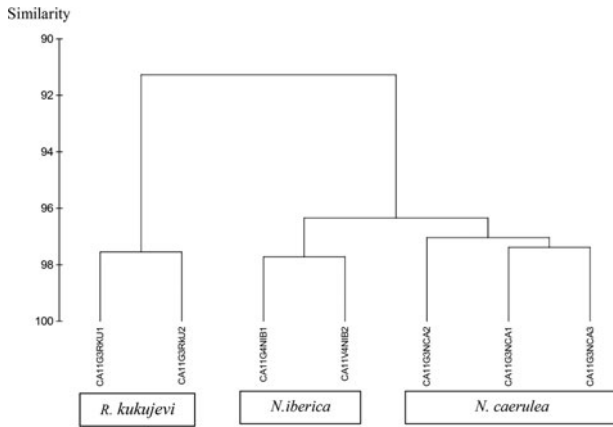


Fig. 4. Cluster of similarity between the Rajidae species found in Avilés Canyon System based on morphometric characters. Codes refer to specimens data described in Table 5.

1000–1265 m) (Anonymous, 2001). These records from the southern Bay of Biscay confirm the southern range and broad distribution of this species. Half of the specimens caught were close to the maximum observed size of 49.1 cm (Iglésias *et al.*, unpublished data). Magnusson *et al.* (2000) reported that the species reached 85 cm; however, this maximum size as well as the maximum depth reported by these authors (656–1731 m) needs to be revised because there could have been taxonomic confusion with other species (IUCN, 2009). Compagno *et al.* (2005) provisionally

estimated the maximum depth as 1250 m, thus the current records extend the bathymetric range to 1457–1650 m (deepest haul). Regarding the water masses at this location the presence of Labrador Sea Water characterized by a deep salinity minimum is noteworthy (Lavin *et al.*, 2006).

Rajella kukujevi was described for the first time by Dolganov (1985) based on a specimen caught on the Faraday Seamount (northern part of the Mid-Atlantic Ridge). It has recently been found elsewhere in the North Atlantic: the Mid-Atlantic Ridge, Iceland, Ireland and the Faroe Islands (Clarke, 2000; Kukuev, 2002; Orlov *et al.*, 2006; Orlov & Cotton, 2011). It has been found at depths between 775 and 1500 m, but it is most commonly recorded at depths greater than 1000 m (Gibson *et al.*, 2008). It was recently recorded close to this area, which extended the known distribution range of this species in the north-east Atlantic towards the south (Rodríguez-Cabello *et al.*, 2012). These captures along with other recent records of this species in the north-east Atlantic do not support the hypothesis that *R. kukujevi* is endemic to the North Atlantic Ridge (Kukuev, 2002). Like *N. iberica* these specimens were caught in the MOW layer (González-Pola personal communication), which suggests that this species could occur in other geographical areas with similar environmental features.

Neoraja iberica was first recorded and described by Stehmann *et al.* (2008) from southern Portuguese waters and the Gulf of Cádiz (southern Spain). The description was based on 50 specimens representing all size-classes of the two sexes. The maximum total length recorded was 31.6 cm for females and 32.7 cm for males, and depths ranged from

Table 6. Dissimilarity index (average 3.66 %) between groups of *Neorajas* and morphometric characters that contributed more to the difference found among them. Results expressed as percentage of total length (L_T).

Morphometric character	<i>N. iberica</i>		<i>N. caerulea</i>		Av.Diss	Diss/SD	Percentage	
	Average	SD	Average	SD			Contribution	Cumulative
Disc width	52.4	0.48	56.8	1.14	0.5	4.3	13.1	13.1
Disc length	44.5	0.68	46.9	1.47	0.3	1.7	7.1	20.1
Cloaca to caudal-fin	59.1	1.57	57.4	0.83	0.2	1.3	5.1	25.3
Distance between fifth gills	5.9	0.22	7.6	0.67	0.2	3.0	5.0	30.2
Length anterior pelvic lobe	12.7	0.52	14.3	0.75	0.2	2.0	4.6	34.8
Head length (dorsal)	17.5	0.24	19.1	0.79	0.2	2.2	4.6	39.4
Length posterior pelvic lobe	16.8	1.58	15.7	0.84	0.2	1.3	4.2	43.5
Distance D1-caudal	11.8	0.62	10.4	0.38	0.1	2.3	3.9	47.5
Snout to cloaca	40.6	0.67	41.8	1.23	0.1	1.2	3.9	51.4
Pelvic base width	8.4	0.50	9.2	1.23	0.1	1.1	3.1	54.4
Snout width	26.7	0.03	26.2	1.13	0.1	1.8	2.7	57.2
Distance between spiracles	6.2	0.18	7.1	0.06	0.1	5.3	2.7	59.8
Snout-spiracle	15.7	0.47	16.5	0.74	0.1	1.4	2.7	62.5
Prenasal length	8.9	0.89	8.6	1.00	0.1	1.3	2.6	65.1
D2 base	5.2	0.28	4.9	0.95	0.1	3.3	2.4	67.5
Head length (to fifth gill)	22.9	0.19	23.7	0.51	0.1	1.7	2.4	69.9
Distance between first gills	12.6	0.12	13.4	0.53	0.1	1.7	2.3	72.2
Snout length (pre upper jaw)	11.0	1.02	11.0	0.59	0.1	1.4	2.2	74.4
Snout length (preorbital)	10.5	0.75	10.7	0.81	0.1	1.5	2.2	76.6
D1 base	4.1	0.62	4.1	0.75	0.1	1.5	1.9	78.4
Distance D2-caudal	7.4	0.40	6.8	0.22	0.1	1.6	1.9	80.3
D2 height	1.9	0.21	2.5	0.15	0.1	2.8	1.8	82.1
Caudal length	3.2	0.15	2.6	0.40	0.1	1.6	1.8	83.8
Nasal width	7.5	0.02	8.1	0.07	0.1	10.1	1.7	85.5
Width of fifth gills	0.8	0.17	1.3	0.03	0.1	4.6	1.5	86.9
Tail at pelvics (height)	2.8	0.01	3.3	0.32	0.1	1.9	1.5	88.4
Tail at D1 (width)	1.2	0.14	1.7	0.37	0.1	1.5	1.5	89.8
Spiracle length	3.0	0.45	3.5	0.19	0.1	1.2	1.4	91.2

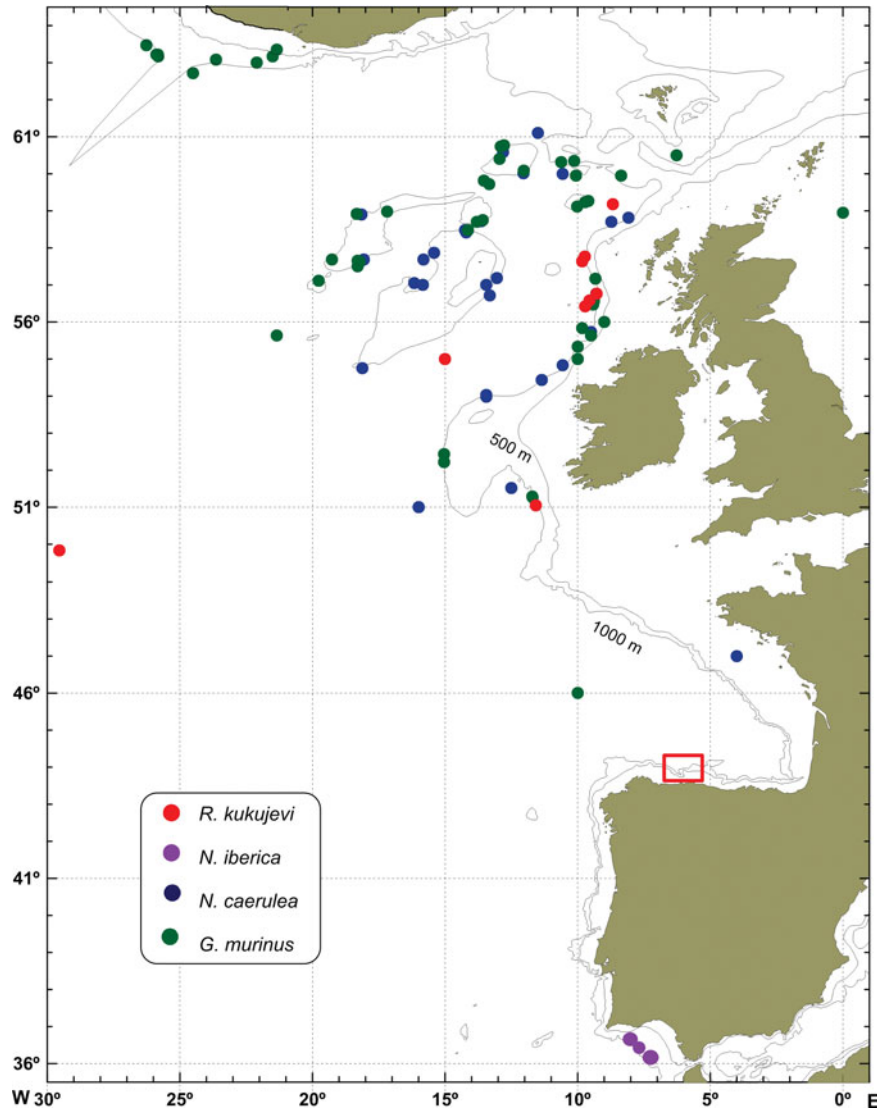


Fig. 5. Geographical distribution of the above species based on published records (Froese & Pauly, 2010). A few records of *Galeus murinus* below 36°N (see text) have not been represented here. The red rectangle shows the area where these four species have been recorded for the first time in the Cantabrian Sea.

172–670 m. Therefore, the present study extends its known northern distribution limits (to northern Spain), bathymetric range (530–784 m) and maximum length (male 35.2 cm). The fact that the water masses where this species was caught corresponded to the MOW could explain this northern distribution and potential occurrences in higher latitudes. The fate of the MOW once it flows out of the Strait of Gibraltar into the Bay of Biscay and further north is uncertain and there is some controversy. However, according to a study by Iorga & Lozier (1999) a continuous signal of Mediterranean Water is tracked northward to 50°20'N into the Rockall Trough and over to Iceland–Scotland. Whether this could have some influence on the distribution range of this species needs to be explored.

Stehmann (1976) originally described *N. caerulea* in the genus *Breviraja*. The geographical distribution of this species is not well known. It was previously considered to be relatively rare and possibly endemic to the north-east Atlantic (Iceland, Rockall Trough, north-west Ireland). However, further records from the Mid-Atlantic Ridge and Bay of Biscay (Stehmann & Burkel, 1984; Quérou *et al.*, 2003)

have expanded its known distribution range. It is commonly found at depths of 600–1260 m. Thus, the present records extend its known bathymetric range (1554 m).

According to external appearance and colour patterns (Figure 2C,D), the *N. caerulea* and *N. iberica* specimens seem to belong to different species; however, the similarity percentage obtained (97%) based on morphological characters is rather high. These similarities were also reported by Stehmann *et al.* (2008), who included two further congeners, *N. stehmanni* and *N. africana*, in their description. As stated by these authors these species resemble each other in several external aspects, such as their general shape, squamation, morphometric proportions, tooth row counts and internal meristics, as well as their scapulocoracoids and pelvic girdles, as demonstrated by McEachran & Compagno (1982).

However, DNA sequences obtained for the two species were identical (Table 3, Figure 3). Similar results were obtained by Serra-Pereira *et al.* (2011), who suggested the possible synonymy between the two taxa. The low number of specimens caught made it impossible to draw any firm conclusions about the morphometric measurements. The fact that

specimens of both sexes belonging to each species were examined indicated that the morphological differences found were probably not due to gender or size. Thus, these results suggest that it could be a case of morphological divergence. This is not so uncommon and can occur among populations living in different habitats with environmental variables that act as potential selective forces responsible for adaptation to divergent habitats (Collin & Fumagalli, 2011). In the present study the two species were recorded at different depths and environmental conditions separated by a distance of 64.8 km.

The application of molecular genetics to study sharks, skates and rays has considerably increased recently, particularly for studies focused on the identification, conservation or fisheries management of elasmobranch species (see references in Tinti *et al.*, 2003; Serra-Pereira *et al.*, 2011; Vélez-Zuazo & Argñarsson, 2011; Dudgeon *et al.*, 2012). Although there is an increasing number of molecular studies that aim to identify cryptic species and species complex issues (Tinti *et al.*, 2003; Ward *et al.*, 2008; Iglésias *et al.*, 2009; Straube *et al.*, 2011; Dudgeon *et al.*, 2012), few studies have found elasmobranch species that have similar DNA sequences but also showing morphological divergence. One similar case was recently described by Serra-Pereira *et al.* (2011) regarding two species of *Raja*, *R. clavata* and *R. maderensis*. The similarity between these two species in the BOLD database was 99.69% and the phylogenetic analysis failed to separate these taxa into different clades. The authors stated that the most probable hypothesis to explain these results is that *R. maderensis* was simply a different morphotype of *R. clavata*. The case of *Neoraja* species may be similar. Clarifying the taxonomic situation will require further studies with other molecular markers and more individuals sampled to validate *N. iberica* as a distinct species. In addition, the fact that all the species of the genus *Neoraja* appear to have localized and rather limited distributions should be explored in order to test the hypothesis of allopatric speciation in this clade.

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REFERENCES

Anonymous (2000) *Fish collection database of the Institut für Seefischerei (ISH)*. BIOLAB Forschungsinstitut, Hohenwestedt, Germany.

Anonymous (2001) *Fish collection database of the Institut de Ciències del Mar (C.S.I.C.)*. Dept. Recursos Marins Renovables Grup d'Ictiologia Marina (G.I.M.). Psg. Joan de Borbo s/n 08039 Barcelona.

Bertrand J.A., Gil de Sola L., Papaconstantinou C., Relini G. and Souplet A. (2002) The general specifications of the Medits surveys. In Abelló P., Bertrand J., Gil de Sola L., Papaconstantinou C., Relini G. and Souplet A. (eds) *Mediterranean marine demersal resources: the MEDITS international trawl survey (1994–1999)*. *Scientia Marina* 66 (Supplement 2), 9–17.

Buchanan J.B. (1984) Sediment analysis. In Holme N.A. and McIntyre A.D. (eds) *Methods for the study of marine benthos*. Oxford: Blackwell Scientific, pp. 41–65.

Castro J., Marín M., Costas G., Abad E., Punzon A., Pereiro J. and Vázquez A. (2011) *Atlas de las flotas de pesca españolas de aguas europeas atlánticas*. Madrid: Instituto Español de Oceanografía and MICIN, 215 pp.

Clarke M. W. (2000) Records of deepwater chondrichthyan fish caught on long-line in the Rockall Trough. *Journal of the Marine Biological Association of the United Kingdom* 80, 377–378.

Clarke K.R. and Warwick R.M. (1994) *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth: Natural Environment Research Council, Plymouth Marine Laboratory, 144 pp.

Collett R. (1904) Diagnoses of four hitherto undescribed fishes from the depths south of the Faroe Islands. *Bulletin of the Zoological Survey of India (for 1904)* 9, 1–7.

Collin H. and Fumagalli L. (2011) Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). *Molecular Ecology* 20, 4490–4502.

Compagno L.J.V. (1984) *Sharks of the world. An annotated and illustrated catalogue of shark species known to date. FAO Fisheries Synopsis* 125, Vol. 4 Part 2.

Compagno L.J.V., Dando M. and Fowler S. (2005) *Sharks of the world*. Princeton and Oxford: Princeton University Press.

Dolganov V.N. (1985) *Raja (Rajella) kukujevi* sp. (Elasmobranchii, Rajidae) from the North Atlantic Ridge. *Zoologicheskij Zhurnal* 64, 304–307.

Dudgeon C.L., Blower D.C., Broderick D., Giles J.L., Holmes B. J., Kashiwagi T., Kruck N.C., Morgan J.A.T., Tillett B.J. and Ovenden J.R. (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology* 80, 1789–1843.

Felsenstein J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.

Froese R. and Pauly D. (eds) (2010) *FishBase*. Available at: <http://www.fishbase.org>, update version 02/2013 (accessed 25 February 2013).

Gibson C., Valenti S.V., Fordham S.V. and Fowler S.L. (2008) *The Conservation of Northeast Atlantic Chondrichthyans: Report of the IUCN Shark Specialist Group Northeast Atlantic Red List Workshop*. Newbury: IUCN Species Survival Commission Shark Specialist Group.

Gil J. (2008) Macro and mesoscale physical patterns in the Bay of Biscay. *Journal of the Marine Biological Association of the United Kingdom* 88, 217–225.

Gómez-Ballesteros M., Druet M., Muñoz A., Arrese B., Rivera J., Sánchez F., Cristobo J., Parra S., García-Alegre A., González-Pola C., Gallastegui J. and Acosta J. (in press) Geomorphology and sedimentary features of the Avilés canyon system. Cantabrian Sea (Bay of Biscay). *Deep-Sea Research II*. (in press).

- González-Quirós R., Cabal J., Álvarez-Marques F. and Isla A.** (2003) Ichthyoplankton distribution and plankton production related to the shelf break front at the Aviles Canyon. *ICES Journal of Marine Science* 60, 198–210.
- Hasegawa M., Kishino H. and Yano T.** (1985) Dating of human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22, 160–174.
- Hebert P.D.N., Cywinska A., Ball S.L. and DeWaard J.R.** (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society, B* 270, 313–321.
- Iglésias S.P.** (2011) *Chondrichthyans from the north-eastern Atlantic and the Mediterranean (a natural classification based on collection specimens)*. Provisional version 05, 01.04.2011. <http://www.mnhn.fr/iccanam> (accessed 25 February 2013).
- Iglésias S.P., Nakaya K. and Stehmann M.** (2004) *Apristurus melanoasper*, a new species of deep-water catshark from the North Atlantic (Chondrichthyes: Carcharhiniformes: Scyliorhinidae). *Cybium* 28, 345–356.
- Iglésias S.P., Toulhoat L. and Sellos D.Y.** (2009) Taxonomic confusion and market mislabelling of threatened skates: Important consequences for their conservation status. *Aquatic Conservation: Marine and Freshwater Ecosystems*. Available at: [http://onlinelibrary.wiley.com/journal/10.1002/\(ISSN\)1099-0755](http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1099-0755) (accessed 25 February 2013).
- Iorga M.C. and Lozier M.S.** (1999) Signatures of the Mediterranean outflow from a North Atlantic climatology 1. Salinity and density fields. *Journal of Geophysical Research* 104, 25985–26009.
- IUCN** (2009) *IUCN Red List of Threatened species (ver.2009.2)*. Available at www.iucnredlist.org (accessed 25 February 2013).
- Kukuev E.I.** (2002) Ichthyofauna research on underwater mountain within the north-Atlantic ridge and adjacent areas. *ICES, CM* 2002/M: 05.
- Last P.R., White W.T., Pogonoski J.J. and Gledhill D.C.** (2008) New Australian skates (Batoidea: Rajoidei) background and methodology. In Last P.R., White W.T., Pogonoski J.J. and Gledhill D.C. (eds) *Descriptions of new Australian skates (Batoidea: Rajoidei)*. CSIRO Marine and Atmospheric Research Paper 021, pp. 1–8.
- Lavín A., Valdés L., Sánchez F., Abauza P., Forest J., Boucher P., Lazure P. and Jegou A.M.** (2006) The Bay of Biscay. The encountering of the Ocean and the shelf. In Robinson A. and Brink K. (eds) *The seas*. Cambridge, MA: Harvard University Press, Vol. 14, pp. 933–1001.
- Louzao M., Anadón N., Arrontes J., Álvarez-Claudio C., Fuente D.M., Ocharan F., Anadón A. and Acuña J.L.** (2010) Historical macro-benthic community assemblages in the Avilés Canyon, N Iberian Shelf: baseline biodiversity information for a marine protected area. *Journal of Marine Systems* 80, 47–56.
- Luchetti E.A., Iglesias S.P. and Sellos D.Y.** (2011) *Chimaera opalescens* n. sp., a new chimaeroid (Chondrichthyes: Holocephali) from the north-eastern Atlantic Ocean. *Journal of Fish Biology* 79, 399–417.
- Magnússon J., Magnússon J.V. and Jakobsdóttir K.B.** (2000) Icelandic contributions to the deep water research project EC FAIR PROJECT CT 95-0655, 1996–1999. *Hafrannsóknastofnun Fjölrit* nr 76.
- McEachran J.D. and Compagno L.J.V.** (1982) Interrelationships of an within *Breviraja* based on anatomical structures (Pisces: Rajoidei). *Bulletin Marine Science* 32, 399–425.
- Moura T., Silva M., Figueiredo I. and Neves A.** (2008) Molecular barcoding of north-east Atlantic deep-water sharks: species identification and application to fisheries management and conservation. *Marine and Freshwater Research* 59, 214–223.
- Orlov A., Cotton C. and Byrkjedal I.** (2006) Deepwater skates collected during the 2004 cruises of R.V. 'G.O. Sars' and M.S. 'Loran' in the Mid-Atlantic Ridge area. *Cybium* 30(Supplement 4), 35–48.
- Orlov A. and Cotton C.** (2011) Sexually dimorphic morphological characters in five north deepwater skates (Chondrichthyes: Rajoiformes). *Journal of Marine Biology*, 2011, Article ID 842821, 18 pp.
- Pérez M. and Presa P.** (2011) FENOSALT: Un método sintético para la extracción de ADN de peces y moluscos. In Estévez J.M. et al. (eds) *Métodos y Técnicas de Investigación Marina*. Madrid: Technos. pp. 81–89.
- Punzón A. and Gancedo R.** (1998) Specific characterization and identification of the fishing gears in use in the Cantabrian Sea (NE Atlantic, northern Spain). *ICES, CM*. 1998/U:7 20 pp.
- Quéro J.C., Porché P. and Wayne J.J.** (2003) *Guide des Poissons de l'Atlantique Européen*. Paris: Delachaux et Niestle.
- Rodríguez-Cabello C., Arronte J.C., Sánchez F. and Pérez M.** (2012). New records expand the known southernmost range of *Rajella kukujevi* (Elasmobranchii, Rajidae) in the North-Eastern Atlantic (Cantabrian Sea). *Journal of Applied Ichthyology* 28, 633–636.
- Ruiz-Villareal M., Coelho H., Díaz del Río G. and Nogueira J.** (2004) Slope current in the Cantabrian: observations and modeling of seasonal variability and interaction with Aviles Canyon. *ICES, C.M.* 2004/N:12. 23 pp.
- Saitou N. and Nei M.** (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406–425.
- Serra-Pereira B., Moura T., Griffiths A.M., Gordo L.S. and Figueiredo I.** (2011) Molecular barcoding of skates (Chondrichthyes: Rajidae) from the southern Northeast Atlantic. *Zoologica Scripta* 40, 76–84.
- Stehmann M.** (1976) *Breviraja caerulea* spec. nov. (Elasmobranchii, Batoidea, Rajidae); eine neue archibenthale Rochenart und zugleich ein Erstnachweis ihrer Gattung im Nordostatlantik. *Archiv für Fischereiwissenschaft* 27, 97–114.
- Stehmann M. and Bürkel D.L.** (1984) Rajidae. In Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J. and Tortonese E. (eds) *Fishes of the north-eastern Atlantic and the Mediterranean*. Paris: UNESCO, pp. 163–196.
- Stehmann M., Séret B., Costa E.M. and Baro J.** (2008) *Neoraja iberica* n. sp., a new species of pygmy skate (Elasmobranchii, Rajidae) from the southern slope of the Iberian Peninsula (Eastern North Atlantic). *Cybium* 32, 51–71.
- Straube N., Kriwet J. and Schliwen U.K.** (2011) Cryptic diversity and species assignment of large lantern sharks of the *Etmopterus spinax* clade from the Southern Hemisphere (Squaliformes, Etmopteridae). *Zoologica Scripta* 40, 61–75.
- Tamura K., Nei M. and Kumar S.** (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America* 101, 11030–11035.
- Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S.** (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.
- Tinti F., Ungaro N., Pasolini P., De Panfilis M., Garoia F., Guarniero I., Sabelli B., Marano G. and Piccinetti C.** (2003) Development of molecular and morphological markers to improve species-specific monitoring and systematics of Northeast Atlantic and Mediterranean skates (Rajiformes). *Journal of Experimental Marine Biology and Ecology* 288, 149–165.
- Vélez-Zuazo X., and Agnarsson I.** (2011) Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Molecular Phylogenetics and Evolution* 58, 207–217.

- Ward R.D., Zemlak T.S., Innes B.H., Last P.R. and Hebert P.D.N.** and
(2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society*, B 360, 1847–1857.
- Ward R.D., Holmes B.H., William A., White T. and Last P.R.**
(2008) DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research* 59, 57–71.
- White W.T. and Last P.R.** (2012) A review of the taxonomy of chondrichthyan fishes: a modern perspective. *Journal of Fish Biology* 80, 901–917.
- Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nielsen J., and Tortonese E. (eds)** (1984) *Fishes of the north-Eastern Atlantic and the Mediterranean*. UNESCO, Paris.
- Zhang Z., Schwartz S., Wagner L. and Miller W.** (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7(1–2), 203–214.

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