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The Second Record of *Gymnesigobius medits* Kovačić, Ordines, Ramirez-Amaro & Schliewen, 2019, the Deepest Benthic Gobiiform Species, and the Additional Records of *Gobius xoriguer* Iglésias, Vukić & Šanda, 2021 (Actinopterygii: Gobiiformes: Gobiidae)

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Abstract: *Gymnesigobius medits* is reported for the first time after a recent description recorded from the Balearic Islands and from the slope of the Gulf of Vera on the Iberian Peninsula coast. The record from the Emile Baudot seamount on the Balearic Islands represents the deepest positive benthic gobiiform species record in general. The presence of the membrane connection between the pelvic fins in *Gymnesigobius medits*, presumed on the damaged fin in the original description, was confirmed. The recently described *Gobius xoriguer* is the first record from the Pitiusas and Columbretes islands and from the Iberian Peninsula coast. It appears to be widely distributed in the circalittoral bottoms, preferentially in red algae beds. Morphological identification of both species was confirmed using molecular analyses based on the sequencing of the mitochondrial cytochrome c oxidase subunit I (DNA barcode) gene. The deepest records of gobiiform fishes in oceans and seas are reviewed. The European seas, a well-studied area with eight gobiid species recorded deeper than 200 m, show high bathyal gobiid species richness compared to other areas. The real worldwide diversity of bathyal gobies, although only a fraction of the shallow water species richness of this taxon, is probably much larger than presently known.

Keywords: Gobiinae; bathyal; red algal beds; rare species; morphology; DNA barcode; depth distribution

Key Contribution: The record of *Gymnesigobius medits* from the Emile Baudot seamount in the Balearic Islands represents the deepest positive benthic gobiiform species record in general. The deepest records of gobiiform fishes by oceans and seas are reviewed.

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1. Introduction

The family Gobiidae, with 1971 presently valid species, is the largest fish family and contains the largest number of new species descriptions in the last 10 years [1]. Gobies are also the only species-rich fish family present in both marine and freshwater environments, while four other fish families with more than one thousand valid species are exclusively freshwater fishes [1]. However, the majority of marine gobiid species are restricted to shallow water and to warm temperate or tropical seas, where the family Gobiidae is often the most species-rich family in the area, e.g., in the Mediterranean Sea [2]. The small number of gobiid species worldwide is present at depths below the shelf break, and gobiid diversity also heavily decreases in the cold temperate seas, with only a few species extending north or south of 50° latitudes, except in Norway, where representatives of this fish family enter the Arctic Circle [3,4]. Until recently, Mediterranean gobiid fauna was also expected to be restricted mostly to shallow waters, as in other seas, with only a few eurybathic species extending their presence down to circalittoral or even bathyal depths. However, in the last decade, six new deepwater gobiid species were described in the Western Mediterranean, showing the presence of original gobiid fauna at the two deep bottom habitats, circalittoral red algae beds and bathyal muds [5–10]. All six species are small sized, even for gobiid standards, and have shape and colouration adaptations to deep habitats. Among them, *Buenia massutii* Kovačić, Ordines & Schliewen, 2017, *Speleogobius llorisi* Kovačić, Ordines & Schliewen, 2016 and *Gobius xoriguer* Iglésias, Vukić & Šanda 2021 were additionally recorded shortly after their description [11–14], while *Buenia lombartei* Kovačić, Ordines & Schliewen, 2018; *Gymnesigobius medits* Kovačić, Ordines, Ramirez-Amaro & Schliewen, 2019 and *Lebetus patzneri* Schliewen, Kovačić & Ordines, 2019 still wait records following the description.

The aim of the present work was to report the new records of two deep Mediterranean gobies, *Go. xoriguer and Gy. medits*, and to review the deepest benthic records of gobiiform species.

2. Materials and Methods

2.1. Sampling

Samples from the DRAGONSAL and LIFE IP INTEMARES projects and the Marine Strategies monitoring programme (CIRCA-LEBA surveys) were collected using a standard beam trawl described by Jennings et al. [15]. The first two projects studied the distribution and characterisation of benthic habitats in the southwest continental shelf of the Mallorca islands and the Mallorca Channel seamounts, respectively, whereas CIRCA-LEBA surveys aimed to characterise and monitor benthic habitats from the continental shelf and slope on the Iberian Peninsula coast from Cabo de Gata to Cap de Creus, including the Balearic Islands (Figure 1). Samples from CANAL surveys were collected in the Menorca Channel using the same beam trawl method. These surveys were carried out within the framework of the MARFISH and SosMed projects, with the objective of assessing the effects of habitat protection on demersal resources. Samples from MEDITS surveys, aimed at assessing the exploitation state of demersal ecosystems and resources in the Mediterranean, were collected using the experimental bottom trawl GOC-73, described in Bertrand et al. [16].

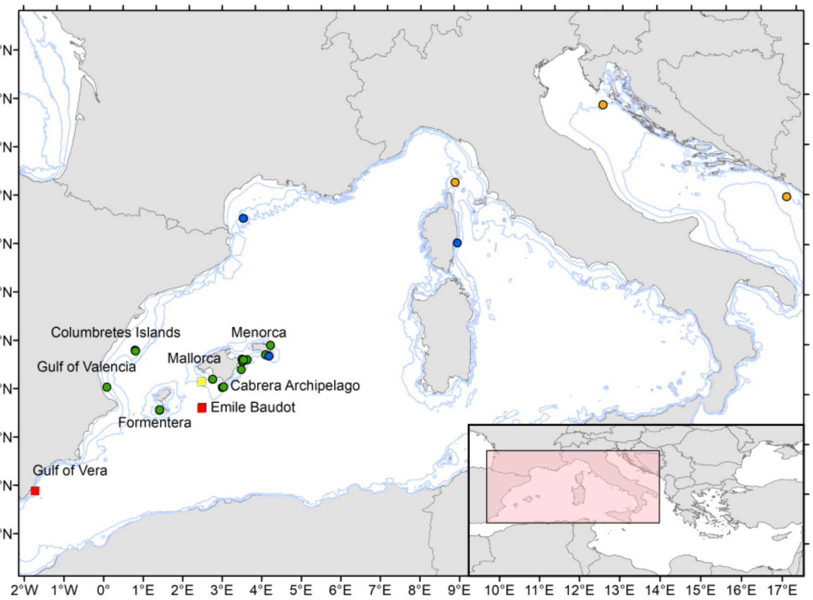

Figure 1. The records of *Gobius xoriguer* and *Gymnesigobius medits* in the study area. For *Go. xoriguer*: (•) material from Iglesias et al. [10], (•) records in Kovačić & Froglia [14], and (•) present records. For *Gy. medits* (■) material from Kovačić et al. [8], and (■) present records. Isobaths represent 50, 100 and 500 m depth.

2.2. Morphological Identification

The morphological data were a combination of characters that positively identified the species among the family Gobiidae in the CLOFNAM area [2,8,10]. The terminology and format style of head canal pores and head rows of sensory papillae followed [17,18]. Morphometric and meristic methods followed the methodology in [8,10]. The material of *Gy. medits* was stained in 2% solution of Cyanine Blue in distilled water [19] for studying scales, pelvic fin membrane and head lateral-line system. Taxonomy followed. [1], and the phylogenetic lineages were from [20]. The material was deposited in the Prirodoslovni muzej Rijeka (PMR) and the Marine Fauna Collection of the Instituto Español de Oceanografía in the Centro Oceanográfico de Málaga (CFM-IEOMA).

2.3. Genetic Analyses

A piece of the right pectoral fin was removed from the fresh specimens and preserved in 96% ethanol. For genetic analyses, a total of five specimens of *Go. xoriguer* and *Go. roulei* and four of *Gy. medits* were used. DNA was extracted from this tissue using the DNeasy Blood and Tissue Extraction Kit (Qiagen, West Sussex, UK). Polymerase chain reaction (PCR) was used to amplify a partial mitochondrial gene, the cytochrome c Oxidase subunit I (COI; DNA barcode), with primers FF2d/FR1d [21]. PCR was performed in a 25 µL volume: 17.7 µL ddH_2, 2.5 µL Mangobuffer (Bioline), 1 µL DNTPs, 1.75 µL $MgCl_2$, 0.5 µL each primer (each 10 pmol), 0.05 µL TAQ (Bioline) and 1 µL DNA. The PCR thermal profile used was: initial stage of 96 °C for 5 min; then 35 cycles at 94 °C for 60 s, 54 °C for 60 s and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. The PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN) and sent for sequencing by the Sanger method to the laboratory of the MACROGEN company in Madrid.

Sequences were imported into BioEdit 7.0.5.2. [22] and checked for quality and accuracy with nucleotide base assignment. Multiple sequence alignments (MSA) were obtained with ClustalW [23]. The DNA sequences obtained were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) (accessed on 27 April 2023) under the following numbers: OQ874680–OQ874687. In the case of *Go. xoriguer*, additional sequences were obtained from [10], while for *Gy. medits*, they were obtained from [8]. The matrix of sequences was complemented with the most similar sequences retrieved from GenBank via BLAST analyses (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 2 January 2023). Genetic distance (*p*-distance) and the number of base differences between the pairs of sequences of each mitochondrial fragment were calculated with MEGA v.7.1 [24]. The average values of the COI indices between our study samples and GenBank sequences were compared.

3. Results

3.1. Gobius xoriguer Iglésias, Vukić & Šanda 2021

3.1.1. Studied Material

PMR VP3587, female, 33.6 + 7.0 mm, and four males, 35.4 mm, damaged caudal fin, to 41.2 + 10.1 mm, Balearic Islands, South-west Mallorca, 39.41° N, 2.452° E, 75–79 m depth, R/V Francisco de Paula Navarro, Cruise DRAGONSAL_0914, St. 15, 5 September 2014. PMR VP3829, female, 37.3 + 9.2 mm and two males, 44.1 + 9.5 mm and 40.8 mm, damaged caudal fin, Balearic Islands, East Mallorca, 39.5183° N, 3.508° E, 74–76 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2016, St. 188, 10 June 2016. PMR VP3831, female, 35.2 + 7.5 mm, and two males, 39.7 + 10.0 mm and 36.7 + 8.1 mm, Balearic Islands, South Menorca, 39.8558° N, 4.0873° E, 64-65 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2016, St. 192, 11 June 2016. PMR VP3835, female, 40.2 + 9.9 mm (Figure 2), Balearic Islands, South Menorca, 39.8233° N, 4.1823° E, 54-59 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2016, St. 193, 11 June 2016. CFM-IEOMA 7777, male, 38.4 mm, damaged caudal fin, Balearic Islands, Menorca Channel, 39.699° N, 3.5575° E, 62 m depth, R/V Ramon Margalef, Cruise CANAL_04_2023, St. 9, 14 April 2023. CFM-IEOMA 7778, male, 39 + 8.9 mm, Balearic Islands, Menorca Channel, 39.7247° N, 3.5755° E, 64 m depth, R/V Ramon Margalef, Cruise CANAL_04_2023, St. 16, 15 April 2023. CFM-IEOMA 7779 (GenBank

ID: OQ874682 for female 42.8 + 9.2 mm), male, 26 + 6.5 mm, and two females, 31.9 + 7.1 mm and 42.8 + 9.2 mm, Balearic Islands, Menorca Channel, 39.728° N, 3.6735° E, 82 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1121, St. 68, 26 November 2021. CFM-IEOMA 7780, two males, 25.7 + 6.1 mm and 26.5 + 6.5 mm, Balearic Islands, Menorca Channel, 39.736° N, 3.5343° E, 59 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1121, St. 69, 27 November 2021. CFM-IEOMA 7781, two females, 40.5 + 9.7 mm and 40.3 + 9.4 mm (GenBank ID: OQ874683- OQ874684, both specimens sequenced), Balearic Islands, Menorca Channel, 39.69° N, 3.5458° E, 62 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1121, St. 70, 27 November 2021. CFM-IEOMA 7782, female, 32.7 + 6.9 mm, Balearic Islands, Cabrera Archipelago, 39.1548° N, 2.9998° E, 76–77 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. NI-12, 27 November 2022. CFM-IEOMA 7783, male, 46.3 + 9.9 mm, Iberian Peninsula, Gulf of Valencia, 39.1173° N, 0.0781° E, 56-61 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. 16, 8 November 2022. CFM-IEOMA 7784, female, 31.9 mm, damaged caudal fin, male, 22.5 + 6.3 mm, Balearic Islands, North Menorca, 40.0186° N, 4.3071° E, 77 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. 61, 24 November 2022. CFM-IEOMA 7785, female, 32.5 + 7.4 mm, Balearic Islands, South Menorca, 39.8261° N, 4.1731° E, 60 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. 69, 25 November 2022. CFM-IEOMA 7786, two females, 31.5 + 7.5 mm and 32 + 7.1 mm, Balearic Islands, South Mallorca, 39.3206° N, 2.7426° E, 62 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. 71, 26 November 2022. CFM-IEOMA 7787, two females, 34.2 + 7.7 mm and 36.6 + 8.5 mm, Iberian Peninsula, Columbretes Islands, 39.9005° N, 0.6483° E, 64 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. NI-7, 22 November 2022. CFM-IEOMA 7788, female, 37.4 + 8.5 mm, two males, 33.7 + 8.2 mm and 36.5 mm, damaged caudal fin, Iberian Peninsula, Columbretes Islands, 39.8928° N, 0.6555° E, 60 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. NI-8, 22 November 2022. CFM-IEOMA 7789, female, 33.1 + 7.6 mm, Iberian Peninsula, Columbretes Islands, 39.8828° N, 0.6573° E, 64 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. NI-9, 22 November 2022. CFM-IEOMA 7790, female, 39.5 + 8.5 mm, Balearic Islands, Cabrera Archipelago, 39.161° N, 3.0338° E, 73 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1122, St. NI-10, 27 November 2022. CFM-IEOMA 7791, male, 35.6 + 8.2 mm, Balearic Islands, South Menorca, 39.8576° N, 4.0853° E, 64 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2022, St. 239, 2 July 2022. CFM-IEOMA 7792, male, 36.5 + 8.2 mm, female, 32.8 + 7.7 mm (GenBank ID: OQ874680–OQ874681, both specimens sequenced), Balearic Islands, Pitiusas Islands, West Formentera, 38.6726° N, 1.3391° E, 65-68 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2021_PITIUSES, St. 265, 24 August 2021. CFM-IEOMA 7793, male, 43.3 + 9.9 mm, Balearic Islands, Pitiusas Islands, West Formentera, 38.674° N, 1.3398° E, 67 m depth, R/V Miguel Oliver, Cruise MEDITS_ES_2022_PITIUSES, St. 279, 19 August 2022. All collected by F. Ordines, M.T. Farriols and S. Ramírez-Amaro.

3.1.2. Identification

The genus and species were identified by the following combination of characters [2,10]: (1) suborbital sensory papillae without row a below the eye; (2) all three head canals present, anterior oculoscapular canal with pores σ, λ, κ, ω, α, β, ρ, posterior oculoscapular with pores $\rho1$, $\rho2$, and preopercular canal with pores γ, δ, ε; (3) anterior dorsal row g of sensory papillae ends behind row o; (4) seven suborbital transverse row c of sensory papillae; (5) pre-dorsal area scaled; (6) anterior oculoscapular head canal with pore α at rear of orbit; (7) oculoscapular row x^1 of sensory papillae ending forward behind pore β; (8) midlateral scale count 47–51 scales; (9) suborbital row d of sensory papillae discontinuous; (10) anterior dorsal rows o of sensory papillae separated; (11) elongate first dorsal fin spines in adult males, with third spine the longest, reaching backward when folded down to 2nd to 5th rays of second dorsal fin (Figure 2B); (12) pelvic disc emarginate i.e., posterior edge concave (Figure 2C).

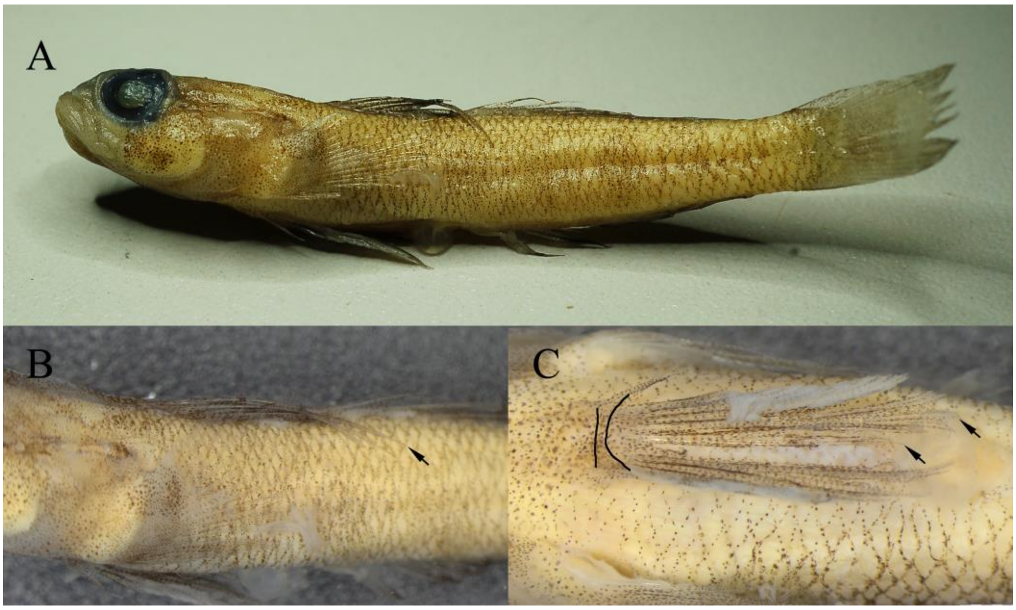

Figure 2. PMR VP3831, *Gobius xoriguer*, male, 39.7 + 10.0 mm. (**A**) Lateral view. (**B**) First dorsal fin elongated. The posterior most tip of the first dorsal fin spines marked with arrow. (**C**) Emarginated pelvic disc with low anterior membrane. Anterior membrane edges outlined and the tips of right rays 4 and 5 of pelvic fin marked with arrows.

3.1.3. Genetics

A total of 609 base pairs (bp) for the COI fragment were sequenced (4 variables sites, 1 informative site, no gaps) for five specimens identified as *Go. xoriguer* under deposit numbers CFM-IEOMA 7779 (GenBank ID: OQ874682), CFM-IEOMA 7781 (GenBank ID: OQ874683–OQ874684) (2 specimens) and CFM-IEOMA 7792 (GenBank ID: OQ874680–OQ874681) (2 specimens).

All specimens showed a close genetic distance (0.16–0.66%; 1–4 pb differences; Table 1) to the two previously published sequences of *G. xoriguer*. The interspecific genetic distances between *G. xoriguer* and the closely related goby species ranged from 8.7 to 14.12% (Table 1). *Gobius roulei* showed the largest genetic distance (13.46–14.12%; 82–86 pb differences; Table 1).

Table 1. Pairwise comparisons between *Gobius xoriguer* and closely related species based on the COI fragment. Genetic distances (%) and number of base differences are presented, below and above the diagonal, respectively. GenBank ID's are indicated next to species name.

N	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Gobius xoriguer* OQ874680		2	3	1	3	4	3	56	57	57	57	83	84	81	83	85	85	83	83
2	*Gobius xoriguer* OQ874681	0.33		1	1	1	2	1	54	55	55	55	83	84	81	83	85	85	83	83
3	*Gobius xoriguer* OQ874682	0.49	0.16		2	2	3	2	55	56	56	56	84	85	82	84	86	86	84	84
4	*Gobius xoriguer* OQ874683	0.16	0.16	0.33		2	3	2	55	56	56	56	82	83	80	82	86	86	84	84
5	*Gobius xoriguer* OQ874684	0.49	0.16	0.33	0.33		3	2	53	54	54	54	82	83	80	82	84	84	82	82
6	*Gobius xoriguer* KR914773	0.66	0.33	0.49	0.49	0.49		3	55	56	56	56	82	83	80	82	86	86	84	84
7	*Gobius xoriguer* KR914774	0.49	0.16	0.33	0.33	0.33	0.49		55	56	56	56	84	83	82	84	84	84	82	82
8	*Gobius gasteveni* KR914770	9.20	8.87	9.03	9.03	8.70	9.03	9.03		3	4	3	89	90	87	89	89	89	86	88
9	*Gobius gasteveni* MT670218	9.36	9.03	9.20	9.20	8.87	9.20	9.20	0.49		1	0	88	89	86	88	88	88	85	87
10	*Gobius gasteveni* MT670220	9.36	9.03	9.20	9.20	8.87	9.20	9.20	0.66	0.16		1	87	88	85	87	87	87	84	86
11	*Gobius gasteveni* MT670222	9.36	9.03	9.20	9.20	8.87	9.20	9.20	0.49	0.00	0.16		88	89	86	88	88	88	85	87
12	*Gobius niger* MT670242	13.63	13.63	13.79	13.46	13.46	13.46	13.79	14.61	14.45	14.29	14.45		12	8	11	75	75	77	75
13	*Gobius niger* MT670243	13.79	13.79	13.96	13.63	13.63	13.63	13.63	14.78	14.61	14.45	14.61	1.97		6	9	78	78	78	76
14	*Gobius niger* MT670244	13.30	13.30	13.46	13.14	13.14	13.14	13.46	14.29	14.12	13.96	14.12	1.31	0.99		5	75	75	75	73
15	*Gobius niger* MT670245	13.63	13.63	13.79	13.46	13.46	13.46	13.79	14.61	14.45	14.29	14.45	1.81	1.48	0.82		79	79	79	77
16	*Gobius roulei* MT670259	13.96	13.96	14.12	14.12	13.79	14.12	13.79	14.61	14.45	14.29	14.45	12.32	12.81	12.32	12.97		2	4	4
17	*Gobius roulei* MT670260	13.96	13.96	14.12	14.12	13.79	14.12	13.79	14.61	14.45	14.29	14.45	12.32	12.81	12.32	12.97	0.33		4	2
18	*Gobius roulei* MT670261	13.63	13.63	13.79	13.79	13.46	13.79	13.46	14.12	13.96	13.79	13.96	12.64	12.81	12.32	12.97	0.66	0.66		2
19	*Gobius roulei* MT670262	13.63	13.63	13.79	13.79	13.46	13.79	13.46	14.45	14.29	14.12	14.29	12.32	12.48	11.99	12.64	0.66	0.33	0.33	

3.1.4. Remarks

Among the present specimens of *Go. xoriguer*, twelve specimens were originally stored in the PMR collection as *Go. gasteveni*. However, *Go. xoriguer* differs from *Go. gasteveni* in several morphological characters [[10,25], this study]: pelvic fin with emarginate posterior end and low anterior membrane (vs. pelvic fin with truncate posterior end and with well-developed anterior membrane (Figure 3D); first dorsal fin spines elongated in males, reaching backward when folded down to second dorsal fin rays 2 to 5 (vs. first dorsal fin spines not elongated in both sexes, reaching backward in males when folded down barely to the second dorsal fin spine (Figure 3C); lateral-line system suborbital row *b* ending anteriorly behind suborbital row *5* (vs. ending anteriorly before or at suborbital row *5*); midlateral scale count 47–51 scales (vs. 37–45 scales). However, *Go. xoriguer* specimens examined in this study showed a wider range of midlateral scale counts compared to *Go. xoriguer* data shown in the original description (50–51 scales, [10]). Additionally, compared to the data in Iglesias et al. [10], the studied comparative material of *Go. gasteveni* and the original description of *Go. gasteveni* [25] had lateral-line system anterior dorsal rows *o* separated and not joint (Figure 3B) and suborbital row *b* ending anteriorly before or at suborbital row *5*, not only before suborbital row *5*.

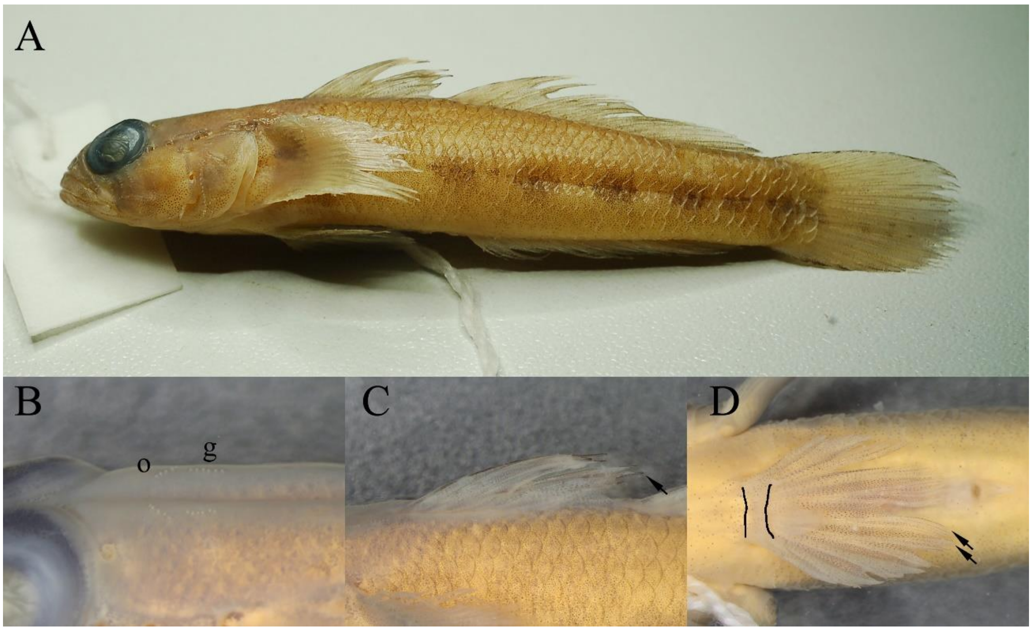

Figure 3. PMR VP3703, *Gobius gasteveni*, male, 48.8 + 10.6 mm. (**A**) Lateral view. (**B**) Pre-dorsal area, lateral-line system anterior dorsal rows *o* and *g* marked. (**C**) First dorsal fin. The posterior most tip of the first dorsal fin spines marked with arrow. (**D**) Truncated pelvic disc with low anterior membrane. Anterior membrane edges outlined and tips of left rays 4 and 5 of pelvic fin marked with arrows.

3.1.5. Ecology and Geographic Distribution

The specimens were collected by trawl from 54 to 82 m depth, primarily on red algae beds. All samples where this species was recorded, except CFM-IEOMA 7783 from the Gulf of Valencia, were collected from red algae species, mainly dominated by coralline algae species, such as *Spongites fruticulosa*, *Lithothamnion valens* and *Lithothamnion corallioides*, but also non-calcareous species, such as *Peyssonnelia* spp., *Phyllophora crispa* and *Osmundaria volubilis*. The specimens were recorded at Balearic Sea localities and from the Iberian Peninsula, Columbretes Islands and Gulf of Valencia (Figure 1).

3.2. *Gymnesigobius Medits* Kovačić, Ordines, Ramirez-Amaro & Schliewen 2019

3.2.1. Studied Material

PMR VP4960 (GenBank ID: OQ874685), female, 25.7 + 6.1 mm, Balearic Islands, Emile Baudot seamount, 38.71017° N, 2.461167° E, 501–520 m depth, R/V Ángeles Alvariño, Cruise INTEMARES-A22B-0720, St. 38, 26 July 2020. PMR VP4961 (GenBank ID: OQ874688), female, 38.3 mm, damaged caudal fin, Balearic Islands, Emile Baudot seamount, 38.71017° N, 2.461167° E, 501–520 m depth, R/V Ángeles Alvariño, Cruise INTEMARES-A22B-0720, St. 38, 26 July 2020. PMR VP4963 (GenBank ID: GenBank ID: OQ874686), male, 31.2 + 7.7 mm, Balearic Islands, Emile Baudot seamount, 38.73167° N, 2.468667° E, 391–413 m depth, St. 166, R/V Ángeles Alvariño, Cruise INTEMARES-A22B-1019, 28 October 2019 (Figure 4). CFM-IEOMA 7775 (GenBank ID: OQ874687), male, 30.1 mm, damaged caudal fin, Iberian Peninsula, Gulf of Vera, 36.901° N, 1.8337° W, 328–334 m depth, R/V Ramon Margalef, Cruise CIRCA-LEBA-1121, St. 2, 10 October 2021. All collected by F. Ordines, M.T. Farriols and S. Ramírez-Amaro.

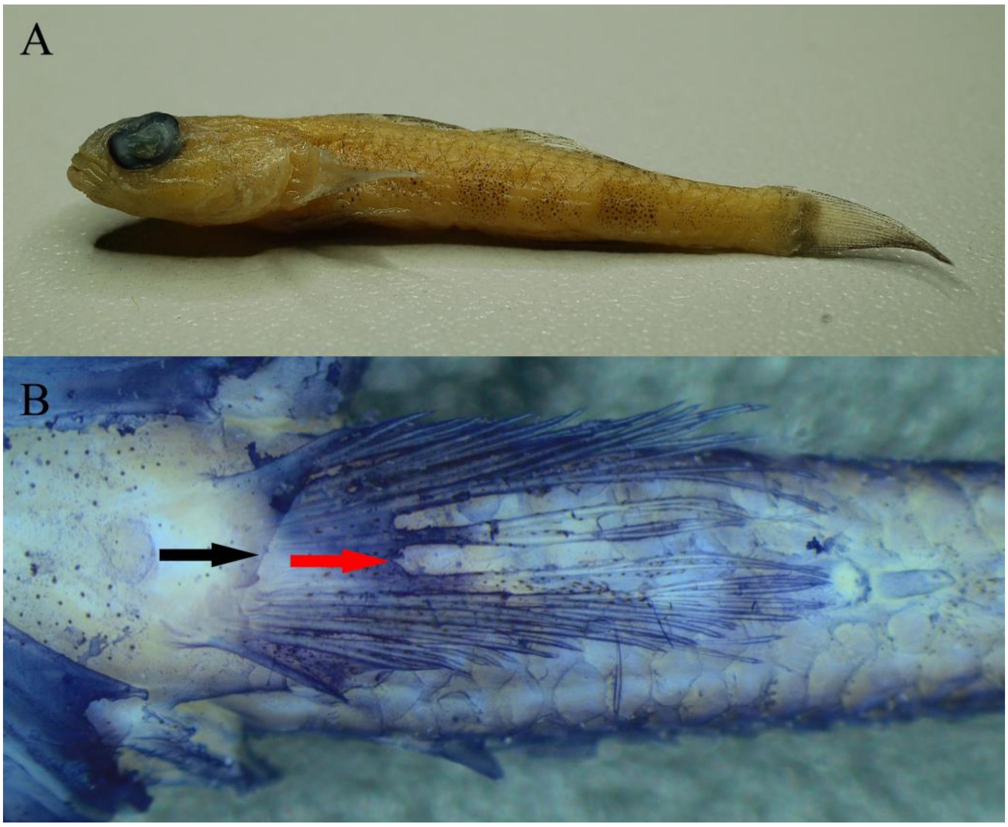

Figure 4. PMR VP4963, *Gymnesigobius medits*, male, 31.2 + 7.7 mm. (**A**) Lateral view. (**B**) The edge of the anterior membrane is marked with a black arrow, and the edge of the still connected membrane between fifth rays is marked with a red arrow.

3.2.2. Identification

The genus and species were identified by the following combination of characters [8]: (1) chin without fold or barbels; (2) mouth terminal, oblique, with anterior tip above horizontal level of lower eye edge; (3) pre-dorsal area and first dorsal fin base naked; (4) pelvic fin anterior membrane (frenum) well developed; (5) head with anterior oculoscapular and preopercular canals, with pores σ, λ, κ, ω, α, β, ρ and γ, δ, ε respectively, posterior oculoscapular canal absent; (6) pores of head canals enlarged e.g., pores α and ρ larger or about same size as interspaces to pore β; (7) six transverse suborbital rows of sensory papillae, four continuous suborbital rows in front of row *b*, fifth divided in three parts but in front of row *b*, sixth just as superior part above row *b* and below pore α; (8) longitudinal suborbital row *b* barely reaching forward to the vertical from posterior edge of eye in PMR VP4961, ending shortly behind the vertical in PMR VP4960, PMR VP4963 and CFM-IEOMA 7775.

3.2.3. Genetics

A total of 570 pb were sequenced (8 variables sites, 1 informative site, no gaps). The four specimens identified as *Gy. medits* showed a close genetic distance (0.35–0.88%; 2–5 pb differences), with the only published sequence belonging to this species. Interspecific distances between *Gy. medits* sequences and the closely related goby species ranged from 16.84 to 18.95 (Table 2). The largest genetic distance was found with *Wheelerigobius maltzani* (18.6–18.95%; 106–108 pb differences, Table 2).

Table 2. Pairwise comparisons between *Gymnesigobius medits* and closely related species based on the COI fragment. Genetic distances (%) and number of base differences are presented below and above the diagonal, respectively. GenBank ID's are indicated next to species name.

N	Species	1	2	3	4	5	6	7	8	9
1	*Gymnesigobius medits* OQ874685		6	5	5	5	100	97	104	108
2	*Gymnesigobius medits* OQ874686	1.05		5	5	3	101	98	105	106
3	*Gymnesigobius medits* OQ874687	0.88	0.53		2	0	100	98	104	108
4	*Gymnesigobius medits* OQ874688	0.88	0.53	0.35		2	100	97	104	108
5	*Gymnesigobius medits* MK628514	0.88	0.53	0.00	0.35		98	96	102	106
6	*Odondebuenia balearica* MK628520	17.54	17.72	17.54	17.54	17.19		90	88	98
7	*Vanneaugobius dollfusi* MK628516	17.02	17.19	17.19	17.02	16.84	15.83		104	102
8	*Wheelerigobius wirtzi* MK628521	18.25	18.42	18.25	18.25	17.89	15.44	18.16		68
9	*Wheelerigobius maltzani* MK628522	18.95	18.60	18.95	18.95	18.60	17.19	17.85	11.93	

3.2.4. Remarks

Kovačić et al. [8] described *Gy. medits* having pelvic fins with the fifth ray being longest and with visible remaining broken membrane present along the fifth rays at least to the half, although the connection was broken all the way to the base of the rays. They concluded that the pelvic fins were therefore probably not divided, but with the fin emargination, which degree of emargination is unknown due to the damaged membrane. In the present material, we recorded pelvic fins with fifth rays still interconnected by membrane at least to some degree in all specimens, confirming the presumption of Kovačić et al. [8] but with most of the connection again broken and so with unknown original length of connection and depth of emargination. The fifth rays were still connected by membrane to at least $\frac{1}{4}$ of the fifth ray length, visible in PMR VP4963 and CFM-IEOMA 7775, and the membranes were more broken in the other two specimens (Figure 4B). The anterior pelvic membrane (frenum) was well developed, without lateral lobes and with depth in the midline of about 1/3 of the spinous ray, as in the original description (Figure 4B). The connected pelvic fins and the presence of well-developed anterior pelvic membrane (frenum) is interesting for this species, which is nested deep inside the phylogenetic clade of species exclusively having completely divided pelvic fins, which was discussed in Kovačić et al. [8].

3.2.5. Ecology and Geographic Distribution

The specimens were collected by trawls from 328 to 520 m depth on bathyal muds. The specimens were recorded at the Emile Baudot seamount in the Balearic Sea and Iberian Peninsula, Gulf of Vera (Figure 1).

4. Discussion

Gobius xoriguer and *Gy. medits*, despite being lately described and known from a few records [present data, [8,10,14]], are probably not rare in their preferred habitats. The morphological similarity between *Go. xoriguer* and *Go. gasteveni* and the bathyal distribution of *Gy. medits* could explain their late, 21st century, discovery and description, sharing the destiny of all other deep-water small size Mediterranean gobiid species described in the last decade from bathyal mud or from circalittoral red algae beds [5–10]. Only the intensive sampling effort, developed during the projects and scientific monitoring included here (INTEMARES, DRAGONSAL, MARFISH, SosMed, Marine Strategies and MEDITS), on poorly studied benthic habitats and their associated communities has allowed these results to be produced. *Gobius xoriguer* was misidentified as *Go. gasteveni* before the species description, so it is probably very abundant at the circalittoral bottoms. Before the description, *Go. xoriguer* had been commonly caught during the MEDITS and was often seen in commercial catches by F. Ordines, recognisable by its elongate first dorsal fin spines in adult males (F. Ordines unpublished data).

The three new Mediterranean species that have been recorded from bathyal depths, *Gy. medits*, *B. lombartei* and *Go. xoriguer*, are among a few gobiid species that are present at the continental slope in bathyal conditions, based on the published records (Table 3). The marine gobies are almost all and everywhere continental shelf species, and most of the marine gobiid species are even more depth restricted, just to shallow infralittoral depths [1,8]. The two gobiid records (Table 3) at around one thousand metres depth from the Western Pacific are unconfirmed and contradictory to other known depths and habitats of those two species, which are limited to the continental shelf [26,27] (Table 3). Murdy [26] reported one lot (USNM 113201) of *Karsten totoyensis* (Garman, 1903) collected at a depth of 608 fathoms (1122 m), while, according to him, all other known samples of this species were from less than 30 fathoms (55 m). Okiyama [27] considered the record of *Platygobiopsis tansei* Okiyama, 2008 from 960–970 m depth problematic, since all other specimens were collected exclusively from shallower bottoms between 65 and 128 m, and no other gobioids have ever been recorded from depths greater than 500 m. Therefore, excluding the two problematic records of shelf species recorded at depths twice the maximum depth of any known bathyal goby and 10–20× deeper than other conspecific records, the present record of *Gy. medits* from at least 501 m depth, which was the shallowest depth of the trawl pull, is the deepest positive benthic record of any Gobiinae, Gobiidae or Gobiiformes fish. The second and third deepest verified records of Gobiiformes are also from the Mediterranean Sea. The two species of *Lesueurigobius* were recorded deep by Goren et al. [28] (Table 3). Bathyal gobies were also recorded in the Western Atlantic, Red Sea, North and Southeastern Atlantic, Western Pacific and Caspian Sea (Table 3 and references therein), while for the Indian Ocean and the Eastern Pacific, the deepest recorded gobies are restricted only to the continental shelf (Table 3 and references therein). Among the pelagic Gobiidae, the two deepest records so far known are those of *Schindleria* sp. in the Western Pacific, reported at about 500 and 1000 m [29].

Interestingly, all three known lineages of European gobies sensu Agorreta et al. [20] evolved bathyal presence, compared to only a few lineages of Gobiiformes fish in total having bathyal representatives in general (Table 3). The number of gobiid species with positive records deeper than 200 m in European seas, including the Caspian Sea, is comparable only to the Western Atlantic bathyal goby species richness. In both areas, eight gobiid species have been recorded at bathyal depths (Table 3) [30,31]. In the Western Atlantic, a number of new species and genera have been described recently from bathyal depths collected by the manned submersible [30,31]. The samples used in the present work were collected from Mediterranean research projects and monitoring programmes aimed at characterising benthic habitats and the exploitation state of demersal ecosystems and resources. Despite not being specifically focused on small fishes, these surveys have produced a substantial improvement in fish biodiversity knowledge. Therefore, the number of bathyal gobiid species and their phylogenetic diversity from a small sea like the Mediterranean may more likely be the result of a higher research effort applied in this sea compared to other regions than a real overrepresentation of bathyal gobies in the Mediterranean Sea compared to other regions. The real worldwide diversity of bathyal gobies, although far from the shallow water species richness, is probably much larger than presently known.

Table 3. The deepest bathyal published records of gobiid species in the European seas and the deepest published records of benthic Gobiiformes species from other regions. Based only on published data on benthic gobies. Taxonomy follows Fricke et al. [1] and the phylogenetic lineages are from Agorreta et al. [20]. The records are sorted by decreasing depth. For the trawl depth ranges, the minimum of the range is considered as the positive maximum depth.

Species	Maximum Confirmed Depth	Collecting Method	Region of Collecting Site	Taxonomy & Phylogeny	Comment on Depth	Species Usual Depth Zones and Habitat	Reference(s)
European seas confirmed bathyal records and the confirmed deepest records in other oceans and seas							
Gymnesigobius medits Kovačić, Ordines, Ramirez-Amaro & Schliewen, 2019	501 m (501–520 m)	Beam trawl	Mediterranean	Gobiidae, Gobiinae, *Gobius*-lineage	The deepest positive record of Gobiiformes, Gobiidae and Gobiinae in general, the deepest gobiid record in the Mediterranean	Restricted to bathyal mud	Present results
Lesueurigobius friesii (Malm, 1874)	440 m	Trawl	Mediterranean	Gobiidae, Gobiinae, *Aphia*-lineage		Circalittoral and bathyal mud	[28]
Lesueurigobius suerii (Risso, 1810)	440 m	Trawl	Mediterranean	Gobiidae, Gobiinae, *Aphia*-lineage		Circalittoral and bathyal mud	[28]
Varicus adamsi Gilmore, Van Tassell & Tornabene, 2016	435 m	Manned submersile	Western Atlantic	Gobiidae, Gobiinae, *Gobiosomatini*-lineage	The deepest positive gobiid record in the Western Atlantic	Bathyal sand and rubbles	[30]
Obliquogobius turkayi Goren, 1992	434 m (434–496 m)	Trawl	Red Sea	Gobiidae, Gobiinae, lineage unknown	The deepest positive gobiid record in the Red Sea	Bathyal soft sediment	[32]
Obliquogobius cirrifer Shibukawa & Aonuma, 2007	394 m (394–404 m)	Beam trawl	Western Pacific	Gobiidae, Gobiinae, lineage unknown	The deepest positive gobiid record in the Western Pacific	Bathyal fine sand	[33]
Buenia lombartei Kovačić, Ordines & Schliewen, 2018	375 m	Trawl and beam trawl	Mediterranean	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage	The deepest positive record of Gobionellinae in general	Restricted to bathyal mud	[7]
Gobius xoriguer Iglésias, Vukić & Šanda, 2021	374 m	Grab	Mediterranean	Gobiidae, Gobiinae, *Gobius*-lineage		Eurybathic, from coralline algae sea bed to bathyal	[14]
Gobius roulei De Buen, 1928	320 m (320 –385 m)	Trawl	North Eastern Atlantic	Gobiidae, Gobiinae, *Gobius*-lineage	The deepest positive gobiid record in the North-Eastern Atlantic	Eurybathic, from infralittoral sands to bathyal	[34]
Anatirostrum profundorum (Berg, 1927)	294 m	Trawl	Caspian Sea	Gobiidae, Gobiinae, *Gobius*-lineage	The deepest positive gobiid record in the Caspian Sea	Circalittoral and bathyal mud	[35]
Thorogobius rofeni Miller, 1988	288 m (288–294 m)	Trawl	South Eastern Atlantic	Gobiidae, Gobiinae, *Gobius*-lineage	The deepest positive gobiid record in the South-Eastern Atlantic	Bathyal, no data on the bottom composition	[36]
Lebetus scorpioides (Collett, 1874)	242 m	Trawl	North Eastern Atlantic	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage		From infralittoral to bathyal at various bottoms	[3]
Trypauchen vagina (Bloch & Schneider, 1801)	200 m	Trawl	Mediterranean	Gobiidae, Amblyopinae, *Periophthalmus*-lineage	The deepest positive record of Amblyopinae in general, the deepest alien gobiid record in the Mediterranean	Continental shelf (circalittoral) to bathyal mud	[28]
Obliquogobius eptactis Fujiwara, Psomadakis, Swe & Motomura, 2021	181 m (181–184 m)	Trawl	Indian Ocean	Gobiidae, Gobiinae, lineage unknown	The deepest positive gobiid record in the Indian Ocean, continental shelf	Deep shelf, no data on the bottom composition	[37]
Pinnichthys atrimela (Bussing, 1997)	137 m (137–146 m)	Otter trawl	Eastern Pacific	Gobiidae, Gobiinae, Gobiosomatini-lineage	The deepest positive gobiid record in the Eastern Pacific, continental shelf	Deep shelf, no data on the bottom composition	[38]
Lythrypnus lavenbergi Bussing, 1997	137 m (137–146 m)	Otter trawl	Eastern Pacific	Gobiidae, Gobiinae, Gobiosomatini-lineage	The deepest positive gobiid record in the Eastern Pacific, continental shelf	Deep shelf, no data on the bottom composition	[38]
Worldwide deepest doubtful records							
Karsten totoyensis (Garman, 1903)	1122 m	No data	Gulf of Boni, Sulawesi, East Indian Arcipelago (Indonesia), Western Pacific	Gobiidae, Amblyopinae, *Periophthalmus*- lineage	The deepest record of Gobiiformes, Gobiidae and Amblyopinae in general, the recorded depth is doubtful since it is so different from conspecific records from continental shelf at depths 30–55 m	Circalittoral sand mud	[26]
Platygobiopsis tansei Okiyama, 2008	960 m (960–970 m)	No data	Southern Japan, Western Pacific	Gobiidae, Gobiinae, lineage unknown	The deepest record of Gobiinae. The recorded depth has already been questioned by Okiyama (2008) and is doubtful since it is so different from conspecific records from the continental shelf at depths 65–128 m	Circalittoral muddy sand bottoms	[27]
European seas unconfirmed bathyal records							
Crystallogobius linearis (Düben, 1845)	400 m	No data	North Eastern Atlantic	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage	The deepest gobiid record in Eastern Atlantic, the depth not positively confirmed, mentioned for the species by Miller (1986) without any exact data, details or cited reference	From infralittoral to bathyal, at various bottoms, from coarse sand to mud	[18]

Table 3. *Cont.*

Species	Maximum Confirmed Depth	Collecting Method	Region of Collecting Site	Taxonomy & Phylogeny	Comment on Depth	Species Usual Depth Zones and Habitat	Reference(s)
Lebetus scorpioides (Collett 1874)	375 m	No data	North Eastern Atlantic	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage	The depth not positively confirmed, mentioned for the species by Miller (1986) without any exact data, details or cited reference	From infralittoral to bathyal at various bottoms	[18]
Buenia jeffreysii (Günther, 1867)	330	No data	North Eastern Atlantic	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage	The depth not positively confirmed, mentioned for the species by Miller (1986) without any exact data, details or cited reference	From infralittoral to bathyal at various bottoms	[18]
Pomatoschistus norvegicus (Collett, 1902)	325 m	No data	North Eastern Atlantic	Gobiidae, Gobionellinae, *Pomatoschistus*- lineage	The depth not positively confirmed, mentioned for the species by Miller (1986) without any exact data, details or cited reference	From infralittoral to bathyal, at various bottoms, from coarse sand to mud	[18]

5. Conclusions

The recently described *Gobius xoriguer* and *Gymnesigobius medits* appear to be widespread in their preferred habitats in the Mediterranean. The record of *Gymnesigobius medits* from the Emile Baudot seamount in the Balearic Islands represents the deepest positive benthic gobiiform species record in general. The known bathyal gobiid species richness of European seas is high compared to other areas, except for the Western Atlantic. However, in the Mediterranean, this richness may more likely be the result of the higher research effort applied in this sea compared to other regions than of the real overrepresentation of bathyal gobies in the Mediterranean Sea compared to other regions.

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Institutional Review Board Statement: The sampling scheme followed a standardised protocol approved by international authorities (EU/DG Mare, FAO/GFCM). If a live specimen of a species subject to conservation measures was caught, it was quickly sampled (4–5 min) and returned back to the sea unharmed, giving it a chance for survival, following the recommendation GFCM/36/2012/3 (http://www.gfcmonline.org/decisions/) (accessed on 27 April 2023) on fisheries management measures for conservation of sharks and rays in the GFCM area. In the cases the animal was alive when it arrived on the vessel during the scientific survey, it was suppressed by administering an overdose of anaesthetic in compliance with the recommendation of Decree Law n. 26 of 4 March 2014. All efforts were made to minimise suffering.

Informed Consent Statement: Not applicable.

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