Mediterranean Marine Science

Vol 20, No 1 (2019)

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doi: 10.12681/mms.19307

To cite this article:

BAÑÓN, R., TEJERINA, R., MORALES, X., ALONSO-FERNÁNDEZ, A., BARROS-GARCÍA, D., & DE CARLOS, A. (2019). Unusual occurrences of fishes along the Northeast Atlantic: new biological and distributional data. *Mediterranean Marine Science*, *20*(1), 189–196. https://doi.org/10.12681/mms.19307

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 19/07/2025 07:54:03

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.19307

Unusual occurrences of fishes along the Northeast Atlantic: new biological and distributional data

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Handling Editor: Argyro ZENETOS

Received: 11 December 2018; Accepted: 23 January 2019; Published on line: 30 April 2019

Abstract

The occurrences of 13 specimens belonging to 9 species of little known marine fishes in the Northeast Atlantic in 2017 and 2018 are reported. Information about the morphology and new findings of *Decapterus tabl* and *Decapterus macarellus* (Carangidae), *Kyphosus vaigiensis* (Kyphosidae), *Remora osteochir* (Echeneidae), *Antigonia capros* (Caproidae), *Hyperoglyphe perciformis* (Centrolophidae), *Halobatrachus didactylus* (Batrachoididae), *Parapristipoma octolineatum* (Haemulidae) and *Zu cristatus* (Trachypteridae) is reported from different locations, where they are categorized as unusual or rare. Morphometric and meristic parameters confirmed the identification to species level of each specimen, also supported by DNA barcoding. Histological examination of reproductive tissue was carried out in four specimens to determine the sex and reproductive stage. All species are known from other areas of the Atlantic Ocean, but these findings are an important contribution to understanding their biology and distribution. These include new northernmost records of *Remora osteochir* and *Decapterus tabl* in the eastern Atlantic and the first records of *Antigonia capros*, *Hyperoglyphe perciformis*, *Halobatrachus didactylus* and *Parapristipoma octolineatum* from Galician waters.

Keywords: Ichthyofauna; Teleostei; Distribution; Biogeography.

Introduction

The term “rare fishes” and analogous terms such as “unusual fishes”, “little-known fishes” and “odd fish” have been used for a long time in the ichthyological literature to describe the occurrence of new, unknown or scarcely known fishes in a given marine area (Phillipps, 1926; Prokofiev, 2017). These reports allow us to either substantially expand their known distribution range or to refine information on morphological variation of the given species in the given area (Prokofiev, 2017). Information on species distributions is of value in support of obligations faced by regulatory authorities and those promoting conservation and biodiversity (Baldock & Kay, 2012).

The Northeast Atlantic is characterised by the presence of coastal upwelling regimes associated with high rates of primary production and important coastal fisheries. Knowledge of the fish biodiversity in this vast area can be generally considered good, given the existence of many ichthyological studies and identification guides (Whitehead *et al*., 1986; Quéro *et al*., 2003; Carpenter & De Angelis, 2016). However, at present, there are many rapid human-induced changes in fish species composition related to overfishing, introduction of alien species and climate change (Afonso *et al*., 2013; Bañón *et al*., 2018a).

A continuous assessment of local marine fish biodiversity is necessary to improve our understanding of the variety and distribution of the marine fish community (Bañón *et al*., 2018b). Effective conservation requires the routine monitoring of coastal waters (e.g. under the EU Marine Strategy Framework Directive); monitoring species distribution for climate change; tracking potentially invasive species and the collection of baseline data on ecology and distribution (Baldock & Kay, 2012).

The purpose of this research is to provide new records of rare fish caught over a wide area of the Northeast Atlantic, stretching from the Madeira Islands in the south to the south of Ireland in the north, many of them in the vicinity of or at the edge of their distribution range.

Materials and Methods

The specimens were caught by fisheries observers on board commercial and oceanographic vessels or by spearfishermen at different locations in the Northeast Atlantic, and frozen afterwards. In the laboratory specimens were defrosted at room temperature and identified at species level, mainly following Quéro *et al*. (2003) and Lloris (2015). The main morphometric and meristic characters were recorded according to the literature as follows: Total length (TL), Standard length (SL), Fork length (FL); Head length (HL); Pre-orbital length (PO); Eye diameter (ED); Post orbital Length (POL); Inter-orbital length (IO); Predorsal length (PD); Pre-first dorsal length (PD1); Pre-second dorsal length (PD2); Prepectoral length (PP); Pre-anal length (PA); First dorsal fin base length (DB1); Second dorsal fin base length (DB2); Anal fin base length (AB); Disc length (DL); Pre-pectoral length (PP); Pre-pelvic length (PV); Pectoral fin length (PL); Pelvic fin length (VL); Maximum body depth (BH); Caudal peduncle depth (CP); Number of rays in first dorsal fin (D1); Number of rays in second dorsal fin (D2); Number of rays in pectoral fin (P); Number of rays in anal fin (A); Number of rays in caudal fin (C); Branchiostegal rays (BR); Gill-rakers (GR); Number of disc laminae (LN). With the exception of TL and SL, measurements are distances perpendicular to the length of the fish measured with a digital calliper to the nearest mm. All measurements are expressed as the percentage of standard length (%SL). Descriptive data are reported individually for species represented by one or two specimens and ranges are reported when there are three.

A muscle sample from each specimen was collected in order to extract DNA and sequence the standard 5´ barcoding region of the mitochondrial *COI* gene, following procedures described elsewhere (Bañón *et al*., 2016). The identity of most barcodes was confirmed by comparison with those deposited in the Barcode of Life Database (BOLD; www.boldsystems.org) by means of the Identification System (IDS) tool, which returns species-level identification when one is possible. In the case of the *Remora osteochir* (Cuvier, 1829) specimens, identification was carried out separately by constructing a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) with *p*-distances (Nei & Kumar, 2000) and using a bootstrap resampling procedure (Felsenstein, 1985). Voucher specimens were deposited in the Museo de Historia Natural da Universidade de Santiago de Compostela (MHNUSC, Santiago de Compostela, Spain). All information regarding these specimens as well as their barcodes, images, places of capture and other complementary data are available in the projects “Marine Fishes from Galicia” (code FIGAL) and “Unusual Atlantic Fishes” (code UNAFI), both in the BOLD database.

Only the gonads of four specimens corresponding to four species could be examined. After morphometric measurements were taken, the gonads were removed and fixed immediately in 10% formalin buffered with $Na_2HPO_4*2H_2O$ (0.046 M, molar concentration) and $NaH_2PO_4*H_2O$ (0.029 M). One central section from the gonad of each specimen was extracted, dehydrated, embedded in paraffin, sectioned at 3 µm and stained with haematoxylin-eosin for histological examination. The specimen was sexed and classified within its corresponding maturity phase using histological criteria when possible (Grier, 1981; Brown-Peterson *et al*., 2011). In two additional specimens belonging to *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) and *Hyperoglyphe perciformis* (Mitchill, 1818), sex was determined based on macroscopic criteria. It should be mentioned that the gonad samples had been previously frozen, which affected the quality of the histological slide. Therefore, in some cases it was not possible to ascertain the reproductive stage.

Results

Unusual fish species

A total of 13 specimens belonging to nine species and eight families were found in a wide area of the Northeast Atlantic (Fig. 1).

Decapterus tabl Berry, 1968 (Fig. 2a)

Material examined: MHNUSC 25116-1, 162 g, 261 mm TL, 240 mm FL, 227 mm SL, 6th July 2017, Madeira islands, 32.631 ºN, -17.036 ºW, 135 m depth; MHNUSC 25116-2, 202 g, 277 mm TL, 253 mm FL, 242 mm SL, 31th July 2017, Madeira islands, 32.618 ºN, -17.023 ºW, 162 m depth; MHNUSC 25116-3, 249 mm TL, 127 g, 227 mm FL, 214 mm SL, 9th October 2017, Madeira Islands, 32.617 ºN, -17.028 ºW, 150 m depth. Morphology: HL: 26.0-27.1; PO: 8.4-9.1; POL: 11.0-11.2; ED: 6.2-7.0; IO: 6.2-7.4; PD1: 32.2-34.1; PD2: 49.2-52.3; DB2: 36.0-38.3; PA: 55.1-58.4; AB: 28.5-33.0; PP: 23.3-26.4; PV: 27.3-29.0; PL: 17.2-18.2; VL: 9.7-11.6; BH: 17.8-19.8; D1: VIII; D2: I+31-34; A: II+I+24-26; P: 22-23; V: I+5; BR: 6; GR: 10-12+30-31. Distribution: Amphiatlantic, in the western Atlantic from Bermuda and North Carolina to Venezuela whereas in the eastern Atlantic it is only known in Ascension and St Helena islands (Smith-Vaniz, 2016); also in the Indian Ocean and the Indo-West Pacific to Hawaii.

Decapterus macarellus (Cuvier, 1833) (Fig. 2b)

Material examined: MHNUSC 25117, 53.7 g, 190 mm TL, 175 mm FL, 165 mm SL, 9th October 2017, Madeira Islands, 32.617 ºN, -17.028 ºW, 150 m depth. Morphology: HL: 23.6; PO: 7.3; POL: 10.3; ED: 6.1; IO: 5.5; PD1: 32.1; PD2: 49.7; DB2: 38.2; PA: 53.3; AB:

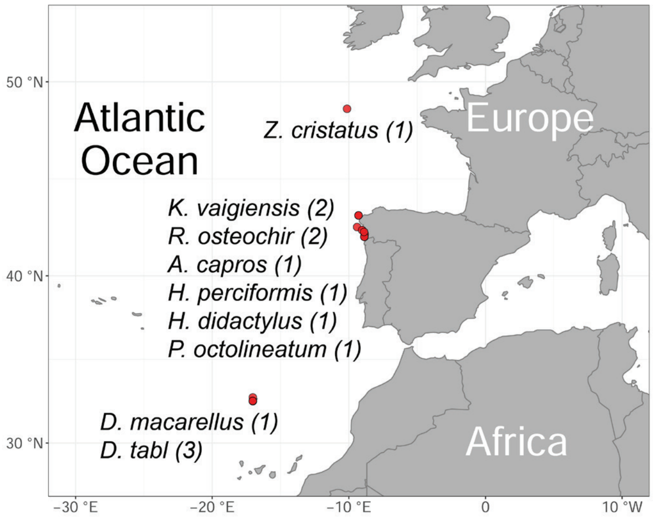

Fig. 1: Map showing the sampling areas in the North Atlantic Ocean, including species captured and number of specimens (shown in brackets).

34.5; PP: 24.2; PV: 25.5; PL: 13.3; VL: 9.7; BH: 17.0; D1: VIII; D2: I+36; A: II+I+30; P: 23; V: I+5; BR: 6; GR: 10+39. Distribution: Circumtropical; although its distribution in the eastern Atlantic is not well known, it is reported from the Azores, Madeira, Canary (rare), Cape Verde, Ascension and St Helena islands, and the Gulf of Guinea (Smith-Vaniz, 2016).

Kyphosus vaigiensis (Quoy & Gaimard, 1825) (Fig. 2c)

Material examined: MHNUSC 25118-1, ♀, 2480 g, 519 mm TL, 463 mm FL, 416 mm SL; 23th May 2017, Galician waters, 42.148 ºN, -8.860 ºW, 5 m depth; MHNUSC 25118-2, ♂, 1434 g, 450 mm TL, 405 mm FL, 372 mm SL; 20th Mars 2018, Galician waters, 42.292 ºN, -8.839 ºW, 7 m depth. Morphology: HL: 24.0, 24.2; D: XI+13, XI+14; A: III+12, III+13; P: 19; V: I+5; BR: 7; GR: 9+20, 8+20. PO: 6.5, 7.3; POL: 12.7, 12.1; ED: 4.8; IO: 11.5-9.9; PD: 33.7, 32.5; DB: 50.2, 49.7; PA: 59.9-58.3; AB: 25.5, 23.9; PP: 23.1, 23.9; PV: 29.8, 31.7; PL: 18.0, 15.9; VL: 14.7, 12.9; BH: 40.1, 39.8. Distribution:

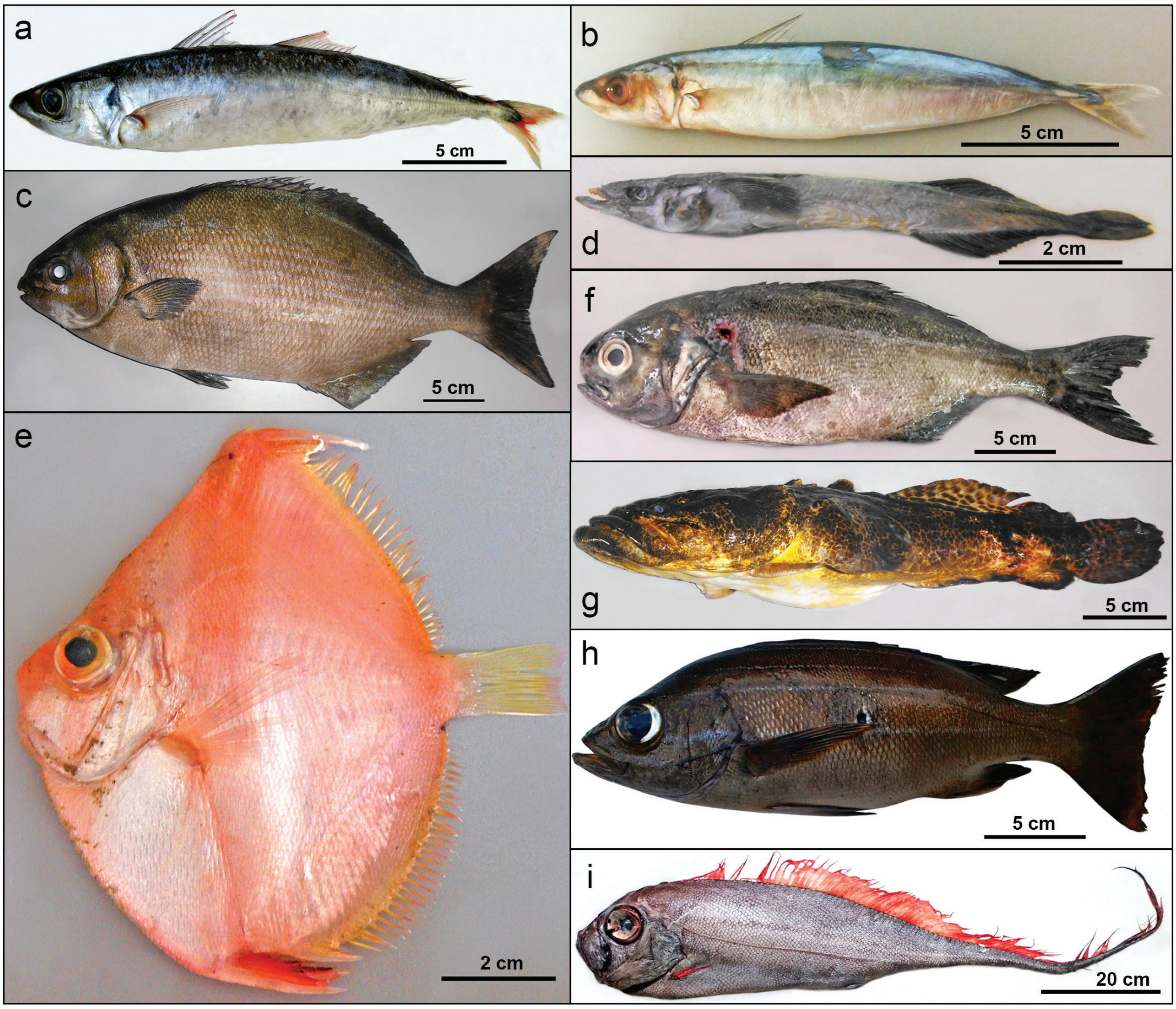

Fig. 2: Rare fishes collected along the Northeast Atlantic: a: *Decapterus tabl*, 277 mm TL; b: *Decapterus macarellus*, 190 mm TL; c: *Kyphosus vaigiensis*, 450 mm TL; d: *Remora osteochir*, 106 mm TL; e: *Antigonia capros*, 102 mm TL; f: *Hyperoglyphe perciformis*, 376 mm TL; g: *Halobatrachus didactylus*, 367 mm TL; h: *Parapristipoma octolineatum*, 293 mm TL; i: *Zu cristatus*, 1109 mm TL.

Widely distributed in the Pacific, Indian and Atlantic Oceans, and also in the Mediterranean Sea (Knudsen & Clements, 2013). In the eastern Atlantic it is distributed from Galicia (Northwest Spain) to Angola (Sakai & Nakabo, 2016; Bañón *et al*., 2017b). Reproduction: The bigger specimen of 519 mm TL, with an ovary weight of 8.23 g, was a female probably in immature phase, as the presence of only primary growth oocytes indicates (Fig. 3a). The 450 mm TL specimen was classified macroscopically as a male.

Remora osteochir (Cuvier, 1829) (Fig. 2d)

Material examined: Two specimens over a *Xiphias gladius* Linnaeus, 1758 of 121 cm TL; MHNUSC 25119-1, 8.4 g, 114 mm TL, 98 mm SL; 24th September 2017, Galician waters, 43.302 ºN, -9.294 ºW, 169 m depth; MHNUSC 25119-2, 6.2 g, 106 mm TL, 91 mm SL; 24th September 2017, Galician waters, 43.302 ºN, -9.294 ºW, 169 m depth. Morphology: HL: 22.4, 22.0; PO: 10.2, 8.8; POL: 9.2, 9.9; ED: 3.1, 3.3; IO: 12.2-12.1; PD: 61.2, 58.2; DB: 34.7, 36.3; PA: 59.2-58.2; AB: 34.7, 36.3; DL: 41.8, 37.4; PP: 24.5, 23.1; PV: 28.6; PL: 17.3, 16.5; VL: 13.3, 13.2; BH: 10.2, 9.9. D: 23, 22; A: 23, 20; P: 21, 20; V: I+5; BR: 8; GR: 1+12, 2+12; LN: 17, 18. Distribution: Epipelagic, worldwide in all warm seas (Collette, 2016).

Antigonia capros Lowe, 1843 (Fig. 2e)

Material examined: MHNUSC 25120, 39.6 g, 102 mm TL, 79 mm SL; 11th May 2018, Galician waters, 42.679 ºN, -9.399 ºW, 170 m depth. Morphology: HL: 38.0; PO: 10.1; POL: 12.7; ED: 15.2; IO: 10.1; PD: 51.9; DB: 43.0; PA: 57.0; AB: 39.2; PP: 36.7; PV: 46.8; PL: 35.4; VL: 29.1; BH: 125.3; D: VIII+33; A: III+33; P: 13; V: I+5; BR: 6; GR: 6+14. Distribution: Worldwide in subtropical and tropical oceans except the eastern Pacific. In the eastern Atlantic Ocean occurs from France to Namibia, including the Azores Archipelago and Madeira Islands (Zidowitz & von Westernhagen, 2004).

Hyperoglyphe perciformis (Mitchill, 1818) (Fig. 2f)

Material examined: MHNUSC 25121, ♂, 604 g, 376 mm TL, 339 mm FL, 296 mm SL; 07th July 2018, Galician waters, 42.510 ºN, -9.005 ºW, 5 m depth. Morphology: HL: 29.4; PO: 6.1; POL: 17.6; ED: 5.7; IO: 11.1; PD: 30.4; DB: 51.7; PA: 58.1; AB: 24.7; PV: 29.1; PP: 27.7; PL: 25.0; BH: 33.1; D: VIII+22; A: III+18; P: 21; V: I+5; BR: 6; GR: 7+16. Distribution: Occurs on both sides of the North Atlantic, from Nova Scotia to the Florida Keys, in the western North Atlantic, and from Iceland to the Azores in the eastern Atlantic, including the western Mediterranean (Quigley, 1986).

Halobatrachus didactylus (Bloch & Schneider 1801) (Fig. 2g)

Material examined: MHNUSC 25122, ♂, 786 g, 367 mm TL, 310 mm SL; 11th July 2018, Galician waters, 42.151 ºN, -8.868 ºW, 20 m depth. Morphology: HL: 35.2; PO: 9.7; POL: 21.6; ED: 3.9; IO: 15.5; PD1: 36.1; PD2: 46.1; DB1: 5.5; DB2: 46.1; PA: 58.1; AB: 31.3; PV: 25.2; PP: 33.9; VL: 19.0; PL: 21.0; CP: 8.4; BH: 16.8; D1: III; D2: 20; A: 15; P: 24; V: I+2; BR: 6. Distribution: Eastern Atlantic, from Portugal to the South to at least Ghana and perhaps as far as Nigeria, and western Mediterranean. However, some authors extend it northwards to south of the Bay of Biscay and there is an old northern record from the Kattegat, in Norway (Roux, 1986), conserved in the Lund Museum of Zoology (MZLU: L820/3004). Reproduction: The specimen was classified as a mature male, likely to be in spawning capable phase (Fig. 3b).

Parapristipoma octolineatum (Valenciennes, 1833) (Fig. 2h)

Material examined: MHNUSC 25124, ♀, 308 g, 293 mm TL, 235 mm SL; 21th July 2018, Galician waters, 42.415 ºN, -8.887 ºW, 7 m depth.

Morphology: HL: 31.9; PO: 6.5; POL: 11.3; ED: 7.1; IO: 6.5; PD: 23.5; DB: 40.0; PA: 50.6; AB: 9.0; PV: 25.5; PP: 23.9; VL: 17.4; PL: 19.4; CP: 8.7; BH: 25.5; D: XIII+14; A: III+7; P: 17; V: I+5; BR: 6; GR: 4+24. Distribution: Eastern Atlantic, from Angola northwards to the South of Spain and Portugal, including Cape Verde, Madeira and Canary Islands, and the western Mediterranean (Carpenter & Johnson, 2016); one specimen was recently observed *in situ* for the first time on the French coast of the Bay of Biscay (Casamajor, 2016). Reproduction: The specimen, with an ovary weight of 3.8 g, was classified as a developing female, by the presence of cortical alveoli oocytes in the ovary (Fig. 3c).

Zu cristatus (Bonelli, 1819) (Fig. 2i)

Material examined: MHNUSC 25123, ♀, 1109 mm TL, 983 mm SL; 29th January 2018, south of Ireland, 48.716 ºN, -10.133 ºW, depth unknown. Morphology: HL: 17.7; PO: 3.1; POL: 8.0; ED: 6.6; IO: 3.0; PD: 12.4; DB: 85.7; PV: 16.6; PL: 6.9; BH: 19.5; D: 126; P: 11; V: I+5; BR: 6; GR: 3+9. Distribution: Mesopelagic, circumglobal in tropical and temperate waters. In the eastern Atlantic it is occasionally found from the North Sea to South Africa and also in the Mediterranean (Olney & Hartel, 2016). Reproduction: The status of preservation and fixation of the reproductive tissue of this specimen hamper the classification of the maturity phase; however there was evidence of female reproductive tissue (Fig. 3d).

Molecular Identification

A *COI* sequence was obtained from each of the morphologically identified specimens. These sequences were used as queries in the IDS tool. Table 1 shows the results summary after searching the Species Level Barcode Records database, which included 3,205,173 sequences, 193,748 species and 78,906 interim species. The return consists of every *COI* barcode record with a species level identification and a minimum sequence length of 500 bp. This includes many species represented by only one or two specimens as well as all species with interim taxonomy. Most of the *COI* sequences obtained a maximum

Table 1. BOLD IDS results summary after searching the *COI* species database.

Morphology ID	Query ID[1]	Best ID (100% identity)
Antigonia capros	FIGAL006-18	*Antigonia capros*
Decapterus macarellus	UNAFI004-18	*Decapterus macarellus*
Decapterus tabl	UNAFI001-18	*Decapterus tabl*
Decapterus tabl	UNAFI002-18	*Decapterus tabl*
Decapterus tabl	UNAFI003-18	*Decapterus tabl*
Halobatrachus didactylus	FIGAL008-18	*Halobatrachus didactylus*
Hyperoglyphe perciformis	FIGAL007-18	*Hyperoglyphe perciformis*
Kyphosus vaigiensis	FIGAL002-18	*Kyphosus vaigiensis*
Kyphosus vaigiensis	FIGAL003-18	*Kyphosus incisor*
Parapristipoma octolineatum	FIGAL009-18	*Parapristipoma octolineatum*
Remora osteochir	FIGAL004-18	*Remora* sp.
Remora osteochir	FIGAL005-18	*Remora* sp.
Zu cristatus	UNAFI005-18	*Zu cristatus*

[1]Query barcode sequences identified with BOLD Process ID codes

identity value of 100% with voucher specimens of the same species present in the database, and also give the same result in the morphological examination.

Regarding the *R. osteochir* specimens, a species level match could not be made and the queried sequences were likely to be either *R. osteochir* or *Remora brachyptera* (Lowe, 1839); therefore, they were initially named as *Remora* sp. In order to obtain an unambiguous molecular identification, an alignment of barcode sequences was created, including those obtained from the captured specimens and others of public access in the BOLD repository belonging to several species of the *Remora* genus. Figure 4 shows a NJ tree in which the two *R. osteochir* sequences of the specimens are grouped with others of the same species. Both nucleotide sequences are equal to each other except for a single position.

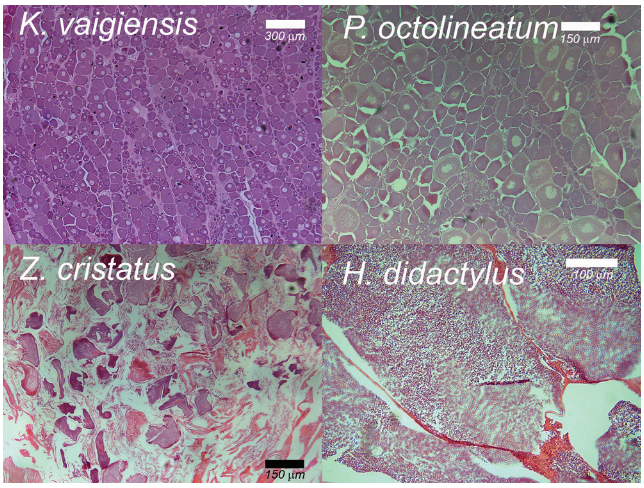

Fig. 3: Histological slides. a: female reproductive tissue of *K. vaigiensis*; b: male reproductive tissue of *H. didactylus*; c: female reproductive tissue of *P. octolineatum* and d female reproductive tissue of *Z. cristatus*. PG=Primary growth oocyte, CA=cortical alveoli.

Discussion

Morphological data, including meristic and biometric measures, are in agreement with previous diagnosis of each species, with the molecular identification based on DNA barcodes supporting the reliability of the morphological one. DNA barcoding is recognized as an important new tool that can be usefully applied to help resolve taxonomic issues in fishes (Ward *et al*., 2009), based on the development of a reference library of barcode sequences from vouchered specimens. The molecular identification by means of IDS agrees with the same nominal species in all cases excepting three specimens. The *K. vaigiensis* barcode FIGAL003-18 is identified at 100% with a *Kyphosus incisor* (Cuvier, 1831), but this species has been recently synonymized with *K. vaigiensis* (Knudsen & Clements, 2013). The two *R. osteochir* sequences were not identified at species level by the IDS, but this could be due to the use of restricted access (non-public) barcodes from incorrectly identified or pending validation. However, a NJ analysis grouped these two sequences in one highly supported cluster with other nine previously assigned to *R. osteochir* voucher specimens and well differentiated from other clusters.

The observation of new species in a new area represents the first, and sometimes the only opportunity to follow and study the dynamics of colonization. Monitoring the spread is crucial to understand how a new species arrives, what are its movements and developments and the impact that it may have (Azzurro, 2010). Unusual occurrences may testify to substantial extensions of geographical ranges of tropical species which are moving to northern and colder sectors of the eastern Atlantic

(Kaimuddin *et al*., 2016). In the case of mesopelagic fishes such as *H. perciformes* and *Z. cristatus*, they are rarely caught because mesopelagic habitats are poorly sampled, so it is difficult to relate their presence to changes in geographical distribution. The difficulty of sampling many marine habitats could cause fish to be considered rare when in fact they could be common or abundant (Swaby & Potts, 1990). However, the occurrence of coastal and tropical species such as *K. vaigiensis* or *P. octolineatum* would more probably be related to a northward displacement related to global warming (see Bañón *et al*., 2017b for *K. vaigiensis*).

The records reported here add new biological information and interesting biogeographic locations for the eastern Atlantic. Regarding *Decapterus* fishes, only two species are known in the Madeira Islands: *Decapterus punctatus* (Cuvier 1829) and *D. macarellus* (Wirtz *et al*., 2008). These results confirm the presence of *D. macarellus* and add *D. tabl* as a new species for the Madeira Islands. The current records of *K. vaigiensis* confirm its increasingly frequent presence in Galician waters, at their northern limit of distribution in the eastern Atlantic.

These are also the first confirmed records of *R. osteochir*, *A. capros*, *H. perciformis, H. didactylus* and *P. octolineatum* from Galician waters. The location mark of *A. capros* drawing in front of Galicia by Quèro (1986) really corresponds to an unspecified Portuguese record (Quéro & Duron, 1980). The *H. perciformis* length, 339 mm FL, agrees well with the general size composition reported in the eastern Atlantic, composed of young specimens up to 410 mm FL (Filer & Sedberry, 2008). The size of *H. didactylus*, 367 mm TL, is greater than the maximum previously reported (Collette & Greenfield, 2016) of 277 mm TL, but in range with Roux (1986), up to 500 mm SL. Moreover, according to two of the biometric measures (ED 3.9%SL, IO 15.5%SL), the specimen of *H. didactylus* belongs to the small-eyed European morphotype, characterized by ED 5.3–7.9% SL and IO 12.2–16.1% SL, which could represent a separate species of the large-eyed African morphotype, with ED 7.8–11.1% SL and IO 8.8–12.1% SL (Collette *et al*., 2006). The location of *P. octolineatum* constitutes the second northernmost record for the East Atlantic. The most northerly specimen found so far was photographed but not analysed in the South of the Bay of Biscay (Casamajor, 2016). *Zu cristatus* is a very rare species in the Atlantic European waters and here constitutes the second northernmost record from the eastern Atlantic, only surpassed by a specimen recorded in the Rockall Bank, at 56°N (Quigley & Henderson, 2014).

Regarding the two *R. osteochir* specimens, both were very similar in size, colour and morphological data to a previous specimen of *R. brachyptera* (MF038134) also reported in Galician waters (Bañón *et al*., 2017a). Both species can be differentiated mainly by the lower number of dorsal fin rays and a greater disc length in *R. osteochir* respect to *R. brachyptera* (Collette, 2016). In the eastern Atlantic, *R. osteochir* is distributed northwards off the mainland of Portugal and the Azores Islands (Carneiro *et al*., 2014). The Galician records suppose therefore a new northernmost limit for the eastern Atlantic.

All these findings allow us a more detailed knowledge of the spatial distribution of these species. This is necessary to know its changes linked to anthropological, physical or oceanographic factors, such as global warming and tropicalization processes. In the concrete case of *H. didactylus*, this species shows some difficulties in the dispersal and colonisation of new territories due to its ecological characteristics: low fecundity, presence of benthic eggs and larvae, parental care of the offspring and marked sedentary lifestyle of the adults (Costa *et al*., 2003). However, there are some old vagrant specimens found beyond its northern limit sited in the Tagus River: a specimen in Norway (Nilsson, 1832), a doubtful record (Quéro pers. communication) from the Loire estuary (Desvaux, 1843) and an unpublished record in Galician waters. This latter was a specimen of *H. didactylus* found in the fish collection of the Museo Natural de Ciencias Naturales de Madrid, in Spain (MNCN_ICTIO 13294), labelled as “caught off the coast of Galicia” in June 1926, without any further information. As far as we know, there are no records further north until now since this last year, which seems to support the low dispersal ability of this species.

Of the four individuals sampled to collect reproductive tissue, only *H. didactylus* was a male, which seemed to be in spawning capable condition, in agreement with previous studies (Palazón-Fernández *et al*., 2001). Regarding females, *K. vaigiensis* were classified as immature, with all the oocytes in primary growth stage. However, previous reports of this species revealed smaller maturation lengths, for instance, 42.8 cm TL in the Caribbean (Mora, 2013) or 36 cm TL in Papua New Guinea (Longenecker *et al*., 2012). The regenerating phase can be a source of misclassification, even using histological techniques, and more samples would be convenient to validate current classification (Brown-Peterson *et al*., 2011). Ovaries of

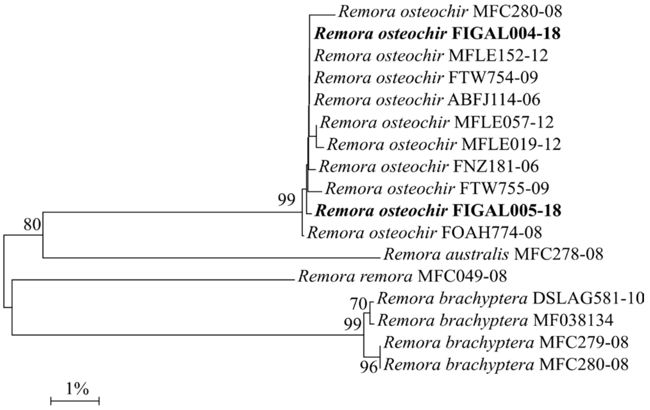

Fig. 4: Neighbor-Joining tree of *Remora COI* barcode sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the nodes. The tree is drawn to scale; with branch lengths computed using the *p*-distance method and are in units of the number of base differences per site. The analysis involved 17 nucleotide sequences and 655 nucleotide positions.

P. octolineatum presented cortical alveoli oocytes, which are evidence of developing maturity phase, so, it was classified as a mature female. Fish in the reproductive cycle and gonadal growth become gonadotropin dependent, and will reproduce the coming spawning season.

The study of reproductive biology can provide clues about the key factors that determine the seasonal occurrence of unusual species (Bañón *et al*., 2008). It can also be particularly informative in early settled populations, as a means of knowing if colonizers attain final gonad maturation stages and have the potential for successful reproduction (Azzurro, 2010). Due to the small number of samples and the poor condition of some of them, we cannot draw clear conclusions on the reproductive aspects and more sampling effort is needed.

Acknowledgements

The authors would like to thank Jose Manuel Gonzalez Juste (vessel "Mª Jose") for kindly donating the specimen of *H. didactylus*. The specimens of *R. osteochir* were obtained during the "Demersales 2017" bottom trawl surveys and therefore Alberto Serrano and Marián Blanco (Instituto Español de Oceanografía, C.O. de Santander) are thanked. Also thanks to the spear fishermen of the Federación Galega de Actividades Subacuáticas and especially to Abraham Padín for providing various specimens here reported. Also thanks to Lucía Sánchez-Ruiloba (IIM-CSIC) for the help with the fish photographic composition. This study was partially financed by the agreement between CSIC and Xunta de Galicia to analyse fisheries-dependent data from the monitoring program of small-scale fisheries in Galicia (Agreement No. 070401150009). RT was financially supported by the Oceanic Observatory of Madeira Project (M1420-01-0145-FEDER-000001-Observatório Oceânico da Madeira-OOM).

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