

## NEW RECORDS OF TWO SOUTHERN FISHES FOUND IN NORTHERN WATERS OF THE IBERIAN PENINSULA

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**Abstract.** The first records of the spearfish remora, *Remora brachyptera* (Lowe, 1839) (Actinopterygii: Echeineidae), and the white grouper, *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817) (Actinopterygii: Serranidae), from northern Spanish waters are reported. Both findings are, respectively, the second and the first northernmost reports, for both fish species, in the eastern Atlantic. Morphometric and meristic parameters, along with DNA barcoding, confirmed the identification of the remora specimen. Results of the histological examination of the gonads of *R. brachyptera* are also reported, showing a mature male at the developing maturity phase. The grouper specimen was identified taking into account its characteristic coloration based on a photograph.

**Keywords:** NE Atlantic, tropicalisation, morphology, DNA barcoding, fish identification

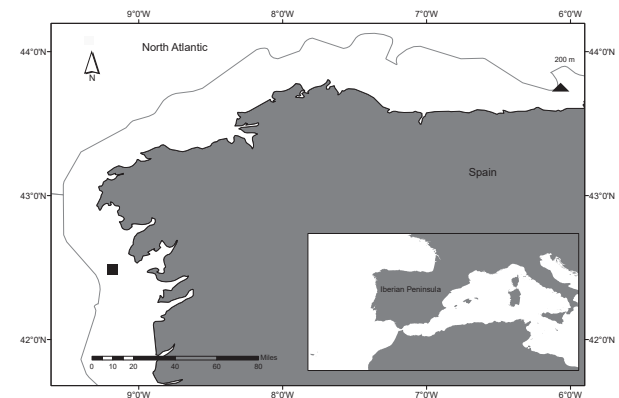
Two unusual fish species, the spearfish remora, *Remora brachyptera* (Lowe, 1839) (Actinopterygii: Perciformes: Echeineidae), and the white grouper, *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817) (Actinopterygii: Perciformes: Serranidae), were caught in the north of Spain and are reported herewith for the first time (Fig. 1).

*Remora brachyptera* is a globally distributed, epipelagic species, found to a maximum depth of 200 m in tropical to warm temperate seas, frequently associated with billfishes, molids, sharks, and occasionally free swimming (Collette 2010).

*Epinephelus aeneus* is a demersal, protogynous hermaphrodite and usually solitary species distributed in the eastern Atlantic, along the west coast of Africa down to southern Angola. It is present also in the Mediterranean. Juveniles occur in coastal lagoons and estuaries whereas adults are found on rocky, muddy, and sandy bottoms at depths from 20 to 200 m (Heemstra and Randall 1993, Heemstra 2016).

A single specimen of *R. brachyptera* of 141 mm TL (Fig. 2) was captured alive on 7 March 2017

near the coast of Corrubedo (Galicia, NW Spain), at 42°31'13"N; 09°10'45"W by commercial fishers, using gillnets, at 80 m depth. The specimen was found detached on the deck of the ship after hauling and, therefore, it was not possible to assign it to an associate ('host') species.



**Fig. 1.** Sampling locations of *Remora brachyptera* (square) and *Epinephelus aeneus* (triangle)

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The specimen was preserved alive in the installations of O Grove Aquarium facility until its death, one month later. Afterwards, the specimen was fixed in 10% formalin, transferred to 70% ethanol, and finally deposited in the fish collection of the Museum Luis Iglesias de Ciencias Naturais of Santiago de Compostela (Galicia, Spain) with the reference number MHNUSC 25049.

One specimen of *E. aeneus* 6.5 kg in weight (Fig. 2) was caught by a bottom trawler on 24 February 2017 in a fishing ground called Mar del Medio in the Cantabrian Sea (Asturias, North of Spain), at 43°42'44"N; 006°04'01"W at a depth of 290 m (Fig. 3). The fish was landed in the port of Gijón and sold at the fish market, which prevented its preservation, but a photograph was taken.

The main morphometric and meristic characters were taken on the *R. brachyptera* specimen following Myoung et al. (2015) (Table 1). The *E. aeneus* specimen was identified based on the two or three prominent oblique white stripes on the head behind eye characteristic to this species (Heemstra 2016). A muscle sample from fresh *R. brachyptera* specimen was collected and used for DNA purification and sequencing of the standard 5' barcoding region of the mitochondrial COI gene, following previously described procedures (Bañón et al. 2016). PCR amplification was carried out with Thermo Scientific Phire Green Hot Start II PCR Master Mix and the primer set C\_FishF1t1-C\_FishR1t1 (Ivanova et al. 2007). A 658 nucleotides-long sequence was submitted to the GenBank

repository and given the accession number MF038134. In order to explore the taxonomic status of the sample, its sequence was aligned with other phylogenetically-related barcodes using the MEGA6 software (Tamura et al. 2013), and the Neighbor-Joining method (Saitou and Nei 1987) was employed to construct a tree diagram. The divergence among sequences was calculated in the units of the number of base differences per site (p-distances). Confidence limits of this analysis were tested through a bootstrap procedure with 2000 replicates (Felsenstein 1985). The tree was saved as an Enhanced Metafile (emf) and edited using the free and open-source vector graphics editor Inkscape (inkscape.org).

After morphometric measurements were taken, the gonads of *R. brachyptera* were removed and immediately fixed in 10% formalin buffered with  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (0.046 M, molar concentration) and  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (0.029 M). Gonads were dehydrated, embedded in paraffin, sectioned at 3  $\mu\text{m}$ , and stained with haematoxylin-eosin for histological examination. The specimen was sexed and classified within its correspondent maturity phase using histological criteria (Grier 1981, Brown-Peterson et al. 2011).

Morphological data of *Remora brachyptera* are consistent with measurements and counts reported by other authors describing this species (Myoung et al. 2015, McEachran and Fechhelm 1998), with minor differences. On the other hand, the molecular identification supports the reliability of the morphological analysis. The resulting



**Fig. 2.** Specimens caught in northern waters of the Iberian Peninsula: *Remora brachyptera* caught in Galician waters with a scale bar (top) and *Epinephelus aeneus* in Asturian waters (bottom)

Neighbor-Joining tree (Fig. 3) clustered the Galician sequence MF038134 together with other two publicly available barcodes of *R. brachyptera* from Panamá (GU440496) and South Africa (DSL A G581-10) with a mean nucleotide divergence value of 0.41% among them. This value rose to 15.05% when the sequence was compared to the ones from the other species included in the alignment, belonging to the family Echeneidae. These figures are concordant with the accepted values described for the respective taxonomic levels in fishes (Ward et al. 2009).

Studies on the reproductive biology are essential for understanding fish population dynamics and histology is an important tool to study routinely basic reproductive parameters (Blazer 2002, Brown-Peterson et al. 2011). Histological examination of the gonads revealed testes with a presence of spermatocysts and spermatids in different development stages evident along lobules. Therefore, the *R. brachyptera* was sexed as a mature male at developing maturity phase. Information about the reproductive biology of *R. brachyptera* is poorly documented. Mature females of this species are reported in the Gulf of Guinea, between July and August, from 11 to 26.5 cm TL (Pampillón 1996). Our specimen with 14.1 cm TL falls into this size range, which represents, as far as we know, the first information regarding maturation of males for the species.

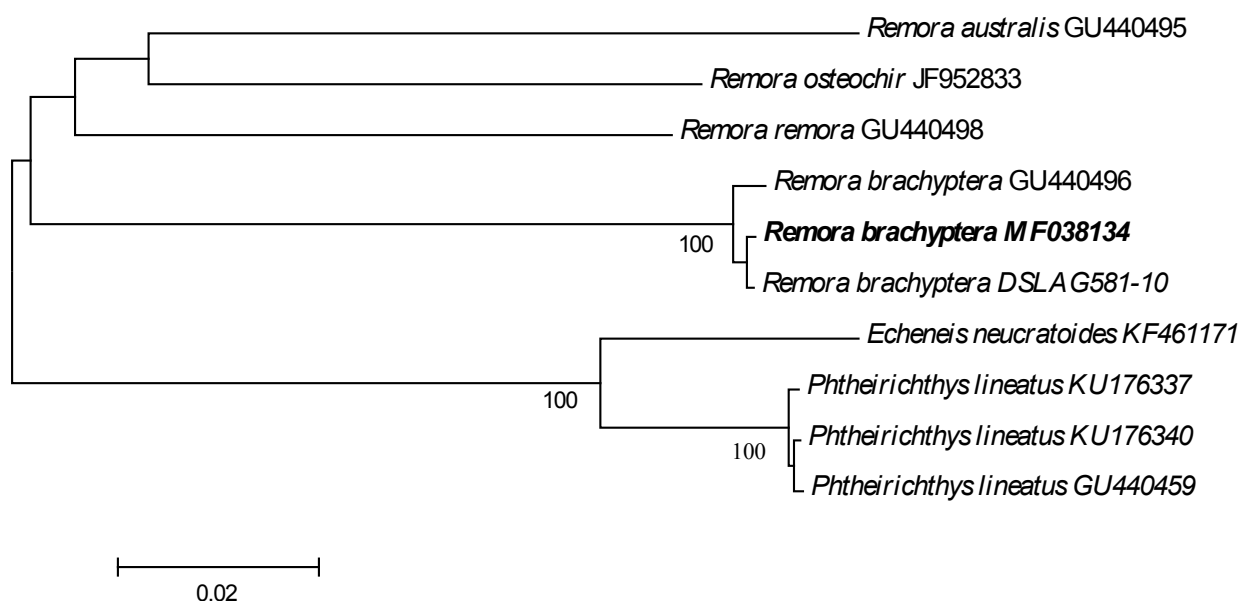
Regarding the presence of *R. brachyptera* in Atlantic European waters, Quéro et al. (2003) reported this species from southern Spain and Portugal. Afterwards, Quéro et al. (2007) extended this species northward, to the Bay of Biscay at 43°53'N, 002°07'W, establishing the northernmost limit in the distribution of this species from the eastern Atlantic. Until now, the presence of

*R. brachyptera* in northern Spanish Galician waters was doubtful. Solórzano et al. (1988) reported specimens attached to swordfish individuals caught by the Galician pelagic longliners. However, this fleet traditionally operated in Atlantic waters out of Spanish Exclusive Economic Zone, and the exact record location points were unknown. Therefore, the validity of these records was questioned by Bañón et al. (2010). The presently reported discovery confirms the occurrence of this species in Galician waters and establishes the second northernmost record from the eastern Atlantic.

The observed coloration in the specimen of *Epinephelus aeneus*, mainly the presence of two oblique white stripes on the head behind the eye, considered a diagnostic character (Heemstra 2016), supports, without doubt, its correct identification. An old record of *E. aeneus* in British waters, reported as '*Perca robusta*' by Couch (1832), was subsequently questioned (Heemstra 1991, Heemstra and Randall 1993). Nowadays, the distribution map of the species establishes its northern limit at the latitude of Lisbon, Portugal (Craig 2015). Therefore, we consider the present report as the northernmost confirmed record of this species from the Eastern Atlantic.

The rise in the ocean temperature due to global climate change is causing poleward shifts in the latitudinal abundance and distribution ranges of fish species (Horta e Costa et al. 2014). A rise by 0.24°C per decade has been observed in the in the NW Iberian Peninsula since 1974 (Gómez-Gesteira et al. 2011) and a strong warming of ~0.2°C per decade for the period 1965–2004 is apparent from the surface down to the 200-m depth in the Bay of Biscay (Michel et al. 2009).

The occurrence of southern, warm-water fishes has been well documented in the last decades, possibly



**Fig. 3.** Neighbor-Joining tree of the Galician specimen of *Remora brachyptera* (in bold letters) and related Echeneidae species based on *p*-distances. Numbers at the main nodes are bootstrap percentages after 2000 replicates; only values higher than 70% are shown; the GenBank accession number or BOLD sequence ID accompanies each taxon name; there were a total of 652 nucleotide positions in the final dataset

**Table 1**  
Morphometric and meristic characters of the presently described specimen of *Remora brachyptera* compared with the previously reported values (see Myoung et al. 2015, McEachran and Fechhelm 1998, and Collette 2016)

Character	Morphometry		Meristic count	Weight [g]	Previously reported values [%SL]
	Absolute value [mm]	%SL			
Total length (TL)	141				
Standard length (SL)	121				
Head length	30	24.8		24.0–27.7	
Preorbital length	13	10.7		10.0–13.5	
Eye diameter	5	4.1		3.2–4.1	
Postorbital length	12	9.9		—	
Interorbital distance	14	11.6		—	
Predorsal length	68	56.2		52.4–62.8	
Dorsal fin base length	43	35.5		32.5–42.4	
Preanal length	73	60.3			
Anal fin base length	38	31.4		31.5–33.4	
Pectoral length	18	14.9		11.6–16.0	
Ventral length	15	12.4		9.8–13.0	
Disc length	35	28.9		29.4–35.0	
Disc width	15	12.4		14.9–19.2	
Body depth	14	11.6		13.9–17.4	
Body width	10	8.3		—	
No. of dorsal fin rays			28	26–37	
No. of anal fin rays			23	22–34	
No. of pectoral fin rays			22	23–28	
No. of ventral fin rays			I + 5	I + 5	
No. of disc laminae			16	15–18	
Branchiostegal rays			8	9	
Gill rakers			1 + 11	10–17	
Total weight [g]				13.6	

resembling a tropicalisation process of the North Atlantic Spanish waters ichthyofauna (Bañón et al. 2010). The two species here reported could suppose new cases of this phenomenon, mainly *Epinephelus aeneus*, a well-known coastal species targeted by the local fisheries, whereas *Remora brachyptera* is an oceanic non-commercial species less reported in the literature. In fact, a northward displacement of *E. aeneus* in the Mediterranean Sea was previously observed (Dulčić et al. 2006) and it is suggested that this species is in process of colonisation of new areas in the northern Mediterranean and Adriatic seas (Glamuzina et al. 2000).

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