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First confirmed record of the spearfish remora *Remora brachyptera* (Lowe, 1839) in the Mediterranean Sea

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Abstract

One specimen of spearfish remora *Remora brachyptera* (Lowe, 1839) was caught in a bottom trawl in the eastern Adriatic Sea (Croatia) in June 2021. Morphological analysis and genetic barcoding were used for species identification. Despite references to the species inhabiting the Mediterranean Sea, no documented records have been available to substantiate its presence in the basin. This finding represents the first documented record of this species from the Adriatic and Mediterranean Seas.

Keywords: Adriatic Sea; suckerfish; Echeneidae.

Introduction

The Echeneidae or remoras are a family of marine fish species distributed worldwide. They usually hitchhike by attaching themselves to a variety of hosts via a suction disc but some can be found swimming freely (Lachner, 1986). Five of eight known species occur in Mediterranean waters and belong to two genera, *Echeneis* (*E. naucrates*) and *Remora* (*R. australis*, *R. brachyptera*, *R. osteochir* and *R. remora*). Except for *R. brachyptera*, all mentioned members of the Echeneidae family have also been recorded in the Adriatic Sea, with *R. osteochir* being the most recently documented species in the area (Dulčić *et al*., 2021).

The spearfish remora *Remora brachyptera* (Lowe, 1839) is an epipelagic species found in tropical to warm waters and recorded down to a maximum depth of 200 m. It can reach a total length of 50 cm, with 25 cm being the most common length of the species. It is frequently found attached to the gills of various billfishes and swordfishes and the mouths of various shark species (Froese & Pauly, 2023). The spearfish remora, being a migrant species, has a circum-global distribution where it occurs in warm waters of all oceans, i.e., in the eastern Pacific from California to Chile and to New Zealand in the western Pacific while in the western Atlantic its distributional range extends from Nova Scotia to Uruguay (Collette, 2010; Anislado-Tolentino *et al*., 2022). In the Pacific, its presence was recently documented for the first time by Myoung *et al*. (2015) in Korean waters and by Anislado-Tolentino *et al*. (2022) in Mexican waters. This species is also present in the eastern Atlantic where it is known to occur around the Madeira Islands (Wirtz *et al*., 2008) and the northern waters of the Iberian Peninsula (Bañón *et al*., 2017).

Although the presence of *R. brachyptera* in the Mediterranean is mentioned in the literature, the absence of documented records or specimens in zoological collections resulted in exclusion of this species from the latest evidence-based checklist of Mediterranean fishes (Kovačić *et al*., 2021). According to Kovačić *et al*. (2021) the species' mentions in the more recent checklists of Mediterranean fishes (for example Quignard & Tomasini, 2000; Psomadakis *et al*., 2012) were probably based on earlier works that included the Mediterranean as its distributional area.

In this paper, we report the first confirmed record of the Spearfish remora, *R. brachyptera*, in the Mediterranean Sea.

Material and Methods

The examined specimen of *R. brachyptera* was caught by a fisherman in the central Adriatic Sea (approx. 43.575129 N, 15.811749 E). It was collected on 18 June 2021 by bottom trawling near Blitvenica Island operating at 150–200 m depth. Morphological identification of the species was done according to the species key guide and taxonomic features by Fischer *et al*. (1987) and Lachner & Post (1990). In the laboratory, the specimen was weighed to the nearest gram and measured to the nearest millimetre. Main morphometric measurements and meristic counts were recorded. Morphometric measures were taken with a digital calliper to the nearest mm and soft rays of all fins were counted with the help of a stereo microscope observed under a reflected light. A small tissue sample of anal fin was taken and stored in 96% ethanol for subsequent molecular taxonomy analysis. After laboratory analysis, the specimen was stored in the Ichthyological collection at the Institute of Oceanography and Fisheries (Split, Croatia) under the catalogue number IOR-Remora32023.

DNA extraction, amplification and analysis

Genomic DNA was extracted using a DNeasy 96 Tissue Kit (Qiagen) according to the manufacturer's instructions. The cytochrome oxidase subunit I (*COI*) gene segment was amplified using the primer pair FishF1/FishR1 (Ward *et al*., 2005). PCR was performed in 25 µL volume according to the preparation conditions of QIAGEN HotStart Taq (Qiagen, Germany) with conditions as follows: 35 cycles of 94 °C for 45 s, 54 °C for 45 s and 72 °C for 1 min, with the last extension lasting 10 min at 72 °C. PCR products were visualised on a 1% agarose gel using a UV transilluminator. Product purification and sequencing was performed by Macrogen Inc (Amsterdam, The Netherlands) on an ABI 3730 automated sequencer. The quality of the chromatograms was assessed with the programme BioEdit 7.2 (Hall *et al*., 2011). The Blast Local Alignment Search Tool (NCBI, available online) was used to compare the sequence obtained with other known vertebrate sequences and with sequences deposited in the Barcode of Life Database (BOLD; www.boldsystems.org). Using the ClustalW tool in MEGA X software (Kumar *et al*., 2018), the DNA sequence was also compared with the *COI* sequences of other known Remora species available in the database BOLD, i.e., *R. brachyptera*. The phylogenetic tree was also constructed with MEGA 7 using the HKY+G model and the Maximum likelihood method. The branches were validated by bootstrap analysis from 1000 replicates. The *COI* sequence obtained was deposited in GenBank (Accession Number: PP346364).

Results

The specimen was found as a solitary individual in the trawl catch, not attached to a host fish. Also, billfishes on which this species is frequently found attached to were not collected in the same catch. All morphometric and meristic measurements of the specimen are presented in Table 1. Total length (TL) of the caught specimen was 17.3 cm, while the fish weighed 21.7 g (Fig 1.). Species identification was confirmed both morphologically and by genetic barcoding. Description of the specimen was as follows: body is elongated, anteriorly broader; scales cycloid and very small, tightly clustered all over the body; scales absent under fleshy part of the sucking disc; head dorsally flattened; eyes relatively small; lower jaw rounded anteriorly and protruding; numerous caniniform teeth in the lower jaw, organized in several rows anteriorly reducing to one row on the sides with larger teeth on the outer margins; teeth in the upper jaw also caniniform appearing more scattered and rarer than in the lower jaw; teeth also present on the vomer; on the outer edge of upper jaw, a row of dense, comb-like projections visible (with second row overlapping first on the sides of upper lip); posterior margin of pectoral fins rounded; dorsal and anal fins similar in shape, with the dorsal fin originating slightly before the anal fin; caudal fin slightly concave; colour of the body is dark brown; in the fresh specimen, sucking disc, lower jaw, branchiostegal region and base of the ventral fins were void of pigmentation and were bloodshot, turning white after preservation in ethanol. Sucking disc with two rows of lamellae each containing two rows of comb-like projections (Fig 2a). Between lamellae with projections, a smaller and shorter lamella is present (Fig 2b). It should be noted that counting soft rays

Fig. 1: Specimen of *Remora brachyptera* from the Adriatic Sea photographed a couple of hours after the catch.

Table 1. Morphometric measurements (in mm) and meristic counts of *R. brachyptera* from the Adriatic Sea with comparisons of measurements and counts from other studies. Values outside ranges of previous studies are marked with *.

Morphometric character	This study	Percent in SL	Myoung *et al.* (2015) (% in SL)	Paulin & Habib (1982) (% in SL)	Hatooka & Kai (2013)	Bañón *et al.* (2017) (% in SL)	Anislado-Tolentino *et al.* (2022) (% in SL)
Total length (TL)	173	123.6	-	-	-	141	210.6
Standard length (SL)	140	100.0	180.2–209.0	120–244	-	121	182.6
Head length (HL)	34	24.3*	26.4–27.7	25.4–27.7	-	24.8	27.6
Predorsal length	86	61.4	56.1–62.8	52.4–60.8	-	56.2	58.5
Preanal length	90	64.3	-	-	-	60.3	62.1
Pre-pectoral distance	38	27.1	-	-	-	-	-
Pre-ventral distance	40	28.6	-	-	-	-	-
Dorsal fin length	56	40.0	38.4–39.7	32.5–42.4	-	35.5	37
Anal fin length	37	26.4*	31.5–33.4	-	-	31.4	32.6
Pectoral fin length	21	15.0*	11.6–14.2	-	-	14.9	14
Ventral fin length	19	13.6*	9.8–12.6	-	-	12.4	12.1
Caudal fin length	29	20.7*	19.2–20.2	-	-	-	19
High body depth	20	14.3	15.7–17.4	13.9–17.1	-	11.6	13.7
Low body depth	9	6.4	-	4.5–7.1	-	-	6.5
Sucking disc width	24	17.1	14.9–15.5	15.2–19.2	-	12.4	16.9
Sucking disc length	45	32.1*	29.3–31.1	29.4–33.1	-	28.9	32.6
Orbit diameter	4.8	3.4	3.4–4.2	3.2–4.1	-	4.1	3.2
Interorbital width	21	15	15.4–16.2	14.3–18	-	11.6	-
Post-orbital length	15	10.7	-	-	-	9.9	-
Pre-orbital length	14	10	-	-	-	10.7	-
Meristic character							
Dorsal fin soft rays	27		30	-	27–37	28	27
Anal fin soft rays	23		25–27	-	22–34	23	25
Pectoral fin soft rays	27		25	-	23–28	22	25
Pelvic fin rays	I, 5		I, 5	-	-	I,5	I, 5
Caudal fin soft rays	16		-	-	-	-	-
Sucking disc lamellae	16		16	-	-	16	17

in all fins was challenging due to the thick fin membrane and skin on the fin base, which made distinction among individual rays difficult.

To avoid possible confusion and verify the identity of the specimen, sequencing of the cytochrome oxidase subunit fragment (*COI*) confirmed that the specimen indeed belonged to *Remora brachyptera* (*R. brachyptera*, Ass. No. PP346364). Namely, the 525 bp long mtDNA *COI* fragments of the studied specimen produced significant alignments with sequences of *R. brachyptera* from the BOLD and the GenBank database with 99% similarity. Moreover, phylogenetic reconstruction (Fig. 3) clearly aggregated our specimen with sequences of *R. brachyptera* in a separate clade.

Discussion

Data presented in this paper for the first time confirm the presence of *Remora brachyptera* in Mediterranean waters. According to Kovačić *et al*. (2021) this species has been previously mentioned as a member of Mediterranean ichthyofauna, most notably in the work of Lachner (1973), on which subsequent checklists were probably based. However, in some previous works that provided information about the distribution of this species, such as Fowler (1936), the Mediterranean region was not mentioned in its distribution range, despite the mention of specimens from the Atlantic region. Additionally, Lachner (1986) identifies the Alboran and Balearic Seas as part of the species' distributional range in the Mediterranean, although without providing detailed indications of the records.

Morphological description and meristics of the studied specimen agree with species descriptions provided by other authors (i.e., Lachner, 1986; Myoung *et al*., 2015; Bañón *et al*., 2017). However, it should be noted that our specimen did slightly differ in some morphometric characters, specifically in relative proportions of certain characters, versus those determined by other authors

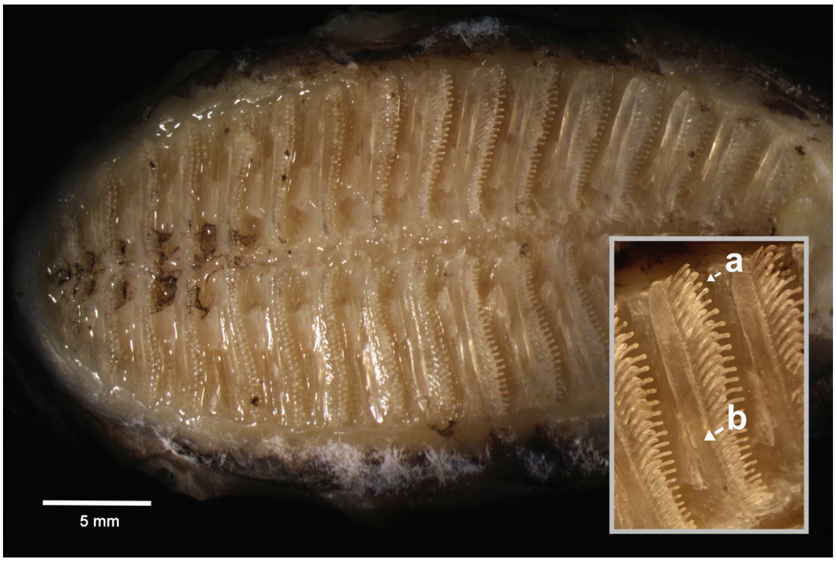

Fig. 2: Sucking disc of *Remora brachyptera*. Close-up photo shows projections on lamellae (a) and smaller lamellar structure between attachment lamellae (b).

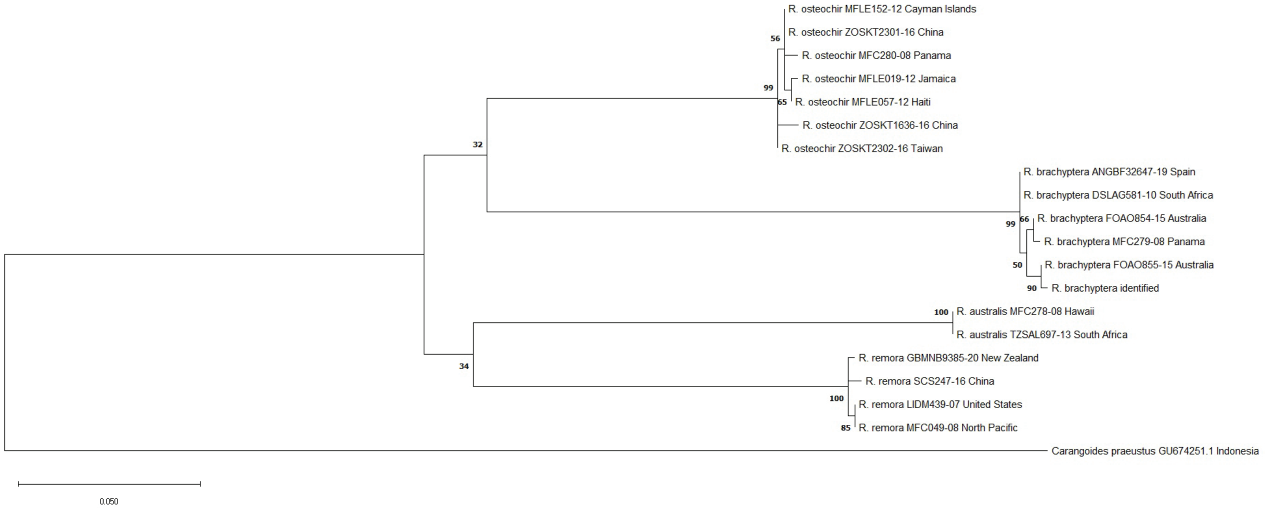

Fig. 3: Maximum likelihood (ML) phylogenetic tree of the genus *Remora* inferred from the cytochrome I (*COI*) region. Sequences from the BOLD database are included for *R. osteochir*, *R. brachyptera*, *R. australis* and *R. remora*, and are represented by different haplotypes. The percentage of trees in which the associated taxa were clustered together is shown next to the branches after 10,000 replicates.

(Table 1). These differences can be expected given that morphological data are based on a small number of analysed specimens. Additionally, a note should be made regarding the colour variations of this species, namely, among documented colorations of analysed specimens, both all white or whitish coloration was observed as well as totally brown specimens similar to the one observed in the present study. For example, in Bañón *et al*., (2017) the colour of the body appeared brown and very similar to that of our specimen, while Anislado-Tolentino *et al*., (2022) reported a white-coloured specimen, and Myoung *et al*, (2015) reported both brown and white specimens. Such notable differences in body coloration raise questions about colour variability across its distribution range or whether some short-term adaptive mechanisms influence coloration of the specimens (i.e., host colour-dependent coloration).

Adriatic waters being a confirmed area for the presence of *Remora brachyptera* in the Mediterranean can imply that the species was previously overlooked in other Mediterranean areas as has already been suggested for various fish species in the Mediterranean and other areas (i.e., Wright *et al*., 2016; Doumpas *et al*, 2020; Tiralongo *et al*., 2020). The appearance of a new species of remora in the Adriatic Sea contributes to the recent trend of alterations in the fish fauna of this area. These alterations have been marked by significant changes due to various processes, including anthropogenic introductions, increased research efforts and climate change. This is evidenced by the emergence of alien and neonative species, species new to science, as well as some typically rare species (Dulčić & Dragičević, 2023). Remoras are typically associated with warmer waters (Wright *et al*., 2016), indicating that the shifting sea conditions in the Adriatic and Mediterranean Seas (Grbec *et al*., 2018) may offer increasingly favourable habitats for their presence. Additionally, in recent years, many new species in the Adriatic waters were detected thanks to efforts focused on citizen science and this present record could have easily gone unnoticed had it not been reported by fisherman who was aware of the importance of reporting unusual species. In conclusion, this record and the recent record of *Remora osteochir* in Adriatic waters (Dulčić *et al*., 2021) provide new insights into the distributional extensions of Echeneidae species, contributing valuable data to our understanding of their geographic range.

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