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# A Stranded *Mola alexandrini* (Ranzani, 1839) from the North-Western Adriatic Sea: Integrating Morphological and Molecular Evidence with a Preliminary Morphometric Framework

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**Abstract**

Recent taxonomic revisions within the family Molidae have clarified the validity and diagnostic characters of the bump-head sunfish *Mola alexandrini* (Ranzani, 1839), a species historically confused with *Mola mola*. Despite its circumglobal distribution, confirmed records of *M. alexandrini* in the Mediterranean Sea remain limited, particularly in the Adriatic Sea. Here, we report the occurrence of an adult female *M. alexandrini* stranded along the north-western Adriatic coast (Ravenna, Italy) in March 2026. Species identification was assessed through an integrative approach combining external morphology, morphometric measurements, and mitochondrial COI sequencing. The specimen exhibited diagnostic morphological traits consistent with *M. alexandrini*, including a rounded clavus lacking lobes and back-fold, a deep laterally compressed body, and characteristic coloration patterns. Phylogenetic analysis based on COI sequences clustered the specimen within the *M. alexandrini* clade, clearly separating it from *M. mola*. This finding provides further evidence of the occurrence of *M. alexandrini* in the Adriatic Sea and highlights the importance of combining molecular and morphological approaches to resolve taxonomic uncertainty within the genus *Mola*. The study also highlights that historical misidentification may have contributed to an underestimation of the occurrence of *M. alexandrini* in the Mediterranean basin.

**Keywords:** bump-head sunfish; Mediterranean Sea; COI barcoding; molecular taxonomy; morphometric analysis; integrative specimen identification



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## 1. Introduction

In recent years, taxonomic revisions based on both morphological and molecular evidence have substantially clarified the systematics of the genus *Mola* Koelreuter, 1766 [1–3]. Historically, the taxonomy of the family Molidae was first comprehensively reviewed by

Fraser-Brunner [4], who re-examined all nominal taxa and recognized a limited number of valid forms, including the ocean sunfish *Mola mola* (Linnaeus, 1758) and *M. ramsayi* (Giglioli, 1883). This classification remained widely accepted for decades and was later largely retained by subsequent works. In his comprehensive revision of the family Molidae, Parenti [5] reviewed 19 nominal genera and 57 nominal species, of which only four were recognized as valid species.

More recently, integrative taxonomic approaches have led to significant advancements. Nyegaard et al. [6], combining morphological and molecular data, confirmed the presence of three distinct species within the genus *Mola*: *M. mola*, *M. ramsayi*, and the newly described hoodwinker sunfish *M. tecta*, representing the first discovery of a new *Mola* species in over a century. Subsequently, Sawai et al. [1] revised this framework by rejecting the validity of *M. ramsayi* and resurrecting the bump-head sunfish *M. alexandrini* (Ranzani 1839), previously considered a junior synonym of *M. mola* [4], treating it as the senior synonym of *M. ramsayi*. This revalidation was based on the re-examination of a rediscovered dried holotype, along with additional fresh and preserved material, and was accompanied by the designation of a neotype for *M. mola* to resolve longstanding taxonomic ambiguity. The validity of *M. alexandrini* was later questioned by Britz [7], who identified inconsistencies between morphometric data, published images, and the original illustration by Ranzani [8]. However, Sawai and Nyegaard [9] subsequently addressed these issues and confirmed the species as valid. Accordingly, the genus *Mola* is currently considered to include three valid species: *M. mola*, *M. alexandrini*, and *M. tecta* [1,6,9,10].

Within this updated framework, *M. alexandrini*, originally described from the Adriatic Sea by Ranzani [8], had long been overlooked in the Mediterranean due to its historical synonymy with *M. mola*. Only recent systematic studies have re-established its validity, leading to a reassessment of the diversity and distribution of ocean sunfishes in the Mediterranean basin. Despite its circumglobal distribution in temperate and tropical waters, *M. alexandrini* remains relatively poorly documented in the Mediterranean Sea, where only a limited number of confirmed records are available. This scarcity is even more pronounced in the Adriatic Sea, where the species has been only occasionally reported and remains largely under-documented. Within the Adriatic Sea, the family Molidae is represented by the ocean sunfish and the bump-head sunfish, as well as by the slender sunfish *Ranzania laevis* (Pennant, 1776), whose seasonal occurrence and ecology differ significantly from those of *Mola* spp. [11,12]. The apparent rarity of this species may be partly attributable to historical misidentifications, as several specimens previously classified as *Mola mola* could actually correspond to the bump-head sunfish. This species is distinguished by a deep, laterally compressed body, a rounded clavus lacking lobes and back-fold, and opposite spineless dorsal and anal fins, whereas reliable identification primarily depends on clavus morphology and associated meristic characters. Fresh individuals generally display a dark gray dorsal coloration, lighter ventral surfaces, and irregular pale patches distributed across the body, particularly near the clavus [13,14].

In this context, the integration of multiple lines of evidence has become essential for achieving reliable specimen identification within the genus *Mola*. Furthermore, the classification has important implications for biodiversity assessments, biogeographic reconstructions, and conservation strategies, as accurate species assignment underpins reliable ecological data and effective management policies. In this study, we report the finding of a stranded ocean sunfish along the north-western Adriatic coast, an area where records of *M. alexandrini* remain scarce. Given the taxonomic complexity of the genus and the potential for historical misidentifications, we applied an integrative approach combining molecular analyses, morphometric characterization, and necropsy data to accurately determine its species identity, thereby contributing to a better understanding of the occurrence and

distribution of *Mola* species in the Adriatic Sea, and, consequently, in the Mediterranean basin.

## 2. Materials and Methods

On 10 March 2026, the local Coast Guard of Ravenna reported a citizen sighting of a dead stranded ocean sunfish specimen (Figure 1A–D) on the beach of Marina di Ravenna, within the Ravenna municipality, Italy, in the northwestern Adriatic Sea (Geographical Sub-Area-GSA 17; geographic coordinates: 44.486254 N, 12.285843 E). Researchers from "Centro Sperimentale per la Tutela degli Habitat" (CESTHA) conducted a field inspection to document the event, including photographic records, morphometric measurements, and muscle tissue sampling. In coordination with the Istituto Zooprofilattico Sperimentale dell'Emilia-Romagna e della Lombardia, the Department of Comparative Biomedicine and Food Science (BCA) of the University of Padua was involved in the recovery of the carcass for further post-mortem investigations at their facilities. The specimen was recovered on the day of stranding (10 March 2026), transported to the necropsy facility, and stored in a cold room at 4 °C until the post-mortem examination, which was performed on the morning of 11 March 2026.


**Figure 1.** Stranded specimen of *Mola* sp. ((**A**)—ph. Andrea Fiumana) Close-up of the head and snout region showing the anterior body profile; ((**B**)—ph. Guido Pietroluongo) specimen during necropsy examination; ((**C**)—ph. Diletta Pasini) aerial view of the entire specimen obtained by drone; ((**D**)—ph. Andrea Fiumana) lateral view of the specimen during the transport operation.

Morphometric measurements were obtained directly from the specimen, and additional observations were derived from high-resolution photographs. Photographic materials were recorded using a Fujifilm GFX100S II (Fujifilm corporation, Tokyo, Japan) equipped with a 55 mm f1.7 telephoto lens, a Sony a7IV (Sony Corporation, Tokyo, Japan) equipped with a 28–105 mm f2.8 and a DJI Air 2S drone (SZ DJI Technology Co., Ltd., Shenzhen, China). Sex was determined during the necropsy through macroscopic assessment of gonadal development. Morphometric measurements were collected according to the definitions reported for *Mola* spp. by Jawad [15] and Phillips et al. [16]. Based on these previous studies, a set of 12 primary morphometric measurements was selected, operationally defined, and recorded. The measurement definitions proposed for each variable are reported in Table 1, while their anatomical landmarks and measurement positions are schematically illustrated in Figure 2. These measurements are herein proposed as the basis for a preliminary morphometric framework for species of the genus *Mola*, for which no broadly adopted measurement protocol is currently available. The establishment of a framework is intended to facilitate consistent specimen characterization and improve the comparability of data derived from necropsies, stranding events, fisheries bycatch records, and research collections. Such standardization may also support future taxonomic, morphological, and comparative studies across the geographic range of the genus, particularly in light of the growing international scientific interest in this taxon.

**Table 1.** Preliminary morphometric measurements proposed for species of the genus *Mola*, including measurement definitions and values recorded for the examined specimen. The proposed set of measurements is intended to provide a consistent framework for morphological characterization and comparison among species of the genus *Mola*.

| | Measurement Definition | Measurement (cm) |
|---|---|---|
| **1** | TL: Total Length: horizontal distance from tip of snout/rostrum to posterior edge of clavus | 237 |
| **2** | FW: Fin Width: vertical distance from dorsal fin tip to anal fin tip | 277 |
| **3** | BW: Body Width: vertical distance at the widest points of the body margin | 142 |
| **4** | DFH: Dorsal Fin Height: vertical distance from the base to the tip of the dorsal fin | 82 |
| **5** | DFBW: Dorsal Fin Base Width: horizontal distance between the anterior and posterior insertions of the dorsal fin base | 61 |
| **6** | AFH: Anal Fin Height: vertical distance from the base to the tip of the anal fin | 82 |
| **7** | AFBW: Anal Fin Base Width: horizontal distance between the anterior and posterior insertions of the anal fin base | 53 |
| **8** | PFL: Pectoral Fin Length: distance from the pectoral fin insertion to the distal tip of the fin | 35 |
| **9** | PFW: Pectoral Fin Width: maximum width of the pectoral fin measured perpendicular to its longest axis | 28 |
| **10** | CW: Clavus Width: maximum horizontal distance between the lateral margins of the clavus | 42 |
| **11** | ED: Eye Diameter: distance between the anterior and posterior edges of the orbit | 7 |
| **12** | MW: Mouth Width: horizontal distance between the lateral corners of the closed mouth | 16 |

Total genomic DNA was isolated from approximately 20 mg of muscle tissue obtained from this single specimen, using an RBC Bioscience extraction kit (RBC Bioscience Corp., New Taipei City, Taiwan) according to the manufacturer's instructions. A 652 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene from this individual was amplified using the universal primers FishF2 and FishR2 [17]. PCR amplification was performed in a final volume of 25 µL, containing 2.5 µL of genomic DNA template derived from the specimen, 5× PCR buffer, 2.5 mM $MgCl_2$, 0.05 mM dNTPs, 0.1 µM of each primer, and 0.25 U of GoTaq G2 Flexi DNA polymerase (Promega Corporation, Madison, WI, USA).

Thermal cycling was carried out on a TAdvanced Biometra thermocycler, with an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 1 min at 72 °C, and a final extension of 7 min at 72 °C. Amplification success was verified by electrophoresis on a 2% agarose gel. The resulting PCR product from the single specimen was purified using EXOSAP and sequenced in both directions by an external provider (Macrogen Europe B.V., Milan, Italy).

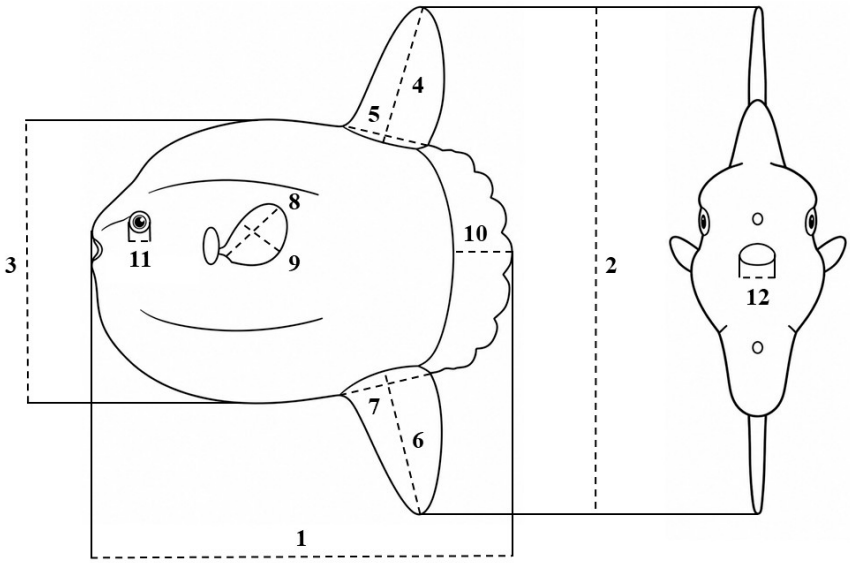


**Figure 2.** Proposed preliminary morphometric measurements for *Mola* spp. Left lateral (**left**) and cranial (**right**) views illustrating the twelve external measurements proposed for specimen characterization and inter-study comparisons. The numbers correspond to the morphometric measurements, which are fully defined in Table 1. Illustration by Guido Pietroluongo.

The sequence obtained from this specimen was compared against publicly available sequences in NCBI GenBank and BOLD Systems [18] using the BLAST algorithm [19] and the BOLD Identification Engine, respectively. A Maximum Likelihood (ML) phylogenetic tree was reconstructed in MEGA 11 [20] under the Kimura 2-parameter model (K2P), using 29 reference COI sequences of *Mola* sp. and *Masturus lanceolatus* from Nyegaard et al. [6], together with 27 additional COI sequences retrieved from GenBank. Species assignments for sequences included in Nyegaard et al. [6] followed the identifications reported in their Supplementary Table S2. The nomenclature adopted in this study follows the taxonomic revision of Sawai et al. [1]. All sequences used in the phylogenetic analysis, together with their GenBank accession numbers and corresponding species assignments, are reported in Table S1. The COI sequence generated for this specimen was deposited in GenBank under accession number PZ386888.

## 3. Results

The stranded specimen was identified as an adult female *M. alexandrini* with an estimated total body weight of approximately 400 kg. External morphometric measurements collected during necropsy are reported in Table 1. The specimen exhibited the diagnostic external morphological traits attributed to *M. alexandrini* including a deep and laterally compressed body, a rounded clavus replacing the caudal fin, and opposite dorsal and anal fins lacking fin spines. Fresh coloration was dark gray dorsally and lighter ventrally, with irregular pale patches particularly evident in the clavus region.

BLAST analysis identified the obtained 652 bp COI sequence as *M. alexandrini*, with 99.84% sequence identity and 100% query coverage. The BOLD Identification Engine returned the same species as the best match, with 100% sequence similarity. The ML

phylogeny recovered well-supported species-specific clades (bootstrap support >70%; Figure 3). The sequence obtained from the stranded individual clustered within the *M. alexandrini* clade, with strong bootstrap support, and was clearly separated from the *M. mola* and *M. tecta* clades.

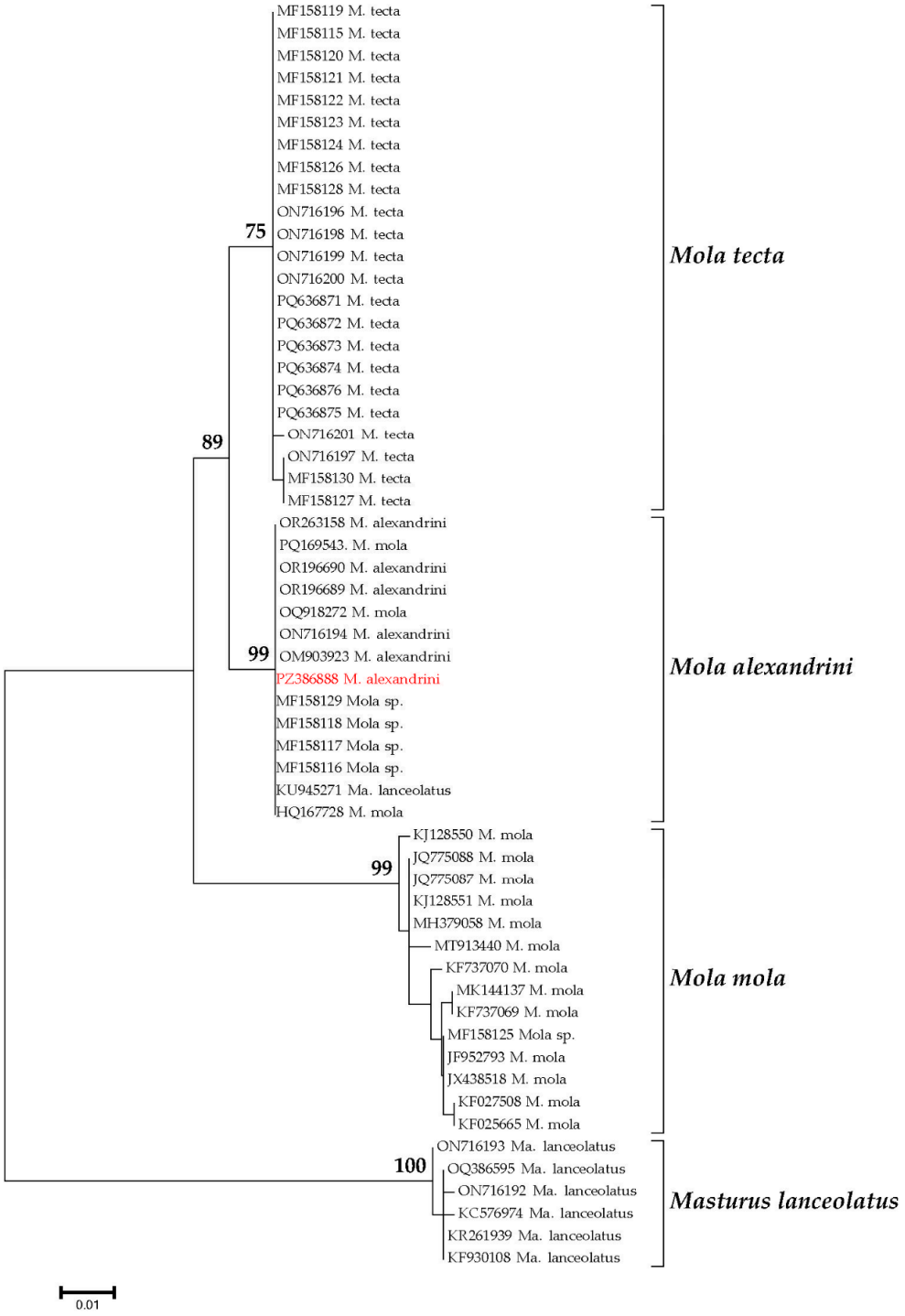


**Figure 3.** Maximum Likelihood (ML) tree of mitochondrial Cytochrome c Oxidase Subunit I (COI) gene sequences of *Mola* spp. The analysis included the COI sequence generated in this study (highlighted in red), together with reference sequences from Nyegaard et al. [6] and additional GenBank sequences (the complete list is provided in Table S1). *Masturus lanceolatus* sequences were used as the outgroup. Bootstrap support values (>70%) are shown at the nodes.

## 4. Discussion

The external morphology observed in the stranded specimen was consistent with the diagnostic characteristics reported for *M. alexandrini* [14,21]. The combination of body proportions, clavus morphology, fin configuration, and coloration allowed differentiation from the closely related *M. mola*. In particular, the rounded and unlobed clavus with marginal ossicles, together with the predominantly rectangular scales, matched descriptions previously reported for large individuals of *M. alexandrini*. Similar morphological features were also reported for the Adriatic specimen re-evaluated by Insacco et al. [13], further supporting the consistency of these characters in Mediterranean individuals of the species. Some morphological features traditionally considered useful for species recognition, such as the head bump and chin protuberance, were only weakly developed in the examined specimen. This observation agrees with previous studies indicating considerable intraspecific variability in these traits and confirms that visual identification alone may be insufficient for reliable discrimination among species of the genus *Mola* [1,6,22].

The molecular analysis further confirmed the specimen identification. The Maximum Likelihood reconstruction recovered a topology consistent with previous studies, particularly that of Nyegaard et al. [6], with the sequence generated in this study clustering within the *M. alexandrini* clade. The clear separation from the closely related M. tecta clade, as well as from the *M. mola* clade further supported the taxonomic assignment. Notably, several reference sequences retrieved from GenBank retain original annotations that differ from their current taxonomic assignment. As previously recognized by Nyegaard et al. [6], some sequences originally deposited as *Mola* sp., *M. mola*, or even *Masturus lanceolatus* were assigned to *M. alexandrini*. These discrepancies reflect historical misidentifications and the substantial taxonomic revisions of Molidae proposed by Nyegaard et al. [6] and stabilized in subsequent nomenclatural revisions [1,9].

The present specimen can be compared with the Adriatic individual re-evaluated by Insacco et al. [13], which represents one of the few confirmed records of *M. alexandrini* in the basin. Both specimens shared the main diagnostic morphological characters described for the species, including a head bump, a protruding chin, and a rounded, unlobed clavus. However, while the specimen reported by Insacco et al. [13] was a large adult male stranded in the north-western Adriatic Sea, the present individual was an adult female found stranded along the same coastline. Despite these differences in sex and body size, both findings support the occurrence of *M. alexandrini* in Adriatic waters. Nevertheless, the currently available records remain too scarce to determine whether the species represents a resident component of the Adriatic ichthyofauna or only an occasional visitor. Additional molecularly confirmed records will be necessary to clarify its status within the basin.

The present record provides additional evidence of the occurrence of *M. alexandrini* in the Adriatic Sea and supports previous observations from the region [12,13]. Confirmed records of *M. alexandrini* in the Mediterranean Sea remain scarce and geographically scattered. In the Adriatic Sea, only a few ascertained occurrences have been documented to date, including the historical specimen originally described by Ranzani [8] and the specimen re-evaluated by Insacco et al. [13]. Outside the Adriatic basin, additional confirmed records have been reported only sporadically, such as the individual documented from the northeastern Levantine Sea along the Turkish coast [23]. The apparent rarity of this species in the Mediterranean basin may partly reflect its historical synonymy with *M. mola*, which likely resulted in numerous misidentifications before the recent taxonomic revision of the genus [1,6,9]. In addition, the limited availability of specialists able to reliably distinguish species within the genus *Mola*, together with the still limited use of molecular tools in routine species identification, may have further contributed to the scarcity of confirmed records. Consequently, the actual distribution of *M. alexandrini* in the Mediterranean Sea

may be broader than currently documented, and additional integrative studies combining morphological and molecular approaches are needed to clarify its occurrence and distribution patterns. The integration of molecular analyses with standardized morphological examination therefore appears essential for accurate specimen identification when dealing with taxonomically challenging groups such as *Mola*.

Accurate recognition of *M. alexandrini* in the Mediterranean Sea represents an important prerequisite for future studies on its taxonomy, ecology, population dynamics, and conservation status. Improved species-level identification will also support more reliable assessments of distribution patterns and potential demographic risks, ultimately contributing to the development of species-specific conservation strategies. More broadly, this study reinforces the value of an integrative taxonomic approach for evolutionary and biogeographic investigations within the family Molidae.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/hydrobiology5030023/s1. Table S1: COI reference sequences included in the analysis, with their GenBank accession numbers, original GenBank annotations, species assignments reported by Nyegaard et al. [6], and the nomenclature adopted in the present study following Sawai et al. [1].

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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