Mediterranean Marine Science

Vol 26, No 1 (2025)

Mediterranean Marine Science

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doi: 10.12681/mms.38595

Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.38595

New records of the spotfin burrfish *Chilomycterus reticulatus* (Linnaeus, 1758) along the Italian coasts

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Contributing Editor: Paraskevi K. KARACHLE

Received: 07 August 2024; Accepted: 02 January 2025; Published online: 06 February 2025

Abstract

Here we report two new records of the spotfin burrfish, *Chilomycterus reticulatus*, in the Mediterranean Sea, detected through a public awareness campaign designed to inform citizens about the presence of hazardous alien fish species. The first specimen was recorded by a professional fisherman in January 2023, stranded along the coast of Lazio, central Tyrrhenian Sea, Italy. The specimen was collected and identified through morphological and molecular methods as *C. reticulatus.* Moreover, its gut content was analyzed showing mollusc fragments, some of which belonged to *Bolinus brandaris*. A second specimen of *C.* cf. *reticulatus* was observed and filmed in Sardinia on 29 June 2024 in the framework of another citizen science initiative.

Keywords: Diodontidae; citizen science; alert campaign; Mediterranean Sea; non-indigenous species; early detection.

Introduction

The Mediterranean Sea is one the most invaded marine regions in the world, with over 1,000 Non Indigenous Species (NIS) recorded so far (Galanidi *et al.*, 2023). Some of these species can be dangerous for human health with emerging risks of intoxication and injuries along the coasts (e.g., Balasubashini *et al.*, 2006; Ulman *et al.*, 2024).

More specifically, species belonging to the order Tetraodontiformes can be toxic if consumed, whilst painful stings can be caused by siganid and scorpaeniform fishes (Galil, 2018). This has raised awareness of the need to develop an early warning communication strategy (EWCS) with the aim of promulgating the rapid detection, the identification and risk assessment of introduced or undesired species, followed by the rapid alerting of the authorities with intervention capabilities (Andaloro *et al.*, 2016).

The scientific community has recognised that the public can contribute to knowledge and support research through the collection of data in citizen science initiatives (Pocock *et al*., 2023). Furthermore, the recent adoption of information and communications technology (ICT), such as internet and/or mobile application-based interfaces for citizen training and data generation, has contributed remarkably to the increase of initiatives involving citizens for monitoring Invasive Alien Species (IAS) (Johnson *et al*., 2020). In the marine environment, the involvement of its main users, i.e., fishers and divers, has proved essential for the collection of data on the presence of NIS/IAS (Mannino & Balistreri, 2018; Maggio *et al*., 2020; Tiralongo *et al*., 2020; Perzia *et al*., 2023). This is particularly relevant when toxic species are early reported, since unpleasant consequences due to their unknowing consumption can be avoided. We report here the result of collaboration with a fisherman, who enabled the recovery of a toxic fish during an alert campaign for hazardous species. The fish, collected in January 2023, was previously found only once in the Mediterranean Sea (Follesa *et al.*, 2009). Another, more recent record was provided by a citizen science initiative from Sardinia, which reported and filmed a specimen on 29th June 2024, which can be assigned to the same species.

Materials and Methods

In August 2022, a team of researchers from different Italian institutions launched an informative campaign called "Attenti a quei 4!" (Beware of those 4!) to alert citizens about the presence of four hazardous alien fish in the Italian seas: the silver-cheeked toadfish *Lagocephalus sceleratus*, the lionfish *Pterois miles*, the dusky spinefoot *Siganus luridus*, the marbled spinefoot *Siganus rivulatus*. The four species were selected because they pose a threat to human health: *L. sceleratus* is toxic to consumption, while the other three fish have poisonous spines that can cause harm when in contact. At the end of January 2023, the researchers were contacted at the campaign phone number by a professional fisherman who claimed to have found a specimen of *L. sceleratus.* The fish was stranded on the beach of Santa Marinella (42.033701° N, 11.857179° E) on the Lazio coast, near Civitavecchia Harbour, in the Central Tyrrhenian Sea, Italy. The fisherman documented the record with photos and videos from which it was immediately understood that it was a porcupine fish. After a few days the specimen was collected and transferred to the ISPRA Laboratory for subsequent morphological, morphometric and molecular analyses. It was measured with an ichthyometer to the nearest 1 mm total length (TL) or with a calliper to the lowest 0.1 mm. Meristic data were also recorded, when possible.

Molecular analysis was conducted in order to confirm/validate species classification. A small portion of fin tissue (30-50 mg) was used for total DNA extraction following the protocol of Wizard® Genomic DNA Purification Kit (Promega; www.promega.com). The target COI region was amplified using four primer sets combinations of FishF1-FishF2/FishR1-FishR2 (Ward *et al*., 2005). PCR reactions were carried out in a 25 µl reaction volume containing 14.4 µl ddH_20, 5 µl PCR buffer (5x), 2,5 µl $MgCl_2$ (25 mM), 0.5 µl dNTP mix (10mMx4) 0.25 µl of each primer (10 µM), 0.125 Taq polymerase (5 U/ml), 2 µl DNA template (10-50 ng). The thermocycling profile for all primers sets consisted of one cycle of 2 min at 94° C and 35 cycles of 30 s at 94° C, 40 s at 52° C, and 60 s at 72° C, with final exposure for 10 min at 72° C. Amplification product obtained with primers FishF1/FishR1 showed the highest quantity of DNA with neat bands on agarose gel, it was therefore used for following sequencing reactions on an ABI 3730xl automated sequencer (Applied Biosystems; www appliedbiosystems.com) using the same PCR primers for forward and reverse sequences.

Gut contents were analysed by both macroscopic observation and molecular analyses. Portions of tissue remains attached to opercula were used for barcode analysis. DNA extraction was performed on three samples separately using E.Z.N.A. Mollusc DNA kit (Omega Bio-tek Inc., Norcross, GA, USA), following the protocol provided by the manufacturer. The target COI region was amplified using two primer sets LCO1490/HCO2198 (Folmer *et al*., 1994) and jgLCO1490/jgHCO2198 (Geller *et al*., 2013). PCR reactions were carried out in a 25 µl reaction volume containing 11.05 µl ddH_20, 5 µl PCR buffer (5x), 0.25 µl BSA, 1,5 µl $MgCl_2$ (25mM), 0.5 µl dNTP mix (10mMx4) 1.25 µl of each primer (10 µM), 0.2 Taq polymerase (5 U/ml), 4 µl DNA template (10–50 ng). The thermocycling profile for both primers sets consisted of one cycle of 1 min at 94° C and 35 cycles of 30 s at 94° C, 90 s at 45° C, and 90 s at 72° C, with final extension for 5 min at 72° C. Amplification product obtained with primers LCO1490/HCO2198 showed the highest quantity of DNA with neat bands on agarose gel, and was therefore used for following sequencing reactions on an ABI 3730xl automated sequencer (Applied Biosystems; www appliedbiosystems.com) using the same PCR primers for forward and reverse sequences.

The obtained nucleotide sequences were aligned with ClustalW. Basic sequence analyses and amino acid composition were carried out with MEGA 3.1. The taxonomic identification of sequences was conducted through both the BOLD Identification System (https://www.boldsystems.org) and the Basic Local Alignment Search Tool (https://www.ncbi.nlm.nih.gov)

A second specimen was observed and filmed on 29th June 2024 in Sardinia (Torre del Pozzo, Oristano, Italy; 40.08533° N, 8.48902° E), at a depth of 4 m on rocky bottom ((https://youtu.be/FwpH0rgjrHc) by one of the Authors (C.M.) from the citizen science initiative *Subacquei per la Scienza* group (Grech *et al.,* 2023). The specimen was filmed by a GoPro HERO4 Silver equipped with 2000 Lumens Scubalamp PV22 Video Light. The video was carefully analysed to ascertain the salient characteristics useful for species identification (e.g., colour pattern, presence of spines in specific body parts, shape of spines, etc.).

Results

At the moment of the collection, the specimen found in the Lazio coast was very dehydrated since it had remained beached for several days (Fig. 1A). Based on morphometric and meristic characteristics, the individual was classified as *Chilomycterus reticulatus* (Linnaeus, 1758) according to Leis (2002; 2006). The fish measured 595 mm TL, and presented a big head and a rounded body covered by sturdy spines under skin and short fixed spines with three roots. The head (Fig. 1B) had spines with three roots and some spines with four roots (Fig. 1C). The eyes were well developed and the teeth of the upper and lower jaws were fused into a unique plaque. The colouration appeared brownish on the back with paler shades tending to pink on the head and on the caudal peduncle; sides and belly showed beige colouration. Numerous small black spots were present on the body and on the dorsal, pectoral and caudal fins. Meristic data were collected only for the dorsal and pectoral fins, since the caudal and anal fins were damaged.

Morphometric and meristic characteristics are reported in Table 1 and compared with those available in the literature for specimens collected in the Mediterranean Sea (Follesa *et al*., 2009; Zava *et al*., 2024).

The analysis of gut contents showed 9 gastropod oper-

Fig. 1: The specimen of *Chilomycterus reticulatus* stranded along the Santa Marinella beach (A), with a detail of the head (B) with spines with three roots and some spines with four roots, such as the one sampled (C).

cula bearing tissue pieces attached and shell fragments belonging to Muricidae and Pectinidae species (Fig. 2).

Following molecular analyses, a COI partial sequence of 637 bp nucleotides and 212 amino acids was obtained from the fish sample. The sequence matched with the species *C. reticulatus* with a percent identity ranging from 99.06% to 100%, similarly to the specimen collected in August 2023 in Sicilian waters (Zava *et al*., 2024).

The COI partial sequence obtained from the gut content is a transcriptable sequence of 614bp nucleotides and 204 amino acids. The sequence matched with the species *Bolinus brandaris* with a percent identity ranging from 99.02% to 100%.

The second specimen, observed alive *in situ* in June 2024, can also be attributed to *C. reticulatus* (Fig. 3A). Although the meristic characters could not be accurately evaluated, the general morphology, colour pattern (e.g., black spotted fins) and the presence of a spine on the

Fig. 2: Gut contents of *Chilomycterus reticulatus* stranded along the Santa Marinella beach (A) and opercula with tissue pieces of *Bolinus brandaris* (B).

Table 1. Morphometric and meristic data for *Chilomycterus reticulatu*s stranded along the Santa Marinella beach, compared with specimens reported in the Mediterranean Sea by Follesa *et al.,* (2009) and Zava *et al.,* (2024). SL = standard length.

	Follesa *et al*., 2009		Zava *et al*., 2024		Present study	
Morphometric data	**mm**	**%SL**	**mm**	**%SL**	**mm**	**%SL**
Total length	470		310		595	
Standard length	405		255		515	
Head length			86	33.7	162	31.46
Head depth	120	29.63	95	37.3	123	23.88
Head width			107	42.0	170	33.01
Snout length			22	8.6	83	16.12
Eye diameter (maximum)	45	11.11			58	11.26
Eye diameter (minimum)	40	9.88			33	6.41
Interorbital length			91	35.7	112	21.75
Gill opening length			37	14.5	55	10.68
Preorbital length					68	13.20
Pre-pectoral length			105	41.2	192	37.28
Pre-dorsal length			197	77.3	397	77.09
Pre-anal length	330	81.48	210	82.4	421	81.75
Body depth	180	44.44	77	30.2	120	23.30
Length of caudal peduncle	40	9.88	31	12.2	80	15.53
Dorsal fin height			40	15.7	83	16.12
Dorsal fin base length			27	10.6	35	6.80
Pectoral fin base length			40	15.7	50	9.71
Anal fin base length			18	7.1	36	6.99
Caudal fin length	75	18.52	46	18.0	90	17.48
Number of fin rays						
Dorsal	13		12		12	
Anal	12		11			
Pectoral	20		21-21		21	
Caudal	10		10			

dorsal surface of the caudal peduncle (Fig. 3B), correspond to those typical of the species (Leis, 2002; 2006). The estimated total length of the fish was about 30 cm. The fish was slowly swimming away from the observer, feeling disturbed by his presence. It had all its fins more or less abraded, especially the dorsal one which was missing some distal parts, probably due to abrasion with hard and sharp surfaces or fishing gears, or bites from other fish species.

Discussion

The stranded specimen on the Italian beach initially found and reported by a fisherman as *L. sceleratus,* turned out to be an adult of spotfin burrfish *C. reticulatus*, as revealed by morphological and molecular analyses. The available meristic data correspond with those reported in the literature (Smith & Heemstra, 2003) as well as those of the specimen described by Zava *et al.* (2024). Instead a certain variability of morphometric characters, i.e., head depth, head width, snout length and interorbital length as a percentage of the standard length, has been observed. These dissimilarities may be due to unavoidable differences in measurements in a dried sample, as the specimen of this study and that reported by Follesa *et al.* (2009), compared to those of a flaccid and thawed sample, as also assumed by Zava *et al.* (2024). In addition, the morphometric differences found between the three specimens having different sizes (470, 310, 595 mm TL) could also be related to ontogenetic changes.

The spotfin burrfish is a Diodontidae species with a

Fig. 3: The specimen of *Chilomycterus* cf. *reticulatus* observed and filmed in Torre del Pozzo (Sardinia) waters (A) with the detail of the spine, indicated by the red arrow, on the dorsal surface of the caudal peduncle (B).

circumglobal distribution (Leis, 2006), from temperate to tropical waters (Follesa *et al*., 2009). In the Mediterranean Sea, it was recorded for the first time in 2008, when an individual was found stranded on the southern Sardinian coast in Italy (Follesa *et al.,* 2009) and it is currently considered as not established in this region (Zenetos *et al*., 2010; Golani *et al*., 2021). Another individual of *C. reticulatus* has recently been found near the port of Mazara del Vallo, southwestern Sicily, Italy, representing the third confirmed record of the species in the Mediterranean Sea (Zava *et al*., 2024). Integrating the confirmed records with the sighting of the documented specimen of *C. reticulatus* in Sardinian waters, the updated Mediterranean distribution of the species highlights that it is currently present only in Italian seas. Two other records reported for the Mediterranean waters (Zava *et al*., 2024) remain anecdotal: the first, catalogued in the collection of the Swedish Museum of Natural History, concerns a specimen from Trieste (north Adriatic Sea) and dates back to the second half of the 19th century, but no details about its origin are available. The second record was reported from France in 2012, without any documentary material.

Juveniles up to about 20 cm standard length are pelagic, while adults are epibenthic, inhabiting reefs and soft bottoms at depths between 20 and 100 m, although they may occur deeper in tropics (Sommer *et al*., 1996; Leis, 2002). Eggs and juveniles of spotfin burrfish are pelagic in oceanic waters and thus their distribution is heavily influenced by currents (Sommer *et al*., 1996). The species reaches a maximum standard length of about 75 cm, though the most common sizes range between 30 and 40 cm TL (Leis, 2002; Espino *et al*., 2019). Little is known about the diet of the species: it is a predator on hard-shelled invertebrates (Follesa *et al.,* 2009) as well as on large sea urchins (Brito & Falcón, 1990). Gut contents found in the specimen from Lazio confirmed the feeding on hard-shelled invertebrates and revealed that this individual spent an unspecified amount of time in Mediterranean waters, actively feeding on local species, as revealed by tissue pieces of *Bolinus brandaris*. The current work may contribute to increase knowledge on this topic, especially regarding the food supply in the Mediterranean Sea. In fact, knowledge of feeding habits, and in general of the biology and ecology of *C. reticulatus* is still scarce, despite the wide distribution of the species.

Spotfin burrfish is not of commercial interest, nevertheless accidental captures can occur (Espino *et al*., 2019). Some countries have implemented conservation measures for the species: in Spain, this species is listed as Vulnerable in the National Catalogue of Threatened Species, and it is protected by national and regional legislation (Leis *et al*., 2015; Espino *et al*., 2019); in Cape Verde *C. reticulatus* has also been listed as Threatened since 2002 (Leis *et al*., 2015). In the Canary Islands spotfin burrfish has been considered an iconic reef species and has been included in a citizen science program project, the results of which showed a geographical range extension of the species, with longitudinal displacement from the western to the center of the Canarian archipelago in the last three decades (Espino *et al*., 2019). Range expansion of this species was also confirmed by Castro *et al*. (2021) for the Madeira Archipelago.

One reasonable hypothesis for the occurrence of *C. reticulatus* in the Central Tyrrhenian Sea is that the individual specimen could have actively entered the Mediterranean through the Strait of Gibraltar. Indeed, natural range expansion of Atlantic fishes through the Strait of Gibraltar has been documented for many other fish (Golani *et al*., 2021; Azzurro *et al*., 2022; Tiralongo *et al*., 2023) including Tetraodontidae (Virgili *et al*., 2024). Interestingly, many of these species showed recent geographical expansions along both the Atlantic and the Mediterranean coasts, a phenomenon which is usually attributed to climate warming (Azzurro, 2008; Tiralongo *et al.*, 2023). Nevertheless, we cannot exclude other human-mediated vectors, such as ship transport or aquarium release (Zenetos *et al*., 2016), though *C. reticulatus* presents some difficulties in being raised in aquaria and is not among the most popular aquarium fish (see list of species in https://www.frankbaensch.com/marine-aquarium-fish-culture/captive-bred-species-list/).

The discovery of the stranded specimen of *C. reticulatus* in the Lazio coast, along with the sighting of the Sardinia specimen, highlights the importance of informative campaigns on alien marine species and of the involvement of citizens in surveillance activities.

In conclusion, the number of documented observations of this species in recent years, following its first sighting in 2008, suggests an increasing presence of *C. reticulatus* in Mediterranean waters. This trend appears to be driven by the warming of the sea waters, considering that spotfin burrfish is a thermophilic species.

Acknowledgements

The authors are grateful to the fisherman Alberto Corvace who contacted us and provided the specimen of *C. reticulatus*. We wish to express our sincere gratitude to Professor Douglas Mark Ponton native speaker and Associate Professor of English Language, Translation, and Linguistic (Department of Political and Social Sciences, University of Catania) for his professional review of the English language. DG was funded by "ALIEM VIGIL - Actions de limitation, de gestion et de vigilance liées à l'introduction d'espèces exotiques envahissantes dans les zones de transports en Méditerranée", project financed under Axis 2 of Interreg Italia - France Maritime 2021 - 2027 (https://www.aliem-network.eu/). EA for this study was supported by the @CNR USEIt project - funded by the Italian National Research Council.

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