Acta Ichthyologica et Piscatoria 54, 2024, 157–163 | DOI 10.3897/aiep.54.121303

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ACTA ICHTHYOLOGICA ET PISCATORIA

Chilomycterus reticulatus (Actinopterygii: Tetraodontiformes: Diodontidae) in the southern Sicilian waters, central Mediterranean Sea

Bruno ZAVA[1,2], Maria CORSINI-FOKA[3], Danilo SCANNELLA[4], Gianni INSACCO[2], Alan DEIDUN[5], Valentina CROBE[6], Fausto TINTI[6]

1 *Wilderness Studi Ambientali, Palermo, Italy*
2 *Museo Civico di Storia Naturale, Comiso (Ragusa), Italy*
3 *Institute of Oceanography, Hellenic Centre for Marine Research. Hydrobiological Station of Rhodes, Rhodes, Greece*
4 *Italian Institute for Environmental Protection and Research (ISPRA), Department for the Monitoring and Protection of the Environment and for the Conservation of Biodiversity, Palermo, Italy*
5 *Oceanography Malta Research Group (OMRG), Department of Geosciences, University of Malta, Msida, Malta*
6 *Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, Italy*

https://zoobank.org/04A58C9F-015E-475E-8FC0-4E7321489406

Corresponding author: Danilo Scannella (danilo.scannella@isprambiente.it)

Academic editor: Paraskevi Karachle ♦ **Received** 20 February 2024 ♦ **Accepted** 28 May 2024 ♦ **Published** 19 July 2024

Citation: Zava B, Corsini-Foka M, Scannella D, Insacco G, Deidun A, Crobe V, Tinti F (2024) *Chilomycterus reticulatus* (Actinopterygii: Tetraodontiformes: Diodontidae) in the southern Sicilian waters, central Mediterranean Sea. Acta Ichthyologica et Piscatoria 54: 157–163. https://doi.org/10.3897/aiep.54.121303

Abstract

A recently discovered fish specimen representing the family Diodontidae has been documented in the waters off Mazara del Vallo in southwest Sicily, Italy. A detailed description of the morphological and meristic characteristics of the specimen is reported. Molecular identification was performed through sequence analysis of the Cytochrome Oxidase subunit I (COI). The specimen was identified as *Chilomycterus reticulatus* (Linnaeus, 1758) both morphologically and genetically. The finding marks the third confirmed record of *Chilomycterus reticulatus* in the Mediterranean Sea and Italian waters. The significance of the presence of this uncommon fish in the basin is briefly discussed, shedding light on its occurrence and potential implications.

Keywords

Citizen scientists, cryptogenic species, DNA barcoding, Mediterranean Sea, non-indigenous species, range expanding species, rare species, spotfin burrfish

Introduction

In the Mediterranean Sea, four distinct species, representing the family Diodontidae have been identified: the spotfin porcupinefish, *Diodon hystrix* Linnaeus, 1758; the spotbase burrfish, *Cyclichthys spilostylus* (Leis et Randall, 1982); the spotfin burrfish, *Chilomycterus reticulatus* (Linnaeus, 1758) (see Kovačić et al. 2021); and the Guinean burrfish, *Chilomycterus mauretanicus* (Le Danois, 1954) (see Evans et al. 2020). The species *Diodon hystrix* and *Chilomycterus reticulatus* are both distributed globally in warm waters areas of the Pacific, Indian, and Atlantic oceans (Froese and Pauly 2023). In the Mediterranean, the first valid record for *Diodon hystrix* dates back to 1953 from the waters of the Gulf of Taranto, Ionian Sea, Italy, central Mediterranean (Anonymous 1954; Torchio 1963). Subsequently, in 2016, the species was reported in the Balearic Islands, Spain, the

western Mediterranean (Ordines et al. 2018) and additionally, in the eastern Mediterranean, notably in 2017 off the coast of Cyprus (Kleitou et al. 2020) and in 2019 in the waters off Lebanon (Bariche et al. 2020).

To date, two records of *Chilomycterus reticulatus* have been reported in the basin. These records are based on two specimens found dried on the shoreline: one in 2008 in the south Sardinian Sea, specifically on S. Antioco's Island in the western Mediterranean (Follesa et al. 2009), and the second in March 2023 in Santa Marinella near Civitavecchia harbor in the Central Tyrrhenian Sea. The latter was reported solely in an official press release by the Italian Institute for Environmental Protection and Research (ISPRA 2023). Notably, the species was also sighted in 2012 during scuba diving in the Mediterranean waters off France, according to Casassovici and Brosens (2022). Furthermore, an additional specimen of *Chilomycterus reticulatus*, originating from Trieste in the north Adriatic Sea, Italy, and dating back to either July 27, 1858, or July 27, 1868, is listed in the collection of the Swedish Museum of Natural History (Catalogue number NMR 8907) (https://artedi.nrm.se/nrmfish/index.php).

The spotbase burrfish, *Cyclichthys spilostylus*, is distributed in the Indo–Pacific Ocean, including the Red Sea (Golani and Fricke 2018); to date, it has been recorded in the Mediterranean waters off Israel in 1992 and 2009 (Golani 2010; Golani et al. 2010), and in Turkey in 2011 (Erguden et al. 2012). Finally, the Guinean burrfish, *Chilomycterus mauretanicus*, with a tropical west African natural range, has been recorded once in the Alboran Sea, western Mediterranean, in 2008 (Garrido et al. 2014). Among the four diodontids mentioned above, only *Cyclichthys spilostylus* is considered a non-indigenous species introduced in the Mediterranean via the Suez Canal from the Red Sea (Lessepsian immigrant) (Golani 2021), while the possible pathway of arrival in the basin of the other three species is under discussion.

In the present study, the occurrence of the spotfin burrfish, *Chilomycterus reticulatus*, is documented for the third time in the whole Mediterranean Sea and in Italian waters, based on a morphological and molecular study carried out on a specimen collected during summer 2023 in the shallow waters of southwest Sicily, central Mediterranean. The possible provenance of this uncommon species in the Mediterranean is briefly discussed.

Material and methods

A strange spiny balloon-like fish was caught alive on 31 August 2023, with a fishing rod from the shore by a young amateur angler near the port of Mazara del Vallo, southwestern Sicily, Italy (37.645677°W, 012.578223°E) at about 5 m of depth on a rocky substrate, sea surface temperature approximately 27°C. Photos of the fish were shared on Facebook. One of the authors (BZ) soon contacted the angler and alerted him about possible danger if the fish was consumed. The specimen was immediately shipped (frozen) to the facilities of the Wilderness Studi Ambientali in Palermo. The sample was photographed and weighed, morphometric measurements were taken with a caliper (accuracy 0.1 mm), and the meristic data were determined. For the taxonomic identification of the sample, Leis (1986, 2001, 2002, 2006, 2016), Fischer and Bianchi (1984), and Fahay (2007) were consulted.

Samples of muscle and fins preserved in 99% ethanol were sent to the Department of Biological, Geological, and Environmental Sciences of the University of Bologna for genetic analysis. DNA was extracted using the Wizard® SV Genomic DNA Purification System by Promega, according to the manufacturer's protocol. The quality of the extracted gDNA was assessed on a 1% agarose gel electrophoresis. The mitochondrial gene Cytochrome oxidase subunit 1 (COI) was amplified and sequenced with the M13-tailed primer cocktail (COI-3; C_FishF1t1–C_FishR1t1) described by Ivanova et al. (2007). The PCR reaction was performed in a 25 µL total volume containing 3 µL of gDNA template, 1 × PCR buffer, 25 µM of $MgCl_2$, 0.5 mM dNTPs, 0.1 µM of each primer cocktail, and 0.25 U GoTaq G2 Flexi DNA polymerase (Promega). Amplification was performed in a T-gradient thermocycler (Biometra) with an initial denaturation of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C, 60 s at 72°C, and a final extension of 10 min at 72°C. The PCR product was evaluated on a 2% agarose gel.

From the NCBI database (https://www.ncbi.nlm.nih.gov), 66 available COI sequences belonging to 12 species of the family Diodontidae were retrieved. The molecular identification was performed with a neighbor-joining (NJ) method (Saitou and Nei 1987) using MEGA 11 (Tamura et al. 2021) with p-distance (Collins and Cruickshank 2013) and pairwise deletion. To estimate support for the nodes, 1000 bootstrap replicates (Felsenstein 1985) were performed.

The specimen is currently deposited at the Museo Civico di Storia Naturale di Comiso, Italy (inventory number MSNC 4918).

Results

The thawed specimen, with a total length of 310 mm and a weight of 1160 g, presented the following main characteristics: body moderately robust, head width 2.4 in standard length; almost rounded anal and caudal fins; a single (unsutured) tooth in each jaw; body covered with immovable spines (Fig. 1A, 1A-inset, 1B, 1C, 1D), with three roots under the skin (Fig. 1B-inset); one spine on the dorsal surface of caudal peduncle (Fig. 1D); 9 spines from snout to dorsal fin base; nostrils positioned in front of eyes; nostrils not closed at the top, but each having the appearance of two lips (Fig. 1C). Dorsal-fin rays 12, pectoral-fin rays 21–21, anal-fin rays 11, and caudal-fin rays 10; approximately 14 spines from lower jaw to anus (Fig. 1A-inset, B); 61 spines sparse on the whole belly. Color: greyish background with numerous small

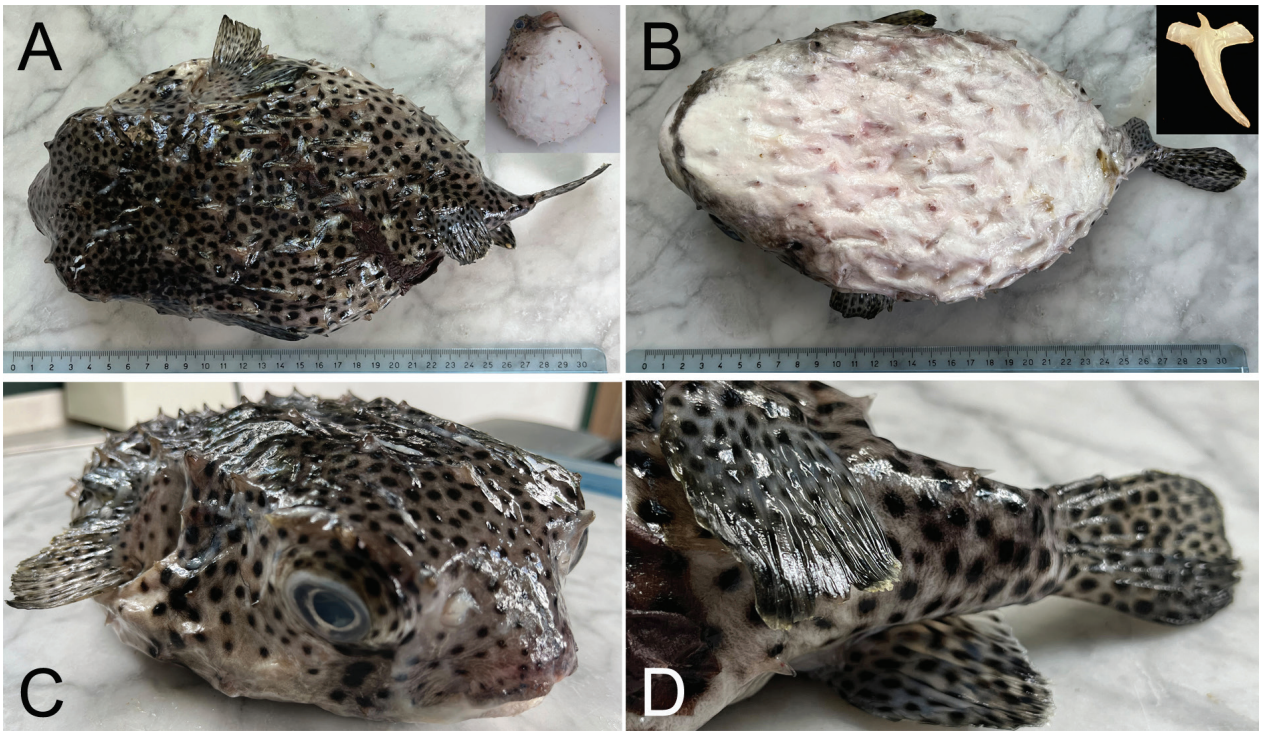

Figure 1. *Chilomycterus reticulatus* from Mazara del Vallo, Sicily, Italy. **A**: dorsal view (inset A: freshly caught specimen, inflated), **B**: ventral view (inset B: a three-rooted body spine), **C**: head profile, **D**: tail with a single spine on its upper side. (Photos A, B, C, D by G. Polizzi, inset in A by R. Figuccia).

dark spots that characteristically extend to cover the majority of the fins (Fig. 1A, 1C, 1D), whitish ventrally (Fig. 1B), a dark band on the chin, under the lower jaw (Fig. 1A-inset, D). The values of the main measurements of our specimen and their percentage of standard length are presented in Table 1.

The results of genetic analysis validated the morphological identification of the specimen as *Chilomycterus reticulatus*. In the reconstruction of the NJ tree (Fig. 2), the 652 bp COI sequence (Accession Number: PP267988) clustered within the *Chilomycterus reticulatus* clade, exhibiting robust support with a bootstrap value of 99%.

Table 1. Morphometric characters of a specimen of *Chilomycterus reticulatus* caught in Mazara del Vallo, Sicily, Italy, August 2023.

Character	Value	
	Absolute [mm]	Relative [% SL]
Total length	310	
Standard length (SL)	255	
Head length	86	33.7
Head width (HW)	107	42.0
Head depth	95	37.3
Eye diameter	20	7.8
Snout length	22	8.6
Gill opening length	37	14.5
Body depth	77	30.2
Interorbital length	91	35.7
Postorbital length	50	19.6
Pectoral fin height	41	16.1
Prepectoral length	105	41.2
Pectoral fin base length	40	15.7
Predorsal length	197	77.3
Dorsal fin base length	27	10.6
Dorsal fin height	40	15.7
Preanal length	210	82.4
Anal fin base length	18	7.1
Anal fin height	36	14.1
Precaudal length	243	95.3
Caudal peduncle length	31	12.2
Caudal peduncle depth	25	9.8
Caudal fin length	46	18.0

Discussion

The morphological and morphometric characters, as well as the coloration observed in the Diodontidae specimen from Sicily, as described above, agreed with the description of *Chilomycterus reticulatus* by Leis (1986, 2001, 2002, 2006, 2016). The accuracy of this identification was unequivocally confirmed by genetic analysis. The shape of the nostrils of our sample is characteristic of the genus *Chilomycterus* Brisout de Barneville, 1846. In particular, when comparing the main measurements of our specimen, as a percentage of the standard length, they partially differed from those reported by Follesa et al. (2009). This discrepancy is likely due to the inherent challenges of accurately comparing measurements from a dried specimen with those from a flaccid, defrosted specimen, as noted in the study. The spotfin burrfish, *Chilomycterus reticulatus*, reaches a maximum standard length of 75 cm. In its life stages, from eggs and larvae to juveniles of about 20 cm, it tends to be pelagic, often associated with floating weeds. Larger fish are benthic, inhabiting mostly rocky reefs,

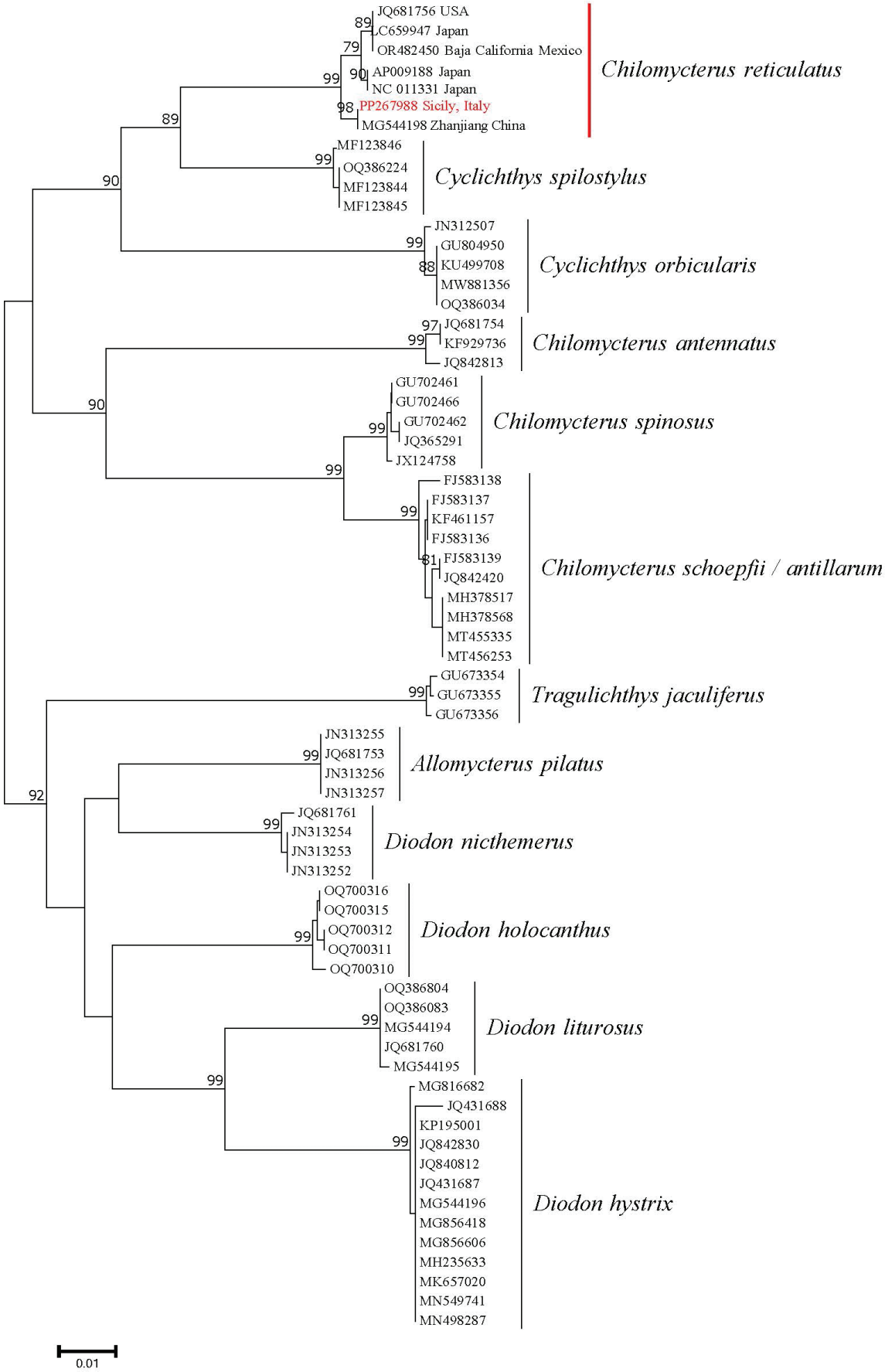

Figure 2. Neighbor-Joining tree based on COI genetic p-distances of 12 Diodontidae taxa. Numbers near nodes indicate bootstrap values (≥70%). A distance scale bar is given.

coral reefs, and sandy bottoms down to 100 m of depth or more (Leis 2016; Castro et al. 2021; Froese and Pauly 2023). Usually solitary, spotfin burrfish adopt a bracing posture against the substrate to sleep at night, while they appear to be more active during the day, feeding predominantly on hard-shelled invertebrates (Espino et al. 2019; Arndt and Evans 2022; Froese and Pauly 2023).

Spotfin burrfish are preyed upon by large carnivorous fish such as dorados, sharks, and wahoo (Snow 2024). Toxicity for *Chilomycterus* spp. has been reported (Halstead 1965) and *Chilomycterus reticulatus* is listed as a poisonous fish in Australia (Froese and Pauly 2023), posing a potential risk to human health if consumed. It is listed as edible in Japan (Noguchi and Arakawa 2008). Generally considered of no commercial value in fishery, the species has only limited interest in the ornamental fish trade, since it is suitable only for large display tanks (public aquaria or zoo) (Meerwasser-Lexikon Team 2022).

According to Leis (1986), the pelagic stage of *Chilomycterus reticulatus* is blue dorsally with dark spots also on the back and belly, while the benthic adults are grey to brown with small black spots on the upper surface and fins and a black gill band. The capture method, the color, and the size of the specimen of *Chilomycterus reticulatus* from Sicily allowed us to consider it as a benthic adult, smaller than the specimen of 470 mm total length washed up on the Sardinian beach (Follesa et al. 2009).

The presently reported finding constitutes the third confirmed record of *Chilomycterus reticulatus* in the Mediterranean and in Italian waters, after that of Follesa et al. (2009) and ISPRA (2023). These findings, with the addition of an underwater sighting from France (Casassovici and Brosens 2022), could indicate that the occurrence of the species, although rare, is no longer casual. Details on the 19th-century specimen of *Chilomycterus reticulatus* collected in the northeastern Adriatic and preserved at the Swedish Museum of Natural History were not available.

Relini and Orsi Relini (1995) emphasized that the presence of fishes representing the order Tetraodontiformes in the basin could have historical roots, possibly extending back to remote times. This suggests the plausible existence of a small native population of this diodontid in the western basin. The pelagic early life and juvenile stages of *Chilomycterus reticulatus* could facilitate its dispersion in the Mediterranean regions, aided by currents, but the cryptic habits of the adults and the probable rarity of individuals could render its observation or capture difficult. In fact, the first individual of *Chilomycterus reticulatus* was found stranded on a beach (Follesa et al. 2009), while in our case it was caught by fishing rod from the shore, demonstrating a lack of catchability by commercial fleets in the Mediterranean so far, as highlighted by Ordines et al. (2018) for the cofamilial *Diodon hystrix*.

The development of marine research, combined with the intensification of fishermen and citizen scientists' input in marine biodiversity observations, the diffused use of social media platforms, and in general the increasing speed of information exchange and dissemination due to innovative technological instruments could probably explain the recent density of records of *Diodon hystrix* in 2016, 2017, 2019 (Bariche et al. 2020) and the present one of *Chilomycterus reticulatus* in 2023 in the Mediterranean. On the other hand, the warming of Mediterranean waters linked to global climate change (Galil 2023) may favor the enhancement and development of the population of this warm-water species. The increase in sightings and spread of *Chilomycterus reticulatus* in the Canary Islands and Madeira Archipelago observed in recent decades could be associated with the ongoing seawater warming observed in both regions (Espino et al. 2019; Castro et al. 2021).

The casual finding of this species in 2008 (Follesa et al. 2009) and in 2023 (ISPRA 2023) led to the suggestion that this fish, exhibiting tropical affinities, might be a vagrant visitor or a range-expanding newcomer (or neonative), naturally expanding its distribution from the Atlantic through the Strait of Gibraltar (Psomadakis et al. 2012; Zenetos et al. 2022).The locations of first finding off southern Sardinia (Follesa et al. 2009), followed by subsequent sightings in the central Tyrrhenian Sea (ISPRA 2023), and the presently reported one in south Sicily, as well as the underwater observation in south France (Casassovici and Brosens 2022), could corroborate a possible arrival from the Atlantic and potential establishment of a small population. It is to be noted that our *Chilomycterus reticulatus* was captured from the shallow waters of southwest Sicily, located along the Sicily Strait, a hydrological, geological, biogeographical transitional zone between the western and eastern sub-basins of the Mediterranean, where range expanding species from the Atlantic and non-indigenous species from the Red Sea are detected (Azzurro et al. 2014; Deidun et al. 2021).

The Mediterranean Sea is the hot-spot region in the world for biological invasions due to the introduction of non-indigenous species (NIS), mainly through the man-made Suez Canal (Galil 2023). Although widely distributed in the Indo–Pacific oceans, *Chilomycterus reticulatus* is unreported in the Red Sea (Golani and Fricke 2018), consequently an introduction via the Suez Canal corridor appears unlikely at present.

Prudently, Evans et al. (2020) included *Chilomycterus reticulatus* among the cryptogenic fish species of the Mediterranean, denoting species that cannot be definitively classified as native or introduced (sensu Carlton 1996). The circumtropical nature of this fish, coupled with minimal genetic differentiation between populations in different regions of the world, as confirmed by the genetic analysis described in the present work, adds to the challenge of determining the potential origin of Mediterranean specimens. A similar complexity has recently been recognized for *Diodon hystrix* (see Kleitou et al. 2020).

Scientific research aimed at monitoring any increase in the Mediterranean population of this toxic warm-water fish, possibly with the support of citizen-scientist observations, is important, not only to minimize the risk to human health (Malloggi et al. 2023), but also to evaluate its

role in biological assemblages and the food web, and to assess potential impacts on Mediterranean biodiversity.

Acknowledgments

The authors warmly thank the young angler Kevin Bibiche Nour, who captured the *Chilomycterus reticulatus* reported in this paper, and Roberto Figuccia for sharing the first photos of the fish and providing the authors with the specimen and information on its collection. They also acknowledge the support of Marcello Bascone (Mazara del Vallo) and Giovanna Polizzi (Wilderness Studi Ambientali, Palermo). They are grateful to anonymous reviewers for useful and constructive comments and suggestions on the first version of the manuscript.

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