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Acanthocybium solandri (Actinopterygii, Scombriformes, Scombridae) first record from Malta with notes on using its parasites as biological tags

Alan DEIDUN[1], Maria CORSINI-FOKA[2], Alessio MARRONE[1], Gianni INSACCO[3], Jean Paul SCHEMBRI[4], Arnold SCIBERRAS[5], Mario SANTORO[6], Flavia OCCHIBOVE[6], Antonio DI NATALE[7], Bruno ZAVA[3,8]

1 *Department of Geosciences, University of Malta, Msida, Malta*
2 *Institute of Oceanography, Hellenic Centre for Marine Research, Hydrobiological Station of Rhodes, Rhodes, Greece*
3 *Museo Civico di Storia Naturale di Comiso, Comiso (Ragusa), Italy*
4 *Department of Fisheries and Aquaculture, Marsa, Malta*
5 *The Exterminator Ltd., Naxxar, Malta*
6 *Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy*
7 *Aquastudio Research Institute, Messina, Italy*
8 *Wilderness studi ambientali, Palermo, Italy*

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Corresponding author: Maria Corsini-Foka (mcorsini@hcmr.gr)

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Abstract

The wahoo, *Acanthocybium solandri* (Cuvier, 1832), an uncommon scombrid species for the Mediterranean, is hereby reported for the first time from Maltese waters. Details of this unusual capture, and characteristics of the specimens recovered are provided, as well as a brief discussion on dynamics of its introduction in the Mediterranean Sea, and its distribution in this area. In addition, two individuals of the parasitic nematode *Anisakis pegreffii* Campana-Rouget et Biocca, 1955 (Anisakidae) were found from the stomach of one of the specimens.

Keywords

Anisakis pegreffii, Mediterranean Sea, new introduction, uncommon species record, wahoo

Introduction

The scombroid *Acanthocybium solandri* (Cuvier, 1832), commonly known as wahoo (FAO code WAH), is a species circumglobally distributed in tropical and subtropical areas. Its occurrence in the Mediterranean Sea is based on few occasional records, of which the first goes back to a specimen collected in the tuna trap of Solanto near Palermo (Sicily, Italy). This individual was initially described as a new species, namely *Cybium verany*, by Doderlein (1872), but later ascertained as *A. solandri* Cuv. (cf. Doderlein 1881; Tortonese 1949). The origin of this individual, preserved at the Museo di Zoologia of the University of Palermo (catalogue number P 363) (Di Palma 1979; Sarà and Sarà 1990) is still unknown. It likely entered the Mediterranean through the Strait of Gibraltar, if coming from the Atlantic, while

it seems unlikely that it arrived through the then recently opened (1869) Suez Canal. The species was later recorded in Sicily after more than a 100-year period in the Strait of Messina, in 1990 (Costa 2012) and in 2004 (Romeo et al. 2005). More recently, wahoo occurrences were reported in Libyan waters (Elbaraasi et al. 2019), as well as in the Levantine Sea, where it was recorded for the first time in Lebanese waters (Fatfat et al. 2024) and off Iskenderun and Antalya, Turkey (Gökoğlu et al. 2024).

In the presently reported study, two new occurrences of *A. solandri*, an uncommon species for the Mediterranean, are reported from Maltese waters, complemented by morphometric measurements and meristic characters, together with a parasitological investigation.

Materials and methods

The first specimen (A) of *Acanthocybium solandri* was caught on 16 October 2024 by a professional fisherman off the island of Malta (35.2503°N, 13.5846°E, depth 518 m) (Fig. 1), using a Fishing Aggregated Device (FAD) deployed for the capture of *Coryphaena hippurus* (Linnaeus, 1758) (Maltese common name "lampuki, lampuka") and subsequently encircled at the surface with a boat seine net. A second specimen (B) was captured on 15 November 2024, at an approximate location of 42 nautical miles SW off the coast of Malta (approximate depth 350 m), through trolling baited with artificial lure stuck to a feather; this was caught close to the surface, next to "lampuki" FADs, as the first specimen. A few weeks earlier, fishermen reported sightings of 5–6 similar fish, swimming close to the surface.

The specimens collected were brought to the laboratory, photographed, measured, and weighed. Identification and measurements were carried out following Collette and Nauen (1983), Collette (2016), and Collette and Graves (2019). Fahay (2007) was also consulted. Sex and gonadal maturity were determined at the dissection by macroscopic observation.

During dissection, viscera of both individuals were examined for metazoan parasites following the methods described in Santoro et al. (2023). Briefly, each organ (gills, heart, liver, gonads, stomach, and intestine) was cut and the surfaces examined under a dissecting microscope. Then, each organ was individually washed in a basin, and the washed-out material examined under a dissecting microscope. When present, parasites were collected, counted, and preserved in 70% ethanol.

The anterior extremity of nematode larvae was cut and clarified in Amman's lactophenol for a tentative morphological identification using a compound microscope, according to available identification keys, while a portion of the middle body tract was used for molecular analysis.

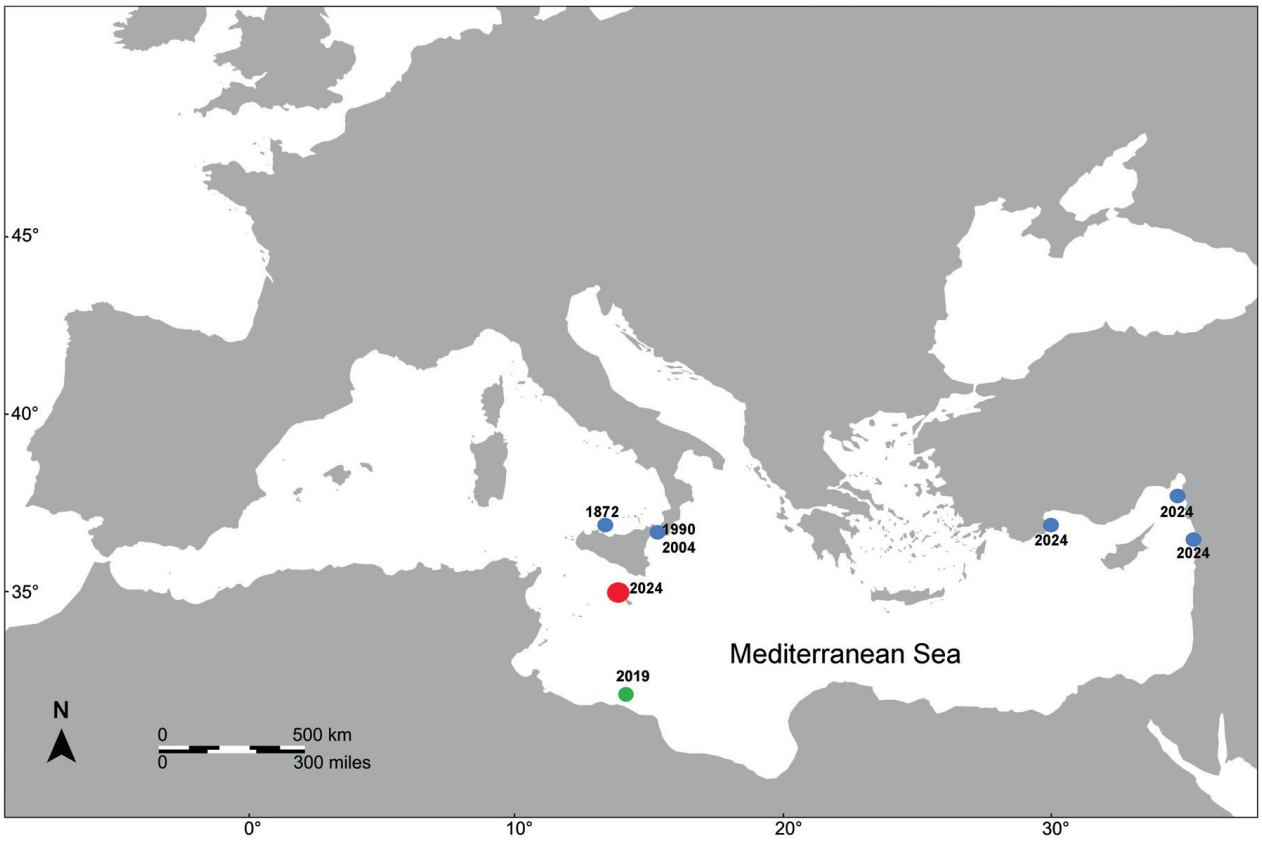

Figure 1. Locations of records of *Acanthocybium solandri* in the Mediterranean Sea. [blue circles, previous records with defined geographical location: off Solanto, Palermo, Sicily, Italy, 1872 (Doderlein 1872); Strait of Messina, Italy, 1990 (Costa 2012) and 2004 (Romeo et al. 2005); Antalya and Iskenderun, Turkey, 2024 (Gökoğlu et al. 2024); Al-Arida, Lebanon, 2024 (Fatfat et al. 2024); green circle, previous record without defined geographical location: Libya (Elbaraasi et al. 2019); red circle, presently reported study: Malta].

Genomic DNA of larvae was extracted using Quick-gDNA Miniprep Kit (Zymo Research, USA) following the standard manufacturer-recommended protocol, with the exception of the addition of a grind buffer to the Proteinase K (Thermo Scientific, USA) in the digestion step (incubation for 2 h at 56°C).

The mitochondrial cytochrome *c* oxidase subunit II gene (mtDNA *cox2*) was amplified using the primers 211F (5′-TTTTCTAGTTATATAGATTGRTTTYAT-3′) and 210R (5′-CACCAACTCTTAAAATTATC-3′) (Nadler and Hudspeth 2000). Polymerase chain reactions (PCRs) were carried out in a 25 µL volume containing 0.5 µL of each primer 10 µM, 3 µL of MgCl2 25 mM (Promega, USA), 5 µL of 5× buffer (Promega), 0.5 µL of DMSO 0.3 mM, 0.5 µL of dNTPs 10 mM (Promega), 0.3 µL of Go-Taq Polymerase (5 U/µL) (Promega), and 2 µL of total DNA template, adding ultrapure water to reach the final volume. PCR time-temperature conditions were the following: 94°C for 3 min (initial denaturation), followed by 34 cycles at 94°C for 30 s (denaturation), 46°C for 60 s (annealing), 72°C for 90 s (extension), followed by post-amplification at 72°C for 10 min. PCR was carried out according to the procedures provided by Timi et al. (2014).

All successful PCR products were purified using Agencourt AMPure XP (Beckman Coulter, USA), following the standard manufacturer-recommended protocol. Clean PCR products were submitted to Sanger sequencing from both strands, utilizing the above primers, through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, USA) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA). The obtained contiguous sequences were assembled and edited using UGENE v51.0 (Okonechnikov et al. 2012). Sequence identity was checked using BLASTn (Morgulis et al. 2008).

Results

Both specimens of *Acanthocybium solandri* were immature males; specimen A and B measured 57.8 cm and 61.2 cm of total length and weighed 1227 g and 1043 g, respectively. They presented the following main features (Fig. 2A, B): body elongate, fusiform and only slightly compressed; mouth large with strong, triangular teeth set in a single series; pointed snout about as long as the rest of head; gillrakers absent; posterior part of maxilla concealed under preorbital bone; lateral line single, curving downward under first dorsal fin; body covered with small scales; no anterior corselet developed; caudal peduncle slender, with a well-defined lateral keel between the two small ones on each side; swim bladder present. Meristic counts, respectively for specimen A and B: first dorsal fin XXVI and XXV; second dorsal fin 11 and 11, followed by 9 finlets; anal fin 12 and 11, followed by 9 finlets; pectoral fin 21 and 23; ventral fin I + 5 and I + 5; a bifid inter-pelvic process; caudal fin approximately 50 rays. Main measurements and their proportions expressed as % of the total length are given in Table 1. Color after death: back bluish dark; snout silvery dark; sides silvery with more than 20 dark grey vertical bars which extend lower the lateral line, some doubled or y shaped. Two dorsal fins, pectoral, ventral and anal fins dark, caudal fin dark with whitish lobes' tips.

Figure 2. Specimens of *Acanthocybium solandri* caught off Malta on 16 October 2024 (**A**) and on 15 November 2024 (**B**). Scale bars: 20 cm.

Table 1. Selected biometric characters (cm) of the two specimens of *Acanthocybium solandri* caught off Malta described in this study.

Character	Specimen A		Specimen B	
	Value		Value	
	Absolute [cm]	Relative [% TL]	Absolute [cm]	Relative [% TL]
Total length (TL)	57.8		61.2	
Fork length	56.5	97.8	59.4	97.1
Head length	12.1	20.9	12.5	20.4
Predorsal fin length	12.9	22.3	13.8	22.5
First dorsal fin base length	19.2	33.2	18.4	30.1
Dorsal interspace	2.1	3.6	1.5	2.5
Second dorsal fin base length	4.8	8.3	3.6	5.9
Caudal peduncle length	4.2	7.3	3.9	6.4
Eye diameter	1.2	2.0	1.3	2.1
Preorbital length	5.5	9.5	5.9	9.6
Preopercle length	10.7	18.5	10.6	17.3
Prepelvic length	13.0	22.5	13.9	22.7
Pelvic fin length	3.7	6.4	4.0	6.5
Prepectoral length	12.8	22.1	13.7	22.4
Pectoral fin length	4.9	8.5	4.8	7.9
Preanal length	35.3	61.1	36.6	59.8
Anal fin base	4.2	7.3	4.7	7.7
Caudal fin height	13.5	23.4	11.9	19.4
Mouth length	6.5	11.3	6.8	11.1
Mouth width	5.8	10.0	6.0	9.8
Body depth	8.4	14.5	7.3	11.9

At the dissection, specimen A presented in the stomach two nematode larvae representing the family Anisakidae and remains of digested fishes. The stomach of individual B contained eight specimens of *Cavolinia tridentata* (Forsskål, 1775) (Mollusca: Pteropoda), a widely distributed species in the Atlantic and Pacific oceans, and the Mediterranean Sea (Palomares and Pauly 2024).

Molecular analysis allowed the identification of the two nematode larvae as *Anisakis pegreffii* Campana-Rouget et Biocca, 1955. According to BLASTn, both larvae showed > 99.5% identity (and > 99% query coverage) with several sequences of that species previously deposited in GenBank [The first two hits were the following: OP525266, *A. pegreffii* found in *Scomber japonicus* Houttuyn, 1782 from Turkey (Aldik et al. 2023); MG076944 collected from *Kogia breviceps* (de Blainville, 1838) from the Tyrrhenian Sea (Santoro et al. 2018)]. The consensus sequence, of 608 bp, here obtained was deposited in GenBank under the PQ686358 accession number.

Discussion

Based on morphological description and biometric characters of both specimens, these were identified as *Acanthocybium solandri*, following Collette and Nauen (1983), Collette (2016), and Collette and Graves (2019). Proportions of measurements were comparable with those reported by Romeo et al. (2005).

In the Mediterranean, the species that most closely resembles the wahoo is *Scomberomorus commerson* (Lacepède, 1800) (Fig. 3), a Lessepsian migrant introduced into the basin via the Suez Canal (Hornell 1935; Golani 2021). The narrow-barred Spanish mackerel *S. commerson* is frequently caught in Levantine waters (Di Natale et al. 2020; Al Mabruk et al. 2021; Salah et al. 2023), and possible misidentification with *A. solandri* has been emphasized by Fatfat et al. (2024) and Gökoğlu et al. (2024). However, *A. solandri* can be distinguished from *S. commerson* at least by the absence of gillrakers, as well as having the posterior end of the maxilla concealed, the snout being about as long as the rest of the head, and the lateral line abruptly curving downward under the first dorsal fin. In fact, in *S. commerson*, the gillrakers are present, the posterior end of the maxilla is exposed, the snout is shorter than the rest of the head, and the lateral line abruptly bends downward below the end of the second dorsal fin.

The wahoo is an epipelagic oceanic species, globally distributed at depths lower than 200 m in tropical and subtropical waters of the Atlantic, Indian and Pacific oceans, including the Caribbean and Mediterranean seas (Collette and Graves 2019; Froese and Pauly 2024). In the Red Sea, the occurrence of this species was only recently documented, in 2015, from the Gulf of Aqaba, and later from Saudi Arabian waters (Williams et al. 2022). Due to its very large distribution, *A. solandri* has been assessed as "Least Concern (LC)" by the IUCN (Collette et al. 2023).

Among small tunas caught in tropical oceans, the wahoo is one of the most important species, having a high commercial value, especially in the Atlantic and Pacific, with its catches increasing worldwide (Triay-Portella et al. 2023). This species reaches a maximum fork length of 210 cm and 96 kg of weight. It is a generalist top predator, preying near the surface mostly upon pelagic fishes, cephalopods and crustaceans; it presents high fecundity with pelagic eggs and larvae; large individuals are able to reach a swimming speed of ~45 km/h (Collette and Nauen 1983; Collette 2016, 2022; Travassos et al. 2021). It is considered very valuable in recreational fishery, captured through light to heavy tackle, surface trolling with spoon, feather lure, strip bait, flying fish or halfbeak; it also represents a longline bycatch (Collette and Nauen 1983; Froese and Pauly 2024).

The *A. solandri* specimens previously recorded from the Mediterranean were captured through a variety of fishing gears: tuna trap off Palermo (Doderlein 1872), harpoon from a traditional swordfish fishing vessel called 'Passerella' in the Straits of Messina, Sicily (Romeo et al. 2005; Costa 2012), nets for tunas and mackerels in Lebanon (Fatfat et al. 2024), rod and reel in Antalya, and underwater speargun in Iskenderun Bay (Gökoğlu et al. 2024). The specimens from Malta were caught with a boat seine net, the first, and a trolling the second, both near a FAD. The latter was based on a Maltese traditional method for the capture of pelagic fish, similarly to what has been traditionally used in many other Mediterranean

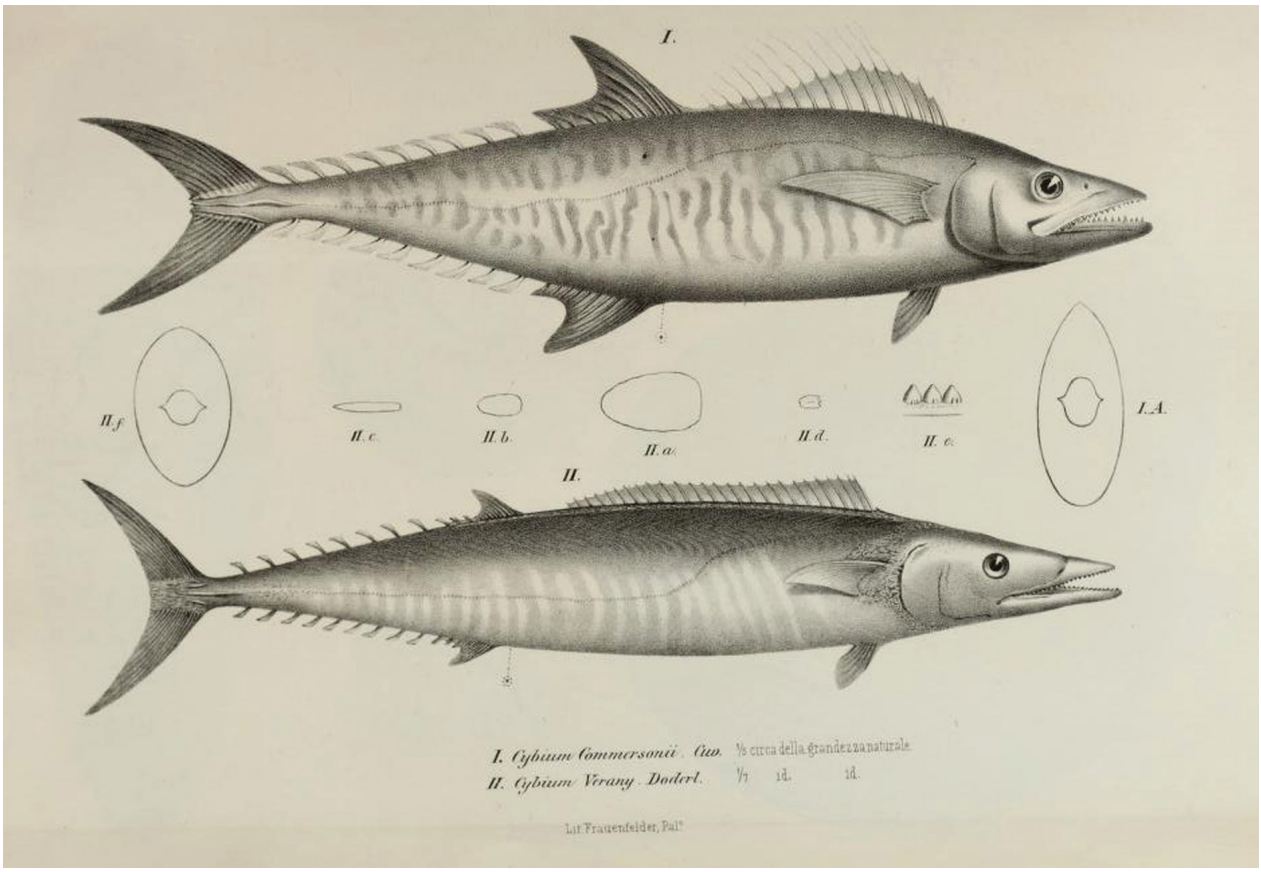

Figure 3. The plate showing *Scomberomorus commerson* (as *Cybium commersonii* Cuv.) above, and *Acanthocybium solandri* (as *Cybium verany* Doderl.) below, reproduced from Doderlein (1872). The drawing of *S. commerson* was obtained by Doderlein from Rüppell. (1828).

countries (Di Natale 1988; Economidis 2000; Vassilopoulou and Anastasopoulou 2007).

To date, specimens from Malta are the smallest among the wahoos caught in the Mediterranean; the total length of previously caught specimens ranged from 90 cm to 144 cm, and their weight from 4 kg to 26 kg. The majority of the Mediterranean captures, such as those from Sicily and Turkey, were recorded in summer; the wahoo from Lebanon was captured in January 2024, while ours in the fall.

In 2024, a relatively high frequency of species occurrences was recorded from various Mediterranean regions (Turkey, Lebanon, Malta) (Fig. 1), compared to the scarcity of records over the previous years. A greater fishing effort by professional and recreational fishers, facilitated by the extended period of fair weather, the exceptionally high seawater temperatures recorded in the whole basin between 2023 and 2024 (see von Schuckmann et al. 2024), as well as the ease of access to information posted on social media by scientists, could have contributed to the recent, unexpected, number of records of *A. solandri* within the Mediterranean.

Given the confirmed occurrence of the cosmopolitan *A. solandri* in the Red Sea, a potential additional recent introduction into the Mediterranean through the Suez Canal cannot be excluded, as hypothesized by Fatfat et al. (2024), at least for individuals found in Levantine waters.

The present parasitological study only revealed two *A. pegreffii* larvae recovered from the stomach of one of the two wahoos. *Anisakis pegreffii* is the most common species of the genus occurring in the Mediterranean Sea. It matures in the gastric chambers of odontocetes, with crustaceans and small fish serving as intermediate hosts, and many fish and cephalopod species as paratenic hosts (Mattiucci et al. 2018; Cipriani et al. 2022). According to Mattiucci et al. (2018), the wahoo represents a new host record for *A. pegreffii*.

The wahoo is known to harbor a wide range of metazoan parasites (Williams and Bunkley-Williams 1996; Zischke et al. 2013). However, the absence of parasite species from the host native geographical range might suggest that both individuals only recently entered the Mediterranean basin. According to Torchin et al. (2003), newly introduced species have lower parasite diversity than conspecifics in their native range, since host-parasite interactions are mostly species-specific, and, in case of heteroxenous parasites, the intermediate hosts are usually absent in the newly invaded areas. Likewise, infections by parasites native to the newly invaded areas do not easily occur in the early stages of colonization (Torchin et al. 2003; Virgili et al. 2024), as confirmed by the present study, which registered the absence of trophically transmitted helminth parasites, with the exception of

A. pegreffii. Furthermore, as reported by Zischke et al. (2013), in wahoos, anisakid species are usually localized in/on viscera of the body cavity. Hence, the occurrence of as little as two larvae, free in the gastric lumen, suggested a recent acquisition. Indeed, in intermediate and paratenic hosts, anisakid larvae acquired by ingestion of an infected prey tend to quickly leave the stomach lumen crossing the stomach wall, initially reaching the body cavity before localizing in/on a visceral organ.

The present finding represents the first record of *A. solandri* for Malta and adds another species to the checklist of marine fishes reported for the Maltese archipelago in Borg et al. (2023). These new records improve the current knowledge about wahoo distribution in the Mediterranean, as well as the general knowledge of the ichthyofaunal diversity of Malta. Additionally, this study expands the parasitological records of *A. solandri*, adding *A. pegreffii* to the two previously recorded parasite species found in a host from the Mediterranean Sea, the trematode *Hirudinella ventricosa* (Pallas, 1774) and the monogenean *Neothoracocotyle acanthocybii* (Meserve, 1938) (Romeo et al. 2005).

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