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Rapid Communication

A bonanza of angelfish (Perciformes: Pomacanthidae) in the Mediterranean: the second documented record of *Holacanthus ciliaris* (Linnaeus, 1758)

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Abstract

The second record of the Queen angelfish, *Holacanthus ciliaris* (Linnaeus, 1758), is hereby documented for the Mediterranean, through a single individual spearfished within Maltese coastal waters. Considerations on the potential introduction pathway for the species, which is popular in the aquarium trade, are made.

Key words: Mediterranean Sea, first record, range-expanding species, ballast water, Malta-Sicily Channel, Non-Indigenous Species (NIS), western Atlantic

Introduction

There is an increasing body of evidence underscoring the addition of new arrivals to the marine biodiversity of the Mediterranean Sea, mediated through an array of different introduction pathways (Rilov and Galil 2009; Mannino et al. 2017) and supported by an increasing warming trend of the Mediterranean Sea (Raitsos et al. 2010). In terms of its exotic contribution to Mediterranean fish diversity, the Pomacanthidae fish family (marine angelfishes) is represented, to date, by *Pomacanthus maculosus* (Forsskal, 1775), *Pomacanthus imperator* (Bloch, 1787), *Holacanthus ciliaris* (Linnaeus, 1758) and *Holacanthus africanus* Cadenat, 1951 (Zenetos et al. 2016).

Holacanthus ciliaris is a marine, non-migratory resident of coral reefs of sub-tropical and tropical regions of the western and central Atlantic, at depths ranging between 1 and 70 m (Lieske and Myers 1994), with its native range extending from Bermuda, Florida and the Gulf of Mexico to the north (Allen 1985), to Brazil and St. Paul's Rocks in the south (Lubbock and Edwards 1981). The species, known with the common name of "Queen angelfish", is common in waters around the Bahamas and Florida (Froese and Pauly 2020) and is, to date, known from the Mediterranean from just one record, made in Trogir Bay within central swathes of the Eastern Adriatic Sea in 2001 (Dulčić and Dragičević 2013). A vagrant specimen

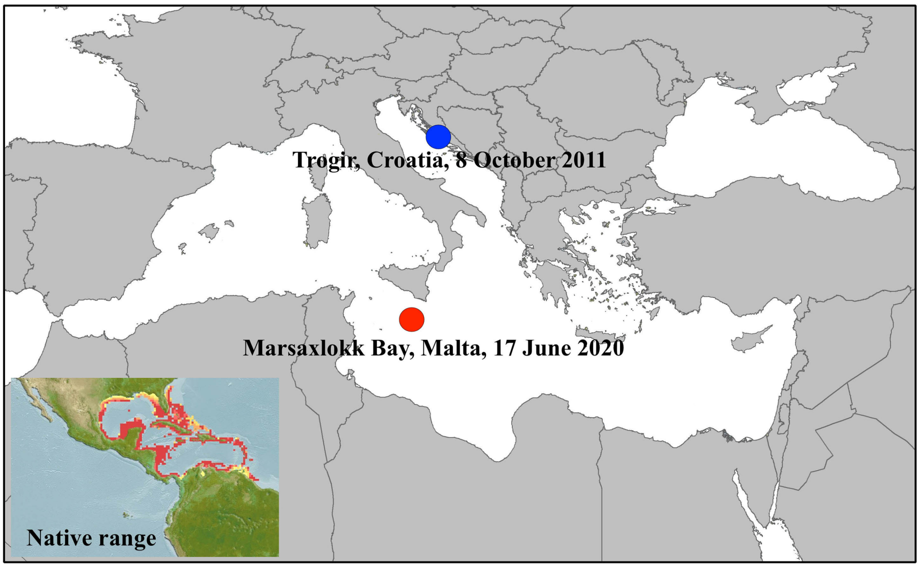

Figure 1. Updated map of known Mediterranean coastal sites at which *H. ciliaris* has been recorded to date. The native range of the species is included as an inset (Source: Froese and Pauly 2020).

has been recorded off the island of Taiwan (Shao 1997), although the validity of this report has been questioned (Froese and Pauly 2020). The species is commonly caught in traps when destined to marine aquarium trade and is not important for human consumption, although it is edible (Carpenter 2002). Adults of the species are known to have a varied diet, which consists mainly of sponges, algae and bryozoans (Reis et al. 2013), with juveniles of the species known to pick parasites off other fish individuals (Carpenter 2002).

Yet another *Holacanthus* species—*Holacanthus africanus*—having a tropical eastern Atlantic native distribution, is known from the Mediterranean through just two individuals caught within Maltese waters in the central Mediterranean (Deidun et al. 2017), with its taxonomic identity being further confirmed through genetic analysis (Deidun et al. 2020). This study represents the first record of *H. ciliaris* from Maltese waters and the second record of the species for the Mediterranean Sea.

Materials and methods

On the 17th June 2020, the "*Spot the Alien Fish*" citizen science campaign, implemented since 2017 within the Department of Geosciences at the University of Malta, was contacted by a sports fisherman in connection with an interesting catch he had made. The catch was made through spearfishing at a depth of 14 m, along the outer margin of the breakwater shielding the Freeport cargo-handling port within Marsaxlokk Bay (35.812675°N; 14.544060°E, Figure 1). The individual was photographed, weighed and characterised in terms of its meristic and morphometric attributes, whilst detailed notes about its livery were also taken. The same individual is currently preserved in a frozen state within the Department of Geosciences at the University of Malta.

DNA was extracted from a piece of fresh frozen muscle tissue 4 mm × 2 mm cut off from the specimen, using a QIAGEN® kit (QIAamp®), following the associated protocol (QIAGEN 2014). A total volume of 200 ml was collected in Buffer AE. The total DNA concentration was found to be 40 ng ml^{-1} using NanoDrop™ 2000 spectrophotometer (Thermo Scientific™).

The cytochrome c oxidase subunit 1 (COI) mitochondrial gene was used as a marker for species-level DNA barcoding identification (Viret et al. 2018; Zemlak et al. 2009). A 680 bp fragment (at 5' end of the COI mitochondrial gene) was amplified using the primers FishCoxI-F2 (5'-TCGACTAATCA TAAAGATATCGGCAC-3') and FishCoxI-R2 (5'-ACTTCAGGGTGACC GAAGAATCAGAA-3') (Ward et al. 2005). The 10 µl of PCR mix included: 40 ng of template DNA, 4.25µl of 2× Dream *Taq* buffer (Thermo Scientific™), 2.5×10^{-9} mol of primers, and 4.25 µl of DNAase free water. The PCR cycling protocol comprised an initial step of 2 min at 95 °C, followed by 35 cycles of 0.5 min at 95 °C, 0.5 min at 50 °C, and 1 min at 75 °C, for 10 min.

Sequencing reactions of the PCR product were carried out, using the forward and reverse directions, at Eurofins Genomics (Ebersberg, Germany) using cycle sequencing technology (dideoxy chain termination / cycle sequencing) on ABI 3730XL sequencing machines, using the same primers as used for the PCR reaction (i.e. FishCoxI-F2 and FishCoxI-R2). The forward and reverse sequence chromatograms were visually checked for any artefacts or heterogeneity. No double/superimposed peaks were detected. The sequence was compared with barcode sequences on GenBank® using BLAST®.

Results

The spearfished individual was taxonomically attributed to *Holacanthus ciliaris*, on the basis of the description of the species provided by Carpenter (2002). Consistent with this description, our individual presented a deep and laterally-compressed body which was laterally tinged with blue, with yellow-orange edges and scales and a yellowish, relatively-small head (Figure 2A). A highly conspicuous black notch circled and spotted with blue (the so-called “crown” pattern) was evident at the nape (Figure 2B), whilst the mouth, chin, throat, chest and the abdomen all presented a purplish-blue colouration. A dark blue hue was evident just above the eyes as well as on the eyes, snout, pre-opercular spine and the opercle (Figure 2B). The spines of the pre-opercle region and of the opercle itself were blue, whilst the colour of the dorsal and anal fins changes to orange towards the edges, which, in turn, were light blue, whilst their extended tips were yellow (Figure 2B). The pectoral fins were yellow, presenting a black blotch spotted with light blue at its base, whilst the pelvic and the caudal fins were yellow (Figure 2B). The caudal fin was slightly curved at the edge, lacking filaments along the upper and lower edges.

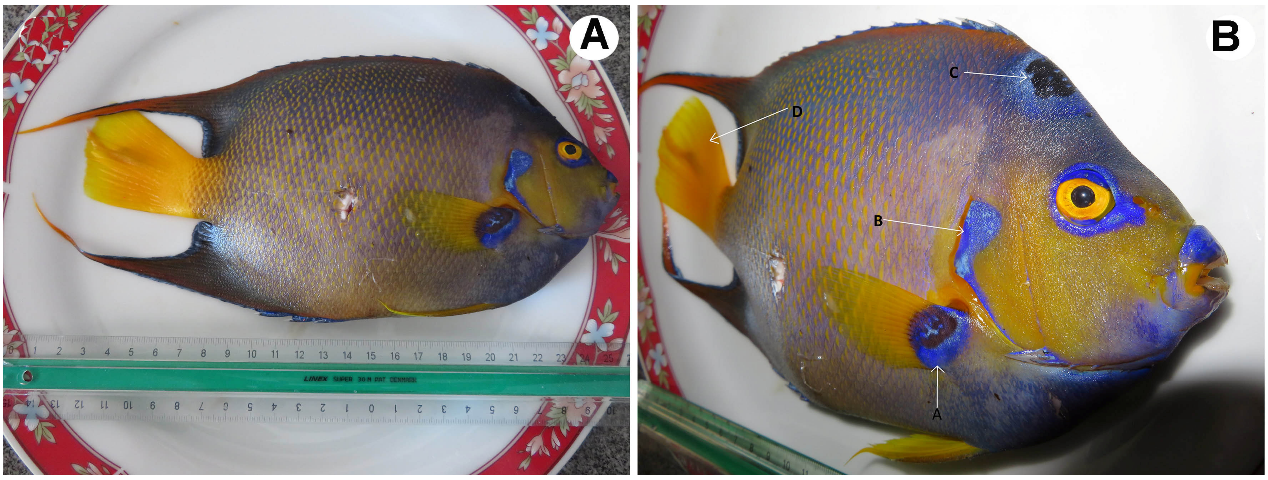

Figure 2. A: The *H. ciliaris* individual recorded in the current study. B: Different aspects of the habit and livery for the *H. ciliaris* individual reported in the current study. Arrows A–D underscore a number of diagnostic livery characteristics for *H. ciliaris* evident in our individual. Photographs by Alan Deidun.

Holacanthus ciliaris is occasionally misidentified taxonomically as *H. bermudensis* Goode, 1876, but this normally occurs at juvenile stage, given that the livery of the two species at adult stage is distinctive and easy to separate. However, these sister species hybridize on a regular basis, producing all sorts of intermediate colour patterns (Carpenter 2002).

The meristic counts of the sample were: Dorsal fin XIV-19, Anal fin III-20, Pectoral fin 16, Pelvic fin I-5, Caudal fin 17. The Total length (TL) was 22.3 cm, the head length (HL) 5.2 cm and the body depth 11.4 cm, the weight was 365.5 g. The following main proportions of morphometric measurements were obtained: Standard length 80.7, Pre-dorsal length 20.6, Pre-anal length 53.8, Pre-pectoral length 24.2, Pre-pelvic length 19.7 as % of TL; Eye diameter 21.2, Inter-orbital width 42.3 as % of HL. The meristic counts and morphometric attributes obtained in this study were consistent with the values cited in Carpenter (2002).

The individual reported upon in this study is considered to be an adult of *H. ciliaris*, given the distinctive body livery characterising the juvenile stages of the species, consisting mainly of three primary blue-wide bars (Carpenter 2002). However, the same individual is not considered to have attained its full maturity, given the disparity between its total length value and the average total length reported for adults of the species (22.3 cm and 30.0 cm, respectively).

The COI sequence extracted from this *H. ciliaris* specimen aligned 99.56% with the various *H. ciliaris* sequences, available on GenBank®, extracted from specimens collected from the Caribbean Sea (Steinke et al. 2009; Weigt et al. 2012; Shen et al. 2015; Sanciangco et al. 2016).

Discussion

Although the range-expanding hypothesis explaining the entry of the individual being recorded in this study cannot be ruled out completely, given that the native range of *H. ciliaris* is located within the Atlantic, this prospect is considered to be unlikely. The motivation behind this opinion include the physical distance between the native range of the species and the Mediterranean Sea, a lack of existing reports of the species from eastern Atlantic regions contiguous to the Mediterranean and the slow-moving and non-migratory habits of the same species.

Two alternative plausible introduction pathways include the aquarium/pet industry and a ballast water-mediated transfer. Dulčić and Dragičević (2013) attribute the first record of *H. ciliaris* from the Mediterranean to the shelter provided by slow-moving, towed oil platforms, which act as de facto reefs (Galil 2006). A similar introduction pathway has been implicated for yet another *Holacanthus* species recorded recently to the Mediterranean—*H. africanus*—which has been linked with the towing of an oil from tropical western Africa to a Maltese harbour (Deidun et al. 2017).

Conversely, Zenetos et al. (2016) describe the species as a probable introduction through the aquarium industry. We tend to support the latter hypothesis for the following reasons. The fish is stocked by at least one aquarium company in Italy, which sells imported individuals of the species at 180–200 euros each (Franco Mannelli, Palermo, *pers. comm.*) and the native range of the species does not correspond, as is the case for *H. africanus*, with the origin of most oil platforms towed into the Mediterranean. The Mediterranean and, in particular, Maltese waters, are not new to introduced species through the aquarium industry. Most of these, including *P. maculosus* (originally misidentified as *P. asfur*, Karachle et al. 2016), *Acanthurus chirurgus* (Bloch, 1787), *Acanthurus coeruleus* Bloch and Schneider, 1801 and *Chrysiptera hemicyanea* (Weber, 1913), all previously recorded for Maltese waters (Evans et al. 2017; Deidun et al. 2018), are vagrant species, further suggesting releases of the species through the aquarium trade.

However, a ballast-mediated introduction of the *H. ciliaris* individual involving a vessel rather than a towed oil platform cannot be completely discounted, considering the location at which the same individual was caught. This location is in fact in close proximity to the Freeport, a key Mediterranean transhipment hub which handles over 3 million containers per year and which services a number of ports in North America, including those sited in Jamaica and Guadeloupe, which lie within the species' native range.

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