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-RESEARCH ARTICLE-

The first record of the longjaw squirrelfish, *Holocentrus adscensionis* (Osbeck, 1765) (Holocentriformes: Holocentridae), in the Mediterranean Sea

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Abstract

Research and monitoring of coastal habitats and biodiversity is increasingly contributing to the discovery of new alien species, which highlights the use of long-term monitoring for timely assessment and management due to marine environmental changes. Research work undertaken through coastal snorkelling surveys and working with fishermen allow for additional monitoring effort to record biodiversity changes and new alien species presence. One such new alien fish species was observed during spring snorkelling research while a specimen was collected in August 2016 to undertake detailed morphological, meristic and genetic analyses. Mitochondrial DNA barcoding analyses were undertaken to confirm the species' identity as the family Holocentridae is composed of a number of species that have very similar and overlapping morphological characters which may lead to misidentification. This research led to the identification of the first record of a specimen of Longjaw squirrelfish, *Holocentrus adscensionis* (Osbeck, 1765), in the Mediterranean Sea. This is a subtropical reef-associated species native to the Atlantic Ocean and may compete with native Mediterranean reef species.

Keywords:

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Introduction

Holocentridae is a family composed of over 84 soldier fishes and squirrelfishes, most of which are primarily nocturnal carnivorous reef-dwelling fishes which inhabit mainly tropical

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and temperate habitats (Nelson, 2006; Froese & Pauly 2016). This family is not native to the Mediterranean Sea, however specimens of genus *Sargocentron* have been collected and analysed from various regions in the Eastern Mediterranean. The first record of this genus in the Mediterranean Sea was along the Palestine coast and was reported in Haas and Steinitz (1947), and by 2009 it became one of the commonest fish landed in Lebanon with a 3% contribution to the total landings by artisanal Lebanese fishermen as it is commonly caught on gill-nets and long-lines (Carpentieri et al., 2009). Despite all Mediterranean records point to *S. rubrum*, recent genetic evidence shows that specimens from Turkey match closely to sequences of *S. rubrum* (Keskin and Atar, 2013), however specimens from Lebanon diverge from any currently genetically barcoded *Sargocentron* species (Bariche et al., 2015). This indicates that possibly more than one species of *Sargocentron* have entered the Mediterranean Sea (Bariche et al., 2015), however prior to this study there have been no records of *Holocentrus adscensionis* or any other species from the genus *Holocentrus* in the Mediterranean Sea.

Holocentrus adscensionis is a subtropical reef-associated species native to the western Atlantic Ocean from North Carolina (USA) and the islands of Bermuda down to Brazil, including the Gulf of Mexico and several islands in the region; the mid-Atlantic ridge at St. Paul's Rocks, Ascension Island and St. Helena; and the eastern Atlantic Ocean from Gabon to Angola, including Sao Tome Island (Carpenter, 2002; Nelson, 2006; Froese & Pauly 2016). This squirrelfish is known to have a prolonged pelagic period, which includes pelagic eggs, followed by pelagic larvae and 'rhynchichthys' pelagic stage, having a total mean of 48 days (43 days to 56 days). The 'rhynchichthys' stage is then followed a 'meeki' stage which can either transform quickly to a benthic juvenile or extends its pelagic duration by an average of an additional 13 days (Tyler et al., 1993), allowing ample time for dispersal prior to settlement.

Methods

Snorkelling surveys along the Maltese coast, side by side with research with fishermen, allow for useful long-term monitoring of coastal biodiversity. A new alien fish species was observed during snorkelling field research work in May 2016, followed by the collection of a specimen on the 24th of August, photo of which is supplied in Figure 1. This specimen was collected from shallow coastal waters in Marsascala, Malta [GPS: 35°52'5.56"N, 14°34'29.28"E] (Figure 2). Once captured the specimen was kept for scientific analyses. The diagnostic features used in its identification followed Carpenter, 2002; Nelson, 2006; Froese & Pauly, 2016. All body length measurements were taken to the nearest 0.1 mm using electronic calipers and mass was recorded to the nearest 0.01 g.

A tissue biopsy was taken and preserved in 95% ethanol. The specimen was subsequently deposited in the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta with reference code number CBRG/F.160824/HA001. For genetic analyses, the tissue was digested with Proteinase K and the total DNA was extracted using GF-1 Tissue DNA Extraction Kit (Vivantis Technologies). PCR amplifications were carried out for: cytochrome c oxidase I gene (COI), cytochrome b (Cyt b) and 16S rRNA (16S) using primers sets as described in Vella et al., (2016a) and the 12S rRNA gene (12S) using H1478 and L1091 primers (Kocher et al., 1989). PCR amplifications were carried out following the amplification protocols described in Vella et al., (2016b). PCR products were purified and sequenced via ABI3730XL sequencer using both

the forward and reverse primers. The sequences, at both nucleotide and amino acid level, were analysed using Geneious v6 (http://www.geneious.com, Kearse et al., 2012). The sequences obtained were deposited in GenBank, under accession numbers KX961690-3 for COI, Cyt b, 12S and 16S respectively. These sequences were compared to other sequences available in genomic databases using BLASTn.

Various COI gene sequences from different Holocentridae species available in GenBank and BOLD allowed for phylogenetic analyses of this specimen, and for comparative analyses of the current specimen to the *Sargocentron* haplotypes from the Mediterranean Sea. The sequences chosen were aligned using Geneious v6 (http://www.geneious.com, Kearse et al. 2012), and were trimmed to the size of the smallest homologous sequence. The genetic divergences between the 575 bp COI sequences selected were calculated using the Kimura 2-parameter distance model (Kimura, 1980), while the phylogenetic tree was constructed using 1500 bootstraps with Maximum Likelihood utilizing MEGA v6 (Tamura, 2013).

Results

Morphometrics

The specimen caught weighs 310.82 grams and has a total body length of 301.0 mm. Its appearance, morphology and meristic counts (Table 1 and Figure 1) match the descriptions of *H. adscensionis* given by Carpenter, 2002; Nelson, 2006; Froese & Pauly 2016. The specimen has a moderately compressed oblong body and a long slender caudal peduncle, while the head has two very conspicuous strong spines on the sides. The anterior dorsal fin soft rays and upper caudal fin rays are elongated. The back and the upper sides of the body are reddish with some golden reflections and yellowish stripes on its back. Lighter coloured stripes get broader on the lower scale rows. The lower sides and belly are white. The snout and top of head are red, with a white streak running diagonally across the cheek. The dorsal fin spines and the interspinal membranes adjacent to the spines are yellowish-green, while the rest of the dorsal fin has a reddish pink colour. The first three anal fin spines are white while the fourth spine and the anal fin soft rays have a pink colouration (Figure 1).

Figure 1. The *Holocentrus adscensionis* specimen caught from Marsascala, Malta on the 24th August, 2016. (Photo of the specimen on a 1x1cm scaled background).

Table 1. Measurements and meristic counts of the *Holocentrus adscensionis* specimen caught in Maltese waters.

Parameter	Measurements	Proportion
Total length	301.0 mm	
Fork length	238.9 mm	
Standard length	224.0 mm	
Maximum body depth	79.2 mm	35.4 % (SL)
Length of dorsal fin base	127.1 mm	56.7 % (SL)
Pectoral fin base	11.4 mm	5.1 % (SL)
Anal fin base	32.1 mm	14.3 % (SL)
Depth of caudal peduncle	17.4 mm	7.8 % (SL)
Pre-pelvic length	74.0 mm	33.0 % (SL)
Pre-anal length	156.0 mm	69.6 % (SL)
Head length	70.3 mm	31.4 % (SL)
Eye diameter	22.6 mm	10.1 % (SL)
	Meristic Counts	
Dorsal fin spines	11	
Dorsal fin soft rays	15	
Pelvic fin spines	1	
Pelvic fin soft rays	7	
Anal fin spines	4	
Anal fin soft rays	10	
Pectoral fin soft rays	15	
Lateral line scales	50	
Gill rakers (total)	24	
Gill rakers on the lower limb of first gill arch	18	

Genetic analyses

The sequence lengths obtained were 655 bp, 792 bp, 387 bp and 578 bp for COI, Cyt b, 12S and 16S respectively. Each sequence was run via BLASTn to identify matches in GenBank. The COI sequence of the currently analysed specimen, did not match the sequences of *S. rubrum* from Turkey (gb:KC501233-52 - Keskin and Atar, 2013) and *Sargocentron* sp. from Lebanon (gb:KR861551 - Bariche et al., 2015), but rather it matched between 99% to 100% with a number of GenBank sequences belonging to *H. adscensionis* (Figure 3). This result matched the morphological analyses and thus confirmed the current specimen as the first record of *H. adscensionis* in the Mediterranean. Genetic data from the Cyt b also matches completely with the most common haplotype for *H. adscensionis* (DQ380026) as recorded in various regions across the Atlantic Ocean (Bowen et al., 2006). Data on the 12S and 16S genes of *H. adscensionis* is absent and thus it was not possible to use these genes for identification down to the species level although they confirmed the genus by matching with *H. rufus*, KC441975 and KC442001 respectively (Chen et al., 2014).

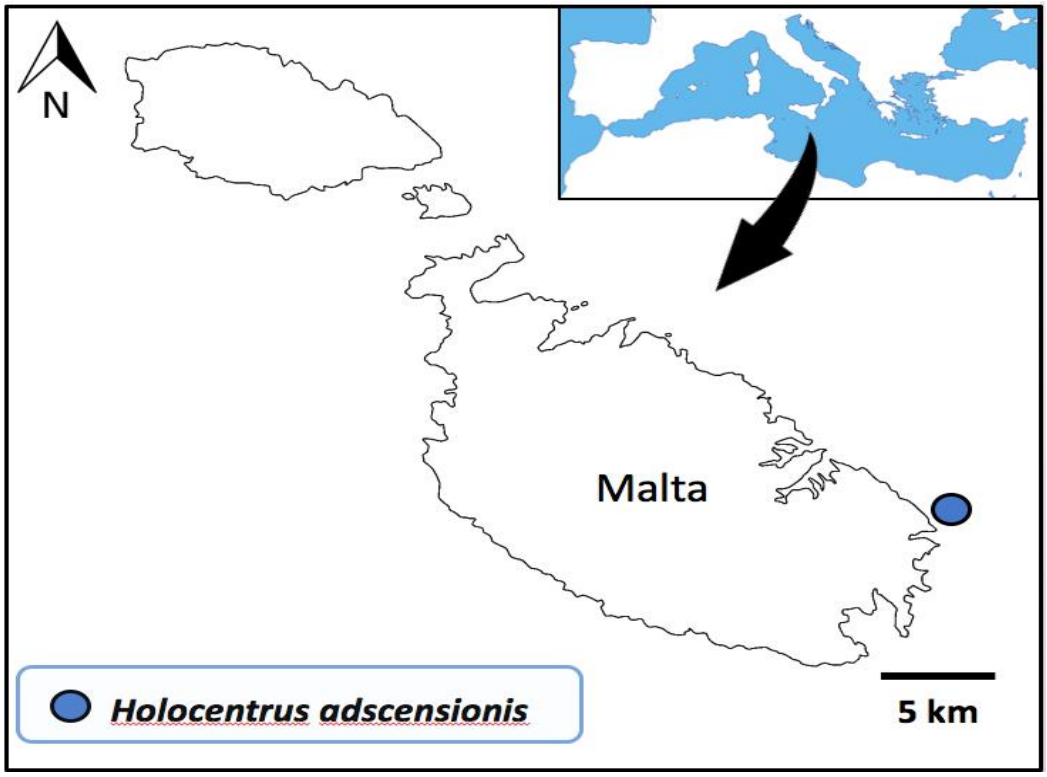

Figure 2: Map showing the location from where the first recorded Mediterranean *Holocentrus adscensionis* specimen was caught.

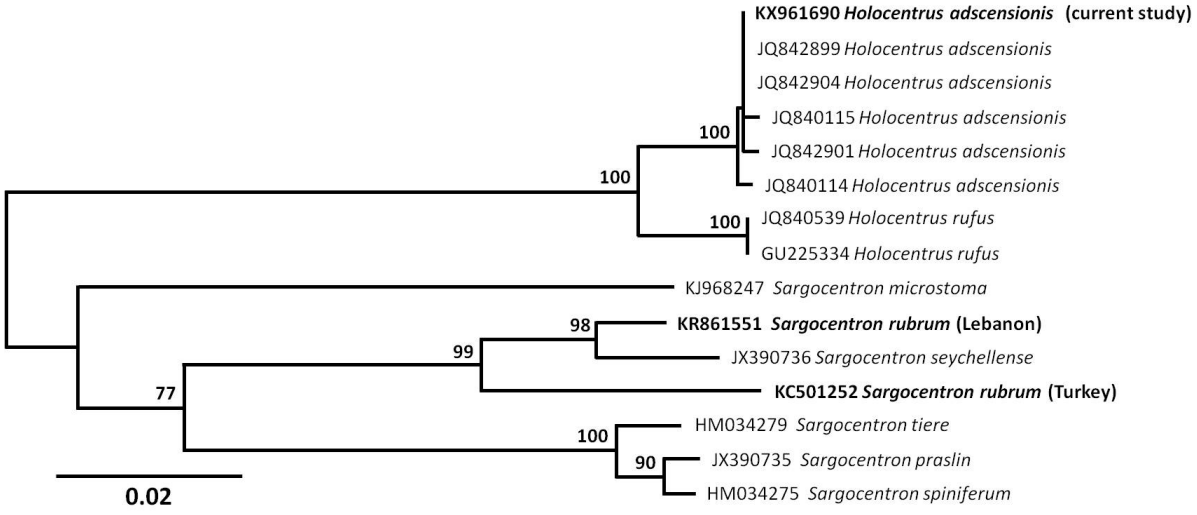

Figure 3. Maximum Likelihood tree of a homologous 575 bp sequence of the COI gene from some Holocentridae species using K2P distances and 1500 bootstraps. The codes indicate the accession numbers used in the tree construction while labels in bold indicate the sequences and sampling locations for Mediterranean specimens of the family Holocentridae.

Discussion

The results presented here provide the relevant scientific data related to the first record of *H. adscensionis* in the Mediterranean Sea. The number of recorded non-native species in the Mediterranean Sea are constantly increasing, with species coming in from both the Atlantic and the Indo-Pacific Ocean (Golani, 2010; Golani et al., 2013). Alien fish records in the Mediterranean have been mostly reported as Lessepsian migrants (Golani, 2010) through the Suez Canal, followed by some years of adaptation in the Levantine Sea prior to spreading westerly throughout the rest of the Mediterranean Sea (Golani, 2010). However, in the past three years there has been an increasing number of new Mediterranean records of fish which are first recorded from the central Mediterranean, such as the Maltese Islands, including the occurrence of *Stegastes variabilis*, *Lutjanus fulviflamma* and *Abudefduf hoefleri* (Vella et al., 2015 a & b; 2016b), and the reappearance of *A. vaigiensis* (Vella et al., 2016c).

Like *S. variabilis* and *A. hoefleri* (Vella et al., 2015a & 2016b), *H. adscensionis* is of Atlantic origin and lacks records in other regions of the Mediterranean Sea prior to its first record in the central Mediterranean. At this stage we cannot exclude the presence of *H. adscensionis* in the western Mediterranean especially from areas that lack nocturnal ichthyological investigation given that by day this species prefers to hide in crevices. However, it is highly possibly that this species is not a natural migrant but has been introduced in the area through anthropogenic marine activities. At Mediterranean level, shipping activity has been considered as a main vector, transporting alien marine species in the region (Galil, 2006). Similar to previously mentioned local first records (Vella et al., 2015 a, b & 2016 a, b, c), the specimen presented here had been captured from an area that lies between the two local major ports which offer a wide variety of marine related services in central Mediterranean, including ship repair, oil platform servicing, transhipment activities and berthing of large marine vessels including commercial vessels, cruise-liners and super-yachts. This intensive marine activity assisted with marine currents might have easily been the cause of transportation of this species to the area.

The occurrence of *H. adscensionis* in the Mediterranean Sea must be closely monitored. At first glance this species can be morphologically confused with *S. rubrum* or a number of other Holocentridae species, as this family is composed of several species that have a number of overlapping external morphological characters (Carpenter, 2002; Nelson, 2006; Dornburg et al., 2012; Froese & Pauly 2016), thus DNA barcoding would play an important role in tracking this species. In fact, this technique has proven to be valuable in detecting morphologically very similar non-native species (Vella et al., 2015b, 2016a, b & c). The life-history and ecology of *Holocentrus* are very similar to those of the closely related *Sargocentron* species (Carpenter, 2002), thus the establishment of a population of the *H. adscensionis* assisted with the gradual warming of the Mediterranean Sea (Occhipinti-Ambrogi and Galil 2010; Turan et al. 2016) may lead to an expansion in the range of this non-native species, similar to what was noted for *Sargocentron rubrum* in the Eastern Mediterranean (Carpentieri et al., 2009; Keskin and Atar, 2013), where the latter is one of the most invasive non-native species in the area (Azzurro et al., 2014). Moreover, it has to be noted that the pelagic presettlement stage of *H. adscensionis* is fairly long, even longer than for some *Sargocentron* species (Tyler et al., 1993), thus once it establishes itself, the proliferation of this new alien species is expected to be more rapid.

This study represented the first record and DNA barcoding of *H. adscensionis* in the Mediterranean Sea. Close monitoring of this subtropical nocturnal-feeding reef fish species is useful to assess its impacts on local reef fish communities.

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References

Azzurro, E., Victor, M.T., Lombarte, A., Maynou, M., Simberloff, D., Rodríguez-Pérez, A., Solé, R.V. (2014). External morphology explains the success of biological invasions. *Ecology Letters*, 17, 1455-63.

Bariche, M., Torres, M., Smith, C., Sayar, N., Azzurro, E., Baker, R., Bernardi, G. (2015). Red Sea fishes in the Mediterranean Sea: a preliminary investigation of a biological invasion using DNA barcoding. *Journal of Biogeography*, 42, 2363-73.

Bowen, B.W., Bass, A.L., Muss, A., Carlin, J., Robertson, D.R. (2006). Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine Biology*, 149(4), 899-913.

Carpenter, K.E. (ed.) (2002). *The living marine resources of the Western Central Atlantic. Volume 2: Bony fishes part 1 (Acipenseridae to Grammatidae*). FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5. Rome, FAO, 601-1374.

Carpentieri, P., Lelli, S., Colloca, F., Mohanna, C., Bartolino, V., Moubayed, S., Ardizzone, G.D. (2009). Incidence of Lessepsian migrants on landings of the artisanal fishery of South Lebanon. *Marine Biodiversity Records*, 2:e71.

Chen, W.J., Santini, F., Carnevale, G., Chen, J.N., Liu, S.H., Lavoue, S., Mayden, R.L. (2014). New insights on early evolution of spiny-rayed fishes (Teleostei: Acanthomorpha). *Frontiers in Marine Science*, 1:e53.

Dornburg, A., Moore, J.A., Webster, R., Warren, D.L., Brandley, M.C., Iglesias, T.L., Peter, C., Wainwright, P.C., Near, T.J. (2012). Molecular phylogenetics of squirrelfishes and soldierfishes (Teleostei: Beryciformes: Holocentridae): Reconciling more than 100 years of taxonomic confusion. *Molecular Phylogenetics & Evolution*, 65, 727-38.

Froese, R., Pauly, D. (2016) FishBase. URL http://www.fishbase.us. Accessed 15th September 2016.

Galil, B.S. (2006). Shipwrecked – Shipping impacts on the biota of the Mediterranean Sea. Chapter 3 in Davenport, J. and J. L. Davenport (eds.), *The Ecology of Transportation: Managing Mobility for the Environment.* Springer publishers, The Netherlands.

Golani, D. (2010). Colonization of the Mediterranean by Red Sea fishes via the Suez Canal - Lessepsian migration. In: Golani, D., Appelbaum-Golani, B. (eds), *Fish Invasions of the Mediterranean Sea: Change and Renewal*. Sofia: Pensoft.

Golani, D., Orsi-Relini, L., Massuti, E., Quignard, J.P., Dulčić, J., Azzurro, E. (2013). CIESM-Atlas of Exotic Fishes - List [WWW Document]. 2013. URL. http://www.ciesm.org/atlas/appendix1.html. Accessed 15th September 2016.

Haas, G., Steinitz, H. (1947) Erythrean fishes on the Mediterranean coast of Palestine. *Nature*, 160, 28.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647-9.

Keskin, E., Atar, H.H. (2013). DNA barcoding commercially important fish species of Turkey. *Molecular Ecology Resources*, 13, 788-97.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-20.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America*, 86, 6196-200.

Nelson, J.S. (2006). *Fishes of the World*, 4th edn. John Wiley and Sons, Inc., New York.

Occhipinti-Ambrogi, A. & Galil, B. (2010). Marine alien species as an aspect of global change. *Advances in Oceanography and Limnology*, 1, 199-218.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.

Turan, C., Erguden, D., Gürlek, M. (2016). Climate change and biodiversity effects in Turkish Seas. *Natural and Engineering Sciences*, 1(2), 15-24.

Tyler, J., Johnson, G., Brothers, E., Tyler, D., Smith, L. (1993). Comparative early life histories of western Atlantic squirrelfishes (Holocentridae): Age and settlement of *rhynchichthys, meeki*, and juvenile stages. *Bulletin of Marine Science*, 53, 1126-50.

Vella, A., Agius Darmanin, S., Vella, N. (2015 a). Morphological and genetic barcoding study confirming the first *Stegastes variabilis* (Castelnau, 1855) report in the Mediterranean Sea. *Mediterranean Marine Science*, 16(3), 609-12.

Vella, A., Vella, N., Agius Darmanin, S. (2015 b). First record of *Lutjanus fulviflamma* (Osteichthyes: Lutjanidae) in the Mediterranean Sea. *Journal of the Black Sea & Mediterranean Environment*, 21(3), 307-15.

Vella, N., Vella, A., Agius Darmanin, S. (2016 a). The first record of the lowfin chub *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) from Malta. *Journal of the Black Sea & Mediterranean Environment*, 22(2), 175-81.

Vella A, Vella N, Agius Darmanin S. (2016 b). The first record of the African sergeant, *Abudefduf hoefleri* (Perciformes: Pomacentridae), in the Mediterranean Sea. *Marine Biodiversity Records*, 9(1), 1-5.

Vella, A., Agius Darmanin, S., Vella, N. (2016 c). The first records of Indo-Pacific sergeant *Abudefduf vaigiensis* (Quoy & Gaimard, 1825) and further notes on the occurrence of sergeant major *A. saxatilis* (Linnaeus, 1758) in Malta: expanding populations of an invasive genus in the Mediterranean Sea. *Journal of the Black Sea & Mediterranean Environment*, 22(1), 1-15.