

MARINE RECORD

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# Morphological and genetic analyses of the first record of the Niger Hind, *Cephalopholis nigri* (Perciformes: Serranidae), in the Mediterranean Sea and of the African Hind, *Cephalopholis taeniops*, in Malta

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## Abstract

**Background:** Non-native marine species, including tropical eastern Atlantic fish species are on the increase in Malta, with shipping activities being the main vector for the movement of these alien species from the Atlantic into the Mediterranean Sea. This calls for cooperation and collaboration between various sea-users and researchers to ensure continuous monitoring of coastal biodiversity.

**Methods:** Research methods involving local fishermen cooperation in monitoring efforts to identify and track populations of alien species in the Central Mediterranean has led to new records for the genus *Cephalopholis* (Perciformes: Serranidae) in Malta. Morphological characteristics, meristic counts and mitochondrial DNA sequences from specimens of both species sampled from Maltese waters were analysed to confirm their species identify accurately, essential for tracking their respective population expansions in the Mediterranean.

**Results and conclusion:** Results from this study have led to confirmation of the first record of the Niger Hind, *Cephalopholis nigri* (Günther, 1859), in the Mediterranean Sea and of the establishment of the African Hind, *Cephalopholis taeniops* (Valenciennes, 1828) in Maltese waters.

**Keywords:** *Cephalopholis nigri*, *Cephalopholis taeniops*, Non-native, New record, Mediterranean

## Background

Groupers are economically important species and are caught by commercial, artisanal and recreational fishermen. In the Mediterranean, the subfamily Epinephelinae is represented by six native species, *Epinephelus aeneus*, *E. caninus*, *E. costae*, *E. marginatus*, *Hyporthodus haifensis* and *Mycteroperca rubra* (Heemstra and Randall 1993; Froese and Pauly 2016). In addition to these, there are non-native tropical Epinephelinae species, including six Indo-Pacific *Epinephelus* species, *E. malabaricus*, *E. coioides*, *E. merra*, *E. fasciatus*, *E. geoffroyi* and *E. areolatus*, that were first recorded in 1966 (Heemstra and Randall 1993), 1969 (Ben-Tuvia and Lourie 1969;

Heemstra and Golani 1993), 2004 (Lelong 2005), 2011 (Bariche and Heemstra 2012), 2015 (Golani et al., 2015) and 2015 (Rothman et al., 2016) respectively. Additionally, another two Atlantic species, *Cephalopholis taeniops*, first noted in 2002 (Ben Abdallah et al., 2007), and *Mycteroperca fusca*, reported in 2010 (Heemstra et al., 2010) were also found in the Mediterranean Sea. The increase in new records of non-native Epinephelinae species in this region follows the trend noted for a number of other tropical fish groups (Golani 2010; Golani 2013; Vella et al., 2015a, b & 2016a).

The genus *Cephalopholis* Bloch & Schneider, 1801 is composed of 24 species, 19 of which occur in the Red Sea and the Indo-Pacific region, one is from the eastern Pacific, two from the western Atlantic and two from the eastern Atlantic (Heemstra and Randall 1993; Froese and

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Pauly 2016). These species are primarily tropical and sub-tropical species, none of which are native to the Mediterranean Sea. The two species analysed in this study are *C. nigri* and *C. taeniops*, both of which are of tropical eastern Atlantic Ocean origin, with their native range extending from Angola to the Canary Islands and Western Sahara respectively (Heemstra and Randall 1993; Froese and Pauly 2016). While specimens of *C. taeniops* have been morphologically analysed in Libya (Ben Abdallah et al., 2007) and Israel (Salameh et al., 2009), prior to this study there have been no records of *C. nigri* in the Mediterranean Sea.

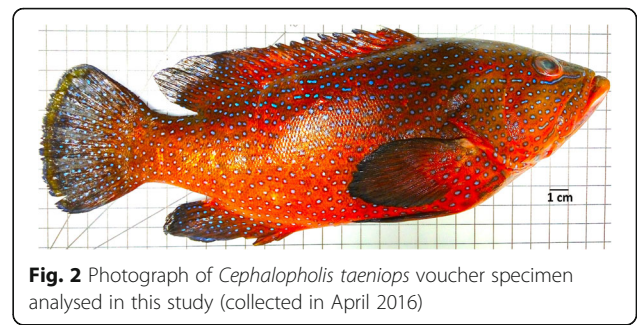
**Results and discussion**

**Morphometrics**

The voucher specimen of *C. nigri* sampled weighed 36.43 grams and had a total length of 140.0 mm (Fig. 1) while the voucher specimen of *C. taeniops* weighed 402.42 grams and had a total length of 288.0 mm (Fig. 2). The appearance, morphology and meristics are presented in Table 1 and Figs. 1 and 2. These match the descriptions of *C. nigri* and *C. taeniops* given by Heemstra and Randall (1993) and Froese and Pauly (2016). The *C. nigri* specimen had a meristic formula with a dorsal fin count of IX + 14; pectoral fin count of 14; pelvic fin count of I + 5; and anal fin count of III + 8. The pectoral fin length was 59% the head length, the pelvic fins reached the anus and were 53% the head length, while the caudal fin was rounded. The specimen had 45 scales on the lateral line and 22 gill rakers. The colour of the fish was brown, with reddish orange reticulated spots. Four darker brown bars were noted over the body extending over the dorsal fin, and another two bars on the caudal peduncle. The margin of the distinctly indented membrane on the dorsal spines was orange. Unlike the rest of the Epinephelinae species, the continuous dorsal fin of *Cephalopholis* species has 9 hard dorsal spines, a feature that is important especially in identifying *C. nigri*, given that there are other groupers such as *E. coioides* that have similar orange spots and banding patterns (Froese and Pauly 2016). The *C. taeniops* specimen collected had a meristic formula with a dorsal fin count of IX + 15; pectoral fin count of 18; pelvic fin count of I + 5; and anal fin count of III + 9. The pectoral fins were longer than the pelvic fin, and their length was



**Fig. 1** Photograph of the first record of *Cephalopholis nigri* from the Mediterranean Sea (collected in July, 2016)



**Fig. 2** Photograph of *Cephalopholis taeniops* voucher specimen analysed in this study (collected in April 2016)

64% the head length. The specimen had 72 scales on the lateral line and 23 gill rakers. The colour of the fish was reddish orange and its body, including the head, was covered in small blue spots. The fins had a darker blue colouration.

**Genetic analyses**

A total of 2026 bp were sequenced from the mtDNA of both specimens. The sequence lengths obtained were 412 bp, 604 bp, 585 bp and 425 bp for cytochrome b

**Table 1** Measurements and meristic counts of the first record of *Cephalopholis nigri* in the Mediterranean Sea and of a *Cephalopholis taeniops* both specimens caught from Maltese waters

Parameter	<i>Cephalopholis nigri</i>		<i>Cephalopholis taeniops</i>	
	Measurements (mm)	% SL	Measurements (mm)	% SL
Total length	140.0		288.0	
Standard length	116.0		247.0	
Maximum body depth	36.9	31.8	81.3	32.9
Length of dorsal fin base	14.6	12.6	113.8	46.1
Pectoral fin base	8.9	7.7	17.8	7.2
Anal fin base	19.1	16.5	43.5	17.6
Pre-pelvic length	41.0	35.3	86.9	35.2
Pre-anal length	77.0	66.4	143.4	58.1
Head length	43.1	37.2	88.1	35.7
Pre-orbital length	10.6	9.1	29.3	11.9
Eye diameter	9.1	7.8	12.7	5.1
	Counts		Counts	
Dorsal fin spines	9		9	
Dorsal fin soft rays	14		15	
Pectoral fin soft rays	14		18	
Pelvic fin spines	1		1	
Pelvic fin soft rays	5		5	
Anal fin spines	3		3	
Anal fin soft rays	8		9	
Scales on lateral line	45		72	
Gill rakers	22		23	

(Cytb), cytochrome c oxidase I (COI), 16S rRNA (16S) and 12S rRNA (12S) genes respectively. Each sequence was run via BLASTn to identify sequence matches.

The two studied specimens were genetically confirmed to species level at the 12S and the 16S genes at >99.3% matches with Craig and Hastings (2007). The 12S matched AY949451 and AY949387, while 16S matched AY947604 and AY947589 for *C. nigri* and *C. taeniops* respectively. Cytochrome B gene of *C. taeniops* also confirmed the species with a 100% match to EF455990-1 specimens from Mauritania (Gonzalez-Sevilla et al., unpublished). It was not possible to compare CytB in *C. nigri* and COI for both species with any other sequences as there is no publically available data for them. This study presents the first sequences for these alien species collected from the Mediterranean Sea.

#### Other specimens

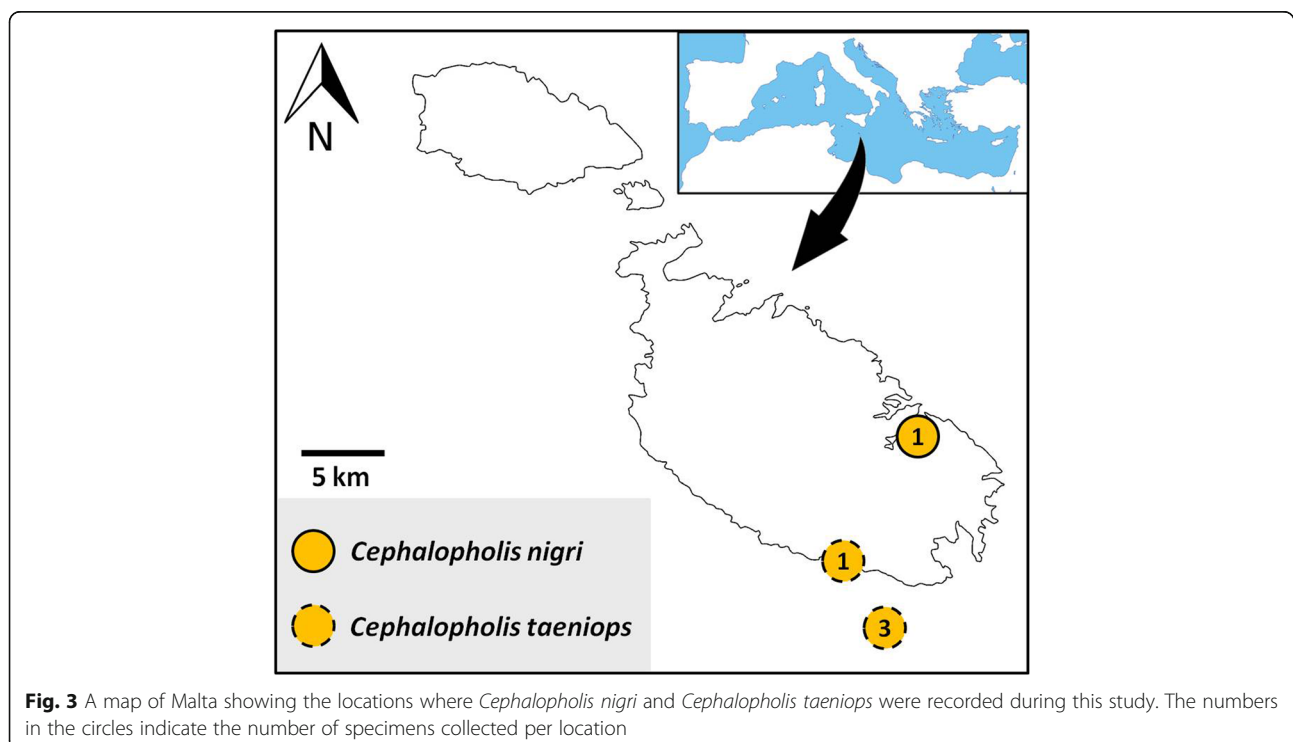
In December, 2015, an additional three specimens of *C. taeniops* have been caught in close proximity to each other (Fig. 4). The three recorded individuals included one specimen with a colouration similar to the one described in Fig. 2, and another two specimens of the less common darker variety (Seret, 1981), one of which can be seen in Fig. 4.

#### Discussion

The number of alien species in the Mediterranean Sea are on the increase (Golani 2010; 2013), including species of both Atlantic and Indo-Pacific origin. In fact

the list of non-native species extends across several taxa, including groupers from the family Epinephelinae. *Cephalopholis nigri* is the latest record of a non-native grouper in the region. The species is not known to be a natural migrant as there are no records of this species elsewhere in the Mediterranean Sea. Moreover, given that Epinephelinae species are highly prized catches, it is very unlikely that records of this species would have gone unnoticed by divers and fishermen. This first Mediterranean record of *C. nigri* is from a busy Maltese harbour which caters for dockyards, oil platform servicing, transshipment activities and berthing of large marine vessels including cruise-liners and super-yachts. Like elsewhere in the region (Galil, 2006; Katsanevakis et al., 2014), such maritime activity can be considered as the main vector in the introduction of alien species. This new record of alien species, follows others from areas that are characterized by intensive marine activity in Malta, such as the first Mediterranean records of *Stegastes variabilis*, *Lutjanus fulviflamma* and *Abudefduf hoefleri* (Vella et al., 2015a, b & 2016a). Nonetheless, one cannot exclude the possibility that *C. nigri* was an aquarium release given that members of the genus *Cephalopholis* are exported as ornamental fish (Monteiro-Neto et al., 2003) and in recent years this industry has led to an increase in alien species within the Mediterranean Sea (Guidetti et al., 2016; Zenetos et al., 2016).

Prior to this study there have only been isolated sighting reports for *C. taeniops* in Maltese waters without the



**Fig. 3** A map of Malta showing the locations where *Cephalopholis nigri* and *Cephalopholis taeniops* were recorded during this study. The numbers in the circles indicate the number of specimens collected per location

analyses of any voucher specimen for this alien grouper species. This study has therefore presented the first scientific morphometric and genetic analyses of this species from Maltese waters, while reporting an increasing number of sightings and photographic records. The four new records of *C. taeniops* indicate the establishment of a population in Maltese waters. Ongoing scientific monitoring would be required to further study the expanding range of this species, although the permanence of its population is threatened by the local fishing industry that is looking at *C. taeniops* as another grouper that may be exploited for commercial means (pers. comm. with fishermen). This latter activity can prove to be beneficial in controlling the spread of this non-native species.

### Conclusion

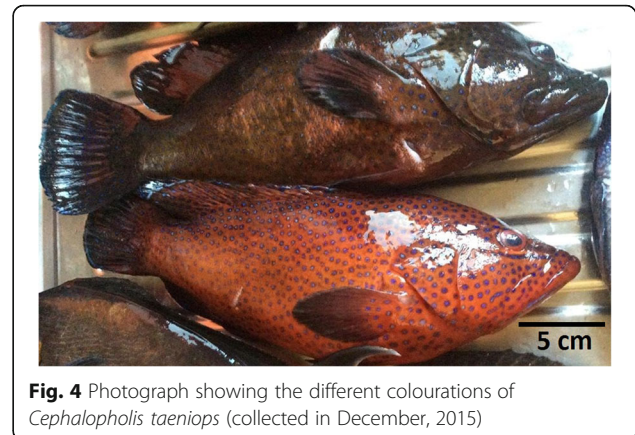
The occurrence and proliferation of these carnivorous species need monitoring as one cannot exclude the possibility that expanding populations of these species might lead to interspecific competition for resources with other already vulnerable native Epinephelinae species in the Mediterranean and in Maltese waters. However, since groupers are species under pressure from over-exploitation these alien species are also being exploited for local consumption, keeping their numbers low.

### Methods

As part of ongoing research with fishermen to study species caught in Maltese waters, voucher specimens of two alien grouper species were collected and analysed morphologically and genetically. On 10th July 2016 a voucher specimen of *C. nigri* (Fig. 1) was caught at 8 m depth from Senglea, Malta [GPS: 35°53'14.26"N, 14°30'55.90"E] (Fig. 3). On 9th April 2016 a voucher specimen of *C. taeniops* (Fig. 2) was caught from Żurriq [GPS: 35°49'8.88"N, 14°27'8.66"E] (Fig. 3). Various sea-users provided sightings and photographic records of *C. taeniops* around Malta (Figs. 2, 3 and 4) since 2015. These two voucher specimens have been deposited in the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta with reference code number CBRG/F.160710/CN001 and CBRG/F.160409/CT001 respectively.

The diagnostic features used in the morphological identification of both specimens followed Heemstra and Randall (1993) and Froese and Pauly (2016). All length measurements were taken to the nearest 0.1 mm using electronic calipers and mass was recorded to the nearest 0.01 g.

DNA was extracted using GF-1 Tissue DNA Extraction Kit (Vivantis Technologies). PCR amplifications were carried out for Cytb, COI and 16S using the primers sets as described in Vella et al., (2016a, b), and for the 12S using H1478 and L1091 primers (Kocher et al., 1989). PCR amplifications were carried out following the amplification



**Fig. 4** Photograph showing the different colourations of *Cephalopholis taeniops* (collected in December, 2015)

protocols described in Vella et al., (2016a). 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 analyzed using Geneious v6 (<http://www.geneious.com>, Kearsse et al., 2012). The sequences obtained were deposited in GenBank, with accession numbers of *C. taeniops* KX758563-6 and *C. nigri* KX758567-70 for Cytb, COI, 16S and 12S respectively. These sequences were compared to other sequences available in genomic databases using BLASTn.

### Abbreviations

12S: 12S rRNA gene; 16S: 16S rRNA gene; COI: Cytochrome c oxidase I gene; Cytb: Cytochrome b gene

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### Availability of data and materials

The genetic sequence data supporting the results of this article are available in GenBank, as accession numbers KX758563-6 (*C. taeniops*) and KX758567-70 (*C. nigri*) for Cytb, COI, 16S and 12S genes respectively. While the voucher specimens CBRG/F.160710/CN001 and CBRG/F.160409/CT001 were collected from Malta and have been deposited in the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta.

### Authors' contributions

NV and AV have contributed to all aspects of the research work presented here including the conception and design of the molecular genetics research, analyses and interpretation of both genetic and morphological data and were involved in finalizing the manuscript. SAD contributed to the morphological research work of the specimens collected. All three authors were involved in the drafting of the manuscript and gave approval for publication.

### Authors' information

All three authors are researchers of the Conservation Biology Research Group, Department of Biology, University of Malta.



**Competing interests**

None of the authors of this paper have financial or non-financial competing interests associated with this research work.

**Consent for publication**

Authors gave consent to publish this MS.

**Ethics approval and consent to participate**

This research did not require any ethics approval or consent to participate.

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