

# First record of two squirrelfishes, *Sargocentron spinosissimum* and *Sargocentron tiereoides* (Actinopterygii, Beryciformes, Holocentridae) from the Egyptian Mediterranean coast

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<http://zoobank.org/C2BAD19D-9895-4C09-A2E8-92600C32E378>

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**Academic editor:** Paraskevi Karachle ♦ **Received** 21 November 2020 ♦ **Accepted** 16 January 2021 ♦ **Published** 31 March 2021

**Citation:** Deef LEM (2021) First record of two squirrelfishes, *Sargocentron spinosissimum* and *Sargocentron tiereoides* (Actinopterygii, Beryciformes, Holocentridae) from the Egyptian Mediterranean coast. Acta Ichthyologica et Piscatoria 51(1): 107–112. <https://doi.org/10.3897/aiep.51.63216>

## Abstract

Holocentrinae and Myripristinae (Holocentridae) are among the most apparent species in the nocturnal reef fish community. However, there is no clear assent regarding their phylogenetic relations, which is reported in their complicated taxonomic history. In this study, *Sargocentron spinosissimum* (Temminck et Schlegel, 1843) and *Sargocentron tiereoides* (Bleeker, 1853) were reported from the Mediterranean coast of Egypt (Damietta coast). This is the first record of these species which is greatly distributed across Indo-Pacific regions and eastern Africa showed the success of these species to migrate to the Mediterranean water with a good adaptation to the new habitats.

In the presently reported study, 26 morphometric measurements were recorded and cytochrome c oxidase subunit I (COI) barcodes were recovered for a total of 20 specimens (8 from *S. spinosissimum* and 12 from *S. tiereoides*).

The specimens from the Damietta coast, Egypt show character states diagnostic of *S. spinosissimum*: Head is curved. Spinous dorsal fin base straight. Soft dorsal fin base slightly raised. Spinous dorsal fin membranes red. Anterior margin of pelvic and anal fins white. Other fin rays are red. While character states diagnostic of *S. tiereoides*: Head is straight and pointed. Spinous dorsal fin base almost straight. Soft dorsal fin base not raised and spinous dorsal fin membranes vaguely red. Sequences of both species were submitted to the GenBank and Barcode of Life Database (BOLD) publication database which displayed 99%–100% similarity value *S. tiereoides* from GenBank and BOLD databases but, *S. spinosissimum* has not any deposited sequences from GenBank or BOLD.

DNA barcoding based on COI gene was demonstrated as a powerful and useful molecular marker in the identification and differentiation of *S. spinosissimum* and *S. tiereoides* fish species.

## Keywords

COI gene, DNA barcoding, fish, Holocentrinae, phylogeny, taxon

## Introduction

The squirrelfishes and soldierfishes (family Holocentridae Bonaparte, 1833) are widespread from tropical to warm temperate waters in shallow water on coral reefs or rocky

bottoms. The Holocentridae is divided into two subfamilies, Holocentrinae Bonaparte, 1833 and Myripristinae Nelson, 1955, based on the communication between the swim bladder and skull (Nelson 1955). Woods and Sonoda (1973) recognized these subfamilies in a recent review

of the western Atlantic holocentrids. These two stems are recognized in fossil beryciforms (see Dunkle and Olsen 1959) and fossil otoliths (Frizzell and Lamber 1961; Lamber unpublished<sup>1</sup>). Woods (1955) classified Holocentrinae into four subgenera: *Holocentrus* Scopoli, 1777, *Flammeo* Jordan et Evermann, 1898, *Sargocentron* Fowler, 1904, and *Adioryx* Starks, 1908. *Flammeo* has been displaced to synonymy with *Neoniphon* Castelnau, 1875, due to the uncertain status of the type species for *Neoniphon* (see Woods 1955). Then, *Adioryx* was elevated to the generic level (Woods 1965). However, Randall and Heemstra (1985, 1986) recognized *Flammeo* as a junior synonym of the *Neoniphon* depending on the identification of the type species of *Neoniphon* as *Neoniphon sammara* (Forsskål, 1775).

Similarly, Matsuura and Shimizu (1982) reported that the species of *Adioryx* and *Sargocentron* could not be identified by features of the swim bladder and auditory bulla, and then they classified all *Adioryx* as *Sargocentron*. Hubert et al. (2010) took eight *Sargocentron* species and one species of *Neoniphon* for the mtDNA cytochrome c oxidase subunit I (COI) gene and postulated that *Sargocentron* is paraphyletic to *Neoniphon*. Moreover, phylogenetic analyses of rhodopsin amino acid sequences resolved a paraphyletic *Sargocentron* to *Neoniphon* (see Yokoyama and Takenaka 2004; Yokoyama et al. 2008).

Genus *Sargocentron* has a great diversity among its species, which belongs to the family Holocentridae known as squirrelfish. This genus includes about 33 species (Froese and Pauly 2019, eight of which were found in the Red Sea (Golani and Bogorodsky 2010). *Sargocentron rubrum* (Forsskål, 1775), is one of the oldest migratory Red Sea species that have entered the Mediterranean Sea via the Suez Canal (Golani and Ben-Tuvia 1985). Until recently, *Sargocentron rubrum* was considered to be the only representative of the squirrelfish family in this basin, with a single record from Libyan waters (Štirn 1970). *Sargocentron rubrum* has been recorded from Egyptian waters (Ibrahim and Soliman 1996; Alwany 2011; Bakhroum 2018; Farrag et al. 2018), and it was mentioned in the marine ichthyofauna of Egypt (Akel and Karachle 2018). *Sargocentron* was proved as a genus without comment (Fowler 1944). Starks (1908) realized differences in the formation of the swim bladder with respect to the back of the skull in several holocentrids and then separated *Holocentrus adscensionis* (Osbeck, 1765) from *Holocentrus suborbitalis* Gill, 1863, grouping the latter into a new genus, *Adioryx*. Whitley (1933) added two subgenera to *Holocentrus*: *Faremusca* Whitley, 1933 for the Indo-Pacific *Holocentrus punctatissimum* Cuvier, 1829 and *Cephalofarer* Whitley, 1933 for the western Atlantic species *Holocentrus vexillarium* Poey, 1860. In this study, *Sargocentron spinosissimum* (Temminck et Schlegel, 1843) and *Sargocentron tiereoides* (Bleeker, 1853) were reported for the first time from the Mediterranean coast of Egypt (Damietta coast). These species which are greatly distributed across Indo-Pacific regions and eastern Africa showed the successful of these species to migrate to the Mediterranean water with a good adaptation to the new habitats.

## Methods

### Study area and sample collection

Twenty specimens of the North Pacific squirrelfish, *Sargocentron spinosissimum*, and the pink squirrelfish *Sargocentron tiereoides*, (Fig. 1A, B) were collected from the Damietta coast of the north of Egypt, in the south-eastern part of the Mediterranean Sea at 31°46'48.0"N, 31°40'48.0"E.

### Morphological data

Twenty-six morphometric measurements were recorded with vernier calipers to the nearest 0.05 mm. Body proportions were expressed in percentage of standard length (SL). All measurements are presented in Table 1 and abbreviations for measurements are as follows: Total length (TL); Fork length (FL); Standard length (SL); Prepectoral fin length (PPL); Predorsal fin length (PDL); Prepelvic fin length (PVL); Preanal fin length (PAL); Caudal peduncle length (CPL); Head length (HL); Body depth (BD); Eye diameter (ED); Preorbital length (POL); Pectoral fin base length (PBL); Dorsal fin base length (DBL); Pelvic fin base length (VBL); Anal fin base length (ABL); Caudal fin length (CL). Pectoral fin length (PL); Dorsal fin length (DL); Pelvic fin length (VL); Anal fin length (AL); Pectoral fin count (PC); Dorsal fin count (DC); Pelvic fin count (VC); Anal fin count (AC); Lateral line scale (LS).

### DNA extraction PCR amplification and sequencing

Liver tissue was obtained from *Sargocentron* species, then preserved in 95% alcohol and stored in a deep freezer at -4°C, where the *Sargocentron* spp. DNA was extracted using a GeneJET kit Genomic DNA Kit#K0721 following the manufacturer's protocol.

Cytochrome c oxidase subunit I gene was amplified using primers FF-5' TTC TCC ACC AAC CAC AAR GAY ATY GG -3' and FR-5' CAC CTC AGG GTG TCC GAA RAA YCA RAA -3' (Ivanova et al. 2007). The polymerase chain reactions (PCR) consisting of approximately 50 ng of template DNA were carried out in volumes of 15 µL with 1 × PCR Buffer, 2 mM MgCl, 0.5 µM of each FF and FR, 0.2 mM of dNTP, and 0.6 U of Taq DNA Polymerase. The thermal program started with an initial denaturation at 94°C for 5 min, followed by 10 cycles of 1 min at 94°C, 30 s at 60°C, 1 min at 72°C, and a final extension of 5 min at 72°C.

PCR product was visualized in a 2.0% agarose gel stained with ethidium bromide and photographed under UV transillumination. PCR product was purified using a GeneJET kit (Thermo K0701) according to the manufacturer's recommendations. A purified PCR sample was sent for sequencing to a GATC Company in England that uses an ABI 3730xl DNA sequencer.

<sup>1</sup> Lamber CK (1963) Fossil and recent beryciform otoliths: an adjunct to ichthyological classification. M. S. Thesis, Univ. Rolla, 134.

## Molecular analysis

The resulting sequences were confirmed as being derived from *Sargocentron* species DNA using the GenBank Blast algorithm. The DNADynamo software version 1.459 was used for editing the sequences and they were aligned using Clustal W. Finally, the phylogenetic analyses used were Maximum Evolution, Neighbor Joining, and Maximum Likelihood in MEGA 6.0 software (Tamura et al. 2013).

## Results

Fish species belonging to the family Holocentridae were collected and identified based on traditional morphotaxonomy and further confirmed by molecular marker using DNA sequencing. In this study, it was recorded for the first time two different fish species such as; eight specimens of *Sargocentron spinosissimum* and twelve specimens of *Sargocentron tiereoides* of the order Beryciformes, from the Damietta coast. The morphometric as well meristic data of both species were presented in Table 1.

**Table 1.** Morphometric and meristic counts of *Sargocentron spinosissimum* ( $n = 8$ ) and *Sargocentron tiereoides* ( $n = 12$ ), collected from the Damietta coast, Egypt.

	<i>Sargocentron spinosissimum</i>	<i>Sargocentron tiereoides</i>
Morphometric variables	Measurement [mm]	
Total length (TL)	172	186
Fork length (FL)	149	152
Standard length (SL)	134	130
Head length (HL)	42	47
Body depth (BD)	50	53
Eye diameter (ED)	17.5	15
Pre orbital length (POL)	8	17
Pre dorsal length (PDL)	48	59
Pre pectoral length (PPL)	44	60
Pre pelvic length (PVL)	51	67
Pre anal length (PAL)	101	122
Dorsal fin base length (DBL)	76	87
Pectoral fin base length (PBL)	7.6	6
Pelvic fin base length (VBL)	7	10
Anal fin base length (ABL)	17	21
Dorsal fin length (DL)	95	100
Anal fin length (AL)	26	29
Pectoral fin length (PL)	19	35
Pelvic fin length (VL)	24	34
Caudal fin length (CL)	38	56
Caudal peduncle length (CPL)	11	14
<b>Relative characters:</b>		
SL/BD	2.86	2.45
SL/HL	3.19	2.76
SL/ED	7.65	8.66
SL/PDL	2.79	2.20
SL/PAL	1.32	1.06
HL/ED	2.4	3.1
<b>Meristic counts</b>		
Dorsal fin count (DC)	XI + 13	XI + 14
Anal fin count (AC)	IV + 9	IV + 9
Pelvic fin count (VC)	I + 7	I + 8
Pectoral fin count (PC)	14	14
Lateral line scale (LS)	36	40

## Key to the subfamilies

Holocentrinae has anal fin rays 7–10 (soft rays). A stout long spine was found in the angle of preopercle which is longer than its width while, Myripristinae species have anal fin rays more than 10 (soft rays). Angle of preopercle without spine in adult (Atlantic species *Corniger spinosus* Agassiz, 1831 has one or two strong spines).

## Key to the genera

Last dorsal fin spine located at middle between the first dorsal fin ray and the penultimate dorsal fin spine in *Sargocentron*. In *Neoniphon* last dorsal fin spine located nearer to the first dorsal fin ray than the penultimate dorsal fin spine.

## Morphological data

The following morphometric characters of *Sargocentron spinosissimum* were registered: scales are very rough ctenoid and large; ridges and mucous channels dorsally on rounded red head and edges of external bones of head serrate or with spines. Opercle with two sharp spines, almost equal in length. Preopercular spine moderate, about a half of orbit; very large eyes; dorsal fin with 11 spines and 12 soft rays; 13 anal fin rays (4 spines and 9 soft); 14 pectoral fin rays; pelvic fin with 1 spine and 7 soft rays; standard length 134 mm; fork length 149 mm; head length 42 mm; eye diameter 17.5 mm. Body depth 50 mm; length of caudal peduncle length 11 mm; length from tip of snout to origin of dorsal fin 48 mm; length from tip of snout to origin of pectoral fin 44 mm; length from tip of snout to origin of anal fin 101 mm; length from tip of snout to origin of pelvic fin 51 mm. Base of dorsal fin 76 mm; base of pectoral fin 7.6 mm; base of pelvic fin 7 mm. Base of anal fin 17 mm. Caudal fin forked. Morphometric data are given as percentages of standard length in Table 1. Coloration of body is brilliant reddish. Body with 9 red stripes alternating with 9 narrower white stripes that pass along the middle of the longitudinal scale rows; head is red with a white bar along the posterior margin of preopercle (Fig. 1A).

The following morphometric characters of *Sargocentron tiereoides* was registered: scales are very rough ctenoid and large; ridges and mucous channels dorsally on rounded red head and edges of external bones of head serrate or with spines. Opercle with two spines, the upper slightly longer. Preopercular spine long, slightly longer than two-thirds of orbit; very large eyes; dorsal fin with 11 spines and 14 soft rays; 13 anal fin rays (4 spines and 9 soft); 14 pectoral fin rays; pelvic fin with 1 spines and 8 soft rays; standard length 130 mm; fork length 152 mm; head length 47 mm; eye diameter 15 mm. Body depth 53 mm; length of caudal peduncle length 14 mm; length from tip of snout to origin of dorsal fin 59 mm; length from tip of snout to origin of pectoral fin 60 mm; length from tip of





**Figure 1.** *Sargocentron* species, (A) *Sargocentron spinosissimum*, 134 mm SL and (B) *Sargocentron tiejeoides*, 130 mm SL, specimens caught from Coast of Damietta, Egypt

snout to origin of anal fin 122 mm; length from tip of snout to origin of pelvic fin 67 mm. Base of dorsal fin 87 mm; base of pectoral fin 6 mm; base of pelvic fin 10 mm. Base of anal fin 21 mm. Caudal fin forked. Morphometric data are given as percentages of standard length in Table 1. Coloration of body is brilliant reddish orange. Body with 9 reddish orange stripes alternating with 9 narrower white stripes that pass along the middle of the longitudinal scale rows; head is reddish with a white bar along the posterior margin of preopercle (Fig. 1B).

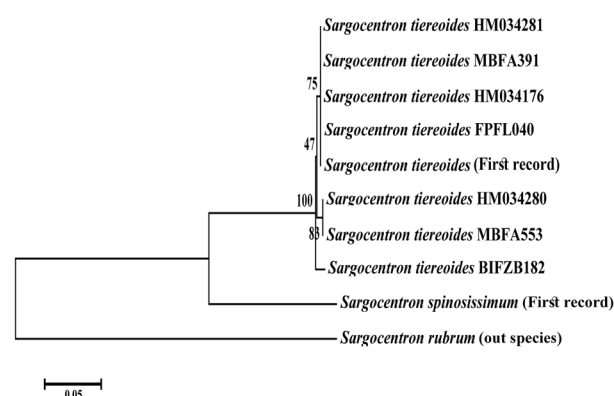
## Sequencing analysis

Cytochrome c oxidase subunit I (COI) barcodes were recovered for a total of twenty specimens (eight from *Sargocentron spinosissimum* and twelve from *Sargocentron tiejeoides*) of the family Holocentridae. No insertions/deletions, heterozygous sites or stop codons were discovered, accepting the view that all of the amplified sequences form functional mitochondrial COI sequences. BLAST outcomes of all nucleotide sequences succeeded to identify sequence similarity of *Sargocentron tiejeoides* species under study. While *Sargocentron spinosissimum* has not any similarity of sequence deposited in GenBank. The COI sequence analysis of *Sargocentron tiejeoides* resulted the average nucleotide frequencies as 25.7% (A), 30.3% (T), 26.9% (C), and 17.1% (G). Similarly, in *Sargocentron spinosissimum* the nucleotide frequencies are 25.8% (A), 29.8% (T), 26.9% (C) and 17.6% (G).

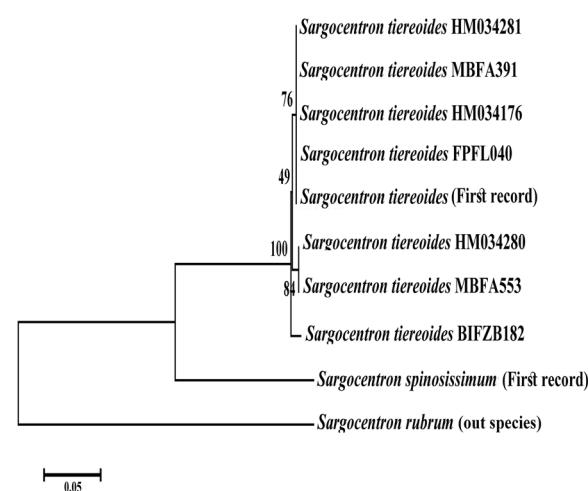
Phylogenetic analysis revealed a well-determined hypothesis of relations at the species level. Overall Maximum Evolution, Neighbor Joining, and Maximum Likelihood trees analyses (Figs 2–4 respectively), new sequences from

the two species grouped in different clusters. Moreover, where applicable, the sequences from the same species (newly obtained in this study and deposited from Barcode of Life Database (BOLD) and GenBank) were grouped, resulting in homology and more or less conspecific distances between them. Furthermore, sequences from *Sargocentron tiejeoides* species retrieved from National Center for Biotechnology Information (NCBI) grouped in the same cluster.

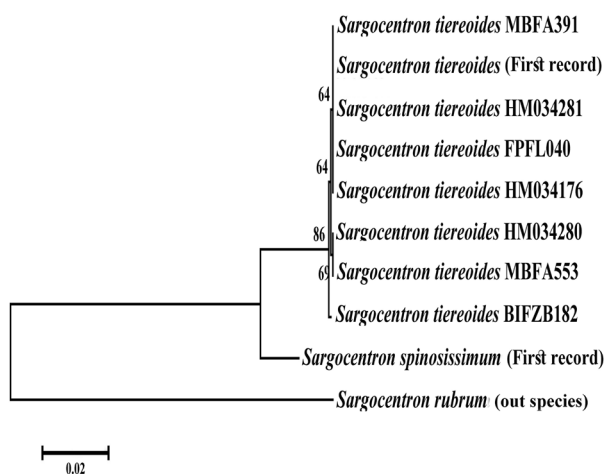
The phylogenetic trees generated through Maximum Evolution, Neighbor Joining, and Maximum Likelihood analyses shows same topology (Figs 2, 3, 4). Sequences from above specimens of *Sargocentron tiejeoides* were submitted to the NCBI GenBank Barcode database and BOLD with accession and BIN numbers HM034176, HM034280, HM034281, BOLD: BIFZB182, FPFL040, MBFA391 and MBFA553 respectively.



**Figure 2.** Maximum Evolution phylogenetic of COI variation for reconstruction for *Sargocentron spinosissimum* and *Sargocentron tiejeoides* using Kimura 2-Parameter distances and values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.



**Figure 3.** Kimura 2-Parameter distances Neighbor Joining tree of COI variation for *Sargocentron spinosissimum* and *Sargocentron tiejeoides*. Values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.



**Figure 4.** Phylogenetic tree of Maximum Likelihood analyses based on COI gene from *Sargocentron spinosissimum* and *Sargocentron tiereoides* using Kimura 2-Parameter distances and values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.

## Discussion

Species of the genus *Sargocentron* have compressed and an elongated body with thin caudal peduncle; ridges and mucous channels dorsally on head and have very large eyes. They have a single spine in pelvic fin with soft rays 5–8. Although dorsal fin is long with 10–13 spines but, a notched soft-rayed part is found with 11–17 rays. Anal fin has four spines and 7–16 soft rays. Forked caudal fin has 18 or 19 rays. Scales are very rough ctenoid and large. The majority of them are brilliant reddish in color. Usually are nocturnal. Usually cryptic during the day in beneath ledges of reefs or crevices. *Sargocentrons* feed on worms and small fishes (Randall 1998).

The specimens from the Damietta coast, Egypt show character states diagnostic of *Sargocentron spinosissimum*: Head is curved. Spinous dorsal fin base straight. Opercle with two sharp spines, almost equal in length. Preopercular spine moderate, about a half of orbit. Soft dorsal fin base slightly raised. Spinous dorsal fin membranes red. Anterior margin of pelvic and anal fins white. Other fin rays are red.

On the contrary, opercle with two sharp spines, the upper longer. Preopercular spine long, about two-thirds of orbit in *Sargocentron rubrum* and its ground color of head and body crimson to violet. Each longitudinal body scale row with a wide white stripe. Dorsal fin spines red. Spinous dorsal fin membranes white. Anterior margin of soft dorsal fin red, the rest of fin yellow. The doubt on the distinction between *Sargocentron spinosissimum* and *Sargocentron rubrum* was the reason for Re-examination of *Sargocentron spinosissimum* indicates that this species has spinules on the posterior portion of the nasal bone. On the other hand, the posterior portion of the nasal bone in *Sargocentron rubrum* is quite smooth without any supination. Therefore, both are regarded as distinct species.

While character states diagnostic of *Sargocentron tiereoides*: Head is straight and pointed. Spinous dorsal fin base almost straight. Opercle with two spines, the upper slightly longer. Preopercular spine long, slightly longer than two-thirds of orbit. Soft dorsal fin base not raised and spinous dorsal fin membranes vaguely red. Besides morphology, morphological measurements are agreed to Shimizu and Yamakawa (1979) which aid in identifying these *Sargocentron* species.

DNA barcoding is a recent and greatly used molecular-based recognition system that aims to recognize biological specimens. Studies on the biodiversity of coral reefs inevitably require taxonomic coverage. DNA barcoding is a method for taxonomic identification of organisms that is entirely based on the 5' portion of the mitochondrial gene cytochrome oxidase subunit I (COI-5). It can be a beneficial gene for identification of fish species (Neigel et al. 2007). Under any form of anthropogenic change, species will have to move, adapt or die. Progress in genetic studies of adaptation until recently had been constrained by the lack of resolution and absence of genomic perspective. Genetic tools can give us crucial insights into these processes. At the largest scale, molecular tools can identify cryptic species and their introductions, as in this study.

The presently reported study is based on the first occurrence of two fish species *Sargocentron spinosissimum* and *Sargocentron tiereoides* from the Damietta coast, Egypt. DNA barcoding uses a genetic marker (often a single gene) to assign an individual to a particular known species. It has also been suggested that barcoding can be used to identify unknown species based on the expectation that interspecific genetic divergence considerably exceeds intraspecific variation to form a clear “barcode gap”. In addition to that, these fish species have never been reported earlier, hence this happens to be the first record from the Damietta coast, Egypt.

Ward et al. (2009) revealed that the simplest method of seeking the recognition of an unknown specimen is to place its cytochrome b sequence into the BOLD identification engine. In the presently reported study, COI gene sequence was corresponding with *Sargocentron* species base which displayed 99%–100% similarity value *Sargocentron tiereoides* from GenBank database but, *Sargocentron spinosissimum* has not any deposited sequences from GenBank or BOLD. Overall COI constructed Maximum Evolution, Neighbor Joining, and Maximum Likelihood trees placed *Sargocentron spinosissimum*, *Sargocentron tiereoides* and *Sargocentron rubrum* (as out species of this study) in three different clades due to these are three distinct species. While *Sargocentron tiereoides* and all deposited COI sequences from GenBank and BOLD formed high bootstrap-supported clusters without any overlap between species.

## Conclusion

This study contains novel findings of *Sargocentron spinosissimum* species that is distributed Northwest Pacific:

southern Japan to Taiwan; also reported from Hawaii and *Sargocentron tiereoides* is distributed in Indo-Pacific regions and East Africa showed the success of the migration of these species to the Mediterranean water with a

good adaptation to the new habitats. In this work, DNA barcoding based on the COI gene was demonstrated as a powerful and useful molecular marker in identifying fish species.

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