AMERICAN UNIVERSITY OF BEIRUT

INVESTIGATING THE GENETICS OF THE INVASIVE SOLDIERFISH *SARGOCENTRON RUBRUM* IN THE MEDITERRANEAN SEA

by
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ABSTRACT
OF THE THESIS OF

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Title: Investigating the Genetics of the Invasive Soldierfish *Sargocentron rubrum* in the Mediterranean Sea

The opening of the Suez Canal led to the influx of marine organisms from the Indo-Pacific to the Mediterranean Sea (Lessepsian migration). A significant number of Lessepsian species have already established large populations in the region, and many are exhibiting invasive behavior. One of which, *Sargocentron rubrum* has established large populations in most of the eastern basin, particularly the Levant. This species has a vast native geographic range in the Indo-Pacific realm, from the Red Sea to New Caledonia.

In this work, *Sargocentron rubrum* individuals were collected from Lebanon and Turkey. They were genetically barcoded for COI mitochondrial gene. Phylogenetic trees were constructed by including available sequences from online databases and genetic distances were calculated.

Phylogenetic trees showed that the species known as *Sargocentron rubrum* (Forsskål, 1775) formed two distinct clusters, where one of which represents samples from the Mediterranean Sea and the Red Sea. The second cluster includes samples from the Indian Ocean, as well as some from the Mediterranean coast of Turkey. Several hypotheses have been proposed to explain the results from this study, as solid conclusions cannot be drawn due to the limiting data for *S. rubrum*.

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CHAPTER I

INTRODUCTION

A. Mediterranean Sea

The Mediterranean Sea is a body of water connected to the Atlantic Ocean at the Strait of Gibraltar, enclosed by narrow continental shelves. It covers an area of 2,969,000 km^2 and has an average depth of 1,460 m, making it the largest and deepest sea on the planet (Coll *et al.*, 2010; 2011). The Mediterranean is divided into two main basins, known as the Western and Eastern Mediterranean, separated by the Strait of Sicily. In each basin, several regions are delineated, such as the Ionian or Levantine seas (Astraldi *et al.*, 1999).

1. Messinian Salinity Crisis

Looking back a few million years, the present Mediterranean Sea had been under extreme geological pressures which resulted in a catastrophic incident known as the Messinian Salinity Crisis. The start of this event occurred in the late Miocene around 5.96 million years ago (Mya), when the Mediterranean Sea became isolated from the eastern Atlantic Ocean. This resulted in a drop in sea levels and a tectonic uplift of one kilometer at the presently known Strait of Gibraltar (Duggen *et al.*, 2003; Govers, 2009; Garcia-Castellanos & Villaseñor, 2011). Severance from the Atlantic Ocean not only changed the physical geography of the sea, but also completely altered its water chemistry, which consequently had a tremendous impact on its marine ecosystems (Roveri *et al.*, 2014). One of which is the desiccation of the Mediterranean basin due to its high evaporative rate, subsequently transforming it into a series of brackish lakes within a relatively short period of time (less than 700,000 years) (Carnevale *et al.*, 2008; Roveri *et al.*, 2014). Towards the end of the crisis, euryhaline species, including fishes, were able to inhabit these brackish lakes, which were presumably being enriched via drainage from European rivers and streams (Hsü *et al.*, 1977; Carnevale *et al.*, 2006).

2. Biodiversity

Around 5.33 Mya, the Messinian Salinity Crisis then ended with the opening of the Strait of Gibraltar, followed by an influx of Atlantic water into the desiccated basin (Loget *et al.*, 2005; Gargani & Rigollet, 2007). Consequently, the Mediterranean changed yet again due to the arrival of marine biota from the Atlantic. As such, its marine life is currently similar to the eastern Atlantic, and today this biota is considered native to the present Mediterranean Sea (Coll *et al.*, 2010; Bianchi *et al.*, 2012). The Mediterranean Sea holds more than 17,000 marine species, which accounts for about 7% of the world's biodiversity (Coll *et al.*, 2011; Mannino *et al.*, 2017). A decreasing gradient in species richness runs from the western to the eastern parts of the Mediterranean basin (Katsanevakis *et al.*, 2014). One explanation for this is that the Atlantic Ocean is closer in proximity to the western basin and the eastern Mediterranean is oligotrophic (Mannino *et al.*, 2017). Moreover, human activity is a recent issue affecting the biodiversity of the Mediterranean. While overexploitation and habitat loss are the main drivers of change, pollution, coastal urbanization and species introductions are just as important (Coll *et al.*, 2011; Mannino *et al.*, 2017). Climate change may also act as a catalyst for the transformation of the Mediterranean biodiversity, especially since surface water temperatures have been rising for the past two decades

(Katsanevakis *et al.*, 2014), affecting both abiotic and biotic entities. On the long run, this may influence biochemical cycles, water flow and circulation patterns, indirectly altering dispersal abilities of marine organisms (Bianchi & Morri, 2003; Katsanevakis *et al.*, 2014). Sea warming impacts all biological levels, ranging from members of a species to the whole ecosystem (Moullec *et al.*, 2019). Survival rate, reproductive success, dispersal patterns and behavior are factors directly dependent on climate (Bianchi & Morri, 2003), and organisms may ultimately cope with rising temperatures by shifting their biogeographical ranges (Burrows *et al.*, 2011). The most notable consequence of climate change is the process of "tropicalization" in the Mediterranean. This trend is characterized by the general poleward shift of thermophilic organisms due to increased sea temperatures (Bianchi *et al.*, 2017). These range shifts typically move from the southeastern towards the northwestern sector in the Mediterranean Sea as a result of expanding temperature gradients, which normally play a role in defining biotic boundaries (Lo Brutto *et al.*, 2011). This in turn favors most native and non-native species found in lower latitudes to inhabit northern regions of the sea, due to their tropical tendencies and eurythermal nature (Azzurro *et al.*, 2011; Katsanevakis *et al.*, 2014). Given that the rise in sea temperature continues, it is possible that biotic homogenization of native flora and fauna will be observed, where the distinct biodiversity between the two Mediterranean basins will gradually disappear in the near future (Bianchi *et al.*, 2017). On the other hand, cold water species present in the Mediterranean, may not have the capabilities to adapt, eventually leading to their extinction (Mannino *et al.*, 2017).

B. Suez Canal and Lessepsian migration

Geologically, the ancient Mediterranean was also once connected to the presently known Indian Ocean mainly through the Mesopotamian Seaway (Bialik *et al.*, 2019), where it harbored tropical marine biota (Bianchi *et al.*, 2012). However around 13.6 Mya, the formation of the Isthmus of Suez closed this connection. As the Messinian Salinity Crisis progressed, almost all the tropical biota that once inhabited the Mediterranean slowly diminished to extinction. At the end of the crisis, rather than being recolonized by tropical marine species that once flourished there, species of Atlantic origin repopulated the Mediterranean basin due to the opening of the Strait of Gibraltar (Por, 2009; Mannino *et al.*, 2017). It was not until the fifth Century B.C. when efforts were made to connect the Mediterranean to the Indian Ocean (Galil, 2006). This started with Necho II, the king of Egypt (609-594 B.C.), who was the first to venture in building a canal into the Red Sea. There have only been a few records of successful attempts, namely by the Persian King, Darius the Great (521-486 B.C.), and the Egyptian pharaoh Ptolemy II Philadelphus (285-246 B.C.), as most scholars advised against connecting the two water bodies. Since the Red Sea had a higher sea level than the Mediterranean, they believed that it could potentially flood Egypt if a canal were to be constructed (Galil, 2006).

The French engineer Ferdinand de Lesseps was the first to successfully create a functioning waterway cutting through Egypt, commonly known as the Suez Canal, in 1869 (Galil, 2006). This canal was constructed to facilitate international trade, connecting the Far East to Europe and providing a much shorter alternative than the usual shipping route via the Cape of Good Hope (Schøyen & Bråthen, 2011; Essallamy *et al.*, 2019).

Considered the longest marine corridor in the world, the 193-km long Suez Canal linked two major biogeographical regions, the eastern Atlantic and the Indo-west Pacific (Dávid & Piala, 2016; Sabonge & Sánchez, 2020). Consequently, it served as a pathway for non-native species of Indo-Pacific origin to enter the Mediterranean from the Red Sea (Katsanevakis *et al.*, 2013). Reports of Red Sea species in the Mediterranean started in 1902, about 30 years after the canal's construction (Arndt & Schembri, 2015). At first, this problem was insignificant since the canal passed through two major hypersaline lakes, the Great Bitter Lake and the Little Bitter Lake, which may have acted as barriers against the movement of species. However, the salinity of the Bitter Lakes gradually decreased over time (from 70 ppt to 43-48 ppt) (Spanier & Galil, 1991). Moreover, the construction of the Aswan Dam in the 1970's reduced the Nile River's outflow, which resulted in increasing the salt concentration of the northern section of the canal (Bianchi, 2007). The loss of the saline barriers, as well as the input of freshwater, homogenized the overall salinity across Suez Canal, which enabled the influx of marine species from the Red Sea to the Mediterranean (Por, 1978; Bianchi, 2007). It was Por (1978) who first named this movement as "Lessepsian migration" after Ferdinand de Lesseps and defined it as the "unidirectional migration of the Red Sea species to the Mediterranean via the Suez Canal". Over the years, the Suez Canal was continuously widened and deepened, and by the end of 2015, an additional waterway was opened (Galil *et al.*, 2017; Mannino *et al.*, 2017).

Boudouresque (1999) proposed that one reason behind the arrival of Lessepsian migrants is the relatively low species diversity in the eastern Mediterranean basin, as opposed to the species-rich Red Sea. However, crossing the Suez Canal does not necessarily ensure the success of the species, since there are several characteristics that are correlated with the settlement and spread of Lessepsian migrants in the Mediterranean (e.g., Belmaker *et al.*, 2013; Samaha *et al.*, 2016).

The Suez Canal is considered as one of the principal vectors for introduced non-native species, however there are other pathways that are also relatively important (Galil *et al.*, 2018). These include water ballast and the ornamental aquarium trade, which are among the most significant (e.g., Galil *et al.*, 2018; Katsanevakis *et al.*, 2013; Ojaveer *et al.*, 2018).

Human-mediated introductions have been documented and recognized as significant threats to marine ecosystems (Ojaveer *et al.*, 2018). Introduced species, especially Lessepsian migrants, have reshaped the native biodiversity of the Mediterranean Sea. Opposite to their endemic counterparts, these non-native species form a gradient along the sea in which a higher richness is present in the eastern basin compared to the western basin, mainly due to its connection to the Red Sea (Katsanevakis *et al.*, 2014; Mannino *et al.*, 2017). There have been more than 800 records of marine non-native species, where almost half of which are believed to be Lessepsian migrants (Galil *et al.*, 2015; Bonanno & Orlando-Bonaca, 2019).

C. Invasive Species

Invasive species are defined as introduced species that have established a large population in a new environment, where they may bring negative impacts at both ecological and socio-economic levels (Lee, 2002). Ecological interactions, either being direct (i.e., competition, predation, symbiotic relationships) or indirect (i.e., habitat alteration, trophic cascades), could eventually restructure the population biology of native species or the invaded habitat (Sakai *et al.*, 2001). Such events may result in the displacement of native species from their ecological niches (Bariche *et al.*, 2004). Resource availability can also be distorted by invasive species, possibly changing communities, where the relative abundance of several species may either be suppressed or intensified (Didham *et al.*, 2005), and may eventually lead to some evolutionary changes. Surprisingly, there has yet to be any evidence of extinctions from the observed reduction of local populations in the Mediterranean, and due to the rising number of introductions, the species richness in the basin is increasing (Azzurro *et al.*, 2014).

Typically, there is a substantial period of time between the arrival of an introduced species and the establishment and expansion of the population. Lag times may be necessary for successful invasions, as they provide the required time for the adaptation and invasive characteristics to a new habitat (Sakai *et al.*, 2001). These could be dependent on the ecological status of the new environment, as well as the invader's innate capability of evolutionary adaptation. Nevertheless, a series of variables, whether biotic or abiotic, determine the success of an invasive species (Bernardi *et al.*, 2010). Moreover, several stochastic introductions are often associated with the success of establishment (Sakai *et al.*, 2001; Lee, 2002), and it is possible that newer waves of invasions replenish existing populations, making it difficult to determine a specific time of the initial introduction (Bernardi *et al.*, 2010).

Since invasions often involve genetically dynamic events, both native and invasive populations are capable of evolutionary change either from natural selection or genetic drift. Responses to ecological stress, such as climate, photoperiod or interspecific interactions, often involve changes in morphology, physiology, phenology or plasticity, and the lack of adaptation to these pressures are believed to be reasons as to why many invasions have failed. A decrease or absence of competitive forces and other interspecific interactions is advantageous for invasive species during establishment, as it may facilitate their ability to exploit resources and dominate local communities (Sakai *et al.*, 2001; Lee, 2002). With a relatively “vacant” or “unsaturated” sea such as the Mediterranean, invasive species would easily have the opportunity to occupy the ecological space available. These “niche opportunities” would be suitable for those invasive species possessing traits already adapted to the specific habitat in question (Azzurro *et al.*, 2014). Source populations with high levels of genetic variation often carry diverse traits that facilitate invasions. One is phenotypic plasticity, which is common among invading species and desirable for colonization, as it enables species to tolerate a wide range of new and unfamiliar environmental conditions (Sakai *et al.*, 2001; Lee, 2002). This form of genetic drift mainly involves small colonizing populations and may actually reduce the overall genetic variation once established. This could have consequences, one being the limitation of population growth due to inbreeding depression, which could lower the overall probability of the population’s survival. Another ramification is the restrictions concerning the evolution of a population due to the decline of genetic diversity. Invasive species preadapted only to a specific environment would not be able to expand and establish in different habitats. With this in mind, it is safe to say that adaptive evolution is just as important as the initial colonization process for invasive species. If gene flow between invading populations were to occur, invasive genotypes would be able to spread. However, locally beneficial alleles could dissipate and prevent a species’ invasiveness in a specific habitat (Lee, 2002).

D. Molecular Techniques

Today, molecular techniques have become commonly used instruments for phylogenetic and phylogeographic studies, as well as taxonomic identifications. In particular, DNA barcoding is a procedure that has a novel approach in the classification of species. Short DNA sequences were recommended to be utilized for the purpose of species identification and recognition, eventually enabling the classification of unknown specimens (Bucklin *et al.*, 2011). This technique was refined by Hebert *et al.* (2003), who proposed that the mitochondrial cytochrome c oxidase I gene (COI) should be the standard sequence used for DNA barcodes. The use of mitochondrial genomes is preferred over nuclear genomes for analysis due to their lack of introns and haploid nature, therefore preventing the chances of recombination. Moreover, universal primers for COI are very resilient, allowing vast coverage from the 5' end of the sequence in almost all animal taxa. The COI gene also seems to have the strongest phylogenetic signal out of any other mitochondrial gene, since it has a relatively rapid molecular evolution that results in several variations observed in numerous species, exhibiting detectable divergences even within intraspecific phylogeographic groups (Hebert *et al.*, 2003).

DNA barcoding has been crucial when linking life stages and dealing with cryptic species, therefore surpassing the capabilities of taxonomy in these cases. Several thousands of DNA barcode records have been uploaded on online databases (e.g., GenBank (NCBI, 1988), The Barcode of Life Data System (Ratnasingham & Hebert, 2007) for the general public, and this library is continuously growing. This accessible data can be easily obtained and used for an array of molecular analyses (Hebert & Gregory, 2005; Bucklin *et al.*, 2011).

In a study by Bariche *et al.* (2015), Lessepsian fishes from the eastern Mediterranean (Lebanon) were collected and sequenced in order to create a DNA barcode reference library. In their findings, two species, namely *Sargocentron rubrum* (Forsskål, 1775) and *Plotosus lineatus* (Thunberg, 1787), showed high genetic divergence between Indo-Pacific and Mediterranean individuals. Interpreting these results, it is highly possible that there is the presence of cryptic species or species complexes in the Mediterranean, where morphologically identical populations are genetically different.

E. *Sargocentron rubrum*

Sargocentron rubrum, commonly known as the Redcoat, is a species belonging to Holocentridae, a large family comprising approximately 83 species of soldierfishes found in tropical marine waters in the Atlantic and Indo-Pacific regions (Nelson *et al.*, 2016). Soldierfishes are known to be a highly diverse group with lineages stemming back to the Cretaceous period, around 93 Mya (Dornburg *et al.*, 2012). Species of this family hold a strong fossil record, notably in deposits of the ancient Mediterranean (Dornburg *et al.*, 2014).

Sargocentron rubrum is native to the Indo-West Pacific, ranging from the Red Sea to the coasts of India, Japan, Australia, as well as other countries in the Indo-Pacific (Froese & Pauly, 2021). It was first recorded in the Levantine region of the Mediterranean Sea as *Holocentrum rubrum* by Haas and Steinitz (1947) and is considered as one of the earliest documented Lessepsian migrants (Golani & Ben-Tuvia, 1985). Like all holocentrid species, *S. rubrum* is characterized by having a general red color, spines on the edge of the operculum and large eyes, an adaptation to its nocturnal behavior (Nelson *et al.*, 2016). In the Mediterranean, this species primarily lays hidden in dark crevices and caves during the day and becomes active at night, mainly feeding on benthic crustaceans (Golani *et al.*, 1983, Froese & Pauly, 2021). Golani *et al.* (1983) showed that the majority of their diet consisted of nocturnal decapods native to the Mediterranean, exemplifying the success of *S. rubrum*'s establishment. While individuals of this species are found in rocky habitats down to a depth of about 40 m in the Mediterranean, this is not the case in their native environment (Golani *et al.*, 1983). In the Indo-Pacific, this species is less associated with coral reefs compared to other holocentrids and reside within a larger depth range (Golani & Ben-Tuvia, 1985). The same goes for average lifespans when comparing Lessepsian migrants to Red Sea individuals. While the general lifespan of tropical species is between two to three years, *S. rubrum* migrants are able to live up to four years, which could be explained by favorable conditions present in the Mediterranean (Farrag *et al.*, 2018). Finally, Golani (1987) documented morphometric and meristic differences between Indo-Pacific and Mediterranean populations. These included a significantly lower number of lateral line scales and vertebra in Mediterranean populations, as well as variations in the suborbital spines and shape of the nasal bone. Golani (1987) even proposed that this could ultimately lead to the rise of a new taxon.

F. Aims of the Study

The purpose of this work is to investigate the genetics of the soldierfish, referred to as *Sargocentron rubrum* (Forsskål, 1775), that has invaded the Mediterranean Sea. Sequenced samples from the Mediterranean and the comparison to those available on online databases were used to unveil the identity of the species present in the

Mediterranean and show phylogenetic relationships between the various congenerics in the native distributional range.

CHAPTER II

MATERIALS AND METHODS

A. Sampling

During the spring of 2019, individuals of *Sargocentron rubrum* (N=29) were collected from the fish market from various locations off the coast of Lebanon (Table 1; Figures 1, 2). Specimens of *S. rubrum* (N=20) were also sampled between the years 2020 and 2021 from the Gulf of İskenderun, Turkey (Table 2; Figures 1, 3).

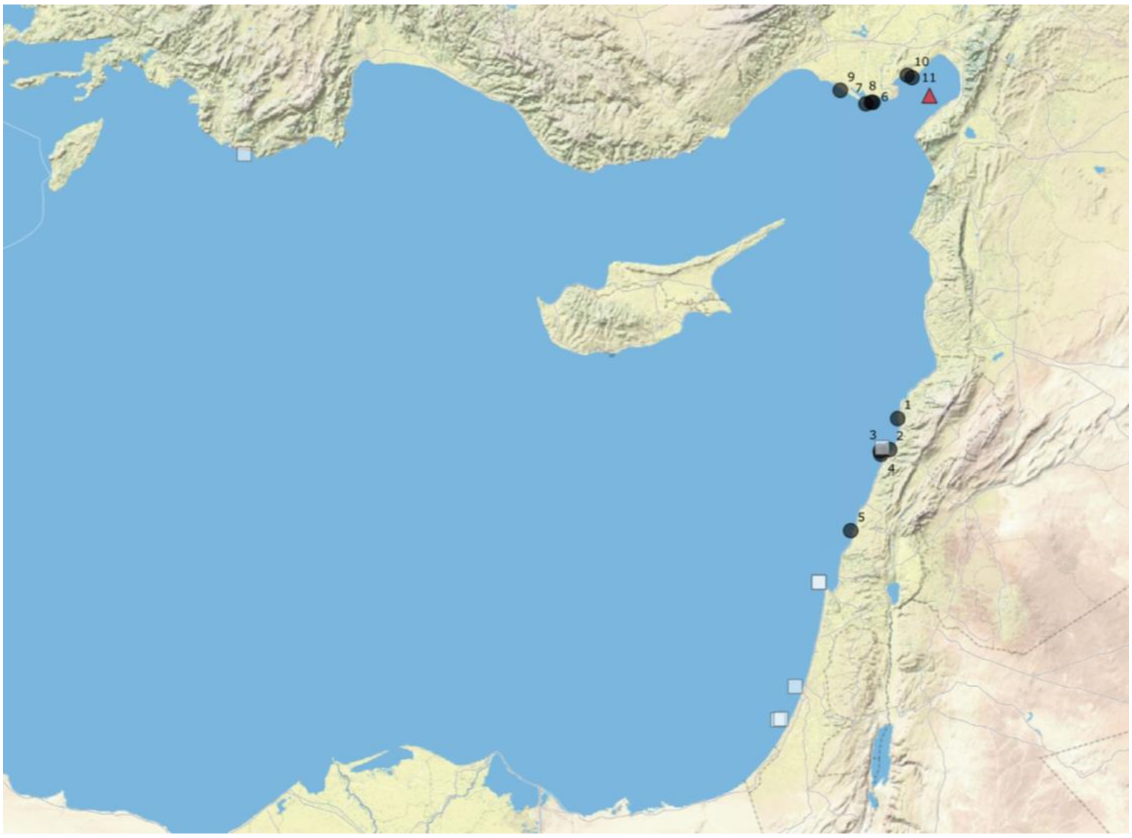


Figure 1: Geolocations of all Sargocentron rubrum sequences from the Mediterranean Sea. Circles: samples collected from Lebanon and Turkey for this study, where location numbers are given in Tables 1 and 2; Squares: sequences available on databases; Triangles: sequences available on databases (FE taxon).

The total lengths (length from the tip of the snout to the tip of the upper caudal fin) were taken from each fish individually (Supplementary Material I). All fish were tagged by placing a piece of numbered paper in the right operculum of the fish, fastened by crochet string looped through the buccal cavity (Figure 4). Fin clips were taken from the right side (pectoral and pelvic fins) of each specimen and preserved in 100% ethanol. Fin clips from Lebanon were added to the Sample Tissue (ST) collection at the AUB Natural History Museum (Table 1). The samples were fixed afterwards in buffered formalin (10%) for two weeks, then preserved in 70% ethanol for long-term storage (Figure 5).

Figure 2: *Sargocentron rubrum* specimens from Lebanon with their corresponding labels.

Table 1: List of *Sargocentron rubrum* fin clip samples collected from Lebanon and used in this study. N: number of specimens; ST: Sample tissue numbers as deposited in the marine collection of the AUB; Location numbers are given in Figure 1.

Fin Clip No.	N	Collection Date	Location	Location No.
ST796 – ST801	6	2 March 2019	Tyre	5
ST807 – ST811	5	9 March 2019	Amchit	1
ST812 – ST814	3	19 March 2019	Manara	3
ST816 – ST820	5	April 2019	Dora	2
ST821 – ST826	6	1 May 2019	Dora	2
ST840 – ST843	4	April 2019	Ouzai	4

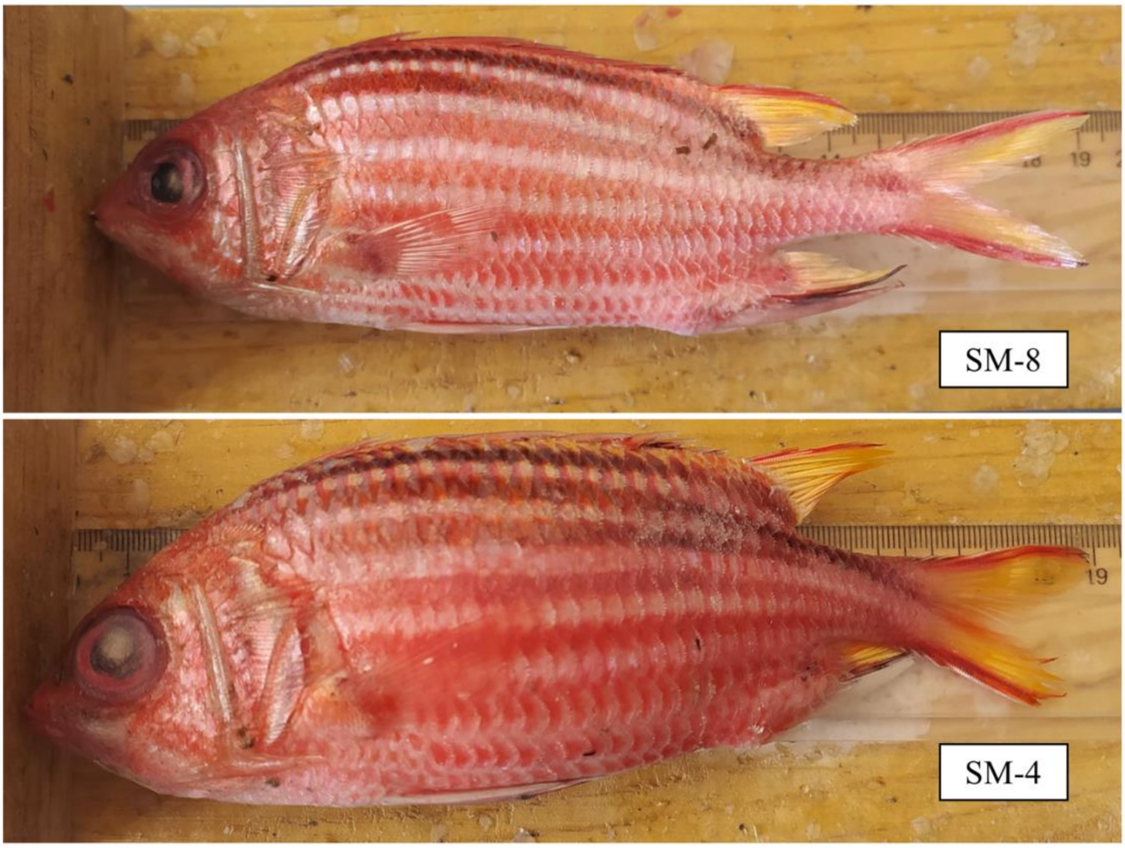


Figure 3: *Sargocentron rubrum* specimens collected from Turkey for this study.

Table 2: List of *Sargocentron rubrum* fin clip samples collected from the Gulf of İskenderun, Turkey, and used in this study. N: number of specimens; SM: Sample tissue numbers; Location numbers are given in Figure 1.

Fin Clip No.	N	Collection Date	Location	Location No.
SM-1 – SM-3	3	21 January 2021	Karataş Fishery Port	6
SM-4 – SM-6	3	21 January 2021	Karataş, Harbiş	7
SM-7 – SM-8	2	20 January 2021	Karataş Ada	8
SM-9	1	15 December 2020	Karagöçer	9
SM-10 – SM-14	5	15 July 2020	Yumurtalık, Abdülkerim	10
SM-15 – SM-20	6	15 September 2020	Yumurtalık, Barınak	11

Figure 4: Labeled specimen in the operculum.

Figure 5: *Sargocentron rubrum* specimens submerged in 70% ethanol for long-term storage.

B. Molecular Work

1. DNA Extraction

DNA from fin clips was extracted using two methods. The first method involved the use of the DNeasy® Blood and Tissue Kit by QIAGEN (Cat. No. 69504), in which the manufacturer's recommended protocol was followed: Fin clips (≤ 25 mg) were submerged in 180 µl Buffer ATL and 20 µl proteinase K and incubated at 56°C until they were completely dissolved. 200 µl Buffer AL was added and mixed thoroughly by vortexing, followed by the addition of 200 µl 100% ethanol. The solution was then transferred to a DNeasy Mini spin column. This was followed by the addition of different buffers, centrifuged sequentially. With the final addition of Buffer AE, a total of 400 µl extracted DNA was obtained. The second method was done using an STE Buffer (0.1 M NaCl, 10 mM Tris-HCl pH 8.0, and 1 mM EDTA, pH 8.0). Fin clips were submerged and mixed in 100 µL of the buffer using a tissue grinding pestle until emulsification. This was followed by incubation at 95°C for 10-15 minutes and then centrifugation at 14000 rounds per minute for 5 minutes, where the supernatant would be used for later reactions. DNA integrity was checked either by quantifying 1-2 µl of sample on a Nanodrop or by running 5 µl in 1% agarose gel. The resulting DNA extract was stored at -20°C.

2. Gene Amplification

Following DNA extraction, polymerase chain reaction (PCR) was used to amplify the 655 base-pair region of the mitochondrial gene, cytochrome c oxidase subunit I (COI), with four primers constructed by Ward *et al.* (2005): FishF1 – 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3', FishF2 – 5'-TCG ACT AAT CAT

AAA GAT ATC GGC AC-3', FishR1 – 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3', and FishR2 – 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3'. PCR was carried out using the *Taq* PCR Core Kit by QIAGEN (Cat. No. 201223). Each reaction consisted of 76.5 µl sterilized nanopure water, 10 µl Buffer (10x), 10 µl primers (2.5 µl each), 2 µl dNTPs, 0.5 µl Taq DNA polymerase, and 1 µl DNA template, thus having a total of 100 µl per sample. PCR samples were then placed in a thermocycler under the following program: one cycle at 95°C for 2 minutes; 35 cycles at 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute; one cycle at 72°C for 10 minutes. To check whether gene amplification was successful, 5 µl of PCR products were visualized in 1% agarose gel under UV light, along with a negative control and DNA ladder (Figure 6). Remaining samples were stored at -20°C.

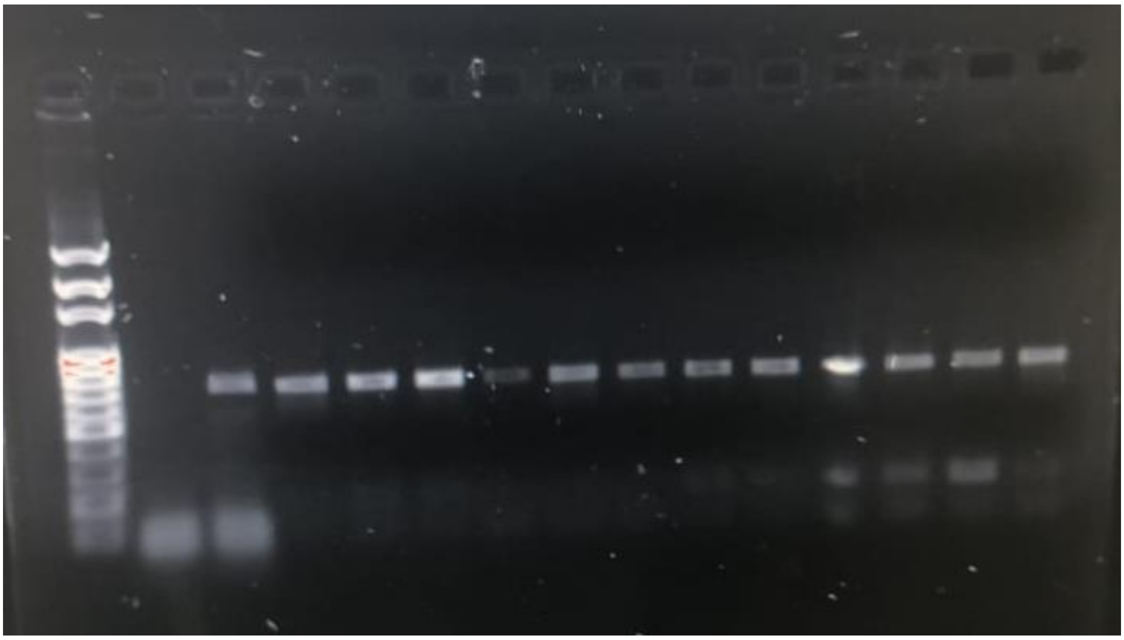


Figure 6: Gel electrophoresis in 1% agarose of PCR products.

3. PCR Purification

PCR products were then purified by using one of two kits by QIAGEN: either the QIAquick® PCR Purification Kit (Cat. No. 28104) or the QIAquick® Gel Extraction Kit (Cat. No. 28704). Protocol for purification was done as written in the instruction handbook for each kit. In both kits, a series of buffers were used to purify PCR products. However, when using the gel extraction kit, 40 µl PCR products were run in a 1% agarose gel, later to be excised and dissolved for further processing. In order to confirm that the PCR samples were in fact purified, the resulting products were also viewed under UV light using 1% agarose gel and then stored at -20°C.

4. Sequencing

Purified PCR products were sent to two facilities for sequencing. Samples were sequenced at the Genetics Core Facility in the American University of Beirut, in which the prepared solutions consisted of 10 µl of purified PCR products, 1 µl primers (10 µM) and 5 µl of sterilized nanopure water. Additional sequencing was done through the services of Macrogen Inc. (Seoul, South Korea), where 20 µl of purified PCR products and 5 µl primers were sent in separate tubes. For both cases, a combination of FishF1 and FishF2 primers was used for sequencing (sequences are found in Supplementary Material IV).

C. Data Analysis

All sequences available for *Sargocentron rubrum* on GenBank and BOLD (N=81) were extracted on 26 May 2021. In addition, sequences for every species belonging to each genus were collected, where sequences for 21 of the 34 species were obtained. These were *Sargocentron bullisi* (N=1), *S. caudimaculatum* (N=49), *S. cornutum* (N=5), *S. coruscum* (N=13), *S. diadema* (N=59), *S. inaequalis* (N=1), *S. ittodai* (N=17), *S. lepros* (N=5), *S. melanospilos* (N=4), *S. microstoma* (N=36), *S. praslin* (N=7), *S. punctatissimum* (N=32), *S. seychellense* (N=5), *S. spiniferum* (N=19), *S. suborbitale* (N=3), *S. tiere* (N=23), *S. tiereoides* (N=4), *S. vexillarium* (N=12), *S. violaceum* (N=2) and *S. xantherythrum* (N=1). All sequences were put into format and aligned in Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7 using CLUSTALW (Thompson *et al.*, 1994, Kumar *et al.*, 2016). Trees were constructed using two methods, Neighbor-Joining (NJ) and Bayesian Markov Chain Monte Carlo (MCMC). They were generated using an improved version of the NJ algorithm (Gascuel, 1997) via the Analysis of Phylogenetics and Evolution package (ape; Paradis & Schliep, 2018) on R (R Core Team, 2020). Furthermore, the MCMC method was implemented on MrBayes (Ronquist & Huelsenbeck, 2003). On R, pairwise distance matrices were generated using the Kimura 2-parameter model (K2P; Kimura, 1980). These matrices were used to determine the genetic distances between phylogenetic groups. Both phylogenetic models were then analyzed to compare the relationships between the different taxa.

CHAPTER III

RESULTS

The phylogenetic trees produced using the NJ and MCMC methods, which includes all available sequences of the congeneric species of *Sargocentron* (Supplementary Materials II, III), show that *S. rubrum* specimens form a separate cluster with a few different species. These are *S. praslin* (Lacepède, 1802), *S. melanospilos* (Bleeker, 1858), *S. cornutum* (Bleeker, 1854), and *S. seychellense* (Smith & Smith, 1963). Within this cluster, the same analysis was done with all *S. rubrum* sequences and the four congeneric species (*S. cornutum*, *S. coruscum*, *S. melanospilos,* and *S. praslin*). Sequences of *S. rubrum* collected in the Mediterranean Sea fall in two distinct groups (Figure 7). For practical reasons, they will be referred to as the “Near East taxon” (NE taxon) and the “Far East taxon” (FE taxon).

All specimens collected from the Levant region are present in the NE taxon, except for those from Turkey, which are found in both the NE and FE taxa. Out of the 42 sequences from Turkey, 21 individuals belong to the NE taxon. They include 20 specimens from the current study sampled from İskenderun Bay (Table 2), and another sequence from Antalya (Kaş) obtained from Genbank. On the contrary, the other 21 individuals, also from İskenderun Bay, belong to the FE taxon, as are most sequences available from the eastern part of the Indo-Pacific. Figure 1 shows the locations of the various samples from the İskenderun Bay. Interestingly, a single sequence from the Kingdom of Saudi Arabia (KSA) in the Red Sea also falls in the NE taxon (Figure 7). The MCMC method showed similar results with some minor variations (Figure 8).

The trees generated using the two methods (NJ and MCMC) show that individuals from the NE taxon are closer to *Sargocentron seychellense* than members of the FE taxon. The genetic distance between *S. seychellense* and members of the NE taxon is 2.25%, and the branch length probability at the point of their bifurcation is 1, as seen in the phylogenetic tree constructed using the MCMC method (Figures 7, 8).

Individuals from the FE taxon also form a separate cluster, more similar to *Sargocentron melanospilos*, *S. cornutum* and *S. praslin* (Figures 7, 8). The average genetic distance between the NE and FE taxa is 5.73% when using the pairwise matrix from the NJ method, and the branch length probability between them, generated when applying the MCMC method, is 1 (Figure 8).

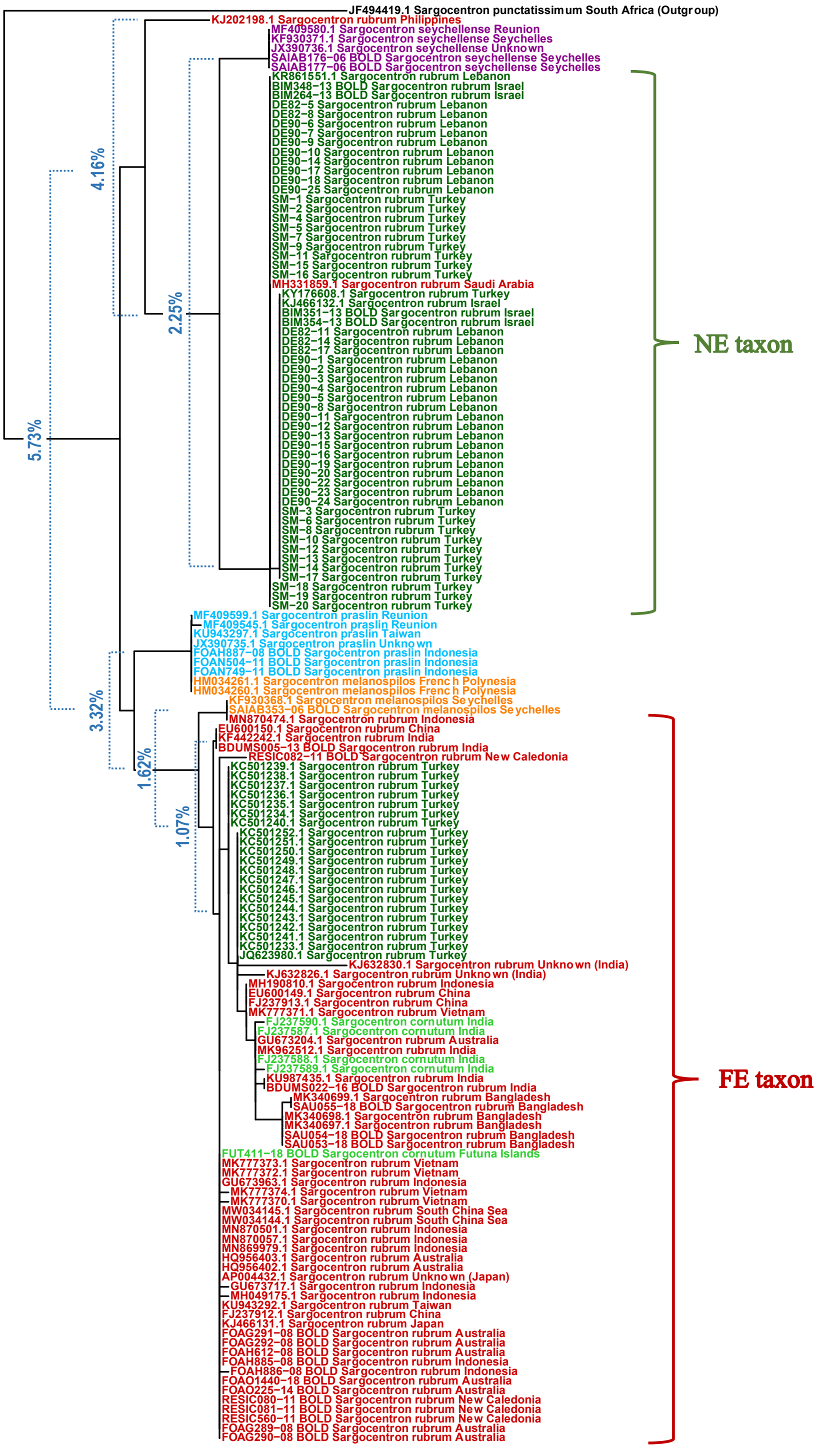


Figure 7: Neighbor-Joining tree of all available *Sargocentron rubrum* sequences, along with the selected congenerics that were closely related in Supplementary Materials II, III. The genetic distances between different taxa are annotated in blue. Color of tip labels correspond to the following: Purple: *Sargocentron seychellense*, Light Blue: *Sargocentron praslin*, Orange: *Sargocentron melanospilos*, Light Green: *Sargocentron cornutum*, Red: *Sargocentron rubrum* captured from the Indo-Pacific region, Green: *Sargocentron rubrum* captured from the Mediterranean Sea. Two separate clusters are annotated with a green (NE taxon) and red bracket (FE taxon).

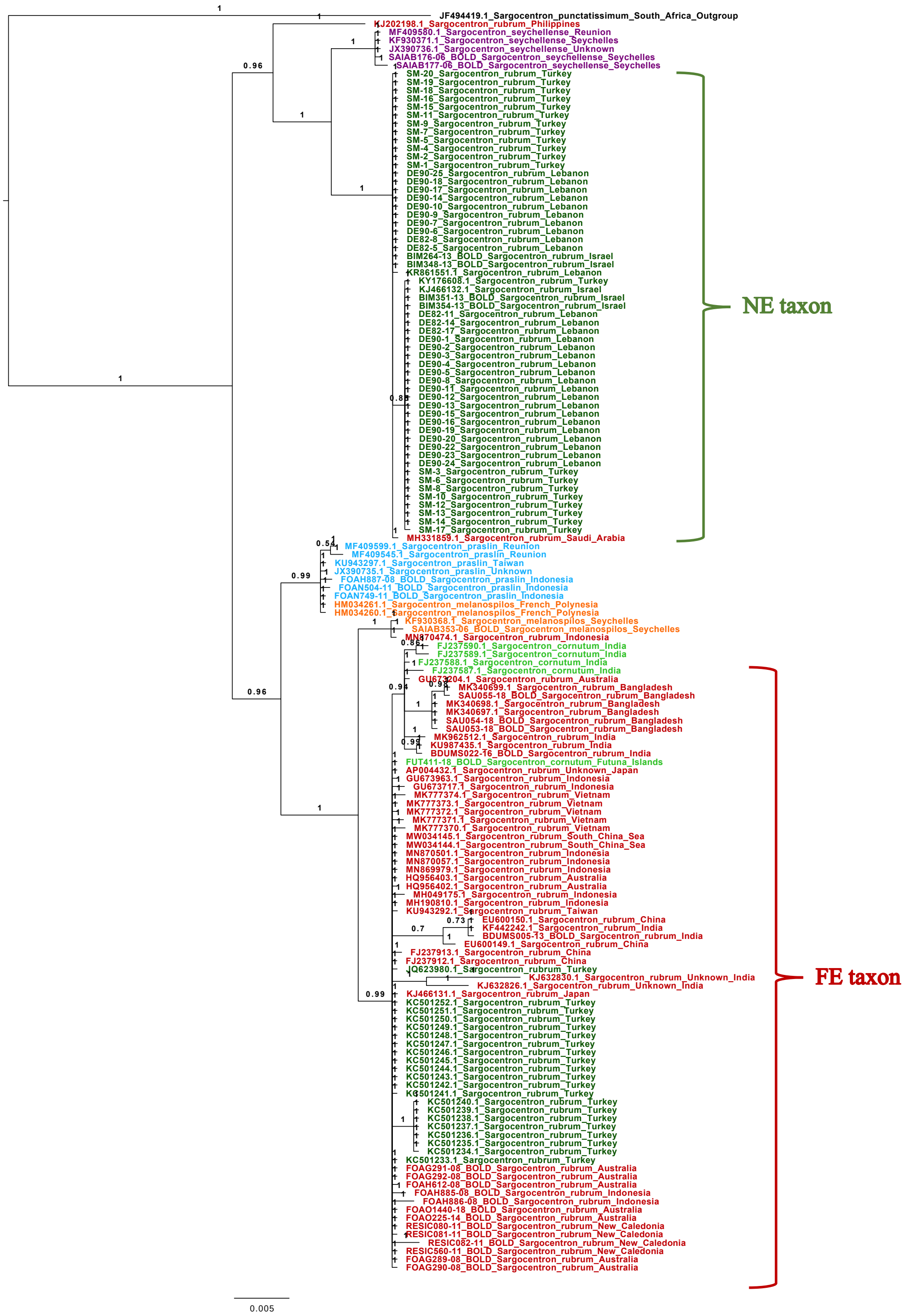


Figure 8: Markov Chain Monte Carlo tree of all *Sargocentron rubrum* sequences, along with the selected congenerics that were closely related in Supplementary Materials II, III. Color of tip labels correspond to the following: Purple: *Sargocentron seychellense*, Blue: *Sargocentron praslin*, Orange: *Sargocentron melanospilos*, Light Green: *Sargocentron cornutum*, Red: *Sargocentron rubrum* captured from the Indo-Pacific region, Green: *Sargocentron rubrum* captured from the Mediterranean Sea. The Near East taxon (NE taxon) is annotated with a green bracket and the Far East taxon (FE taxon) is annotated with a red bracket.

CHAPTER IV

DISCUSSION

Sargocentron rubrum belongs to a family of tropical fishes that are diverse and relatively hard to identify. Taxonomic characters based solely on meristics and morphometrics are not easily distinguished, specifically within members of the same genus. Furthermore, the wide geographic distribution of Holocentridae certainly allows for a wide variety of haplotypes, each specific to a different region.

A. *Sargocentron rubrum*, a species complex

A high intraspecific genetic distance for *Sargocentron rubrum* was previously detected in Bariche *et al.* (2015), where the authors suspected the presence of a species complex. In the current study, we collected additional samples from the eastern Mediterranean, determined where the sequences from this region fall in the phylogenetic tree and deduced the relationship between taxa (Figures 2, 5). The species "*Sargocentron rubrum* (Forsskål, 1775)" is present in two genetically distant taxa (5.73%), labeled as NE and FE (Figure 7). Based on the accepted 2% genetic distance threshold for delineating species, a genetic difference of more than 5% between the two detected taxa generally implies the presence of more than one species (Hebert *et al*., 2003b). In this study, all sequenced individuals from Lebanon and Turkey (Tables 1, 2) belong to the NE taxon. They are closely related to *Sargocentron seychellense* (2.25%) sampled from Seychelles and Reunion Island, which fall in the known native range of the species (Froese & Pauly, 2021). Our sequences are also similar to others from the eastern Mediterranean, as well as a single sequence from the Red Sea (Kingdom of

Saudi Arabia) (Tikochinski *et al.*, 2013; Bariche *et al.*, 2015; GenBank Accession Nos. KY176608, MH331859). This implies that the species populating the eastern Mediterranean is also found in the Red Sea; no other sequences available on BOLD and Genbank from the vast Indo-Pacific match our sequences.

B. *Sargocentron rubrum, S. seychellense* or a new species in the Mediterranean?

Based on our results, it remains unclear which species of *Sargocentron* constitutes the large population present in the eastern Mediterranean Sea. The type specimen of *S. rubrum* was described from the Red Sea as *Sciaena rubra* (Forsskål, 1775 in Niebuhr, 1775). It is interesting to know whether the species described by Forsskål is the one that invaded the Mediterranean. An examination of the type specimen of Forsskål, and ideally sequencing its DNA, would clarify the identity of the Mediterranean population. In this case, the invader would be the true *Sargocentron rubrum* (Forsskål, 1775).

The genetic difference that surpasses the standardized 2% threshold between the NE taxon and *Sargocentron seychellense* (2.25%, Figure 7), as per Hebert *et al.* (2003b), should also be taken into consideration. With this in mind, these two entities should be distinct. Thus, the population in the Mediterranean could either be Forsskål's *S. rubrum* also present in the Red Sea, or a "new" *Sargocentron* sp. to be described. The known native range for this new species would be in the Red Sea, since an identical sequence is present in KSA (GenBank Accession No. MH331859) (Figures 2, 5).

However, in several cases, intraspecific distances among individuals may exceed the 2% threshold and yet they are considered the same species (Geiger *et al.*, 2014). Characterizing a species solely based on its genetic barcode may be misleading and thus increase the risk of misidentification. Therefore, it is also possible that the population in the Red Sea is actually *Sargocentron seychellense* with a genetic distance exceeding the 2% threshold. The known range for *S. seychellense* is in the western Indian Ocean, knowing that the species has never been recorded from the Red Sea (Golani & Bogorodsky, 2010; Froese & Pauly, 2021). In that case, the detected genetic difference between NE taxon and *S. seychellense* (Figure 7) may not be significant and both would belong to the same species, with a high intraspecific variability of 2.25%. If so, *S. seychellense* and the NE taxon should be lumped into one species and possibly renamed according to the principle of priority (ICZN, 1999). This species would be present in the western Indian Ocean, the Red Sea and the Mediterranean, and may also include Forsskål's *S. rubrum*. Levent Artüz and Golani (2018) have stated that *S. rubrum* and *S. seychellense* are extremely similar morphologically and could easily be mistaken.

C. Two haplotypes in Turkey

Due to the additional and unexpected sequences reported from Turkey which belong to both the NE and FE taxon (Supplementary Material V), it was also anticipated to find the two haplotypes in Lebanon (NE and FE), as this is the case in Turkey (Keskin & Atar, 2013; GenBank Accession Nos. JQ623980, KY176608). Despite an intensive search and increased sample size, no specimens that belong to the FE taxon were found in the sampled locations (Lebanon and Turkey). Results clearly show that only one haplotype is present in Lebanon, similar to previously reported cases in the Mediterranean (Tikochinski *et al.*, 2013; Bariche *et al.*, 2015; GenBank Accession No. KY176608; BOLD Accession Nos. BIM348-13, BIM351-13, BIM354-13, BIM264-13).

Furthermore, all sampled individuals from the same area in Turkey where the FE haplotype was previously detected (Keskin & Atar, 2013; GenBank Accession No. JQ623980) also belonged to the NE taxon.

For this, two explanations: (a) the FE taxon haplotype is present in Lebanon but was not sampled, despite the search effort, or (b) this second haplotype is not present in Lebanon.

1. Present in Lebanon

Since it is generally accepted that Lessepsian immigrants enter the Mediterranean from the Suez Canal and tend to spread northwards along the Levant coast, they are theoretically expected to establish a population in Lebanon before reaching farther countries, like Turkey. The most plausible explanations for not sampling them could be the presence of a very small population or the mode of capture used for sampling. All "*Sargocentron rubrum*" individuals were purchased from the fish market and were thus mainly caught using fishing nets. It is possible that sampling using other methods is a way to diversify the sample and include different habitats. Other methods include spearfishing or even angling from the shore using hook and line. Although unlikely, broadening our sampling methods may have allowed us to find individuals from the FE taxon (if present), where they might have a different biology and ecology than the widespread individuals belonging to the NE taxon.

2. Not present in Lebanon

One reason behind the fact that the FE taxon is not present in Lebanese waters could be that the early settlers did not arrive as Lessepsian immigrants via the Suez

Canal. The early propagule may be the result of an aquarium release event, since soldierfishes are common aquarium pets. The aquarium trade is a multi-billion global industry and often an overlooked pathway of introduction in the eastern Mediterranean (Padilla & Williams, 2004). Thousands of tropical and ornamental species have been transported outside their native range worldwide and in the Mediterranean (Katsanevakis *et al.*, 2013). For various reasons, many aquarium species are disposed of or released, and may have a high change of survival in the new environment (Zenetos *et al.*, 2016; Geburzi & McCarthy, 2018; Olden *et al.*, 2021).

The presence of individuals from the FE taxon in Turkey could also be explained by an arrival from the Indo-Pacific through maritime transport, particularly ship ballast waters. Ballast water is another source for such kind of introduction. In fact, another soldierfish *Holocentrus adscensionis*, native to the tropical Atlantic, was recently and unexpectedly found in two different locations, the Maltese waters (Marsascala) and the Mediterranean coast of France (Cap d'Antibes). Among the few plausible explanations were an aquarium release event or an arrival through ballast water (Vella *et al.*, 2016; Iglésias *et al.*, in press).

Whether or not individuals from the FE taxon have arrived by means of one of these pathways, there is a possibility that the population (FE taxa) is present as a very small population within the vicinity of İskenderun, Turkey (or elsewhere), or also may have failed to establish a permanent population.

Finally, the presence of the unexpected FE sequences could simply be due to errors in sequencing. Problems when sequencing could occur, such a cross contaminations or sample mix-ups. However, this seems unlikely due to the relatively large number of published sequences that fall into one clade with the same species; otherwise, these sequences would have formed a third separate taxon in the phylogenetic tree with no contiguity to all other *S. rubrum* individuals. The authors of these sequences (Keskin & Atar, 2013) have been contacted for some clarifications regarding their results without success.

D. Conclusion

This study showed that the case of *Sargocentron rubrum* in the Mediterranean Sea is fairly difficult to solve. This is because of the relatively scarce data for this species in the Indo-Pacific region. Crucial information is also missing from the Red Sea for *S. rubrum* and its congenerics, thus it is not clear which species of soldierfishes are actually present in the region. In addition, it is not possible to draw conclusions on the reported members of the FE taxon from Turkey and the absence of similar sequences from the recent specimens collected in the same vicinity. This will remain unsolved unless new sequences falling within the FE taxon are found in the Mediterranean.

This study also showed that a full revision of the genus may be necessary to undertake, where both genetic and morphological analyses should be covered. This in turn could allow for the delineation of various congeneric species, which may also solve the identity of *S. rubrum* in the Red Sea and the Mediterranean.

APPENDIX A

SUPPLEMENTARY MATERIAL I

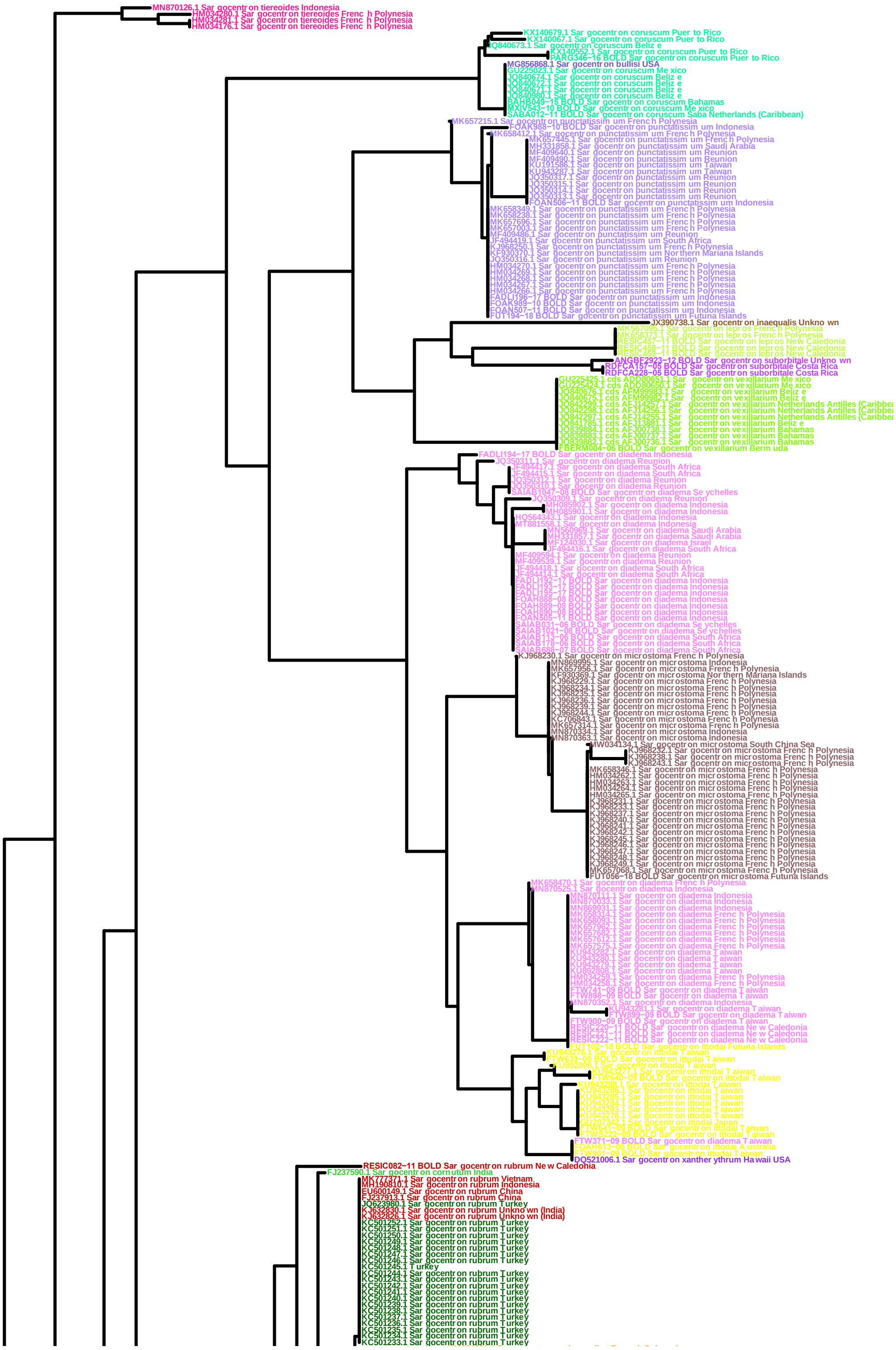
List of specimens with their corresponding total lengths:

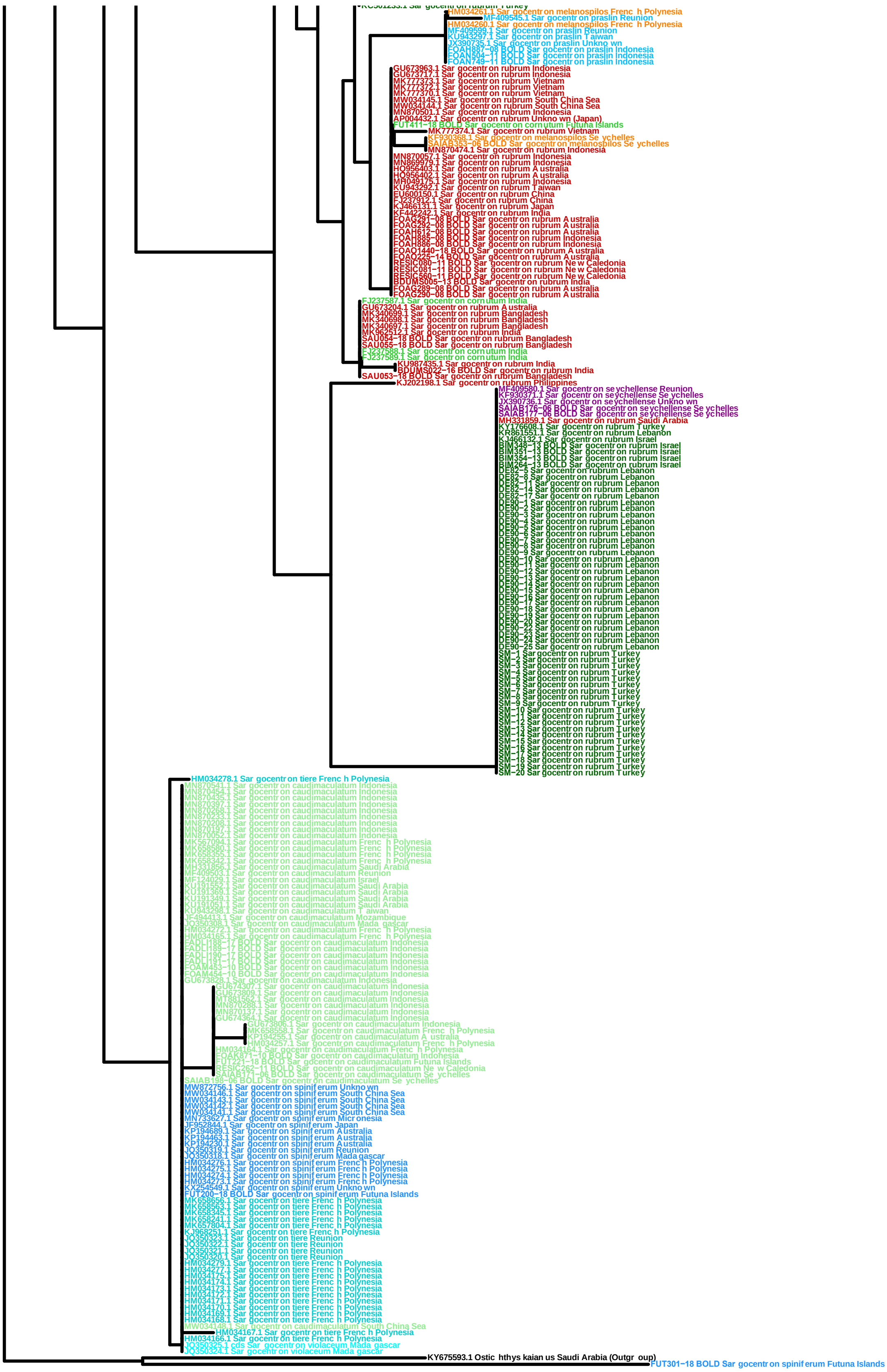
Sample Tissue	Total Length (cm)
ST796	16.2
ST797	15.6
ST798	16.2
ST799	15.7
ST800	14.7
ST801	15.8
ST807	15.0
ST808	14.9
ST809	15.7
ST810	16.6
ST811	15.7
ST812	17.8
ST813	16.8
ST814	17.6
ST816	16.0
ST817	16.8
ST818	16.8
ST819	17.1
ST820	16.4
ST821	16.1
ST822	16.8
ST823	16.1
ST824	19.3
ST825	17.1
ST826	15.3
ST840	16.6
ST841	16.0
ST842	15.0
ST843	15.5
SM-1	14.9
SM-2	13.1
SM-3	14.3
SM-4	18.8
SM-5	19.1
SM-6	14.3
SM-7	14.2
SM-8	19.4
SM-9	17.1
SM-10	17.4
SM-11	18.3
SM-12	16.9
SM-13	13.1
SM-14	12.7
SM-15	13.0
SM-16	10.3
SM-17	12.3
SM-18	10.9
SM-19	10.8
SM-20	11.6

APPENDIX B

SUPPLEMENTARY MATERIAL II

Neighbor-Joining tree of all available sequences of the congeneric species of *Sargocentron*:

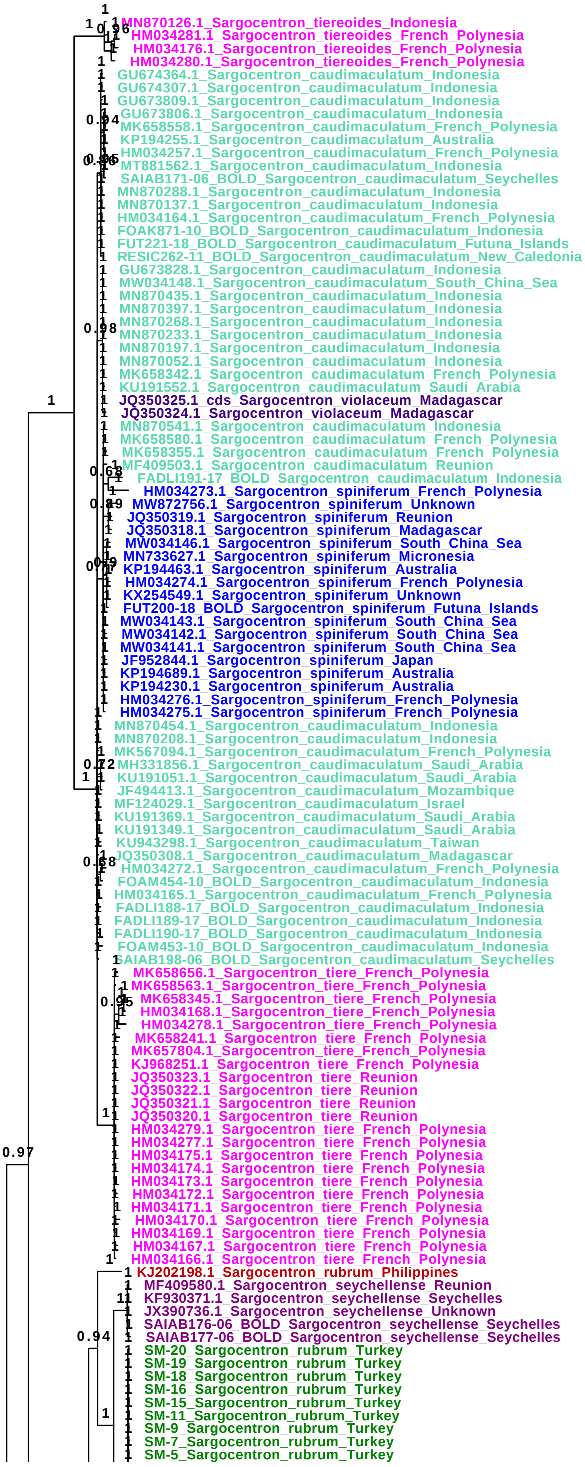


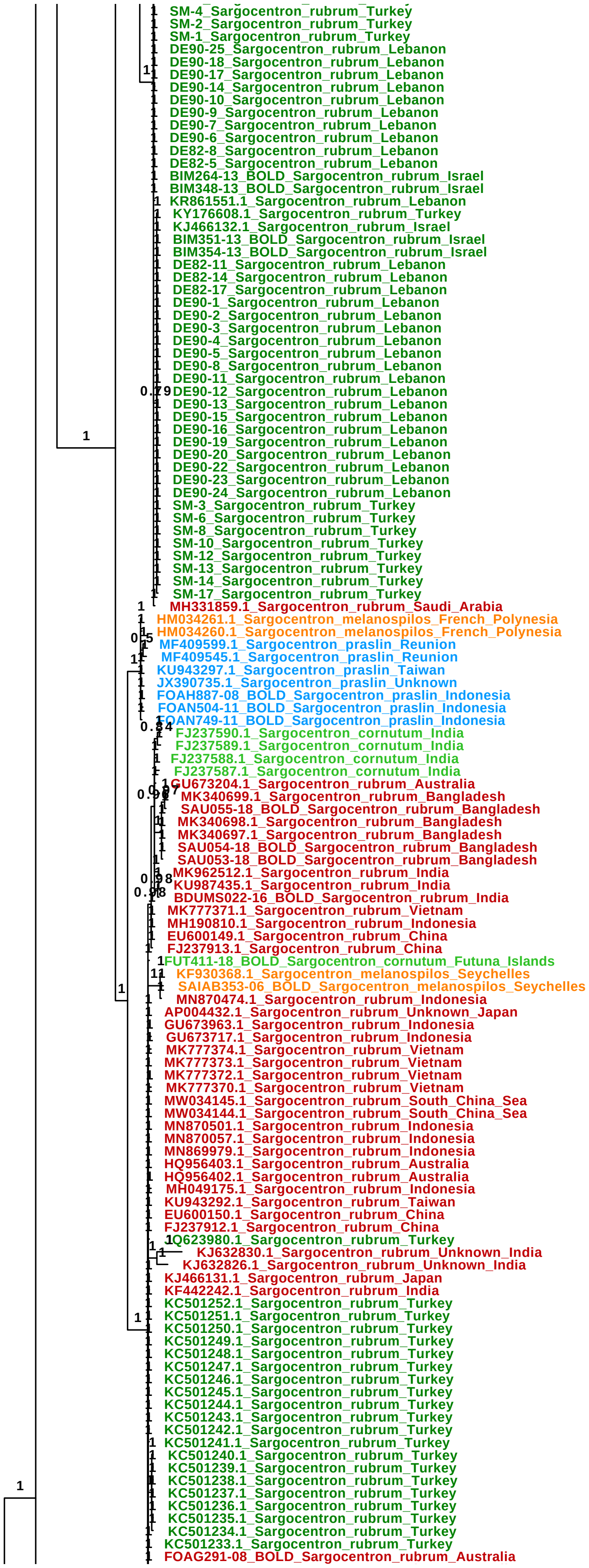


APPENDIX C

SUPPLEMENTARY MATERIAL III

Markov Chain Monte Carlo tree of all available sequences of the congeneric species of *Sargocentron*:




SM-4_Sargocentron_rubrum_Turkey
SM-2_Sargocentron_rubrum_Turkey
SM-1_Sargocentron_rubrum_Turkey
DE90-25_Sargocentron_rubrum_Lebanon
DE90-18_Sargocentron_rubrum_Lebanon
DE90-17_Sargocentron_rubrum_Lebanon
DE90-14_Sargocentron_rubrum_Lebanon
DE90-10_Sargocentron_rubrum_Lebanon
DE90-9_Sargocentron_rubrum_Lebanon
DE90-7_Sargocentron_rubrum_Lebanon
DE90-6_Sargocentron_rubrum_Lebanon
DE82-8_Sargocentron_rubrum_Lebanon
DE82-5_Sargocentron_rubrum_Lebanon
BIM264-13_BOLD_Sargocentron_rubrum_Israel
BIM348-13_BOLD_Sargocentron_rubrum_Israel
KR861551.1_Sargocentron_rubrum_Lebanon
KY176608.1_Sargocentron_rubrum_Turkey
KJ466132.1_Sargocentron_rubrum_Israel
BIM351-13_BOLD_Sargocentron_rubrum_Israel
BIM354-13_BOLD_Sargocentron_rubrum_Israel
DE82-11_Sargocentron_rubrum_Lebanon
DE82-14_Sargocentron_rubrum_Lebanon
DE82-17_Sargocentron_rubrum_Lebanon
DE90-1_Sargocentron_rubrum_Lebanon
DE90-2_Sargocentron_rubrum_Lebanon
DE90-3_Sargocentron_rubrum_Lebanon
DE90-4_Sargocentron_rubrum_Lebanon
DE90-5_Sargocentron_rubrum_Lebanon
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DE90-11_Sargocentron_rubrum_Lebanon
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DE90-19_Sargocentron_rubrum_Lebanon
DE90-20_Sargocentron_rubrum_Lebanon
DE90-22_Sargocentron_rubrum_Lebanon
DE90-23_Sargocentron_rubrum_Lebanon
DE90-24_Sargocentron_rubrum_Lebanon
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SM-6_Sargocentron_rubrum_Turkey
SM-8_Sargocentron_rubrum_Turkey
SM-10_Sargocentron_rubrum_Turkey
SM-12_Sargocentron_rubrum_Turkey
SM-13_Sargocentron_rubrum_Turkey
SM-14_Sargocentron_rubrum_Turkey
SM-17_Sargocentron_rubrum_Turkey
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HM034261.1_Sargocentron_melanospilos_French_Polynesia
HM034260.1_Sargocentron_melanospilos_French_Polynesia
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MF409545.1_Sargocentron_praslin_Reunion
KU943297.1_Sargocentron_praslin_Taiwan
JX390735.1_Sargocentron_praslin_Unknown
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FOAN504-11_BOLD_Sargocentron_praslin_Indonesia
FOAN749-11_BOLD_Sargocentron_praslin_Indonesia
FJ237590.1_Sargocentron_cornutum_India
FJ237589.1_Sargocentron_cornutum_India
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FJ237587.1_Sargocentron_cornutum_India
GU673204.1_Sargocentron_rubrum_Australia
MK340699.1_Sargocentron_rubrum_Bangladesh
SAU055-18_BOLD_Sargocentron_rubrum_Bangladesh
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EU600149.1_Sargocentron_rubrum_China
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KF930368.1_Sargocentron_melanospilos_Seychelles
SAIAB353-06_BOLD_Sargocentron_melanospilos_Seychelles
MN870474.1_Sargocentron_rubrum_Indonesia
AP004432.1_Sargocentron_rubrum_Unknown_Japan
GU673963.1_Sargocentron_rubrum_Indonesia
GU673717.1_Sargocentron_rubrum_Indonesia
MK777374.1_Sargocentron_rubrum_Vietnam
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MK777370.1_Sargocentron_rubrum_Vietnam
MW034145.1_Sargocentron_rubrum_South_China_Sea
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MN870057.1_Sargocentron_rubrum_Indonesia
MN869979.1_Sargocentron_rubrum_Indonesia
HQ956403.1_Sargocentron_rubrum_Australia
HQ956402.1_Sargocentron_rubrum_Australia
MH049175.1_Sargocentron_rubrum_Indonesia
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EU600150.1_Sargocentron_rubrum_China
FJ237912.1_Sargocentron_rubrum_China
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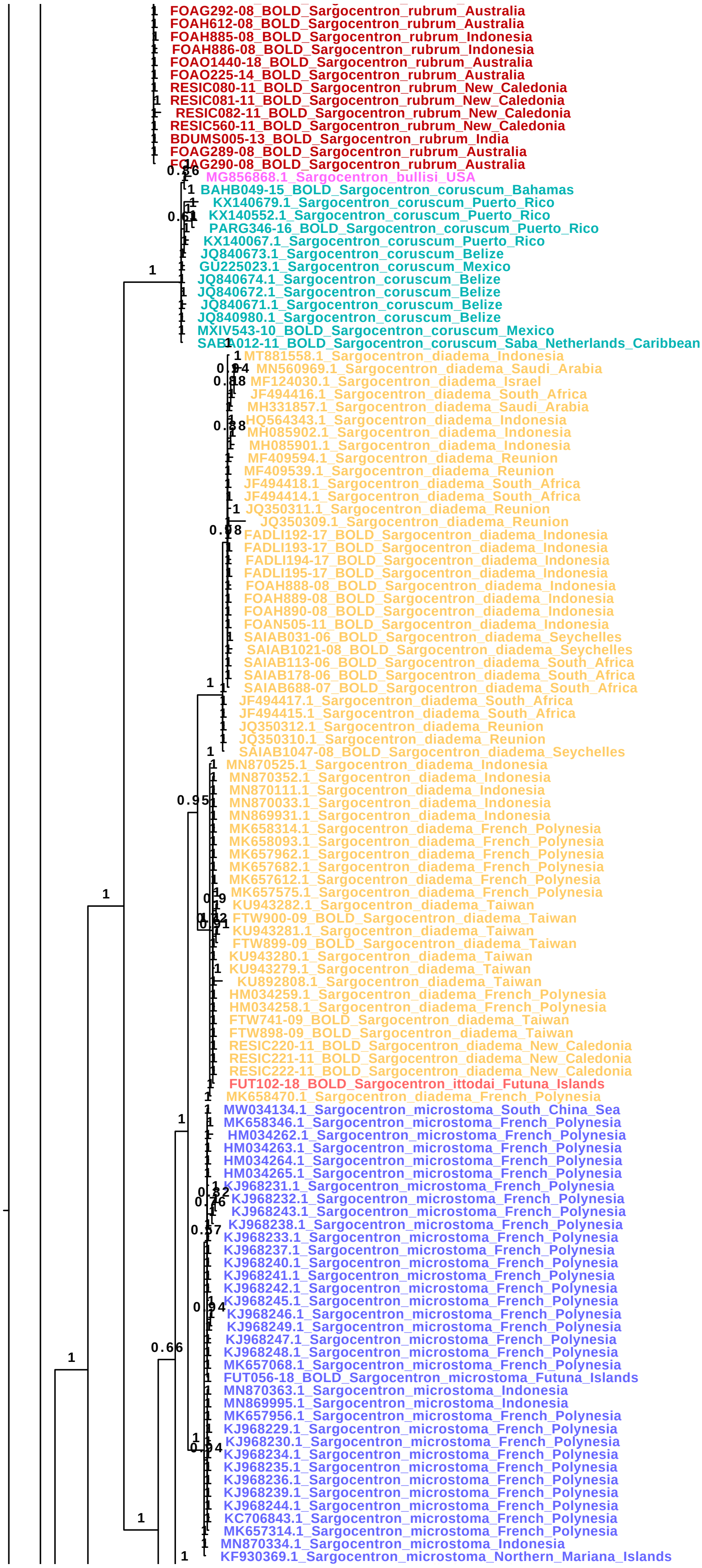
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RESIC560-11_BOLD_Sargocentron_rubrum_New_Caledonia
BDUMS005-13_BOLD_Sargocentron_rubrum_India
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MF124030.1_Sargocentron_diadema_Israel
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HQ564343.1_Sargocentron_diadema_Indonesia
MH085902.1_Sargocentron_diadema_Indonesia
MH085901.1_Sargocentron_diadema_Indonesia
MF409594.1_Sargocentron_diadema_Reunion
MF409539.1_Sargocentron_diadema_Reunion
JF494418.1_Sargocentron_diadema_South_Africa
JF494414.1_Sargocentron_diadema_South_Africa
JQ350311.1_Sargocentron_diadema_Reunion
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FADLI195-17_BOLD_Sargocentron_diadema_Indonesia
FOAH888-08_BOLD_Sargocentron_diadema_Indonesia
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FOAH890-08_BOLD_Sargocentron_diadema_Indonesia
FOAN505-11_BOLD_Sargocentron_diadema_Indonesia
SAIAB031-06_BOLD_Sargocentron_diadema_Seychelles
SAIAB1021-08_BOLD_Sargocentron_diadema_Seychelles
SAIAB113-06_BOLD_Sargocentron_diadema_South_Africa
SAIAB178-06_BOLD_Sargocentron_diadema_South_Africa
SAIAB688-07_BOLD_Sargocentron_diadema_South_Africa
JF494417.1_Sargocentron_diadema_South_Africa
JF494415.1_Sargocentron_diadema_South_Africa
JQ350312.1_Sargocentron_diadema_Reunion
JQ350310.1_Sargocentron_diadema_Reunion
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MN870033.1_Sargocentron_diadema_Indonesia
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FTW741-09_BOLD_Sargocentron_diadema_Taiwan
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MK658470.1_Sargocentron_diadema_French_Polynesia
MW034134.1_Sargocentron_microstoma_South_China_Sea
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FUT056-18_BOLD_Sargocentron_microstoma_Futuna_Islands
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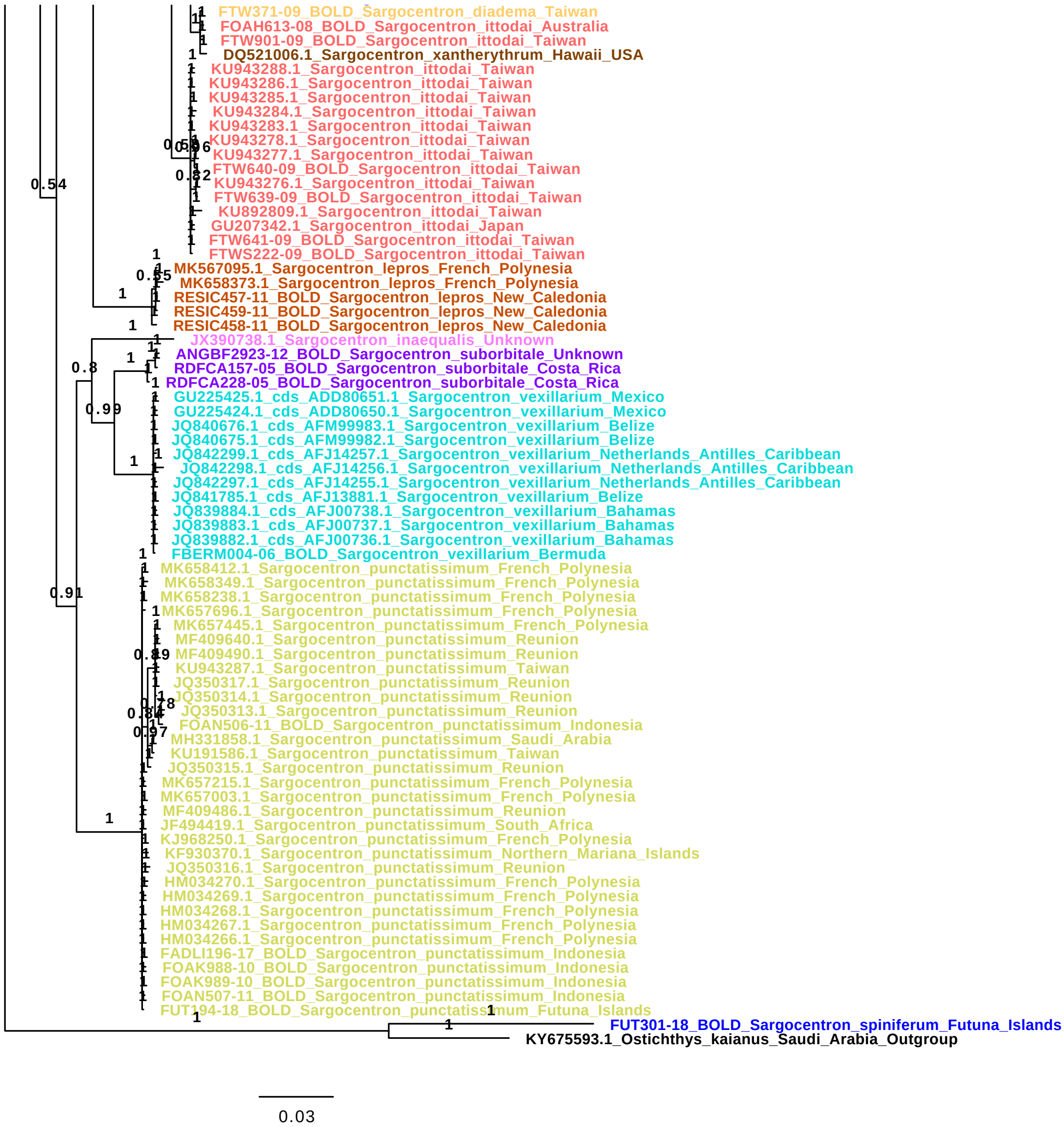
KJ968239.1_Sargocentron_microstoma_French_Polynesia
KJ968244.1_Sargocentron_microstoma_French_Polynesia
KC706843.1_Sargocentron_microstoma_French_Polynesia
MK657314.1_Sargocentron_microstoma_French_Polynesia
MN870334.1_Sargocentron_microstoma_Indonesia
KF930369.1_Sargocentron_microstoma_Northern_Mariana_Islands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FTW901-09_BOLD_Sargocentron_ittodai_Taiwan
DQ521006.1_Sargocentron_xantherythrum_Hawaii_USA
KU943288.1_Sargocentron_ittodai_Taiwan
KU943286.1_Sargocentron_ittodai_Taiwan
KU943285.1_Sargocentron_ittodai_Taiwan
KU943284.1_Sargocentron_ittodai_Taiwan
KU943283.1_Sargocentron_ittodai_Taiwan
KU943278.1_Sargocentron_ittodai_Taiwan
KU943277.1_Sargocentron_ittodai_Taiwan
FTW640-09_BOLD_Sargocentron_ittodai_Taiwan
KU943276.1_Sargocentron_ittodai_Taiwan
FTW639-09_BOLD_Sargocentron_ittodai_Taiwan
KU892809.1_Sargocentron_ittodai_Taiwan
GU207342.1_Sargocentron_ittodai_Japan
FTW641-09_BOLD_Sargocentron_ittodai_Taiwan
FTWS222-09_BOLD_Sargocentron_ittodai_Taiwan
MK567095.1_Sargocentron_lepros_French_Polynesia
MK658373.1_Sargocentron_lepros_French_Polynesia
RESIC457-11_BOLD_Sargocentron_lepros_New_Caledonia
RESIC459-11_BOLD_Sargocentron_lepros_New_Caledonia
RESIC458-11_BOLD_Sargocentron_lepros_New_Caledonia
JX390738.1_Sargocentron_inaequalis_Unknown
ANGBF2923-12_BOLD_Sargocentron_suborbitale_Unknown
RDFCA157-05_BOLD_Sargocentron_suborbitale_Costa_Rica
RDFCA228-05_BOLD_Sargocentron_suborbitale_Costa_Rica
GU225425.1_cds_ADD80651.1_Sargocentron_vexillarium_Mexico
GU225424.1_cds_ADD80650.1_Sargocentron_vexillarium_Mexico
JQ840676.1_cds_AFM99983.1_Sargocentron_vexillarium_Belize
JQ840675.1_cds_AFM99982.1_Sargocentron_vexillarium_Belize
JQ842299.1_cds_AFJ14257.1_Sargocentron_vexillarium_Netherlands_Antilles_Caribbean
JQ842298.1_cds_AFJ14256.1_Sargocentron_vexillarium_Netherlands_Antilles_Caribbean
JQ842297.1_cds_AFJ14255.1_Sargocentron_vexillarium_Netherlands_Antilles_Caribbean
JQ841785.1_cds_AFJ13881.1_Sargocentron_vexillarium_Belize
JQ839884.1_cds_AFJ00738.1_Sargocentron_vexillarium_Bahamas
JQ839883.1_cds_AFJ00737.1_Sargocentron_vexillarium_Bahamas
JQ839882.1_cds_AFJ00736.1_Sargocentron_vexillarium_Bahamas
FBERM004-06_BOLD_Sargocentron_vexillarium_Bermuda
MK658412.1_Sargocentron_punctatissimum_French_Polynesia
MK658349.1_Sargocentron_punctatissimum_French_Polynesia
MK658238.1_Sargocentron_punctatissimum_French_Polynesia
MK657696.1_Sargocentron_punctatissimum_French_Polynesia
MK657445.1_Sargocentron_punctatissimum_French_Polynesia
MF409640.1_Sargocentron_punctatissimum_Reunion
MF409490.1_Sargocentron_punctatissimum_Reunion
KU943287.1_Sargocentron_punctatissimum_Taiwan
JQ350317.1_Sargocentron_punctatissimum_Reunion
JQ350314.1_Sargocentron_punctatissimum_Reunion
JQ350313.1_Sargocentron_punctatissimum_Reunion
FOAN506-11_BOLD_Sargocentron_punctatissimum_Indonesia
MH331858.1_Sargocentron_punctatissimum_Saudi_Arabia
KU191586.1_Sargocentron_punctatissimum_Taiwan
JQ350315.1_Sargocentron_punctatissimum_Reunion
MK657215.1_Sargocentron_punctatissimum_French_Polynesia
MK657003.1_Sargocentron_punctatissimum_French_Polynesia
MF409486.1_Sargocentron_punctatissimum_Reunion
JF494419.1_Sargocentron_punctatissimum_South_Africa
KJ968250.1_Sargocentron_punctatissimum_French_Polynesia
KF930370.1_Sargocentron_punctatissimum_Northern_Mariana_Islands
JQ350316.1_Sargocentron_punctatissimum_Reunion
HM034270.1_Sargocentron_punctatissimum_French_Polynesia
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FADLI196-17_BOLD_Sargocentron_punctatissimum_Indonesia
FOAK988-10_BOLD_Sargocentron_punctatissimum_Indonesia
FOAK989-10_BOLD_Sargocentron_punctatissimum_Indonesia
FOAN507-11_BOLD_Sargocentron_punctatissimum_Indonesia
FUT194-18_BOLD_Sargocentron_punctatissimum_Futuna_Islands
FUT301-18_BOLD_Sargocentron_spiniferum_Futuna_Islands
KY675593.1_Ostichthys_kaianus_Saudi_Arabia_Outgroup
0.54
0.8
0.99
0.91
0.89
0.84
0.97
0.03

APPENDIX D

SUPPLEMENTARY MATERIAL IV

COI sequences of *Sargocentron rubrum* sampled for this study. Those labeled with “DE” are from Lebanon and those with “SM” are from Turkey:

DE82-5
AGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAAT
TTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACT
TCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTC
TGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAA
ACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACAT
TCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

DE82-8
CAGCCCTTAGCCTTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTT
ATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCT
CCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGAT
CTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCT
CTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGAC
CCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

DE82-11
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
TAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTC
CCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATC
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TTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACC
CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCT

DE82-14
TGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTT
CTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCT
ACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGT
TGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACT
CCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCT
TTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATT

DE82-17
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
TAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTC
CCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATC
TTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTC
TTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACC
CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

DE90-1
TAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAA
TTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGAC
TTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTT
CTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAA
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TCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-2
AGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTAT
AGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCC
CTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCT
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AGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-3
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AGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

DE90-4
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AGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCC
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TACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCT
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DE90-5
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DE90-6
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TACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCT
TTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCC
AGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-7
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TATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTG
TATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAG
CAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-8
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AGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCC
CTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCT
TACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCT
TTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCC
AGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-9
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ATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTAC
TATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTG
TATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAG
CAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-10
CTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTG
TAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATAGCATTCCCTCGAATAAATAACATAAGCT
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GGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAA
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CAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-11
GTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATT
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CTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-12
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
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CCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATC
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TTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACC
CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-13
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
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CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-14
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
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CCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATC
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TTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACC
CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-15
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DE90-16
CAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTA
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CCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATC
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CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-17
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DE90-18
GGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTC
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TGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-19
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CAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-20

CCCTTAGCCTTCTTATTCGAGCTGACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTA
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DE90-22
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TTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACC
CAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATT

DE90-23
CCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGT
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CAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

DE90-24
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CTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

DE90-25
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TATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTG
TATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAG
CAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-1
GACATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGAC
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CCTGATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACA
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CAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCA
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SM-2
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CAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCC
CCTGATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACA
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CAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCA
GGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTTT

SM-3
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TCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGC
AGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTAATATG
AAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGC
TAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAA

SM-4
CACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTA
TAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATAT
AGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCC
ACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATT
ATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTA
CTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCT

SM-5
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATT

SM-6
TGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAAT
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CCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTA
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ACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-7
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
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SM-8
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GATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTT
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CTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGG
GATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

SM-9

ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-10
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SM-11
CATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCA
AATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCT
GATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTT
TACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAA
CTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGG
GATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-12
CATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCA
AATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCT
GATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTT
TACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAA
CTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGG
GATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTTT

SM-13
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-14
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-15
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-16
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGA

SM-17
GCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTT
ATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATA
TGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACC
CACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTAT
TATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATT
ACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-18
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-19
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-20
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

APPENDIX E

SUPPLEMENTARY MATERIAL V

COI sequences of all *Sargocentron rubrum* individuals from Turkey, including those sequenced for this study:

KY176608.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTGATATGGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCC
CTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATTATTA
ATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATAT

JQ623980.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501252.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501251.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501250.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501249.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501248.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501247.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501246.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501245.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501244.1 *Sargocentron rubrum* Turkey
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501243.1 *Sargocentron rubrum* Turkey

CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501242.1 ***Sargocentron rubrum*** **Turkey**
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501241.1 ***Sargocentron rubrum*** **Turkey**
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501240.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501239.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501238.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501237.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501236.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501235.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501234.1 ***Sargocentron rubrum*** **Turkey**
CTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTGATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACTACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

KC501233.1 ***Sargocentron rubrum*** **Turkey**
CTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACGGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGGGACGATCAAATTTATAAT
GTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGACTAATCCCCCTAATAATCGGAGCTCCTGACATAGCA
TTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTTCTGCTAGCTTCTTCAGGAGTAGAAGCCGGTGCCGGAACAGGGTGAACAGTTTACCCACCC
CTTGCAGGTAATTTAGCCCACGCAGGGGCTTCTGTTGATCTTACTATTTTCTCACTCCATTTAGCTGGTATTTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATCATTA
ATATGAAACCCCCTGCCATCTCCCAATATCAGACCCCTCTTTTCGTATGGGCTGTCCTAATTACAGCTGTCCTTCTTCTCCTGTCCCTACCTGTACTTGCAGCAGGTATTACTAT
GCTGCTAACAGACCGAAATCTAAACACAACATTCTTCGACCCAGCAGGAGGTGGAGACCCTATTCTATATCAACACCTGTTT

SM-1
GACATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGAC
CAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCC
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CAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCA
GGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCT

SM-2
GACATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGAC
CAAATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCC
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GTTTACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTA
CAACTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCA
GGGATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTTT

SM-3

TTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTATAATGTCAT
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TCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCCACCCCTTGC
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AAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATTACTATGCTGC
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SM-4
CACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAATTTA
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AGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACCC
ACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTATT
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SM-5
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
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ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
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ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATT

SM-6
TGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAAAT
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TTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATT
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SM-7
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ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTG

SM-8
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GATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTT
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CTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGG
GATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTC

SM-9
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ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-10
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ATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTAC
CCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAACTA
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ACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-11
CATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCA
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CTATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGG
GATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-12
CATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCA
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SM-13
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-14
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ATATGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
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SM-15
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
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SM-16

ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
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TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGA

SM-17
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TGGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTTACC
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TATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGGATT
ACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-18
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-19
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

SM-20
ATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCTGGAATAGTTGGAACAGCCCTTAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGAGCCCTTCTGGGAGACGACCAA
ATTTATAATGTCATTGTTACAGCGCACGCGTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGGAGGCTTTGGGAACTGATTAATTCCCTTAATAATCGGAGCCCCTG
ATATAGCATTCCCTCGAATAAATAACATAAGCTTTTGACTTCTACCTCCCTCATTCCTTCTCCTTCTAGCTTCTTCAGGAGTAGAAGCCGGTGCTGGAACAGGATGAACAGTTT
ACCCACCCCTTGCAGGTAACTTAGCCCACGCGGGGGCTTCTGTTGATCTTACTATCTTCTCTCTCCATCTGGCTGGTATCTCCTCAATCCTAGGGGCCATTAATTTTATTACAAC
TATTATTAATATGAAACCCCCTGCCATCTCTCAATATCAAACTCCTCTCTTTGTATGAGCTGTCCTAATTACAGCTGTTCTTCTTCTCCTATCCCTACCTGTACTTGCAGCAGGG
ATTACTATGCTGCTAACAGACCGAAATCTAAACACAACATTCTTTGACCCAGCAGGAGGTGGAGACCCGATTCTATATCAACACCTATTCTGATTCTT

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