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ORIGINAL PAPER

Anti-Lessepsian migration rectified: the Comber *Serranus cabrilla* (L. 1758) existed in the Red Sea prior to the Suez Canal opening

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Abstract

Since the opening of the Suez Canal in 1869, a plethora of Red Sea fishes have entered the Mediterranean Sea (Lessepsian migration). The Comber *Serranus cabrilla* is considered to have moved in the opposite direction as anti-Lessepsian migrant and has an established population in the northern Red Sea. Mitochondrial (COI) and nuclear DNA from 49 individuals from Red Sea (Gulf of Suez) and Mediterranean populations (Cyprus, Egypt and Lebanon), as well as GenBank sequences from other Mediterranean and Atlantic populations were analyzed to study phylogenetic relationships. COI sequencing resulted in a phylogenetic tree that isolated the Red Sea population from all Mediterranean and Atlantic populations. Furthermore, sequence divergence within the Red Sea population was significantly lower than in Atlantic and Mediterranean populations. Moreover, none of the Mediterranean haplotypes were present in the Red Sea. SNPs and MIG-seq analyses of nuclear DNA confirmed that the Red Sea population is genetically different from the Mediterranean populations. A rough estimate of divergence time suggested that Mediterranean and Red Sea populations separated 194,055 years ago, at the latest. *Serranus cabrilla* must, therefore, have existed in the Red Sea prior to the opening of the Suez Canal and can no longer be considered as an anti-Lessepsian migrant.

Introduction

The Suez Canal was officially opened in 1869, connecting the Red Sea to the Mediterranean Sea, and resulted in an unprecedented exchange of organisms. The majority of organisms have moved from the Red Sea to the Mediterranean Sea, so-called Lessepsian migration (Galil 2009; Por 1978), while few have moved in the opposite direction, via anti-Lessepsian migration (Por 1978; Golani 1999). The taxonomic diversity of invasive organisms is broad, including, though not limited to, seagrass (Gambi et al. 2009), zooplankton (Spanier and Galil 1991; Zakaria 2015), echinoderms (Tzomos et al. 2010) and fish (Mavruk and Avsar 2008). Most fish are considered to have actively migrated through the Canal, but some taxa may have been introduced by other means (Por 1978; Katsanevakis et al. 2020). Examples of invasive fish include the well-established lionfish, *Pterois miles* (Bariche et al. 2017; Bos et al. 2018), and the blue spotted cornetfish, *Fistularia commersonii* (Golani 2000), or more recently discovered autochthone species such as the Red Sea spotted grouper, *Epinephelus geoffroyi* (Golani et al. 2015), and the variable-lined fusilier, *Caesio varilineata* (Bos and Ogwang 2018).

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The predominantly unidirectional Lessepsian migration of fish through the Suez Canal has been attributed to two main factors. First, the higher water level of the Red Sea facilitates water movement toward the Mediterranean Sea (Golani 1999). Second, the Mediterranean Sea has more favorable conditions for successful establishment, such as a lower salinity and a lower species diversity, possibly providing unoccupied niches (Chanet et al. 2012; Belmaker et al. 2013). Furthermore, these factors may further discourage anti-Lessepsian migration: Higher salinity as well as niche saturation in the Red Sea may present major establishment challenges to organisms adapted to conditions in the Mediterranean Sea (Chanet et al. 2012).

The Comber *Serranus cabrilla* (Linnaeus 1758) is one of few fishes that were documented to have migrated from the Mediterranean Sea to the Red Sea (Ben-Tuvia 1971; Goren and Klausewitz 1978; Randall and Golani 1995; Chanet et al. 2012). *Serranus cabrilla* is a relatively small demersal bass (Serranidae), commonly found on rocky bottoms at depths ranging from 5 to 70 m (Tortonese 1986). It is found distributed throughout the Mediterranean Sea, western Black Sea, and has also been reported along Atlantic coasts from southwestern Europe to South Africa, including the Canary Islands, Madeira, and the Azores (Tortonese 1986; Heemstra 2012).

While genetic studies comparing Lessepsian migrant fishes to their native populations are plentiful (e.g. Azzurro et al. 2006; Golani et al. 2007; Bariche et al. 2015; Bernardi et al. 2016; Bariche and Fricke 2018; Ogwang et al. 2020), anti-Lessepsian migrants have not been studied. The analysis of the genetic diversity of the Mediterranean populations of *Serranus cabrilla* revealed considerable levels of gene flow based on mitochondrial DNA (Vella and Vella 2016). The Red Sea population, however, has never been included in genetic analyses. Apart from using relative conservative mtDNA for genetic comparison (Ward et al. 2009), the inclusion of a nuclear marker generally strengthens a study's conclusions. Restriction site-Associated DNA sequences (RAD-seq), allowing population genomic analyses of non-model organisms using high-throughput sequencing technology (Baird et al. 2008), were recently extended with the development of multiplexed ISSR genotyping by sequencing (MIG-seq). The ISSR regions are initially amplified by PCR using universal primer sets, indexed by a second PCR, and then sequenced to obtain up to several thousand SNPs. MIG-seq targets obtaining putatively neutral loci adjacent to microsatellite regions in which relatively fast molecular evolution may be detected (Suyama and Matsuki 2015). While the number of available SNPs from MIG-seq is generally lower than from RAD-seq (Miller et al. 2007), MIG-seq can be performed with low quality and/or small amounts of DNA. As this is often the case for marine specimens, this relatively new technique has been successfully applied to species delimitation of non-modal marine organisms (Richards et al. 2018; Takata et al. 2019). For the above-mentioned reason, as well as for the lower operating cost (Takata et al. 2019), it was decided to use MIG-seq for the analysis of nuclear DNA.

The current study aimed at analyzing the genetic diversity of the *Serranus cabrilla* population from the Gulf of Suez (Red Sea) and hereby examine the history of its possible migration from the Mediterranean to the Red Sea.

Materials and methods

Sample collection

Twenty-two individuals of *Serranus cabrilla* (Linneaus, 1758) were bought from a trawler fisher (29.900° N 32.468° E) or caught with hook and line (29.717° N 32.392° E) in the Gulf of Suez, Egypt between June and September 2014 (Fig. 1). Additional tissue of Mediterranean origin was collected from 5 individuals bought at a local fish market east of Limassol, Cyprus (34.724° N 33.342° E) in April 2018, from 12 individuals bought at a local fish market in Beirut, Lebanon (34.486° N 35.916° E) in December 2017, and from 10 individuals bought at a local fish market in Alexandria, Egypt (31.211° N 29.880° E) in September 2019. All samples were preserved in 98% ethanol, transported to the American University in Cairo, Egypt and stored in a freezer at −20 °C until DNA extraction. For comparison, GenBank sequences representing Mediterranean and Atlantic populations of *S. cabrilla* were used (Online Resource 1; Keskin and Atar 2013, Landi et al. 2014, Shirak et al. 2016, Vella and Vella 2016). *Serranus baldwini* (Evermann and Marsh 1899) and *S. subligarius* (Cope 1871) were used as outgroup species (Online Resource 1).

DNA extraction and sequencing

DNA was purified from gill-raker or muscle tissue (20–45 mg) using Qiagen's QIAmp® DNA Mini Kit (Cat. No.: 51304) following the manufacturer's protocol. The optical concentration of each pure DNA sample was measured on a Nanodrop machine and its integrity checked on 1% agarose gel. The purified DNA was stored at −20 °C.

For mitochondrial analysis, the 5′ end (~ 600 bp) of cytochrome c oxidase subunit I (COI), a gene that encodes the catalytic unit of cytochrome c oxidase, an enzyme acting as terminal electron receptor in the aerobic respiratory pathway (García-Horsman et al. 1994), was amplified. MyTaq™ DNA Polymerase (Cat. No.: BIO-21105) was used to amplify COI sequences with the forward primer FishF1: TCAACCAACCACAAAGACATTGGCAC and the reverse primer FishR1: TAGACTTCTGGGTGGCCAAAGAATCA

Fig. 1 Map of the Mediterranean Sea and northern Red Sea (Gulf of Suez). Three Mediterranean sample locations and one Red Sea sample location are represented by inverted triangles. Genbank sequences were used from four additional Mediterranean populations (il, it, mt, tk) and one Atlantic population (pt)

(Ward et al. 2005). Each polymerase chain reaction (PCR) had a total volume of 25 µl and consisted of 5 µl MyTaq™ Red DNA reaction buffer, 1 µl of 10 µM forward and reverse primer, 0.5 µl of DNA polymerase, and varying volumes of template DNA equivalent to 0.25–1 µg DNA. The remaining volume was topped up with nuclease-free water. Cycling conditions were set as follows: 95 °C for initial denaturation (5 min), and 35 cycles of 95 °C (30 s), 45 °C (45 s), 72 °C (1 min), a final extension at 72 °C (7 min) and a final hold at 4 °C, following Bos (2014). Five microliters of each reaction were loaded to 1% agarose to visualize COI bands. Samples with clear bands were cleaned using Qiagen's MinElute™ PCR Purification Kit (Cat. No.: 28004) and the pure products were checked on 1% agarose gel before being stored at −20 °C. The purified PCR products were subjected to a standard two-way cycle sequencing with the above described primers using BigDye™ terminator enzyme (Macrogen Inc., Seoul, South Korea).

For a genome-wide SNP analysis, we applied multiplexed Inter-Simple Sequence Repeat (ISSR) genotyping by sequencing called MIG-seq (Suyama and Matsuki 2015). This is useful for examining phylogenetic relationships and genetic connectivity among populations of a particular species. MIG-seq amplifies genome-wide ISSRs by PCR. Eight pairs of multiplex ISSR primers (MIG-seq primer set 1) were used for initial PCR (Suyama and Matsuki 2015). After indexing each sample by a second PCR, they were pooled and sequenced using MiSeq (sequencing control software version 2.0.12; Illumina) with MiSeq Reagent v3 150-cycle kit (Illumina).

Data analysis

Our samples, containing 49 COI sequences and 18 GenBank COI sequences were aligned online using the MAFFT platform, allowing enhanced visual interaction with the input sequences (Kuraku et al. 2013; Katoh et al. 2017). The Minimum Evolution (ME) algorithm applied in MEGA7 (Kumar et al. 2016) was used to construct the phylogenetic tree using Tamura-3-parameter model with site variation rate shape 5; the T92 + G model (Tamura 1992). Mean intrapopulation and interpopulation genetic divergence based on *p*-distance were calculated using MEGA7 version 7.0.26 by applying the Kimura 2-parameter model (Kimura 1980). In addition, the mean interpopulation genetic distance based on *p*-distance was used to estimate divergence time between the Red Sea and Mediterranean populations. The Atlantic population was excluded from some analyses, because it was represented by one GenBank sequence. We applied the highest divergence known for fishes based on *p*-distance (0.1144 changes site^{-1} million years^{-1}; Burridge et al. 2008) to estimate a minimum divergence time.

Population structure was examined by analyzing the haplotype and nucleotide diversity. Haplotype diversity measures the probability that two alleles picked at random are different, while nucleotide diversity measures the probability

that a point in aligned DNA sequences is different between two randomly chosen sequences (Excoffier et al. 1992). These two parameters were determined by single-nucleotide polymorphism (SNP) analysis using DnaSP (Rozas et al. 2017). To visualize haplotype distributions, minimum spanning networks (Bandelt et al. 1999) were drawn using popART (Leigh and Bryant 2015).

The FASTX-toolkit version 0.0.14, with fastq-quality-filter setting of –Q 33 –q 30 –p 40, was used to eliminate low-quality reads and primer sequences from the MIG-seq raw data (Gordon and Hannon 2012). Adapter sequences for Illmina MiSeq were removed from both 5′ end (GTC AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC) and 3′ end (CAGAGATCGGAAGAGCGTCGTGTAGGG AAAGAC) using Cutadapt version 1.13 (Martin 2011). Any reads with less than 80 bp were excluded. Subsequently, Stacks version 1.4 was used to demultiplex, filter, and stack the reads for extraction of SNPs (Catchen et al. 2013). First, the U-stacks were used with 'minimum depth of coverage required to create a stack (*m*)' = 3, 'maximum distance allowed between stacks (*M*)' = 1, 'maximum distance allowed to align secondary reads to primary stacks (*N*)' = 1, and the deleveraging (*d*) and removal (*r*) algorithms enabled. Second, the C-stacks were used with 'number of mismatches allowed between sample loci when build the catalog (*n*)' = 4, followed by S-stacks. We restricted data by setting *r*, the minimum percentage of individuals required to process a locus across all data, to be 0.5 using the Population Pipeline implemented in Stacks version 1.4. Stacks parameters were set as suggested by previous genotyped MIG-seq studies (Richards et al. 2018; Takata et al. 2019). The population parameters were adjusted by altering the *r*-value to between 0.5 and 0.6 (controlling the proportion of missing data) to avoid allelic drop-out, to avoid outcome inconsistencies. If loci were not found in all individuals, they were removed from the analysis.

STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to detect genetic admixture of different populations of *Serranus cabrilla* excluding low-quality data. Ten independent runs (testing number of clusters $K = 1$–5) were conducted in STRUCTURE using the admixture model and allele frequency-correlated model. Both, the length of burn-in period and the number of Markov Chain Monte Carlo analysis (MCMC) were set at 100,000. The most likely number of clusters ΔK (Evanno et al. 2005) was estimated using Structure Harvester (Earl 2012), summarized and visualized using CLUMPAK (Kopelman et al. 2015) and DISTRUCT (Rosenberg 2004). ΔK is an ad hoc statistic representing the rate of change in log probability between successive K values and enables detecting the true number of clusters (K) in a data set when the pattern of dispersal among populations is not homogeneous (Evanno et al. 2005). In addition, we calculated pairwise F_{ST} among four populations (Cyprus, Lebanon, Egypt-Med, and Egypt-Suez) as well as F_{ST} between Red Sea and Mediterranean populations using GeneAlex version 6.5 (Peakall and Smouse 2006) with 9999 permutations. The Hardy–Weinberg Equilibrium was tested for each population using FSTAT version 2.9.3.2 (Goudet 2002) and its significance was tested adjusting $p < 0.05$ with Bonferroni correction. To visualize the genetic relationship among different individuals in two dimensions, Principle Coordinate Analysis (PCoA) was also conducted using GeneAlex version 6.5. PGDspider version 2.0.8.3 was used for the data conversion (Lischer and Excoffier 2011). Samples with low reads (< 28,000) and/or lacking common loci were excluded from the analysis.

Results

Phylogenetic relationships (COI)

The aligned COI sequence dataset consisted of 639 positions where 572 (89.5%) were conserved, 67 (10.5%) were variable, 41 (6.4%) were parsimony informative and 26 (4.0%) were singletons. The populations from the Red Sea and Turkey had the lowest intraspecific divergence of 0.4%; whereas, this ranged from 0.8 to 1.2% for the other Mediterranean populations. COI sequence divergence between the Red Sea and Mediterranean populations was consistently higher than 2.0%; while, it was 1.8% between the Red Sea and Atlantic population (Table 1). Sequence divergence

Table 1 COI sequence divergence (%) of *Serranus cabrilla* between 8 Mediterranean/Atlantic populations and the Gulf of Suez population (Egypt-Suez)

	Egypt-Med	Cyprus	Israel	Italy	Lebanon	Malta	Portugal	Turkey
Cyprus	1.02							
Israel	0.98	0.88						
Italy	0.86	1.06	1.01					
Lebanon	0.90	1.04	0.95	0.99				
Malta	0.77	1.00	0.95	0.86	0.90			
Portugal	1.06	0.74	0.50	1.03	0.96	0.99		
Turkey	1.24	0.95	0.73	1.22	1.15	1.17	0.24	
Egypt-Suez	2.57	2.29	2.12	2.57	2.52	2.52	1.77	2.01

between the Atlantic and various Mediterranean populations varied between 0.24 and 1.2% (Table 1).

The Minimum Evolution tree (Fig. 2), based on COI sequences, had a sum of branch lengths of 0.4352 and clustered into three clades. One clade isolated all samples from the Gulf of Suez (Red Sea); while, the other clades represented both a combination of the eight Atlantic and Mediterranean locations (Fig. 2).

Haplotype and nucleotide diversity (COI)

The Atlantic and Mediterranean populations of *Serranus cabrilla* were represented by a total number of 20 haplotypes while the population in the Gulf of Suez was represented by 15 haplotypes (Table 2). Although the haplotype diversity was higher in the Suez population, the nucleotide diversity in the Gulf of Suez was the second lowest among eight populations (Table 2). Although haplotypes were generally well mixed among the Mediterranean populations, no haplotypes were shared between the Atlantic/Mediterranean and Red Sea populations (Fig. 3). The Mediterranean haplotype closest to a Red Sea haplotype was separated by 7 mutations and unique to Cyprus (Fig. 3). The single haplotype found in the Atlantic population (Portugal) was the most common Mediterranean haplotype (Fig. 3). The Red Sea population had the highest negative Tajima's D value (Table 2) suggesting bottleneck establishment, which was confirmed by the star-shape haplotype network found for this population (Fig. 3). The low gene flow (Nm = 0.11) and the high fixation index (F_{ST} = 0.7042), as measures of genetic differentiation, were significantly different between the Mediterranean and Red Sea populations (Chi-square, $p < 0.001$) underlining genetic isolation.

The estimated divergence between the Red Sea and Mediterranean populations was 0.0222 (± 0.0048). The rough estimate of minimum divergence time for the populations of the two seas was 194,055 years with a range of 152,000–236,013 years.

Nuclear DNA

In total, 276 loci were obtained from 37 individuals (22 from the Mediterranean locations and 15 from the Red Sea) using the $r = 0.5$ criterion. Seventy-six loci were removed from the analysis, because these loci were not found in all individuals of the populations. Structure Harvester analysis indicated that the most likely number of clusters (K) was 2 (Online Resource 2). For the remaining 200 loci, the analysis indicated clear genetic distinction between the Red Sea and Mediterranean populations with only two individuals from Cyprus and the Gulf of Suez showing some admixture (Fig. 4). Pairwise F_{ST} comparison showed no significant genetic differentiation among the Mediterranean

Fig. 2 Minimum Evolution tree for *Serranus cabrilla* (Secab) based on COI sequences from individuals from Mediterranean (A# = Egypt—Mediterranean, C# = Cyprus, L# = Lebanon; black diamond) and Red Sea (S# = Egypt—Suez; white diamond) populations with *S. baldwini* and *S. subligarius* as outgroup (white circle). Eighteen GenBank sequences from Atlantic (red triangle) and Mediterranean populations (red diamonds) are represented by their accession numbers (for details see Online Resource 1)

populations, while a significant genetic differentiation was found between the Red Sea and Mediterranean populations (Table 3). The F_{ST} between Red Sea and combined Mediterranean populations was high (0.221) and significant

Table 2 Haplotypes diversity, nucleotide diversity and Tajima's D for *Serranus cabrilla* populations from the Red Sea (Gulf of Suez) and seven Mediterranean Sea locations

Location	N	S	H	h ± SD	π	Tajima's D
All populations	107	51	35	0.896 ± 0.0003	0.01364	−1.0075
Suez, Egypt	22	24	15	0.948 ± 0.0013	0.00533	−2.1074
Mediterranean/Atlantic	85	18	20	0.741 ± 0.0009	0.00861	−1.1055
Egypt	10	11	5	0.800 ± 0.0100	0.00739	0.3845
Cyprus	5	17	5	1.000 ± 0.0160	0.01436	0.0360
Israel	7	14	5	0.905 ± 0.0107	0.00946	−0.0676
Italy	8	14	5	0.857 ± 0.0117	0.00992	0.6337
Lebanon	12	21	9	0.939 ± 0.0033	0.01221	0.0100
Malta	20	14	6	0.768 ± 0.0039	0.00847	0.6358
Turkey	22	7	4	0.697 ± 0.0034	0.00398	1.0419

'All populations' and 'Mediterranean/Atlantic' present accumulated data for the respective locations including the single sample from the Atlantic Ocean

N number of sequences, *S* number of polymorphic sites, *H* number of haplotypes, *h* haplotype diversity (with standard deviation) and π = nucleotide diversity

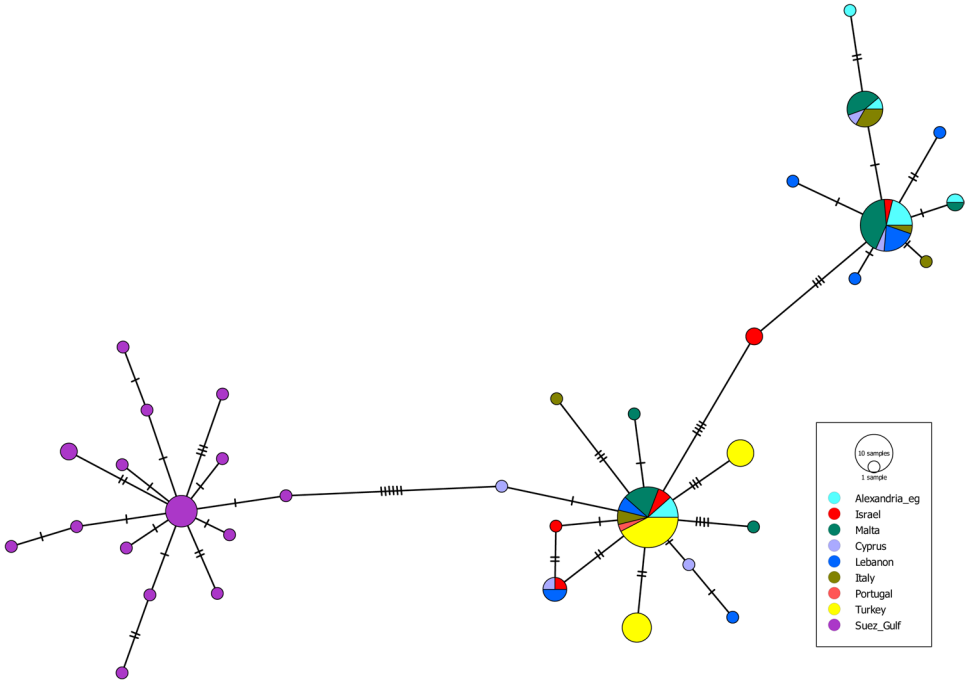

Fig. 3 COI haplotype network for *Serranus cabrilla* from the Red Sea (Gulf of Suez) and one Atlantic and seven Mediterranean locations. Each colored pie represents one haplotype with its size being proportional to frequency. Number of mutations between neighboring haplotypes are represented by hatch marks

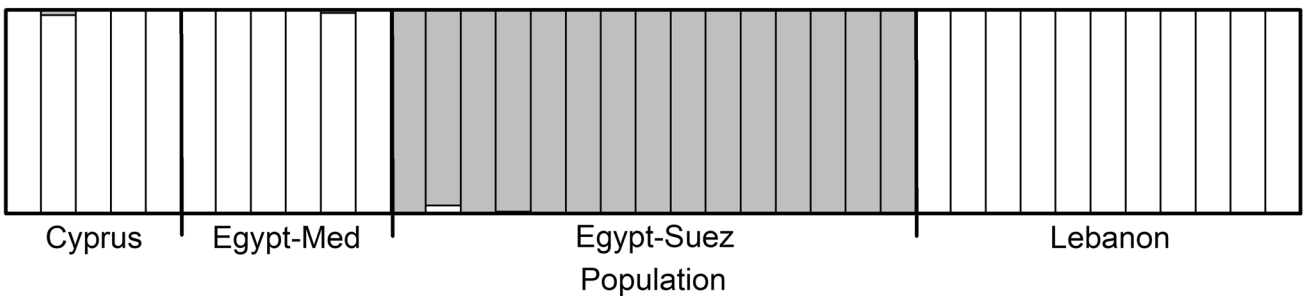

Fig. 4 Nuclear MIG-seq analysis ($K = 2$). Vertical bars represent individuals of *Serranus cabrilla* from three Mediterranean populations (Cyprus, Egypt-Mediterranean, Lebanon) and the Red Sea population (Egypt-Suez). The *Y*-axis indicates the proportion of different genetic compositions by gray scale

Table 3 Pairwise comparison of F_{ST} values among sampled populations based on nDNA (below diagonal) and probability based on 9999 permutations (above diagonal)

	Cyprus	Egypt-Med	Lebanon	Egypt-Suez
Cyprus	–	0.180	0.453	0.000
Egypt-Mediterranean	0.029	–	0.458	0.000
Lebanon	0.000	0.000	–	0.000
Egypt-Suez	0.198	0.240	0.195	–

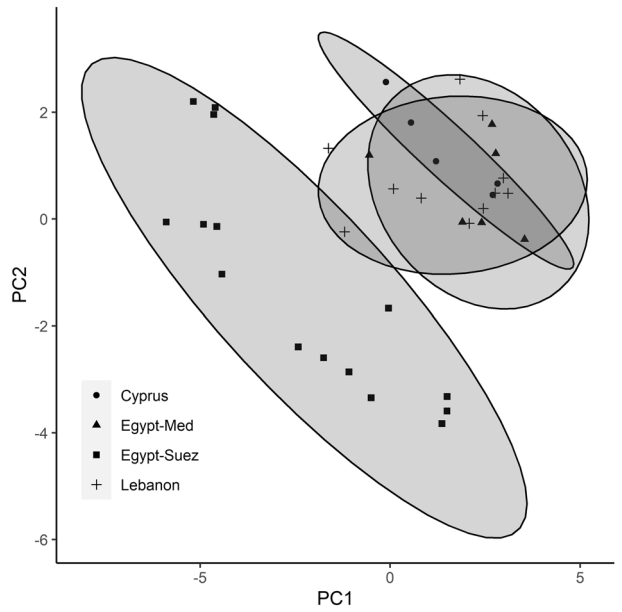

Fig. 5 Principal Coordinates Analysis (PCoA) using nuclear MIG-seq data for 43 individuals of *Serranus cabrilla* from the Mediterranean (Cyprus, Egypt-Mediterranean, Lebanon) and the Red Sea (Egypt-Suez). PC1 and PC2, respectively, explain 23.3 and 9.6% of variance in the data set

(AMOVA, $p < 0.001$). The PCoA with PC1 and PC2 explaining 23.3 and 9.6% of variance of the data clearly isolated the Red Sea population from the three Mediterranean populations with minimum overlap between the 95% confidence ellipses (Fig. 5).

Discussion

Analysis of mitochondrial and nuclear DNA showed that the *Serranus cabrilla* population in the Gulf of Suez (northern Red Sea) is genetically isolated from the populations in the Atlantic and Mediterranean Sea. Mitochondrial COI sequence divergence between Red Sea and Mediterranean populations of *S. cabrilla* was consistently higher than 2.01%, while this was less than 1.24% among Atlantic and Mediterranean populations (Table 1). COI sequence divergence of > 1% has been used to identify serranids, including *S. cabrilla*, in the Mediterranean Sea (Vella and Vella 2016). The authors further found extensive mtDNA haplotype sharing among Mediterranean populations of *S. cabrilla*. Similarly, we found considerable COI haplotype sharing among Atlantic and Mediterranean populations, but none of the haplotypes were shared with the population in the Red Sea (Fig. 3). These results, based on mtDNA, were underlined by genome-wide nuclear SNPs data, which also showed genetic differentiation between the Red Sea and Mediterranean populations. STRUCTURE analysis suggested a relatively large genetic difference with almost no admixture between Red Sea and Mediterranean individuals (Fig. 4). Furthermore, nuclear MIG-seq analysis did not detect a spatial genetic structure within the three Mediterranean populations from Cyprus, Egypt and Lebanon (Table 3 and Fig. 5).

The present study revealed that the *Serranus cabrilla* population from the Gulf of Suez has a relatively high haplotype diversity and a low nucleotide diversity (Table 2). Both could be indicators of rapid population expansion after a genetic bottleneck (Grant and Bowen 1998). Furthermore, the relatively high negative value of Tajima's D (Table 2) as well as the radiating shape of the haplotype network in the Red Sea (Fig. 3) may indicate that rare alleles emerged from rapid population growth following a bottleneck event (Grant and Bowen 1998; Hahn et al. 2002). It is, therefore, logically concluded that *S. cabrilla* migrated from the Mediterranean to the Red Sea (Gulf of Suez). However, not one haplotype from 85 new and published Mediterranean COI sequences was shared between the Mediterranean and Suez populations (Fig. 3), emphasizing the existence of two genetically fully isolated populations. Moreover, gene flow between Mediterranean and Suez populations was almost negligible ($F_{ST} = 0.7042$, $Nm = 0.11$). Gene flow among western Mediterranean populations of *S. cabrilla* was highly associated with ocean currents accommodating larval dispersal (Schunter et al. 2011). Water currents in the Gulf of Suez and Suez Canal may not have supported population establishment in the Red Sea, because the flow is predominantly northward (Por 1978) and, consequently, must have counteracted an anti-Lessepsian migration of *S. cabrilla*.

In the 1970s, Por (1978) questioned that the Red Sea population of *Serranus cabrilla* was of Mediterranean origin, opposing the generally accepted assumption that this fish is an anti-Lessepsian migrant (e.g., Ben-Tuvia 1971). Por (1978) argued that 1875, the year in which *S. cabrilla* was initially reported in the Red Sea, was too early for even the fastest-adapting organism to establish a population in the invaded habitat. Moreover, high fish diversity and complex biodiversity in the Red Sea provide newcomers with challenging settlement conditions because most ecological niches are occupied (Belmaker et al. 2013). Therefore, it seems unlikely that *S. cabrilla* was able to establish a

population in the Gulf of Suez within six years after the opening of the Suez Canal.

Results from both mitochondrial and nuclear DNA suggested that the populations from either side of the Suez Canal have been genetically separated for a long time. The minimum divergence time estimate for populations of *S. cabrilla* in the Mediterranean and Red Sea, using the fastest divergence rate published for fishes (Burridge et al. 2008), was approximately 200,000 years. Therefore, it is likely that these two populations diverged prior to the opening of the Suez Canal in 1869 and that temporal gene flow has been extremely low or entirely absent. The relatively high number of haplotypes and high haplotype diversity may exemplify that the separation happened hundred thousands of years ago.

About 5000 years ago, Pharaonic people maintained boat connections between the main water bodies in Egypt (Creasman and Doyle 2010). Although partly over land, they may have unintentionally carried organisms (e.g., fish eggs or larvae) from the Mediterranean to the Red Sea. This, however, seems too recent to explain the wide genetic gap between the populations of *S. cabrilla*. Tectonic activity and sea-level changes have accommodated connections between the Mediterranean and Red Sea throughout geological times with the last major cut off about 5 million years ago (Rasul and Stewart 2015). It seems unlikely that the *Serranus cabrilla* population in the Red Sea is a remnant of this particular connection. However, considering the small sea-level difference between the two seas (Eid et al. 1997) and continued tectonic activity in the region, geologically insignificant water connections may have allowed individuals to reach the Red Sea more recently.

Serranus cabrilla covers a large geographical distribution area including the western Black Sea, the Mediterranean Sea and the eastern Atlantic coastal waters ranging from southwestern Europe, along West-Africa to South Africa (Froese and Pauly 2019). The existence of a Pan-African population was suggested in the past, based on a record of *S. cabrilla* in the Indian Ocean near Madagascar (Fricke et al. 2017). However, this isolated record was reported in 1904 and no individuals have been found in the Indian Ocean since. As we found that the population of *S. cabrilla* in the Red Sea is genetically isolated, other local populations along the western and southern African coasts may also appear to be genetically different. If so, isolated populations of *S. cabrilla* may proof to be remnants of a once Pan-African population. Whether the isolated population of *S. cabrilla* in the Red Sea represents a sub-species, or possibly a different fish species, needs further morphological study.

Lessepsian migration has facilitated many Indo-Pacific organisms to reach the Mediterranean Sea and many of them have become successful invaders. Anti-Lessepsian migration has been of less relevance, because few Mediterranean organisms were able to reach the Red Sea and establish themselves. The present study shows that *Serranus cabrilla*, often used to exemplify anti-Lessepsian migration, already existed in the Red Sea prior to the opening of the Suez Canal and that its ecological status in the Red Sea, as well as its taxonomic status, needs reconsideration.

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Author contributions ARB and JO designed the study. Samples were collected by ARB, JO, MB and MM. Genetic analyses were developed and conducted by AO, JO, MH and NY. The first draft of the manuscript was written by ARB and JO with contributions from MB and NY. MM collected additional samples and updated figures and text before resubmission. All authors approved the final manuscript.

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Data accessibility DNA Sequences were submitted to GenBank, where they are freely available.

Conflict of interest The authors have no conflict of interest to declare.

Ethical standards This article does not present any data resulting from experiments with live animals. Tissue samples were collected from fish purchased on local markets.

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