1 **Population genetic diversity of green turtles, *Chelonia mydas*, in the Mediterranean revisited**

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17 **Abstract**

18 The Mediterranean green turtle regional management unit is one of the 17 management units of green turtles
19 considered a global conservation priority. However, previous studies using different genetic markers revealed very
20 little diversity and differentiation across populations due to the overdominance of one haplotype (CM-A13) in the
21 Mediterranean. We, therefore, used a more informative marker, mitochondrial short tandem repeats (mtSTRs), in
22 431 samples collected along the eastern Mediterranean coasts of Turkey and Northern Cyprus. In addition, we
23 added the mtSTR haplotypes of previous studies and reached a total of 980 samples covering 12 nesting beaches
24 (almost 100% of the populations in the region). We identified 42 haplotypes, 4 of which were recorded for the first
25 time in the region. The species has a genetic diversity in the region higher than previously thought, ranging from
26 0.54 (Sugözü, Turkey) to 0.934 (Israel) and with the most common haplotypes being 6-8-8-4 (26.5%), 6-8-5-4
27 (17.3%), and 6-8-6-4 (14.9%). The analysis of a more extensive data set of mtSTRs supported recognizing at least
28 three management units in the Mediterranean. Furthermore, we used the new data to assess the origin of the turtles
29 foraging in Israel. We determined that Samandağ (Turkey) was the population of origin of most of the individuals.
30 Overall, we show that mtSTRs highly improve the resolution to detect population structuring and source for this
31 species and region.

32 **Keywords:** *Chelonia mydas*, genetic structure, Mediterranean, mitochondrial DNA, short tandem repeats.

Introduction

Defining biologically relevant population units for monitoring and management is an essential first step in conservation. This is significantly more critical for migratory species since they couple biodiversity and ecosystem functioning worldwide (Bauer and Hoye 2014). The complex life cycle of marine turtles, wide range dispersal, and high migratory ability through oceanic and neritic foraging habitats make the direct observation of these charismatic animals difficult (Avise 1998). In this respect, genetic tools played a vital role in defining natal homing, population boundaries, connectivity between populations and foraging habitats, stock structure, and genetically important management units in sea turtles (Jensen et al. 2013; Komoroske et al. 2017). Genetics is essential for determining the origin of stranded or foraging sea turtles, using Mixed Stock Analysis (MSA) to discover their natal beaches (Monzón-Argüello et al. 2012; Turkozan et al. 2018; Shamblin et al. 2018). However, robust results require adequate sampling, informative genetic markers, and the genetic characterization of potential source nesting beaches. In the last two decades, studies aiming to determine the population genetic structure of nesting colonies of sea turtles in the Mediterranean used short (~380bp) or long sequences (~800bp) of the control region (CR) of mitochondrial DNA (mtDNA) or microsatellites (Carreras et al. 2007; Yılmaz et al. 2011; Bagda et al. 2012; Clusa et al. 2013). While CR haplotypes provided better resolution for the loggerhead turtles (Carreras et al. 2007; Yılmaz et al. 2011; Clusa et al. 2013), they did not provide the exact resolution for green turtles, as single haplotype (CM-A13) accounted for 97% of the individuals (Bagda et al. 2012). However, using four consecutive mitochondrial short tandem repeats (mtSTR), Tikochinski et al. (2012) determined that the dominant haplotype, CM-A13, in the Mediterranean could be subdivided into 33 variants. Thus, indicating that the genetic diversity could be greater than expected within the region. Subsequent studies proved that mtSTR markers could better resolve the population genetic structure of green turtles (Bradshaw et al. 2018; Tikochinski et al. 2018; Shamblin et al. 2020) and a promising tool to perform MSA (Tikochinski et al. 2018).

The global green turtle population is declared endangered by IUCN (Seminoff 2004). However, recent studies suggest a population increase in some regions (Stokes et al. 2014; Mazaris et al. 2017; Casale et al. 2018). The Mediterranean green turtle population is one of the most essential 17 management units described as a global priority for green turtle conservation (Wallace et al. 2010), considering both the strength of the threads in the region (direct and indirect anthropogenic impacts) and the risk of extinction (population viability assessment). The nesting beaches of green turtles are confined to Turkey, Cyprus, Lebanon, Israel, Syria, and Egypt in the eastern Mediterranean (Turkozan and Kaska 2010; Casale et al. 2018). Turkish and Cypriot nesting colonies almost comprise 99% of the overall nesting activity (Casale et al. 2018, Table 1).

63 The Mediterranean populations of green turtles have been suggested to be a recent colony from the Atlantic Ocean,
64 probably after the last glacial interval (Bowen et al. 1992; Encalada et al.1996). A recent study suggested a warm-
65 water corridor hypothesis for the eastward geneflow of green turtles during the last interglacial to the last glacial
66 period (van der Zee et al. 2021). However, the absence of Atlantic CR haplotypes and mtSTRs in a recent study
67 with a considerable sample size (Bradshaw et al. 2018) pointed to the reproductive isolation of the green turtles
68 and further supported the subpopulation status.

69 In the most recent study, Tikochinski et al. (2018) provided samples from some of the green turtle nesting beaches
70 of the Mediterranean, and using mtSTRs, they suggested four distinct management units but with a strong emphasis
71 on the need of doing extensive genotyping of mtSTRs, mainly from Turkey and Syria, to have a clearer picture of
72 Mediterranean green turtle genetic structuring. Given the importance of comprehensive and representative
73 sampling of individuals for the accurate assessment of population structure and to facilitate precise estimates of
74 the fine-scale genetic differentiation among rookeries (Komoroske et al. 2017), we added 431 novel samples,
75 including two unsampled beaches (Sugözü, Davultepe). The primary objectives were (i) to determine discrete
76 populations concerning female natal homing, (ii) to provide robust baseline data for stranded and foraging green
77 turtles to identify their population of origin (iii) to define the extent of the natal neighborhood by nesting females.

78 **Material and Methods**

79 ***Sample collection and DNA extraction***

80 During the nesting seasons of 2017-2019, a total of 431 dead hatchlings of green turtles were sampled from six
81 nesting beaches (Alata, Davultepe, Kazanlı, Akyatan, Sugözü, and Samandağ) along the Mediterranean coast of
82 Turkey (Figure 1, Table 1). To prevent sampling hatchlings from multiple nests of the same females, we collected
83 the samples only from the clutches laid within a 10-day window, as females do not nest at intervals shorter than
84 this period. Furthermore, we reanalyzed our North Cyprus samples used in the Bagda et al. (2012) study. In
85 addition, we added 549 mtSTRs genotypes of the previously published studies (Bradshaw et al. 2018; Tikochinski
86 et al. 2018) to our dataset to better represent the Mediterranean green turtle population. The final dataset comprised
87 a total of 980 samples (Table 1). We refrain from including control region sequences due to the overdominant
88 presence of the CM-A13 (Bagda et al. 2012) that will provide trivial information following the procedures in
89 previous studies (Tikochinski et al. 2018).

90 A piece of muscle tissue was cut from the dead hatchlings and kept in 99% ethanol. Total DNA extractions were
91 performed according to the manufacturer's protocol using the Invitrogen PureLink Mini genomic DNA isolation

92 kit (ThermoFisher). Approximately 25-30 mg of tissue was used. The purity and quantity of each DNA sample
93 were measured on a NanoDrop 2000 spectrophotometer using 1 µl of DNA. DNA samples were then visually
94 inspected by electrophoresis in 1% agarose gel using 1XTBE buffer (Tris-borate-EDTA) and Safeview dye. The
95 DNA quality measured on a spectrophotometer was determined by the ratio 260/280 nm. We used DNA samples
96 with 260/280 nm ratios in the 1.6-1.9 range for the polymerase chain reactions. DNA samples that did not have
97 the desired absorbance values (260/280 nm) determined by spectrophotometer were included in the study after
98 they were cleaned with ethyl alcohol precipitation.

99 ***Polymerase chain reaction and sequencing***

100 For the mtDNA 3′-STR region analysis, the forward primer CM-D-1 F 5′- AGCCCATTTACTTCTCGCCAAACC
101 CC-3′ and the reverse primer CM-D-5 R 5 GCTCCTTTTATCTGATGGGACTGTT-3 were used (Tikochinski et
102 al. 2012) to amplify approximately a 400 bp fragment of mtDNA control region by polymerase chain reaction
103 (PCR).

104 PCR was carried out in a 25-µl reaction containing: 0.75µl (20 µM) of each primer, 12.5µl Taq DNA Polymerase,
105 2X MixRED (Ampliqon), and 50 ng DNA. PCR cycling program was: 4 min at 94 °C, 30 cycles of 45 s at 94 °C,
106 60 s at 62 °C, and 1 s at 72 °C, followed by 5 min at 72 °C (Tikochinski et al. 2012) using Veriti, Applied Biosystem
107 thermal cycler. PCR products were visualized and checked by electrophoresis in 2% agarose gel using 1X TBE
108 buffer and Safeview dye. The obtained PCR products were cleaned using the Invitrogen PureLink Quick PCR
109 cleaning kit (ThermoFisher) and then sent to Macrogen Inc. (Seoul, South Korea) for sequencing (3730XL
110 automatic capillary sequencer, Applied Biosystem). Each sample was sequenced in forward and reverse directions
111 using the same primers described above. The sequences were aligned using the BioEdit 7.2.6 program ClustalW
112 multiple alignments (Thompson et al. 1994). The mtSTR haplotypes were coded as the number of repeats of each
113 of the four mtSTRs, as described in previous studies (Tikochinski et al. 2012; Bradshaw et al. 2017; Tikochinski
114 et al. 2018).

115 ***Data analysis***

116 We used Arlequin 3.5.2 software to calculate the haplotype diversity (H) for each population as well as genetic
117 distances (F_{ST}) values between population pairs that will reveal the difference between the genetic structure of the
118 beaches (Excoffier and Lischer 2010). Inter-population F_{ST} values were calculated by entering haplotype
119 frequencies for each population, and the default setting was used; 1000 for the number of permutations and 0.05

120 for the p-value. All multiple comparisons were corrected using a false discovery rate (FDR) approach (Narum,
121 2006). The resulting frequency based F_{ST} matrix is represented with heatmaps and dendrograms with the "gplots"
122 R package (Warnes et al. 2016). We performed a Principal Coordinate Analysis (PCoA) based on F_{ST} values using
123 GeneAlEx 6.5 (Peakall and Smouse 2012). We also built a network of population connectivity using the F_{ST}
124 dissimilarity distance matrix performed in the software EDENetworks v.2.18 (Kivelä et al. 2015). Each network
125 was constructed based on the parameters of susceptibility and sensibility of the network size using the F_{ST}
126 dissimilarity matrix as the significant percolation threshold value to construct a connection between populations.
127 Subsequently, based on the F_{ST} distance matrix, BARRIER v 2.2 (Manni et al., 2004) was used to assess the
128 relative order of importance of genetic breaks that could limit gene flow between populations. Finally, we tested
129 the significance of the proposed structure among MUs using an analysis of molecular variance (AMOVA) as
130 implemented in Arlequin version 3.5.2

131 To test the potential of our dataset as a robust baseline to perform Mixed Stock Analysis (MSA), we reanalyzed
132 the Israeli strandings data of Tikochinski et al. (2018). A Bayesian Mixed Stock Analysis (MSA) was used to
133 assess the composition of the Israeli stranding stock through the use of Bayes (Pella and Masuda 2001). This
134 analysis estimates the proportion of individuals of the mixed stock coming from the different nesting populations.
135 We used our dataset comprising all the published STR haplotype frequencies from the Mediterranean nesting
136 populations as the baseline. We performed three different simulations, including a) no weighting factor, b) using
137 an estimate on the size of each rookery (expressed as the mean number of nests per year (see Table 1) as a weighting
138 factor, as suggested by previous studies (Bass et al. 2004), and c) using the minimum distance across the sea
139 (expressed as km) as a weighting factor. Population sizes were taken from the literature (Casale et al. 2018), and
140 the minimum distance across the sea was measured using GoogleEarth®. Iterated chains were considered reliable
141 when the Gelman-Rubin criterion was fulfilled (G-R shrink factor <1.2 for all parameters) as described in the
142 software manual.

143 **Results**

144 ***Description of haplotypes and haplotype diversity***

145 We recorded 42 haplotypes, 4 of which were recorded for the first time. Of these new haplotypes, two were unique
146 to Turkish nesting beaches (6-8-5-5 and 7-7-5-4), while one was unique to North Karpaz (6-8-7-5) and another
147 one shared between Turkish and N. Cyprus nesting beaches (6-1-0-5-5) (Supplement Table 1). The most common
148 haplotypes were 6-8-8-4, 6-8-5-4, 6-8-6-4, and 6-9-6-4, with the highest frequencies constituting almost 66.9% of

all haplotypes. Among these most common haplotypes, only 6-8-8-4 and 6-8-5-4 were represented in all nesting beaches. (Supplement Table 1). The haplotype 7-8-7-4 mainly pertained to Cypriot beaches and outside this region was recorded only on Samandağ beach of Turkey. The haplotype diversity ranged from 0.54 (Sugözü) to 0.934 (Israel) (Supplement Table 1).

Management Units

F_{ST} pairwise differences (Table 2), PCoA analysis based on F_{ST} values (Figure 2), and clustering dendrogram (Figure 3) showed significant genetic structuring for the Mediterranean green turtle populations. Most of the pairwise comparisons were significant. However, this was not true for the comparisons involving Israel or Davultepe, probably due to the low sample size of these populations (Table 2). Combining F_{ST} based heatmap clusters, the statistical importance of pairwise comparisons, and geographic context, we propose the identification of a minimum of 3 management units (MUs) named MED1 (Akamas and Akdeniz), MED2 (Alagadi), and MED3 (North and South Karpaz, Israel, Samandağ, Akyatan, Sugözü, Kazanlı, Alata, Davultepe). Akamas was considered an isolated unit according to a significant F_{ST} value with all the remaining populations (except for those with low sample size), and an isolated position in both the dendrogram and PCoA; the same applies to Akdeniz. However, these two had non-significant F_{ST} and therefore grouped together as MED1. MED2 (Alagadi) showed significant F_{ST} differences except for DVL (low sample size) and NKAR but grouped in heatmap separately from other MUs. Furthermore, MED2 does not show connectivity with other management units (Figure 4). Within MED3, NKAR and SKAR had non-significant F_{ST} and were geographically very close. KAZ does not offer a significant F_{ST} with most other sites from Turkey, only with SGZ (but the value is low). Furthermore, some populations (Samandağ, Alata, North Karpaz, and Israel) showed high levels of connectivity (being Samandağ and Alata hubs of connectivity), non-significant F_{ST} values and were grouped in the dendrogram. The PCoA analysis based on F_{ST} values explained 76.27% of the variation among the localities (Figure 2). The first coordinate discriminated Israel and Cyprus nesting beaches from the Turkish nesting beaches, explaining the 47.97% of the variance and points to a possible north-south clustering, while the second coordinate discriminated the AKA, SGZ DVL, and remaining nesting colonies from the others, explaining an additional 28.3% of the variance. Additionally, the clustering dendrogram using the F_{ST} pairwise matrix showed that some of the populations, such as SAM and NKAR, seem to act as connectors among regions, probably because of their location. According to the low values of F_{ST} displayed among populations from different dendrogram branches, Samandağ connects ALT, NKAR, and ISR with AKY, KAZ, SGZ, and DVL while North Karpaz connects with SGZ and DVL. The network analyses (Figure 4) showed high levels of connectivity but restricted mainly to populations within the proposed

179 management units. Furthermore, the populations of Israel, Samandağ, and Alata were positioned as hotspots of
180 connectivity due to their high betweenness centrality values, either within or between the proposed Management
181 Units. On the other side, the relative order of barriers detected by BARRIER (Supplementary Figure 2) confirmed
182 the isolation of some locations, such as North Karpaz or Akamas.

183 ***Haplotypes and haplotype diversity within management units***

184 Considering our proposal of Management Units, twelve haplotypes (Supplement Table 3) were represented in all
185 management units. The management unit of MED3 had 16 private haplotypes, while 1 and 2 were unique to MED1
186 and MED2, respectively (Supplement Table 2). Haplotype diversity was the highest in MED3 (0.855) and the
187 lowest in MED1 (0.723) (Supplement Table 2, Figure 2). The MED1 and MED3 were represented with haplotype
188 6-8-8-4 with the highest frequency in these management units, while in MED2, haplotype 6-8-6-4 was the highest.
189 (Supplement Table 2). The pairwise genetic distance was the highest between MED1-MED3 (F_{ST}=0.059), and all
190 pairwise comparisons among the proposed 3 MUs were significantly different ($p<0.0001$) (Supplement Table 3).
191 The total variation among groups was 5.85%, while 94.15% within the group (Overall F_{ST}=0.058, $p<0.0001$).
192 Heteroplasmy was detected in approximately 14% of samples.

193 ***Mixed stock analysis***

194 Considering the new data on nesting populations provided in the present study, the number of 'orphan haplotypes'
195 (e.g., haplotypes from a foraging ground not found in any nesting area) was 6 out of 30 haplotypes, representing
196 only 6.14% of the total samples. As indicated in a previous study, most stranded individuals came from Turkey
197 nesting beaches (Tikochinski et al. 2018). However, our new data on the Turkish nesting area allowed us to
198 determine the origin of the individuals with more precision as most of them came from the nesting beach of
199 Samandağ, the Turkish nesting area closest to the region of the stranded individuals (Figure 5). Some minor
200 contribution was also detected from the Karpaz region in Northern Cyprus and Kazanlı.

201 **Discussion**

202 A comprehensive and representative sampling of individuals for the accurate assessment of population structure
203 and facilitating precise estimates of the fine-scale genetic differentiation among rookeries is crucial (Komoroske
204 et al. 2017). In this respect, the present work added two nesting colonies (DVL and SGZ) of green turtles, which
205 were not represented in the previous work (Tikochinski et al. 2018). Furthermore, we added 431 novel samples,
206 fulfilling a critical gap, especially for the most vital nesting colonies of green turtles in the Mediterranean, such as

207 Akyatan (AKY) and Samandağ (SAM) beaches, represented in the previous study by 3 and 27 samples,
208 respectively. These efforts resulted in 4 novel haplotypes totaling 42 haplotypes for the Mediterranean green
209 turtles. The previous studies using ~380 bp or ~860 bp of CR recorded up to 10 haplotypes being all populations
210 genetically homogeneous due to the dominant haplotype CM-A13 (accounting for 97% of the samples) (Encalada
211 et al. 1996; Bagda et al. 2012). The use of mtSTRs, a novel method introduced by Tikochinski et al. (2012), showed
212 that Mediterranean green turtle populations are much genetically diverse than previously thought and deliver levels
213 of genetic differentiation, allowing us to determine the genetic structure and finer scale in the region. In contrast
214 to Tikochinski et al. (2018), our improved sample size also allowed us to find genetic structuring among Turkish
215 nesting colonies. This resulted in the characterization of 3 genetically distinct management units for Akamas, the
216 most differentiated with a high haplotype frequency 6-8-8-4. Akamas' lower diversity (0.592) was attributed to
217 more recent colonization or bottleneck caused by a reduction in the number of nesting females (Tikochinski et al.
218 2018).

219 The interchange of nesting individuals between Akyatan-Sugözü and Samandağ-Syria were determined with
220 mark-recapture studies (Sönmez et al. 2017). This suggested the possibility that unsampled Syria may remain in
221 the northern cluster. However, this hypothesis needs further sampling from Akamas and Syrian nesting beaches.
222 Bradshaw et al. (2018) used high-resolution haplotype sets (the mtSTRs concatenated to the end of mtDNA control
223 region haplotype sequence) and showed a significant stock structure in green turtle rookeries of Northern Cyprus.
224 They emphasized a considerable differentiation between Akdeniz - South Karpaz and North Karpaz – Akdeniz.
225 More or less, a similar structure was defined in the present study as Akdeniz constitutes MED1 with Akamas,
226 Alagadi MED2, and remaining nesting colonies comprise MED3. Thus, MED3 contains a set of populations
227 partially connected, thus boosting the overall genetic diversity of this management unit in comparison with
228 management units composed of single isolated populations (such as MED2).

229 On the other hand, the absence of shared mtSTRs with Atlantic rookeries (Shamblin et al. 2015a, 2015b) was
230 supposed to support the complete isolation of the Mediterranean group (Bradshaw et al. 2018) and its status as a
231 management unit (Wallace et al., 2010). Although previous studies suggested that the Mediterranean populations
232 originated from Atlantic colonizers (Bowen et al. 1992; Encalada et al. 1996), the differences found in haplotype
233 composition and frequencies indicate that the populations of the two regions have been isolated for a long time.
234 However, a recent study from the Atlantic coast of the USA (Shamblin et al. 2020) provided evidence of 4 shared
235 haplotypes (6-8-5-4, 5-7-6-4, 5-8-6-4, and 5-8-5-4). These shared STRs are not found in combination with the
236 CM-A13.1 in the Atlantic, but the similarity of haplotypes (in a phylogeographic context) suggests that the

Mediterranean populations were originated by Atlantic colonizers as happened with other species (e.g., *Caretta caretta*). The only CR haplotypes known to occur in both regions are CM-A13.1 and CM-A27.1 (Encalada et al. 1996; Bagda et al. 2012), which was attributed to a limited gene flow over an ecological time scale (Bradshaw et al. 2017).

Some of the haplotypes that we thought to be novel initially have also been found in Israeli populations in a study exploring the heteroplasmy of the mtDNA in marine turtles. This suggests that a hidden diversity in other populations may be found within individual low frequent haplotypes. Although we found heteroplasmy on a few individuals (14%), it is probably more widespread and can be found with genomic approaches (Tikochinski et al. 2020). For instance, rare haplotypes found in low frequencies such as haplotype 7-8-9-4 and 9-7-7-4, which were supposed to be novel in the present study, were determined to be present at low frequencies in heteroplasmic individuals of Israel.

The new data on Turkish nesting populations in the present study allowed us to determine the origin of the individuals with more precision, as most of them came from the nesting beach of Samandağ, the Turkish nesting area closest to the region of the stranded individuals.

In conclusion, the application of mtSTRs in the present work confirmed better structure than control region sequences in the Mediterranean and the other regions (Shamblin et al. 2015c; Bradshaw et al. 2018; Tikochinski et al. 2018; Shamblin et al. 2020). Our study further supports the natal homing hypothesis (Lohmann et al. 2013; Bradshaw et al. 2018; Shamblin et al. 2020) and nest-site fidelity on a regional basis, not for specific beaches. Furthermore, this study provides robust baseline data for future mixed stock analyses for stranded and foraging turtles.

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390 **Fig. 1** Sampling and nesting locations of recent study and previous studies: ALT (Alata), DVL (Davultepe), KAZ
391 (Kazanlı), AKY (Akyatan), SGZ (Sugözü), SAM (Samandağ), AKA (Akamas), AKD (Akdeniz), ALG (Alagadi),
392 NKAR (North Karpaz), SKAR (South Karpaz), and ISR (Israel)

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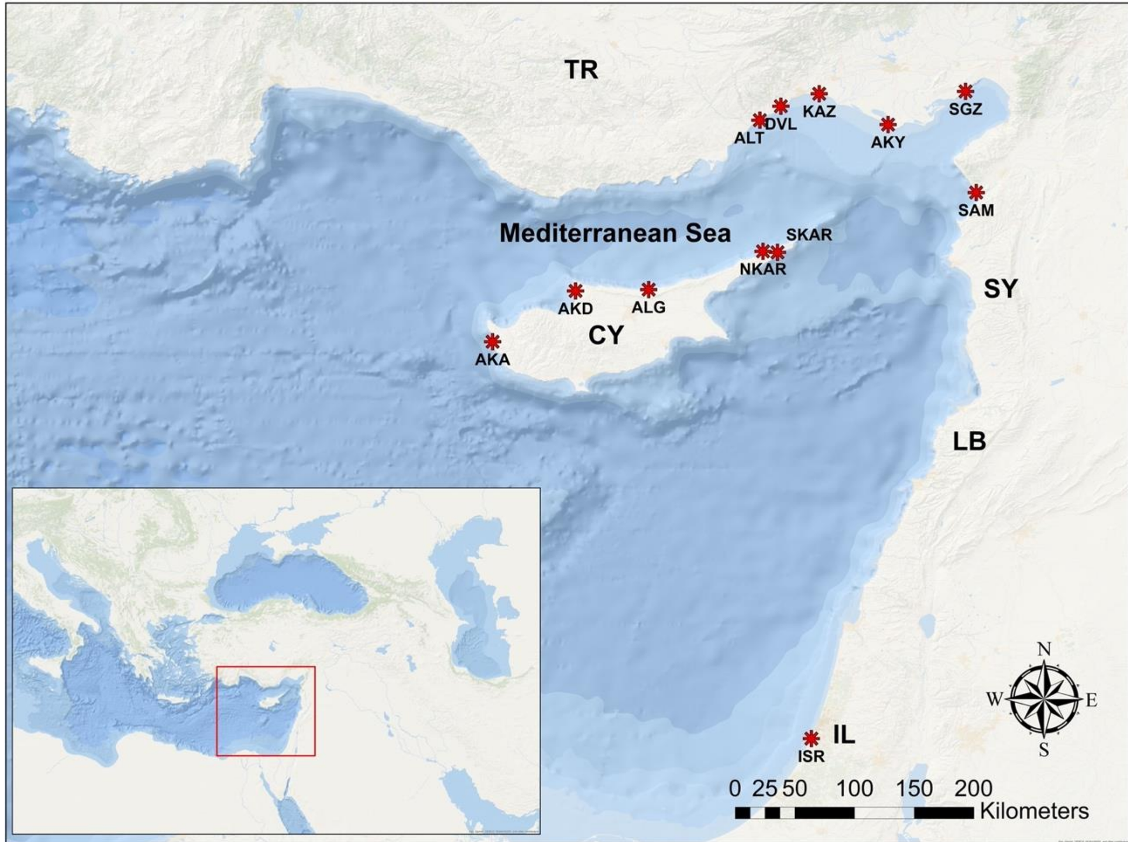

408 **Fig. 2** Principal coordinate analysis (PCoA) based on genetic distances (F_{ST}) among the sampling locations of
409 *Chelonia mydas* in the Mediterranean. **AKA:** Akamas, **ALG:** Alagadi, **AKD:** Akdeniz, **SKAR:** South Karpaz,
410 **NKAR:** North Karpaz, **ISR:** Israel, **SGZ:** Sugözü, **DVL:** Davultepe, **SAM:** Samandağ, **ALT:** Alata, **KAZ:**
411 Kazanlı, **AKY:** Akyatan. The blue squares group the locations sampled either in Turkey (right) on Cyprus (left).

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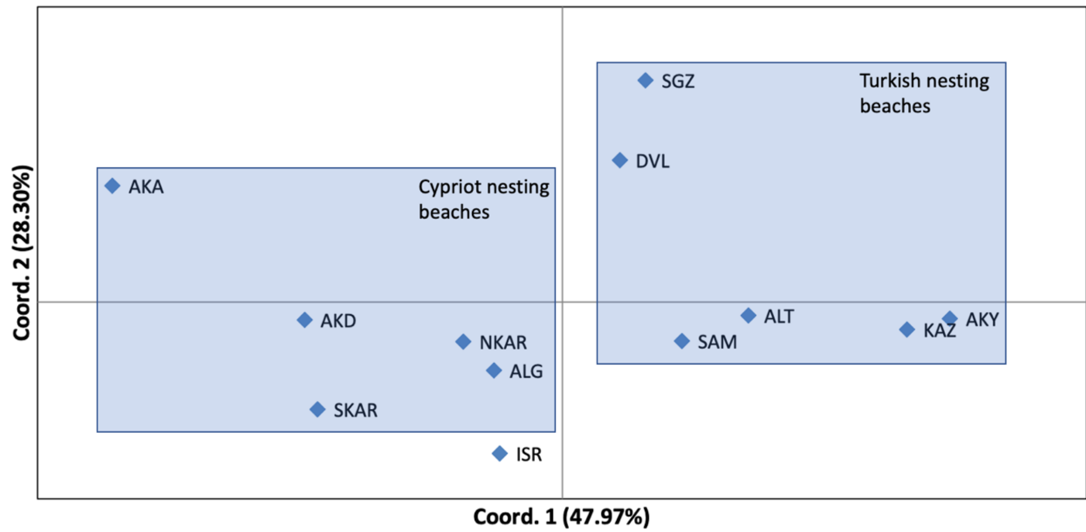

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Fig 3 Heatmaps and dendrograms based on F_{ST} pairwise distances among green turtle nesting beaches. The low F_{ST} values shown among some of the populations belonging to different dendrogram clusters suggest connectivity points among management units. The asterisks within cells indicate pairwise comparisons that are significant after FDR correction (as in Table 2). **AKA:** Akamas, **ALG:** Alagadi, **AKD:** Akdeniz, **SKAR:** South Karpaz, **NKAR:** North Karpaz, **ISR:** Israel, **SGZ:** Sugözü, **DVL:** Davultepe, **SAM:** Samandağ, **ALT:** Alata, **KAZ:** Kazanlı, **AKY:** Akyatan

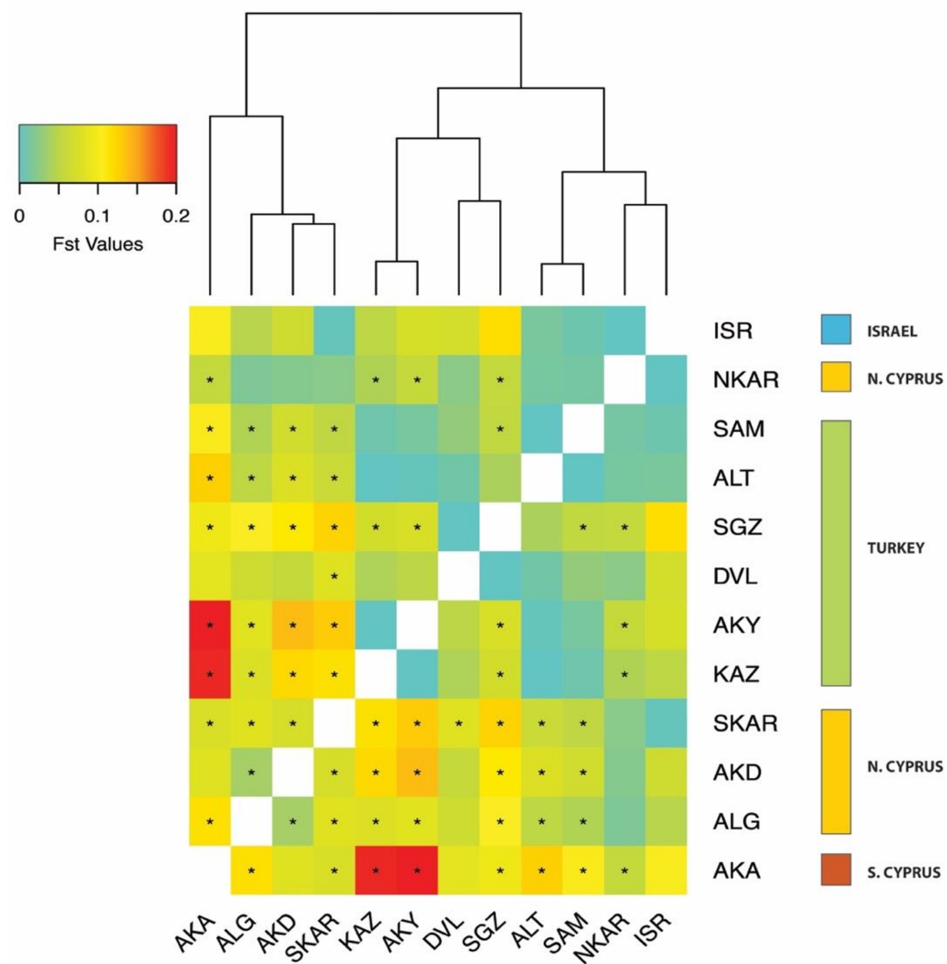

Fig 4 Network of connectivity among Mediterranean populations of the green turtle (*Chelonia mydas*). Lines represent significant connectivity links among populations according to EDENetworks. The width of the lines represents the strength of this connectivity. The size and the colour of the nodes (populations) represent the betweenness value. AKA: Akamas, **ALG:** Alagadi, **AKD:** Akdeniz, **SKAR:** South Karpaz, **NKAR:** North Karpaz, **ISR:** Israel, **SGZ:** Sugözü, **DVL:** Davultepe, **SAM:** Samandağ, **ALT:** Alata, **KAZ:** Kazanlı, **AKY:** Akyatan

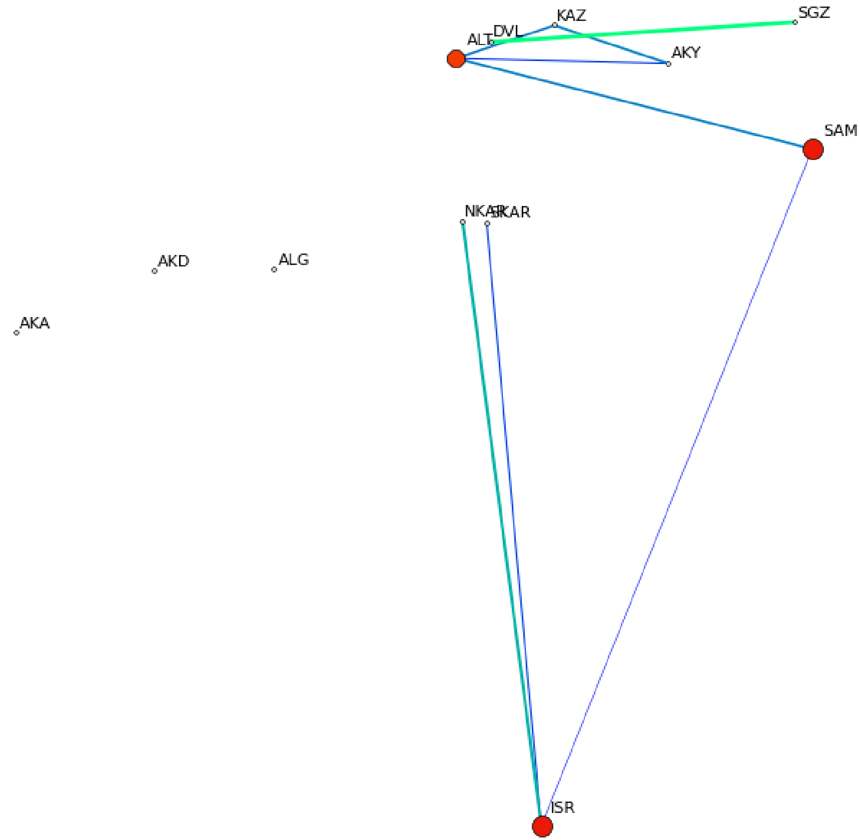

Fig 5 Mixed stock analysis (MSA) of the stranded turtles found along the Israeli coast. Each bar represents the percentage of turtles that originate from nesting populations as indicated by the analysis using three different settings a) with no weighting factor, b) using population size as weighting factor and c) using the minimum distance at sea as a weighting factor. Error bars represent 95% confidence intervals

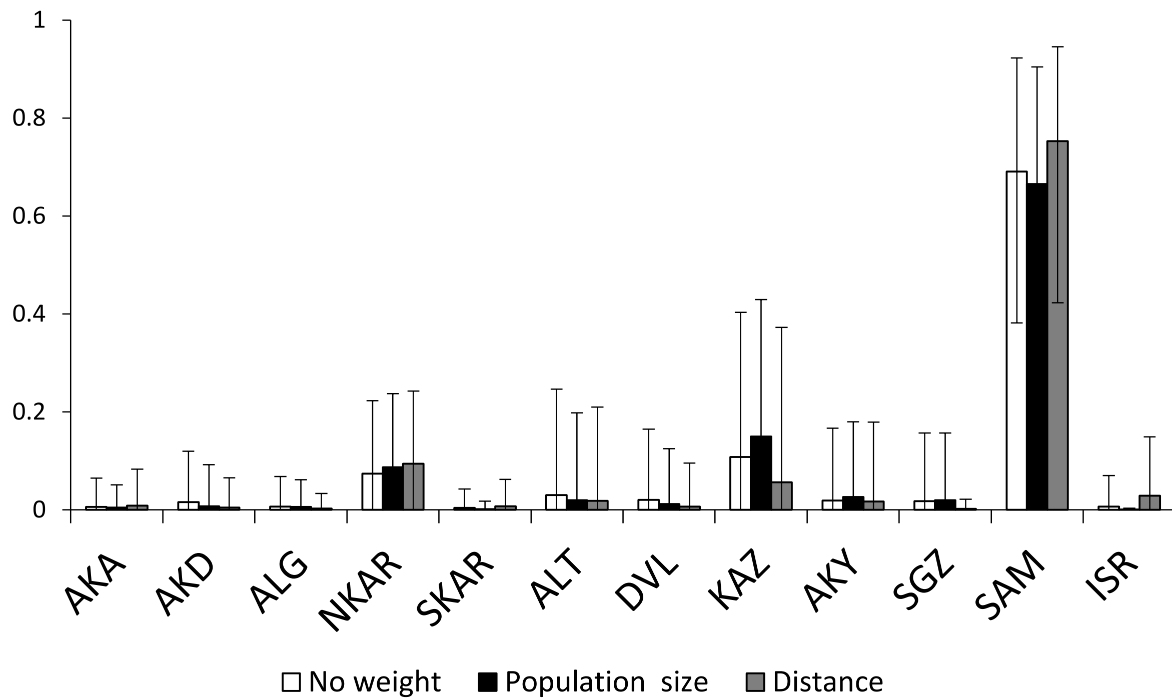

Table 1 Detailed information about the sampling locations in the Mediterranean, including the present study and published information. Population sizes of the nesting areas are expressed as mean nests per year, as found in the literature (Casale et al., 2018). *Population sizes from Israel is from Tikochinski et al 2018

Sampling Site	Acronym	Average nests yr^{-1}	No of the samples included from Tikochinski et al. (2018)	No of the samples included from (Bradshaw et al. 2018)	Recent Study	Total sample size
Akyatan	AKY	322	3	-	110	113
Alata	ALT	125	27	-	28	55
Kazanlı	KAZ	365	28	-	48	76
Samandağ	SAM	306	27	-	140	167
Sugözü	SGZ	213	-	-	40	40
Davultepe	DVL	113	-	-	29	29
Akamas	AKA	108	29	-	-	29
Akdeniz	AKD	70	-	84	-	84
Alagadi	ALG	154		234	-	234
North Karpaz	NKAR	220	-	54	36	90
South Karpaz	SKAR	59	-	46	-	46
Israel	ISR	18*	17	-	-	17
Total			**131**	**418**	**431**	**980**

Table 2 Pairwise genetic distances (F_{ST}) among the nesting sites of green turtles in the Mediterranean. Cells below the diagonal show genetic distances and those above the diagonal show p values. We highlighted in bold the significant values after FDR correction (for 66 comparisons, corrected p= 0.0105)

	SAM	AKY	KAZ	ALT	SGZ	DVL	NKAR	SKAR	AKD	ALG	AKA	ISR
SAM		0.02539	0.09570	0.56055	**0.00684**	0.04688	0.03906	**0.00000**	**0.00000**	**0.00098**	**0.00000**	0.25977
AKY	0.01367		0.65625	0.26953	**0.00391**	0.01660	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	0.01367
KAZ	0.00744	-0.00401		0.48242	**0.00586**	0.04785	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	0.06543
ALT	-0.00352	0.00238	-0.00262		0.02637	0.22168	0.09180	**0.00000**	**0.00000**	**0.00586**	**0.00098**	0.21387
SGZ	0.05369	0.07458	0.06938	0.04046		0.530027	**0.00391**	**0.00098**	**0.00293**	**0.00195**	**0.01562**	0.01758
DVL	0.02866	0.04837	0.04193	0.00992	-0.01495		0.07031	**0.00391**	0.02148	0.02051	0.02148	0.04395
NKAR	0.01164	0.05781	0.04295	0.0131	0.05619	0.02377		0.05078	0.01855	0.03223	**0.00879**	0.54102
SKAR	0.05246	0.12972	0.11616	0.06092	0.12484	0.08085	0.02236		**0.00195**	**0.00000**	**0.00879**	0.35938
AKD	0.06745	0.1375	0.12199	0.07761	0.11142	0.05816	0.02018	0.07097		**0.00195**	0.01074	0.03125
ALG	0.04326	0.08307	0.07722	0.05025	0.10521	0.06507	0.01673	0.08201	0.03694		**0.00684**	0.06543
AKA	0.10778	0.21047	0.1931	0.12704	0.09594	0.08541	0.05666	0.07415	0.08083	0.11725		0.01172
ISR	0.00721	0.07323	0.05047	0.01455	0.11907	0.06958	-0.00807	0.00174	0.06535	0.04645	0.10422	

Figure 1

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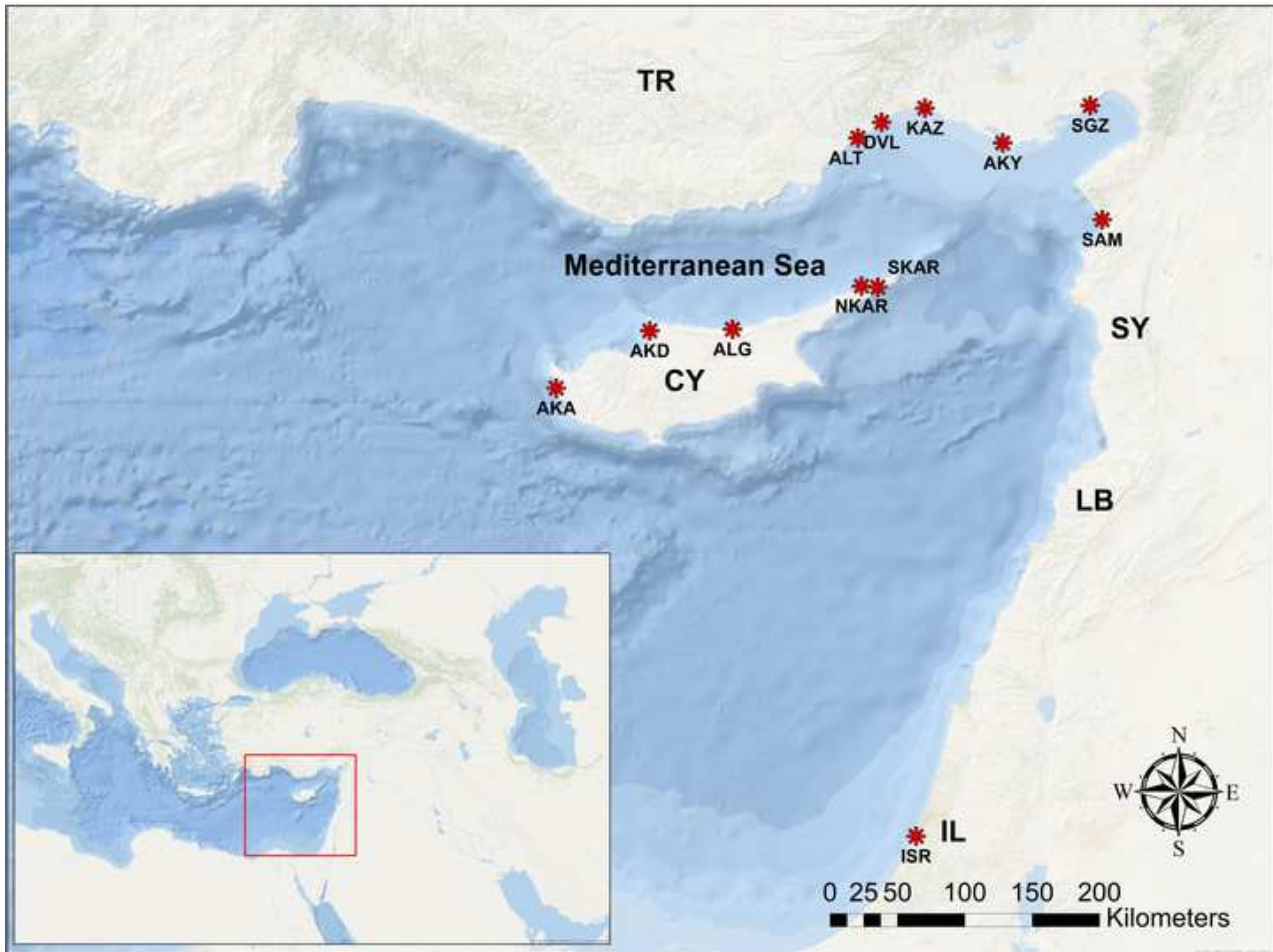

Figgure 2

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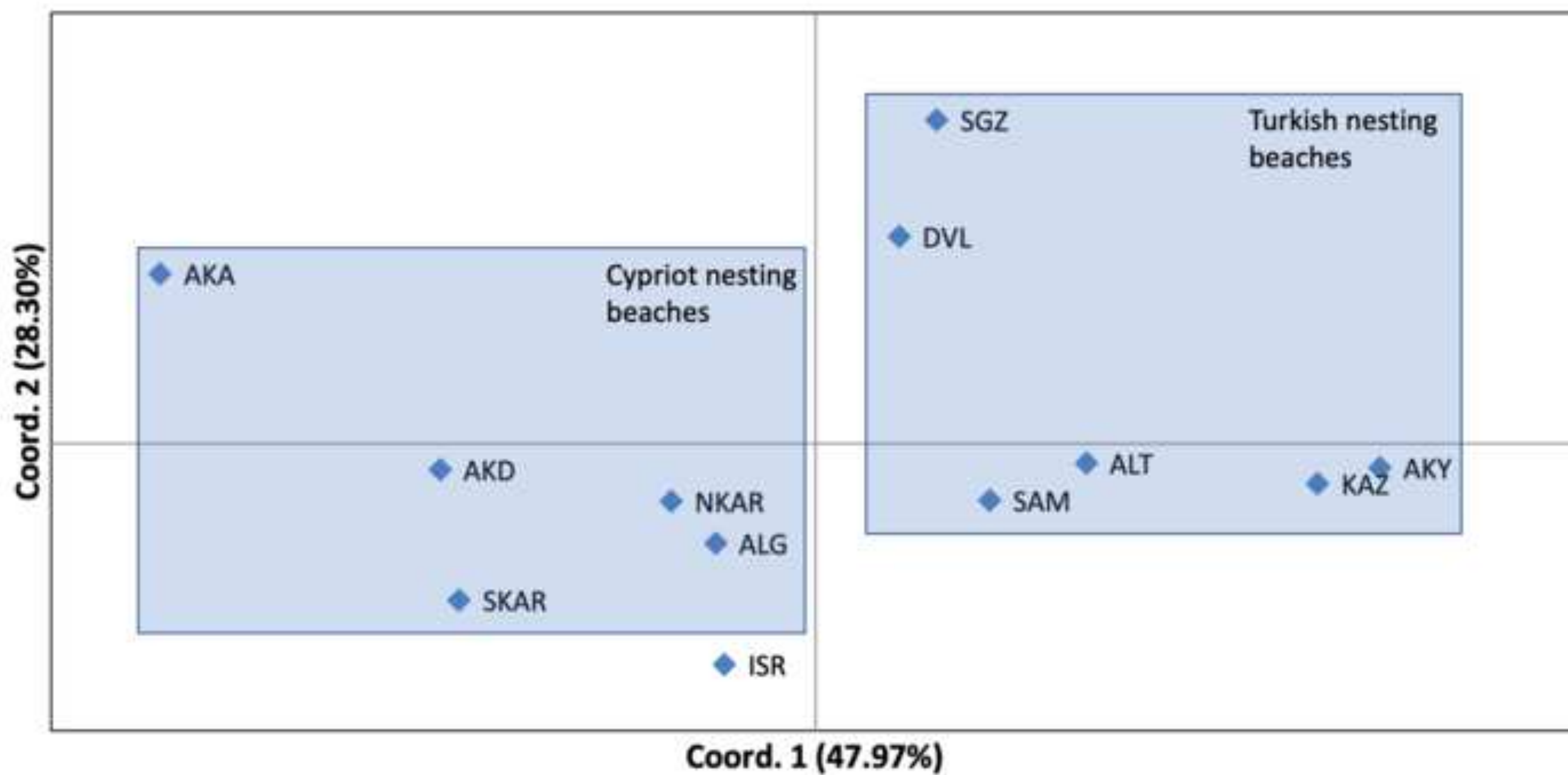

Figure 3

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Fst Values

ISR
NKAR
SAM
ALT
SGZ
DVL
AKY
KAZ
SKAR
AKD
ALG
AKA

ISRAEL
N. CYPRUS
TURKEY
N. CYPRUS
S. CYPRUS

AKA ALG AKD SKAR KAZ AKY DVL SGZ ALT SAM NKAR ISR

Figure 4

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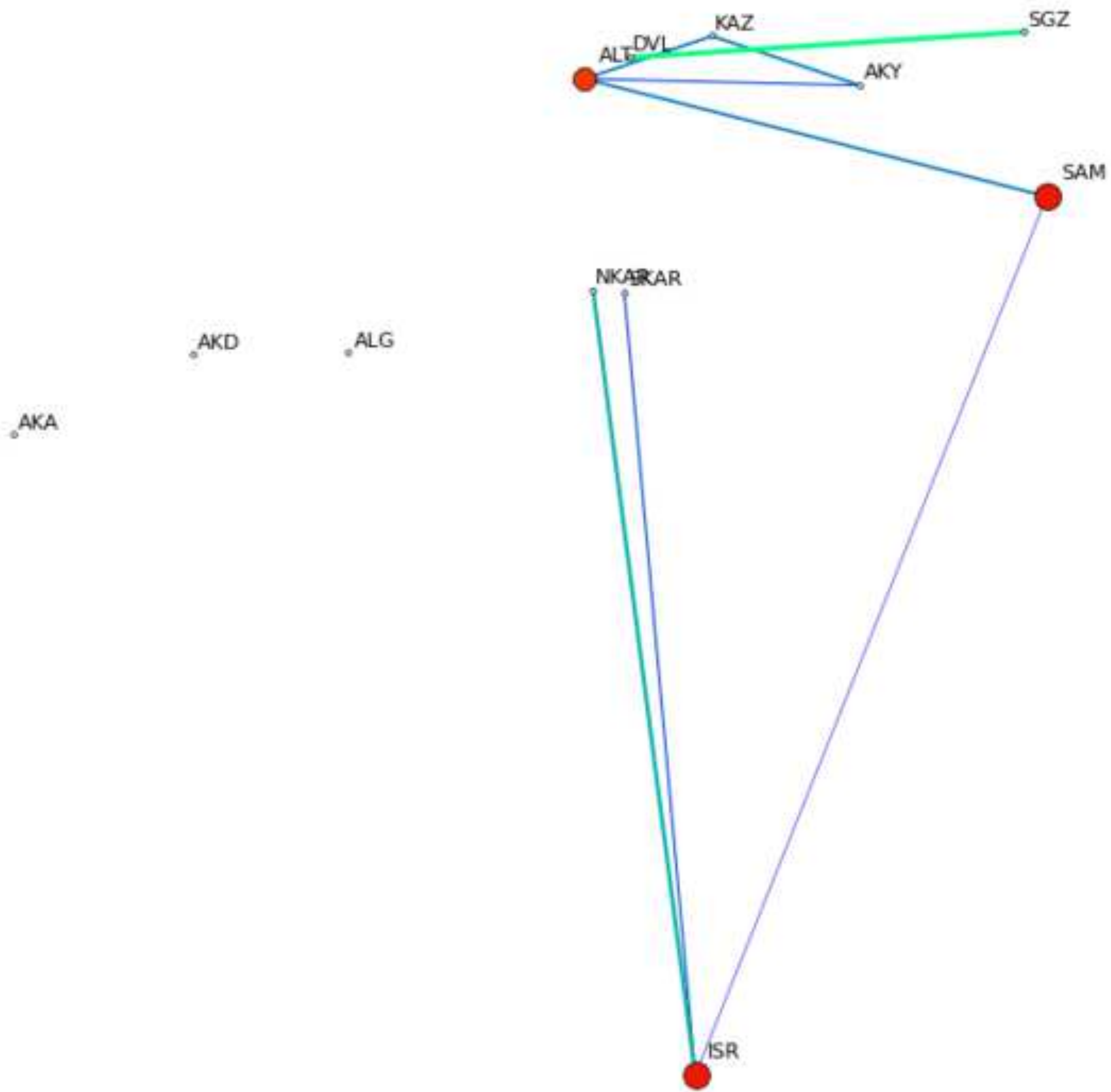

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