

## HAPLOTYPE ANALYSES OF NEWLY IDENTIFIED NESTING BEACH FOR LOGGERHEAD SEA TURTLE: YAKACIK BEACH, TÜRKİYE

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### Abstract

Sea turtles can be genetically differentiated for their nesting beaches or regions because they show natal homing behaviour. This may be a single beach or as a management unit of several beaches. Mediterranean coast of Türkiye has many small pocket beaches and other inaccessible remote sandy beaches. In this study, we investigated the genetic structure of a newly identified loggerhead turtle (*Caretta caretta*) nesting site, Yakacık beach in the border of Antalya and Mersin provinces. The CC-A2.1 and CC-A3.1 haplotypes were defined within the 16 samples collected from the nests during the 2020 and 2021 nesting seasons. One sample per nest were collected as we are interested in mtDNA inherited only from mothers. The beach is identified in the Eastern Türkiye Management Unit for the presence of these haplotypes. There are 23 nesting beaches along the Mediterranean coast of Türkiye and these beaches are very important nesting sites for the loggerhead and the green turtles (*Chelonia mydas*) in the Mediterranean. In this study, we analysed the genetic structure of a newly identified nesting beach and the importance of genetic diversity for Mediterranean Sea turtles was emphasized.

**Keywords:** *Caretta caretta*, Haplotype, Mediterranean, mtDNA, Nesting site expansion

## İRİBAŞ DENİZ KAPLUMBAĞASI İÇİN YENİ BELİRLenen YUVALAMA KUMSALININ HAPLOTİP ANALİZLERİ: YAKACIK KUMSALI, TÜRKİYE

### Özet

Deniz kaplumbağaları, doğumları kumsala tekrar yuva yapmak için dönme davranışlarından dolayı kumsallarına veya bölgelerine göre genetik olarak farklılık göstermektedirler. Bu farklılık, tek bir kumsal veya kumsallardan oluşmuş bir yönetim birimi olabilir. Türkiye'nin Akdeniz kıyılarında çok sayıda küçük kumsallar ve erişilemeyen uzak kumsallar bulunmaktadır. Bu çalışmada, Antalya ve Mersin il sınırındaki yeni bir yuvalama kumsalı olan Yakacık kumsalında yuvalayan *Caretta caretta* türü kaplumbağaların genetik yapısı araştırılmıştır. 2020 ve 2021 yuvalama sezonunda yuvalardan toplanan 16 örnekte CC-A2.1 ve CC-A3.1 olmak üzere iki haplotip tespit edilmiştir. Annelerden kalıtım yoluyla geçen mtDNA araştırıldığı için her yuvadan sadece bir adet örnek toplanmıştır. Bu haplotiplerin varlığı sonucunda, yakacık kumsalının Doğu Türkiye Yönetim Birimi'nde yer aldığı tespit edilmiştir. Türkiye, İribaş deniz kaplumbağaları (*Caretta caretta*) ve yeşil deniz kaplumbağalarının (*Chelonia mydas*) Akdeniz'deki en önemli yuvalama alanlarından birine sahip olup, yakın zamanda belirlenen kumsallarla birlikte toplamda 23 yuvalama kumsalı olmuştur. Bu çalışmada, yeni belirlenen yuvalama kumsallarının genetik özelliklerinin belirlenmesi ve Akdeniz deniz kaplumbağaları için genetik çeşitliliğin de araştırılmasının da önemine değinilmiştir.

**Anahtar Kelimeler:** Akdeniz, *Caretta caretta*, Haplotype, mtDNA, Yuvalama kumsalı

### Cite

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### 1. Introduction

The Loggerhead sea turtles are widely distributed in the world. Two species of sea turtles, the loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*),

colonized in the Mediterranean [1]. The loggerhead sea turtle, which is known as a charismatic species due to its characteristics such as being well known by the public and assuming a facilitating role in the dissemination of

wildlife conservation activities, is most common sea turtle species in the Mediterranean and categorized as “Vulnerable” (VU) globally [2], while the Mediterranean subpopulation is categorized as the “Least Concern” (LC) as it is conservation dependent. Although the loggerhead turtle’s conservation status has been downgraded and the population is considered to be in good condition, it is not possible to say clearly whether this is really the result of population growth or other environmental reasons. For this reason, it is underlined as a conservation dependent species in the species assessment. It is known that ecosystems undergo transformation towards a state that is no longer suitable for some living organisms [3]. Assuming that ecosystems altered by anthropogenic impact will not recover any time soon, these organisms need to rapidly adapt to new environmental conditions or move to new suitable habitats to avoid extinction, and so some species are shifting their distribution by establishing new populations [4]. On the other hand, it has been reported that organisms with limited adaptability may be more vulnerable and more prone to extinction [5].

Sea turtles are known to show philopatry behaviour, defined as returning to natal beaches for reproduction [6] and their tendency to establish new populations is therefore thought to be limited [7, 8]. Nevertheless, a limited number of studies in the Mediterranean have shown that the distribution of sea turtle nesting areas may change [9, 10]. Philopatry limits the potential of organisms to establish new populations, while it strengthens the ability of organisms that are able to do so to persist in suitable habitats and achieve population growth [11]. It is known that our knowledge of sea turtles is far from complete [12], and also that new nesting sites can always be found [13-15]. Although it is not easy to determine the origin of a new nesting population, genetic studies can be used to determine to which of the previously identified populations it belongs [16-18]. Mitochondrial DNA (mtDNA) transferred from mother to the hatchlings and the control region is usually used for genetically different haplotypes [16,19-21]. Mitochondrial DNA has been widely used for the last 30 years as a marker showing the demographic and evolutionary history of both populations and species [16,18,22,23]. In the Mediterranean, the nesting populations are well known and identified as seven independent Management Units (MUs) (Calabria, Western Greece, Crete, Dalyan-Dalaman, Western Turkey, Eastern Mediterranean and Libya-Tunisia) [24]. Genetic differences seen between beaches generally focus on mtDNA, that is, maternal gene transfers, but there may be only gene transfers via fathers when males are also shifting from one beach to another [8, 16, 17, 25]. The beaches of Türkiye and other beaches in the Mediterranean have been the subject of genetic analyses [17, 18, 26-28]. Kaska [20] carried out the first sequencing study in Türkiye with short mtDNA control region. Yılmaz et al. [17] collected samples from all the known nesting beaches and described both previously

identified and new haplotypes on Turkish beaches. It has been shown that by increasing the sample sizes, there is a possibility of the identification of new haplotypes and the connectivity among the nesting beaches better understood [26]. There were 17 nesting beaches previously identified and evaluated [29-32] but later Çıralı, Yumurtalık-Sugözü, Alata, Davultepe, ODTÜ-Karahasanlı and Yakacık beaches were identified as nesting sites [14, 33-37]. In this paper we describe the genetic structure of a newly identified beach by using examples of samples collected on Yakacık beach located at the border of Mersin and Antalya provinces.

## 2. Materials and methods

### 2.1. Sample collection

Dead hatchlings’ tissues, found in the nest, were collected during the sporadic checks on the beaches that potential nesting activities that also discovered during the nesting season. Tissues stored in Eppendorf tubes with 95% ethanol during the hatching seasons of 2020 and 2021. The location of Yakacık beach together with other known nesting sites in Türkiye were given in Figure 1.

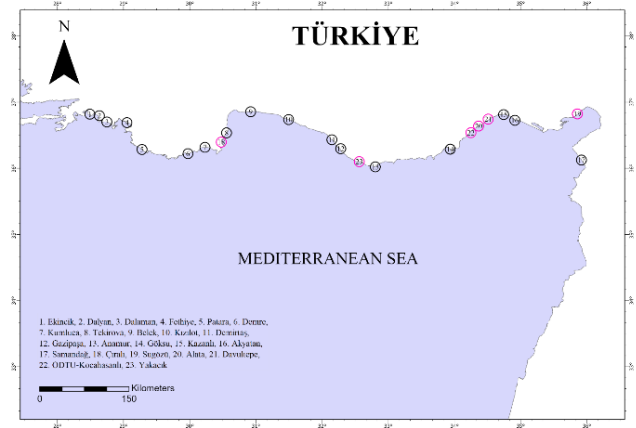


Figure 1. The nesting beaches in Türkiye and the location of Yakacık beach.

### 2.2. DNA Isolation and Amplification

Tissue samples were cut into small pieces (~0.010 mg). DNA isolation was carried out by the salt method [26]. The amount of the isolated DNA sample was measured with the Qubit Flex Fluorometer and isolated DNA was stored at -20°C.

The DNA fragment (~800 bp) of the mitochondrial DNA control region was amplified with LCM15382 and H950, commonly used primers for loggerhead sea turtles, primers. For the mtDNA region analysis primers used: Forward LCM15382 (5'-GCTT AACCTAAGCATTGG-3') and Reverse H950 (5'-GTCTCGG ATTTAGGGGTTT-3') [38, 39]. We performed DNA amplification by applying appropriate PCR solutions and procedures. PCRs can be conducted with ReadyMix™ Taq Sigma (2X), primer (final concentration at 0.5 pM), pure water and DNA [26]. PCR products obtained after the amplification step were visualized by electrophoresis using 1XTBE (Tris-borate-

EDTA) buffer and ABM SafeView dye. The visualized PCR products were examined under UV light.

### 2.3. Sequencing and Alignment

Amplification samples were sent to Genartek (Istanbul, Türkiye) for DNA purification and Sanger sequencing. The sequences were aligned by using the BioEdit version 7.2.5 [40] and compared with haplotypes found in the Archie Carr Center for Sea Turtle Research database (ACCSTR; [http://accstr.uf.edu/fles/cclongmtdn\\_a.pdf](http://accstr.uf.edu/fles/cclongmtdn_a.pdf)) and the sequence comparison tool, GenBank BLAST (<http://ncbi.nlm.nih.gov/Blast.cgi>). Haplotype diversity (Hd), nucleotide diversity ( $\pi$ ), number of haplotypes (k) and number of polymorphic sites in DnaSP version 5.10.01 were used in order to obtain MtDNA control region polymorphism data [41]. Mixed stock analysis (MSA) was performed to estimate the proportion of individuals from different recruits. Mixed stock analysis was performed in BAYES software using the Markov Chain Monte Carlo (MCMC) method [42].

### 3. Results

The beach surveys were carried out in 1<sup>st</sup> of September in 2020 and 13<sup>rd</sup> of September in 2021 and one dead embryo/hatchlings per nest were taken from the excavated nests. The haplotype network of 16 samples collected from Yakacık beach shows that two haplotype (CC-A2.1(n=15) CC-A3.1 (n=1)) network is given in Figure 2. The mixed stock analyses and contribution of other loggerhead population to Yakacık beach is given in Figure 3. As it can be seen from this figure, main contribution to Yakacık beach estimated from Bayesian statistics is from western Greece (WGR) and Libya (Mısırata).

The nucleotide diversity ( $\pi$ ) were calculated as 0.00230061 for 815 nucleotides in 16 samples. Tajima's D statistics (D) were also calculated as 10.3699 by using DnaSP.v. 5.10.01. Tajima's D is a statistic used to compare an observed nucleotide diversity against the expected diversity under the assumption that all polymorphisms are selectively neutral and constant population size. Tajima's D is similar to chi-square and described as the ratio between an observed and expected haplotypes under the constant population with selectivity is neutral. This is commonly used for genetic diversity. Mixed stock analysis for Yakacık samples, although located within the Turkey east Mediterranean population, might be related to Libya and Tunisian population (16%), Italy (9%) and western Greece (43 %) and Crete (7%) (Table 1), showing this beach is mainly contributed from south and western Mediterranean populations, only a total of 26% from all Turkish beaches and 4% from Cyprus.

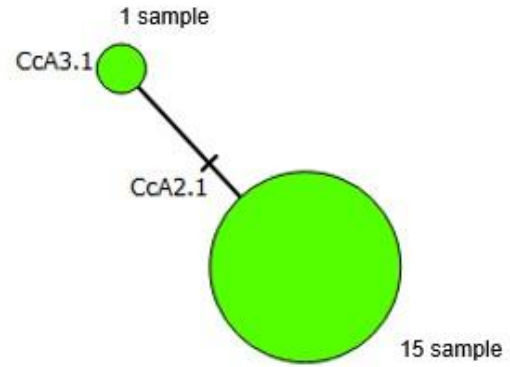


Figure 2. The haplotype distances of two haplotypes found among the samples from Yakacık beach, Antalya Türkiye.

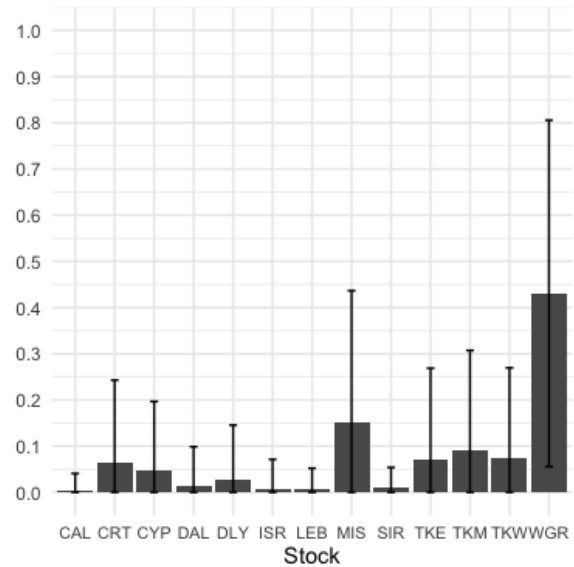


Figure 3. The contribution of loggerhead populations to Yakacık beach.

CAL: Calabria, CRT: Crete, CYP: Cyprus, DLY: Dalyan, DAL: Dalaman, ISR: Israel, LEB: Lebanon, MIS: Misurata, SIR: Sirte, TKE: Eastern Türkiye, TKM: Middle Türkiye, TKW: Western Türkiye, WGR: Western Greece

Table 1. Bayesian estimates for Mediterranean stocks. LIBYTUN: Libya and Tunisia, EMED: Eastern Mediterranean (including Central and Eastern Türkiye, Cyprus, Israel and Lebanon), TKW: Western Türkiye, DLYDAL: Dalyan-Dalaman-Türkiye, CRT: Crete-Greece, WGRC: Western Greece, CAL: Calabria-Italy.

Stock	Mean	SD	2.5%	MEDIAN	97.5%
MIS	0.1515	0.2851	0.0000	0.0002	0.9491
SIR	0.0107	0.0432	0.0000	0.0000	0.1145
ISR	0.0080	0.0633	0.0000	0.0000	0.0420
LEB	0.0054	0.0468	0.0000	0.0000	0.0324
CYP	0.0477	0.1490	0.0000	0.0001	0.6136
TKE	0.0722	0.1965	0.0000	0.0006	0.8259
TKM	0.0910	0.2163	0.0000	0.0002	0.8861
TKW	0.0749	0.1945	0.0000	0.0001	0.8177
DAL	0.0133	0.0854	0.0000	0.0000	0.1305
DLY	0.0268	0.1187	0.0000	0.0000	0.4167
CRT	0.0654	0.1775	0.0000	0.0000	0.7218
WGR	0.4306	0.3750	0.0001	0.3487	0.9961
CAL	0.0028	0.0380	0.0000	0.0000	0.0001

#### 4. Discussion

In our study, the mtDNA control region haplotypes found at of Yakacık, a newly identified nesting beach, were investigated and it was found that CC-A2.1 and CC-A3.1 haplotypes, which are common in the Turkish population, were also observed on this beach. The CC-A2.1 haplotype has been reported from all nesting beaches in Türkiye, whereas the CC-A3.1 haplotype has been previously reported from western beaches in Türkiye (TKW) and a few specimens with this haplotype were found in central (TKM) and eastern Türkiye (TKE) populations [17, 26]. The findings of this study are consistent with the general genetic structure of the loggerhead turtles in Türkiye. The CC-A3.1 haplotype on the nesting beaches (19.8 % in the entire Mediterranean) were reported by Kaska et al [26] as 50% in Dalyan-Dalaman management Unit, 26,8% in the western Türkiye, while they found this haplotype on a small number of TKE (8,1%) nesting beaches. Yakacık beach is located right between the units defined as TKM and TKE. Although the presence of a single sample belonging to CC-A3.1 is not sufficient to make a definite judgement, we can assume that Yakacık beach may be close to the beaches of the TKE unit but mainly contributed from south and western Mediterranean populations.

The loyalty of female sea turtles to their nesting sites can be resolved by maternally inherited mtDNA control region due to its high rate of evolution [43, 44]. Differences in mtDNA haplotype frequencies among nesting populations have been used as genetic markers to associate feeding associations with the nesting associations from which they originated.

Previous genetic studies indicate that nesting populations in the Mediterranean began to become isolated from the Atlantic in the late Pleistocene and early Holocene (~10 thousand years ago) [6, 44]. It is suggested that the low genetic diversity observed in the

mtDNA d-loop region is due to the colonization of the Mediterranean by the migration of a few individuals from the Atlantic Populations about 12 thousand years ago [6]. Therefore, the Mediterranean population is considered as an independent unit and consists of 7 different genetic structures [24]. Geographically, in terms of mtDNA, Dalyan, Dalaman, West (Fethiye, Patara, Kale, Kumluca and Çıralı), Central (Tekirova, Belek, Kızılot and Gazipaşa) and East (Anamur, S. Delta, Alata, Kazanlı, Akyatan, Ağyatan and Samandağ) to make up to 5 groups were previously described by Yılmaz et al. [17]. Later these were classified only as western and eastern Türkiye. The Mediterranean population is a population whose value is increasing due to the genetic isolation of the Mediterranean population from the Atlantic population and the decrease in genetic diversity due to genetic drift and inbreeding effects [16]. Laurent et al. [45] stated that the mtDNA haplotype frequencies of *C. caretta* nesting populations in Türkiye were significantly different.

The frequencies of haplotypes CC-A2.1 and CC-A3.1 are high in Turkish nesting beaches but the CC-A3.1 haplotype is found only in the Eastern Mediterranean nesting beaches. The CC-A2.1 and CC-A3.1 haplotypes were defined as the ancestral haplotype [16, 17, 45]. A haplotype parsimony network analysis of published mtDNA control region haplotypes of Atlantic and Mediterranean *C. caretta* populations were analysed and the haplotypes detected in the Mediterranean and Türkiye's nesting beaches originated from the CC-A2 haplotype [16, 17, 45]. The Mediterranean coast of Türkiye is the place with the highest genetic diversity for *Caretta caretta* after Calabria [17]. The Turkish population differs from the Mediterranean populations in terms of CC-A3 haplotype and its frequency of haplotypes derived from this CC-A3.1, CC-A3.2 and CC-A3-3. Therefore, nesting beaches that contribute CC-A3 haplotype to the Turkish population are the beaches that should be protected [17]. Yakacık is a newly defined nesting beach. Barbanti et al. [46], has shown that females concentrate nesting activity in specific areas and exhibit a high degree of nest site fidelity within and between nesting seasons. However, some research has shown that long-distance nesting appears to be more common than previously thought [47, 48]. It is known that there are wintering and foraging areas in the eastern Mediterranean coast of Türkiye [49]. Yakacık beach was not reported during the detailed studies conducted by Baran and Kasperek [29] on the Turkish coasts suggests that this beach was not visited at that time, there was no nesting at that time, or no significant nesting was detected. Nevertheless, this beach is now reported to be an important nesting site and the nesting size seems to be very important [37]. It is still an open question whether the population breeding on this new beach the result of the expansion of a small population is already breeding on the beach, or whether it is the result of the expansion of populations on different beaches. To determine the origin of this new population, studies with

new markers and larger sample sizes are needed. The mtDNA control region sequences can be used for the identification of the haplotypes and if there are any differences among the beaches. The full genome can also be analysed as it is becoming more informative in recent times in identifying the individuals [50]. We presented here the haplotypes and their frequencies with the limited sample size for a new nesting site that never being sampled before. There may be more nesting occurring outside of the beaches already identified for loggerhead sea turtles in Türkiye. The sporadic and new nesting sites should be monitored and genetically analysed to determine whether they came from other coasts within the Mediterranean or entered the Mediterranean from other populations.

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