Conservation Genetics Resources (2023) 15:67–75
https://doi.org/10.1007/s12686-023-01303-9

ORIGINAL RESEARCH

Increased sample size provides novel insights into population structure of Mediterranean loggerhead sea turtles

Arzu Kaska[1] · Gizem Koç[2,20] · Doğan Sözbilen[3] · Diryaq Salih[4] · Ashraf Glidan[4] · Abd Alati Elsowayeb[19] · Almokhtar Saied[4] · Dimitris Margaritoulis[5] · Panagiota Theodorou[5] · ALan Rees[5] · Robin Snape[6] · Annette Broderick[6] · Brendan Godley[6] · Damla Beton[7] · Meryem Ozkan[7] · Imed Jribi[8] · Manel Ben Ismail[9] · Ahmed Ben Hmida[10] · Ali Badreddine[11] · Enerit Sacdanaku[12] · Vicky Rae[13] · Mohamed Said Abdelwarith[14] · Nahla Naguib[14,15] · Lobna Ben Nakhla[16] · Atef Limam[16] · Marwan Abderrahim[16] · Julien Sémelin[17] · Yakup Kaska[2,18]

Received: 4 July 2022 / Accepted: 23 April 2023 / Published online: 13 May 2023

Abstract

The loggerhead sea turtle (*Caretta caretta*), has the widest distribution among sea turtle species in the Mediterranean, requiring regional and international collaborations in addition to local efforts to better inform conservation actions. Molecular techniques are powerful tools for the assessment of population dynamics at large scales, especially in determining the connectivity among different nesting and foraging sites, and genetic diversity. In this study, a large sample was collected synchronously in the nesting areas located in the north, south and eastern Mediterranean. Recently confirmed nesting sites from Albania and other nesting sites represented by lower sample sizes were also included in order to better assess the genetic composition of the region's rookeries. Samples from 698 individuals were collected and the longer (815 bp) mtDNA D-loop fragment of these samples was sequenced. We recorded 15 haplotypes, three of which were novel. In addition, our results show that some haplotypes considered of Atlantic origin, have a wider dispersal in the Mediterranean than previously thought, albeit with low levels of representation. Our results contribute to determining the likely origin of haplotypes that were previously only recorded from foraging sites. They highlight the utility of broad-scale sampling, with increased sample sizes and the longer mtDNA sequence to determine genetic diversity and connectivity. This study also demonstrates the importance of continued monitoring for the contribution of Atlantic-origin haplotypes to the Mediterranean population and the effects of climate change on the resident Mediterranean population, which is expected to expand its geographical range for reproduction. This work is important for, mixed stock analyses (MSA) that seek to determine the natal regions of stranded or accidentally caught sea turtles and those purposefully obtained from foraging sites. In doing so biogeographic linkages between areas of the Mediterranean can be elucidated for conservation purposes.

Keywords *Caretta caretta* · Genetic structuring · mtDNA · Phylogeography · Conservation

Introduction

The loggerhead turtle (*Caretta caretta*) is the most common sea turtle species in the Mediterranean Sea (Casale et al. 2018). Nesting occurs mainly in the Eastern Mediterranean basin, with the highest number of clutches in Greece, Turkey, Libya and Cyprus (Casale et al. 2018). Lower nesting numbers are recorded in Egypt, Israel, Lebanon, Syria and Tunisia. Minor nesting also occurs in the western basin in Italy, Spain and France (Casale et al. 2018).

Globally, sea turtle populations are under many anthropogenic pressures. However, as a result of conservation efforts, the Mediterranean loggerhead population has recovered substantially. It is currently classified as *Least Concern* by the International Union for Conservation of Nature (IUCN) due to increasing nest numbers at major Mediterranean nesting sites, but, is considered as *Conservation Dependent* since various threats still exist (Casale 2015).

It is worth noting that the loggerhead turtle is a highly mobile species. Recent studies have shown that the Mediterranean loggerhead population exhibits a wide dispersal

✉ Yakup Kaska
yakupkaska@gmail.com

Extended author information available on the last page of the article

from nesting sites to different foraging sites (Cerritelli et al. 2022; Haywood et al. 2020; Schofield et al. 2010). In addition to conservation efforts at local levels, studies based on international cooperation and the determination of regional-based conservation measures have been adopted recently (Kot et al. 2022). Consequently, this step will provide high benefits for sea turtle conservation studies.

Sea turtles exhibit a behavior known as philopatry, defined as a tendency to return to their natal beaches (Bowen and Karl 2007). Accordingly, this behavior results, over time, in certain regions forming populations with genetic structuring. Those populations are defined as Management Units (Moritz 1994).

The Mediterranean loggerhead turtle population is identified globally as one of the 10 Regional Management Units (RMU) based on studies of nesting sites, population abundances and trends, population genetics, and satellite telemetry (Wallace et al. 2010). Although the Mediterranean loggerhead turtle population is defined as a single RMU, there is genetic diversity across the region (Carreras et al. 2006, 2014; Yilmaz et al. 2011; Saied et al. 2012; Clusa et al. 2014, 2018; Rees et al. 2017; Tolve et al. 2018). However, the Mediterranean loggerhead metapopulation is composed of three independent RMUs (1 Mediterranean RMU and 2 Atlantic RMUs) (Casale et al. 2018). Recently, loggerhead sporadic nesting events have become more frequent in the western Mediterranean, and Atlantic origin turtles contributed to these nesting events (Carreras et al. 2018). Therefore, genetic diversity differs and is evolving at both foraging and nesting sites.

Mixed-stock analysis (MSA) based on mitochondrial DNA (mtDNA) sequences are used to assess a combination of haplotype diversity and frequency among different nesting sites and estimate the origins of individuals at foraging sites (Bowen and Karl 2007). Due to unsampled breeding sites and incomplete sampling of large, well-known sites, complete genetic characterization is so far unachieved for the Mediterranean. Furthermore, mainly when "orphaned haplotypes" exist (i.e. haplotypes recorded from an individual of unknown breeding site), the MSA has lower power (Tolve et al. 2018). To have a more comprehensive knowledge on the Mediterranean loggerhead turtle population structure, there was a need for simultaneous studies with an increased number of samples from areas that were previously represented with few or no samples.

In this context, and with the contribution of researchers from seven countries in the Mediterranean, this study aimed to increase the sample size of rookeries from different countries to contribute to better understanding of the genetic structure of *Caretta caretta* and answer the following questions:

1. Did we attain the maximum knowledge available for the loggerhead turtle population structure?
2. Do the Atlantic loggerhead turtles contribute to the breeding population in the eastern Mediterranean?
3. What is the origin of the individuals at new nesting sites in the Mediterranean?

Materials and methods

Sample collection

Skin biopsies were taken from adult females and tissues from dead hatchlings and preserved in 70% alcohol until required for analysis. For hatchling samples, one hatchling was sampled per nest, from nests made within a 15-day interval, which is a typical internesting interval for the loggerhead turtles, to prevent pseudo-replication.

A total of 698 samples were collected between 2018 and 2019 along the beaches of Cyprus, Greece, Lebanon, Libya, Tunisia and Turkey. The sites from Libya and Lebanon had not been previously sampled, while larger sample sizes were collected from the other, previously investigated sites. The sample size in Turkey was 374, Libya 134, Greece 104, Tunisia 41, Cyprus 27, and Lebanon eight. A total of 8 Samples from Albania and 2 samples of stranded turtles from Egypt were included to identify their haplotypes in the analyses. Six Albanian samples were collected from live bycatch turtles at Ishmi stavnik, Patok area (Drini Bay). A further two were collected from dead hatchlings found in the first officially documented nest in Albania in Divjaka beach area, in 2018 (Piroli and Haxhiu 2020).

Laboratory analysis

DNA extraction was performed using a standard phenol–chloroform protocol (Kaska et al. 2001) or a QuickGene DNA tissue kit (KURABO). Representatives of each research team trained for the same protocol for extraction of DNA and PCR amplification at DEKAMER Lab in Turkey. An approximately 815 base pair (bp) long fragment of the non-coding mitochondrial DNA (mtDNA) control region was amplified by Polymerase Chain Reaction (PCR). The primer pair used was LCM15382 (5′-GCTT AACCCTAAG CATTGG-3′) and H950 (5′-GTCTCGG ATTTAGGGGTTT -3′) (Abreu-Grobois et al. 2006). PCR was performed in a total volume of 30-µL mastermix, 0.5 µM of each primer, and 2 µL of DNA. Thermal conditions consisted of an initial denaturation at 95 °C for 3 min (min), followed by 34 cycles of 30 s (s) at 95 °C, 1 min at 55 °C and 30 s at 72 °C, with a final extension step at 72 °C for 10 min. PCR products were visualised on a 1% agarose gel stained with Safeview™ for amplification evaluation. After completing the

DNA extraction and amplification the products were sent to Genartek (Istanbul, Turkey) for DNA purification and Sanger sequencing with both forward and reverse primers.

Data analysis

Obtained sequences were, if needed, edited in Chromas (v.2.6.6) and aligned in Bioedit (v.7.2.5) (Hall 1999). Haplotype classification was conducted through comparing the sequences with haplotypes already presented in the Archie Carr Center for Sea Turtle Research database (ACCSTR; https://accstr.ufl.edu/wp-content/uploads/sites/98/cclongmtdna-2.pdf) and the sequence comparison tool, GenBank BLAST (http://ncbi.nlm.nih.gov/Blast.cgi). Novel haplotypes found were submitted to ACCSTR for assigning the international nomenclature and their sequences were sent to GenBank. To highlight the relationship between different haplotypes identified, haplotype networks based on the median-joining algorithm were created using the program POPART version 1.7 (Leigh and Bryant 2015). Polymorphism data was obtained by estimating the haplotype diversity (h), nucleotide diversity (π), number of haplotypes (k) and number of variable sites (p) in DnaSP version 5.10.01 (Librado and Rozas 2009).

Haplotype networks of mtDNA for loggerhead turtles from different Mediterranean countries were created with the connecting lines between haplotypes representing single mutations (Fig. 1). The software MEGA v. 7 (Kumar et al. 2016) was used to create a phylogenetic tree (Supplement Fig. 1). The best-fit substitution model (ML) was chosen on the basis of the lowest Bayesian information criterion (BIC) value which was the Tamura 3-parameter with Gamma distribution (T92 + G). For the phylogenetic analysis the neighbour-joining (NJ) method was used including the selected substitution model. We carried out 1000 bootstrap replications to obtain valid results for the nodes. Bootstrap values greater than or equal to 50% were considered statistically significant (Margush and McMorris 1981). For node calibration, D-loop sequences from two olive ridleys (*Lepidochelys olivacea*, GenBank AM258984 and JX454991) and a Kemp's ridley sea turtles (*Lepidochelys kempii*, GenBank JX454981) were included in the alignment as outgroups.

Results

Among the 698 mtDNA sequences analysed, 14 variable sites were observed defining 16 haplotypes of which three were previously undescribed. Previously described haplotypes found in this study are presented in Table 1. Both, CC-A2.1 and CC-A3.1 are the most common haplotypes at different Mediterranean rookeries. The highest number of haplotypes found were from Libya (9), followed by Greece (6) and Turkey (5) (Table 2). The haplotype diversity was highest in the previously established Libyan (together with Tunisian MU) Management Unit, followed by western Turkey and Crete, Greece. The most frequently found haplotypes were CC-A2.1 (67.0%) and CC-A3.1 (19.8%), both were present at all nesting sites, followed by CC-A2.9 (6.7%) most found in Libya and 3.2% with haplotype CC-A26.1, also originating from Libya. One hatchling and the bycatch samples from Albania and 2 samples from Egypt were found of the haplotype of CC-A2.1. The genetic polymorphism

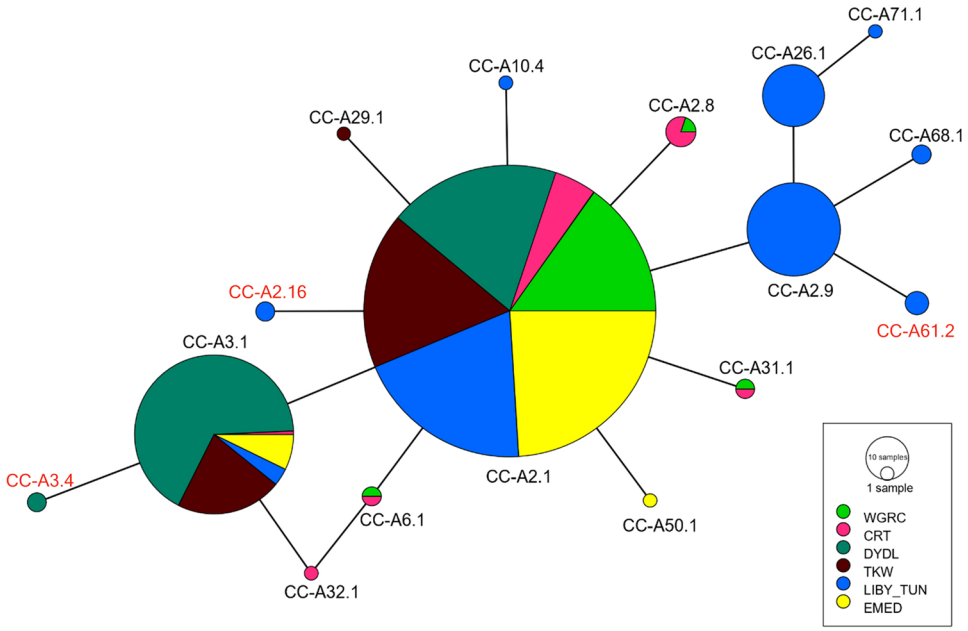

Fig. 1 Haplotype network of mtDNA for loggerhead sea turtles from different management units. Connecting lines between haplotypes represent single mutations. The circle areas of the haplotypes are proportional to the sample size carrying the specific haplotype. New haplotypes were given in red

Table 1 The haplotypes and the sample size (n) of *Caretta caretta* nests sampled at different Mediterranean rookeries and their rookery of origin (Clusa et al.2014)

Haplotype	n	Source rookeries found in this study	Rookery previously identified
CC-A2.1	468	DLM (7), DLY (83), TKE (46), TKM (35), TKW (81), MIS (4), SIR (49), CRT (22), KOR (18), KOT (8), LAK (26), ZAK (19), TUN (39), CYP (26), LEB (5)	Mediterranean: MIS, SIR, ISR, LEB, CYP, ETU, MTU, DLM, DLY, CRE, WGR, CAL Atlantic: CEF, SEF, SAL, DRT, QMX, SWF, CWF, NWF, CPV
CC-A2.8	5	CRT (4), LAK (1)	Mediterranean: CRE Atlantic: –
CC-A2.9	47	SIR (45), MIS (1), TUN (1)	Mediterranean: MIS, SIR, ISR Atlantic: –
CC- A3.1	138	DLM (36), DLY (55), TKE (6), TKW (30) (128), SIR (4), CRT (1), TUN (1), CYP (1), LEB(3)	Mediterranean: MIS, SIR, LEB, ETU, WTU, WGR, DLM, DLY Atlantic: CEF, SEF, SAL, QMX, SWF, CWF, NWF
CC-A6.1	2	CRT (1), KOT (1)	Mediterranean: WGR Atlantic: –
CC- A10.4	1	MIS (1)	Mediterranean: Tuscany [a], Campania [a] Atlantic: CEF
CC-A26.1	22	MIS (1), SIR (21)	Mediterranean: SIR Atlantic: –
CC-A29.1	1	TKW (1)	Mediterranean: ISR Atlantic: –
CC-31.1	2	CRT (1), KOR (1)	Mediterranean: CAL, WGR, Sicily [a] Atlantic: –
CC-A32.1	1	CRT (1)	Mediterranean: WGR Atlantic: –
CC-A50.1	1	TKE (1)	Mediterranean: CYP
CC-A68.1	2	SIR (2)	Mediterranean: SIR
CC-A71.1	1	SIR (1)	Unknown
CC-A61.2	3	SIR (3)	Novel haplotype
CC-A2.16	2	SIR (2)	Novel haplotype
CC-A3.4	2	DLY (2)	Novel haplotype

Mediterranean rookeries: *MIS* Misurata, Libya, *SIR* Sirte, Libya, *ISR* Israel, *LEB* Lebanon, *CYP* Cyprus, *ETU* Eastern Turkey, *MTU* Middle Turkey, *WTU* Western Turkey, *DLM* Dalaman, Turkey, *DLY* Dalyan, Turkey, *CRE* and *CRT* Crete, Greece, *WGR* Western Greece, *KOR* Koroni, Western Greece, *KOT* Kotychi, Western Greece, *CAL* Calabria, Italy

Table 2 Genetic polymorphism measures of loggerhead sea turtles from different management units

Management unit	n	k	p	S	Hd	π
WGRC	74	4	3	3	0.0800	0.00007
CRT	30	6	4	4	0.4552	0.00053
DLYDLM	184	3	2	2	0.5132	0.00064
TKW	112	3	2	2	0.4088	0.00051
LIBY_TUN	175	9	8	8	0.6362	0.00107
EMED	123	3	2	2	0.1655	0.00020
Total_Med	698	16	14	14	0.5077	0.00078

n number of turtles sampled, k number of haplotypes, p number of polymorphic (segregation) sites, S Number of variable sites, Hd haplotype diversity, π nucleotide diversity

measures data (Table 2) and frequencies of haplotypes (Table 3) were given according to Management Units.

One new haplotype detected in Sirte, Libya, differed by one substitution from CC-A2.9 which is an exclusively Mediterranean haplotype (Tolve et al. 2018) and common in the Libyan nesting sites (Splendiani et al. 2017). The novel sequence was named CC-A61.2 after the nomenclature rules published on the Archie Carr Center for Sea Turtle Research (ACCSTR) website. The second new haplotype also identified from Sirte differed by one substitution from CC-A2.1, which is found in both Mediterranean and Atlantic populations (Shamblin et al. 2014) and was named CC-A2.16. The

Table 3 Haplotype number and frequencies (%) found in different MU

Haplotype	WGRC	CRT	DLYDAL	TKW	LIBYTUN	EMED	Overall
CC-A2.1	71 (95.9%)	22 (73.3%)	90 (48.9%)	81 (72.3%)	92 (52.6%)	112 (91.1%)	468 (67.0%)
CC-A2.8	1 (1.4%)	4 (13.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (0.7%)
CC-A2.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	47 (26.9%)	0 (0.0%)	47 (6.7%)
CC-A3.1	0 (0.0%)	1 (3.3%)	92 (50.0%)	30 (26.8%)	5 (2.9%)	10 (8.1%)	138 (19.8%)
CC-A6.1	1 (1.4%)	1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)
CC-A10.4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.1%)
CC-A26.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	22 (12.6%)	0 (0.0%)	22 (3.2%)
CC-A29.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
CC-31.1	1 (1.4%)	1 (3.3%)	0 (0.0%)	0 (0.0%)	0(0.0%)	0 (0.0%)	2 (0.3%)
CC-A32.1	0 (0.0%)	1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
CC-A50.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	1 (0.1%)
CC-A68.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.1%)	0 (0.0%)	2 (0.3%)
CC-A71.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.1%)
CC-A77.1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.7%)	0 (0.0%)	3 (0.4%)
CC-A2.16	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.1%)	0 (0.0%)	2 (0.3%)
CC-A3.4	0 (0.0%)	0 (0.0%)	2 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)

third previously undiscovered haplotype, named CC-A3.4, was detected at a Turkish nesting site (Dalyan) and is one substitution separated from CC-A3.1, which is also found both in the Mediterranean and the Atlantic (Shamblin et al. 2014). The phylogenetic tree of *C. caretta* generated with the NJ method clearly showed the two different mitochondrial lineages (known as Haplogroup I and Haplogroup II) highlighted in Shamblin et al. (2014). The three new haplotypes (CC-A2.16, CC-A3.4 and CC-A61.2) are representatives of Haplogroup II. The haplotype network of mtDNA for loggerhead turtles from different Mediterranean management units is shown in Fig. 1, the phylogenetic trees were also provided (Supplement Fig. 1).

Discussion

Molecular genetic investigation for differentiated populations has been characterised as a powerful tool for conservation purposes (Moritz 1994; Crandall et al. 2000). However, length of the sequences, selected markers and sample size play an important role in differentiating populations (Garofalo et al. 2009; Monzón-Argüello et al. 2010; Clusa et al. 2013). Using extended mtDNA 815 bp haplotype sequences, independent management units (MUs) have been identified within the Mediterranean RMU (Shamblin et al. 2014), namely: (i) Calabria-Italy (CAL), (ii) western Greece (WGRC), (iii) Crete-Greece (CRT), (iv) Libya (LIBY), (v) Dalyan-Dalaman-Turkey (DLYDAL), (vi) western Turkey (TKW), (vii) eastern Mediterranean (EMED, including middle and eastern Turkey, Cyprus, Israel, and Lebanon).

To date, the highest diversity with seven haplotypes has been reported from nesting sites in Turkey (Yilmaz et al. 2011; Carreras et al. 2014; Clusa et al. 2014 and references therein). It is followed by Libya with five haplotypes (Said et al. 2012), Greece with four haplotypes (Yilmaz et al. 2011; Carreras et al. 2014), Cyprus (Clusa et al. 2013) and Lebanon (Yilmaz et al. 2011) with two haplotypes, and Tunisia with one haplotype (Chaieb et al. 2010). Although recent sporadic loggerhead turtle nest records were available from Albania (Piroli and Haxhiu 2020), genetic characterization was not available at the time of this study.

The Libyan coastal region and information about it's nesting sites are identified as the major knowledge gap in the Mediterranean (Hamza 2010; Casale et al. 2018). In addition, Libya is significant as the first site in the Mediterranean to be colonised by the loggerhead turtle (Clusa et al. 2013). The results show that more sampling effort is needed, especially along the largely unexplored Libyan coasts as two novel haplotypes were found in this area in this study. In total nine haplotypes were found. The second most frequent haplotype found in Misratah and Sirte, Libya was CC-A2.9 which occurs frequently in Israel and Libyan rookeries (Saied et al. 2012; Clusa et al. 2014). One specimen from a nesting site in Misratah, Libya, was detected carrying the rare haplotype CC-A10.4. This haplotype has only been recorded occasionally at Tyrrhenian nesting sites (Garofalo et al. 2016a; Mafucci et al. 2016). It derives from the CC-A10 haplotype (380 bp mtDNA sequence) which has only been observed once in Greece (Laurent et al. 1998). For CC-A10.4 it was also predicted that this haplotype originated from both Tyrrhenian rookeries or other Mediterranean colonies (Tolve et al. 2018). The origin of the specimens found in the Adriatic (Yilmaz et al. 2012; Tolve et al. 2018; Bertuccio et al. 2019) could be Libya. Both haplotypes, CC-A29.1 and CC-A10.4 were only found once in Turkey and Libya,

respectively which is why the sampling size in these rookeries needs to be increased. Another very rare haplotype is CC-A68.1, which was found only once at the nesting location in Sirte, Libya in 2009. During this study two individuals were recorded as carrying CC-A68.1 from Sirte, Libya. Splendiani et al. (2017), who obtained samples from rescued *Caretta* found along the Southern Adriatic coast of Italy, described the haplotype CC-A71.1 for the first time which is why they were not able to determine it's origin. However, by creating a phylogenetic tree it became possible to see the phylogenetic relationship to CC-A26.1, which is exclusive to Libya (Shamblin et al. 2014). For this reason, Splendiani et al. (2017) suggested that CC-A71.1 could be from a Libyan rookery. Our findings supports this, as one sample from Sirte, Libya carried this haplotype.

The 104 samples analysed from Greece, including the Management Units of Western Greece and Crete, contained all known haplotypes in these rookeries. In Western Greece 71 (95.9%) and in Crete 22 (73.3%) of the loggerheads carried the haplotype CC-A2.1. Four individuals from Chania, Crete carried CC-A2.8 while one individual from Mavrovouni, Lakonikos Bay carried this haplotype, which was known to be endemic to the Cretan rookery. One individual from Crete carried a haplotype CC-A3.1, another one CC-A32.1. Haplotype CC-A6.1 was carried by loggerheads from Kotychi, western Greece and Crete. Both haplotypes, CC-A6.1 and CC-A32.1 were known to be endemic to Western Greece. One specimen from Crete and one from Koroni had haplotype CC-A31.1 originating from Greek and Calabrian rookeries but also found in sporadic Sicilian nesting sites (Garofalo et al. 2016b).

In this study a total of five haplotypes were detected at main Turkish nesting sites of which two individuals belong to the same, new haplotype. The most frequent haplotype in Turkey, which is also the most common in the Mediterranean, was CC-A2.1 followed by CC-A3.1 also a common haplotype. The discovery of the CC-A29.1 haplotype at a nesting beach in Turkey, more precisely in western Turkey (Çıralı) shows, as has been proposed previously (Tolve et al. 2018), that the origin of this haplotype must not be restricted to Israel alone, but also in other, poorly sampled or unknown nesting sites. This finding reinforces the results of MSA from the Adriatic Sea (Tolve et al. 2018): Western Turkish rookeries, which despite the fact that they are much more abundant and closer to the Adriatic Sea, showed a medium contribution probability to the Adriatic stock. Two samples from Slovenia (northern Adriatic) carried the haplotype CC-A29.1. If CC-A29.1 is also to be included as a haplotype of Western Turkish origin, the probability would most likely increase, and the contribution would not be similar or smaller than the ones of Israeli nesting areas. Another rare Haplotype is CC-A50.1 which was identified for the first time in Cyprus (Clusa et al. 2013) and we are not aware of this haplotype being recorded in other research. During this study one specimen from a Turkish rookery (Kazanlı) carried the haplotype CC-A50.1.

Turkey is also a foraging site for loggerhead sea turtles originating from Cyprus and a review of migratory routes from 63 adult loggerhead turtles released mainly from Greece and Cyprus revealed that only a few individuals oriented to Turkish coasts (Luschi and Casale 2014). The tracking studies from Northern Cyprus showed that only early nesters visited other Turkish rookeries (Snape et al. 2016). An MSA of an eastern Turkish foraging ground performed by Türkozan et al. (2018) showed a local contribution (62%) of Cyprus to the western subdivision.

For Tunisian rookeries (Kuriat islands), only the short mtDNA control region fragment (500 bp) had been used to date, and only CC-A2.1 was found (Chaieb et al. 2010). Analysis using the short fragment has shown no significant differences between Libyan and Tunisian rookeries, but due to several hundred kilometres of separation Shamblin et al. (2014) suggested that these might be demographically isolated nesting populations. A distinction, with the shorter D-loop sequence, between the haplotype CC-A2.9 and the widely distributed haplotype CC-A2.1 is not possible (Splendiani et al. 2017), and given the fact that CC-A2.9 is common in Libya, it has been proposed that reanalysing the Tunisian samples using the longer D-loop fragment is crucial (Shamblin et al. 2014). To date the long sequence has only been used for MSA (Karaa et al. 2011). This study showed that most loggerheads from the Tunisian rookery carry the common haplotype CC-A2.1 (39). One specimen also carried haplotype CC-A2.9, and another individual carried CC-A3.1. Based on the detection of the CC-A2.9 haplotype at a Tunisian rookery, classification of Tunisia and Libya as one Management Unit (MU) is reasonable (see Shamblin et al. 2014).

In Albania, evidence of the occurrence of nesting was apparent with infrequent reports of hatchling sightings along the coastline, but it was not until 2018 that the first official loggerhead nest was confirmed (Piroli and Haxhiu 2020) which is why, until now, limited molecular genetic analyses has been carried out (Yilmaz et al. 2012) but no molecular genetic analysis has been carried out for the nesting population. In this study, one hatchling and the bycatch samples were included in the analysis to detect different haplotypes and all eight samples analysed carried the cosmopolitan haplotype CC-A2.1. We have also included two samples from Egypt to determine the presence of any additional (orphaned) haplotypes present in the Mediterranean as samples were provided by project partners. These two samples also resulted as the most common haplotype CC-A2.1.

Eight loggerheads samples were of Lebanese origin. The haplotypes they carried were consistent with previous findings, CC-A2.1 and CC-A3.1 (Saied et al. 2012; Clusa et al. 2014).

Different polymorphic measurements were observed across the participating Mediterranean countries. While these differences may reflect a discrepancy in sample size for most comparisons, higher values were measured in Libya than in Turkey, even though fewer loggerhead sea turtle samples were used there. Furthermore, two new haplotypes were discovered in Libya, thus our findings support Saied et al. (2012) claim that Libya has an important population carrying a wide range of haplotypes for loggerhead sea turtle, which is why the protection of this assemblage is essential in order to conserve diversity in the Mediterranean stock.

This study has provided new insights into the population structure of the loggerhead sea turtle in the Mediterranean Sea. Haplotypes previously thought to be endemic to certain nesting sites, have been found to be present at other rookeries. Additionally, the assignment of an "orphaned" haplotype to a nesting area, suggests that MSA should be repeated (e.g. Adriatic Sea), as these new findings could change the contribution of some rookeries to these stocks.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12686-023-01303-9.

Acknowledgements We would like to thank the MAVA Foundation for supporting the project of Conservation of Marine Turtles in the Mediterranean. This paper is significant for the loggerhead turtle in the Mediterranean, and for this reason all authors agreed to collaborate by providing data. We all thank our volunteers in collecting the samples in the field.

Author contributions All authors contributed to the study conception and design. The first draft of the manuscript was written by corresponding author and his co-authors from the same affiliation and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding MAVA Foundation, Conservation of Marine Turtles in the Mediterranean.

Declarations

Competing interests The authors declare no competing interests.

References

Abreu-Grobois FA, Horrocks J, Formia A, Dutton P, LeRoux R, Vélez-Zuazo X, Soares L, Meylan P (2006) New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Frick M, Panagopoulou A, Rees AF, Williams K (eds) Proceedings of the 26th annual symposium on sea turtle biology Island of Crete. Greece, Springer, p 179

Bertuccio V, Costantini F, Angelini V, Furii G, Gobic K, Abbiati M (2019) Haplotype and biometric patterns in loggerhead turtles from the Adriatic foraging ground. J Sea Res 147:1–9. https://doi.org/10.1016/j.seares.2019.02.002

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Mol Ecol 16:4886–4907. https://doi.org/10.1111/j.1365-294X.2007.03542

Carreras C, Pont S, Maffucci F, Pascual M et al (2006) Genetic structuring of immature loggerhead sea turtles *(Caretta caretta)* in the Mediterranean Sea reflects water circulation patterns. Mar Biol 149:1269–1279

Carreras C, Rees AF, Broderick AC, Godley BJ, Margaritoulis D (2014) Mitochondrial DNA markers of loggerhead marine turtles (*Caretta caretta*) (Testudines Cheloniidae) nesting at Kyparissia Bay Greece confirm the western Greece unit and regional structuring. Sci Mar 78:115–124. https://doi.org/10.3989/scimar.03865.27B

Carreras C, Pascual M, Tomas J, Marco A, Hochscheid S, Castillo JJ, Gozalbes P, Parga M, Piovano S, Cardona L (2018) Sporadic nesting reveals long distance colonisation in the philopatric loggerhead sea turtle (*Caretta caretta*). Sci Rep 8:1435

Casale P (2015) *Caretta caretta* (Mediterranean subpopulation). The IUCN Red List of Threatened Species 2015: e.T83644804A83646294. www.iucnredlist.org

Casale P, Broderick AC, Camiñas JA, Cardona L et al (2018) Mediterranean sea turtles: current knowledge and priorities for conservation and research. Endanger Species Res 36:229–267. https://doi.org/10.3354/esr00901

Cerritelli G, Casale P, Sözbilen D, Hochscheid S, Luschi P, Kaska Y (2022) Multidirectional migrations from a major nesting area in Turkey support the widespread distribution of foraging sites for loggerhead turtles in the Mediterranean. Mar Ecol Prog Ser 683:169–177

Chaieb O, El Ouaer A, Mafucci F, Bradai MN, Bentivegna F, Said K, Chatti N (2010) Genetic survey of loggerhead turtle *Caretta caretta* nesting population in Tunisia. Mar Biodivers Rec 3:e20. https://doi.org/10.1017/S175526721000014X

Clusa M, Carreras C, Pascual M, Demetropoulos A, Margaritoulis D, Rees AF, Hamza AA, Khalil M, Aureggi M, Levy Y, Turkozan O, Marco A, Aguilar A, Cardona L (2013) Mitochondrial DNA reveals Pleistocenic colonisation of the Mediterranean by loggerhead turtles (*Caretta caretta*). J Exp Mar Biol Ecol 439:15–24. https://doi.org/10.1016/j.jembe.2012.10.011

Clusa M, Carreras C, Pascual M, Gaughran SJ, Piovano S, Giacoma C, Fernández G, Levy Y, Tomás J, Raga JA, Maffucci F, Hochscheid S, Aguilar A, Cardona L (2014) Fine-scale distribution of juvenile Atlantic and Mediterranean loggerhead turtles (*Caretta caretta*) in the Mediterranean Sea. Mar Biol 161:509–519. https://doi.org/10.1007/s00227-013-2353-y

Clusa M, Carreras C, Cardona L, Demetropoulos A et al (2018) Philopatry in loggerhead turtles *(Caretta caretta)*: beyond the gender paradigm. Mar Ecol Prog Ser 588:201–213

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends Ecol Evol 15(7):290–295. https://doi.org/10.1016/S0169-5347(00)01876-0

Garofalo L, Mingozzi T, Mico A, Novelletto A (2009) Loggerhead turtle (*Caretta caretta*) matrilines in the Mediterranean: further evidence of genetic diversity and connectivity. Mar Biol 156:2085–2095. https://doi.org/10.1007/s00227-009-1239-5

Garofalo L, Marsili L, Terracciano G, Baini M, Senese M, Mancusi C, Lorenzini R (2016) Welcome to the North! Genetic makeup of loggerhead *Caretta caretta* females from the northernmost Italian nesting site. In: Menegon M, Rodriguez-Prieto A, Deforian MC (eds) Atti XI Congresso Nazionale Societas Herpetologica Italica. Ianieri Edizioni, Trento

Garofalo L, Insacco G, Scaravelli D, Lorenzini R (2016) On the connectivity between nesting colonies of the loggerhead turtle *Caretta caretta* in the Ionian Region. In: Menegon M, Rodriguez-Prieto A, Deflorian MC (eds) Atti XI Congresso Nazionale Societas Herpetologica Italica. Ianieri Edizioni, Trento, pp 135–136

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hamza A (2010) Libya. In: Casale P, Margaritoulis D (eds) Sea turtles in the Mediterranean: distribution, threats and conservation priorities. IUCN, Gland, pp 157–170

Haywood JC, Fuller WJ, Godley BJ, Margaritoulis D, Shutler JD, Snape RT, Widdicombe S, Zbinden JA, Broderick AC (2020) Spatial ecology of loggerhead turtles: insights from stable isotope markers and satellite telemetry. Divers Distrib 26:368–381

Karaa S, Maffucci F, Imed J, Abderrahmen B, Bradai M, Bentivegna F (2011) Demographic composition of the Tunisian loggerhead foraging aggregation: does the analysis of a longer mtDNA segment provide additional information? In: Bentivegna F, Maffucci F, Mauriello V (eds) Book of Abstracts, 4th Mediterranean conference on marine turtles, Naples, p 74

Kaska A, Bagcı H, Kaska Y (2001) Genetic material collection of sea turtles and PCR amplifications. In: Margaritoulis D, Demetropoulos A (eds) Proceedings of the First Mediterranean Conference on Sea Turtles. Barcelona Convention-Bern Convention-Bonn Convention (CMS). Nicosia, Cyprus, p 147–150

Kot CY, Åkesson S, Amorocho Alfaro-Shigueto J, Llanos DF et al (2022) Network analysis of sea turtle movements and connectivity: a tool for conservation prioritization. Divers Distrib 28(4):810–829

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Laurent L, Casale P, Bradai MN, Godley BJ, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D, Abd El-Mawla EM, et al (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Mol Ecol 7:1529–1542. https://doi.org/10.1046/j.1365-294x.1998.00471.x

Leigh JW, Bryant D (2015) Popart: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinform 25:1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Luschi P, Casale P (2014) Movement patterns of marine turtles in the Mediterranean Sea: a review. Ital J Zool 81:478–495

Maffucci F, Corrado R, Palatella L, Borra M, Marullo S, Hochscheid S, Lacorata G, Iudicone D (2016) Seasonal heterogeneity of ocean warming: a mortality sink for ectotherm colonizers. Sci Rep 6(1):1–9. https://doi.org/10.1038/srep23983

Margush T, McMorris FR (1981) Consensus*n*-trees. Bull Math Biol 43:239–244. https://doi.org/10.1007/BF02459446

Monzón-Argüello C, Rico C, Naro-Maciel E, Varo-Cruz N, López P, Marco A, López-Jurado LF (2010) Population structure and conservation implications for the loggerhead sea turtle of the Cape Verde Islands. Conserv Genet 11:1871–1884. https://doi.org/10.1007/s10592-010-0079-7

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. Mol Ecol 3:401–411. https://doi.org/10.1111/j.1365-294X.1994.tb00080.x

Piroli V, Haxhiu I (2020) Nesting of the loggerhead turtle (*Caretta Caretta*) in the southeast Adriatic confirmed. Nat Croat 29:23–30

Rees AF, Carreras C, Broderick AC, Margaritoulis D, Stringell TB, Godley BJ (2017) Linking loggerhead locations: using multiple methods to determine the origin of sea turtles in feeding grounds. Mar Biol 164(2):1–14. https://doi.org/10.1007/s00227-016-3055-z

Saied A, Maffucci F, Hochscheid S, Dryag S, Swayeb B, Borra M, Oureghi A, Procaccini G, Bentivegna F (2012) Loggerhead turtles nesting in Libya: an important management unit for the Mediterranean stock. Mar Ecol Prog Ser 450:207–218. https://doi.org/10.3354/meps09548

Schofield G, Hobson V, Fossette S, Lilley M, Katselidis KA, Hays GC (2010) Biodiversity research: Fidelity to foraging sites, consistency of migration routes and habitat modulation of home range by sea turtles. Divers Distrib 16(5):840–853. https://doi.org/10.1111/j.1472-4642.2010.00694.x

Shamblin BM, Bolten AB, Abreu-Grobois FA, Bjorndal KA, Cardona L, Carreras C, Clusa M, Monzón-Argüello C, Nairn CJ, Nielsen JT et al (2014) Geographic patterns of genetic variation in a broadly distributed marine vertebrate: new insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. PLoS One. https://doi.org/10.1371/journal.pone.0085956

Snape RTE, Broderick AC, Çiçek BA, Fuller WJ, Glen F, Stokes K, Godley BJ (2016) Shelf life: neritic habitat use of a turtle population highly threatened by fisheries. Divers Distrib 22:797–807

Splendiani A, Fioravanti T, Giovannotti M, D'Amore A, Furii G, Totaro G et al (2017) Mitochondrial DNA reveals the natal origin of *Caretta caretta* (Testudines: Cheloniidae) stranded or bycaught along the Southwestern Adriatic coasts. Eur Zool J 84(1):566–574. https://doi.org/10.1080/24750263.2017.1400597

Tolve L, Casale P, Formia A, Garofalo L, Lazar B, Natali C et al (2018) A comprehensive mitochondrial DNA mixed-stock analysis clarifies the composition of loggerhead turtle aggregates in the Adriatic Sea. Mar Biol 165(4):1–14. https://doi.org/10.1007/s00227-018-3325-z

Türkozan O, Yilmaz C, Uçar A, Carreras C, Ergene S, Aymak C, Karaman S (2018) Local differentiation in the origin of stranded loggerhead turtles, *Caretta caretta*, within an eastern Turkey foraging area. Ocean Coast Manag. https://doi.org/10.1016/j.ocecoaman.2017.12.011

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, Chaloupka MY et al (2010) Regional management units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5(12):e15465. https://doi.org/10.1371/journal.pone.0015465

Yilmaz C, Turkozan O, Bardakci F (2011) Genetic structure of loggerhead turtle *(Caretta caretta)* populations in Turkey. Biochem Syst Ecol 39:266–276

Yilmaz C, Türkozan O, Bardakci F, White M, Kararaj E (2012) Loggerhead turtles (*Caretta caretta*) foraging at Drini Bay in Northern Albania: Genetic characterisation reveals new haplotypes. Acta Herpetol 7(1):155–162

Authors and Affiliations

Arzu Kaska[1] · Gizem Koç[2,20] · Doğan Sözbilen[3] · Diryaq Salih[4] · Ashraf Glidan[4] · Abd Alati Elsowayeb[19] · Almokhtar Saied[4] · Dimitris Margaritoulis[5] · Panagiota Theodorou[5] · ALan Rees[5] · Robin Snape[6] · Annette Broderick[6] · Brendan Godley[6] · Damla Beton[7] · Meryem Ozkan[7] · Imed Jribi[8] · Manel Ben Ismail[9] · Ahmed Ben Hmida[10] · Ali Badreddine[11] · Enerit Sacdanaku[12] · Vicky Rae[13] · Mohamed Said Abdelwarith[14] · Nahla Naguib[14,15] · Lobna Ben Nakhla[16] · Atef Limam[16] · Marwan Abderrahim[16] · Julien Sémelin[17] · Yakup Kaska[2,18]

1 Department of Science and Mathematics, Faculty of Education, Pamukkale University, Denizli, Turkey

2 Sea Turtle Research, Rescue and Rehabilitation Center (DEKAMER), Dalyan, Turkey

3 Acıpayam Vocational School, Department of Veterinary, Pamukkale University, Denizli, Turkey

4 Sirte Environmental Affairs Department, Ministry of Environment, Sirte, Libya

5 ARCHELON, the Sea Turtle Protection Society of Greece, Athens, Greece

6 Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn TR10 9FE, UK

7 SPOT, Society for Protection of Turtles, Nicosia, Northern Cyprus

8 Sfax Faculty of Sciences, University of Sfax, B.P. 1171, 3000 Sfax, Tunisia

9 Notre Grand Bleu Association, Monastir, Tunisia

10 Agence de Protection Et Aménagement de Littoral, Tunis, Tunisia

11 Tyre Coast Nature Reserve- Biology Laboratory, Tyre, Lebanon

12 Research Center of Flora and Fauna, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

13 Mediterranean Association to Save the Sea Turtle (MEDASSET), Athens, Greece

14 Nature Conservation Sector, Biodiversity Department, Cairo, Egypt

15 Genetic Engineering Research Center, Faculty of Agriculture, Cairo Unisversity, Cairo, Egypt

16 Specially Protected, Areas Regional Activity Centre (SPA/RAC), Boulevard du Leader Yasser Arafat - B.P, 337 - 1080 Tunis Cedex, Tunisia

17 MAVA Foundation, Rue Mauverney 28, 1196 Gland, Switzerland

18 Department of Biology, Faculty of Science and Arts, Pamukkale University, Denizli, Turkey

19 Libyan Organization for Conservation of Nature, LOCN, Misrata, Libya

20 Marine Research Institute, Klaipeda University, Klaipeda, Lithuania