diversity

MDPI

Article

Elucidating the Origins of Stranded Loggerhead Sea Turtles (*Caretta caretta*) in the Eastern Mediterranean through Mitochondrial DNA Mixed-Stock Analysis

Arzu Kaska [1], Tugçe Binen [2,3], Dogan Sözbilen [2,4], Robin Snape [5], Annette Broderick [5], Brendan Godley [5], Damla Beton [6], Meryem Ozkan [6], Carlos Carreras [7] and Yakup Kaska [3,*]

1 Department of Science Education, Faculty of Education, Pamukkale University, Denizli 20160, Türkiye; akaska@pau.edu.tr
2 DEKAMER, Sea Turtle Research, Rescue and Rehabilitation Centre, Iztuzu, Dalyan 48600, Türkiye; tugceebinen@gmail.com (T.B.); dsozbilen@pau.edu.tr (D.S.)
3 Department of Biology, Faculty of Sciences, Pamukkale University, Denizli 20160, Türkiye
4 Department of Veterinary, Acıpayam Vocational School, Pamukkale University, Denizli 20160, Türkiye
5 Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn TR10 9FE, UK; r.t.e.snape@exeter.ac.uk (R.S.); a.c.broderick@exeter.ac.uk (A.B.); b.j.godley@exeter.ac.uk (B.G.)
6 SPOT, Society for Protection of Turtles, Nicosia 99010, Cyprus; damlabeton@gmail.com (D.B.); meryemcyprusturtles@gmail.com (M.O.)
7 Department of Genetics, Microbiology and Statistics, Institute for Research on Biodiversity (IRBio), University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain; carerras@ub.edu
* Correspondence: caretta@pau.edu.tr

Abstract: It is widely recognized that the examination of haplotypes among sea turtles inhabiting nesting beaches holds significant importance. Yet, an effective conservation effort of a population also requires an investigation of the origin of sea turtles that strand on the shore, especially as many of these result from interactions with fisheries. In consideration of this, we analyzed the haplotypes of 542 stranded individuals from the Eastern Mediterranean and identified a total of 9 different haplotypes. Two of these were new haplotypes, one individual was found in Marmaris, Türkiye, and the other in a stranded species in northern Cyprus. Mixed-stock analysis demonstrated that the majority of the individuals stranded in western Türkiye originated from nesting beaches in the same area (33%), followed by Dalyan, Türkiye (25%), and Cyprus (21%). The partial mixed-stock analysis of individuals stranded in the Dalyan–Dalaman region of Türkiye revealed that most originated from Dalaman (45%) and Dalyan (21%), followed by western Greece (11%). The partial mixed-stock analysis for the Eastern Mediterranean showed that the majority of individuals originated from western Türkiye (69%), followed by Cyprus (11%) and Dalyan (7%). These findings, by quantifying the relative contributions of each region, provide valuable insights for guiding conservation efforts regarding *Caretta caretta* in the Mediterranean marine environment.

Keywords: genetic diversity; haplotype analyses; genetic structuring; conservation genetics

Citation: Kaska, A.; Binen, T.; Sözbilen, D.; Snape, R.; Broderick, A.; Godley, B.; Beton, D.; Ozkan, M.; Carreras, C.; Kaska, Y. Elucidating the Origins of Stranded Loggerhead Sea Turtles (*Caretta caretta*) in the Eastern Mediterranean through Mitochondrial DNA Mixed-Stock Analysis. *Diversity* **2024**, *16*, 583. https://doi.org/10.3390/d16090583

Academic Editors: Guido Pietroluongo, Tommaso Gerussi, Cinzia Centelleghe and Bert W. Hoeksema

Received: 11 July 2024
Revised: 14 August 2024
Accepted: 19 August 2024
Published: 14 September 2024

1. Introduction

Biodiversity refers to the variety of life and its processes, including the variety of living organisms, the genetic differences among them, and the communities and ecosystems in which they occur, and it is an important factor in ensuring the continuity of life [1]. It is particularly important for the protection of organisms with relatively small populations [2]. As an illustration, despite the fact that the loggerhead sea turtle (*Caretta caretta*) is listed as a species of 'Least Concern' in the Mediterranean according to IUCN criteria [3], its present condition should continue to be regarded as reliant on the sustained conservation efforts [4].

The phenomenon of sea turtles returning to their natal beaches for breeding as a pivotal stage in their life cycle [5] has led to the subdivision of the population into management units over time [6]. The loggerhead sea turtle population is divided into 10 regional management units (RMUs) around the world, one of which is defined to be the Mediterranean [7]. The Mediterranean population is genetically divided into seven subunits: Calabria–Italy (CAL); western Greece (WGRC); Crete–Greece (CRT), Libya (LIBY); Dalyan–Dalaman–Türkiye (DLYDAL); western Türkiye (TKW); and Eastern Mediterranean (EMED, including middle and eastern Türkiye, Cyprus, Israel, and Lebanon [8], as presented in Figure 1A. In the Mediterranean, the loggerhead sea turtle is known to be the most abundant sea turtle in the region [4].

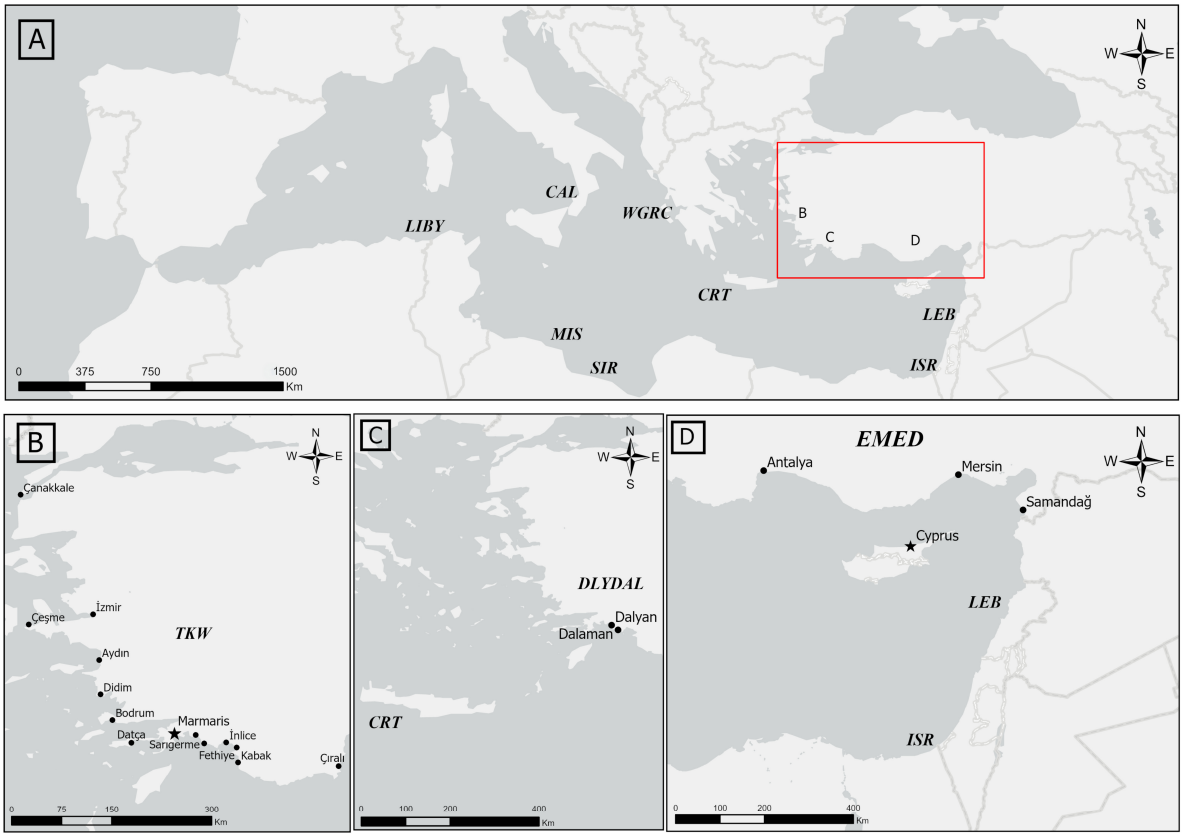

Figure 1. Locations of stranded loggerhead sea turtles sampled in this study: (**A**) management units of the Mediterranean showing the other management units. CAL (Calabria), CRT (Crete), ISR (Israel), LEB (Lebanon), MIS (Misurata), SIR (Sirte), WGRC (western Greece), and LIBY (Libya); (**B**) western Türkiye (TKW); (**C**) Dalyan–Dalaman region (DLYDAL), (**D**) Eastern Mediterranean (EMED).

In order to describe population structures and determine the management units, genetic analysis is the frequently applied method [6,9]. Usually, the mitochondrial DNA (mtDNA) sequences are used in sea turtle genetic studies because they exhibit high haplotype diversity, contributing to inter-intra variation populations and their structure evaluation among regions [10]. With this species, mtDNA haplotype mixed-stock analysis (MSA) is often used to determine the birth areas of juveniles in foraging grounds [5,11]. Genetic analyses of loggerhead sea turtles in the Mediterranean management unit performed thus far have shown that there exists a regional genetic diversity [12–16]. However, it was noted that studying Mediterranean loggerhead sea turtles with more samples from previously under-represented areas is needed to enhance our understanding of the population structure [16].

Nesting occurs in the Eastern Mediterranean, particularly in Türkiye and Greece, followed by Cyprus. Although satellite tracking and bycatch studies have shown that most wintering and feeding areas are in western areas, such as the Tunisian plateau and

the Adriatic Sea [4,17,18], a significant population is also found in the Eastern Mediterranean [13,17,19–21]. However, mixed-stock analysis of the Eastern Mediterranean is limited [14].

The objective of this study is to estimate the origin of stranded individuals from the Eastern Mediterranean using haplotype analysis and MSA. As MSA regarding the Eastern Mediterranean is limited, this will contribute to a better understanding of the threats faced by populations at different life stages of loggerhead turtles and provide more information on the population structure in the entire Mediterranean. Consequently, increased knowledge will shed light on conservation efforts for the globally critically endangered loggerhead sea turtles.

2. Materials and Methods

2.1. Sample Collection

A total of 542 stranded specimens of loggerhead sea turtles (388 adults, 154 subadults) were collected over the period spanning from 2020 to 2021, including 418 specimens sampled from the coasts of Türkiye and 124 specimens from the coasts of northern Cyprus. Samples were taken from the skin or muscle tissue of loggerhead turtles and preserved in 96% ethanol until they were analyzed further. For sea turtles, a carapace length threshold of <65 cm was considered adult, while <65 cm was considered subadult. The data were analyzed in three groups considering previous studies on such units [7,8], namely Dalyan–Dalaman (DLYDAL), western Türkiye (TKW), and Eastern Mediterranean (EMED), as not many studies were carried out previously on these groups [15,16], and the analysis results would provide more information on the genetic diversity of the region (Figure 1B–D).

Considering the sample study sites, TKW included western Türkiye, namely Çıralı (1), Kabak (1), Fethiye (42), İnlice (3), Sarıgerme (2), Ekincik (2), Marmaris (6), Datça (3), Bodrum (10), Didim (1), Aydın (12), Çeşme (1), İzmir (5), and Çanakkale (1) (Figure 1B); DLYDAL included Dalyan (239) and Dalaman (41) (Figure 1C); and EMED included eastern Türkiye and northern Cyprus (Samandağ (1), Mersin (44), and Antalya (2) from Türkiye and 124 individuals from Cyprus) (Figure 1D). Study sites with sample sizes and haplotypes are provided in Table S1.

2.2. DNA Extraction and PCR Amplification

DNA extraction was performed following the standard salt method as the small amount of tissue (<10 mg) tissue was homogenized in 450 µL of sterile salt homogenizing buffer (0.4 M NaCl 10 mM Tris-HCl pH 8.0 and 2 mM EDTA pH 8.0). Then, 100 µL of 10% SDS (2% final concentration) and 50 µL of 20 mg/mL proteinase K were added and mixed well. The samples were incubated at 55–65 °C for at least 1 h or overnight, after which 150 µL of 6 M NaCl (NaCl-saturated H_2O) was added to each sample. Samples were vortexed for 30 s at maximum speed, and tubes were spun down for 30 min. The supernatant was transferred to fresh tubes, and an equal volume of isopropanol was added to each sample and mixed well, and samples were incubated at −20 °C for 1 h. They were then centrifuged for 20 min, 4 °C, at 10,000× *g*. The pellet was washed with 70% ethanol, dried, and finally resuspended in 300–500 µL sterile dH_2O. The quantity and quality of the extracted DNA samples were then assessed using a Qubit Flex Fluorometer (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). For PCR amplification, the primer pair consisting of LCM15382 (5′-GCTT AACCCTAAGCATTGG-3′) as the forward primer and H950 (5′-GTCTCGG ATTTTAGGGGTTT-3′) as the reverse primer was employed. This primer pair is widely recognized in loggerhead sea turtle studies, yielding around 800 bp and a span as much of the d-loop but leaving out the highly repetitive region in the 3′ end of the d-loop, which exhibits variations in the number of repeats and heteroplasmy [22]. The thermal cycling conditions for PCR were set according to the description provided by Kaska et al. [16], and Sanger sequencing samples were dispatched to EYS Medikal (Istanbul, Türkiye). These products were sequenced for both forward and reverse sequencing.

2.3. Data Analysis

Sequence alignment and gene quality assessment were performed using SeqScape3 software. Subsequently, the sequences to be aligned were imported into Bioedit version 7.2.5, as described by Hall [23], and subjected to necessary editing by means of comparison with the requisite sequences utilizing Chromas version 2.6.6. The sequences were then examined in more detail using the Archie Carr Centre for Sea Turtle Research database (ACCSTR; https://accstr.ufl.edu/wp-content/uploads/sites/98/cclongmtdna-2.pdf, accessed on 8 January 2024) and the GenBank BLAST sequence comparison tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify haplotypes. The new haplotypes were submitted to GenBank and ACCSTR for international nomenclature, and the haplotypes of CC-A66.2 and CC-A77.1 were obtained (accession numbers were PQ141706 and PQ141705, respectively). Haplotype networks were constructed using the PopART version 1.7 program [24] to illustrate the relationships among distinct haplotypes. Haplotype diversity (Hd), nucleotide diversity (π), and haplotype number (k) were quantified using DnaSP version 5.10.01 [25], as presented in Table 1. We also calculated heterozygosity (Hs), synonymous substitution rate (Ks) and non-synonymous substitution rate (Kxy), genetic differentiation (Gst), total genetic differentiation (DeltaSt), net genetic differentiation (GammaSt), number of subpopulations (Nst), fixation index (Fst), average pairwise nucleotide difference (Dxy), and net nucleotide substitution rate (Da), and the results are presented in Supplementary Table S2. The haplotype network was built considering only our samples, as is common. Similar haplotype networks from the potential populations of the origin can be found in the literature [15–17]. Haplotype frequencies reported in other studies are also given in Table 1 for comparison. Mixed-stock analysis (MSA) was used to determine the origin and foraging areas of rookeries. BAYES software 1.0 [26] was used in this analysis using the Markov chain Monte Carlo (MMC) method, which focused on estimating the proportions of individuals from different rookery sources. In summary, this analysis removes the haplotypes not found in any nesting area (orphan haplotypes) and then infers the composition of the mixed stock (e.g., the population of origin of the stranded animals) by comparing its haplotype frequencies with those from the nesting populations. The first analysis was performed at the regional level, considering two regional areas (Atlantic and Mediterranean) and revealed that this linkage was negligible, so this was not presented, and the second analysis ignored the Atlantic contribution and considered all recruits for the Mediterranean as independent units (fine-scale level) and presented as previously described [27–30]. Following the manual instructions, the MSA was considered reliable only when the Gelman–Rubin criterion was met (G-R contraction factor < 1.2 for all parameters), indicating that all MMC chains converged [31].

Table 1. Measurements of genetic diversity in loggerhead sea turtles and the number of haplotypes and frequency (%) of haplotypes found in different regions. DLYDAL: Dalyan–Dalaman; TKW: western Türkiye; EMED: Eastern Mediterranean; n: number of sea turtles sampled; k: number of haplotypes; Hd: haplotype diversity; π: nucleotide diversity. New haplotypes identified are presented with *.

	TKW	**DLYDAL**	**EMED**	**Overall**	**Kaska et al. [16]**	**Tolve et al. [14]**	**Turkozan et al. [15]**
Population size	91	280	171	542			
Haplotype diversity (Hd)	0.39243	0.51359	0.27100	0.44820			
Nucleotide diversity (π)	0.00051	0.00067	0.00034	0.0058			
Number of haplotypes (k)	4	5	4	9			
Haplotype							
CC-A2.1	68 (75.6%)	158 (56.4%)	144 (84.2%)	370 (68.3%)	437 (62.6%)	405 (82.9%)	113 (73.8%)
CC-A3.1	21 (23.4%)	116 (41.4%)	25 (14.6%)	162 (29.9%)	169 (24.2%)	59 (12.1%)	19 (12.4%)
CC-A3.4	0 (0.0%)	4 (1.4%)	0 (0.0%)	4 (0.7%)	2 (0.3%)	-	-
CC-A2.8	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	5 (0.7%)	4 (0.8%)	-
CC-A50.1	1 (1.1%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.1%)	1 (0.2%)	-
CC-A31.1	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.2%)	2 (0.3%)	3 (0.6%)	-
CC-A53.1	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	-	2 (0.4%)	1 (0.7%)
*CC-A77.1	1 (1.1%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	-	-	-
*CC-A66.2	0 (0.0%)	0 (0.0%)	1 (0.6%)	1 (0.2%)	-	-	-

3. Results

A total of 9 different haplotypes were found in the 542 samples whose mtDNA was analyzed. Two of these were previously unidentified haplotypes. One of the novel haplotypes was detected (CC-A77.1) in an adult female loggerhead stranded in Marmaris, Türkiye, while the other was also identified in a stranded (CC-A66.2) adult female loggerhead sea turtle in Cyprus. Neither was included in the mixed-stock analysis as their origin accounted for 0.37% of the samples. Haplotype networks were created to show the relationships between different haplotypes (Figure 2). The number of samples and their haplotypes are given in Table S1.

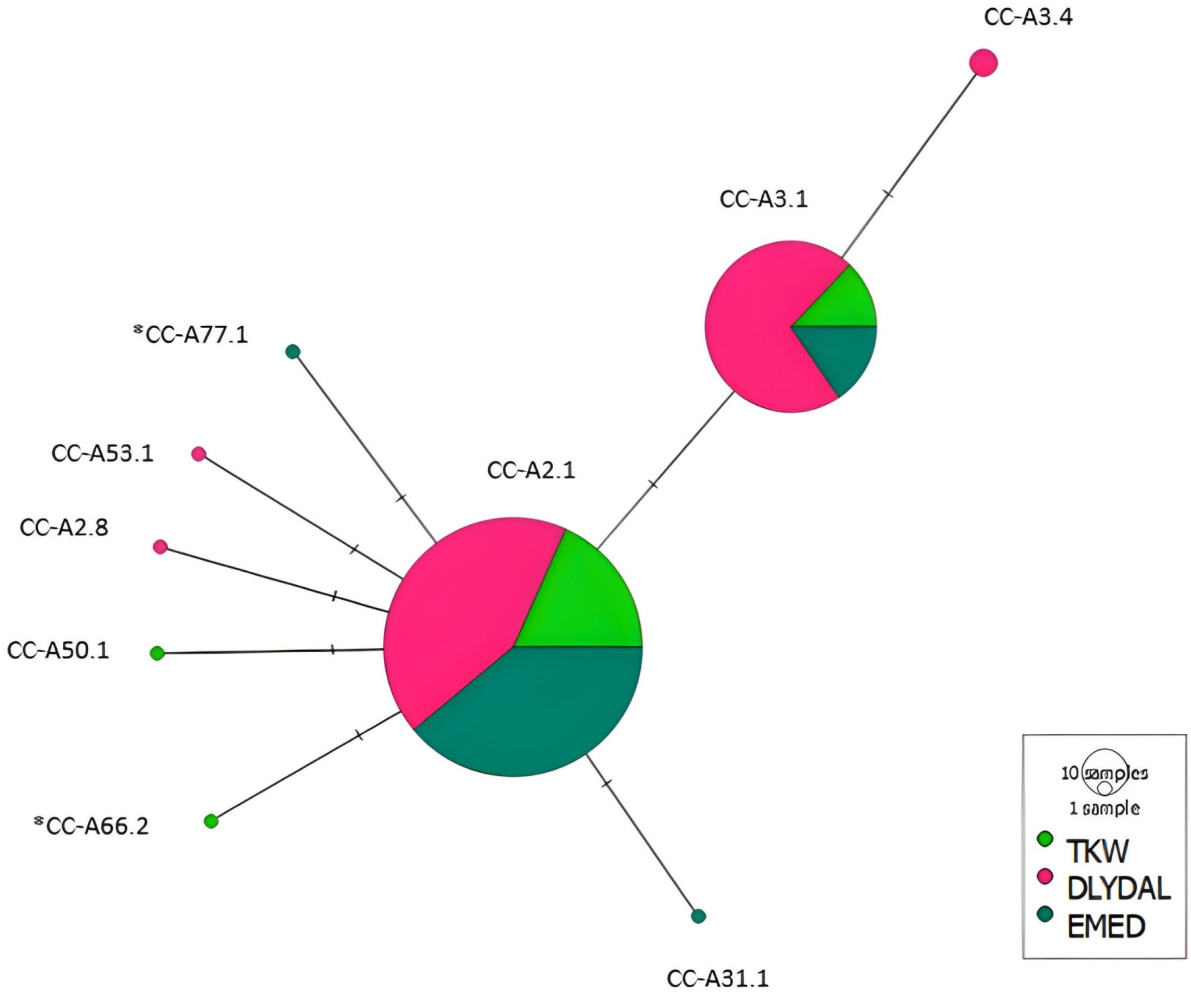

Figure 2. Haplotype network for loggerhead sea turtles in different regions in the Mediterranean. Circle areas shown are proportional to the number of individuals carrying the haplotype. Lines between haplotypes represent single mutations. DLYDAL: Dalyan–Dalaman; TKW: western Türkiye; EMED: Eastern Mediterranean. New haplotypes identified are presented with *.

The region with the highest number of haplotypes in the analysis was the Dalyan–Dalaman region (5). Genetic polymorphism data for loggerhead sea turtles stranded from different regions and haplotype numbers and frequencies (%) are presented in Table 1. Genetic differences between populations are presented in Table S2. Fst values were calculated as 0.16889 for Dalyan–Dalaman when compared with Eastern Mediterranean and 0.07725 when compared with western Türkiye. The Fst value for Eastern Mediterranean when compared with western Türkiye was calculated as 0.01295. As can be seen from these values, the Dalyan–Dalaman region was found to have the highest level of haplotype diversity. The most common haplotype found in all the regions was CC-A2.1 (68.3%), followed by CC-A3.1 (29.9%) (Table 1).

The partial mixed-stock analysis for western Türkiye shows that most of the turtles originated from western Türkiye (33%), followed by Dalyan (25%) and Cyprus (21%). In the partial mixed-stock analysis for Dalyan–Dalaman, it was found that most of the turtles originated from Dalaman (45%) and Dalyan (21%), followed by western Greece (12%). The partial mixed-stock analysis for the Eastern Mediterranean region revealed that the majority of turtles originated from western Türkiye (69%), Cyprus (11%), and Dalyan (7%) (Table S3, Figure 3A–C).

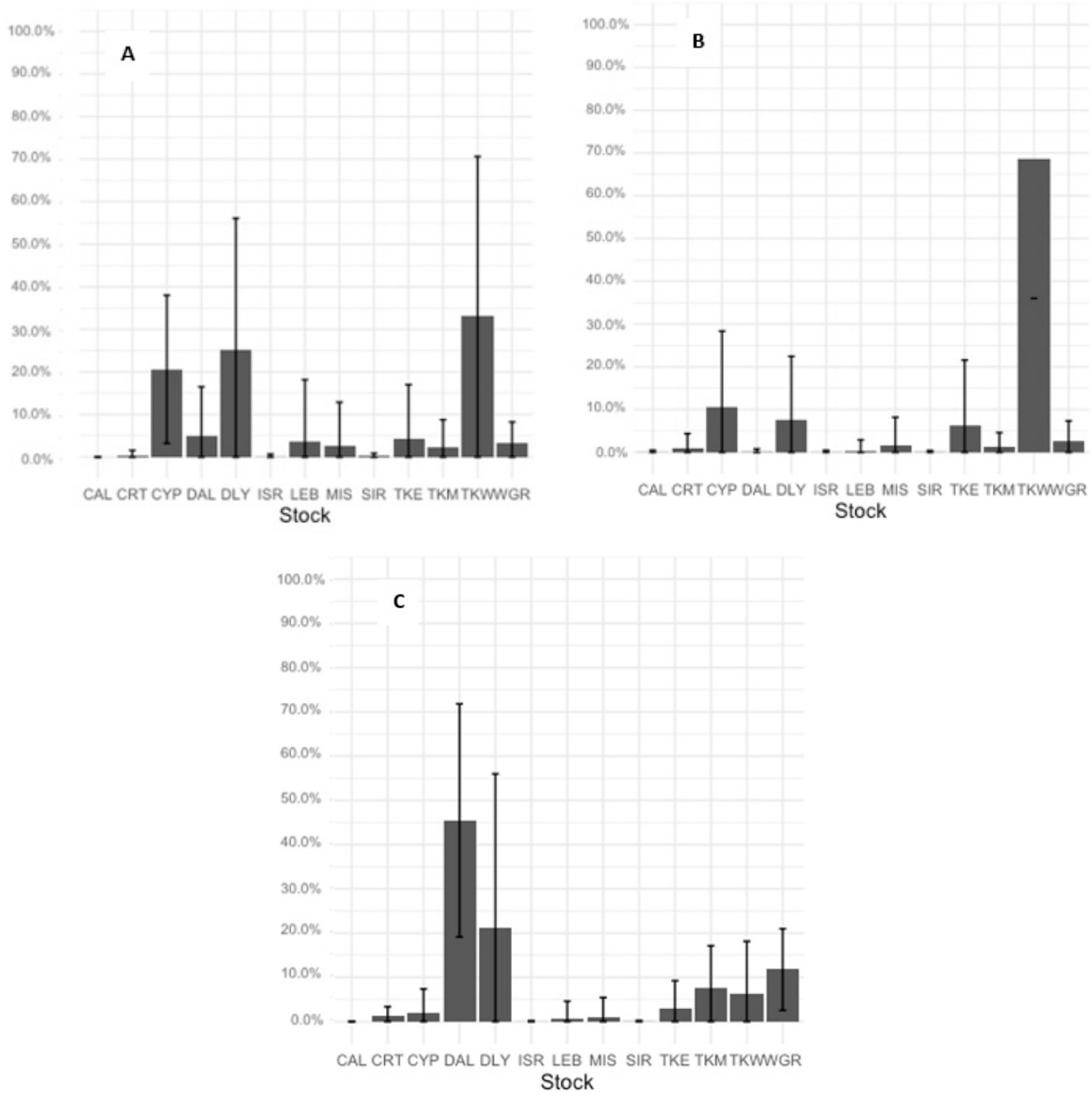

Figure 3. Partial mixed-stock analysis (MSA) of stranded individuals in the Mediterranean Sea: (**A**) stranded individuals in western Türkiye (TKW); (**B**) stranded individuals in the Eastern Mediterranean (EMED); (**C**) stranded individuals in Dalyan–Dalaman (DLYDAL). Bars represent the percentage of individuals in the population. Error bars represent 95% confidence intervals. Mediterranean nesting sites: 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).

4. Discussion

Nesting populations have been well studied, and due to their natal homing, seven independent management units (MUs) have been identified within the Mediterranean region using mitochondrial DNA (mtDNA) markers [7]. These seven independent units comprise individuals contributing from three independent RMUs (the Mediterranean, the Northwest Atlantic, and the Northeast Atlantic) [7]. As far as we know, only individuals from the Mediterranean RMU are breeding in the region, but there may also be some new individuals coming into the Mediterranean. The Mediterranean Sea is frequently visited by nesting and foraging loggerhead turtles, and the origin of these foraging animals has not yet been fully assessed. This is very important for the conservation of sea turtles. Further sampling in nesting areas is needed to identify the origin of these orphan haplotypes as newly identified haplotypes were recorded from nesting beaches [16]. In our previous study, where samples from individuals at nesting beaches were analyzed in order to characterize the haplotype profile of the beaches, we detected a total of 15 distinct haplotypes, 3 of which had not been previously identified. With this study, we also showed that there may be still orphan haplotypes present, as we identified two new haplotypes, and this shows the importance of the studied foraging area for stranded individuals. Nonetheless, apart from determining the haplotype structure of nesting beaches, determining the genetic characterization and origin of loggerhead sea turtles stranded in the Mediterranean is known to be of great importance in guiding conservation efforts, as it enhances our understanding of the range and magnitude of the threats these species are facing in marine habitats.

By examining the origin of stranded individuals using a mixed-stock analysis, we can establish links between conservation efforts on land and in marine habitats, with a focus on foraging grounds, thus building a more comprehensive basis for conservation initiatives. Considering the heavy anthropogenic threats in the Mediterranean [21], combined with the area standing as a pivotal region in terms of its diverse demographic and morphological attributes, distinctive climatic niches, and potential adaptations to local conditions, comprehensive conservation strategies are essential.

In addition to the new haplotypes identified on nesting beaches in previous studies [16], we detected two novel haplotypes from stranded individuals in our study. The identification of novel haplotypes in both nesting beaches [16] and stranded individuals highlights the importance of ongoing comprehensive monitoring efforts, as the detection of rare haplotypes is linked to sample size but also can reflect hidden genetic diversity from unsampled minor nesting areas [16]. The fact that these haplotypes have not been found in the thousands of individuals genotyped in nesting and foraging areas all around the Mediterranean [15–17] in this study as well as previous studies suggests that these haplotypes are very rare. Furthermore, the origins of these novel haplotypes remain ambiguous, raising questions regarding whether they have originated from previously unexamined nesting beaches, or their presence is due to a migration event into the Mediterranean from the Atlantic.

This study investigated the genetic diversity of individuals stranded in the Mediterranean Sea in three management units from western Türkiye, Dalyan–Dalaman, and the Eastern Mediterranean. The CC-A3.4 haplotype, which was first detected in a nesting site in Türkiye (Dalyan) by Kaska et al. [16], was also detected in Dalyan (4) in this study. Additionally, the CC-A2.8 haplotype, which is specific to Cretan rookeries [31], was observed in a stranded sea turtle sampled in Dalyan/Türkiye. The presence of a haplotype associated with an individual from the Cretan rookery in a stranded individual in Dalyan may indicate that the Cretan population comes to Dalyan for wintering.

The CC-A50.1 haplotype, which was first described in Cyprusby Clusa et al. [28] and then documented by Kaska et al. [16] in a rookery in eastern Türkiye (Kazanlı), was documented in a stranded individual in western Türkiye (Milas) in this study. The CCA-31.1 haplotype, which originated from Greek and Calabrian rookeries and was detected at nesting sites in Sicily [31], was found in stranded individuals from Cyprus. The CC-A53.1 haplotype, found in rookeries in the Eastern Mediterranean nesting area [8,15], was found

in a stranded individual sampled from Dalyan–Dalaman in this study. The two most common haplotypes in our study, CCA-2.1 and CCA-3.1, have been previously reported in individuals from the Mediterranean, as these haplotypes are the most common ones (Table 1) in the Mediterranean as a whole [27,28,32].

In conclusion, this study presents new insights into the Mediterranean *C. caretta* population, with implications for the significance of understanding genetic diversity. Presenting new haplotypes from stranded individuals underscores the importance of genetically profiling all nesting grounds, which would allow us to make further predictions. Nevertheless, the performed mixed-stock analysis provides guidance for shaping regional conservation efforts as it establishes links between the rookeries and marine habitats that species inhibit, especially the foraging grounds. The complete genetic diversity of loggerhead populations in the Mediterranean can only be possible by more sampling of nesting sites and foraging habitats as well as investigating new individuals entering the Mediterranean from the Atlantic by using different methods of tracking turtles from the feeding and wintering grounds to the nesting sites or vice versa.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16090583/s1, Table S1. Number of haplotypes of stranded loggerhead sea turtles found in the different regions of the Eastern Mediterranean Sea. DLYDAL: Dalyan–Dalaman, TKW: western Türkiye, EMED: Eastern Mediterranean. Table S2. Demonstration of genetic differences between populations. Hs: heterozygosity, Ks: synonymous substitution rate, Kxy: non-synonymous substitution rate, Gst: genetic differentiation, DeltaSt: total genetic differentiation, GammaSt: net genetic differentiation, Nst: number of subpopulations, Fst: fixation index, Dxy: average pairwise nucleotide difference, Da: net nucleotide substitution rate. Table S3. Bayesian estimates of the contribution of stranded individuals from western Türkiye, Dalyan–Dalaman, and the Eastern Mediterranean to Mediterranean stocks. Table S3. Bayesian estimates of the contribution of stranded individuals from western Türkiye, Dalyan–Dalaman, and the Eastern Mediterranean to Mediterranean stocks. The management units (MUs) referenced are TKW (western Türkiye), DLYDAL (Dalyan–Dalaman), and EMED (Eastern Mediterranean, including Middle and eastern Türkiye, Cyprus, Israel, and Lebanon), as defined by Shamblin et al. [8]. The "Stock" column indicates the locations where the stranded individuals were found. The acronyms used for these locations are as follows: 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), and WGR (western Greece). The higher contributions were given in bold in the table. The color of the stocks refers to the different units described by Shamblin et al. [8].

Author Contributions: A.K., T.B., D.S., R.S., A.B., B.G., D.B., M.O., C.C. and Y.K. were involved in the conceptualization, methodology, software, validation, formal analysis, research, writing—original draft preparation and references, manuscript review and editing, and visualization. All authors have read and agreed to the published version of the manuscript.

Funding: MAVA Foundation (M7-conservation of the marine turtles in the Mediterranean project) provided funding.

Institutional Review Board Statement: Ethical review and approval for this study was waived due to the collection of samples from dead animals.

Data Availability Statement: Original contributions are acknowledged in the manuscript, and additional information is provided in the Supplementary Materials. If further information is required, it can be sent to the author.

Acknowledgments: We would like to thank the MAVA Foundation for their support and funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Penafiel, D.; Lachat, C.; Espinel, R.; Van Damme, P.; Kolsteren, P. A systematic review on the contributions of edible plant and animal biodiversity to human diets. *EcoHealth* **2011**, *8*, 381–399. [CrossRef] [PubMed]
2. Hitchings, S.P.; Beebee, T.J. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: Implications for biodiversity conservation. *Heredity* **1997**, *79*, 117–127. [CrossRef] [PubMed]

3. Casale, P.; Tucker, A.D. Caretta caretta (amended version of 2015 assessment). In *The IUCN Red List of Threatened Species*; IUCN: Gland, Switzerland, 2017; p. e.T3897A119333622. [CrossRef]
4. Casale, P.; Broderick, A.C.; Camiñas, J.A.; Cardona, L.; Carreras, C.; Demetropoulos, A.; Fuller, W.J.; Godley, B.J.; Hochscheid, S.; Türkozan, O.; et al. Mediterranean sea turtles: Current knowledge and priorities for conservation and research. *Endanger. Species Res.* **2018**, *36*, 229–267. [CrossRef]
5. Bowen, B.W.; Karl, S.A. Population genetics and phylogeography of sea turtles. *Mol. Ecol.* **2007**, *16*, 4886–4907. [CrossRef] [PubMed]
6. Moritz, C. Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol.* **1994**, *9*, 373–375. [CrossRef] [PubMed]
7. Wallace, B.P.; Posnik, Z.A.; Hurley, B.J.; DiMatteo, A.D.; Bandimere, A.; Rodriguez, I.; Maxwell, S.M.; Meyer, L.; Brenner, H.; Mast, R.B.; et al. Marine turtle regional management units 2.0: An updated framework for conservation and research of wide-ranging megafauna species. *Endanger. Species Res.* **2023**, *52*, 209–223. [CrossRef]
8. Shamblin, B.M.; Bolten, A.B.; Abreu-Grobois, F.A.; Bjorndal, K.A.; Cardona, L.; Carreras, C.; Clusa, M.; Monzon-Argüello, C.; Nairn, C.J.; Dutton, P.H.; et al. 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* **2014**, *9*, e85956. [CrossRef]
9. Taylor, B.L.; Dizon, A.E. First policy then science: Why a management unit based solely on genetic criteria cannot work. *Mol. Ecol.* **1999**, *8*, S11–S16. [CrossRef] [PubMed]
10. Bowen, B.W.; Bass, A.L.; Soares, L.; Toonen, R.J. Conservation implications of complex population structure: Lessons from the loggerhead turtle (*Caretta caretta*). *Mol. Ecol.* **2005**, *14*, 2389–2402. [CrossRef]
11. Bjorndal, K.A.; Bolten, A.B. Annual variation in source contributions to a mixed stock: Implications for quantifying connectivity. *Mol. Ecol.* **2008**, *17*, 2185–2193. [CrossRef] [PubMed]
12. Yilmaz, C.; Turkozan, O.; Bardakci, F. Genetic structure of loggerhead turtle (*Caretta caretta*) populations in Turkey. *Biochem. Syst. Ecol.* **2011**, *39*, 266–276. [CrossRef]
13. Rees, A.F.; Carreras, C.; Broderick, A.C.; Margaritoulis, D.; Stringell, T.B.; Godley, B.J. Linking loggerhead locations: Using multiple methods to determine the origin of sea turtles in feeding grounds. *Mar. Biol.* **2017**, *164*, 30. [CrossRef]
14. Tolve, L.; Casale, P.; Formia, A.; Garofalo, L.; Lazar, B.; Natali, C.; Novelletto, A.; Vallini, C.; Buzan, E.; Ciofi, C.; et al. A comprehensive mitochondrial DNA mixed-stock analysis clarifies the composition of loggerhead turtle aggregates in the Adriatic Sea. *Mar. Biol.* **2018**, *165*, 68. [CrossRef]
15. Turkozan, O.; Yılmaz, C.; Uçar, A.H.; Carreras, C.; Ergene, S.; Aymak, C.; Karaman, S. Local differentiation in the origin of stranded loggerhead turtles, *Caretta caretta*, within an eastern Turkey foraging area. *Ocean Coast. Manag.* **2018**, *153*, 70–75. [CrossRef]
16. Kaska, A.; Koç, G.; Sözbilen, D.; Salih, D.; Glidan, A.; Elsowayeb, A.A.; Almokhtar, S.; Dimitris, M.; Panagiota, T.; Kaska, Y.; et al. Increased sample size provides novel insights into population structure of Mediterranean loggerhead sea turtles. *Conserv. Genet. Resour.* **2023**, *15*, 67–75. [CrossRef]
17. Patel, S.H.; Morreale, S.J.; Panagopoulou, A.; Bailey, H.; Robinson, N.J.; Paladino, F.V.; Margaritoulis, D.; Spotila, J.R. Changepoint analysis: A new approach for revealing animal movements and behaviors from satellite telemetry data. *Ecosphere* **2015**, *6*, 1–13. [CrossRef]
18. Haywood, J.C.; Fuller, W.J.; Godley, B.J.; Margaritoulis, D.; Shutler, J.D.; Snape, R.T.; Widdicombe, S.; Zbinden, J.A.; Broderick, A.C. Spatial ecology of loggerhead turtles: Insights from stable isotope markers and satellite telemetry. *Divers. Distrib.* **2020**, *26*, 368–381. [CrossRef]
19. Margaritoulis, D.; Rees, A.F. Loggerhead turtles nesting at Rethymno, Greece, prefer the Aegean Sea as their main foraging area. *Mar. Turt. Newsl.* **2011**, *131*, 12.
20. Snape, R.T.E.; Broderick, A.C.; Çiçek, B.A.; Fuller, W.J.; Glen, F.; Stokes, K.; Godley, B.J. Shelf life: Neritic habitat use of a turtle population highly threatened by fisheries. *Divers. Distrib.* **2016**, *22*, 797–807. [CrossRef]
21. Almpanidou, V.; Tsapalou, V.; Chatzimentor, A.; Cardona, L.; Claro, F.; Hostetter, P.; Kaska, Y.; Liu, W.; Mansui, J.; Miliou, A.; et al. Foraging grounds of adult loggerhead sea turtles across the Mediterranean Sea: Key sites and hotspots of risk. *Biodivers. Conserv.* **2021**, *31*, 143–160. [CrossRef]
22. Abreu-Grobois, F.A.; Horrocks, J.; Formia, A.; Dutton, P.; LeRoux, R.; Vélez-Zuazo, X.; Soares, L.; Meylan, P. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In Proceedings of the 26th Annual Symposium on Sea Turtle Biology, International Sea Turtle Society Athens, Island of Crete, Greece, 3–8 April 2006; p. 179.
23. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
24. Leigh, J.W.; Bryant, D. Popart: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]
25. Librado, P.; Rozas, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **2009**, *25*, 1451–1452. [CrossRef] [PubMed]
26. Pella, J.; Masuda, M. Bayesian methods for analysis of stock mixtures from genetic characters. *Fish. Bull.* **2001**, *99*, 151.
27. Garofalo, L.; Mingozzi, T.; Micò, A.; Novelletto, A. Loggerhead turtle (*Caretta caretta*) matrilines in the Mediterranean: Further evidence of genetic diversity and connectivity. *Mar. Biol.* **2009**, *156*, 2085–2095. [CrossRef]

28. Clusa, M.; Carreras, C.; Pascual, M.; Demetropoulos, A.; Margaritoulis, D.; Rees, A.F.; Hamza, A.; Levy, Y.; Turkozan, O.; Cardona, L.; et al. Mitochondrial DNA reveals Pleistocenic colonisation of the Mediterranean by loggerhead turtles (*Caretta caretta*). *J. Exp. Mar. Biol. Ecol.* **2013**, *439*, 15–24. [CrossRef]
29. Carreras, C.; Rees, A.F.; Broderick, A.C.; Godley, B.J.; Margaritoulis, D. 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.* **2014**, *78*, 115–124. [CrossRef]
30. Gelman, A.; Carlin, J.B.; Stern, H.S.; Rubin, D.B. *Bayesian Data Analysis*; Chapman and Hall/CRC: Boca Raton, FL, USA, 1995. [CrossRef]
31. Garofalo, L.; Insacco, G.; Scaravelli, D.; Lorenzini, R. On the connectivity between nesting colonies of the loggerhead turtle Caretta caretta in the Ionian Region. In *XI Congresso Nazionale della Societas Herpetologica Italica*; Ianieri Edizioni: Trento, Italy, 2016; pp. 135–136. [CrossRef]
32. Carreras, C.; Pont, S.; Maffucci, F.; Pascual, M.; Barceló, A.; Bentivegna, F.; Cardona, L.; Alegre, F.; SanFélix, M.; Fernández, G.; et al. Genetic structuring of immature loggerhead sea turtles (*Caretta caretta*) in the Mediterranean Sea reflects water circulation patterns. *Mar. Biol.* **2006**, *149*, 1269–1279. [CrossRef]