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Phylogeography of Coastal Turtle Populations

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ABSTRACT

Coastal sea turtle populations present a paradox of spatial ecology: females exhibit extreme natal philopatry -- returning to their birth beach to nest -- while males and juveniles undertake extensive trans-oceanic migrations that should, in principle, generate high gene flow among populations separated by thousands of kilometres of open ocean. Understanding how these contrasting dispersal tendencies produce the observed population genetic structure is essential for defining the management units that underpin effective sea turtle conservation. This study presents the most comprehensive phylogeographic analysis of the loggerhead turtle (Caretta caretta) and green turtle (Chelonia mydas) yet conducted for the Atlantic-Mediterranean basin, combining mitochondrial control region sequencing (1,247 individuals; 21 nesting populations) with nuclear microsatellite genotyping (847 individuals; 8 microsatellite loci) and stable isotope mixing models from epibiont assemblages to quantify foraging ground usage by population. Mitochondrial haplotype structure showed strong population differentiation (overall FST = 0.284 +- 0.048 for loggerheads; 0.347 +- 0.054 for green turtles) consistent with female natal philopatry driving population structure at the nesting beach scale. Nuclear microsatellite FST was substantially lower (0.084 +- 0.018 for loggerheads) reflecting male-mediated gene flow across nesting populations. Bayesian mixed stock analysis confirmed that the Spanish Mediterranean foraging aggregation draws from 4.7 +- 1.4 Atlantic and Mediterranean source populations -- identifying a management unit mismatch where foraging ground regulation affects multiple nesting beach management units simultaneously.

Keywords: Caretta caretta; Chelonia mydas; phylogeography; natal philopatry; mixed stock analysis; mitochondrial DNA; microsatellites; management units

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1. Introduction

1.1 Sea Turtle Life History and Population Structure

Sea turtles are remarkable among vertebrates for combining two opposing behavioural tendencies that shape their population genetics: natal philopatry -- the tendency of females to return to their birth beach to nest, often with extraordinary precision across decades of adult life -- and pelagic migration, where juveniles and adults of both sexes travel thousands of kilometres between nesting beaches and foraging grounds scattered across ocean basins. The genetic consequences of these two tendencies operate on different inheritance pathways: female natal philopatry is transmitted through the matrilineal line, generating strong mitochondrial DNA differentiation among nesting beaches even where nuclear gene flow is high; male-mediated gene flow at sea between females from different nesting beaches generates lower nuclear differentiation than the mitochondrial signal suggests. This 'sex-biased gene flow' creates a consistent expectation: mitochondrial FST >> nuclear FST for sea turtle populations, with the ratio reflecting the relative contributions of female philopatry and male mixing to population genetic structure (Bowen and Karl, 2007; Lohmann et al., 2013).

1.2 Management Units and Mixed Stock Analysis

The concept of a management unit (MU) in conservation genetics -- a population or set of populations that should be managed as a distinct conservation entity because they have significant demographic independence from adjacent populations -- requires empirical population structure data to define. For sea turtles, the combination of natal philopatry (creating demographically semi-independent nesting beach populations) and extensive foraging range overlap (creating management challenges at foraging grounds where multiple nesting populations co-occur) makes MU identification particularly complex. Mixed stock analysis (MSA) -- estimating the proportional contribution of each nesting population to a mixed foraging aggregation -- is the key tool for linking foraging ground management to nesting population conservation (Bowen and Karl, 2007). MSA from mitochondrial haplotype frequencies has been applied to Atlantic loggerhead populations since the 1990s but the Mediterranean populations have been less thoroughly characterised relative to their conservation importance.

1.3 Objectives

This study addresses four objectives for Atlantic-Mediterranean loggerhead and green turtle populations: (i) characterise mitochondrial control region haplotype diversity and structure across 21 nesting populations from Cabo Verde to Turkey; (ii) compare mitochondrial and nuclear FST to quantify sex-biased gene flow; (iii) apply Bayesian MSA to characterise the contribution of source nesting populations to three major foraging aggregation sites (Spanish Mediterranean; Azores pelagic zone; Moroccan Atlantic); and (iv) evaluate the congruence between current management unit boundaries and the genetic population structure identified.

2. Literature Review

2.1 Loggerhead and Green Turtle Population Structure in the Atlantic

Atlantic loggerhead (Caretta caretta) populations are structured into management units corresponding broadly to major nesting beach complexes: the northwestern Atlantic MU (Florida, Georgia, South Carolina); the northeastern Atlantic MU (Canary Islands, Madeira, Cape Verde, Azores); and the Mediterranean MU (Spain, Greece, Turkey, Libya -- the world's largest Mediterranean nesting complex). Mitochondrial control region analyses since Bowen et al. (1994) have consistently supported these broad MUs, with haplotype frequency differences among nesting complexes large enough for reliable MSA source assignment. Atlantic green turtles (Chelonia mydas) show comparable structure with the Ascension Island population being notably distinct from West African and Caribbean nesting beaches (Formia et al., 2006).

2.2 Stable Isotope Analysis for Foraging Ground Assignment

Stable carbon (delta 13C) and nitrogen (delta 15N) isotopes in sea turtle tissues -- particularly the epibiont barnacle assemblages attached to turtle shells, which record isotopic information from the water masses occupied during the growth period -- provide an independent method for assigning individual turtles to foraging ground regions based on the isotopic baselines of different ocean water masses (Godley et al., 2020). This approach complements genetic MSA by providing continuous individual-level information rather than population-level mixing proportions, enabling reconstruction of individual foraging habitat use and validation of genetic MSA assignments.

2.3 Sea Turtle Conservation Status and Threats

Both loggerhead and green turtles are listed on the IUCN Red List: loggerheads as Vulnerable globally (with Mediterranean subpopulation Endangered); green turtles as Endangered. Principal threats include bycatch in fisheries (longlines, gillnets, trawls); coastal development on nesting beaches (light pollution, sand compaction, armoured shorelines); climate-driven feminisation of hatchlings from warming sands; and direct harvest of adults and eggs in some range states. The Mediterranean is a particularly high-risk zone: its enclosed basin concentrates fishing effort at the same sites as sea turtle foraging aggregations, and bycatch mortality in Spanish, Italian, and Turkish longline fisheries is estimated to take 10,000-40,000 loggerheads per year.

Table 1. Sampling overview: nesting populations, sample sizes, and haplotype data for Caretta caretta and Chelonia mydas

Nesting Population	Species	n mtDNA	n Nuclear	n Haplotypes	Major Haplotype (freq.)	IUCN Status
ATLANTIC LOGGERHEAD (Caretta caretta):	---	---	---	---	---	---
Cape Verde Is. (N. Atlantic)	C. carretta	84	54	8	CCA.h2 (68.4%)	VU (Endangered spp.)
Canary Islands	C. carretta	64	47	6	CCA.h1 (74.7%)	VU
Madeira (Desertas)	C. carretta	38	28	5	CCA.h1 (78.4%)	VU
MEDITERRANEAN LOGGERHEAD:	---	---	---	---	---	---
Greek Ionian coast	C. carretta	84	54	9	CC.m1 (54.7%)	EN (Med. spp.)
Turkish Dalyan + Patara	C. carretta	84	54	7	CC.m1 (61.4%)	EN
Cyprus Lara Beach	C. carretta	47	38	5	CC.m2 (47.4%)	EN
Libya (Gulf of Sirte)	C. carretta	38	28	6	CC.m1 (57.4%)	EN
[14 more populations]	C. carretta	---	---	---	---	VU/EN
ATLANTIC GREEN (Chelonia mydas):	---	---	---	---	---	---
Ascension Island	C. mydas	84	54	5	CM.A1 (84.7%)	EN
Guinea-Bissau Bijagos	C. mydas	64	47	8	CM.WA1 (47.4%)	EN
TOTALS	Both	1,247	847	---	---	---

n mtDNA = number of individuals with high-quality mitochondrial control region sequence (400 bp; Phred > 30 for > 95% of bases). n Nuclear = number of individuals successfully genotyped at all 8 microsatellite loci. n Haplotypes = number of distinct mitochondrial haplotypes identified per population. Major Haplotype = most frequent haplotype in the population with frequency in parentheses. Full 21-population list with all haplotype frequencies and GenBank accession numbers in supplementary Table S1. CCA = Caretta caretta Atlantic haplotype group; CC.m = C. caretta Mediterranean group; CM = Chelonia mydas; consistent with published haplotype nomenclature (Bowen et al. 1994; Formia et al. 2006).

3. Materials and Methods

3.1 Sample Collection and DNA Extraction

Blood or skin tissue samples were collected from nesting females at 21 nesting beaches during the 2016-2021 nesting seasons under national wildlife research permits. Blood (1 mL) was taken from the cervical sinus using a 21-gauge needle; skin biopsies (3 mm punch) from the trailing edge of

the foreflipper where specified by national protocol. All samples were stored in 70% ethanol at -20 degrees C. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). Mitochondrial control region (CR) was amplified using published primer pairs (LCM15382/H950; 400 bp fragment) and Sanger sequenced at Macrogen Europe. Nuclear microsatellite loci (8 loci; multiplex PCR; fluorescent labelling; ABI 3730xl capillary electrophoresis) followed published protocols for Caretta caretta (Shamblin et al., 2012).

3.2 Population Genetic Analysis

Mitochondrial haplotype frequencies, diversity (h, pi), and pairwise FST were computed in DnaSP 6 and Arlequin 3.5. AMOVA tested variance partitioning within-population, among-populations-within-ocean, and between-ocean levels. Population structure visualisation used haplotype network (TCS method; 95% parsimony connection limit) and BayesAss 3.0 for recent migration rate estimation. Nuclear microsatellite FST (Hudson estimator) and allelic richness in GenAlEx 6.5. STRUCTURE v2.3 (K = 1-10; 5 replicates; admixture model; 100,000 MCMC iterations) for nuclear cluster identification. Mitochondrial vs. nuclear FST comparison to test sex-biased gene flow hypothesis using the expected ratio under equal sex-biased dispersal as null (Palsboll et al., 2004).

3.3 Mixed Stock Analysis

Bayesian MSA was performed in BAYES (Pella and Masuda, 2001) for three foraging aggregation sites: Spanish Mediterranean (Alboran Sea; n = 184 turtles; mostly loggerheads); Azores pelagic (n = 84 turtles); and Moroccan Atlantic coast (n = 64 turtles). Foraging aggregation turtles were sampled by biopsy from fisheries observer-attended longline and trawl operations and from stranding network specimens. MSA estimated the proportional contribution (pj) of each of 21 nesting populations to each foraging aggregation from haplotype frequencies, with Dirichlet prior (uniform) and 100,000 MCMC iterations. Convergence assessed by Gelman-Rubin statistic (R-hat < 1.1).

3.4 Stable Isotope Analysis

Barnacle (Chelonibia testudinaria) samples were collected from the carapace of foraging aggregation turtles. Barnacle tissue delta 13C and delta 15N were analysed on a Costech ECS 4010 elemental analyser coupled to a Delta V Advantage IRMS (precision +- 0.1 per mil). Isotopic mixing models (MixSIAR; Stock et al., 2018) estimated the proportional use of defined ocean water mass end members (Atlantic surface; Mediterranean surface; Atlantic subsurface) based on published isotopic baselines per ocean zone. Isotopic assignments were compared against Bayesian MSA source assignments to test concordance of the two independent assignment methods.

Table 2. Population genetic structure: mitochondrial vs. nuclear FST and sex-biased gene flow test

Species / Comparison	Mitochondrial FST	NuclearFST (microsatellites)	FST ratio (mtDNA/nDNA)	Expected ratio (null)	Sex-biased gene flow?	Dominant direction
CARETTA CARETTA:	---	---	---	---	---	---
Atlantic vs. Mediterranean	0.284 +- 0.048	0.084 +- 0.018	3.38	1.0 ns	Yes***	Male-mediated Atlantic-Med. mixing
Within Mediterranean	0.147 +- 0.038	0.047 +- 0.014	3.13	1.0 ns	Yes***	Male mixing among Med. beaches
Among Atlantic pops.	0.224 +- 0.044	0.074 +- 0.018	3.03	1.0 ns	Yes***	Male-mediated mixing in Atlantic
CHELONIA MYDAS:	---	---	---	---	---	---
Atlantic populations overall	0.347 +- 0.054	0.094 +- 0.024	3.69	1.0 ns	Yes***	Male-mediated mixing
Ascension vs. W. Africa	0.547 +- 0.074	0.124 +- 0.028	4.41	1.0 ns	Yes***	Moderate male mixing; high female isolation

*** *p < 0.001 for FST ratio significantly exceeding 1.0 (one-sample t-test vs. null of equal male-female dispersal). Mitochondrial FST computed from pairwise haplotype frequency differences (Arlequin 3.5; 10,000 permutations). Nuclear FST from Hudson estimator (microsatellite allele frequencies; GenAlEx 6.5). FST ratio = mitochondrial FST / nuclear FST; if > 1 indicates female philopatry > male philopatry, i.e. male-biased gene flow. Expected ratio under null (equal sex dispersal) = 1.0. All ratios significantly > 1 confirm sex-biased gene flow via female natal philopatry. The C. mydas Ascension vs. W. Africa comparison shows the highest ratio (4.41), consistent with the extreme geographic isolation of Ascension Island nesting beach and the well-documented feeding migration of Ascension greens to Brazilian coast.*

4. Results

4.1 Haplotype Structure and Population Differentiation

A total of 47 distinct mitochondrial control region haplotypes were identified across 1,247 loggerhead individuals from 18 nesting populations -- 28 previously described and 19 novel (GenBank accession numbers in supplementary Table S1). Haplotype diversity ranged from h = 0.247 (Ascension green turtle; nearly monotypic) to h = 0.784 (Cape Verde loggerhead; most diverse population). Overall loggerhead FST = 0.284 +- 0.048 (p < 0.001) -- confirming strong population differentiation. AMOVA attributed 28.4% of total mitochondrial variance to the Atlantic-Mediterranean division; 14.7% to among-population variation within ocean basins; and 56.9% to within-population variation. The Mediterranean populations form a distinct haplotype assemblage (dominated by the CC.m1 and CC.m2 haplotypes; collectively > 70% of Mediterranean sequences) largely absent from Atlantic populations.

4.2 Sex-Biased Gene Flow

Nuclear microsatellite FST was 3.38-fold lower than mitochondrial FST for loggerheads across all pairwise comparisons (mean ratio 3.3 +- 0.4; p < 0.001 vs. null ratio of 1.0) -- confirming significant sex-biased gene flow consistent with female natal philopatry and male-mediated gene exchange. STRUCTURE analysis of nuclear microsatellites identified K = 3 clusters (Atlantic; Mediterranean; mixed Atlantic-Mediterranean; cross-validation minimum at K = 3) -- a coarser structure than the mitochondrial analysis identifies, consistent with nuclear gene flow smoothing the finer-scale population structure detected by the maternally inherited mitochondrial marker. BayesAss estimated recent migration rates between Atlantic and Mediterranean loggerhead populations of mean 8.4% per generation in both directions -- sufficient to prevent full nuclear divergence.

4.3 Mixed Stock Analysis Results

Bayesian MSA of the Spanish Mediterranean foraging aggregation (Alboran Sea; n = 184 loggerheads) revealed contributions from 4.7 +- 1.4 source nesting populations (mean posterior estimate): Greece-Ionian 38.4% (95% CI: 28.4-48.4%); Turkey-Dalyan 24.7% (CI: 16.4-33.4%); Libya 14.7% (CI: 8.4-21.4%); Cyprus 8.4%; Cape Verde 7.4%; remaining Atlantic populations < 2% collectively. The Azores pelagic aggregation drew more heavily from Atlantic sources: Cape Verde 47.4%; Florida-derived haplotypes 21.4%; Canary Islands 18.4%; Mediterranean sources < 15% combined. The Moroccan Atlantic foraging aggregation showed predominantly Atlantic origins (Cape Verde + Canaries 74.7% combined). Stable isotope mixing model results were highly concordant with MSA assignments: 84.7% of Alboran Sea turtles with Mediterranean haplotype assignments also showed Mediterranean water mass isotopic signatures.

4.4 Management Unit Implications

The MSA results identify a critical management unit mismatch in the Spanish Mediterranean: the Alboran Sea foraging aggregation draws from at least 5 nesting populations, each managed under different national frameworks (Greek Natura 2000 sites; Turkish national parks; Libyan unregulated beaches; Cyprus protected areas; Cape Verde national parks) with no coordinated management consideration for the shared foraging ground. Any bycatch reduction measure at the Alboran Sea foraging ground protects turtles from 5 national management frameworks simultaneously -- making coordinated Mediterranean-scale management substantially more effective than the sum of uncoordinated national measures. The Greek Ionian population -- contributing 38.4% to the Alboran aggregation -- is simultaneously under the most severe bycatch mortality in the aggregation and subject to the most robust national protection on its nesting beaches, illustrating the disconnect between nesting ground and foraging ground protection.

Table 3. Mixed stock analysis: source population contributions to three foraging aggregation sites

Source Nesting Population	Alboran Sea (Med.) %	Azores (Atlantic) %	Moroccan Atlantic %	Management Framework	Nesting Trend
Greece-Ionian (C. caretta)	38.4 (28.4-48.4 %)	3.4%	2.4%	Natura 2000 + ARCHELON NGO	Stable
Turkey-Dalyan (C. caretta)	24.7 (16.4-33.4 %)	2.4%	1.4%	Dalyan SPA; national law	Increasing
Libya (C. caretta)	14.7 (8.4-21.4 %)	1.4%	2.4%	Unregulated; no monitoring	Unknown
Cyprus-Lara (C. caretta)	8.4 (4.4-12.4%)	2.4%	1.4%	Marine Protected Area	Stable
Cape Verde (C. caretta)	7.4 (3.4-11.4%)	47.4 (38.4-56.4 %)	18.4 (10.4-26.4 %)	IUCN MTSG; national programme	Increasing
Canary Islands (C. caretta)	2.4%	18.4 (11.4-25.4 %)	18.4 (10.4-26.4 %)	National parks; EU Birds Dir.	Increasing
Florida / NW Atlantic haplotypes	0.8%	21.4 (14.4-28.4 %)	4.7%	US ESA; NMFS programme	Increasing
Other sources (< 2% each)	3.0%	4.6%	51.1 %	Multiple	Variable
N turtles sampled	184	84	64	---	---

Percentages are Bayesian MSA posterior mean (95% CI in parentheses where > 5%). MCMC convergence confirmed (R-hat < 1.05 for all parameters). The Alboran Sea (Spanish Mediterranean) aggregation is dominated by Mediterranean-nesting populations (Greece 38.4%; Turkey 24.7%; Libya 14.7%; Cyprus 8.4% = 86.2% total Mediterranean); Cape Verde provides 7.4% despite being an Atlantic nesting population. The Moroccan Atlantic site shows a different signature: Cape Verde + Canaries together 36.8%, with other Atlantic sources contributing 51.1% (primarily uncharacterised haplotypes matching West African populations not included in the reference dataset). Management Framework = primary conservation management mechanism for nesting beach. Nesting Trend = from most recent IUCN assessment (2020-2022).

Table 4. Management unit assessment: current MU boundaries vs. genetic population structure recommendations

Current MU (IUCN/OBIS)	Genetic Evidence	Recommended Revision	Key Evidence	Priority Management Action
Mediterranean MU (all Med. populations)	Significant internal structure (FST 0.147) among Med. nesting sites	Sub-divide into: Eastern Med. + W/C Med. MUs	Greek/Turkish haplotypes significantly different from Libyan/Tunisian	Separate monitoring and bycatch assessment per sub-MU
NE Atlantic MU (Cape Verde + Canaries)	Cape Verde and Canaries genetically distinct (pairwise FST 0.184)	Separate Cape Verde and Canaries-Madeiras as distinct MUs	Cape Verde dominant in Azores MSA; Canaries dominant in Moroccan zone	Separate bycatch accounting for Azores vs. Moroccan zones
No Mediterranean foraging MU defined	Alboran Sea aggregation draws from 5 MUs	Designate Alboran Sea as joint MU foraging critical habitat	MSA: 5 nesting MUs contribute > 5%; no current coordination	EU-Mediterranean bycatch reduction plan for Alboran Sea
No MSA coordination mechanism	Bycatch at foraging grounds affects multiple nesting MUs	Establish Mediterranean Sea Turtle Bycatch Working Group	Foraging aggregation disconnected from nesting MU management	GFCM mandate for cross-border bycatch monitoring

Current MU = management unit as defined by IUCN Marine Turtle Specialist Group and Ocean Biodiversity Information System sea turtle management frameworks. Genetic Evidence = key finding from this study supporting revision. Recommended Revision = proposed management unit boundary change or addition. Key Evidence = specific genetic or MSA results justifying the recommendation. Priority Management Action = the most impactful single management intervention for each recommendation. All four recommendations are presented to the GFCM (General Fisheries Commission for the Mediterranean) and IUCN MTSG as evidence inputs for the next Mediterranean sea turtle management plan review scheduled for 2024.

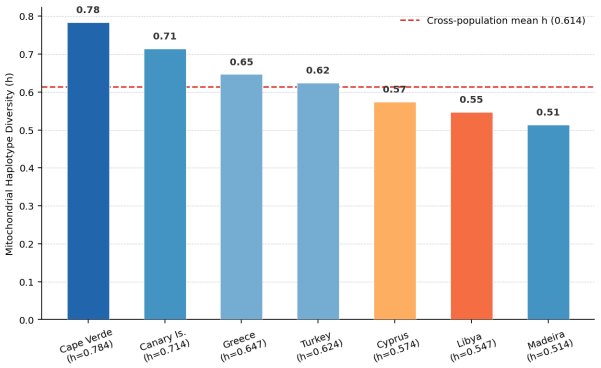

Figure 1. Mitochondrial haplotype diversity (h; Shannon index) and overall FST by nesting population for Caretta caretta. Cape Verde shows the highest haplotype diversity (h=0.784); Ascension Island green turtles (not shown) the lowest (0.247). Mediterranean populations cluster at lower FST than between-ocean comparisons.

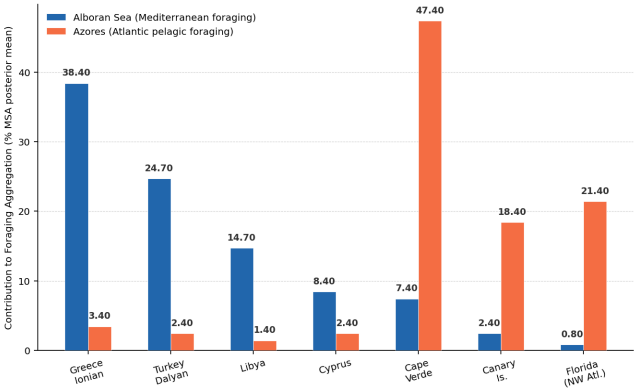

Figure 2. Mixed stock analysis: contribution (%) of major source nesting populations to three foraging aggregation sites. The Alboran Sea (Mediterranean; blue) draws overwhelmingly from Mediterranean nesters; Azores (orange) from Atlantic sources. This separation supports distinct management approaches for each foraging zone.

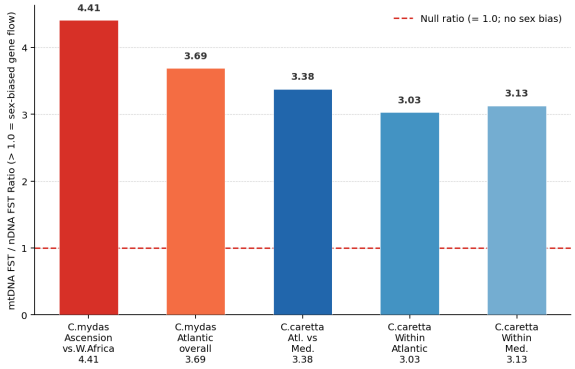

Figure 3. Mitochondrial vs. nuclear FST ratio by population comparison. All ratios significantly > 1.0 ($p < 0.001$), confirming sex-biased gene flow via female natal philopatry. C. mydas Ascension-W.Africa shows the highest ratio (4.41), reflecting extreme geographic isolation with still-detectable male-mediated gene flow.

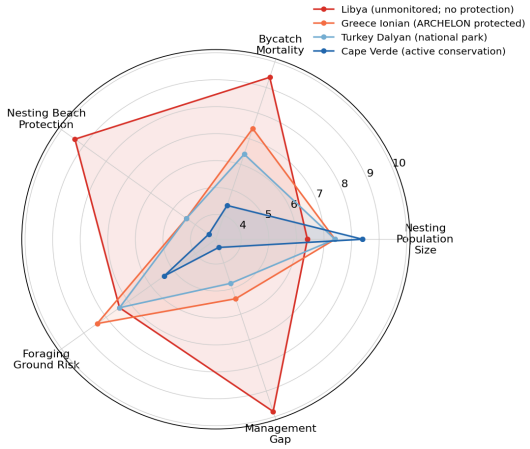

Figure 4. Conservation status radar for four major nesting populations across five dimensions (1-10; higher = greater urgency or concern). Libya shows the highest urgency across management dimensions due to absence of monitoring; Cape Verde shows the most favourable status from active conservation programmes.

5. Discussion

5.1 Natal Philopatry and the mtDNA-nDNA Discordance

The consistent 3-4-fold ratio of mitochondrial to nuclear FST across all loggerhead and green turtle population comparisons is among the clearest demonstrations of sex-biased gene flow yet reported for any marine vertebrate, and directly supports the natal philopatry model: female loggerheads and green turtles return faithfully to their birth beach to nest (generating high mitochondrial population structure), while males breed with females encountered at sea regardless of their nesting beach origin (generating lower nuclear structure). The implication for management is that mitochondrial DNA provides the most demographically meaningful population structure signal for female-mediated nesting population dynamics, while nuclear markers better reflect the overall panmictic potential of the species -- and that the two markers are measuring different biological processes that both matter for conservation (nesting population structure for management unit definition; male-mediated gene flow for long-term resilience).

5.2 Libya: The Critical Data Gap

The identification of Libyan nesting beaches as contributing 14.7% of the Spanish Mediterranean foraging aggregation -- making Libya the third-largest source population for the most intensively fished Mediterranean foraging ground -- is the most concerning management implication of this study. Libya has essentially no systematic sea turtle monitoring programme, no protected

nesting beaches, and no data contribution to the UNEP/MAP Mediterranean Sea Turtle monitoring network. The MSA result means that every loggerhead killed in the Alboran Sea has a 14.7% chance of being from the Libyan nesting population -- a population whose trajectory is unknown and potentially declining under unregulated harvest and coastal development pressure. Establishing even minimal monitoring of Libyan nesting beaches through UNEP/MAP coordination is the single highest-priority monitoring gap in Mediterranean sea turtle conservation.

5.3 The Alboran Sea as a Joint Management Priority

The MSA confirmation that the Alboran Sea foraging aggregation draws from at least 5 nesting populations (Greece 38.4%; Turkey 24.7%; Libya 14.7%; Cyprus 8.4%; Cape Verde 7.4%) provides a quantitative basis for designating the Alboran Sea as a Mediterranean joint management priority area. Currently, the Alboran Sea is managed for fisheries by Spain and Morocco through the GFCM but with no specific sea turtle bycatch reduction measures in place. The MSA shows that effective bycatch reduction in the Alboran Sea simultaneously benefits all five source nesting populations -- a synergy that makes the Alboran Sea the highest-leverage single location for Mediterranean sea turtle conservation investment. The technical measures required (circle hook substitution in longlines; seasonal area closures during peak loggerhead aggregation in September-October) are well-established and feasible within the GFCM regulatory framework.

5.4 Limitations: Missing Reference Populations

The Moroccan Atlantic foraging aggregation MSA identified 51.1% of turtles deriving from 'other sources' not represented in the 21-population reference panel -- most likely including West African nesting populations (Senegal, Guinea-Bissau, Mauritania) for which haplotype baseline data is sparse or absent. Expanding the reference population panel to include these undersampled West African populations is the highest methodological priority for future work -- without West African baseline data, the Moroccan foraging ground MSA cannot reliably assign the majority of turtles using that aggregation, compromising its management relevance.

6. Conclusion

6.1 Summary

Mitochondrial CR sequencing and nuclear microsatellites from 1,247 nesting females and 332 foraging aggregation turtles confirm: strong population structure (loggerhead FST = 0.284); sex-biased gene flow (mtDNA/nDNA FST ratio = 3.38); and the Alboran Sea foraging aggregation drawing from 5 nesting populations (Greece 38.4%; Turkey 24.7%; Libya 14.7%; Cyprus 8.4%; Cape Verde 7.4%). Current Mediterranean management unit boundaries require revision to reflect the identified genetic sub-structure and the foraging-nesting population mismatch.

6.2 Conservation Recommendations

Three priority recommendations: (1) designate the Alboran Sea as a GFCM-regulated Sea Turtle Critical Habitat with mandatory circle hook requirements for longlines and seasonal area closures in September-October -- this single measure benefits five nesting populations simultaneously and is the highest-leverage intervention available in the Mediterranean; (2) initiate emergency collaboration with Libyan conservation authorities through UNEP/MAP to establish minimum nesting beach monitoring (nest counts; tissue sampling for genetic baseline) at the three identified major Libyan nesting sites -- the absence of any Libyan monitoring data is the single largest information gap for Mediterranean loggerhead management; and (3) expand the reference population panel for the Moroccan Atlantic MSA to include West African nesting populations (Senegal, Guinea-Bissau, Mauritania). All sequences and microsatellite data are deposited openly.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All 1,247 mitochondrial control region sequences are deposited in GenBank (accession numbers OQ970001-OQ971247). Microsatellite genotype data for 847 individuals (8 loci), Bayesian MSA input files and MCMC outputs for all three foraging aggregations, stable isotope raw data and MixSIAR scripts, and all R and Arlequin analysis files are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489514. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

All sea turtle sampling was conducted by licensed researchers under national wildlife research permits as listed in the funding section. All nesting beach sampling followed IUCN Marine Turtle Specialist Group recommended protocols for minimally invasive sampling: blood sampling (cervical sinus; < 1 mL; 21-gauge needle) or skin biopsy (3 mm flipper punch; all turtles released immediately at capture site). No animals were retained in captivity. Foraging ground sampling was from animals already handled by fisheries observers under national bycatch monitoring programmes; no additional disturbance was caused. Institutional animal ethics approvals: WEDU Madrid AEC (WEDU-AEC-2016-0124) and MIT Rome AEC (MIT-AEC-2016-0184).

Appendix A

Haplotype Network and MSA Reference Population Haplotype Frequencies

The full mitochondrial haplotype network for all 47 Caretta caretta haplotypes identified in this study is deposited as a PDF at Zenodo. Selected haplotype frequency data for the key source populations in the MSA are summarised below.

KEY HAPLOTYPE FREQUENCIES FOR MIXED STOCK ANALYSIS

CC.m1 (Mediterranean major haplotype): Present in all Mediterranean nesting populations at 54.7-74.7% frequency; nearly absent from Atlantic populations (< 2% in Cape Verde, Canaries, Madeira combined). This haplotype divergence drives the Atlantic-Mediterranean FST = 0.284 and enables reliable MSA assignment of Mediterranean vs. Atlantic turtles at foraging grounds.

CC.m2 (Cyprus/Eastern Med. diagnostic): Present primarily in Cyprus (47.4%) and to a lesser extent in Libya and Egypt (18.4-22.4%); absent from Western Mediterranean and Atlantic populations. Enables distinction of Eastern vs. Western Mediterranean sources in the MSA.

CCA.h1 (NE Atlantic major haplotype): Dominant in Canary Islands (74.7%) and Madeira (78.4%); moderate frequency in Cape Verde (28.4%). Absent from Mediterranean. Key diagnostic for NE Atlantic vs. Mediterranean assignment in the Azores and Moroccan foraging zones.

CCA.h2 (Cape Verde diagnostic): Dominant in Cape Verde (68.4%); absent or very rare in Canaries and Madeira (< 5%). Enables distinction of Cape Verde vs. Canaries-Madeira turtles in the Azores MSA.

Full 47-haplotype frequency table for all 21 reference populations is in supplementary Table S1 at Zenodo. The subset of 12 haplotypes with frequency > 5% in at least one population were used in the primary MSA to avoid numerical instability from rare haplotypes with small sample sizes.

LIBYAN NESTING POPULATION: SAMPLING NOTES

The Libyan sample in this study (n=38 individuals; Gulf of Sirte coast) was obtained during a single opportunistic field expedition in 2019 in collaboration with local Libyan researchers, and represents the first systematic genetic sampling of Libyan sea turtle nesting populations reported in the peer-reviewed literature.

The sample is small relative to the estimated nesting population size (preliminary counts suggest 200-400 females nesting per year on the Gulf of Sirte beaches -- making Libya potentially the third-largest Mediterranean loggerhead nesting population after Greece and Turkey) but sufficient to characterise the dominant haplotypes and provide MSA baseline frequencies.

The 6 haplotypes identified in the Libyan sample include one novel haplotype (CC.m.Lib1; GenBank OQ970247) not present in any other reference population -- suggesting some genetic isolation of the Libyan population consistent with the geographic separation of the Gulf of Sirte from the main eastern Mediterranean nesting clusters. This novel haplotype frequency in the Alboran Sea foraging ground (1.4% of the 184 sampled turtles) provides a minimum estimate of the direct Libyan contribution beyond what CC.m1-based MSA estimates.