Molecular Phylogenetics of Endangered Loggerhead Populations of Crete

Erika Lego [1], **Michael Valahas** [1]

[1] School of Liberal Arts and Sciences, The American College of Greece, 6 Gravias Street, 15342 Athens, Greece

HEL2602- Honors Thesis II

Introduction

- Marine turtles are among the most anatomically unique animals and are divided into two major taxonomical groups: Dermochelyidae and Cheloniidae.
- The loggerhead *Caretta caretta* turtles belong to the Carettini division of the Cheloniidae family of Testudines.

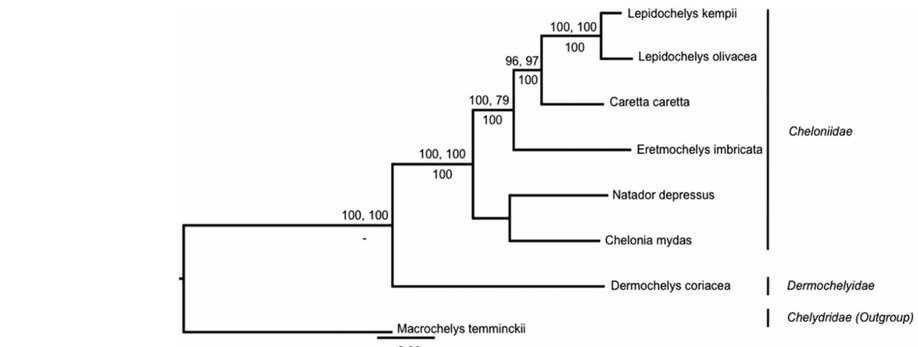


Figure 1: Phylogenetic relationship among the two families of marine turtles based on their taxonomical division [1].

- *C.caretta* are encountered in tropical and subtropical regions with main nesting sites in the eastern Mediterranean abundantly Greece, Cyprus, Tunisia, Turkey, and Libya.

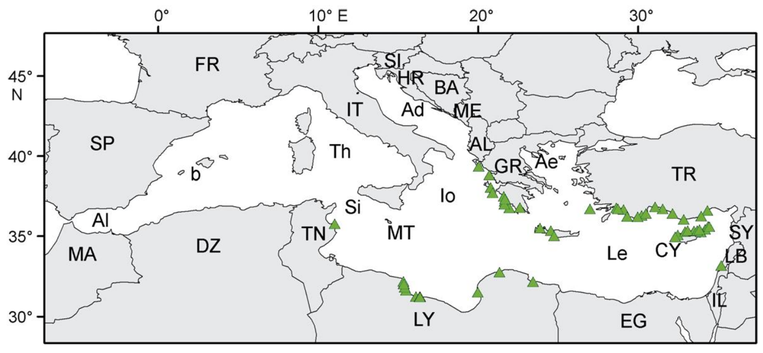


Figure 2: Study site- Crete (35°12.6'N 24°54.6'E / 35.2100°N 24.9100°E) is home to the third largest population of loggerheads [2].

- Globally, the *C. caretta* population is declining and is classified as *Vulnerable* to extinction in the Red List of Threatened Species™, with the primary threats being of human origin.

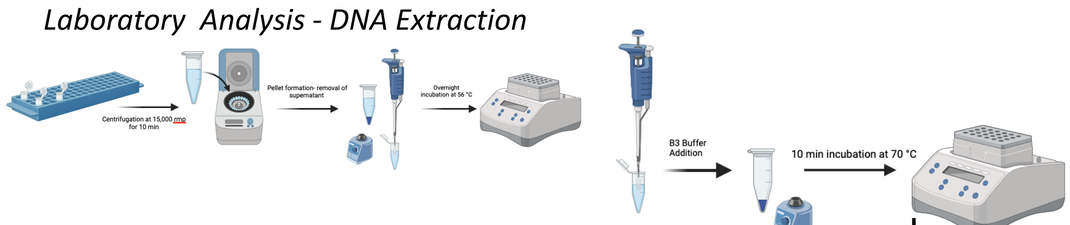
Objectives

- To present a multidisciplinary approach in phylogeny, bioinformatics, ecology, evolutionary and molecular biology through the study of maternally-inherited markers.
- To examine the genetic diversity of a risked species, indigenous to the island of Crete by means of molecular techniques.
- To evaluate nesting preferences while studying six nesting areas in Crete.

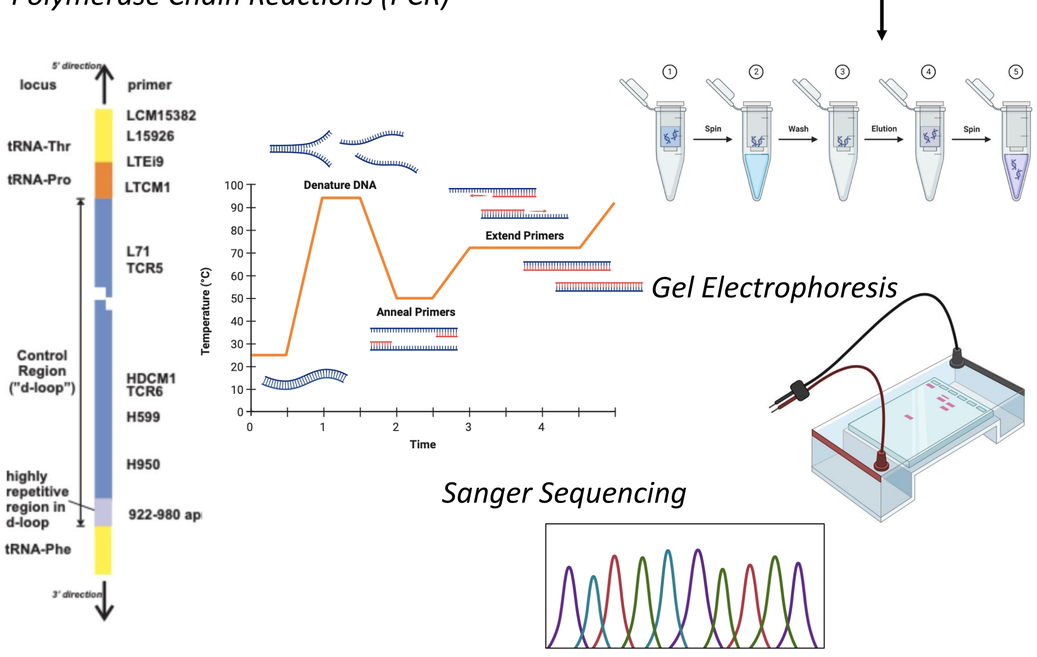
Methods

- Tissue samples of 95 *C.caretta* individuals were preserved in 95% EtOH.
- Samples were coded as 'KOT', 'KOR', 'MESS', 'CHA', 'MAV', 'AMB'.
- The target sequence for amplification was a fragment of mitochondrial DNA (mtDNA) displacement loop (d-Loop) control region of (>800 bp).

Laboratory Analysis - DNA Extraction



Polymerase Chain Reactions (PCR)



Gel Electrophoresis

Sanger Sequencing

Bioinformatics Analysis

Programs and databases used

- FinchTV chromatogram viewer
- Basic Local Alignment Search Tool (BLAST) algorithm
- National Center for Biotechnology Information (NCBI) database

Science figures were generated through BioRender illustration software.

Results

Sequence Match	CC-c-1	CC-A2.1	CC-A6.1
Samples	MAV 22-2a CHA 21 CHA 70 MESS 21	KOR 29 KOR 02 KOR 13 KOR 17 AMB 84 MESS 54-24 MESS 75 KOT 21	KOT 1

Table 1: Sequence match for 13 of the samples through BLAST database with GenBank accession no. MF554690.1, EU179445.1, JQ350705.1 respectively. CC-A2.1 haplotype is generally shared by *C.caretta* individuals migrating through the Mediterranean and the Atlantic, whereas haplotype CC-A6.1 is considered exclusive for the Mediterranean, endemic to western Greece.

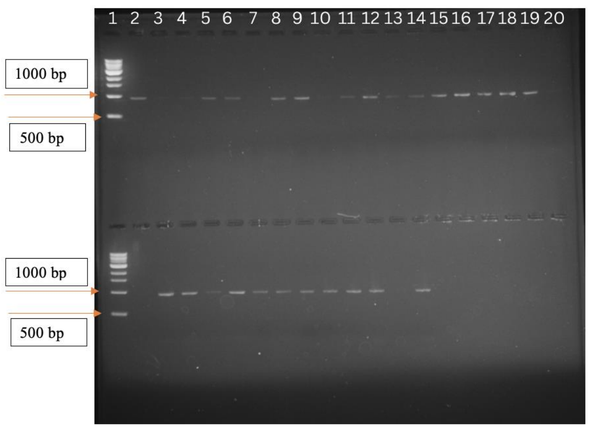
Query 15785 TTATTTCTTAGTTCAGCTCATCACGAGAAATAAGCAATCCTTGTTAGTAAGATACAATAT 15844

Polymorphic site number (Cont)

Sample	22 45 83	22 56 71	22 66 12	22 67 14	22 77 48	22 78 05	22 89 68	22 99 85	22 99 89	22 90 05	23 00 81	33 01 15	33 13 56	33 33 45	33 34 83	33 44 35	33 45 66	33 55 90	33 56 02	33 67 25	33 77 60	33 78 13	33 88 35	33 89 56	33 99 87	33 99 06	44 00 70	44 01 78	44 23 38	44 33 27
MAV 47																														
AMB 60		G			G																				G			G		
MESS 44																				A					G					
MESS 65		G																							G			G		G
CHA 16																														
AMB 52													T						T											
MAV 22-2b																														
MESS 29														G											G			G		G
AMB 04																														
AMB 36	A			A		G																								
AMB 64																														
AMB 44																				A										
MAV 57																														
MAV 17																														
MAV 7															G					C			T							
KOT 21																														
KOT 2																														
KOT 3		C	T			G		C	A	T	G	G	G	T	C	G	T	T		T	C		A	G	G	C	A			C	A			T	T		C	A	T		C	G	T	
KOT 1																														
CHA 42																									G			G		G
CHA 46																														
KOR 5			.			G																								
KOR 39																									G			G		
KOR 22																														

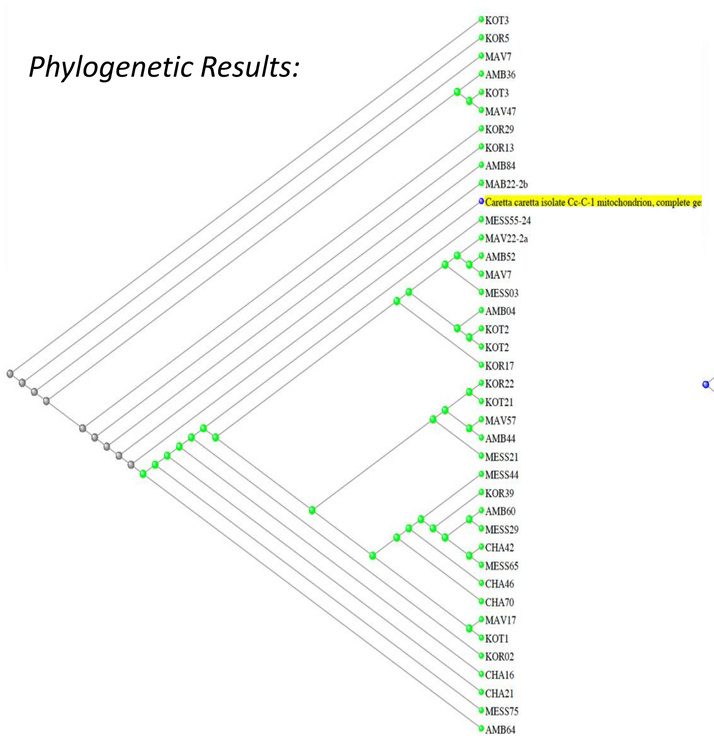
Table 2: Polymorphic sites showcasing variations in the DNA sequences were detected during the multiple sequence alignment step. The reference genome used was the d-Loop region of Cc-C-1. These results indicate rich genetic diversity which is crucial for the adaptability and survival of a species.

Gel Electrophoresis Results:

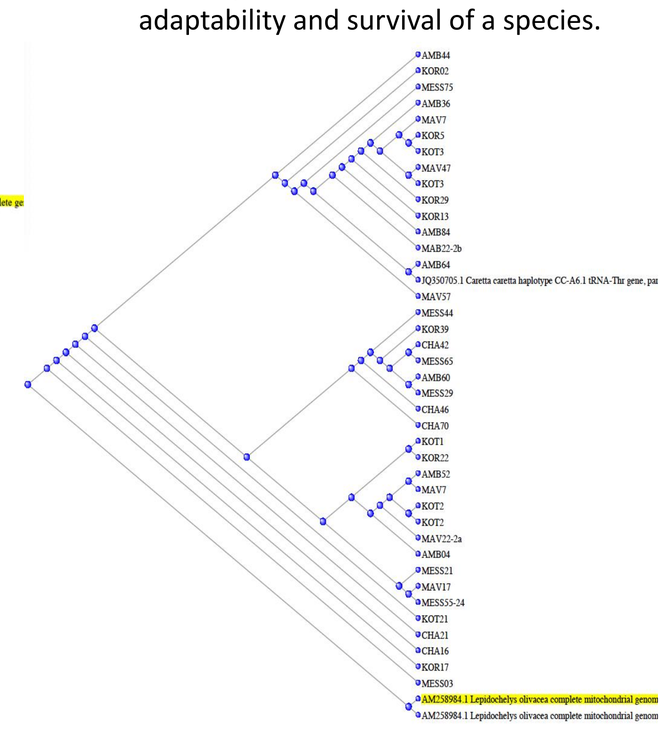


1% agarose gel visualized with a Transilluminator. With a 1 kb ladder as a reference, the lengths of the amplicons were detected at approximately 800bps, confirming the presence of the gene of interest(d-Loop, mtDNA).

Phylogenetic Results:



Cladogram 1: generated through BLAST software, when the samples were evolutionary compared to the complete mitochondrial genome (GenBank accession no. MF554690.1).



Cladogram 2: generated with BLAST software, with *Lepidochelys olivacea* marine turtles as an outgroup (GenBank accession no. AM258984.1).

The cladograms illustrate evolutionary relationships among the six nesting areas, supporting moderate genetic flow and diversity. The study of phylogenetic relations among the Testudines, supports hypothesis that the sequences of samples from Crete belong to *C.caretta* individuals.

Conclusions

- Two distinct haplotypes were found with most turtles carrying haplotype CC-A2.1 generally shared by *C.caretta* individuals migrating through the Mediterranean and the Atlantic and CC-A6.1 endemic to western Greece.
- Polymorphic results show a total number of 90 transitions, with A->G and C-> T transitions being the dominant ones, 122 transversions with T->G, and C->G being abundant and 13 deletions.
- A phylogenetic study further emphasized the evolutionary relatedness of the six areas, adding to the importance of research in molecular phylogenetics of marine turtles for the island of Crete.
- Despite considering ecological importance, management, diversity and economy, conservation projects play an important role in shaping communities as there is a common ethical responsibility to preserve ecosystems and wildlife, part of the legacy for future generations.

Acknowledgments

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