RUPPIN Academic Center
Social Sciences & Management, Engineering, Marine Sciences

School of Marine Sciences

Conservation of Green Sea Turtles through Genetics and Genomics

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Green Sea Turtles in Israel: On the verge of extinction

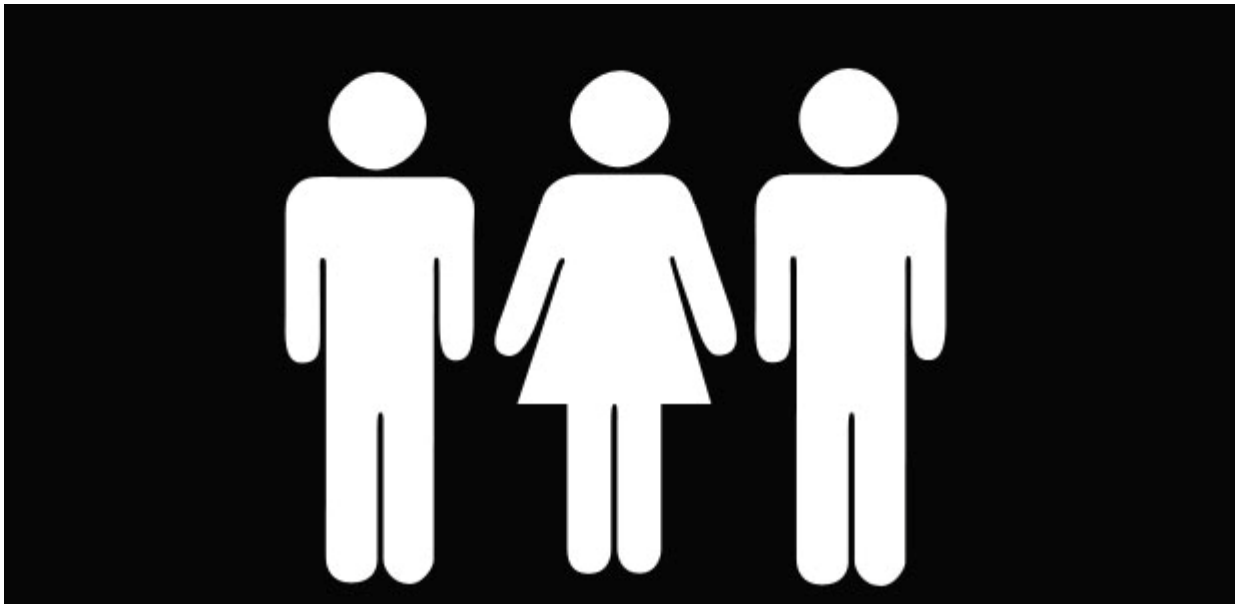
About 10 nesting females along the Israeli shore (about 200 Km)

About Sea Turtles:

- Philopatric

About Sea Turtles:

- polyandry



The Sea Turtle Rescue Center

- Locate
- Heal
- Release

Sea Turtles Rescue Center: Save and Heal

Sea Turtles Rescue Center: Feed and Bread

Let's Increase the Numbers

"I can make my own people"

Jerry Seinfeld

Breeding Stock

The Sea Turtle Rescue Center

A Large Variable Population

"Population with no Variation will not survive Evolution"

C. T. Urtle

A Stable, Strong Population

“My boys can swim!”

George Costanza
(marine biologist)

Genetic Variability of Green Turtles

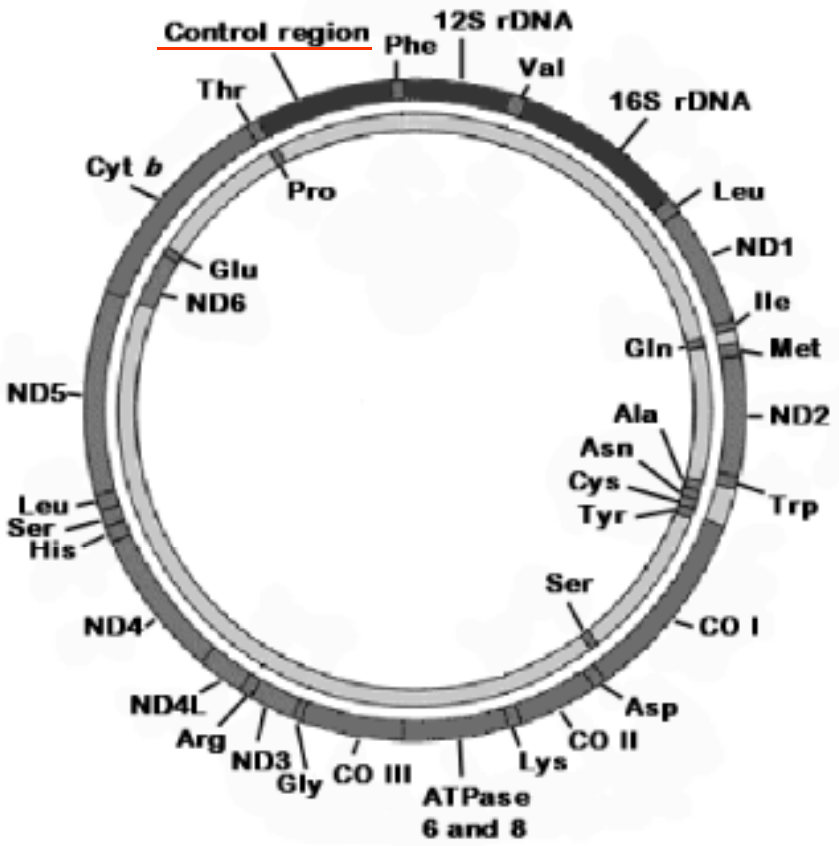


Figure 1 - Schematic representation of the circular molecule of the "conserved" vertebrate mitochondrial genome. Genes outside and inside the circle are transcribed in the H and L strands, respectively. Protein-coding genes are represented as follows: Cyt *b* - cytochrome *b*; CO I, CO II and CO III - subunits I, II and III of the cytochrome oxidase; ND1-6 - subunits 1 to 6 of the NADH reductase. tRNA are represented by their three-letter amino acid abbreviations.

- Mitochondrial DNA D-Loop
- 600bp at the 5'
- 70 haplotypes worldwide
- All Mediterranean (but 2) CM-A13
- Genomic STR's show variability

Genomic STR's

- Genomic STR's show variability
- Difficult to analyze – can't tell a mother's genotype by her offspring

  - Back to mtDNA?
  - Longer Fragments?

The Mitochondrial D-loop

ACACAGGAATAAAAGTGTCCACACAAACTAACTACCTAAATTCTCTGCCGTGCCCAACAGAACAATACCC
GCAATACCTATCTATGTATTATTGTACATCTACTTATTTACCAATAGCATATGACCAGTAATGTTAACAG
TTGATTTGGCCCTAAACATAAAAAATCATTGAATTTACATAAATATTTTAACAACATGAATATTAAGCAG
AGGATTAAAAGTGAAATGACATAGGACATAAAATTAAACTATTATACTCAACCATGAATATCGTCACAGT
AATTGGTTATTTCCTAAATAGCTATTCACGAGAAATAAGCAACCCTTGTTAGTAAGATACAACATTACCA
GTTTCAAGCCCATTCAGTCTGTGGCGTACATAATTTGATCTATTCTGGCCTCTGGTTAGTTTTTCAGGCA
CATACAAGTAACGACGTTCATTCGTTCCCCTTTAAAAGGCCTTTGGTTGAATGAGTTCTATACATTAAAT
TTATAACCTGGCATACGGTAGTTTTACTTGCATATAGTAGTTTTTTTTCTCTCTGTGTTCTCAGGCCCAC
ATAACTGATACCTGCCGATTCAGTGAAACTGGACTTACGTTTAAATATGATTGGCCGTGCAAACTGATTA
ATGGTATTATTAAGTTAATGCTTATAAGACATAGAATTTCACAATTAAACCTAAACAATGATCTACAACC
TAACTCATTATTAACTGTACTTTTTAGCTAAACCCCCCTACCCCCGTTAAAGTCAACACCAGCCCGCTAT
AGCCATTTACTTCTCGCCAAACCCCTAAATCCGAGACTGACCAAACTGACATAATATCAACTGCATAAGC
ATCACACAAATCAATAGGATACTTACACTAATATTTAAAAAGTACTATACAATTCAAAACACCTCTACCA
CACCTCAACCAATATATATATATATTATACATTATATATATATATATATTATATATATTATATATATAAT
AT

DNA Repeats – a Source for Polymorphism

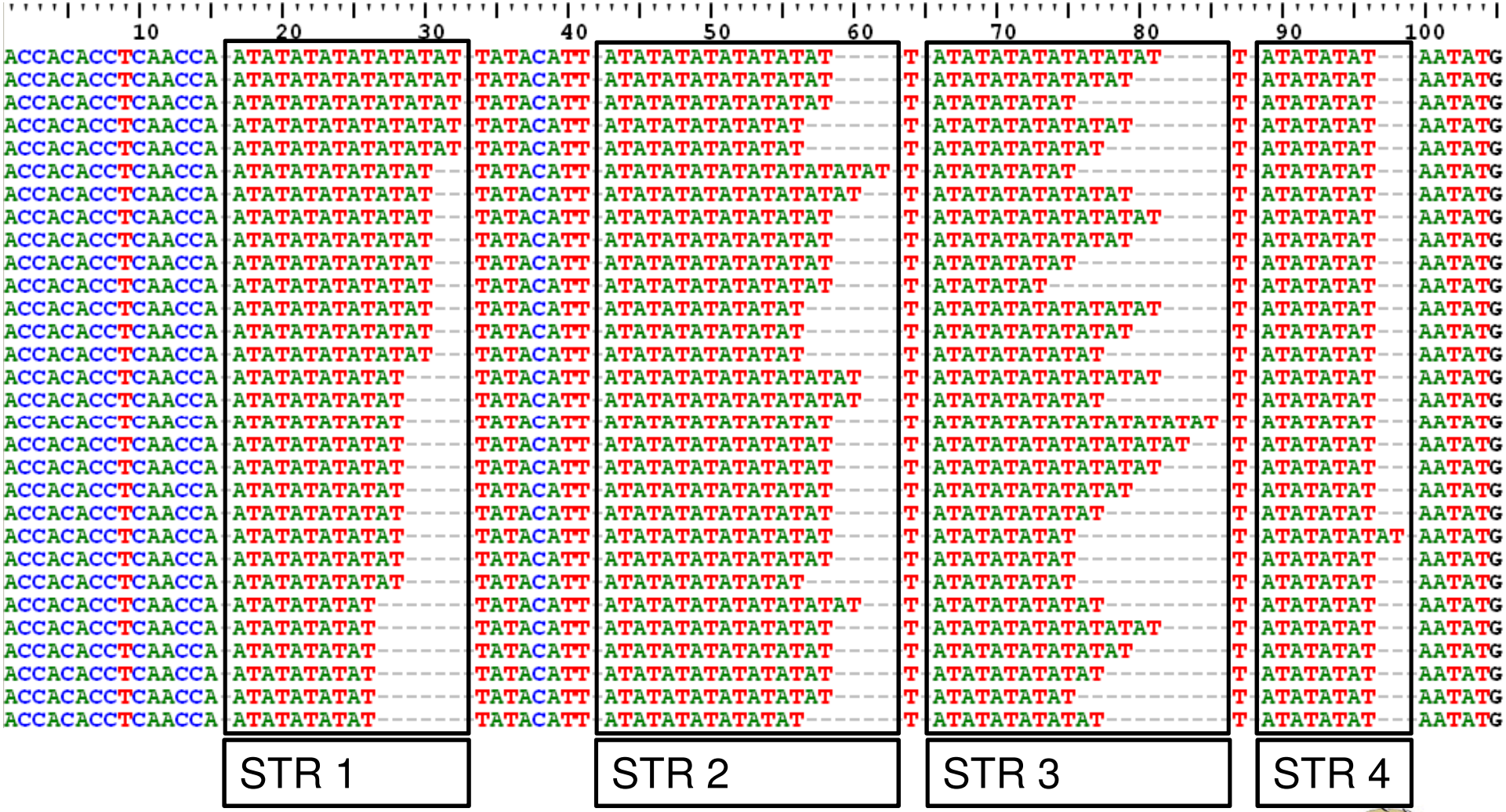
- Mutations' hot spots
- Evolutionary shortcuts

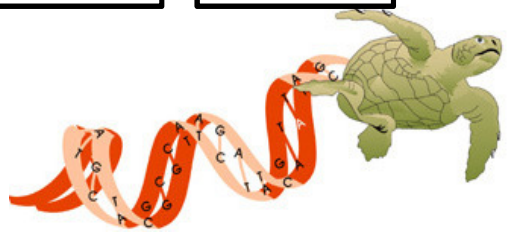
Polymorphism Emerging

The mitochondrial D-loop:
PCR with fluorescent primers.

The 3’ end has length polymorphism:
115, 117, 119, 121, 123, 125, 127 bp

AT Repeats - Aligned



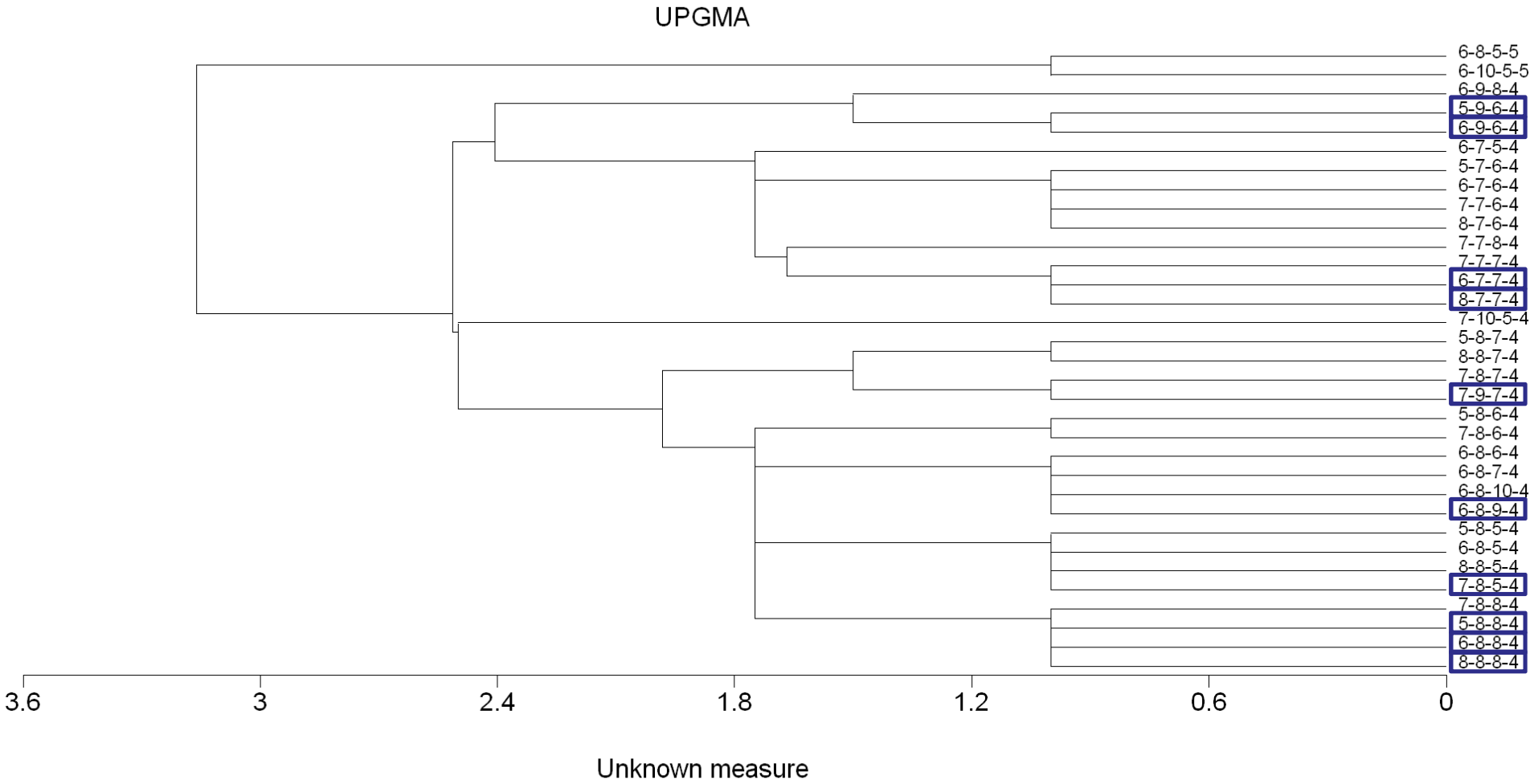


New Haplotyping

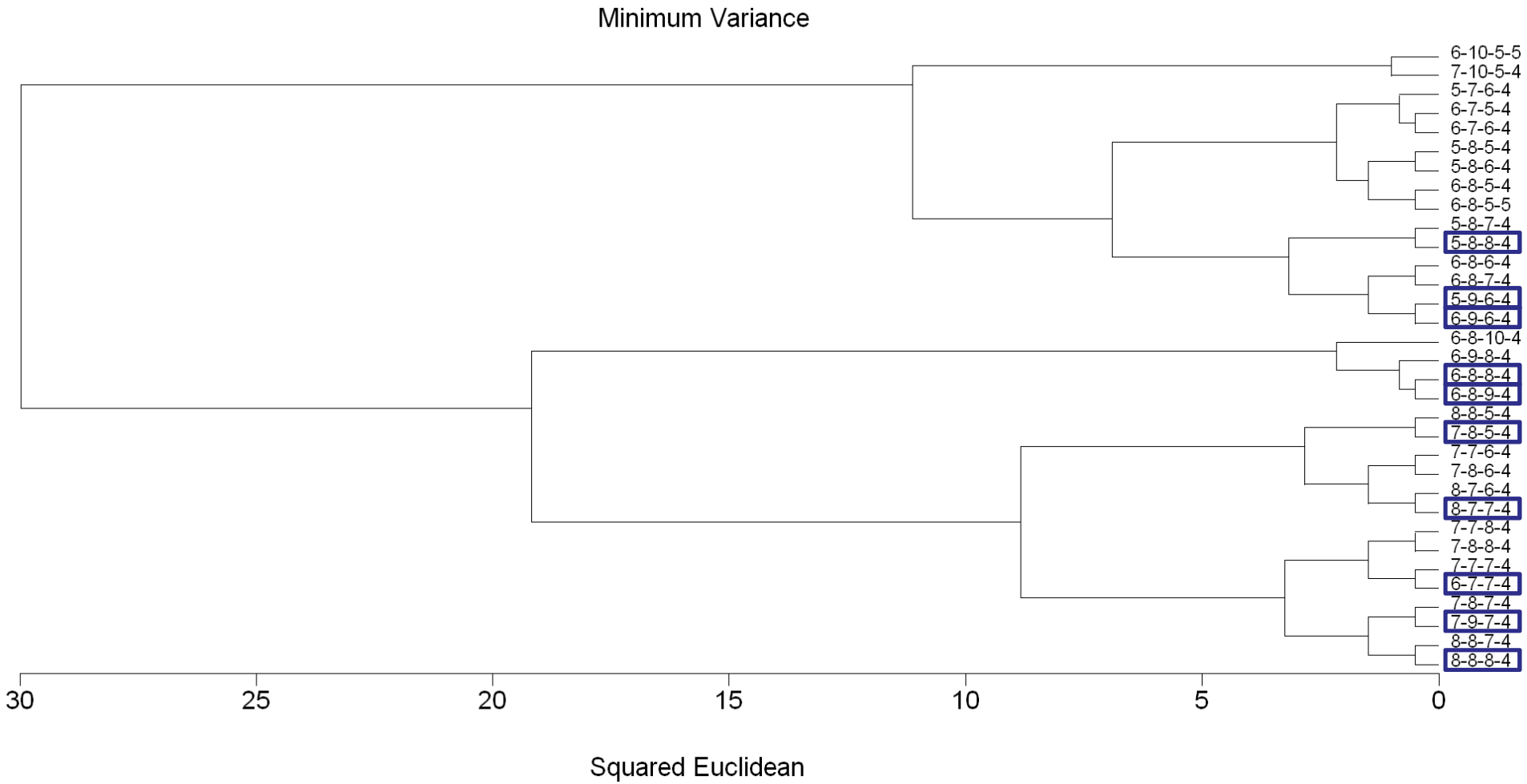
- 34 Haplotypes (+1) (mostly non-Israeli)
- Can we use them?
  - Polymorphic
  - Reliable
  - Reproducible
  - Kinship

STR1	STR 2	STR 3	STR 4	Stranded	Local*
8	8	8	4	0	1
8	7	7	4	16	47
7	9	7	4	0	1
7	8	5	4	3	1
6	9	6	4	4	16
6	8	9	4	6	12
6	8	8	4	37	11
6	7	7	4	0	1
5	9	6	4	0	2
5	8	8	4	3	3
8	8	7	4	2	
8	8	5	4	1	
8	7	6	4	2	
7	10	5	4	2	
7	8	8	4	5	
7	8	7	4	2	
7	8	6	4	4	
7	7	8	4	4	
7	7	7	4	2	
7	7	6	4	4	
6	10	5	5	1	
6	9	8	4	1	
6	8	10	4	2	
6	8	7	4	8	
6	8	6	4	14	
6	8	5	5	2	
6	8	5	4	54	
6	7	6	4	3	
6	7	5	4	2	
5	8	7	4	1	
5	8	6	4	4	
5	8	5	4	4	
5	7	6	4	1	
				194	95

Mediterranean/Israeli Green Turtles Tree



Mediterranean/Israeli Green Turtles Tree



Does it Tell a True Story?

- DNA never lies
- Scientists should always doubt

Analysis

Israeli

Haplotype	Close Linkage*	Stranded turtles
8 8 8 4	5	0
8 7 7 4	4	16
7 9 7 4	2	0
7 8 5 4	7	3
6 9 6 4	4	4
6 8 9 4	5	6
6 8 8 4	9	37
6 7 7 4	5	0
5 9 6 4	3	0
5 8 8 4	6	3
		69

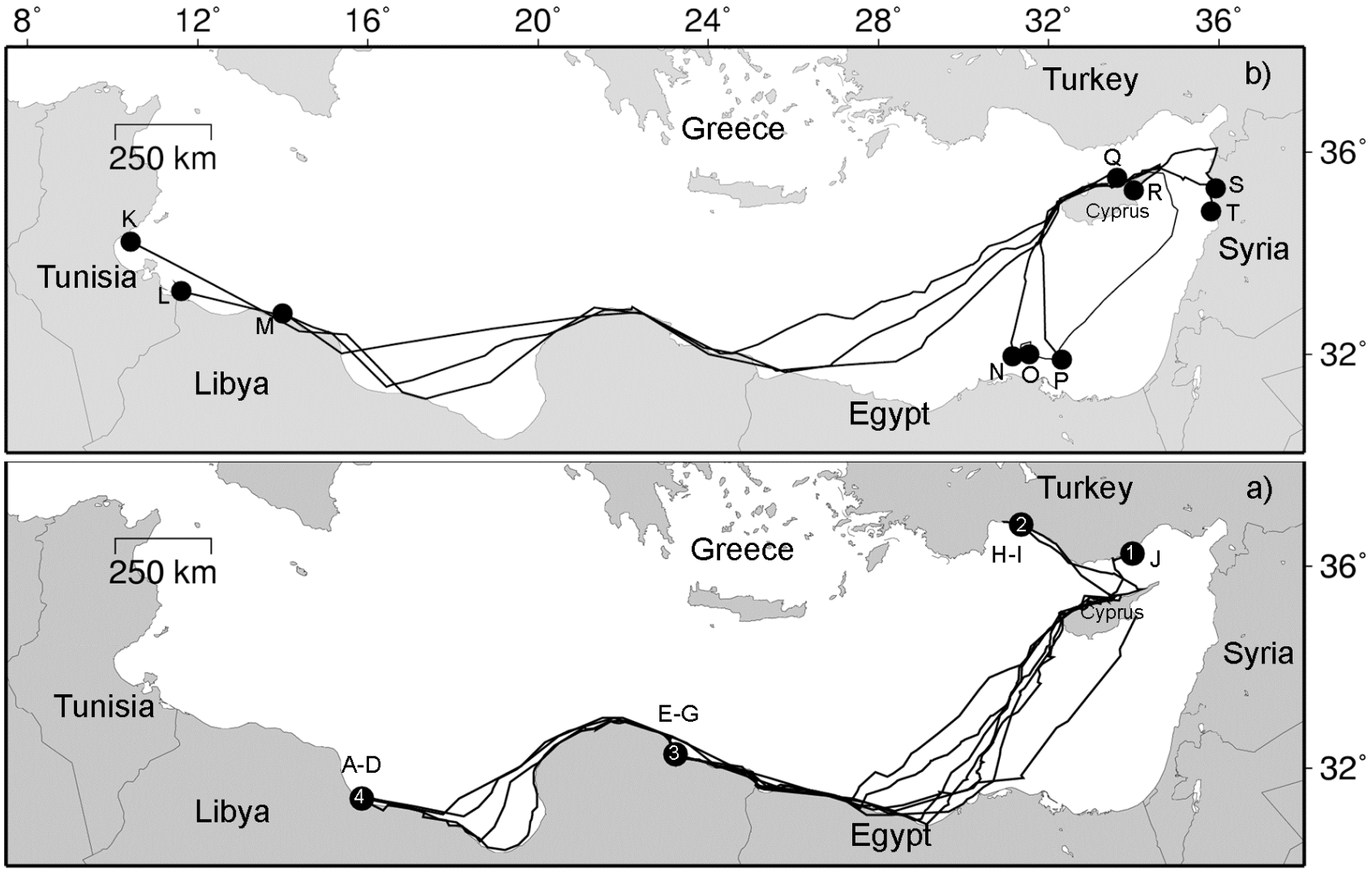
Other

Haplotype	Close Linkage*	Stranded turtles
8 8 7 4	6	2
8 8 5 4	5	1
8 7 6 4	4	2
7 10 5 4	1	2
7 8 8 4	7	5
7 8 7 4	8	2
7 8 6 4	6	4
7 7 8 4	3	4
7 7 7 4	6	2
7 7 6 4	6	4
6 10 5 5	1	1
6 9 8 4	2	1
6 8 10 4	5	2
6 8 7 4	9	8
6 8 6 4	9	14
6 8 5 5	2	2
6 8 5 4	10	54
6 7 6 4	7	3
6 7 5 4	3	2
5 8 7 4	6	1
5 8 6 4	7	4
5 8 5 4	6	4
5 7 6 4	5	1
		125

Haplotype				Israel	Stranded	Turkey	N. Cyprus	S. Cyprus
6	8	8	4	1	37	17	12	20
8	7	7	4	1	16	6	0	0
6	8	7	4	1	8	8	4	0
6	8	9	4	1	6	0	0	0
6	9	6	4	1	4	1	5	1
7	8	5	4	1	3	3	0	0
5	8	8	4	1	3	0	0	0
8	8	8	4	1	0	0	0	0
7	9	7	4	1	0	0	0	0
6	7	7	4	1	0	0	0	0
5	9	6	4	1	0	0	0	0
6	8	5	4	0	54	27	0	0
6	8	6	4	0	14	11	16	0
7	8	8	4	0	5	1	1	1
7	8	6	4	0	4	1	1	0
7	7	8	4	0	4	0	0	0
7	7	6	4	0	4	2	0	0
5	8	6	4	0	4	0	0	0
5	8	5	4	0	4	0	0	0
6	7	6	4	0	3	1	1	0
8	8	7	4	0	2	0	0	0
8	7	6	4	0	2	2	0	0
7	10	5	4	0	2	0	0	0
7	8	7	4	0	2	0	0	0
7	7	7	4	0	2	3	0	0
6	8	10	4	0	2	0	0	0
6	8	5	5	0	2	0	0	0
6	7	5	4	0	2	2	0	0
8	8	5	4	0	1	0	0	0
6	10	5	5	0	1	0	0	0
6	9	8	4	0	1	2	0	1
5	8	7	4	0	1	0	0	0
5	7	6	4	0	1	0	0	0
7	10	6	4	0	0	0	1	0
				11	194	87	41	23

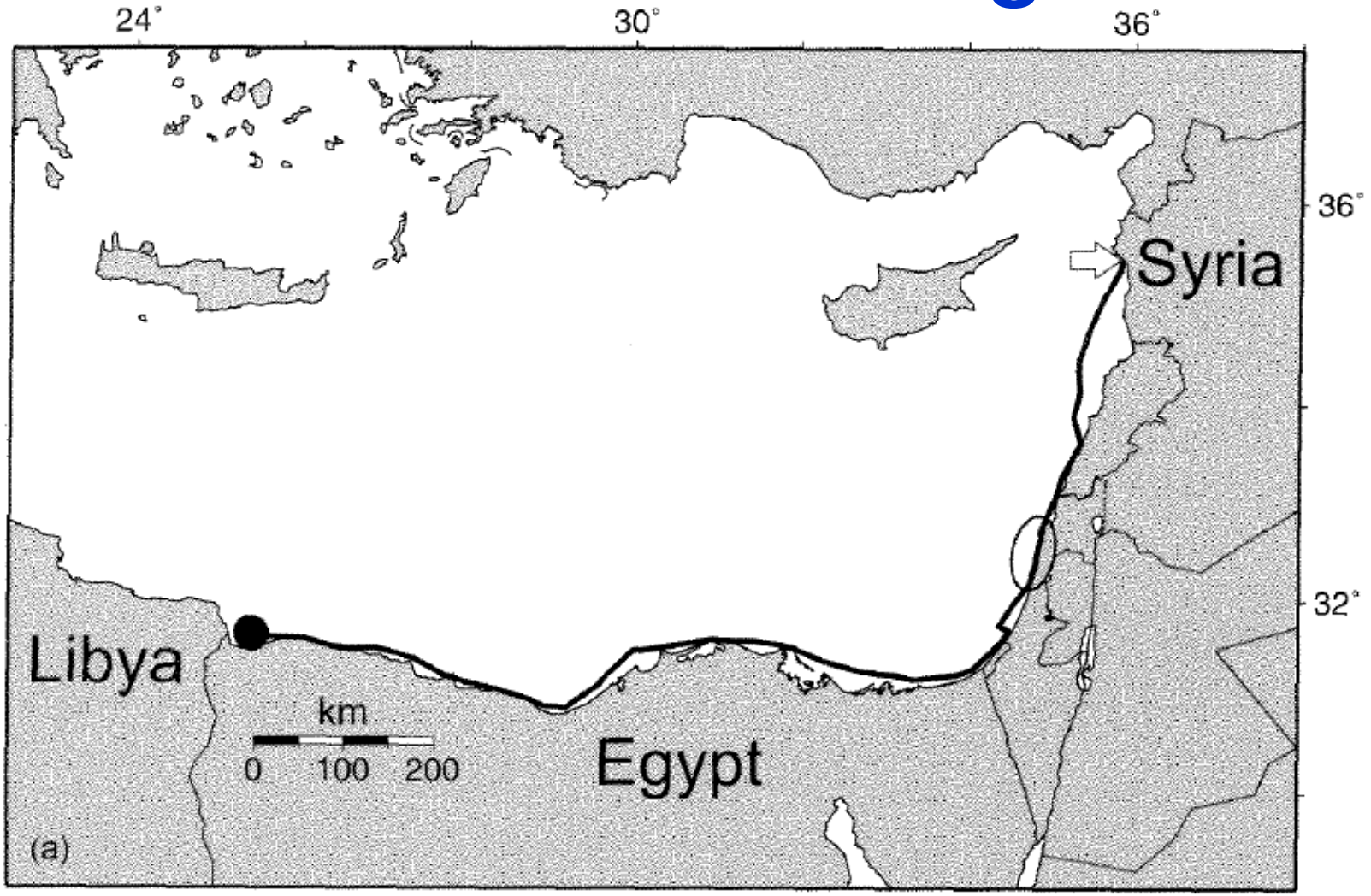
Mediterranean Green Turtles New Haplotyping

Mediterranean Green Turtles Satellite Tracking



Broderick et al., 2007

Mediterranean Green Turtles Satellite Tracking



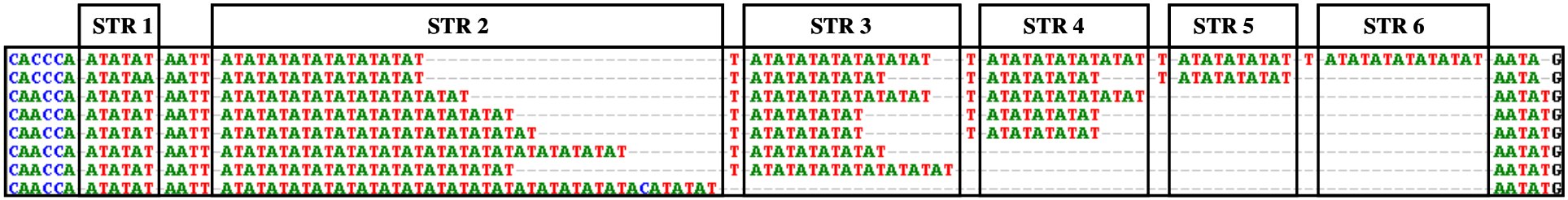
Rees et al., 2008

Can We Use This Storyteller Outside the Mediterranean?

Atlantic – same pattern

Pacific - ?

Genetic Variability of Indo-Pacific Green Turtles



Repeats in Other Sea Turtles

Loggerhead:

ATATT

Conventional:	3 Haplotypes
Repeat Haplotyping:	48-108 repeats
	30 Haplotypes
	(250 turtles)

Repeats in Other Sea Turtles

Hawksbill:

CATATATAT

Conventional:	? Haplotypes
Repeat Haplotyping:	10,23,30 repeats 3 Haplotypes (8 turtles)

Repeats in Other Sea Turtles

Olive Ridley:

ATATT and ATATTATT

MOLECULAR ECOLOGY

Molecular Ecology (2011) 20, 2878–2887 doi: 10.1111/j.1365-294X.2011.05134.x

Variation in repeat length and heteroplasmy of the mitochondrial DNA control region along a core–edge gradient in the eastern spadefoot toad (*Pelobates syriacus*)

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Abstract

Peripheral populations are those situated at the distribution margins of a species and are often subjected to more extreme abiotic and biotic conditions than those at the core. Here, we hypothesized that shorter repeat length and fewer heteroplasmic mitochondrial DNA (mtDNA) copies, which are associated with more efficient mitochondrial function, may be related to improved survival under extreme environmental conditions. We sampled

Defining Aims

Why do we look at the DNA?

Conservation of Biodiversity

Defining Aims

Sea turtles need our help in order to survive as species

A stable population needs genetic variation

Defining Aims

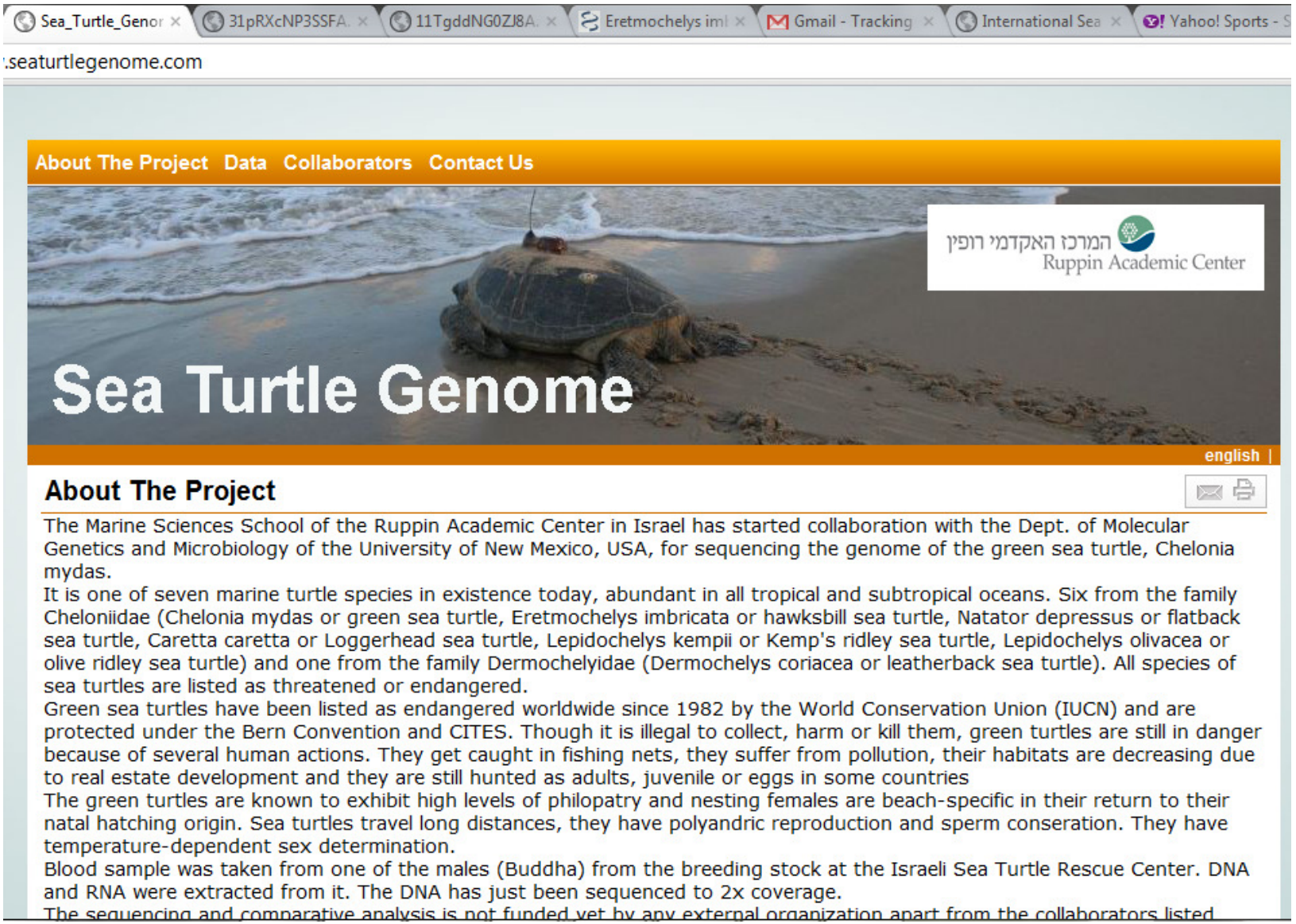
We look at DNA in order to evaluate genetic polymorphism

500bp +300bp +STR's (genomic and mitochondrial)

This is just a glimpse!

Our Initiative – Sea Turtle Genome

www.seaturtlegenome.com



Sea Turtle Genome - Status

We have completed 2X coverage

BGI (China) Published the genome

We are starting a transcriptome

We look for collaborators

What Can We Do With the Sea Turtle Genome

A lot:

Easily find polymorphic sites (STR's)

Genes ⟷ Traits

Genes ⟷ Diseases

Gene expression

Population Studies

Variability
Variability
Variability

Mitochondrial D-loop

Short Tandem Repeats

Library construction for STRs

Extract

Digest

Clone

Find STRs

Screen Population

Can we do it better?

The Alternative

Rational:

One individual will show population polymorphism

The Alternative

2x Genome of 1 specimen

Isolate all STR

Locate site specific pairs

Isolate heterozygote sites

The Algorithm

```
164

276_>gnl|ti|1228074423_171967670 GTGC

CATTTGATGAACTGGCAGAgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgt
gcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtg
cgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgc
gtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcg
tgcgtgcgtgcgtgcgtgcGTGGTCAGGTTTGACTGACC

112_>gnl|ti|1286273056_177629148 GTGC

CATTTGATGAACTGGCAGAgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgt
gcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcgtgcGTGGTCA
GGTTTGACTGACC

//

145

35_>gnl|ti|1236036399_172623525 AACTA

AACACCAGAGAAACTAAACaactaaactaaactaaactaaactaaactaaactaAACAACTGAAAAGCA
ACAGA

180_>gnl|ti|1241113976_173210823 AACTA

AACACCAGAGAAACTAAACaactaaactaaactaaactaaactaaactaaactaaactaaactaaacta
aactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaact
aaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaaactaAACAACTG
AAAAGCAACAGA
```

Primer design

60

8_>gnl|ti|1262525131_175488122 AT

TCTTCAAGTAACTTACTGGTGCCTTTAAGTCCTGTTCAGGCCCAGTCTCatatatatGTATATAAAATT
TCTATTTCATGAAAGAGTGCTGCTGTGCATGCCCAACT

68_>gnl|ti|1203695794_169077269 AT

TCTTCAAGTAACTTACTGGTGCCTTTAAGTCCTGTTCAGGCCCAGTCTCatatatatatatatatatatat
atatatatatatatatatatatatatatatatatatatatatatatatatatatatatatatatatatatGTATATAAAATTTCTATTTCA
TGAAAGAGTGCTGCTGTGCATGCCCAACT

Forward Primer
TTAAGTCCTGTTCAGGCCCAGTCT
Reverse Primer
GCATGCACAGCAGCACTCTTTCA

//

58

88_>gnl|ti|1249504736_174204011 AC

AGCTGATTTTGTGTACTTTTGATAGTATCATCACATTCCTAACAAGTTTacacacacacacacacacacac
acacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacacT
TCCATCCTGCAAATTTGAAGTGAAAGCACCCAGACTTTCTTCTCTACCC

30_>gnl|ti|1203651562_168979272 AC

AGCTGATTTTGTGTACTTTTGATAGTATCATCACATTCCTAACAAGTTTacacacacacacacacacacac
acacacacacTTCCATCCTGCAAATTTGAAGTGAAAGCACCCAGACTTTCTTCTCTACCC

Forward Primer
AGTATCATCACATTCCTAACAAGT
Reverse Primer
GAAAGTCTGGGTGCTTTCACTTC

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Thank you for your attention

Looking beyond the horizon

Thanks:

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