AN INVESTIGATION ON THE EVOLUTION AND CONSERVATION OF

THE HARBOR PORPOISE, *Phocoena phocoena* IN TURKEY

by

Özge Yazıcı

B.Ed. in Biology Teacher Education, Ondokuz Mayıs University, 2008

M.S. in Environmental Sciences, Boğaziçi University, 2015

Submitted to the Institute of Environmental Sciences in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

in

Environmental Sciences

Boğaziçi University

2017

AN INVESTIGATION ON THE EVOLUTION AND CONSERVATION OF THE HARBOR PORPOISE, *Phocoena phocoena* IN TURKEY

APPROVED BY:

Assoc. Prof. Dr. Raşit Bilgin . . . . . . . . . . . . . . . . . . . . .

Dissertation Advisor

Prof. Dr. Bariş Çallı . . . . . . . . . . . . . . . . . . . . .

Prof. Dr. Nüzhet Dalfes . . . . . . . . . . . . . . . . . . . . .

Assoc. Prof. Dr. Başak Güven . . . . . . . . . . . . . . . . . . . . .

Assist. Prof. Dr. Berat Haznedaroğlu . . . . . . . . . . . . . . . . . . . . .

DATE OF APPROVAL: 04/06/2017

**ACKNOWLEDGEMENTS**

Lorem ipsum dolor sit amet, bonorum placerat imperdiet an mea. An vim tale mundi. Nec partem vocent propriae no, vix facilis deleniti repudiare no, aperiri eligendi ei his. Cu sea scripta epicurei necessitatibus, suas graeci vel ut. At mei simul ancillae, eos ut admodum democritum posidonium, pri quot decore intellegat ea.

Lorem ipsum dolor sit amet, bonorum placerat imperdiet an mea. An vim tale mundi. Nec partem vocent propriae no, vix facilis deleniti repudiare no, aperiri eligendi ei his. Cu sea scripta epicurei necessitatibus, suas graeci vel ut. At mei simul ancillae, eos ut admodum democritum posidonium, pri quot decore intellegat ea.

Dicit altera ne vel, sed aliquando conceptam disputando in. Dolore regione nam at. Mei ad alia dictas facilisis. Quo maluisset vituperatoribus ex.

Eu sed altera nemore nominati, quo ex feugait adolescens, mutat recusabo quo id. Duo detracto cotidieque at, vim id deserunt qualisque, quo explicari theophrastus cu. Justo verear cu nec, diam sint solet te est. Ad mea consetetur moderatius honestatis, te aeterno principes eos.

Ullum recusabo oportere pro eu. Impedit tacimates eloquentiam qui id. Ad ferri inermis accommodare mea, eam putent virtute discere ea. Vis quidam prodesset concludaturque et, vim numquam maiorum ex. Munere prompta invenire ad vix, quot minim errem ei ius.

**ABSTRACT**

**AN INVESTIGATION ON THE EVOLUTION AND CONSERVATION OF THE HARBOR PORPOISE, *Phocoena phocoena* IN TURKEY**

In 2008, the species *Phocoena phocoena* was categorized as “least concern” and *Phocoena phocoena ssp. relicta,* as “endangered,” on the IUCN Red List. In the last five decades, the number of harbor porpoises in the Black Sea declined significantly, especially due to mass killings associated with commercial fisheries. Tissue samples of 71 individuals of the *Phocoena phocoena* were collected from 33 locations in Turkey: the western Black Sea (n = 44), the eastern Black Sea (n = 11), the Marmara Sea (n = 14), and the Aegean Sea (n = 2). Samples were either stranded or by-catch from fisheries. Consistent with other studies, none of the haplotypes we found clustered with Atlantic populations. The most common haplotype in the study was found in 49 individuals. The study uncovered five total unique haplotypes from the Black Sea samples. All of them were found in the western Black Sea region. The idea that harbor porpoises from the Aegean Sea first came from the Black Sea through the Istanbul and Dardanelles Straits is supported by our findings. Our data also supports the possibility that there is an isolated population in the Sea of Marmara because four of the individuals we observed shared a unique haplotype with previously studied individuals in the same region. As a result of these findings, it was concluded that the *Phocoena phocoena* population in the Sea of Marmara should be treated of as a management unit (MU) for conservation purposes.

**ÖZET**

**TÜRKİYE’DEKİ MUTUR, *Phocoena phocoena* POPÜLASYONLARININ EVRİM VE KORUNMASI**

2008 yılında, *Phocoena phocoena* (mutur) türü ‘düşük riskli’ ve *Phocoena phocoena ssp. relicta, ‘*tehlikede’ olarak IUCN kırmızı listesinde kategorize edilmiştir. Son beş yılda ticari balıkçılıkla bağlantılı olarak gerçekleşen katliamlar nedeniyle Karadeniz’deki muturların sayısı önemli bir şekilde düşmüştür. Türkiye’nin 33 bölgesinden 71 *Phocoena phocoena* bireyinden alınan deri örnekleri (Batı Karadeniz (n=44), Doğu Karadeniz (n=11), Marmara Denizi (n=14), ve Ege Denizi (n=2)) çalışma kapsamında incelenmiştir. Balıkçılık sırasında yakalanan ya da karaya vuran örnekler kullanılmıştır. Diğer çalışmalarla istikrarlı olarak, bulduğumuz hiçbir haplotip Atlantik popülasyonlarıyla kümelenmemiştir. En çok görülen haplotip 49 bireyde bulunmuştur. Çalışma, Karadeniz örneklerinden toplam beş özgün haplotip ortaya çıkarmıştır. Hepsi Batı Karadeniz bölgesinde bulunmuştur. Ege Denizi’ndeki muturların İstanbul ve Çanakkale Boğazı’nı geçerek ilk olarak Karadeniz’den geldiğine dair fikir bulgularımız tarafından desteklenmiştir. İncelediğimiz bireylerden dördünün, aynı bölgede daha önce incelenmiş bireylerle özgün bir haplotipi paylaşması nedeniyle verilerimiz Marmara Denizi’nde izole bir popülasyon olma olasılığını desteklemektedir. Bu bulguların sonucu olarak, Marmara Denizi’ndeki *Phocoena phocoena* popülasyonunun, koruma amaçları için “idare birimi” olarak kabul edilmesi gerektiği sonucuna varılmıştır.

**TABLE OF CONTENTS**

ACKNOWLEDGEMENTS............................................................................................................ iii

ABSTRACT.................................................................................................................................... iv

ÖZET............................................................................................................................................... v

TABLE OF CONTENTS........................................................................................ ……………… vi

LIST OF FIGURES......................................................................................................................... viii

LIST OF TABLES.................................................................................................. ……………… ix

LIST OF SYMBOLS/ABBREVIATIONS............................................................. ……………… x

1. INTRODUCTION...................................................................................................................... 1

1.1. General Characteristics of Birds, Investigated Along With Their

Phylogenetic Relationships.................................................................................................2

1.2. Turkish Bird Fauna............................................................................................................. 3

1.3. Conservation of Birds......................................................................................................... 4

1.4. General Characteristics of Bird Haemosporidians..................................... ……………… 5

1.5. Life Cycle of Leucocytozoidae Species..................................................... ……………… 7

1.5.1. The Hosts Used in the Different Stages………….………………. ……………… 7

1.5.1.1 Animal Hosts…………………………………………............................. 7

1.6. Host-Switching................................................................................................................... 8

1.7. Objective of the Thesis....................................................................................................... 9

2. LITERATURE REVIEW........................................................................................................... 10

3. MATERIALS AND METHODS....................................................................... ……………… 14

3.1. Collection of Blood Samples.............................................................................................. 14

3.2. DNA Extraction.................................................................................................................. 15

3.3. PCR Screening........................................................................................... ……………… 15

3.4. Sequencing................................................................................................. ……………… 19

3.5. Phylogenetic Analysis................................................................................ ……………… 19

4. RESULTS.................................................................................................................................. 20

4.1. Identification of Samples and Results of PCR Screening.......................... ……………… 20

4.2. Results of Phylogenetic Analysis............................................................... ……………… 22

5. DISCUSSION....................................................................................................……………… 38

6. CONCLUSION.................................................................................................. ……………… 42

REFERENCES................................................................................................................................ 43

APPENDIX A: INFORMATION ON THE SAMPLES OF THE STUDY…...... ……………… 51

APPENDIX B: GEL IMAGES OF PCR REACTIONS AMPLIFIED WITH

THE PRIMER PAIR LEUCOF-LEUCOR………………………………...…….. ……………… 59

APPENDIX C: GEL IMAGES OF PCR REACTIONS AMPLIFIED WITH

THE PRIMER PAIR DW2-DW4…………………………………………...………………….... 67

**LIST OF FIGURES**

Figure 1.1. Taxonomy of cetaceans……………………………………………........................... 1

Figure 1.2. Global distribution map of *Phocoena phocoena*...............................*...........*................9

Figure 2.1. The range map of *Phocoena phocoena* used in the Sea of Marmara

and Black Sea in Turkey…………………………………………………………….. 14

Figure 2.2. A close-up map of *Phocoena phocoena* sampled in the Sea of

Marmara and Western Black Sea in Turkey…………………………………………. 15

Figure 3.1. Haplotype network for the *Phocoena phocoena* sequences…………. ……………... 19

Figure 3.2. Phylogenetic relationships of 32 haplotypes of

*Phocoena phocoena*…………………………………………………. *……………….* 22

**LIST OF TABLES**

Table 3.1. Haplotype numbers of different regions……………………………………………… 20

Table 3.2. The descriptive statistics of *Phocoena phocoena*……………………………………..25

Table 3.3. Corrected (Dxy) and uncorrected P - distance values between

populations……………………………………………………………. ……………... 28

Table 3.4. Фst values of the population calculated by using pairwise

differences method…………………………………………………… ……………... 29

**LIST OF SYMBOLS/ABBREVIATIONS**

**Symbol Explanation Unit**

CH4 Methane mL/day

μl Microliter

π Nucleotide Diversity

Fst Fixation Index

**Abbreviation Explanation**

A Adenine

C Cytosine

D-loop Displacement Loop

DNA Deoxyribonucleic Acid

DNTP Deoxyribonucleotide Triphosphate

G Guanine

G Gamma Distribution

GTR General Time Reversible

h Number of Haplotypes

Hd Haplotype Diversity

I Proportion of Invariable Sites

IUCN International Union for Conservation of Nature

K Average Number of Nucleotide Differences

Kg Kilogram

mtDNA Mitochondrial Deoxyribonucleic Acid

MU Management Unit

n Number of Tissue Samples

nM Nanomolar

PCR Polymerase Chain Reaction

Sd Standard Deviation

T Thymine

**1. INTRODUCTION**

The order Cetacea is one of the most distinctive and highly specialized orders of [mammals](http://www.ucmp.berkeley.edu/mammal/eutheria/placental.html), and includes marine mammals such as whales, dolphins and porpoises (1, 2). The cetaceans comprise three main groups, namely [Mysticeti](http://eol.org/pages/2849458/overview/) (baleen or moustache whales), Odontoceti (toothed whales) and Archeoceti (ancient whales). Mysteceti and Odontoceti still have living representatives, whereas Archeoceti is an extinct group (Figure 1.1) (3, 4).

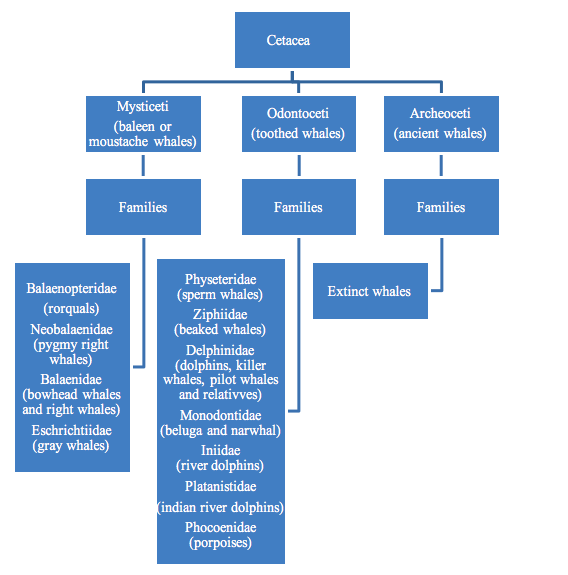


Figure 1.1. Taxonomy of cetaceans.

Although Archaeoceti is an extinct group, [Mysticeti](http://eol.org/pages/2849458/overview/) and [Odontoceti](http://eol.org/pages/2849318/overview/) are assumed to be related to them. Cetacea is a very large order, with around 83 living species, 46 genera and 14 families. The most diverse suborder is [Odontoceti](http://eol.org/pages/2849318/overview/) with around 75 species, 40 genera and 10 families. Cetaceans are derived from terrestrial animals, which evolved to become aquatic (4), and they live, breed and end their lifecycles in the water. Mysticeti are often called baleen whales and [Odontoceti](http://eol.org/pages/2849318/overview/) are often called dolphins and porpoises. The distinguishing characteristic of Mysticeti from Odontoceti is that the former has no teeth, and subsequently prey on small plankton. Odontoceti, toothed whales, on the other hand, prey on fish, cephalopods, small crustaceans, as well as marine mammals (3, 4, 5, 6).

On the International Union for Conservation of Nature (IUCN) Red List, the conservation status of 87 species and 37 subspecies of cetaceans has been evaluated, and five species of Mysticeti and eight species of Odontoceti have been categorized as “under threat” (5, 7, 8). Based on the studies of cetaceans in Turkey (9, 10), ten species exist in the surrounding seas. Geographically speaking, theFin whale *Balaenoptera physalus*, [Risso's Dolphin](http://en.wikipedia.org/wiki/Risso%27s_Dolphin) *Grampus griseus*,Sperm whale *Physeter macrocephalus,* Cuvier's beaked whale *Ziphius cavirostris,* the Long Finned Pilot Whale *Globicephala melas,* the False Killer whale *Pseudorca crassidens,* and the Striped Dolphin *Stenella coeruleoalba* are found in the Aegean and Mediterranean seas (10, 11, 12), and the [Bottlenose Dolphin](http://en.wikipedia.org/wiki/Bottlenose_Dolphin) *Tursiops truncatus* in the Black Sea and the Mediterranean (13).The Short-beaked common dolphin *Delphinus delphis* can be found in all the seas of Turkey (10), especially in the Sea of Marmara and the Black Sea (14). Finally, the [Harbour Porpoise](http://en.wikipedia.org/wiki/Harbour_Porpoise) *Phocoena phocoena ssp. relicta*, the species of interest for this thesis, inhabits the Black Sea, the Sea of Marmara, and the Aegean (15). The taxonomic hierarchy of the cetaceans in Turkey in general, and *Phocoena phocoena* in particular are given in the appendix (16, 17, 18).

Lorem ipsum dolor sit amet, bonorum placerat imperdiet an mea. An vim tale mundi. Nec partem vocent propriae no, vix facilis deleniti repudiare no, aperiri eligendi ei his. Cu sea scripta epicurei necessitatibus, suas graeci vel ut. At mei simul ancillae, eos ut admodum democritum posidonium, pri quot decore intellegat ea. Lorem ipsum dolor sit amet, bonorum placerat imperdiet an mea. An vim tale mundi. Nec partem vocent propriae no, vix facilis deleniti repudiare no, aperiri eligendi ei his. Cu sea scripta epicurei necessitatibus, suas graeci vel ut. At mei simul ancillae, eos ut admodum democritum posidonium, pri quot decore intellegat ea.

**1.1. General Characteristics of Whale Species Found in Turkey’s Coastal**

**Waters**

**1.1.1. Fin Whale, Balaenoptera physalus**

After Blue whales, Fin whales, Balaenoptera physalus are the second largest species of whale, reaching body lengths of up to 22 meters in the Northern Hemisphere, and 26 meters in the Southern Hemisphere. Females are 5 - 10% larger than males. When both sexes mature, their weight can range from 40 to 80 tons. Fin Whales have a distinguishing coloration pattern from light gray to brownish black, and their ventral parts are white. Their body has a smooth and aerodynamic structure. They are commonly found alone or in small groups between three to seven individuals, and use vocalization to communicate. They are also capable of dives of up to 230 meters to primarily prey on krill, small schooling fish, and squid (19, 20).

Fin whales are considered to have cosmopolite behaviour and can migrate between polar and tropical waters. They mostly live in deep ocean waters all around the world. They have also been detected in the central and western parts of the Mediterranean. In Turkey's waters, Fin whales have been observed on the Aegean and Mediterranean coasts. In Adana Yumurtalık, a fin whale was found stranded ashore, and its skeleton was subsequently used for educational displays (10, 19, 20).

**1.1.2. Sperm Whale, Physeter *catodon***

*1.1.2.1. The life history of the sperm whale.* Sperm whale, Physeter *catodon*, is the largest Odontocete species. Adult females reach body lengths of up to 11 meters and weigh 14 tons (13,607 kg). On the other hand, adult males reach body lengths of up to 16 meters and weigh around 45 tons. Sperm whales show sexual dimorphism more than any cetacean species; adult males are 30% longer and almost three times larger than adult females (21, 22). Among odoncetes, sperm whales have a very unusual head anatomy, which is distinguished by its extreme size: its brain is about five times heavier than a human's. They are dark grey in color, with a white section on the interior part of their mouth. Their dorsal fins are small and rounded.

**2. MATERIALS AND METHODS**

**2.1. Sample Collection and DNA Extraction**

Tissue samples of 71 individuals of *Phocoena phocoena* were collected from 33 locations in Turkey: the western Black Sea (n = 44), the eastern Black Sea (n = 11), the Sea of Marmara (n = 14), and the Aegean (n = 2) (Figure 2.1, Figure 2.2). Samples were either stranded or by-catch from fisheries. DNA was extracted from these samples by using Roche High Pure PCR Template Preparation Kit (Mannheim, Germany) using the manufacturer’s instructions. After the extraction, DNA was stored at -20 oC until further processing.

DefaultView_1.tif

Figure 2.1. The range map of *Phocoena phocoena* used in the Sea of Marmara and Black Sea in Turkey (The sequences for Ukraine were retrieved from GenBank).

**2.2. PCR Amplification, Sequencing and Alignment**

Forward and reverse primers, Turs - f (5'-CCATTCCTCCTAAGACTCAAGGAAG-3') and Turs – r (5'-CCTGAAGTAAGAACCAGATGTCTATAAA-3') respectively, were used in order to amplify a 360 base pair D-loop fragment (52). PCR amplification was performed in a 50 μl reaction volume, which was composed of 3 μl DNA, 5 μl of 25mM MgCl2, 5 μl KCl buffer, 1 μl of 10nM DNTP, 1 μl of 10 pmol/μl each primer, 0.3 μl of 5U/ μl Taq buffer and 33.7 μl double distilled water (52). The PCR cycling conditions were 5 minutes at 94 **°**C, 35 cycles of 30 seconds at 94 oC, 1 minute at 59 oC, 1 minute at 72 oC, with a final extension of 1 minute at 72 oC (52). After amplification, presence of DNA was evaluated on a 1% agarose gel. Amplified DNA products and the same primers used for PCR were sent to Macrogen, Korea for commercial sequencing. Sequences were edited and aligned with Sequencer v. 4.8.

**2.3. Data Analyses**

TCS v.1.13 (Clement et al. 2000) (53) was used to construct a haplotype network of the *Phocoena phocoena* samples to reveal the evolutionary relationships among haplotypes. Also, the sequences from 31 individuals of *Phocoena phocoena* were added to our study from Martinez *et al.* (GenBank accession numbers EF063110, EF063646 - EF063675, U09689 - U09691) (52).

Modeltest v. 3.7 (Posada and Crandall 1998) (54) was used to determine the best tree model for our analyses (55). The GTR + I + G tree model had the best fit (-ln likelihood=720.44) for our aligned sequences. Inorder to reveal the relationships of the different populations, maximum parsimony, maximum likelihood, and neighbor-joining trees were constructed with the software Mega v. 5 (56).

The maximum likelihood method is used as a way to estimate parameters in a statistical model. The goal of the maximum likelihood method is to find an evolutionary tree that has the greatest probability of representing the relationships among the haplotypes. The data that represent an individual can be an alignment of protein or DNA sequences. The maximum likelihood method tries to find the best tree by starting at an initial tree, and moves to other closely related trees until it finds the one that most likely represents the relationships among the sequences (57, 58).

The neighbor-joining method uses evolutionary distance data for constructing phylogenetic trees (59). While constructing a tree, again DNA or protein sequences are used. The algorithm begins with an unresolved tree resembling a star network, and then resolves the tree with specific steps until the length of each branch is revealed (60).

The parsimony method is one of the most useful methods in phylogenetics. The parsimony method produces phylogenetic tree estimations from sequence or morphological data. This method might provide information about the phylogeny of the species analyzed, and tries to explain the differences in the observed characters by identifying the phylogeny that has the fewest changes for each alternative (61).

In addition to tree construction, descriptive statistics such as haplotype diversity and nucleotide diversity were computed with DnaSP v. 5 (62). DnaSP v. 5 was also used to plot the mismatch distributions of the populations in the regions (63), to evaluate signatures of expansion and selection. Plots of mismatch distributions help to explain an expansion, if any, in a population by using the data on the differences between sequences, and their frequencies. Under a scenario of expansion, the observed and expected frequencies of pairwise differences should be parallel to each other (63, 64). This analysis (65) is useful to determine signatures of expansion in a population by comparing the observed and expected mismatch distributions to see if they are statistically different from each other (63, 65).

Mega v. 5 was also used to show divergence between populations, if any, by computing the uncorrected P and corrected Dxy genetic distances between populations (66). The differentiation between populations was also evaluated with pairwise Фst comparisons, computed using Arlequin version 3.5 (67). When studying mtDNA regions, Фst is analogous to Fst, for evaluating the structure in a population. If the Фst value is 0, the individuals in populations can interbreed freely, whereas Фst values closer to 1 indicate genetic differentiation (67).

**3. RESULTS AND DISCUSSION**

Consistent with other studies, none of the haplotypes from Turkish coasts in this study, clustered with those from the Atlantic populations. The haplotype network for the samples collected around the Turkish coasts (Figure 3.1) shows a star-like network, indicative of an expansion of the populations. In our study, we aimed to understand whether there was any differentiation among *Phocoena phocoena* populations in Ukraine, the western Black Sea region, the eastern Black Sea region, the Sea of Marmara and the Aegean. When the haplotype network of the samples was analyzed, no obvious differentiation was detected. Looking at the network, our study uncovered five new haplotypes from the Black Sea. All of these (Haplotypes 33, 34, 35, 36, 37) were found in the western Black Sea region. Also, an individual observed in the Sea of Marmara had one haplotype (Haplotype XVI) that was also detected in individuals from the Black Sea and northern Aegean (52).

Our study's most common haplotype (I), was found in 49 individuals: 38 in the Black Sea, one in the southern Aegean (15), one in the Aegean, four in the Sea of Marmara, four in the Istanbul Strait, and one in the Dardanelles Strait. The finding of the haplotype XVI in the Aegean, Ukraine and the Sea of Marmara supports the theory that harbor porpoises in the Aegean originated from the Black Sea (Viaud-Martinez et al., 2007; Rosel et al., 2003) (19, 68) by dispersing through the Istanbul and Dardanelles Straits. Our data also support the possibility that there is an isolated population in the Sea of Marmara because four of the individuals we observed shared a unique haplotype with previously studied individuals from the same sea.

The phylogenetic trees, constructed using the maximum-likelihood (Figure 3.2), neighbor-joining (Figure 3.3) and maximum parsimony (Figure 3.4), methods also support the notion that haplotypes from Turkish coasts cluster separately from those in the Atlantic populations, as shown in the haplotype network (Figure 3.1). The phylogenetic trees (Figures 3.2, 3.3, and 3.4) also show no differentiation in *Phocoena phocoena* populations between the regions of interest, around the Black Sea.

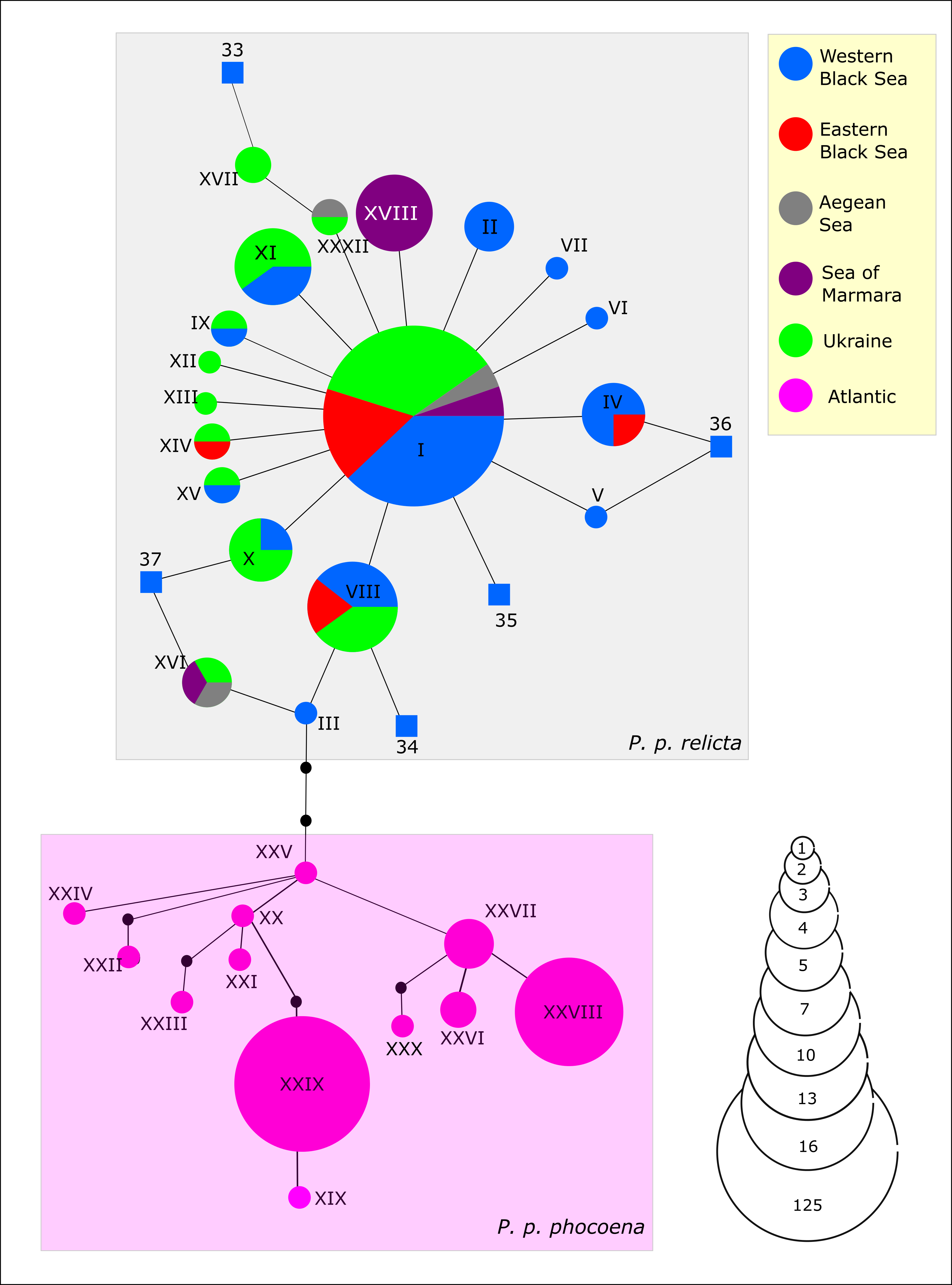


Figure 3.1. Haplotype network for the *Phocoena phocoena* sequences.The sizes of the circles are proportional to the number of individuals. Circles represent the haplotypes found in our study and Viaud-Martinez et al. (51). The five new haplotypes our study uncovered are represented by the boxes numbered 33 - 37. Geographic origins of the haplotypes are represented by different colors.

Table 3.1. Haplotype numbers of different regions. The numbers for sequences taken from Genbank and obtained in this study are on the left and right side of the slash, respectively. (WB, Western Black Sea; EB, Eastern Black Sea; AEG, Aegean Sea; M, the Sea of Marmara; U, Ukraine region; A, Atlantic).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | WB | EB | AEG | M | U | A |
| I |  |  |  |  |  | - |
| II |  | - | - | - | - | - |
| III |  | - | - | - | - | - |
| IV |  |  | - | - | - | - |
| V |  | - | - | - | - | - |
| VI |  | - | - | - | - | - |
| VII |  | - | - | - | - | - |
| VIII |  |  | - | - |  | - |
| IX |  | - | - | - |  | - |
| X |  | - | - | - |  | - |
| XI |  | - | - | - |  | - |
| XII | - | - | - | - |  |  |
| XIII | - | - | - | - |  |  |
| XIV | - |  | - | - |  | - |
| XV |  | - | - | - |  | - |
| XVI | - | - |  |  |  | - |
| XVII | - | - | - | - |  | - |
| XVIII | - | - | - |  | - | - |
| XIX | - | - | - | - | - |  |
| XX | - | - | - | - | - |  |
| XXI | - | - | - | - | - |  |
| XXII | - | - | - | - | - |  |
| XXIII | - | - | - | - | - |  |
| XXIV | - | - | - | - | - |  |
| XXV | - | - | - | - | - |  |
| XXVI | - | - | - | - | - |  |
| XXVII | - | - | - | - | - |  |
| XXVIII | - | - | - | - | - |  |
| XXIX | - | - | - | - | - |  |
| XXX | - | - | - | - | - |  |
| XXXI | - | - | - | - | - |  |
| XXXII | - | - |  | - |  | - |

*See Figure 3.1 for the haplotype codes*

**4. CONCLUSIONS AND RECOMMENDATIONS**

The main findings of our study are as follows:

Consistent with other studies, none of the haplotypes we found clustered with Atlantic populations. Our study's most common haplotype was found in 49 individuals: 38 in the Black Sea, one in the southern Aegean, one in the Aegean, four in the Sea of Marmara, four in the Istanbul Strait and one in the Dardanelles Strait. Our study uncovered five new haplotypes from the Black Sea samples. All of these were found in the west of Black Sea.

The hypothesis that harbor porpoises of the Aegean originated in the Black Sea through the Istanbul and Dardanelles Straits is supported by our findings. The haplotype XVI, found in one individual in the Sea of Marmara was shared with two (one each) found in Ukraine and the Aegean.

Based on the haplotype and nucleotide diversity patterns, *Phocoena phocoena* populations of the western Black Sea and the Sea of Marmara are relatively more stable and could be ancestral. On the other hand, based on the observed and expected mismatch distributions, populations in Ukraine, the Aegean, and eastern Black Sea are more likely to be recent and derived. These results seem to be in concordance with the haplotype and nucleotide diversity patterns mentioned above.

Our data supports the possibility that there is an isolated population in the Sea of Marmara because four of the individuals we observed share a unique haplotype with previously studied individuals in the same region. As a result of these findings, the *Phocoena phocoena* population in this sea should be treated of as a management unit (MU) for conservation purposes. As a follow-up to this study, more samples should be studied, especially from the Sea of Marmara to better understand the isolation of the population inhabiting this body of water.

**REFERENCES**

**Examples of Journal Article Referencing:**

Frimmel, F.H., 1998. Impact of light on the properties of aquatic natural organic matter. Environment International, 24, 559-571.

Hull, C. S., Reckhow, D. A., 1993. Removal of DOX precursors in municipal wastewater treatment plants. Water Research, 27, 419-425.

Rounds, S. A., Tiffany, B. A., Pankow, J. F., 1999. Description of gas/particle sorption kinetics with an interparticle diffusion model: Desorption experiments. Environmental Science and Technology, 27, 366‑377.

*Same authors and same year:*

Tay,T.-H., Liu,Q.-S., Liu,Y., 2002a. Aerobic granulation in sequential sludge blanket reactor. Water Science and Technology, 46, 4-5, 13-18.

Tay,T.-H., Liu,Q.-S., Liu,Y., 2002b. Hydraulic selection pressure-induced nitrifying granulation in sequencing batch reactors. Applied Microbiology and Biotechnology, 59, 332-337.

*Same first author, different co-authors and different years:*

Vodacek, A., Hoge, F., Swift, R.N., Yungel, J.K., Peltzer, E.T., Blough, N.V., 1995. The use of in situ and airborne fluorescence measurements to determine UV absorption coefficients and DOC concentration in surface waters. Limnology and Oceanography, 40, 411-415.

Vodacek, A., Blough, N.V., deGrandpre, D., Peltzer, E.T., Nelson, R.K., 1997. Seasonal variation of CDOM in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnology and Oceanography, 42, 674-686.

*Same first author, different co-authors and same year:*

Vodacek, A., Blough, N.V., deGrandpre, D., Peltzer, E.T., Nelson, R.K., 1997. Seasonal variation of CDOM in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnology and Oceanography, 42, 674-686.

Vodacek, A., Hoge, F., Swift, R.N., Yungel, J.K., Peltzer, E.T., Blough, N.V., 1997. The use of in situ and airborne fluorescence measurements to determine UV absorption coefficients and DOC concentration in surface waters. Limnology and Oceanography, 40, 411-415.

**Example of Book Referencing:**

Belter, P. A., Cussler, E. L., Wei-Shou, H., 1988. Bioseparations: Downstream Processing for Biotechnology, John Wiley and Sons, Inc., U.S.A., 77-98.

Breed, R. S., Murray E. G. D., Smith N. R. (Eds), 1957. Bergley's Manual of Determinative Bacteriology, Seventh Ed., The Williams and Wilkins Company, U.S.A.

Banerjee, P. K., Butterfield, R. (Eds), 1980. Development of Boundary Element   
Methods- I*,* Applied Science Publishers, London.

**Example of Referencing of an Article in a Book:**

Beal, P. T., 1979. Application of Cell Biology to an Understanding of Biological Water. In Drost-Hansen W., Clegg J. S. (Eds.), Cell Associated Water, 271-291, Academic Press, N.Y.

**Example of Referencing of a M.S. Thesis:**

Berk, H., 1986. Heavy Metal Toxicity on Blue Green Alga, M.S. Thesis, Boğaziçi University.

**Example of Referencing of a Ph.D. Thesis:**

Akmehmet, I., 1990. Heterogeneous Photocatalytic Oxidation of Organic Compounds by TiO2, Ph.D. Thesis, Boğaziçi University.

Liu, W. K., 1981. Development of Finite Element Procedures for Fluid-Structure   
Interaction, Ph.D. Dissertation, California Institute of Technology.

**Example of Referencing of a Conference Paper:**

Persson, G. A., 1987. Acid Rain- A Threat to Europe’s Environment, Proceedings of the International Symposium on Environmental Management: Environment’ 87, Istanbul, 5-7 June 1987, 2, 1169-1182.

**Example of Referencing of a Report:**

Nett, A. L. and Trucker, J. D., 1998. A Comparison of Pollutant Transport Models PT1 and PT2, RSRE Memorandum No. 4157, RSRE Malvern.

AWWA Membrane Technology Research Committee, Committee Report, 1992. Membrane Processes in Potable Water Treatment. Journal of American Water Works Association, 84, 59-67.

Calgon Carbon Corporation, 1996. The AOT’s Handbook, 1,1.

CAChE Scientific. 1993. CAChE User Manual. CAChE Scientific Inc.

U.S.EPA., 1993. Perox-pure™ Chemical Oxidation Technology, Peroxidation Systems, Inc.:Applications Analysis Report. Office of Research and Development, SITE Program. Washington, DC. EPA/540/AR-93/501. July.

Ataman, Y., Çaycı, G., Baran, A., Kütük, C., Özaytekin, H., Dengiz,O., 1999. A research on reclamation of physical properties of Yeniçağa-Bolu peat in Turkey as plant growing medium. Project No. TOGTAG-1700. Scientific and Technical Research Council of Turkey.

**Example of Referencing of an Article in Internet:**

Miller, D., 1996. "λProlog: An Introduction to the Language and its Logic", http://www.cis.upenn.edu/~dale/lProlog/index.html.

U.S. Environmental Protection Agency Envirofacts Data Warehouse Home Page. http://www.epa.gov/enviro/index-java.html. (accessed June 2001) .

**Selective examples of citations:**

Standard Methods for the Examination of Water and Wastewater, 1995. APHA, AWWA, WEF, 16th Edition, Washington, D.C.

Conlon, M., Khraisheh, M., 2002. Bioadsorption process for the removal of color from textile effluent. GB Patent WO 0242228.

EC Directice 91/271/EEC, 1991. Concerning Urban Wastewater Treatment.

ISO/TC 147, 2001. Water quality-On line sensors/analyzing equipment for water. specifications and performance tests. Draft International Standard ISO/DIS 15839. International Organisation for Standardization, Geneva.

van Vooren, L., Willems, P., Ottoy, J.-P., Vansteenkiste, G.C., Verstraete, W., 1996. Automatic buffer capacity based sensor for effluent quality monitoring. Water Science and Technology, 33, 1, 81-87.

deClercq, J., Devisscher, M., Bonen, I., Vanrolleghem, P.A., Defrancq, J., 2002. A new one-dimensional clarifier model verification using full scale experimental data. In: Proceedings 3rd IWA World Water Congress April 7-12, Melbourne, Australia.

diPinto, A.C., Limoni, W., Passino, R., Rozzi, A., Tomei, M.C., 1990. Anaerobic process control by automated bicarbonate monitoring. In: Instrumentation, Control and Automation of Water and Wastewater Treatment and Transport Systems. Briggs, R. (Ed) Pergamon Press, London, 51-58.

McCarty, P.L., 1964. Anaeorobic waste treatment fundamentals. Part two. Environmental requirements and control. Public Works, 95, 123-126.

Mac Craith, B., Gratten, K.T.V., Connely, D., Briggs, R., Boyle, R., Avis,M., 1993. Cross comparison of techniques for the monitoring of total organic carbon (TOC) in water resources and supplies. Water Science and Technology, 28, 11-12, 457-463.

WPCF, 1980. Sludge Thickening. Manual of Practice No. FD.1. Water Pollution Control Federation, Washington, D.C.

McTavish, H., Fuchs, j.A., Hooper, A.B., 1993. Sequence of the gene coding for ammonia monooxygenase in *Nitrosomonas europaea*. Journal of Bacteriology, 175, 2436-2442.

Türkiye Çevre Vakfı, 1995. Türkiye’nin Çevre Sorunları. TÇV Yayınları, Ankara.

Metcalf and Eddy, 1991. Wastewater Engineering, Treatment, Disposal and Reuse. McGraw Hill, New York.

Ataman, C., 2002. Personal communication. Director of MEKA Wastewater Treatment Plant, İstanbul.

**APPENDIX A: 360 BP LONG mtDNA D-LOOP SEQUENCES OF *PHOCOENA PHOCOENA* INDIVIDUALS SEQUENCED IN THIS STUDY**

D81 AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAAGCACCACTGTACTATGCCAGTATTAAAAATAACCCGCTCCGAAACATCCCACTGCAACTACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTATGTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAAAGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTACATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTAGATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D82 AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAAGCACCACTGTACTATGCCAGTATTAAAAATAACCCGCTCCGAAACATCCCACTGCAACTACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTATGTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAAAGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTACATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTAGATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D79 AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAAGCACCACTGTACTATGCCAGTATTAAAAATAACCCGCTCCGAAACATCCCACTGCAACTACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTATGTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAAAGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTACATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTAGATCACGAGCTTAATCACCATGCCGCGTGAAACCA