

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

by

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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.

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.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation
%	Percent
oC	Degree Centigrade
μl	Microliter
π	Nucleotide Diversity
Fst	Fixation Index
Abbreviation	Explanation
A	Adenin
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, and includes marine mammals such as whales, dolphins and porpoises (1, 2). The cetaceans comprise three main groups, namely Mysticeti (baleen or moustache whales), Odontoceti (toothed whales) and Archeoceti (ancient whales). Mysticeti and Odontoceti still have living representatives, whereas Archeoceti is an extinct group (Figure 1.1) (3, 4).

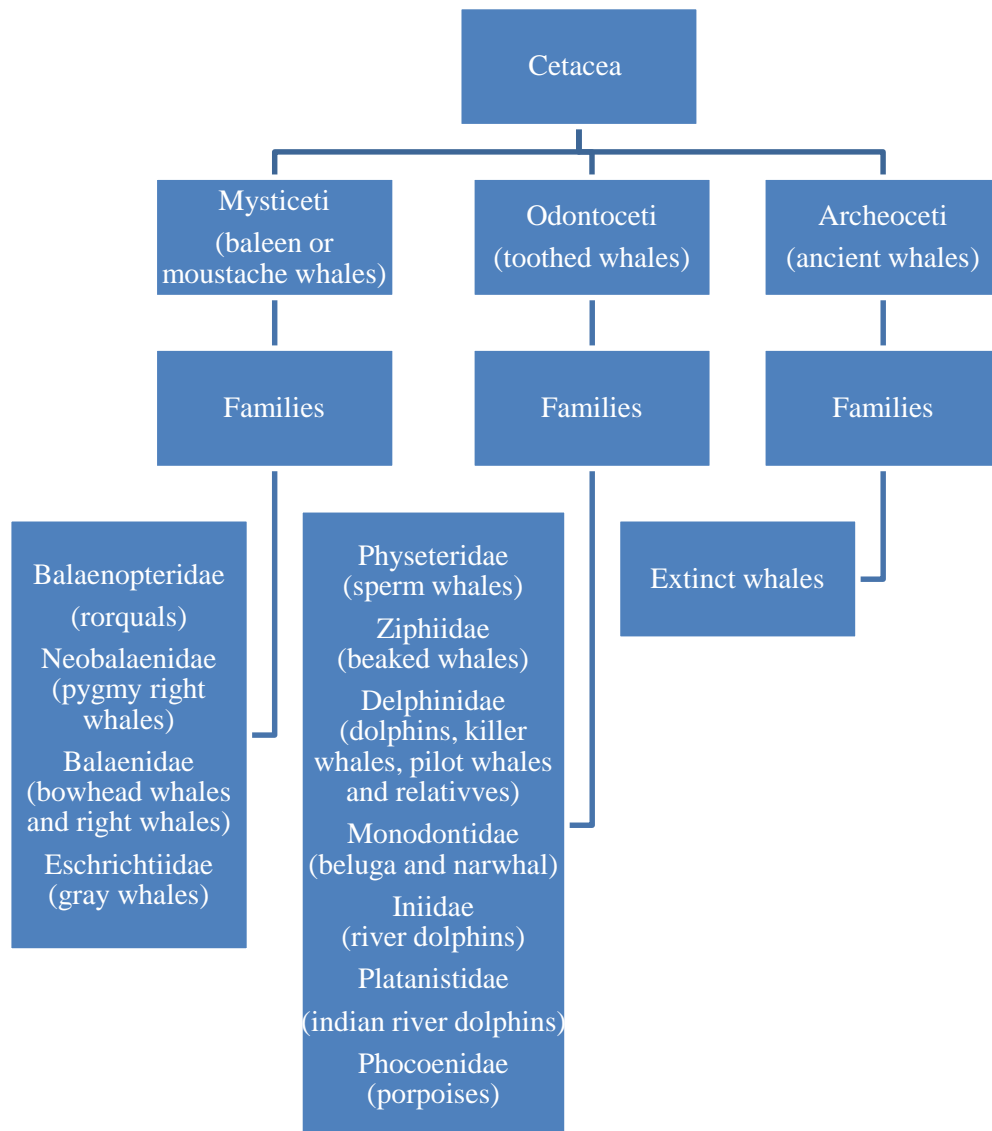


Figure 1.1. Taxonomy of Cetaceans.

Although Archaeoceti is an extinct group, Mysticeti and Odontoceti 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 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 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, the Fin whale *Balaenoptera physalus*, Risso's 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 *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 *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).

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*

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 odontocetes, 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.

They use vocalizations as loud as 230 decibels to communicate, and find their location and prey underwater. They dive to hunt and can remain submerged for up to an hour. Sperm whales are capable of diving up to 2,250 meters, and they primarily feed on large squids, but also on sharks, skates, and fish (21, 22).

In some mid-latitudes, sperm whales have been observed to migrate north and south depending on the seasons; however, no seasonal migration has been recorded in tropical or temperate areas. In Ölüdeniz, Turkey, a female Sperm whale was caught and rescued by Turkish naval forces (10, 21, 22).

1.1.3. Cuvier's Beaked Whale, *Ziphius cavirostris*

Cuvier's beaked whales, *Ziphius cavirostris*, are medium-sized cetaceans. They reach lengths of 4.5 to 7 meters and weigh between 1,845 and 3,090 kg at maturity. The weights of adult Cuvier's beaked whales may reach up to three tons. Their body is rough and rounded. Their dorsal fins are placed close to their backsides. Their body has a coloration pattern that changes between dark gray to a reddish-brown (23).

Cuvier's beaked whales are mostly seen alone or in groups of two to 12 individuals. They can dive very deep, up to 1,000 meters, and can stay underwater for almost 40 minutes to prey mainly on cephalopods, deep sea fish, and some crustaceans (23). They also have cosmopolite behaviour and can be seen in all oceans of the world. They live in deep pelagic waters and are usually observed in temperate and tropical waters (23). In Turkey, they have been observed on the Mediterranean coast (10).

1.1.4. Long-Finned Pilot Whale, *Globicephala melas*

Long-finned pilot whales, *Globicephala melas*, are one of the largest species of dolphins, with adult males reaching body lengths of up to 7.6 meters, and weights of up to 2,300 kg. Adult females reach body lengths of up to 5.8 meters and weigh up to 1,300 kg. The females are smaller than males. These whales typically have dark black color; however, sometimes they can be dark gray or brownish with white marks on their skin. Their long, flat pectoral flippers give them their common name (24).

Long-finned pilot whales are very active and social animals, found commonly in groups of between 10 and 20 individuals. They use a very wide sound range from 3 to 18 kHz, for echolocation to locate and find their prey, which mainly comprise cephalopods, fish, and crustaceans (24, 25). *Globicephala melas* generally prefer temperate waters to sub-polar waters, and are known to be anti-tropical. In Turkey, they have been observed in the Aegean and Mediterranean coasts (10, 24).

1.1.5. False Killer Whale, *Pseudorca crassidens*

One of the larger species of whales is the False Killer Whale, *Pseudorca crassidens*. In this species, females can reach body lengths of up to 4.5 meters, and males can reach body lengths of up to 6 meters. When they both become adults, they can weigh around 700 kg. The adults have a long body with a tall dorsal fin and distinguishing flippers with a hump in the middle of the front part of their body. They have a dark coloration pattern with some lighter patches on their ventral parts (26, 27). Its name “False Killer Whale” refers to the Killer Whale, *Orcinus orca*, because the skulls of the two species are similar. The False Killer Whale, *Pseuda crassidens*, is actually genetically distant from *Orcinus orca* (26, 27).

False Killer Whales can form very large groups but they are often seen in groups of 10 - 20 individuals. They use echolocation underwater to find their way and locate their prey. They can swim for very long distances to prey on fishes and cephalopods (26, 27). False Killer Whales can usually be seen in 1000 meters deep tropical ocean waters, though they have sometimes been spotted in the Mediterranean. In Turkey, a False Killer Whale was stranded in Urla (10, 27).

1.2. General Characteristics of Dolphin Species Found in Turkey’s Coastal Waters

1.2.1. Striped Dolphin, *Stenella coeruleoalba*

Stenella coeruleoalba, known as the Striped Dolphin takes its common name from the striped pattern on its body. The species has a distinct coloration pattern changing from blue

to grey, and have sleek bodies with a tall, hook-like dorsal fin. Males can become 2.7 meters long and weigh as much as 160 kg. Females can grow up to 2.4 meters long and weigh 150 kg (28, 29).

Striped Dolphins are widespread around the world and usually form groups including 50 - 100 individuals. They make clicking and whistling sounds to communicate with each other and to locate their prey. They have the ability to dive to 700 meters deep, to prey on schooling fish and cephalopods (28, 29). Striped Dolphins have a cosmopolite behaviour pattern and they live in deep warm waters. In Turkey, as mentioned above, they can be seen along the Aegean and Mediterranean coasts (10, 28, 29).

1.2.2. Bottlenose Dolphin, *Tursiops truncatus*

Bottlenose dolphin, *Tursiops truncatus*, is one of the most well-known species of marine mammals. Their body has the shape of a torpedo and they have a coloration pattern from light grey to black with brighter ventral parts. The populations living on in-shores have smaller bodies, and those inhabiting offshore waters have larger ones. Their length can be between 1.8 and 3.8 meters. Males grow to be larger than the females. When both sexes become mature, their weight can be between 136 - 635 kg (30, 31).

They are a social species, forming groups of 2-50 individuals. They use echolocation to find their prey, which are mostly fish and squid (30, 31). Bottlenose Dolphins are a wide-ranged species that lives in tropical waters all around the world. They can be easily found in Mediterranean, not only in the open waters but sometimes also inshore. In Turkey, they have been detected in the Black Sea (10, 30, 31).

1.2.3. Short-Beaked Common Dolphin, *Delphinus delphis*

Short-beaked common dolphins (*Delphinus delphis*) have small bodies that are 2.7 meters in length on average. Their weight can reach up to 200 kg. Males can grow larger than females. In the middle of a short-beaked common dolphins's back, there is a tall, hook-like dorsal fin with a triangular shape. Its dorsal parts can be grey to blackish, and its ventral parts are white (32, 33).

Short-beaked common dolphins often form large groups consisting of 10 to 50 individuals, and they are very cooperative while hunting. They have the ability to dive 200 meters to prey on small schooling fish and cephalopods (32, 33). Short-beaked common dolphins can be found in tropical and temperate ocean waters, and they have been observed in all seas surrounding Turkey (10, 32, 33).

1.2.4. Risso's Dolphin, *Grampus griseus*

Risso's dolphins, *Grampus griseus*, are medium-sized dolphins that have a very unique appearance. Different from the other dolphins, they lack a beak and have a blunt, rounded head. They also have a distinct vertical crease in the melon (the organ used for sonar clicks placed in the rounded head) that runs from the forehead to the mouth. Their dorsal fin is tall and curved. These dolphins have a grey coloration pattern when they are born. Over time, their color gets brownish and when they get older their color becomes almost white. On the sides of their bodies, some scars can generally be seen. These scars are thought to result from playing with other dolphins; they may also be squid bites (34, 35). When they become adults, they can become 2.6 - 4 meters long and their weight can be between 300 and 500 kg. Males are usually larger than females (34, 35).

Risso's dolphins usually form groups consisting of 5-50 individuals. They can dive 300 meters to prey and normally hunt at night, waiting for their prey to come to the surface of the water. They feed on fish, krill, and cephalopods— especially squid (34, 35). Risso's dolphins can be seen in temperate and tropical waters of both hemispheres. In Turkey, they have been detected in the Aegean and the Mediterranean (10, 34, 35).

1.3. General Characteristics of the Porpoise Species Found Along Turkey's Coasts

The harbor porpoise, *Phocoena phocoena*, which is the species of interest in this study, has a very wide range of distribution all around the world. It is a member of the Phocoenidae family, which are also called porpoises (36).

Porpoises are small cetaceans related to whales and dolphins (37). Dolphins are bigger, but tend to be generally thinner than porpoises. The heads of porpoises are rounded and small and their jaws are plump. They also do not have beaks like dolphins, and they have spade-shaped teeth instead of the conical teeth of the dolphins. Most of the whales and dolphins have curved dorsal fins, but porpoises have a very unique triangle-shaped dorsal fin. Porpoises also have limp skin with layers containing a spongy material that makes them incredibly fast swimmers. This characteristic of their skin helps them swim in harmony with the movement of the water (38).

The body length of harbor porpoises is around 2.5 meters at most, so they are among the smallest cetaceans. The length of a Vaquita (*Phocoena sinus*) is 1.5 meters at most and is considered the smallest cetacean species. Among the porpoises, the Dall's porpoise (*Phocoenoides dalli*) is the heaviest, weighing between 130 and 200 kilograms; and the least heavy one is the finless porpoise, weighing between 30 and 45 kilograms. In terms of their energy budgets, porpoises lose heat more quickly than their fellow cetaceans because of their small size; however, their stout shape helps them retain heat because it reduces surface area. Their adeps under their skin also protects them from losing body heat and getting cold. Porpoises also eat food more frequently than the other cetaceans because of their small bodies, and as they are generally in need of energy because they do not have large fat reserves (38).

Considering their reproduction, porpoises become sexually mature earlier than dolphins. They are also distinct from dolphins in that they reproduce at a faster rate. Their pregnancy lasts for 11 months. The lifecycle of a porpoise is generally known to be between 8 and 10 years, but some 20-year-old porpoises have also been recorded (38).

Porpoises can be usually seen in small groups of up to 10 individuals. In some cases, they can form big groups of 100 or more individuals. As all toothed whales are capable of finding their way, hunting, and moving with group members using echolocation, porpoises also use this ability for similar purposes. Porpoises can dive 200 meters deep, but instead of hunting in deep waters, they choose to feed on animals around shallow coastal waters. They get their main nutrients from squid, fish and crustaceans (38).

Taxonomically speaking, there are seven porpoise genera. These include Haborophocoena, Neophocoena, Numataphocoena, Phocoena, Phocoenoides, Septemriocetus and Piscalithax. The best known genus is *Phocoena* and the harbor porpoise, *Phocoena phocoena*, can be found across the Northern Hemisphere (38). The harbor porpoise (*Phocoena phocoena*) is a familiar cetacean, widely distributed in the Black Sea, the Pacific and Atlantic parts of the northern hemisphere, along the coasts and continental shelves (Figure 1.2) (39).



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

1.3.1. General Characteristics of the Harbor porpoise, *Phocoena phocoena*

The *Phocoena phocoena*, (Linnaeus, 1758), also referred to as the “harbor porpoise” or the “common porpoise”, is a marine mammal. It has a relatively small body that is thick in the midsection and narrows toward its flukes. It can weigh up to 90 kg and can grow up to 1.9 m in length; however, the typical harbor porpoise does not weigh more than 60 kg or grow longer than 1.5 m. Females are a bit larger on average than males (40).

These mammals feature blunt, rounded heads, which help distinguish them from dolphins (41). Their ventral (or under) side is white, their dorsal (or upper) side is dark gray or brown, and their flanks are a lighter gray. They have dark stripes of gray that run from their mouths or eyes to their small, rudder-like flippers. Their flippers are rounded at the tips. Their flukes (or tail fins) are curved, small, and have a median notch. A harbor porpoise’s triangular dorsal fin is just behind the center of its body. In select porpoises, one can find small nodules on the leading edge of the dorsal fin (40, 42). A harbor porpoise sports between 22 and 26 pointed teeth on each side of its lower jaw, and between 22 and 28 teeth on each side of its upper jaw (40).

The echolocation click sounds used by harbor porpoises are short and have a high frequency, in order to communicate and find their prey, which is composed of capelin, cephalopods, and herring (43). They do not make different kinds of echolocation signals during the day (44).

Harbor porpoises become sexually mature at 3-4 years old. Female porpoises' pregnancies last 10-11 months, and they give birth every one to two years. Their offspring are nursed for 8-12 months, and are called "calves." Calves are generally born between 70 and 90 cm long and weigh between 6.4 kg and 10 kg (40).

Large groups of between 50 and 100 harbor porpoises have been found feeding together; however, they are usually seen singly, paired, or in small groups of up to 10 individuals (40). They usually stay near the surface, coming up to breathe every 25 seconds or so, but they can dive deeper than 200 m (41).

Harbor porpoises often make their habitat in shallow waters (less than 152 m) along the coasts of the Northern Hemisphere, where the climate is mild (40). While they are commonly observed in bays and harbors, they are also known to inhabit rivers, estuaries, inland waters, and tidal channels (41). Their close proximity to shorelines is likely why many porpoises have been stranded. Some stranded porpoises have been found alive and transported to aquariums, but few of them have survived in captivity (40).

Harbor porpoises have a shy temperament and they usually avoid passing boats (40). Their evasive nature has made their behavior in the wild difficult for scientists to study (41). Most of the research on these marine mammals has been confined to dead specimens caught accidentally in commercial fishing nets (41). Harbor porpoises are in rapid decline, largely due to these fishing accidents (40). The species is also harmed by noise and chemical pollution (41). In light of these threats, in 2008, the Black Sea subspecies *Phocoena phocoena ssp. relicta* was categorized as "endangered," on the IUCN Red List (7, 8), and some countries, including the US and Canada, have taken steps to protect it (41). A recent genetic study showed that 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 (39).

1.4. Review of Previous Genetic Studies on the Harbor Porpoise, *Phocoena phocoena*

The genetics of harbor porpoises have been investigated previously, and geographically speaking, the studies span the entire range of the species. Focusing on the Atlantic, 327 individual harbor porpoises in British and adjacent waters were studied to understand their population structure. The 200 base pair section of the mitochondrial DNA control region were amplified (45). The results suggest that there may be considerable interconnectivity among populations through gene flow. However, AMOVA showed that there were significant differences between the northern North Sea porpoises and southern North Sea porpoises, and between the northern North Sea porpoises and the Celtic/Irish Sea porpoises. The frequency of the differences in population genetic structure was directly related to the variation among the females. These results point out that males disperse more freely than females (45).

Several studies were undertaken on the genetics of the harbor porpoise in the North Atlantic. In one study, the datasets of the harbor porpoise in the North Atlantic were combined to understand the degree of genetic interchange that these populations experience (46). The results showed that genetic variability in the Northeast Atlantic was significantly lower than in the Northwest Atlantic, pointing to a new colonization in the Northeast Atlantic. The star phylogeny of northeastern haplotypes also indicated a recent population expansion (46).

Genetic studies on harbor porpoises were also done in eastern Asia and western North America. In one study by Taguchi *et al.* (47), a total of 56 tissue samples were obtained from Japan. In addition, nucleotide sequences in the Northeast Pacific, comprising 40 individuals from Alaska and the Strait of Georgia, 52 individuals from San Juan Island and Vancouver Island, 62 individuals from Washington and the Columbia River, 17 individuals from Oregon, and 52 individuals from San Francisco and Monterey Bay were retrieved from GenBank. The 462 base pair sections of the mitochondrial DNA control region were amplified. The results showed that most of the observed haplotypes were distributed across this extensive geographic range, although some were native to Japan. These results suggest the occurrence of gene flow over the entire distribution range of the species in the North

Pacific. No differences were detected between the Japanese and Alaskan populations, although they are geographically very distant from each other (47).

In another study, polymorphism at 11 microsatellite loci was analyzed in harbor porpoises collected from the Iberian Peninsula north to Arctic waters, and also along the coasts of the Black Sea (48). Based on the results of the multilocus tests, harbor porpoises living in Atlantic and the Black Sea were seen to be genetically isolated from each other. The Black Sea population displayed lower levels of genetic diversity compared to those of the Atlantic (48).

In a study by Rosel *et al.* (49), which included samples from around Turkey, a section of the mitochondrial DNA control region of the harbor porpoise was amplified and sequenced and the variability of the harbor porpoise populations in the Northeast Pacific, North Atlantic, and the Black Sea was examined. The estimated sequence divergence among the three ocean basins was high and no haplotypes were shared among them. Several distinct groups of haplotypes were found in the Northeast Pacific, but there was no relationship between sequence type and geographic location.

A recent study by Fontaine *et al.* (50) showed that a very important founder event had a very significant influence on the population size of harbor porpoises within the past 5 decades. This study shows both a recent event of decline and an ancient event of expansion from contemporary genetic data. Although dolphin fisheries were banned in the countries surrounding the Black Sea, populations of small cetaceans are unlikely to have recovered much since 1983. Incidental catches also had a harmful effect on harbor porpoises in the Black Sea. This study gives support to the theory that the dolphin fishery caused a strong decline in the Black Sea harbor porpoises, and they have not yet started to recover. They continued to decline, especially because of the large-scale mortality in bottom-set gillnets and habitat degradation (39).

Finally, Tonay *et al.* (50) investigated the genetic relationships of three individuals of harbor porpoise (*Phocoena phocoena*) collected from the Black Sea, the Sea of Marmara and the Aegean. Mitochondrial DNA sequences data revealed three polymorphic sites, and haplotypes did not cluster with those in the Atlantic populations. The population structure

of harbor porpoises from the Ukrainian coast of the Black Sea and the Aegean haplotypes helped to understand the relationship between the harbor porpoises in these two seas, by suggesting that they dispersed by using the Istanbul and Dardanelles straits. In this study, a third sample from the Sea of Marmara had the same unique haplotype as the one detected in the samples of the aforementioned study by Viaud-Martinez *et al.* (51). This strengthens the idea that there is an isolated population in the Sea of Marmara (50).

1.5. Thesis Objectives

The main objectives of this thesis are to understand genetic differences in *Phocoena phocoena* species around the seas of Turkey. Although previous studies have been undertaken in the Black Sea, we want to use genetics to understand the relationships of the eastern Black Sea porpoises to the other Black Sea populations with a greater number of samples than previously used in the literature. Also, previous studies indicate a potentially isolated population in the Sea of Marmara, and with the new samples we have collected, it will be possible to evaluate this hypothesis. We also want to investigate the possible origin (Atlantic or Black Sea) of a porpoise from Bodrum, the southernmost location that the species has ever been recorded in the Aegean. All of these investigations will help to define management units (MUs), which will be instrumental in the conservation efforts for this endangered species.

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 °C until further processing.

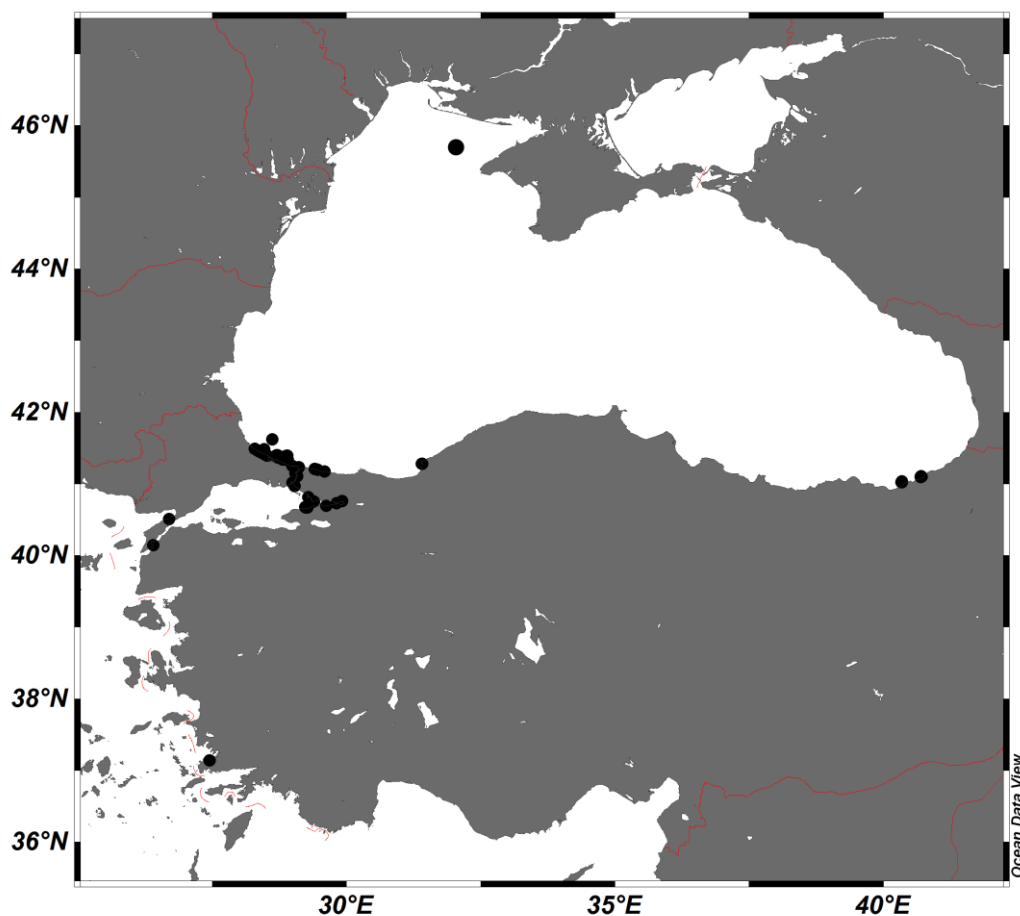


Figure 2.1. The range map of *Phocoena phocoena* used in the Sea of Marmara and Black Sea in Turkey (The sequences for Ukraine sequences were retrieved from GenBank.)

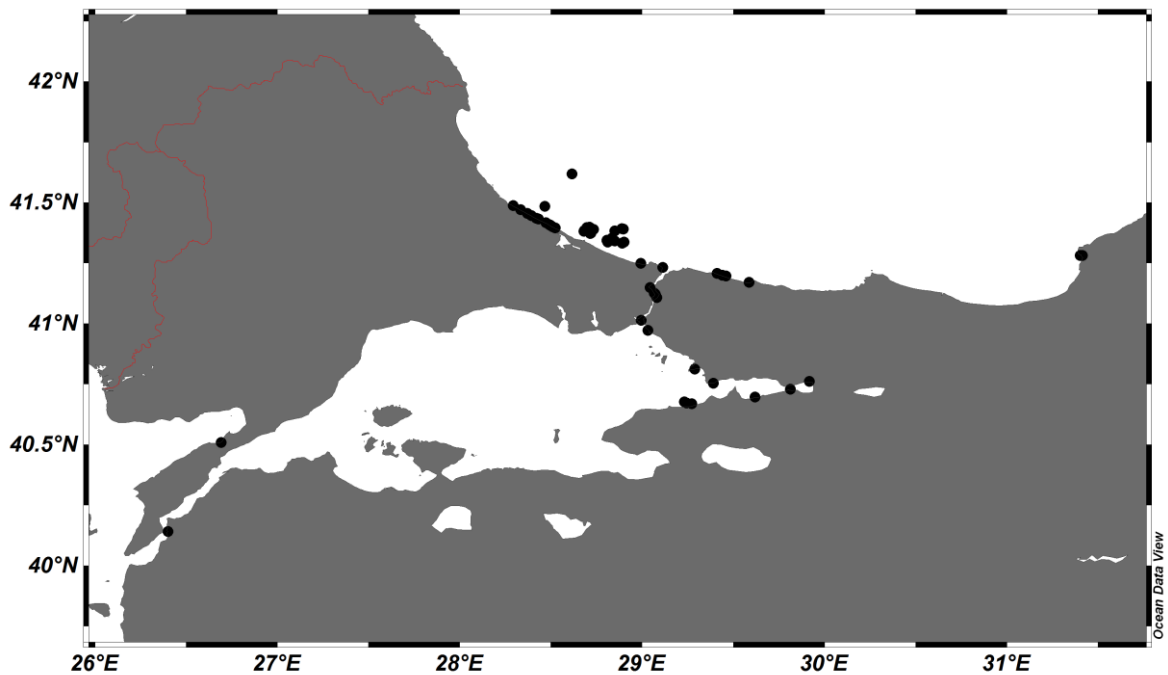


Figure 2.2. A close-up map of *Phocoena phocoena* samples collected from the Sea of Marmara and western Black Sea in Turkey.

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 μ l reaction volume, which was composed of 3 μ l DNA, 5 μ l of 25mM $MgCl_2$, 5 μ l KCl buffer, 1 μ l of 10nM DNTP, 1 μ l of 10 pmol/ μ l each primer, 0.3 μ l of 5U/ μ l Taq buffer and 33.7 μ l double distilled water (52). The PCR cycling conditions were 5 minutes at 94 $^{\circ}C$, 35 cycles of 30 seconds at 94 $^{\circ}C$, 1 minute at 59 $^{\circ}C$, 1 minute at 72 $^{\circ}C$, with a final extension of 1 minute at 72 $^{\circ}C$ (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. In order 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 Φ_{st} comparisons, computed using Arlequin version 3.5 (67). When studying mtDNA regions, Φ_{st} is analogous to F_{st} , for evaluating the structure in a population. If the Φ_{st} value is 0, the individuals in populations can interbreed freely, whereas Φ_{st} 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).

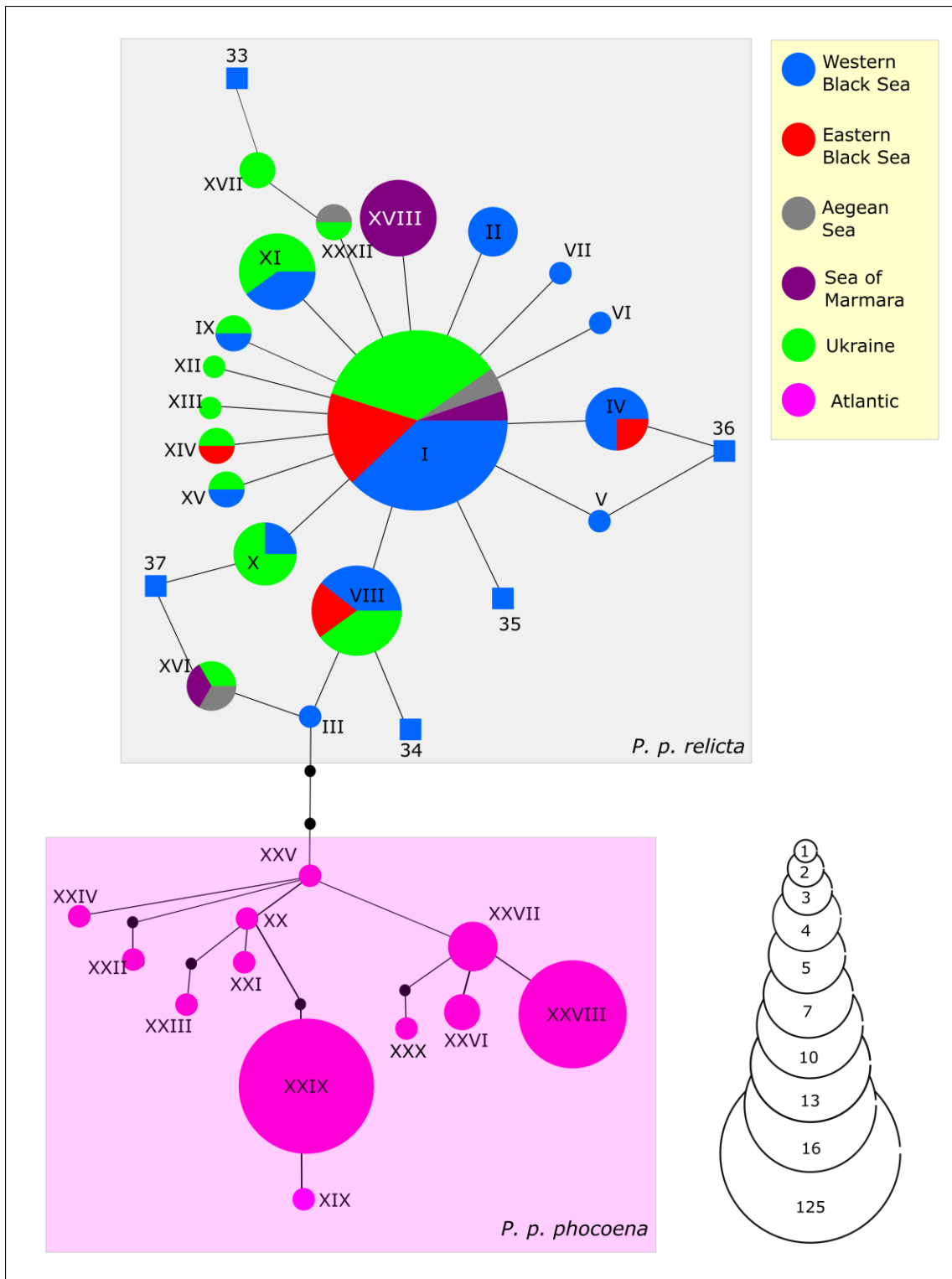


Figure 3.1. Haplotype network for the *Phocoena phocoena* sequences. The sizes of the circles are proportional to the number of individuals. Circles represent the haplotypes found in our study and Viaud-Martinez et al. (51). The five new haplotypes our study uncovered are represented by the boxes numbered 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	25/29	6/9	5/2	0/9	40/0	-
II	1/2	-	-	-	-	-
III	1/0	-	-	-	-	-
IV	3/0	0/1	-	-	-	-
V	1/0	-	-	-	-	-
VI	1/0	-	-	-	-	-
VII	1/0	-	-	-	-	-
VIII	1/6	2/0	-	-	4/0	-
IX	1/0	-	-	-	1/0	-
X	1/1	-	-	-	3/0	-
XI	2/0	-	-	-	3/0	-
XII	-	-	-	-	1/0	-
XIII	-	-	-	-	1/0	-
XIV	-	0/1	-	-	1/0	-
XV	0/1	-	-	-	1/0	-
XVI	-	-	0/1	0/1	1/0	-
XVII	-	-	-	-	2/0	-
XVIII	-	-	-	3/4	-	-
XIX	-	-	-	-	-	1/0
XX	-	-	-	-	-	1/0
XXI	-	-	-	-	-	1/0
XXII	-	-	-	-	-	1/0
XXIII	-	-	-	-	-	1/0
XXIV	-	-	-	-	-	1/0
XXV	-	-	-	-	-	1/0
XXVI	-	-	-	-	-	2/0
XXVII	-	-	-	-	-	3/0
XXVIII	-	-	-	-	-	10/0
XXIX	-	-	-	-	-	16/0
XXX	-	-	-	-	-	1/0
XXXI	-	-	-	-	-	3/0
XXXII	-	-	1/0	-	0/1	-

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.

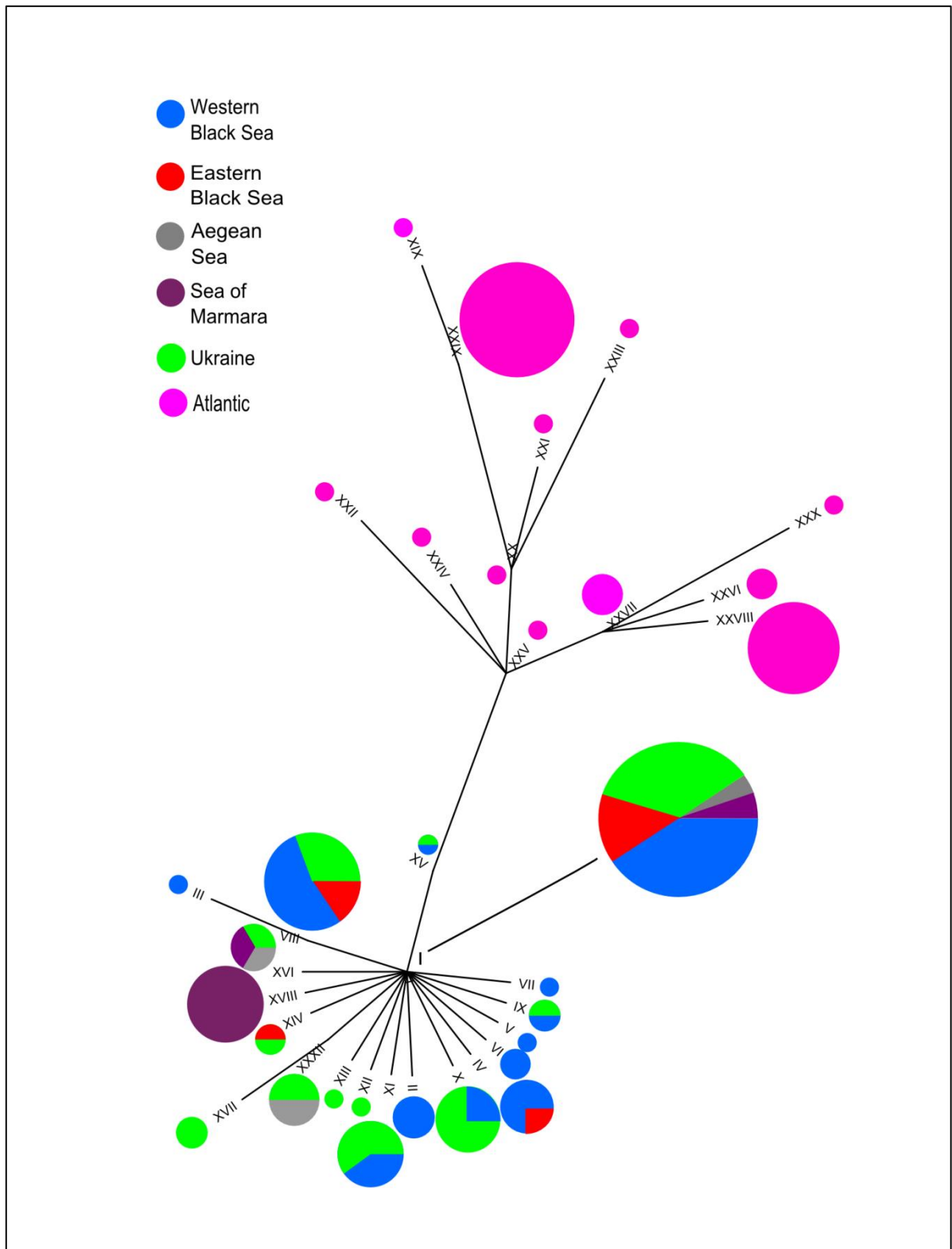


Figure 3.2. Phylogenetic relationships of 32 haplotypes of *Phocoena phocoena*. The phylogenetic tree was constructed using the maximum-likelihood method. The geographic locations of the haplotypes are represented by different colors.

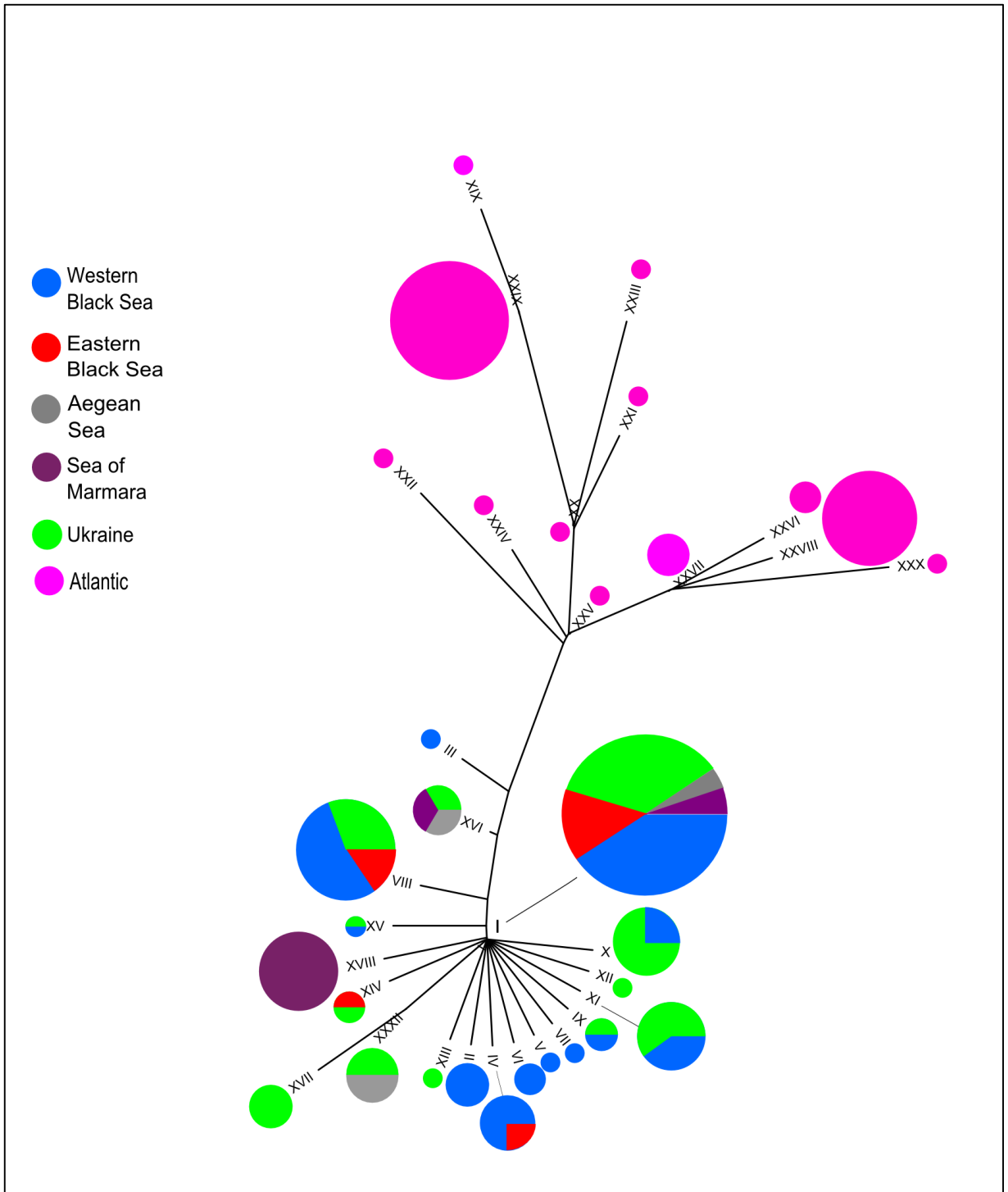


Figure 3.3. Phylogenetic relationships of 32 haplotypes of *Phocoena phocoena*. The phylogenetic tree was constructed using the neighbor-joining method. The geographic positioning of the haplotypes is represented by different colors.

3.1. Genetic Diversity Analyses

Looking at our data, the highest haplotype diversity values were seen in the samples from the Sea of Marmara, Ukraine and western Black Sea samples (Table 3.2). These data indicate that the populations in these areas could potentially be ancestral. In addition, there is a probability that there is an isolated population in the Sea of Marmara, meaning there may be a differentiated population in the Sea of Marmara. The haplotype diversity of Eastern Black Sea and Aegean *Phocoena phocoena* samples were relatively low, suggesting that the populations in these regions might have been founded more recently.

The nucleotide diversity patterns reflected the results and conclusions of the haplotype diversity patterns. The nucleotide diversity values of the Western Black Sea, Ukraine and Sea of Marmara samples were relatively high, and the same values were lower for the Aegean and the eastern Black Sea *Phocoena phocoena* samples (Table 3.2).

Table 3.2. The Descriptive Statistics of *Phocoena phocoena*. (WB, Western Black Sea; EB, Eastern Black Sea; M, the Sea of Marmara, AEG, Aegean Sea; U, Ukraine region).

Region	Number of sequences	Variable sites	Singleton Sites	Parsimony informative sites	Number of Haplotypes (h)	Haplotype diversity Hd (\pm Sd)	Nucleotide Diversity Π (\pm Sd)
All	184	17	5	12	19	0.531 (0.045)	0.00198 (0.00021)
WB	82	12	5	7	13	0.558 (0.065)	0.00203 (0.00030)
EB	19	4	3	1	4	0.380 (0.134)	0.00154 (0.00065)
M	17	2	1	1	3	0.581 (0.068)	0.00189 (0.00033)
AEG	8	1	1	0	3	0.250 (0.180)	0.00075 (0.00054)
U	58	11	6	5	11	0.520 (0.079)	0.00201 (0.00039)

Considering signatures of population expansion, the observed and expected mismatch frequencies were significantly different from each other in the western Black Sea (Figure 3.5) and the Sea of Marmara samples (Figure 3.6). This suggests that *Phocoena phocoena* populations of these two regions are relatively more stable and that they could be ancestral. On the other hand, the observed and expected mismatch distributions in Ukraine (Figure 3.7), the Aegean (Figure 3.8), and eastern Black Sea (Figure 3.9) were not significantly different from each other, indicative of an expansion, and suggesting that these populations are more likely to be recent and derived. These results seem to be in concordance with the haplotype and nucleotide diversity patterns mentioned above.

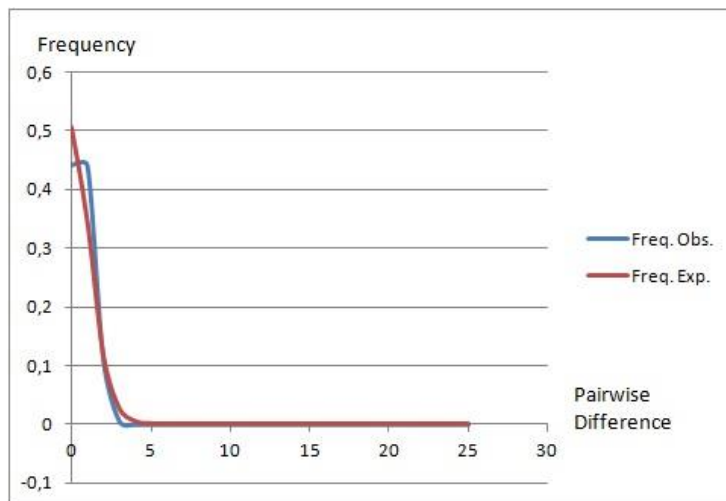


Figure 3.5. The mismatch distribution of Western Black Sea *Phocoena phocoena*.

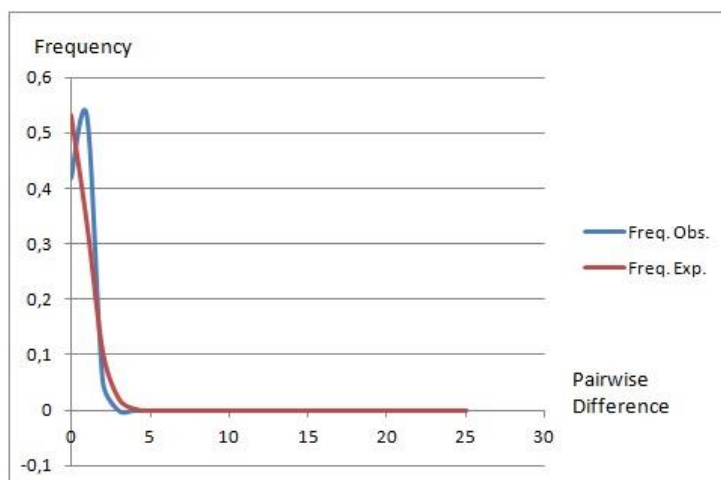


Figure 3.6. The mismatch distribution of the Sea of Marmara *Phocoena phocoena*

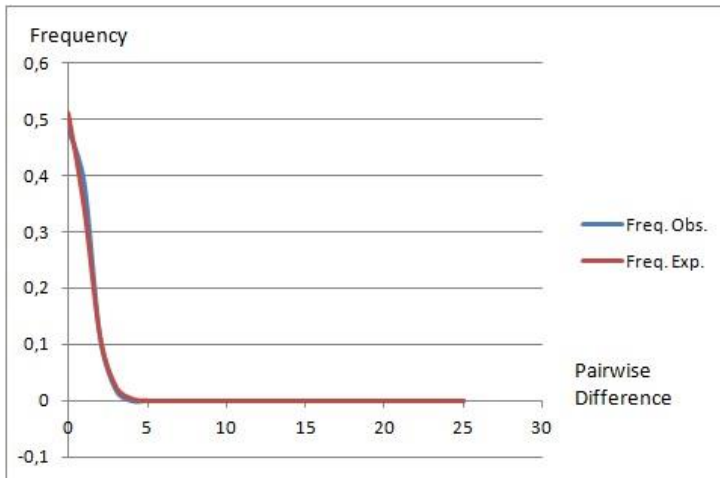


Figure 3.7. The mismatch distribution of the Ukraine region *Phocoena phocoena*.

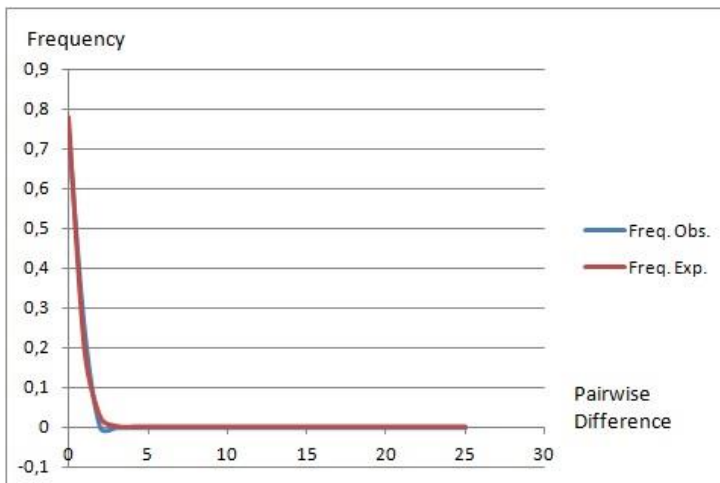


Figure 3.8. The mismatch distribution of the Aegean *Phocoena phocoena*.

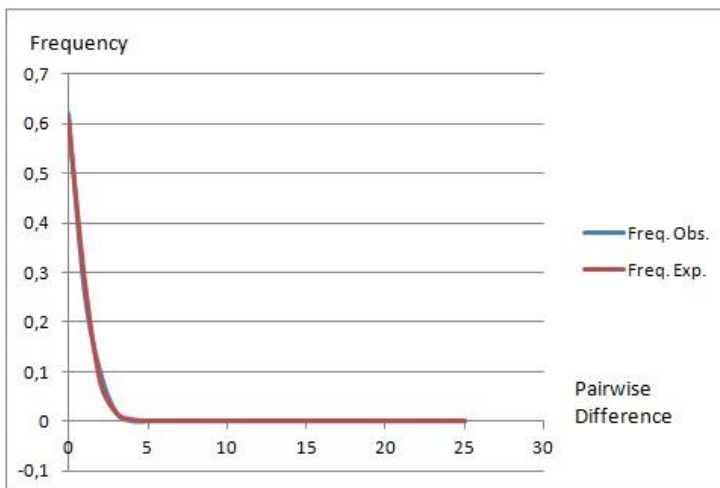


Figure 3.9. The mismatch distribution of Eastern Black Sea *Phocoena phocoena*.

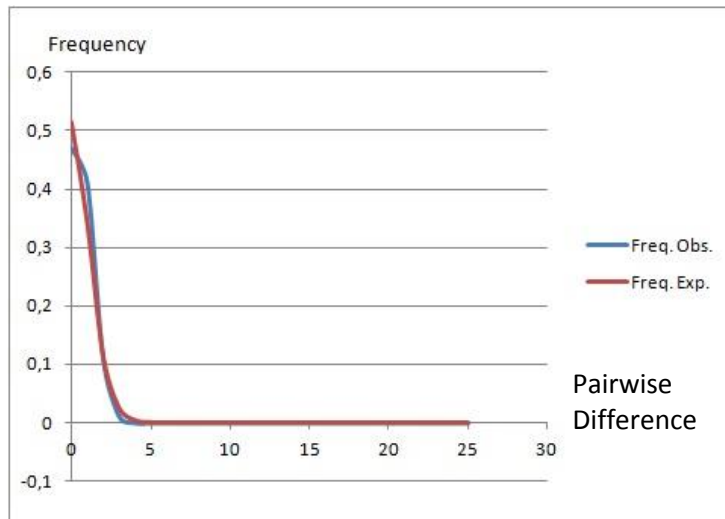


Figure 3.10. The mismatch distribution of all *Phocoena phocoena* samples.

Considering genetic distances (Table 3.3), the populations that had corrected genetic distances greater than zero were observed only in the comparisons with the Atlantic region, and all with a value of 0.003. This indicates that the *Phocoena phocoena* population in the Atlantic region is differentiated from the *Phocoena phocoena* species in other regions. This result is in concordance with the Black Sea vs. Atlantic differentiation observed in the trees and haplotype networks, and also what is known in the literature (48, 49, 50). The genetic distance of the samples taken from all the regions except Atlantic region was zero, again indicating the genetic similarity of the investigated populations.

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

	WB	U	EB	AEG	M	A
WB		0.000	0.000	0.000	0.000	0.003
U	0.001		0.000	0.000	0.000	0.003
EB	0.001	0.001		0.000	0.000	0.003
AEG	0.000	0.000	0.000		0.000	0.003
M	0.001	0.001	0.001	0.001		0.003
A	0.005	0.005	0.005	0.005	0.005	

The differentiation between populations was also evaluated with pairwise Φ_{st} calculations (Table 3.4). The Φ_{st} values marked with an asterisk represent statistically significant values. The Sea of Marmara was significantly differentiated from all of the other populations. These results suggest that all of the different populations, except that in the Sea of Marmara share a common evolutionary history and are possibly connected to each other in terms of gene flow.

Table 3.4. Φ_{st} values of the population calculated by using pairwise differences method.

	WB	EB	U	M	AEG
WB	0.00000				
EB	-0.01402	0.00000			
U	-0.00070	-0.01945	0.00000		
M	0.18686*	0.21985*	0.19421*	0.00000	
AEG	-0.03322	-0.04080	-0.03860	0.21487*	0.00000

Management units (MUs) are defined as populations with significant divergence of allele frequencies at nuclear or mitochondrial regions (69). MU designations take into account the geographic distribution of a population, the population's allele frequencies, and issues with the population's short term management. As MUs are used for monitoring and better management of populations, the above findings of unique haplotypes and high Φ_{st} values in the Sea of Marmara suggest the designation of these populations of *Phocoena phocoena* as a management unit (69).

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. Also next generation sequencing techniques, like RAD-sequencing, can provide higher resolution and help determine the differentiation, if any, of the populations inhabiting different parts of the Black Sea.

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

D81

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
 ACTGTACTATGCCAGTATTA AAAATAACCCGCTCCGAAACATCCC ACTGCAAC
 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D82

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
 ACTGTACTATGCCAGTATTA AAAATAACCCGCTCCGAAACATCCC ACTGCAAC
 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D79

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
 ACTGTACTATGCCAGTATTA AAAATAACCCGCTCCGAAACATCCC ACTGCAAC
 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D53

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
 ACTGTACTATGCCAGTATTA AAAATAACCCGCTCCGAAACATCCC ACTGCAAC
 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D40

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
ACTGTACTATGCCAGTATTAATAAATAACCCGCTCCGAAACATCCCCTGCAAC
TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D116

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
ACTGTACTATGCCAGTATTAATAAATAACCCGCTCCGAAACATCCCCTGCAAC
TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D49

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D121

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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D38

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
ACTGTACTATGCCAGTATTAATAAATAACCCGCTCCGAAACATCCCCTGCAAC
TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D119

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D117

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D118

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTCCATACGACTATGTTAA
AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D101

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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTCCATACGACTATGTTAA
AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D104

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D20

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
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D26

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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D107

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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D110

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 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D55

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 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D057

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D83

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 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCA CTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
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D23

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 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

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D43

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D88

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D9

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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D25

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 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D062

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 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
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 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D100

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 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
 ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
 GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D80

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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 TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
 GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
 AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D059

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D14

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D126

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
ACTGTACTATGCCAGTATTAATAAATAACCCGCTCCGAAACATCCCCTGCAAC
TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
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AGCCCGTATTAAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D6

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGTCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D48

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGTCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D105

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D37

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTTCCATACGACTATGTTAA
AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D50

AATTCTTTATAAACTACTCCTTGAAAAAGCCTATTGTATGATTATTAAGCACC
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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
GTATACATGCTATGTATTATTGTGCATTCATTTATTTCCATACGACTATGTTAA
AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D058

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D08

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D19

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AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D03

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AGTCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D47

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AGTCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D15

AATTCCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTA AAAACTTATTAATCTTACAAAGTACATAATTTGCATGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D28

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAATAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D44

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAATAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D12

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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D127

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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D73

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D70

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D22

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D97

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D89

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA [360]

D102

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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GTATACATGCTATGTATTATTGTGCATTCATTTATTTCCATACGACTATGTTAA
AGTCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D92

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D16

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D93

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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D90

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 AGCCCGTATTAAAACCTATTAATCTTACAAAGTACATAATTTGCACACTCTTAC
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D106

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 CGACTATGTTAAAGCCCGTATTAATAACTTATTAATCTTACAAAGTACATAATTT
 GCACGCTCTTACATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATG
 GCCGCTCCATTAGATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D71

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D95

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D36

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 AGCCCGTATTAATAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC

ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D13

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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D125

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GTATACATGCTATGTATTATTGTGCATTCATTTATTTCCATACGACTATGTTAA
AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D076

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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D51

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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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D27

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D111

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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D129

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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D29

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
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D99

AATTCTTTATAAACTACTCCTTGAAAAAGCCCATTGTATGATTATTAAGCACC
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

D18

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TACCATGTATGTACTCACATACTACAATCCTAGTCTTCCCCTATAAATATTTAT
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AGCCCGTATTAAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
ATATTATATCTCCACTTGTACCTCATATCCATTATATCCTATGGCCGCTCCATTA
GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

EF063110

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EF063646

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EF063647

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EF063648

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EF063649

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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCATGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

EF063650

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AGCCCGTATTA AAACTTATTAATCTTACAAAGTACATAATTTGCACGCTCTTAC
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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

EF063651

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ACTGTACTATGCCAGTATTAATAAATAACCCGCTCCGAAACATCCCCTGCAAC
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EF063652

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EF063653

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EF063675

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GATCACGAGCTTAATCACCATGCCGCGTGAAACCA

**APPENDIX B: THE TAXONOMIC HIERARCHY OF THE
CETACEANS IN TURKEY IN GENERAL, AND *Phocoena phocoena*
IN PARTICULAR**

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Cetacea

A) Suborder: Mysticeti

1) Family: Balaenopteridae

Genus: *Balaenoptera*

Species: *Balaenoptera physalus*

B) Suborder: Odontoceti

1) Family: Physeteridae

Genus: *Physeter*

Species: *Physeter catodon*

2) Family: Ziphiidae

Genus: *Ziphius*

Species: *Ziphius cavirostris*

3) Family: Delphinidae

Genus: *Globicephala*

Species: *Globicephala melas*

4) Family: Delphinidae

Genus: *Pseudorca*

Species: *Pseudorca crassidens*

5) Family: Delphinidae

Genus: *Stenella*

Species: *Stenella coeruleoalba*

6) Family: Delphinidae

Genus: *Tursiops*

Species: *Tursiops truncatus*

7) Family: Delphinidae

Genus: *Delphinus*

Species: *Delphinus delphis*

8) Family: Delphinidae

Genus: *Grampus*

Species: *Grampus griseus*

9) Family: Phocoenidae - Gray, 1825

Genus: Phocoena - G. Cuvier, 1816

Species: *Phocoena phocoena* (Linnaeus, 1758)

Subspecies: *Phocoena phocoena phocoena* (Linnaeus, 1758)

Subspecies: *Phocoena phocoena relicta* Abel, 1905

Subspecies: *Phocoena phocoena vomerina* Gill, 1865 (16, 17, 18)