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Approccio multidisciplinare allo studio di alcune specie di cetacei spiaggiate lungo le coste italiane

Multidisciplinary approach to the study of some cetacean species stranded along the italian coasts

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Riassunto

Questo lavoro si propone di analizzare alcuni aspetti della biologia in specie di Cetacei che si sono spiaggiate lungo le coste italiane. Le specie in questione sono: capodoglio (*Physeter macrocephalus*), kogia (*Kogia sima*), balenottera comune (*Balaenoptera physalus*) e balenottera minore (*Balaenoptera acutorostrata*). Per ognuna di questa specie sono state condotte analisi molecolari sulla regione di controllo del DNA mitocondriale: estrazione, amplificazione e sequenziamento sono state le analisi principali condotte su campioni di tessuto di queste specie. I campioni sono stati ottenuti dalla banca dei tessuti dell'Università degli Studi di Padova. Per quanto riguarda i capodogli, sono state condotte anche delle analisi per la determinazione dell'età mediante l'osservazione di gruppi di strati di crescita (GLG) nei denti mandibolari. Le analisi molecolari hanno permesso di tracciare la provenienza degli individui campionati e di attribuirli ad un determinato aplotipo di provenienza. Risutlati:

- ***Physeter macrocephalus***: i risultati hanno mostrato nel 100% dei casi l'appartenenza all'unico aplotipo presente nel Mediterraneo confermando l'ipotesi dell'isolamento dalla popolazione atlantica e la presenza quindi di una popolazione "stabile". Le analisi per la determinazione dell'età sono state relazionate alla lunghezza totale degli individui spiaggiati sia nei capodogli mediterranei che in quelli atlantici. In quest'ultimo caso le informazioni sono state ricavate dalla bibliografia. Le analisi hanno evidenziato una maggiore longevità dei capodogli mediterranei rispetto agli atlantici, a parità di lunghezza. Il confronto con altre specie di Cetacei permette di ipotizzare che le cause della taglia ridotta degli individui mediterranei possa essere legata alle condizioni fisico-chimiche del bacino, alla disponibilità e al valore nutrizionale della preda preferita da questi animali;
- ***Kogia sima*:** è un animale schivo, gli avvistamenti sono molto rari e lungo le coste italiane sono stati osservati fino ad oggi solo tre individui spiaggiati. Le analisi molecolari hanno attribuito la provenienza di questi esemplari all'Oceano Atlantico; nel Mediterraneo non esiste una popolazione che sia considerata "regolare". Tuttavia, recenti analisi parassitologiche hanno

evidenziato come alcune specie del genere *Anisakis*, che sono caratteristiche dei capodogli mediterranei, sono presenti anche in *Kogia sima*. Ciò suggerisce che entrambe le specie attingano alla stessa rete trofica nel Mediterraneo e considerando il ciclo vitale a step del parassita (riproduzione nell'ospite intermedio-calamari, pesci- incistamento nell'ospite finale-Mammiferi marini), si può ipotizzare la presenza di una popolazione più regolare di *Kogia sima* in questo bacino;

- ***Balaenoptera physalus***: nel Mediterraneo la presenza di una popolazione "regolare" viene supportata dalla presenza di un aplotipo privato (cioè esclusivo) di questo bacino e in particolare del Mar Ligure. In questo lavoro erano disponibili tre campioni di balenottera comune: dalle analisi molecolari due di questi hanno mostrato essere effetivamente balenottere comuni mentre il terzo è risutlato essere un ibrido tra una femmina di balenottera azzurra (*Balaenoptera musculus*) e un maschio di balenottera comune. I motivi di questa ibridazione sono verosimilmente da attribuire alla particolare biologia dei Cetacei, con la formazione di "schooling" misti e alla maggiore propensione all'ibridazione rispetto alle altre specie di Mammiferi, ma non si può escludere che l'ibridazione sia anche conseguenza del crollo demografico cui la balenottera azzurra è andata incontro nel corso del Novecento. La migrazione dell'ibrido dall'Islanda al Mediterraneo è verosimilmente da attribuire a scopi trofici.
- ***Balaenoptera acutorostrata*:** in base agli aplotipi osservati, i due campioni analizzati sono di provenienza atlantica. La balenottera minore è una specie considerata occasionale nelle acque del Mediterraneo. La peculiarità di questi due esemplari risiede nella loro lunghezza: infatti sono entrambi individui di circa 3 m e questo ha permesso di stabilire che fossero esemplari nati da poco. I dati presenti in letteratura riguardo gli spiaggiamenti di questa specie lungo le coste italiane hanno permesso di ipotizzare che la balenottera minore usi il Mediterranaeo come area di nursery dato che tutti gli individui spiaggiati erano nati da poco e questo può implicare la presenza di una popolazione più stanziale nel Mediterraneo.

Aims of this project

Studying cetaceans is not an easy task, their physical conditions and their lifestyle make it difficult to analyze these mammals in their natural environment, most of the knowledges derive from analyzes carried out on individuals stranded along the Italian coasts. Thanks to these strandings we can get to know the species that populate our sea: bottlenose dolphins, short-beaked common dolphins, striped dolphins, sperm whales, large fin and minke whales and some new and rather rare species such as the kogia. Knowing the genetic structure of their populations is crucial for conservation purpose. In fact, geographical and physical features of Mediterranean Sea can promote isolation of their populations, also considering phylopatric behaviour of cetacean species, favouring genetic divergence compared to Atlantic ones: these populaitons could be consequently managed as distinct units, in the light of their uniqueness. This is the case, for example of fin whales, for which unique mitochondrial genotypes were described for Mediterranean populations (Alexander et al., 2016.). on the other hand, cetaeans are very mobile animals, being able to migrate for thousands of kilomerers, thus it may be very important to evaluate exchanges with Atlantic populaiton through Gibraltar straits or with Indo-Pacific ones through the Suez Canal. In the light of climatic changes currently in progress, it is also very interesting to evaluate the possible meaning and geographic origin of species, never reported in the past or only occasional visitors, which are now increasingly observed in the Mediterranean basin. Another significant issue possibly disentangles with the aid of genetic marker is linked to interspecific hybridization, very common both in odontocete and mysticete whales. In this work, genetic studies were carried out on different species of cetaceans stranded on the italian coasts: sperm whales, fin and minke whales and kogia. For all specimens, studies were conducted through the use of nuclear and mitochondrial DNA marker with the aim of tracing the origin of stranded animals. In addition, morphological traits were considered with particular reference to the small size generally reported for the Mediterranean sperm whale population. This aspect of research was faced with skeletochronological approach, by analyzing tooth section that register variation as annual circuli permitting a correlation between age and size of animals. In particular, it was considered if the history of the Mediterranean and the

characteristics of this basin respect to the ocean, may have influenced the average size of these large mammals. All these aspects have been considered in this work in order to enrich our knowledge on cetaceans in the Mediterranean Sea and better understand its role in cetacean evolution.

1. Introduction

1.1. Who are Cetaceans and where are distributed?

Cetaceans are a clade of placental mammals, perfectly adapted to aquatic life. The origin of the name derives from the Greek word "κῆτος" (kētos), which means sea monster and appears for the first time in history mentioned by Aristotle (Rice, 1998). This infraorder includes about 85 different species of which 80 are marine species while 5 are freshwater species. They are further classified into two subclades: Mysticeti and Odontoceti. The name "Mysticeti" refers to baleen: they are made of a substance containing keratin that represent an expansion of the epidermis that the whales use for feeding by filtration, they feed on small crustaceans such as krill and other planktonic organisms. Odontocetes, as the name suggests, have a mineralised teeth and are predators. The cetaceans, while maintaining the characteristics of the class to which they belong, have developed morphological adaptations that have allowed them to colonize the marine environment and in some cases also the river environment. Cetaceans populate all the oceans and seas of the world and in some cases also estuaries and rivers in North America, South America and Asia. Some species, such as the orcas (*Orcinus orca*) are cosmopolitan, others are widely diffused and still others are confined to very restricted areas; this is the case of a species of harbour porpoise (*Phocena sinus*) endemic to the northern part of the Gulf of California and of which to date there are only twelve individuals. Cetaceans are further divided into those living in the neritic province, near the coast, and those found in the oceanic province, in the open sea. In the Italian peninsula, the stretch of sea that is of greatest interest for the wealth of cetaceans is between the western Ligurian Sea, the Corsican Sea and the Gulf of Lion. Their presence in this stretch of sea is favored by the abundance of nutrients and the phenomenon of upwelling. Furthermore, the sea of the Italian peninsula is rich in protected areas and national parks to protect the marine environment, and this creates favorable habitats and conditions for the development of many species of cetaceans. There are ten species in the Italian sea between Misticeti and Odontoceti which, according to their needs, are divided into pelagic species (2000m deep), species of continental slope (between 1000 and 1500m) and coastal species (<200m). The Mysticetes that we can find are

the Fin whale (*Balaenoptera physalus*) and the Minke whale (*Balaenoptera acutorostrata*) both pelagic species. Among the Odontoceti we find both pelagic, continental slope and coastal species. Along the coasts, in the shallow waters it is easy to come across the Bottlenose Dolphin (*Tursiops truncatus*), at increasing depths we find the Dolphins (*Stenella coeruleoalba*). In the continental slope areas it is not uncommon to find Sperm whales (*Physeter macrocephalus*), Risso's dolphins (*Grampus griseus*) and Pilot Whales (*Globicephala melas*); it becomes rarer to spot the common dolphin (*Delphinus delphis*), now reduced to a small population. In past years there have also been sightings of Orcas (*Orcinus orca*), Right Whales (*Eubalena*) and even Humpback Whales (*Megaptera novaeangliae*), Atlantic species that cross the Strait of Gibraltar and reach our waters.

1.2. Anatomical and physiological adaptations to acquatic life

Cetaceans are the largest existing mammals and the primacy they hold was made possible following their conquest of the aquatic environment. In fact, in this habitat the force of gravity is compensated by the buoyancy thrust and both the vital functions that the metabolism of these animals are not affected by living in this conditions (Elsner, 2002). The only force they are required to overcome in order to move is the friction with the water, which is stronger the larger are the body dimensions of the mammal. Their body has a hydrodynamic shape, a property accentuated by the complete absence of hairs which would have generated friction with the water during motion. The skin is three-layered: from the surface to the depths we find epidermis, dermis, hypodermis, each of a different composition. The epidermis is organized in a multi-layered floor of cells, at least 10 times thicker than that of terrestrial mammals; the dermis is composed of dense connective tissue without hair follicles; the hypodermis forms the blubber a layer rich in adipocytes and collagen which creates an insulating area that limits the dispersion of heat (Elsner, 2002). The telescopic head has elongated mandible and maxilla to form a structure called the rostrum: it assumes a certain importance in the perception of sounds and therefore in communication and hunting (in Odontocetes). The caudal fin is in a horizontal position and has a propulsive function for swimming; the dorsal fin may or may not be present depending on the species, is placed vertically with respect to the axis of the body and has a stabilizing function (Nicolosi, 2010). Although they have populated aquatic environments, cetaceans are still mammals and therefore need to breathe air to survive. However, all other biological functions are performed in water, and therefore the physiology of their systems is extremely specific. The breathing system has an external outlet, in a median position on the head: the blowhole. This has a powerful musculature that allows it to tighten when diving and to open up during surfacing. The muscles surrounding this opening are voluntary therefore, unlike other mammals, theirs is voluntary breathing. In the Odontoceti the blowhole consists of a single opening, while in the Misticeti by two. In order to increase the exchange surface, the alveoli consist of two layers of respiratory capillaries. Lung volume is reduced to avoid embolisms during ascents

after apneas; the lungs of cetaceans are able to completely collapse at great depths, in order to avoid the onset of: decompression sicknesses and nitrogen narcosis. During diving, the oxygen supply to vital organs must also be kept under control, and the best way to do this is to adopt a peripheral vasoconstriction in which the organs that are less affected by the absence of oxygen, pass from an aerobic to anaerobic catabolism. Moreover, the cetaceans are bradycardic, in this way they limit the venous flow back to the heart but are able to keep blood pressure constant thanks to the vasoconstriction mechanism (Elsner, 2002). Their circulatory system is composed of "retia mirabilia", a dense branch of arteries that are concentrated in the peripheral regions such as the tips of the pectoral and caudal fins, with the function of reserve of oxygenated blood (Ponganis, 2002). In relation to osmosis, cetaceans have had to evolve specific adaptations: since their body fluids are hypotonic with respect to sea water, they tend to lose them with the diet which, being rich in proteins, creates a significant amount of nitrogen residues. To eliminate these compounds and at the same time avoid dehydration, they produce urine that is hypertonic compared to sea water and their liquids (Elsner, 2002). Cetaceans are warm-blooded animals and are the only ones with this characteristic to live in an aquatic environment, this is because evolution has allowed them to be able to maintain a constant body temperature. To do this, it is necessary that the loss of heat in the external environment due to the dense network of capillaries that covers the surface of the body, and which is more concentrated in the caudal and pectoral fins, is balanced by the production of the heat at the metabolic level (Nicolosi, 2010). Furthermore, the thick layer of fat limits precisely this dispersion. In an environment such as the aquatic one where visibility is poor, cetaceans, in particular Odontocetes, have developed a very effective system to hunt and communicate: echolocation. This consists in using " clicks " produced in the form of sound waves from the nasal sacs. The air is compressed, producing vibrations which are then directed towards the frontal area of the head where there is a lipid organ called " melon ", an oily body that acts as an acoustic lens to amplify sounds. At this point, the amplified sound waves arrive at the object and are reflected. The echo is received through the vibration of the mandibular bone which, like a sounding board, transmits the sound to the eardrum (Nicolosi, 2010). In the

Misticeti the situation is quite different, since the dietary needs are different; this suborder of Cetaceans feeds on planktonic organisms by filtration, and their size allows them to make a complete meal simply by opening their mouth and capturing a certain amount of water which they then push out through the baleen which act as filters, trapping the organic material. This type of feeding does not require a prey detection system, therefore in the Misticeti melon is small (Heyning 1989), possibly vestigial (Milinkovitch, 1995) melon.

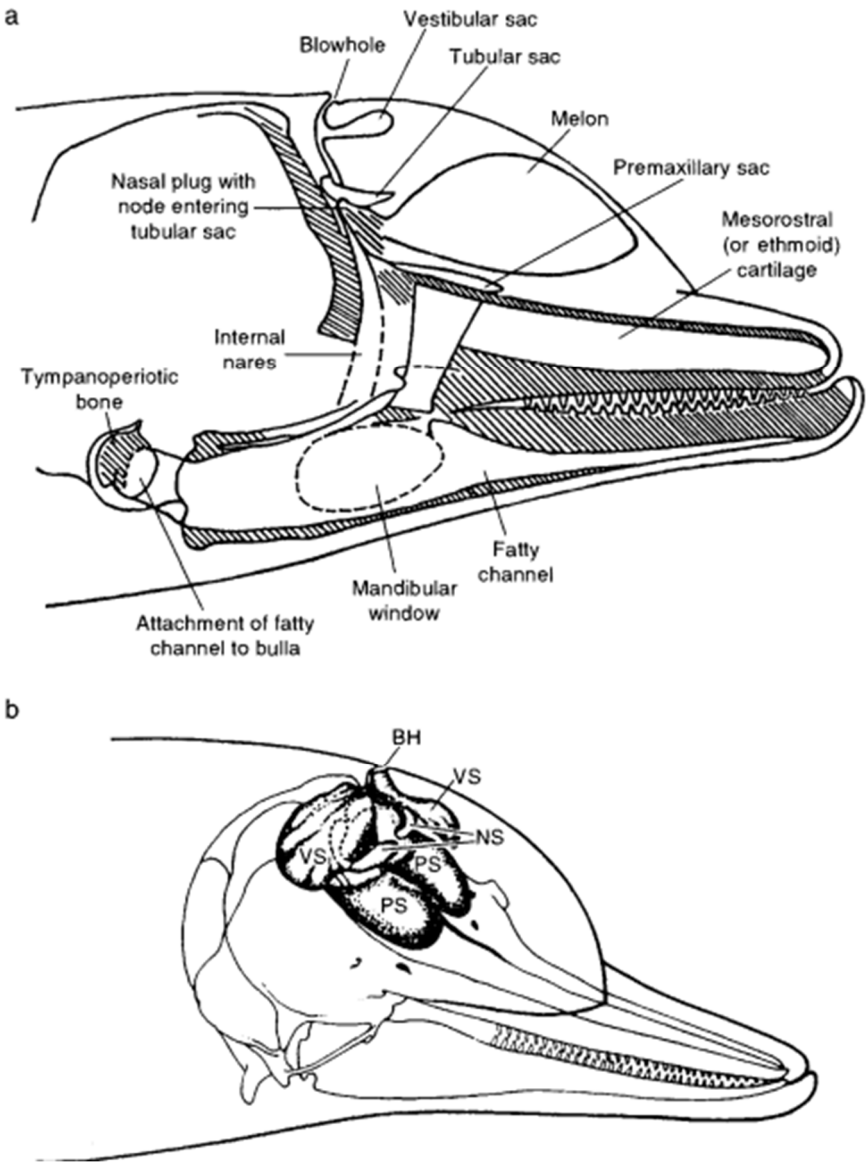


Figure 1 (a) Schematic illustration of a dolphins head (adapted from Norris, 1968) and (b) three-dimensional diagram of the air sacs in a dolphins head. PS, premaxillary sac; VS, vestibular sac; NS, nasofrontal (tubular) sac; AS, accessory sac. Adapted from Purves and Pilleri (1983).

1.3. Threats in Mediterranean Sea

The main threats in Mediterranean Sea for Cetaceans derive from the interactions with human activities: a) global warming (Gambaiani et al., 2008), b) interactions with fishing activities and c) pollution (Giuseppe Notarbartolo di Sciara et al., 1997). Regarding global warming, it has been demonstrated a strong correlation between greenhouse gas emission and the increase in temperature on global scale. The Mediterranean is an oligotrophic sea because of its low nutrient input from rivers and the nutrient depleted Atlantic water inflow through the Strait of Gibraltar. There are different mechanisms of nutrient input into the euphotic zone on which marine productivity is dependent, such as winter vertical mixing, coastal upwelling and river runoff. Therefore, by altering oceanic features, climate change may affect nutrient availability (Gambaiani et al., 2008). The distribution of cetaceans, is closely related to environmental parameters such as oceanographic features and food availability, they are not likely to be able to adapt to rapid shifts in temperatures and environmental conditions, so climate change may represent the most serious long-term threat to cetacean (Burns, 2001). In the Mediterranean Sea, the decline of several cetacean populations has been associated with the reduction of prey resources: for example, a reduction in the abundance of pilchards (*Sardina pilchardus*), could represent a serious problem for some Odontocete species because it is the main food for some of them. Also cephalopods are very important because represent the main food supply for numerous Mediterranean cetacean species and they seem to be particularly vulnerable to environmental changes including pH and temperature. Shifts in prey species availability may force cetaceans to change their feeding strategies and spend more time and energy foraging, which could have drastic consequences on their health and could affect their immune systems (Northridge, 1984; Shane, 1990; Bräger, 1993; Smith & Whitehead, 1993; Agardy, 1996; Stern, 1996; Bearzi, 2002). This feeding problem could also affect the social behaviour of some cetacean species: infact if they spend a lot of time and energy to feeding time, leave less time to social interaction at the expense of sexual reproduction. In addition an increasing temperature, can lead to a strong proliferation of viruses and patogens that can promote epizootic events like morbillivirus infections which are the main causes of stranding and death.

Cetaceans are long-lived, slow-reproducing animals and when a population is severely affected by a virus or other agents, recovery may be slow and such species can relatively easily become endangered (Dhermain et al., 2002; Reeves et al., 2003). The overfishing is another anthropogenic threats that affect cetaceans' survival, in addition to the fact that species such as coastal bottlenose dolphins and common dolphins are competing with fishermen for prey species exploited by fisheries (Di Natale and Notarbartolo di Sciara, 1994, Bearzi, 2002; Abad et al., in press). Bottlenose dolphins in the Mediterranean are known to damage artisanal fine-mesh gear and take fish from nets (Consiglio et al., 1992; Cannas et al., 1994). This led to human hostility which often results in directed mortality (Duguy et al., 1983a; Silvani et al., 1992; Di Natale and Notarbartolo di Sciara, 1994), or the use of high-intensity sound emissions to remove dolphins from the fishing areas (Mhenni,1993). Pollution is another threat for cetaceans in the Mediterranean and it can relate to accumulation of some chemical compounds that can be toxic, like organochlorine compunds such as polychlorinated biphenyls (PCBs) and para-dichlorodiphenyltrichloroethane (DDT). These compounds can migrate down the food web and thus make to the top of the food chain (Giuseppe Notarbartolo di Sciara et al., 1997). Cetaceans, and in particular the odontocetes, are apex predator which means that they can accumulate toxic compounds in their liver or fat. Although the effects of these pollutants at the level of populations is lacking, it is well known that organochlorines can reduce reproductive rates, alter skeleton development, cause cancer, hypertension, stroke and reduce the immune system of mammals (Cummins, 1988; Luster and Faith, 1979). Another important form of pollution that threatens cetaceans in Mediterranean is the presence of floating plastic. This problem is strong in Mediterranean, where peculiar oceanographic conditions can favour the accumulation of floating plastic (Morris, 1980). Plastic ingestion is a known cause of cetacean mortality (Tarpley and Maritz, 1993) because plastic debris can obstruct the digestive tract and lead these animals not to feed. Infact this is one of the most frequent coauses of cetaceans stranding in the Mediterranean (Cagnolaro and Notarbartolo di Sciara,1992). Furthermore, human activity has led to the introduction of noise sources into the marine environment. These sources can be indirect and therefore be a consequence of another activity

such as shipping, or they can be direct such as the use of sonar or other instruments for seismic surveys and oceanographic tomography. This type of noise pollution can alter the behavior of cetaceans both in terms of prey identification techniques and in terms of social communication and orientation (Giuseppe Notarbartolo di Sciara et al., 1997). All that kind of pressures could have synergistic or cumulative impacts with climate change and can influence significantly the presence of cetaceans in Mediterranean Sea.

1.4. **The cetacean species dealt with this work**

1.4.1. Physeter macrocephalus

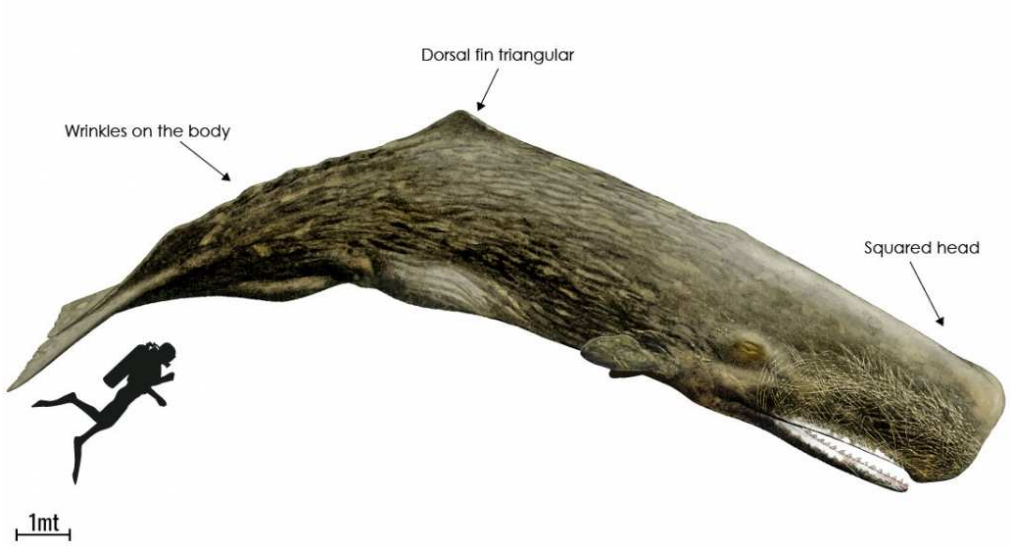
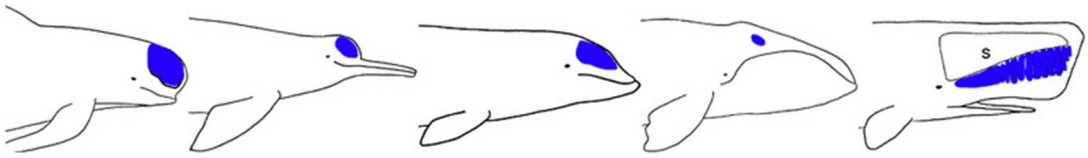


Figure 2 Lateral view of the right side of a sperm whale and size comparison with reference to a human size (Tethys Rsearch Insitute website).

Sperm whales (*Physeter macrocephalus*; Linnaeus, 1758) are the largest of the Odontocete and long ago they were consider closely related to baleen whales than toothed whales. However, more recent genetic and morphological analyses support the original inclusion of sperm whale among Odontocetes. They belong to the family of Physeteridae which is divided into two subfamilies, the extinct Hoplocetinae animals with functional teeth in both upper and lower jaws, and Physeterinae in which the upper dentition is included in the gums and is not used for feeding. The head of the sperm whale and in particular the massive nasal complex, covers up to 1/4 of the total length of the animal's body and is located in the front part of the skull. This portion contains the spermaceti organ, a spongy tissue structure immersed in spermaceti oil. It is believed that it has a fundamental role in maintaining and regulating its attitude both in surface and deep waters (Raven & Gregory 1933; Clarke 1970). Because of the high specialization of the giant sperm whale facial anatomy, it is unclear what structure in this species is homologous to the melon of other cetaceans. Between the spermaceti organ and the upper jaw is the "junk", an agglomeration of spermaceti oil and connective tissue. However, based on the comparison between the facial anatomy of dwarf and pygmy sperm

whales, Heyning (1989) suggested the junk rather than the spermaceti organ as the most likely candidate to be the homologous to the melon. This hypothesis is



supported by comparative CT-scan analyses (Cranford et al. 1996).

Figure 3 The melon is a fatty structure located in the forehead of all cetaceans. In most toothed whales, it serves as an acoustic lens for echolocation sound production. The melon is shown in blue for the genera *Globicephala*, *Inia*, *Ziphius*, *Balaena*, and *Physeter* (from left to right). **S**, spermaceti organ (Michel C. Milinkovitch, 1995).

The colour of the body of sperm whales is mostly dark gray, but there is often a bright white lining to the mouth and sometimes white patches on the belly. The dorsal fin is low, thick, and usually rounded and it may be topped by calluses in mature female: adult males and females can be distinguished not only by size differences, but also by the presence or absence of calluses on the dorsal hump. A large percentage of females (about 85%) have calluses, whereas males almost never have them. Regarding their distribution, sperm whales are considered a species with a very wide distribution range that goes from one pole to another (Jefferson et al., 1993). Furthermore, several studies have highlighted the great ecological importance of these animals: they are apex predators that feed in depth and defecate on the surface, favoring the vertical mixing of substances of extreme importance for primary production. Therefore, their ecological role is essential for the maintenance of a healthy sea. Furthermore, when they die, the carcasses tend to fall down to the deep, constituting an important food resource for the deep environments (Violi, 2020). In Mediterranean, the sperm whale is one of the resident cetacean species and their social distribution is different between age/sex classes: generally, the adult males are distributed in the northernmost part of the Mediterranean while the adult females, calves and juveniles are confined in the southern part of the sea. This organization reflects the same distribution of the sperm whales in the ocean where males, when reach sexual maturity, go away from social unit and migrate to the

higher latitudes. At the mid-latitude we find groups of maturing males that are called "bachelor", at the high latitude we find singleton mature males and at the tropical and subtropical latitude are females, calves and juveniles. The distribution along the latitude that exists in oceanic sperm whales, also exists in the Mediterranean Sea, but is on smaller scale because it has a restricted latitudinal range and probably has not the conditions for a finer segregation (Rendell and Frantzis, 2016). Therefore, the described classes probably inhabit the same areas, but they are not closely associated. This could promote an intraspecific competition for prey resources among age classes. Understanding whether this competition is a consequence of sympatry, is a key point: if lactating female sperm whales are facing competition for resources from subadult males that they do not face in other populations, this could lead to constraints on population growth rate that are not predicted by studies outside the Mediterranean Sea (Rendell and Frantzis, 2016). Indeed, from the morphometric data collected from sperm whales stranded on the Mediterranean coasts, a reduction in size was observed. Age determination studies conducted on some samples of the teeth of these sperm whales have allowed to establish the age of the individuals to be related to the size to support the hypothesis of the reduction in size (Violi,2020). Concerning feeding strategy, sperm whales perform deep dives in search of food, and through echolocation they produce a continuous sequence of clicks to locate prey. Sperm whale clicks are made up of several regularly spaced pulses resulting from multiple reflection of the initial sound within the head of the animal. From the analysis of the stomach contents of beached sperm whales it was possible to trace their type of diet: their "favorite meal"is made up of cephalopods. Mazzariol et al. (2011, 2018) analysed the stomach contents of sperm whales stranded en masse on the coasts of the Adriatic Sea in two different years, in 2009 and 2014, and highlighted the cephalopod families they mainly feed on. Members of the *Histioteuthidae* family have been found which represent the main source of food for animals that feed in deep environments; the representatives of this family in terms of species were 71% *Histiotheutis bonnellii*, 24% *Histiotheutis reversa* followed by *Architeuthis lesueurii* and *Octopoteuthis sicula*. These species are meso or bentho pelagic and are more distributed in the Ionian or southern Adriatic Sea. Concerning the feeding of atlantic sperm whale Clarke et al.

(1993) analysed the stomach content of stranded animals in a range period between 1981-1984. The results hilight that the Atlantic feeding area has a greater variety of prey species and the latter have also a greater energey value than the Mediterranean ones. So the mediterranean sperm whale feed on smaller species which also have a lower energy value and this result in an energy disparity between the Atlantic and Mediteranean populations. Despite the lower energetic value and the lesser variety of their prey, sperm whales are still present in our sea is bacause their feeding area is linked to the deep environment and therefore they are independent from the trophic chain of surface waters. This gives a sort of advantage to sperm whales over other marine species, avoiding interspecific competition for the resource and seems to justify their presence in an oligotrophic environment such as the Mediterranean. The presence of a stable population of sperm whales in the Mediterranean Sea has been discussed for some time: molecular studies conducted with mitochondrial and nuclear markers have highlighted the isolation of the population of these sperm whales from the Atlantic one (Drouot et al., 2004). In very remote times it seems that a part of the Atlantic population of sperm whales broke away to reach the Mediterranean and remain within it. The Strait of Gibraltar represents a physical barrier that led to the isolation of this new population which has adapted and modified according to the characteristics of the new basin. Despite this, there is some evidence that demonstrates the maintenance of a gene flow through the Gibraltar strait and, even if minimal, it is such as to guarantee the absence of inbreeding and the complete decline of the population due to the total lack of genetic variation (Drouot et al., 2004). Considering the Mediterranean sperm whale as a population evolutionary diverging from the Atlantic one has significant conservation consequences: in the Mediterranean, according to the IUCN (International Union for Conservation of Nature), the sperm whale is considered an endangered category (Notarbartolo di Sciara et al., 2012). Initially it was thought that there was also isolation within the Mediterranean population itself, between the eastern and western basin. This hypothesis was subsequently supported by the data of photo-identified specimens documenting their movement between the two basins and by the analysis of the isotope $\delta^{15}N$ and $\delta^{13}C$ in a tooth obtained from a stranded male (Rendell and Frantzis, 2016). It was observed a shift in GLG (growth layer groups)

isotopes levels which occurred as the animal attained 20 years of age. This is around the age in which male sperm whales in other oceans typically make large movements from feeding to breeding grounds (Whitehead, 2003), and indicates a significant change in dietary sources of these stable isotopes. It is known that there are significant variations in isotope levels between the eastern and western basins of Mediterranean sea, and this led to suggest that the observed shift in isotope levels could indicate that the male had moved from the western to the eastern Basin.

1.4.2. Kogia spp.

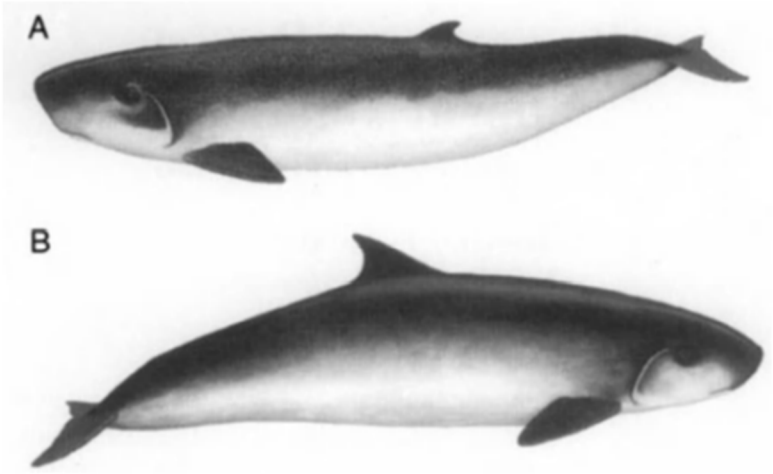


Figure 4 (A) *Kogia breviceps*. The relatively short and more posteriorly positioned dorsal fin is useful to distinguish this species from (B) *K. sima.* PieterA. Folkens/Higher Porpoise DG

The genus *Kogia* includes two different species: dwarf sperm whale (*Kogia sima* Owen, 1966), and pygmy sperm whale (*Kogia breviceps* de Blainville, 1838) and it is only since 1966 that these two species of Kogia have been recognized. Both are small odontocetesthat generally live off the temperate and tropical seas and sometimes strand along the coasts of some regions including the Italian ones. These two species are part of the Physeteridae family even if the morphological characteristics between kogia and sperm whale are quite different. The height and position of the dorsal fin are diagnostic morphological characteristics that allow us to distinguish the two species. Pygmy sperm whales reach a maximum size of about 3.8 m total length and a weight of 450 kg while Dwarf sperm whales are smaller at 2.7 m and 272 kg. Both species, when beacame adult, are dark bluish-gray to blackish-brown dorsally with a light venter. On the side of the head, between the eye and the flipper, there is often a crescent shaped, light-colored mark referred to as a "false gill" (Donald F. McALpine, 2002). *Kogia* spp. have the shortest rostrum among living cetaceans and their skull is asymmetrical; their teeth are small and thin and are present only in the lower jaw for *Kogia breviceps* while in *Kogia sima* there are three pairs of vestigial teeth in the upper jaw. Like sperm whales, *Kogia* spp. have the spermaceti organ and its function is apparently the same that has in *Physeter macrocephalus*. In *Kogia sima*

the sexual maturity is reach at about 2.1 m in lenght while in *K. breviceps* males reach the maturity at about 2.7 m and females at a smaller size The studies conducted on the stomach contents of the stranded specimens made it possible to identify the hunting and living area: along the continental shelf in the epi- and mesopelagic zone. From the analysis of the prey it emerged that *K. sima* feeds deeper than *K. breviceps* and the favorite prey of both are cephalopods, although they also feed on crustaceans and fish. Hunting occurs near the bottom through the use of echolocation, and the hyoid anatomy of *Kogia* spp. suggests feeding by sucking. The causes for the stranding of unhealthy pygmy and dwarf sperm whales have been attributed to degenerative heart disease, as well as being linked to possible immune system problems associated with the thymus gland; furthermore, *Kogia* spp. are infected with intestinal nematodes such as Anisakis spp. and blubber-encysted larval cestodes (*Phyllobothrium delphini*). The studies on the behaviour of *Kogia* spp. are poor, they live in small group of up to 6 (*K. breviceps*) or 10 (*K. sima*) but the stranding involve single animals (McALpine D.F., 2002). The IUCN classifies both *Kogia* species as "Data Deficient". Regarding their presence in Mediterranean Sea, in 1988 and 2002 two cases of stranding of two specimens of *K. sima* on the Italian coasts were recorded respectively at the Foce Chiarone (Larium - Tuscany border, Italy: 42 ° 23'N-11 ° 27'E) and in Eraclea Minoa near Agrigento in western Sicily. According to the criteria indicated by Handley (1966) and Ross (1979, 1984) and recently discussed by Nagorsen (1985), the identification was made up by morphological analysis based mainly upon cranial features: the condylobasal lenght, shape of dorsal cranial fossae, pinched shape of sagittal septum, lenght of mandibular symphysis (45 mm), size (15-25 mm x 3-4.5 mm) and number (10) of mandibular teeth, provided most help in the distinction from the very similar species *Kogia breviceps* (Baccetti et al., 1991). A third stranding along the Italian coasts occurred in 2017 in Baia di Trentova, Agropoli, Salerno. To correctly determine the attribution of a species once the morphological analysis has been used, a molecular analysis must also be performed. Maio et al., (2017) carried out molecular analysis on both this last stranded specimen and the one streanded in 2002 in order to confirm the species determination previously assigned by morphological analysis. They analysed a mitochondrial 16S r RNA gene fragment which was amplified by PCR

using the universal primer pairs 16Sa and 16Sb (Palumbi, 1996) and then was sequenced, confirming identification as *K. sima*. Conversely Chivers et al., (2006) amplified 406 bp of mtDNA control region and 398 bp of the mtDNA cytochrome *b*. Molecular analyses are also very useful identify the origin of a particular specimen in order to better understand its movements and way of life. No stable population of *Kogia* spp. has been identified in the Mediterranean (Notobartolo, 2002).

1.4.3. Balaenoptera physalus

Figure 5 Lateral view of the right side of the Fin whale showing asymmetric coloration of the cephalic region. Pieter A. Folkers/Higher Porpoise DG.

The fin whale (*Balaenoptera physalus* Linneus,1758) is a mysticete, which means that its main feature is the complete absence of teeth and the presence of baleen. It belongs to the Balaenopteridae family and shares the same number of chromosomes with other members of the family (2n=44). This whale has a slender morphology with a narrow rostrum and it has about 400 baleen on each side of the upper jaw and these are up to 70 cm long. The dorsal fin is very curved and shifted cadually and is located at 75% of the total length of the body, the pectoral fins are small and the caudal fin is very large and equipped with powerful muscles that allow it to reach a speed of 37 km / h while swimming. A very peculiar feature is the asymmetric colouration: the right side of the fin whale is white while the left one is dark/gray. Even the baleen are affected by this colour asymmetry in fact those on the whole left side and the rear two-thirds of the right side are gray, whereas those on the front third of the right maxilla are whitish (Aguilar, 2002). The whale's diet includes different organisms and the variability depends on the season and the foraging area. In the northern hemisphere, fin whales have a very varied diet consisting of small school pelagic fish, numerous crustaceans and even small squid, although their favorite food appears to be the krill made up of euphasiid such as *Meganyctiphanes norvegica.* In the southern hemisphere, however, the diet is almost entirely made up of krill and other small pelagic crustaceans. Very often the distribution range of the fin whale and its diet overlap with those of other rorqual species, resulting in mixed schools (e.g., with the blue whale). Cetaceans are social animals and very often live in groups that allow them to improve hunting

techniques and also to protect them from any predation (Crossman et al., 2015). Living in a group, however, also entails risks of inbreeding but also the increase and transmission of parasites. Mixed schools involve the same benefits described above but imply the risk of interspecific hybridization (see. par. 1.5). Between Northen and Southern Hemisphere there is a strong difference concerning the body dimention: fin whales of the southern hemisphere have an average length of 26m for females and 25m for males; in the northern hemisphere, on the other hand, they have an average length of 22.5 m for females and 21 for males. These values highlight a slight sexual dimorphism between males and females and also a substantial difference in size between the specimens of the two hemispheres (Aguilar, 2002). These differences suggest a spatial and genetic isolation between the northen and southern populations with the recognition of three subspecies: *B. physalus physalus* (Linneus, 1758) inhabiting the northern hemisphere, *B. physalus quoyi* (Fischer, 1829) in the southern hemisphere and the pigmy fin whale *B. p. patachonica* (Burmeister, 1865). Fin whales are migratory animals and make a seasonal shift between feeding and breeding areas, respectively at high and low latitudes and the characteristic of these whales to be wide-ranging migratory makes them more vulnerable to the effects of climate change and to anthropic impact (Clapham et al., 2008; MacLeod, 2009; Lascelles et al., 2014). Gauffier et al., (2018) analysed 15 yr of direct observations combining vessels and land-based surveys and discovered a bi-directional migration through the Strait of Gibraltar. The direction of migration is seasonal and seems that all fin whales travel towards Atlantic Ocean between May and October and towards Meditearranean sea between November and April. Furthermore, the observation of young fin whale exiting from this latter sea, highlights that some of these specimens may give birth in the basin. It was found that in Mediterranena there is a subpopulation genetically distinct from those inhabiting the North Atlantic Ocean (Berubè et al., 1998). In the area between the Ligurian Sea and the central Tyrrhenian Sea, were estimate about 500 individuals (Lauriano et al., 2011) whereas in the Santuario Pelagos, the comparison between data from 1992 (Forcada et al., 1995) and 2009 (Panigada et al. 2011) seems to indicate a decline in population size infact the trend of population, according to the information sheet on IUCN site, is “declining”. According to the C2a (ii) criterion this species would be evaluated as

Endangered (EN), but thanks to the immigration of individuals from the Atlantic, is evaluated as Vulnerable (VU) in IUCN Red List of Threatened Species.

1.4.4. Balaenoptera acutorostrata

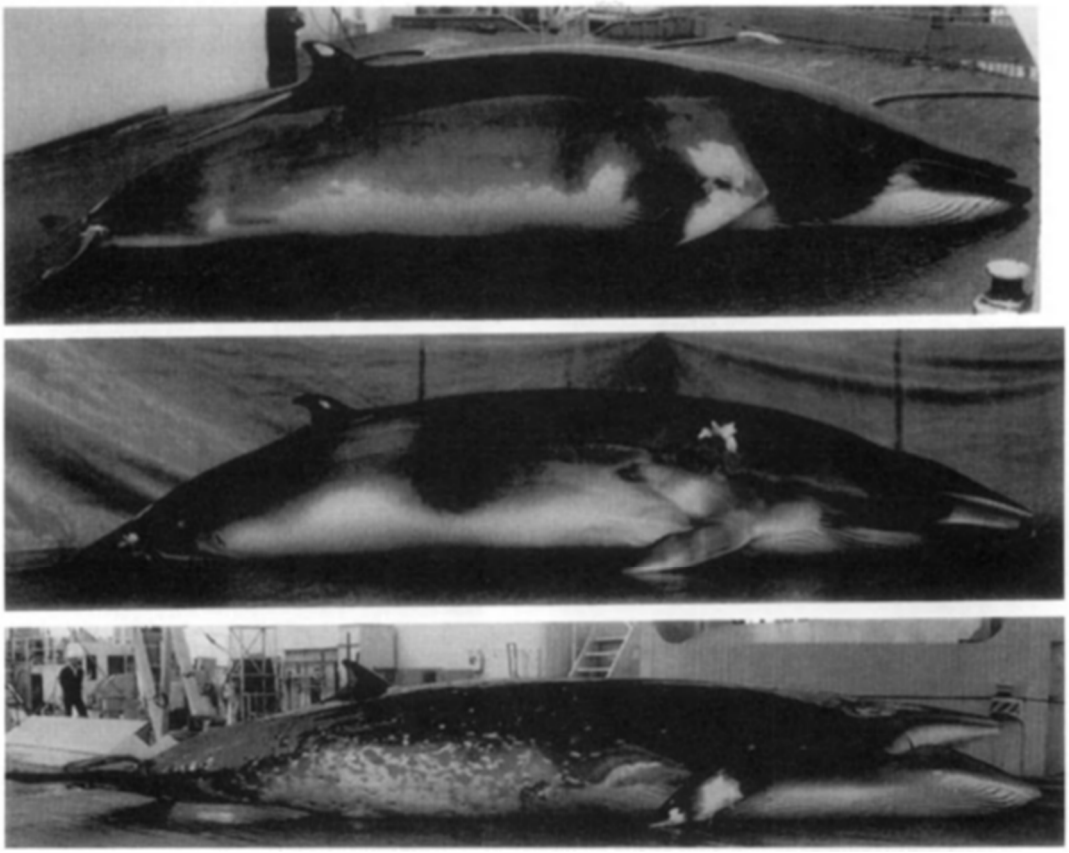


Figure 6 Top) Dwarf minke whale (*Balaenoptera acutorostrata*), (middle) Antarctic minke whale (*B. bonaerensis*), and (bottom) North Pacific minke whale (*B. acutorostrata scammoni*). Photos courtesy of Hidehiro Kato.

The common minke whale, is the smallest species of the rorquals (family of Balaenopteridae) and since the 20th century has been considered single species, *B. acutorostrata* (Lacépède, 1804). Morphological and genetic evidencce allowed to recognize a second species confirmed in the 1990s: *B. boanerensis* (Burmeister, 1867) the Antartic minke whale. Within the species of common minke whale Rice (1998) recognized three subspecies: the North Atlantic minke whale (*B. a. acutorostrata*), the North Pacific minke whale (*B. a. scammoni*, formerly *B. a. davidsoni*), and the unnamed Southern Hemisphere dwarf minke whale that is much smaller than Antartic minke whale and has a white mark on the flipper like a distinctive characteristic of the Northen Hemisphere species while it's absent in the Antarctic specimens. Both the species have a narrow and pointed rostrum, the dorsal fin is tall and falcate and positionated at one-third of the body. In the North Atlantic, mature

minke whales reach 8.5 m in males and 7.8-8.2 m in females, in Antarctica the females reach 9 m while the males a maximum of 8.5 m while the dwarf minke whale is smaller than the Antarctic one by about 2 m. Between northen and southern species there is a differentiation in the colour of baleen: the first one has white baleen while the second one have it dark-grey or brown baleen due to a narrow fringe. In the Antarctic minke whale the baleen plates are black on the left while on the right side are white in the first third and black in the remaining two thirds. Regarding the morphological characteristics, *B. bonerensis* has the skull larger than the other species of *B. acutorostrata* (Perrin et al., 2002). The common minke whale is a cosmopolitan species but it is discontinously distribuited throughout the Northen Hemisphere and is rare at tropical level. On the southern hemispehere the dwarf minke whale and *B. bonerensis* share the same feeding ground so are seasonally sympatric during austral summer and the same could occurs off South Africa during fall and winter. The diet of this species is quite varied and in the North Atlantic ranges from euphasiatic (planktonic component) to fish (herring, cod, mackerel, etc.); in the Antarctic, dwarf minke whales feed mainly on lanternfish but also on some euphausiids (Kato and Fujise, 2000), while Antarctic minke whales feed mainly on euphausiids. Both species of common minke whale could be the preys of Killer whales (*Orcinus orca*): Antarctic minke whales make up 85% of the diet of killer whales in the Southern Ocean (Stewart and Leatherwood, 1985). It is listed as "Vulnerable" at the European level, and classified as "Least Concern" on the IUCN Red List of Threatned Species (vers.2016–2) (Reilly et al., 2008). Regarding the presence of common minke whales in Mediterranean Sea, the ACCOBAMS (Agreement on the Conservation of Cetaceans of th Black Sea, Mediterranean Sea and Countigous Atlantic Area) consider this species as a "visitor", "occasional" in the Italian seas and "vagrant" in Black Sea (Notarbartolo di Sciara and Demma, 1997; Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara and Birkun, 2010; Podestà et al., 2014; Cagnolaro et al., 2015). Genetic studies conducted on individuals stranded along the Mediterranean coasts have shown how their origin was from North Atlantic basin, whereasmorphological and historical studies showed that recently some individuals found in this basin were calves. As soon as they are born, the minke whales are 2.6 to 2.8 m long and remain with their mother

for about 6 months, during which time they migrate towards high latitudes towards cold waters. The recent finding of very young calves in Mediterranean Sea is compatible with the hypothesis that some femalesuse the Mediterranean Sea as a breeding ground (Fraija-Fernández et al., 2015).

1.5. **Hybridization in the Cetacea**

Hybridization is the result of incomplete reproductive isolation between two species considered different from a taxonomic point of view (Mayr, 1963; Arnold, 1992) and that can give birth to fertile or sterile offspring. For the occurrence of this phenomenon three conditions must be present: heterospecific mates that must be genetically and physiologically compatible, predisposition to mating, and overlapping distributional ranges. In cetaceans, hybridization is a very recurrent and natural phenomenon favoured by the biology of these animals (e.g., promiscuous behaviour and prominent karyological uniformity), with the possibility of favouring reticulated evolution and the increase of biodiversity, as suggested by the case of hybrid speciation of *Stenella clymene* (Gray (1846). It derives from ancient events of interspecific hybridization between *Stenella coeruleoalba* and *Stenella longirostris* that gave rise to hybrid stabilized form with its own ecological identity. In fact, ecological divergence has been suggested as one of the main drivers of hybrid speciation, leading to reproductive isolation between the hybrid and the parental species (Amaral et al., 2014). In some cases, hybridization has been shown to increase the genetic diversity of populations providing them with a higher adaptability to environmental change. This is the case of a hybrid between a male beluga (*Delphinapterus leucas*) and a female narwhal (*Monodon monoceros*). These two species have diverged ~5 Ma years ago, have a similar size, similar migratory pattern and both the species reproduce in Spring (Skovrind et al., 2019). It often happens that individuals of narwhals join groups of belugas and *vice versa*. It is interesting to note that the resulting hybrid has a particular set of teeth that are different from the ones of both parents. This allowed to colonize an ecological niche different from the one of its parents. In fact, it feeds on benthic rather than pelagic organisms and this can be understood from tooth morphology and $\delta^{13}C$ isotope analysis (Skovrind et

al., 2019). Cetaceans are social animals and very often live in groups, fact that allows them to improve hunting techniques and also to protect them from any predation (Crossman et al., 2015). However, living in a group also entails risks as regards inbreeding but also the increase and transmission of parasites. It may happen that there are groups formed by different species and this type of aggregation involves the same benefits described above but can promote interspecific hybridization. The hybridization phenomenon can occours also when the equilibrium of a population falters and a demographic decrease occurs and this leads individuals with a lower demographic density to mate with individuals of more numerous populations even if of another species.

Regarding mysticete's hybridization, Cocks (1887), a researcher accompanying the whalers, was the first that noted unusual morphological features in specimens of *B. musculus* (blue whale) and *B. physalus* and identified them as anomalous individuals. These were subsequently considered hybrids by Bérubé and Aguilar (1998). Previously, the analyses for the identification of a hybrid individual were conducted exclusively at the morphological (morphometric) level and by studying vocalizations. Concerning the morphological characteristics of the hybrid between the blue whale and the fin whale, these are halfway between the morphological characteristics of the two parental species. The characters shared by hybrids with the fin whale are the presence of a well defined, moderately high and falcate dorsal fin, whereas a wide rostrum, with the maxilla slightly curved outward, the uniform colour of the jaw and the right portion of the baleen refer to the morphological characteristics of blue whale (Bérubé et al., 1998). To date, molecular techniques allow to establish with greater reliability whether a specimen is hybrid to trace the parental species. The most frequent case of hybrids in nature is within the Balaenopteridae family, the percentage of hybridization between *B. musculus* and *B. physlaus* is equal to 24.6% (Nacimiento et al., 2012).

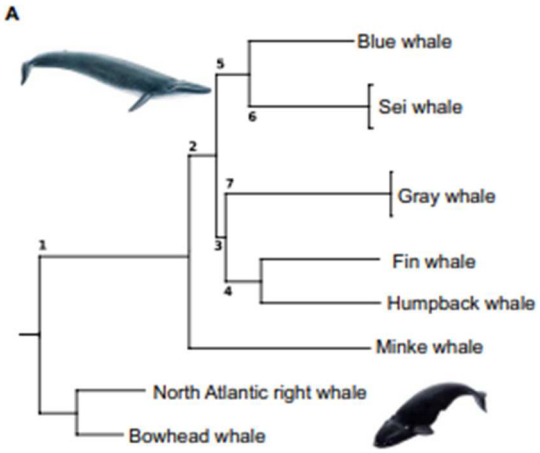


Figure 7 - MSC tree (A) A MSC species tree was constructed from 34,192 individual GFs. Gray whales, family Eschrichtiidae, are placed inside Balaenopteridae as a sister group to fin and humpback whales (Arnason et al., 2018).

In an environment such as the marine one where physical barriers are not the primary cause of speciation and in which migratory behaviors favour gene flow, it is difficult to imagine how these whales could have diverged. Indeed, even after 8 Ma years from their initial divergence, whales can still mix the gene pool by hybridizing, a phenomenon that is facilitated by the uniformity of the karyotype (2n = 44 in all cetaceans). Considering the feeding strategy of baleen whales, it is very important to consider the temporal variation in the territories of grazing on krill, strongly influenced by climatic change starting from the end of Tertiary. Indeed, the radiation of the whales coincides with the cooling of the late Miocene (about 7 Ma years ago): the modern ocean circulation has led to an increase in productivity in the temperate and polar oceans, thus favouring the evolution of whales in different grazing areas (Pastene et al., 2007; Arnason et al., 2018). Driven by these climatic and ecological changes, the evolution of rorquals (Balaenopteridae *sensu lato*) appears a process of gradual divergences that allowed the simultaneous development of three lieneages: a) blue whale plus sei whales, b) gray whale, and c) fin whale plus humpback whales. All the conflict inside the Balaenopteridae phylogenesis have been resolved by molecular analysis (Arnason et al., 2018) (Fig. 7).

Figure 8(A) Blue whales (Balaenoptera musculus) and (B) Fin whales (Balaenoptera physalus), (Crossman et al., 2016).

Using the hybrid similarity index, Crossman et al., (2015) estimated in which aspects two species are more prone to hybridization than others. The extent of sexual dimorphism between species, with the size of the species interval, the body length of both sexes and the vocalization frequencies appear to be fundamental together with the chromosomal similarity for the generation of a hybrid. Indeed, the species that hybridize are very similar from a morphological and ecological point of view compared to the others that do not generate hybrids. Studies on the hybrid specimens between *B. musculus* and *B. physalus* have shown that female individuals were fertile and able to generate second generation hybrids (Pampoulie et al., 2020) while males were infertile. The evolutionary consequence of hybridization phenomenon can be both insignificant or disastrous depending on the size of the population and the frequency of hybridization. An extreme consequence could be genetic introgression: it can lead to the extinction of a species by completely replacing its genome with that of another more abundant species. The presence of

B. physalus in the North Atlantic is equal to ~ 80,000 individuals (Aguilar and Garcia-Vernet 2018) while that of *B. musculus* is about 2,100-4,000 individuals (Sears and Perrin, 2018) and the incidence of hybrids between these two specimens are underestimated (Pampoulie et al., 2020). Between these rorqual species, the hybrids result mainly by female blue whale with male fin whale crossing. The kind of hybridization we have in this case is a unidirectional hybridization, due to sexual selection, which causes a reproductive failure in the blue whale with serious conservation and recovery issues in the Atlantic Ocean. Unidirectional hybridization may occur for different reasons, such as size difference, ecological or behavioral bias, but the main one is "the hypothesis of sexual selection for unidirectional hybridization" (Wirtz, 1999). Generally, males of different species court females of other or closely related species while the females are the more selective sex in interspecific encounters and ignore or reject males that court them (Wirtz, 1999). Infact male of rare species (blue whale) can court female of the common species (fin whale) but, in the presence of their own males, the female of fin whale reject males of the blue whale; so males of the rare species do not produce hybrid offspring (Wirtz, 1999). In the same way females of rare species, as in the case of blue whale, initially reject allospecific males but then can mate with them successfully due to the smallest number of conspecific males. Alternatively, the observed unidirectional hybridization could also be due to size constraints and the result of a purely physical/mechanical impossibilities for blue whale male to sire fin whale female (Pampoulie et al., 2020). All this may represent indirect causes of the decrease in the natural populations of blue whales already demographically impoverished by the previous climatic conditions afer the mid-Pleistocene transition and by subsequent whaling phenomena.

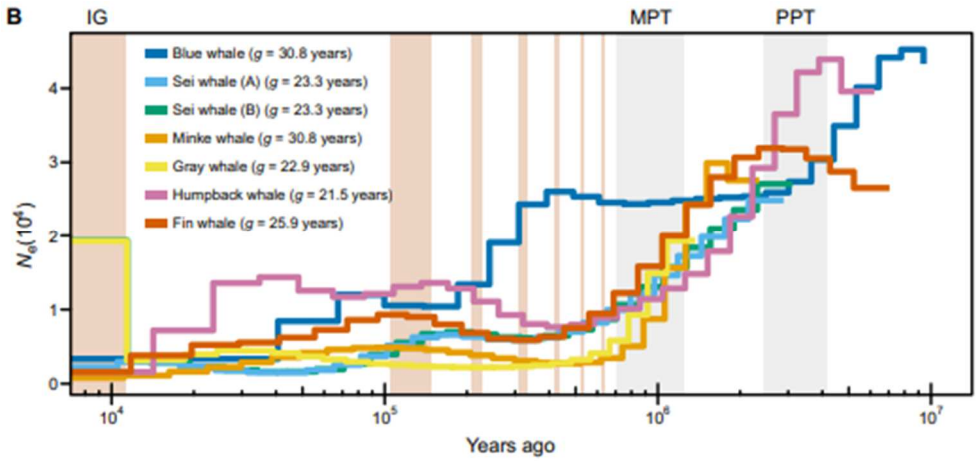


Figure 9 The x axis shows the time, and the y axis shows Ne. Next to the species names there is the generation time. Light brown shading indicates interglacials (IG) in the Pleistocene and Holocene, and gray shading indicates the MPT and the PPT (Arnason et al.,2018).

1.6. Age determination methods in cetaceans

Etsimating the age of the individuals is fundamentale for understanding many aspects of th ebiology of a species, including longevity under natural conditions ("life expectancy"), growth rates, age at sexual maturity and other demographic traits. The study of growth marks in the teeth represents the indirect method most used to estimate age in cetaceans (Klevezal & Kleineberg, 1967; Scheffer & Myrics,1980; Evans et al.,2007; Guarino et al., 2001). This is a method widley accepted and used by the researchers as the incremental layers of growth can be easily identified in sections of mineralized tissues (teeth and bones) although some methodological difficulties associated to the interpretation of the layers are not lacking. In general, according to this method, the individual age can be estimated by counting the annual growth layers' groups (GLGs) that are formed in the mineralised tissues (teeth and bones) of an individual throughout its life cycle; they correspond to deceleration or stopping of the somatic growth, in a similar way to the dendrochronology (i.e., the count of annual growth rings in timber and tree trunks). In particular, one GLG corresponds to one year and is formed by two translucent-thin layers and two thicker-darker or more simply by one dark and one, wide light layer (Evans et al., 2007). The more numerous layers are, the greater the individual age is reached. As early as the 1950', some studies were carried out to

determine the age of *Physeter macrocephalus* using section of teeth (Evans et al., 2001, Pagh et al., 2016). It is useful to remember that in sperm whales, like generally in other mammals, the long bones are not suitable for determining the age of the individual because they undergo remodeling which causes the total disappearance of GLGs. On the other hand, the remodeling process is absent in the case of dentin as well in the cementum and this makes the teeth precious organs for establishing the age estimation methods and their validitation, that is: what is the seasonality in GLG formation? Are they formed annually? In addition, does the number of visible GLGs correspond to the actual LAG (lines of arrested growth) number formed during ageing? The validation techniques generally consist in the use of individuals of known age or labeling experiments with tetracyclines. However, in the case of *Physeter macrocephalus* validation cannot be applied due to the size of individual and consequently to the difficulties of direct observation or manegement of individuals in captivity (Evans et al.,2002). On sperm whales, only the marking and recapturing methods made it possible to estimate the accumulation rate of the growth layers and the calibration of seasonal changes in the thickness of the most recent dentin layer. These studies suggest that GLGs are deposited annually (Ohsumi et al., 1963; IWC, 1967;1971; Best, 1969) and as a result each of these represents one year of the individual's life (Ohsumi, 1971;1977; Lockyer, 1980; Rice ate al., 1986). In addition to the validation problem, there is also teeth modification during aging of the animal: the precision of the count can be lost following the filling of the pulp cavity and therefore the compaction of the most recent layers makes the estimation difficult. Therefore, as a rule, repeated readings are performed by different "readers" to reach a consensus on the estimate of the number of GLGs thus producing an estimate of the most probable individual age. Each reader must not be influenced in any way by others or by photos or by the total lenght of the body of the specimen. Other difficulties in the GLG counting concern: anomalies of mineralization and dentinal resorption (Myrick, 1988; Lockyer, 1993), and the accuracy of the age determination from the teeth of cetaceans associated with the preparation and reading techniques used (Anas, 1970; Hui, 1980; Hohn et al., 1989; Hohn, 1990; Hohn and Fernandez, 1999). Also, the variation of the number of GLG in different teeth of the same individual can be another source of difficulty in the determinations of age. In fact,

in some cases both the mandibular teeth and also the corresponding maxillary ones can fall out and replaced by new teeth; the sperm whales have visible teeth only in the lower jaw because the upper one remain inside the gums. Very often these latter are also analysed because, not being exposed, they are not affected by erosion and therefore can give a more precise reading (Evans et al., 2002).

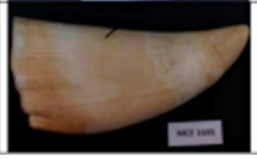
Whale ID and length	Tooth		GLG´s reader 1	GLG´s reader 2	Comment
MCE 1642 12.8 m	Rudimentary non-erupted maxillary tooth		36	31	Pulp stones Average GLG´s: 33.5
	Erupted mandibular tooth A		27	31	Enamel and outer dentine layers worn Average GLG´s: 29.5
	B		29	31	
MCE 1644 14.5 m	Rudimentary non-erupted maxillary tooth		34	33	Average GLG´s: 33.5
	Erupted mandibular tooth A		37	31	Enamel and outer dentine layers worn Pulp stones
	B		43	33	Average GLG´s: 36
MCE 1645 12.8 m	Rudimentary non-erupted maxillary tooth A		38	38	Pulp stones Average GLG´s: 39
	B		41	38	
	Erupted mandibular tooth		-	-	Not sectioned (damaged due to vandalism)

Figure 10. Maxillary and mandibular teeth of 3 individuals of sperm whale streanded along the coast of Denmark in 2010 and 2014 (Pagh et al., 2016).

2. Materials and Methods

2.1. Sample collection

All the samples used for this project were provided by the Mediterranean Marine Mammals Tissue Bank, Department of Biomedicine Comparative and Power supply of the University of Padua, viale University 16, I-35020 Legnaro - Agripolis PD, Italy (BTMMM) and arrived in 50 ml Falcon in a 1:10 ethanol solution. For details on the origin and type of samples used see the table below (Table 1).

Species	ID	Streanding place	Date	Sex	Lenght (m)	Age
Physeter macrocephalus	GP-1	S. Maria di Castellabate (Salerno)	1980	juv	8	8
Physeter macrocephalus	154159	Parghelìa (Vibo Valentia)	26/12/2017	juv	6.10	3
Physeter macrocephalus	45486	San Lucido (Cosenza)	03/04/2018	M	12.16	39-42
Physeter macrocephalus	456	Ischia (Napoli)	26/12/2018	M juv	8.6	11
Physeter macrocephalus	ME15	Contrada Barranca Mare di Acquedolci (Messina)	03/06/2015	F	6.50	25-30
Physeter macrocephalus	BA14	Polignano a Mare (Bari)	29/09/2014	juv	8	NA
Physeter macrocephalus	Ph1	Forio Ischia (Napoli)	1770	M	10	NA
Physeter macrocephalus	400	Bagheria (Palermo)	12/10/2016	F	8.4	NA
Physeter macrocephalus	463	Porto Cervo (Arzachena, Sassari)	28/03/2019	F	8(pregnant)	NA
Physeter macrocephalus	465	Capo Plaia, Cefalù (Palermo)	16/05/2019	F	6.26	NA
Physeter macrocephalus	466	Capo Calavà, Gioiosa Marea (Messina)	21/05/2019	M	5.35	NA
Physeter macrocephalus	467	Acqua dei Corsari, Palermo	19/05/2019	M	8.5	NA

Physeter macrocephalus	172	Cagnano Varano (Foggia)	10/12/2009	M	11.2	NA
Physeter macrocephalus	173	Cagnano Varano (Foggia)	10/12/2009	M	12.14	20
Physeter macrocephalus	174	Ischitella (Foggia)	10/12/2009	M	10.50	15
Kogia sima	KS1	Baia di Trentova (Agropoli, Salerno)	04/02/2017	F	1.95	NA
Kogia sima	KS2	Eraclea Minoa (Agrigento)	11/09/2002	M	2.07	NA
Kogia sima	KS3	Foce Chiarone (Grosseto)	24/05/1988	ind.	2.20	NA
Balaenoptera physalus	531	Cala del Rio (Anacapri, Napoli)	07/11/2020	F	14.2	NA
Balaenoptera physalus	536	Sorrento (Napoli)	14/01/2021	F	19.77	NA
Balaenoptera physalus	553	Albinia	02/09/2021	F	12.1	NA
Balaenoptera acutorostrata	405	Fregene (Fiumicino)	04/05/2020	Ind.	2.9	NA
Balaenoptera acutorostrata	416	Baia S. Antonio (Messina)	10/04/2016	F	3.27	NA

Table1. Place and date of stranding of the analysed samples, their sex, total body length and age. NA = data not available.

2.2. DNA extraction

A standard phenol-chloroform protocol was used to extract DNA from tissue samples. A small amount of tissue was finely chopped in a 1.5 ml eppendorf and added to 400 µl of extraction buffer (MagCore® Nucleic Acid Extraction Kit, RBC Bioscience). 25 µl of proteinase -K (10 mg/ml) was added to the solution and the tissue was left to digest overnight at 37°C in a water-bath or in a thermoblock with agitation at 400 rpm. After the incubation, 40 µl of sodium perchlorate ($NaClO_4$) was added to the solution, and the tubes were then placed vertically on ice for 15'. Subsequently a volume of chloroform (450 µl) was added and the tubes were placed

horizontally in the ice on the orbital shaker at a speed of about 100 for 30'. This step is followed by centrifugation at 10,000 rpm for 10' to favor the separation of the phases. The supernatant was removed and replaced in a new eppendorf tube; at this point a volume of isopropanolom (C_3H_8O) and 1/2 of the volume of sodium acetate ($C_2H_3NaO_2$, pH 2.5) was added to favor the precipitation of the DNA. The solution was then centrifuged for 10' at 14,300 rpm to favor the attachment of the pellet to the bottom of the tube. The solution was then removed and DNA was purified by two successive washes in ice-cold 70% ethanol (EtOH); each wash was followed by a 4' spin at 13,400 rpm. Once purified, the DNA was left to dry in the air overnight to remove ethanol in excess; the resuspension took place in ultrapure sterile water, the quantity of water varied according to the amount of DNA pellet in the eppendorf. To favor the dissolution of the DNA in the water it was placed at 37 ° C on the thermoblock and then the quality and quantity of the DNA extracted were checked using the NanDrop (2 µl of solution). The absorbance at 260 nm was used to estimate the quantity of DNA in solution measured in ng- /- ml, the purity of the DNA was estimated through two ratios:

- 260/280: this ratio refers to absorbance at two different wavelengths. In the first case the absorbance of DNA and in the second one the absorbance of proteins. If this ratio has a value ≥ 1.8 the DNA is pure;
- 260/230: in the same way this ratio indicates the purity of the DNA in relation to the organic compounds that may be present in the solution. The value must be in the range of 2.0 -2.2.

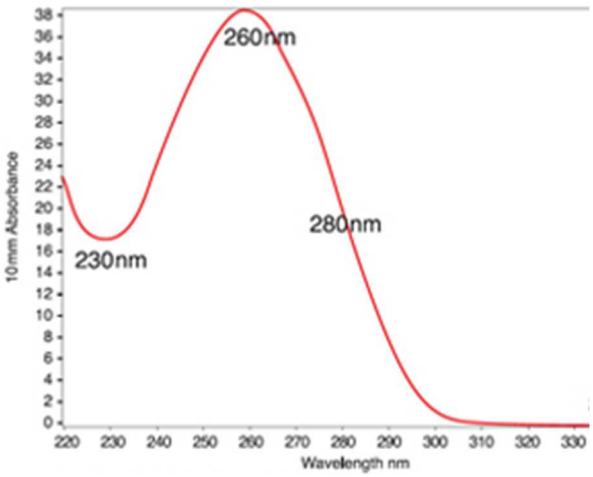


Figure 10 A typical nucleic acid spectrum (Desjardins P., et al., 2010)

2.3. Restriction enzyme digestion and PCR

As reported in Table 3, different primers pairs were used to amplify different mitochondrial or nuclear markers in the various cetacean species here analysed. The PCR protocol used to amplify the trait of interest is the same for all species except for the annealing temperature which is specified in Table 4. The amplification reactionm was performed in a 25 μl solution containing:

- 2.5 μl of primers F+R [5 μM]
- 5 μl of PCR buffer 5X
- 0.4 μl of MyTaq DNA Polymerase [5U/ μl] (Bioline, meridian BIOSCIENCE®)
- 3 or 5 μl of DNA [~40 ng/ μl]
- H_2O to bring up to a final volume of 25 μl

Species	Gene region	Primer sequence (5′-3′)	Product lenght	AT	Source
Physeter macrocephalus	mtDNA CR	Rsh-TTGCAACTAGAGGCCTTGGA Rlg-ACACACAGGTCCGGCTAAGA F-GCACCCAAAGCTGAAATTCT	Short trait: 474bp Long trait: 694bp	55°C	Primer3Plus Synthesized by: Invitrogen by Thermo Fisher Scientific
Kogia sima	mtDNA CR	R-AGATGAAAATGGCCCTGAAG F-CATCAACACCCAAAGCTGAG	CR:498bp	55°C	Primer3Plus Synthesized by: Invitrogen by Thermo Fisher Scientific

Kogia sima	mtDNA Cytb	R-CGGTTGCTCCTCAGAATGAT F-TGGACTCAAACCATGACCAA	Cytb:496bp	55°C	Primer3Plus Synthesized by: Invitrogen by Thermo Fisher Scientific
Balaenoptera physalus	mtDNA CR	R-CCTCAGTTATGTTATGATCATGGGC F-CCTCCCTAAGACTCAAGGAAG	~600bp	54°C	Árnason et al.,1993
Balaenoptera physalus	nuDNA α-lactalbumin	R-CTCACTGTCACAGGAGATGT F-CCAAAATGATGTCCTTTGTC	600-700bp	54°C	Bérubé et al., 1998
Balaenoptera acutorostrata	mtDNA CR	R-GAAGAGGGATCCCTGCCAAGCGG F-CCTCCCTAAGACTCAAGGAAG	~500bp	54°C	Maio et al.,2016

Table 3 Primers used for the amplification of the tracts of the mtDNA **CR** (mitochondrial DNA control region), of the **Cytb** (cytochrome b) and of the nuclear **alpha-lactalbumin** gene. **AT=** annealing temperature.

PCR was performed on a termal cycler "BIORAD T100" with the set PMCR program. To evaluate any contamination during the amplification reaction, in addition to the mix with the template, blank tubes were also prepared where instead of DNA, sterile water is added to the mix in the same proportions.

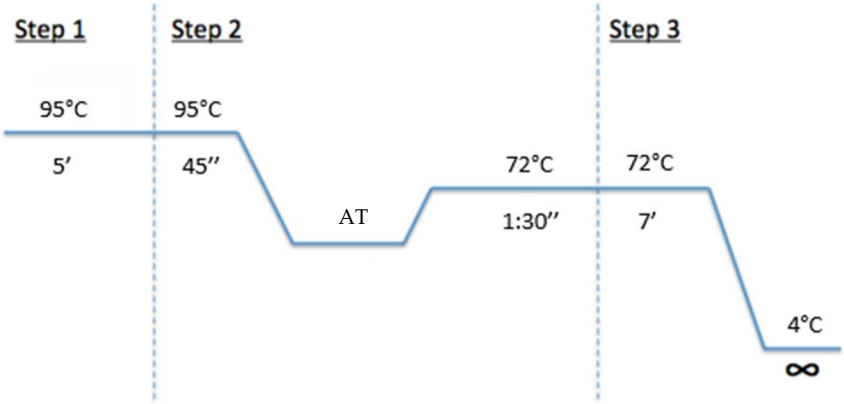


Figure 11 Protocol PMCR used for PCR

To check the quality and quantity of the amplified samples and the possible contamination of the blanks, the amplified samples were loaded on a 2% agarose gel stained with GelRed™. This is a fluorophore that intercalates with the DNA double helix during the electrophoretic run and therefore allows its visualization under the UV lamps of the transilluminator (DiatechMoonLight ML001). Together with the amplified products, a step-ladder of known length is also loaded on the gel that allows us to understand the length of our amplified section (GeneRuler 100pb Plus, ThermoScientific). 3 µl of DNA with 3 µl of Orange Color were loaded onto agarose gel. The electrophoretic run is carried out on a BIO-RAD® PowerPak electrophoretic apparatus, using a TAE1X buffer and applying a potential difference of 75 V for about 45/60 minutes.

For the *B. physalus* samples, the fragment of the α-lactalbumin gene amplified by PCR was digested using the restriction enzyme Fok I (BioLabs®*Inc*) following the protocol proposed by Bérubé et al (1988). The RFLP (Restriction Fragment Length Polymorphism) analysis was performed in a 25 µl solution containing:

- 2.5 µl of buffer 10X
- 0.5 µl of restriction enzime (Fok I 5000 U/ml)
- 5 µl of PCR product
- 17 µl of steril water to bring up to a final volume of 25 µl

The solution was incubated at 37°C for a minimum time of 30' to a maximum of 60'. The restriction fragments were separated by electrophoresis on a 2% agarose gel

stained with GelRed™ (Biotium) and visualized with a transilluminator (MoonLightML001, Diatech Labline).

2.4. Sanger sequencing and sequence analysis

The PCR products were sequenced with the Sanger method (Sanger et al., 1977) by BMR Genomics of Padua. Samples were initially subjected to an exoSAP-IT™ (Affymetrix Inc.) purification process and then sequenced using an ABIPRISM 3730XL (AppliedBiosystems) sequencer. The pherograms were reassembled combining forward and reverse sequences, to obtain a complete sequence of the trait that we have amplified. These sequences were then aligned with BLAST (Altschuel et al., 1990) to check for their accuracy and, then with CLUSTALW (Thompson et al., 1994; Larkin et al., 2007) together with other sequences of the same trait present in Genbank in order to determine the haplotype of the samples of interest. After the alignment, a median-joining haplotype network or a maximum parsimony phylogenetic tree were designed in order to visualize the relationships between all the haplotypes. The haplotype networks were designed using Network 10.2 software (Fluxus Technology Ltd., Colchester, UK, www.fluxusengineering.com) while phylogenetic trees were made using the MEGA11 software (Tamura et al., 2021).

2.5. Tooth analysis for age determination

Age determination of sperm whale was carried out on teeth according to the protocol described by Evans et al., (2001). In brief the steps of this protocol were:

- Removing of soft tissue residues from the tooth: if the sample arrives anchored to the mandible, it must be boiled to soften the tissues and remove them and then the tooth can be extracted from the mandible;
- Cut in the median sagittal plane with a diamond saw to obtain two sections;
- Smoothing the surface of each section with sandpaper, first at 600 and then at 1200 grit, the surface of the tooth previously wet in order to eliminate the cutting lines that are created after cutting with the diamond saw;
- Dip the tooth sections in a strong acid such as formic acid at different concentrations for about an hour and a half: one section is subjected to an acid wash at 20% while the other at 15%;
- Rinse the sample with water for 3 min;
- Rinse with acetone for 3 min;
- Rinse with water for 3 min;
- Leave to dry overnight.

The steps from "smoothing" to "drying overnight" were performed several times until the GLGs were distinct and clearly visible, Then, tooth sections were analyzed using a Leica EZ4 stereo microscope under reflected light and equipped with a digital camera. Different portions of tooth sections were taken at high magnification by digital camera and successively were stitched together into a single image using the AutoStitch64 software. The acquired images of the tooth sections were also optimized with respect to contrast and intensity using Adobe Photoshop 6.0 in order to enhance the distinctiveness of GLGs. The count of GLGs was performed independently by three researchers (FMG, NM, LL). In the case of discrepancies between the GLG count, the sections were read again until final consensus was reached.

3. Results

3.1. *Physeter macrocephalus*

After DNA extraction, amplification and sequencing, the aligment of 619 bp of the mithocondrial DNA control region (mtDNA CR) was carried out using our sequences and those obtained from different works (Lyrholm & Gyllensten 1998; Engelhaupt et al. 2009; Rendell et al. 2012; Mesnick et al. 2011; Whitehead et al. 1998; Richard et al. 1996) highlighting that our samples belong to the haplotype C.001.002 (Table S1), which is characteristic of the Mediterranean sea. The haplotype network (Figure 12) show the relationship between all the mtDNA CR haplotypes observed in populations of sperm whales in all ocean basins. For its realization, sequences of 394 bp in length of the mtDNA control region were used (Alexander et al., 2016) together with the 10 sperm whale sequences obtained in this work. The haplotype network shows that all our sequences, represented by the fuchsia-colored cake portion fall into the haplotype C, a haplotype largely distributed in all the oceans. It wasn't possible to create a network with long sequences of the mtDNA CR (619 bp) because the haplotypic frequencies for that sequences were not reported in the reference works.

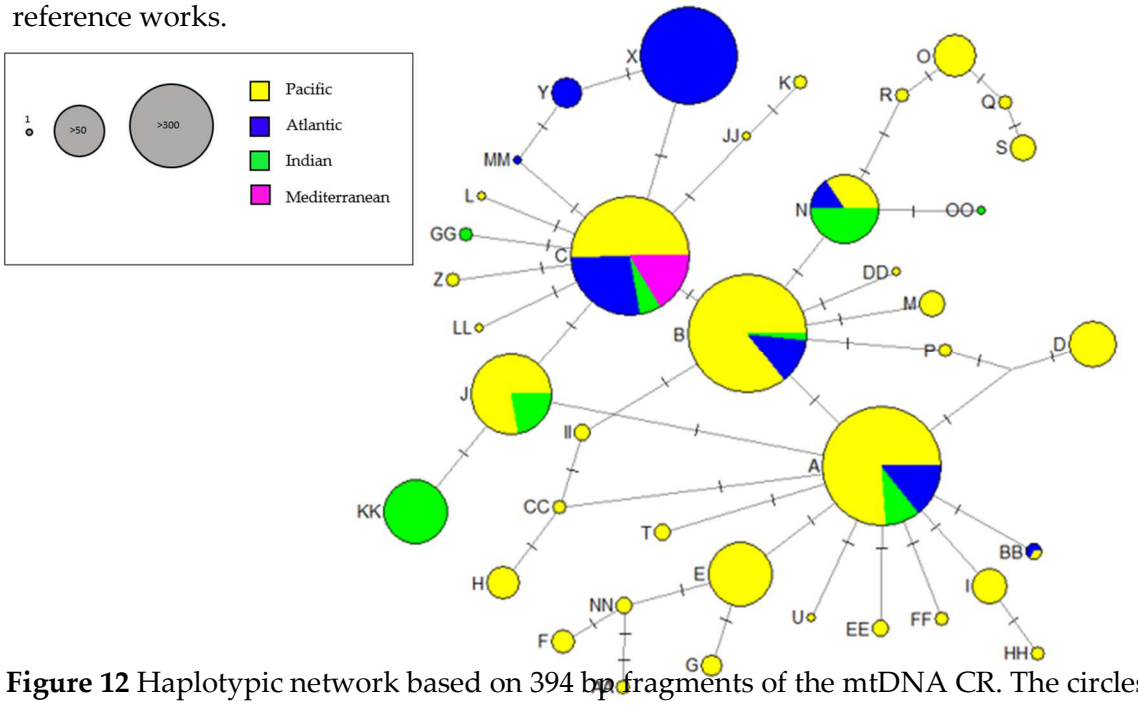


Figure 12 Haplotypic network based on 394 bp fragments of the mtDNA CR. The circles represent the different haplotypes and their size depends on the frequency of the samples for that particular haplotype. Haplotypes are colored based on ocean origin. The horizontal strokes on the lines represent mutational steps between haplotypes.

Figure 13 Composite photograph of longitudinal, acid-etched tooth section of sperm whale estimated to be 24-year-old. Circles show GLGs. **NL**: neonatal line.

Although after the etching process, the GLGS were already visible to a macroscopic examination, however only with the observation under the stereo microscope it was possible to have a reliable reading, with the consensus among the various researchers (Fig. 13). The relationships between age and total body length in sperm whales was based on the data set given in Table 4. As showed in figure 14, there is a significant positive correlation between age and body length both in Atantic and Mediterranean populations (Pearson's correlation coefficent r: = 0.690, df = 13, P <0.01% in Mediterranean sperm whales; r = 0.447, df = 26, P <0.05%, in Atlantic sperm whales). Furthermore, the two regression lines have different slopes (coefficients) and the Mediterranean sperm whales are older than the Atlantic ones of the same size.

Samples	Sex	Total lenght	Age	Sea of origin	Sources
GP-1	juv	8	8	Mediterranean sea	This study
154159	juv	6.1	3	Mediterranean sea	This study
45486	M	12.56	41	Mediterranean sea	This study
456	M juv	8.6	11	Mediterranean sea	This study
ME15	F	6.5	28	Mediterranean sea	This study

173	M	12.14	20	Mediterranean sea	Mazzariol 2011
174	M	10.5	15	Mediterranean sea	Mazzariol 2011
175	M	11.4	20	Mediterranean sea	Mazzariol 2011
176	M	11.3	20	Mediterranean sea	Mazzariol 2011
177	M	11.2	20	Mediterranean sea	Mazzariol 2011
178	M	11.8	24	Mediterranean sea	Mazzariol 2011
1	F	8.95	32	Mediterranean sea	Mazzariol 2018
1b	M	0.98	0	Mediterranean sea	Mazzariol 2018
2	F	8.38	21	Mediterranean sea	Mazzariol 2018
3	F	7.33	14	Mediterranean sea	Mazzariol 2018
2	M	13.1	11	DE	Lonneke L. Ijsseldijk
4	M	12	13	DE	Lonneke L. Ijsseldijk
5	M	12.3	13	DE	Lonneke L. Ijsseldijk
6	M	9.6	10	NL	Lonneke L. Ijsseldijk
7	M	11.1	16	NL	Lonneke L. Ijsseldijk
8	M	10.1	12	NL	Lonneke L. Ijsseldijk
9	M	10.25	10	NL	Lonneke L. Ijsseldijk
10	M	9.7	10	NL	Lonneke L. Ijsseldijk
11	M	10.7	12	DE	Lonneke L. Ijsseldijk
18	M	10.8	12	DE	Lonneke L. Ijsseldijk
19	M	11.7	11	DE	Lonneke L. Ijsseldijk
20	M	11.2	10	DE	Lonneke L. Ijsseldijk
21	M	11	12	DE	Lonneke L. Ijsseldijk
22	M	10.2	10	DE	Lonneke L. Ijsseldijk
23	M	11.3	15	DE	Lonneke L. Ijsseldijk

24	M	11.4	11	DE	Lonneke L. Ijsseldijk
25	M	10.5	12	DE	Lonneke L. Ijsseldijk
27	M	12	11	DE	Lonneke L. Ijsseldijk
28	M	11.4	15	DE	Lonneke L. Ijsseldijk
31	M	12.2	16	NW Spain	Asunción Borrell
32	M	11.6	18	NW Spain	Asunción Borrell
33	F	10.9	18	NW Spain	Asunción Borrell
34	F	9.5	20	NW Spain	Asunción Borrell
35	F	10.1	13	NW Spain	Asunción Borrell
36	M	12.6	24	Denmark	Asunción Borrell
37	M	13.2	27	Denmark	Asunción Borrell
38	M	19.9	22	Denmark	Asunción Borrell
39	M	14	55	Denmark	Asunción Borrell

Table 4 Data set used for the graph age/lenght of sperm whale.

Tables 5A and 5B show the maximum, minimum and average lengths in male and female Atlantic and Mediterranean sperm whales. These tables provide a more intuitive comparison between the sizes of the two populations: the values are derived from Table 4 and the maximum and minimum lengths of only those individuals considered sexually mature have been selected. It is estimated that sexual maturity is reached for males around 11-12 m (18-21 years) and for females between 8-9 m (7-13 years) There is a disparity in the lengths but also in the ages of mature individuals of both sexes compared to the two populations.

A)

Male specimens	Atlantic Ocean	Mediterranean Sea
Maximum lenght	19.9 (22 years)	12.16 (40 years)
Minimum lenght	11.6 (18 years)	11.3 (20 years)
Average lenght	12.5 m	11.7 m

B)

Female specimens	Atlantic Ocean	Mediterranean Sea
Maximum lenght	10.9 m (18 years)	8.95 m (30 years)
Minimum lenght	9.5 m (20 years)	6.50 m (24 years)
Average lenght	10.16 m	7.79 m

Table 5 A) Maximum, minimum and average lenght value for female specimens of sperm whale. The values refer to the lenght of an individual sexually mature: 8-9 m of lenght, corresponding to an edge between 7 and 13 years. **B)** Maximum, minimum and average lenght value for male specimens of sperm whale. The values refer to the lenght of an individual sexually mature: 11-12 m of lenght, corresponding to an age between 18 and 21 years.

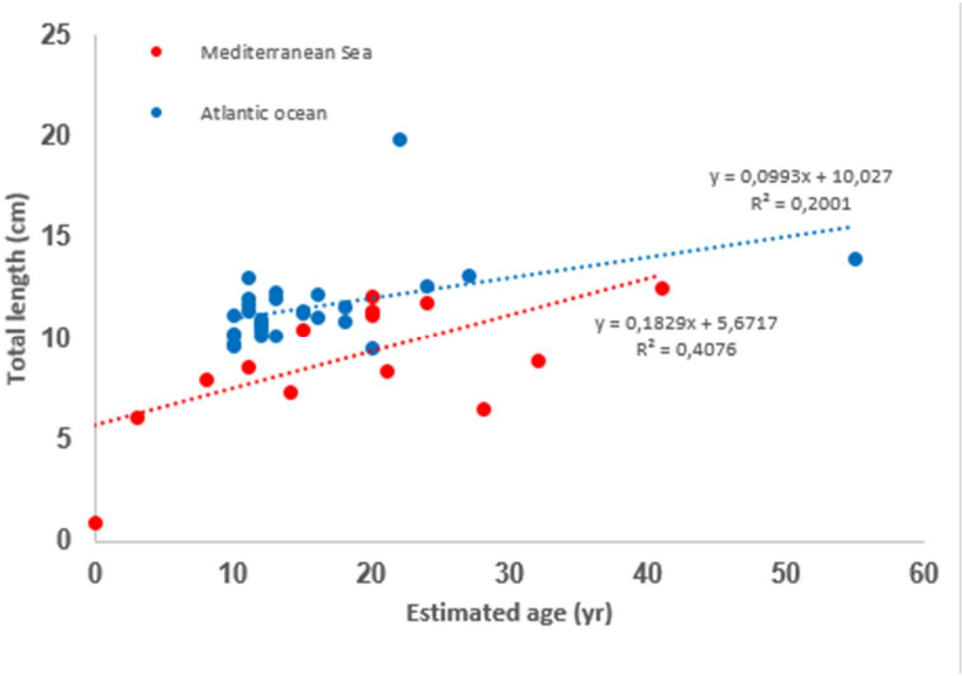


Figure 14. Relatonship between estimated age (years) and total body length (m) in *P. macrocephalus* from Mediterranean Sea and Atlantic Ocean. The red and blu dotted lines represent linear regression for Mediterranean and Atlantic sperm whales, respectively. In the graph are also represented the regression equation (y) and the coefficient of determination R squared (measure of the percentage of variability of y explained by the variable x) from which can also be obtained the coefficient or index of correlation (r).

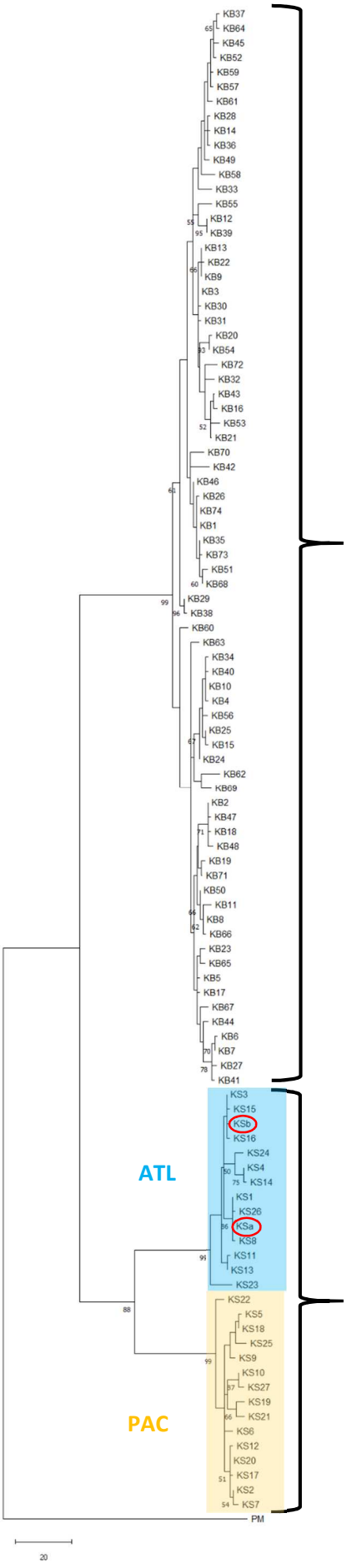
3.2. *Kogia sima*

	CONTROL REGION																			CYTOCHROME b								
	8	28	35	76	78	79	183	191	203	239	266	273	275	276	282	290	302	389	390	30	59	66	177	195	221	224	231	302
KS 4	G	G	T	C	T	C	C	A	C	T	A	C	G	C	T	C	T	G	T	A	C	A	A	G	T	T	G	T
KS 14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	C	.	.	.
KS 24	.	.	.	.	.	.	.	G	.	C	.	.	.	T	C	.	.	A	.	-	.	.	.	.	.	.	A	.
KS a	A	A	C	.	.	.	T	G	.	.	.	.	.	T	.	.	.	A	.	A	T	.	.	A	.	.	.	C
KS 1	A	A	C	.	.	.	T	G	.	.	.	.	.	T	.	.	.	A	.	A	T	.	.	A	.	.	.	C
KS 8	A	A	C	.	.	.	T	G	.	.	.	.	.	T	.	.	.	A	.	A	T	.	G	A	.	.	.	C
KS 26	A	A	C	.	.	.	T	G	.	.	.	.	.	T	.	.	.	A	C	A	T	.	.	A	.	.	.	C
KS 11	A	.	C	.	.	.	T	G	T	.	G	.	.	T	.	T	.	A	.	-	T	.	.	.	C	.	.	.
KS 13	A	.	C	.	.	.	T	G	T	.	.	.	.	T	.	T	.	A	.	A	T	.	.	.	C	.	.	.
KS b	A	.	C	.	.	.	T	G	.	.	.	.	.	T	.	.	C	A	.	A	T	.	.	.	C	.	.	.
KS 3	A	.	C	.	.	.	T	G	.	.	.	.	.	T	.	.	.	A	.	-	T	.	.	.	C	.	.	.
KS 16	A	.	C	.	.	T	T	G	.	.	.	.	.	T	.	.	.	A	.	-	T	.	.	.	C	.	.	.
KS 15	A	.	C	.	.	.	T	.	.	.	.	.	.	T	.	.	.	A	.	-	T	.	.	.	C	.	.	.
KS 23	.	.	.	T	C	.	T	.	.	.	.	T	A	.	.	.	.	A	C	-	T	G	.	.	.	C	.	.

Table 6 Diagnostic sites of CR and cytochrome b of mtDNA of 399 bp and 398 bp, respectively, in *Kogia sima*.

Through DNA extraction, amplification and sequencing it was possibile to determine that our *Kogia*'s samples are precisely *Kogia sima*. The reference sequences of the mtDNA CR and the cytochrome b of *Kogia* spp. were taken from Chivers et al., (2005) and the described haplotypes come from both the Atlantic and Pacific Ocean. In Table 6 there are only the Atlantic haplotypes and our sequences are listed, KSa and KSb correspond respectively to KS1 and KS13 respectively, that are both Atlantic haplotypes. As for the KSb sample, the haplotype of the mtDNA CR would appear to be new but, aligning it with known sequences from GenBank (by means of BLAST nucleotide) we observe that it corresponds to the sequence not published but deposited in GenBank with access number JX403781.1. Figure 15 represents the phylogenetic tree of maximum parsimony of the two known species of *Kogia*, *Kogia*

breviceps and *Kogia sima* with a distinction for the latter between the Atlantic population highlighted in blue and the Pacific one in yellow. The tree was built using the full sequences of both mtDNA CR and Cytochrome by Chivers et al., (2005). Figure 15 allows an overall view of the results for the *Kogia sima* samples. The tree is divided into two distinct portions: in the upper branch we have all the sequences of *Kogia breviceps* while in the lower one we have the sequences of *Kogia sima*. The two samples analysed in this study belong to the latter and, in particular to Atlantic haplotypes.



Kogia breviceps

Kogia sima

Figure 15 The most parsimonious tree with length = 485 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (pg. 126 in Nei and Kumar 2020) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 104 nucleotide sequences. There was a total of 804 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

3.3. *Balaenoptera physalus*

In this study, three presumed fin whale tissue samples ID 531, ID 536 and ID 553 were examined (Table 1). Genetic analyses initially conducted on mtDNA control region confirmed the identification as *Balaenoptera physalus* only for two individuals**.** The mtDNA CR sequences of the individuals genetically identified as *B. physalus* (ID536 and ID553) were compared with the largest dataset of mtDNA CR haplotypes collected so far for the North Atlantic area (Cabrera et al., 2019). The sequence of the sample ID536 was attributed to the haplotype NATL002 while the sequence of the sample ID553 was attributed to the haplotype NATL004 (Table S3). Observing the sampling locations of all the individuals analysed by Cabrera et al., (2019) is evident that the haplotype NATL002 is abundant both in North Atlantic Ocean and in the Mediterranean Sea, while the haplotype NATL004 is mainly present in the Mediterranean basin and extremely rare, observed in only three individuals, in the North Atlantic Ocean (Cabrera et al., 2019). The comparison between sequences of the haplotypes NATL002 and NATL004 (Cabrera et al. 2019) and those described previously by Bérubé et al., (1998) shows that they are identical to haplotypes Bp03 and Bp46 respectively (Table S2). The haplotype Bp03 was described as abundant in both North Atlantic Ocean and Mediterranean Sea, while the haplotype Bp46 was classified as private of the latter basin, in particular of the Ligurian Sea (Bérubé et al., 1998).

In the case of ID 531, the alignment on BLAST (Altschuel et al., 1990) gave a 100% identity with the blue whale, *Balaenoptera musculus*. In addition, the alignment with sequences of *B. musculus* and fin-blue whale hybrids (Pampoulie et al., 2021) show that individual ID531 has a mtDNA CR sequence identical to that of two blue whales (14-99, LK-BM01-2015) and a fin-blue whale hybrid (H2018-2) caught in Icelandic waters (Pampoulie et al. 2021) (Table 7, Figure 19). Erroneous identification is due to the general appearance of the dead specimen, rather similar to a fin whale. However, a proper morphological examination evidenced mixed characters, suggesting a hybrid origin of ID 531 specimen. The individual ID531 was a young female with a body length of 14.20 m. Regarding the body colouration, a clear asymmetry in body colour is apparent, being the right side ostensibly clearer than

the left one as in fin whale (Figure 17 A); however, the grey extends more ventrally on the left side of the animal, so that when observed laterally it appears uniformly dark, as in the blue whale (Figure 17 C). The baleen plates colouration is not uniformly dark, but it appears yellowish anteriorly in the frayed portion as in fin whale (Figure 17 B). The animal resembled a fin whale also in having relatively shorter flipper, corresponding to 9% of body length (compared to 14-15 % in blue whale). Also the dorsal fin shape, moderately concave and pointed, whose height corresponded to 2% of the body length (compared to 0.8-1.3% in blue whale, Cagnolaro et al., 1983), had fin whale appearance (Figure 17 D); however, its insertion, located at about 3/4 back on body (Figure 18, line 2 in table), resembled that of blue whale (white arrow in Figure 17 C). Therefore, ID 531 specimen resulted a hybrid derived from the mating of a female blue whale (as mtDNA is transmitted through the mother) with a male fin whale. In order to confirm the hybrid nature of ID531 sample, the α-lactalbumin nuclear gene was amplified, generating a PCR product of ~ 600 bp in length (Figure 16 **a)**) Their digestion with the FokI endonuclease produced three restriction fragments (Figure 16 **a)**) The comparison of this digestion pattern with those obtained by Bérubé & Aguilar (1998) for a *physalus* x *musculus* hybrid (Figure 16 **a)**), confirmed the hybrid origin of the ID531 individual. Indeed, a ~ 200 bp fragment was present in both fin and blue whales while two restriction fragments of ~ 300 bp and ~ 400 bp in length were typical of blue and fin whales, respectively (Figure 16 **a)**). Accordingly, Sanger sequencing chromatograms revealed the presence of nine double peaks (Figure 16 **b)**) indicating a heterozygous genotype in which the two alleles are inherited from parents of different species. The alignment of the two sequences inferred from chromatograms showed that they match to those described for the two species, *B. physalus* and *B. musculus* (Figure 16 **c)**) confirming the hybrid origin of the analysed individual.

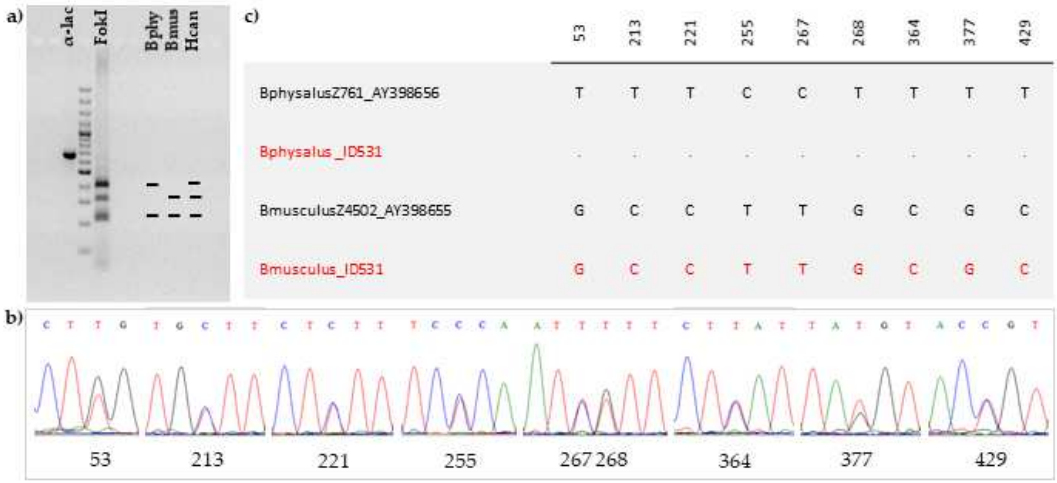


Figure 16 a) α-lac (α-lactalbumin) is the nuDNA trait amplified of ID531. **FokI (endonuclease)** produce the three restriction fragments. Next to the ladder are reported the digestion pattern of ID 531 and of *B. physalus*, *B. musculus*, Caneliñas hybrid from Bérubé et al., (1998). **b)** chromatogram, that highlight the nine double peaks in the hybrid sequence, indicates a heterozygous genotype in which the two alleles are inherited from parents of different species **c)** table with the alignment of the two sequences of ID 531 inferred from chromatograms. The aligment shows that the sequences of hybrid match to those described for the two species, *B. physalus* and *B. musculus*.

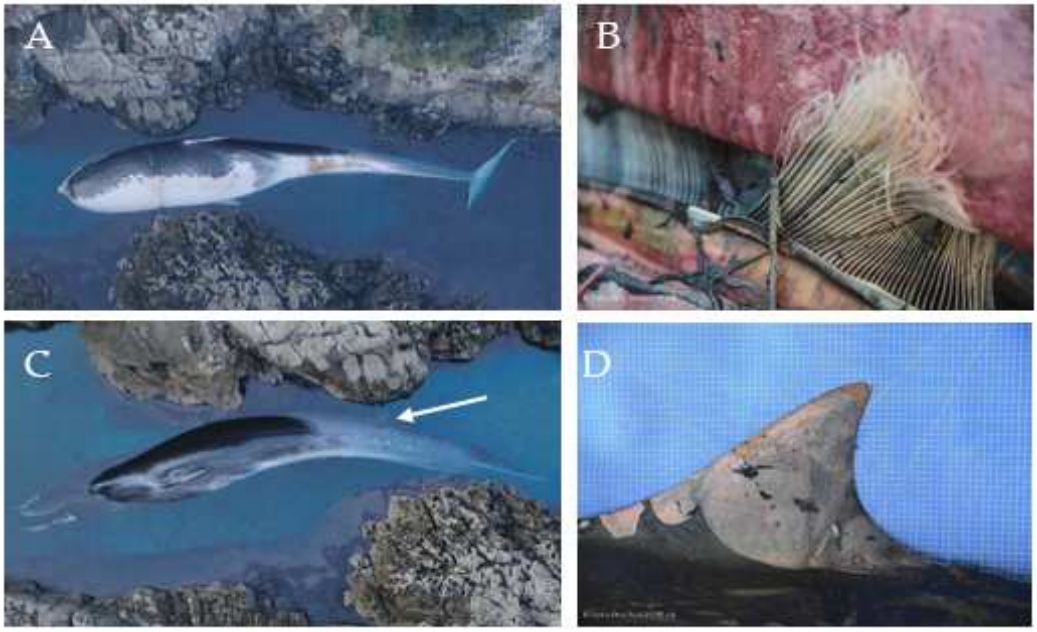


Figure 17 A- asymmetrical colouration between right and left side (drone photo by Ivan Rubino). **B-** not uniformly baleen plates coloration: frayed portion of baleen yellowish (photo by Cristina Oterio Sabio|CERT Italy). **C-** dorsal fin located at about 3/4 back on body, highlight by the white arrow (drone photo by Ivan Rubino) **D-** shape of ID 531 dorsal fin (photo by Cristina Oterio Sabio|CERT Italy).

Measures (expressed as ratio to body lenght if not specified)	Hybrid 531	Caneliñas hybrid	Spilliaert et al., (1991) hybrid	B. physalus	B. musculus
Sex	F	F	F	F	F
1- Total body lenght (m)	14.20	19.40	21.00	19.5±0.78	21-22
2- Tip of snout-anterior insertion of the dorsal fin	73.50	NA	NA	NA	NA
3- Tip of snout-center of eye	20.60	19.50	19.10	19.9±0.69	21.1±0.9
4- Length of dorsal fin (cm)	65.00	80.77	60.22	NA	137.93
5- Height of dorsal fin (cm)	29.00	42.00	53.00	43.9±3.40	40.00
6- Pectoral fin: ratio to body lenght	9.15	NA	NA	8-9	14-15
Dorsal fin: ratio to body lenght	2.04	2.20	2.48	2.3±0.16	1.09±0.27
Dorsal fin: height to lenght of base (ratio)	0.45	0.52	0.88	0.38±0.25	0.29

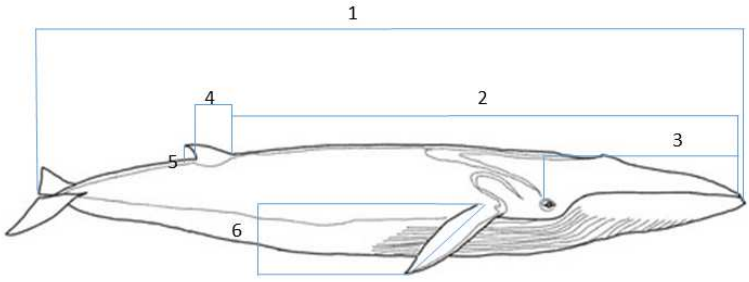


Figure 18 Measures of the diagnostic ratios between the various parts of the rorqual's body. In addition to the hybrid ID 531, the measures of the hybrids of Bérubé's work (1998) are also indicated. **NA**- not available

	DIAGNOSTIC SITES												
SAMPLES	4	82	99	102	190	201	212	241	245	259	277	281	324
14_99	T	T	T	C	G	C	C	T	A	G	T	G	A
Bp531	.	.	.	.	.	.	.	.	.	.	.	.	.
H2018_2	.	.	.	.	.	.	.	.	.	.	.	.	.
LK_BM01_2015	.	.	.	.	.	.	.	.	.	.	.	.	.
8_99	.	.	C	.	.	.	T	.	.	.	.	.	.
4_99	.	.	C	.	.	.	T	.	.	.	.	.	.
11_99	.	.	C	.	.	.	T	.	.	.	.	.	.
S1010	.	.	C	.	.	.	T	.	.	.	.	.	.
EN5_2014_N2	.	.	Y	.	.	.	T	.	.	.	.	.	.
H1986	C	.	.	.	A	.	T	.	.	.	.	.	.
EN_3_2011_21_06_2011	C	.	.	.	A	.	T	.	.	.	.	.	.
2_99	.	.	.	.	.	.	.	.	.	A	.	.	R
S1011	.	.	.	.	.	.	.	.	.	A	.	.	R
BM3_23_06_11	.	.	.	.	.	.	.	.	.	A	.	.	R
12_99	.	.	.	.	.	.	.	.	.	A	.	.	.
H1989	.	.	.	.	.	.	.	.	.	A	.	.	.
BM_07062015	.	.	.	T	.	.	.	.	.	A	.	.	.
2_7_92_N2_SURTSEY	.	.	.	.	.	.	.	.	.	A	.	.	.
BM6_25_6_11	.	.	.	.	.	.	.	.	.	A	.	.	.
BM_26_06_11_Volcano	.	.	.	.	.	.	.	.	.	A	.	.	.
BM1_23_06_2011	.	.	.	.	.	.	Y	.	.	A	.	.	.
H2018_1	.	.	.	.	.	T	T	.	.	A	.	.	.
TAG60010	.	.	.	.	.	.	T	.	.	A	.	.	.
10_99	.	.	.	.	.	.	T	.	.	A	.	.	.
13_99	.	.	.	.	.	.	T	.	.	A	.	.	.
9_99	.	.	.	.	.	.	T	.	.	A	.	.	.
7_99	.	.	.	.	.	.	T	.	.	A	.	.	.
3_99	.	.	.	.	.	.	T	.	.	A	.	.	.
6_99	.	C	C	.	.	.	T	C	G	A	C	A	.
15_99	.	C	C	.	.	.	T	C	G	A	C	A	.
HALIVE	.	C	C	.	.	.	T	C	G	A	C	A	.
H2013	.	C	C	.	.	.	T	C	G	A	C	A	.
BM1_08_06_2011	.	C	C	.	.	.	T	C	G	A	C	A	.
BM2_23_6_2011	.	C	C	.	.	.	T	C	G	A	C	A	.
BM1A_23_06_11	.	C	C	.	.	.	T	C	G	A	C	A	.

Table 7 Alignment of the sequence of ID 531 mt DNA CR vs haplotypes of *Balaenoptera musculus* and hybrids from Pampoulie et al, 2020. Hybrids are indicated in blue, that of this work is indicated in red while in yellow are highlighted the samples in Pampoulie et al, 2020 that have a haplotype identical to that observed for our sample.

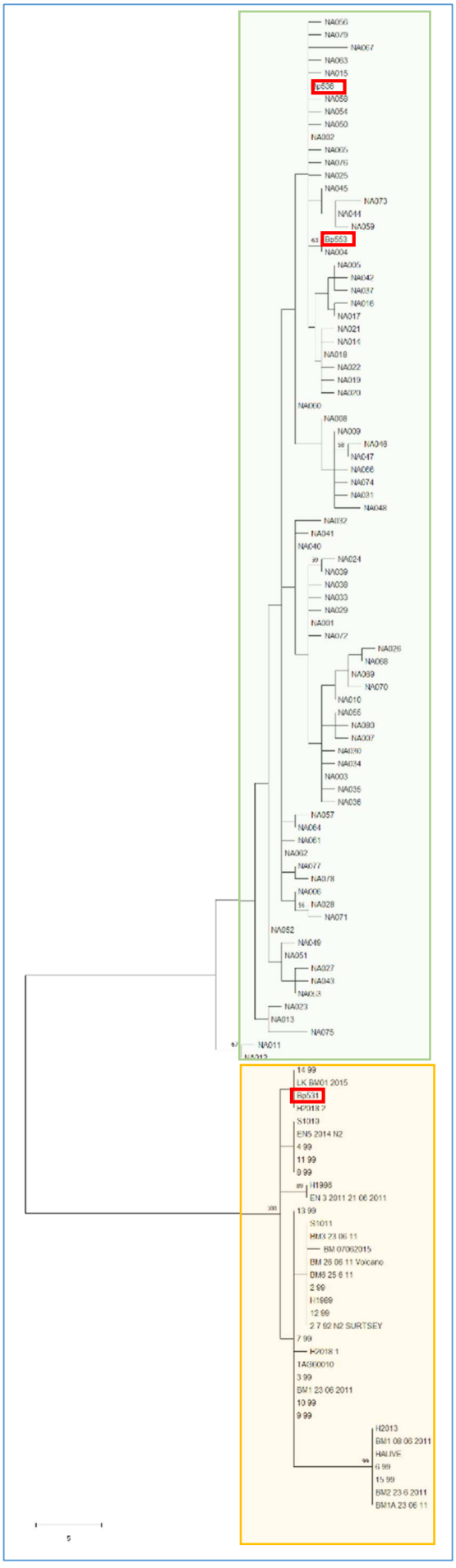


Figure 19 Maximum Parsimony Tree built on MEGA 11 showing the location of our samples, especially the hybrid. The *B. physalus* mtDNA CR sequences were taken from Cabrera et al, 2019, the B. musculus sequences were taken from Pampoulie et al, 2020 and also include other hybrids. The samples of this work ID531, ID536, ID553 are represented in red. The green box shows the haplotypes belonging to *B. physalus* and in the orange box the haplotypes belonging to *B. musculus*.

3.4. *Balaenoptera acutorostrata*

The comparison sequences to identify the mtDNA CR haplotype of the common minke whale samples analysed in this study were taken from Pastene et al., (2007). After the alignment, a table with all variable sites was constructed to show different mtDNA CR haplotypes describe so far for *B. acutorostrata* (Table S4). In addition, using the frequencies of individuals belonging to each haplotype (information obtained from Pastene et al., 2007) and information about their provenance it was possible to build a haplotype network that allows us to better understand the geographic origin of our samples (Figure 20). Individuals ID 405 and ID 416 correspond respectively to the haplotype Ba 187 and Ba 165 which are both representative of the North Atlantic Ocean.

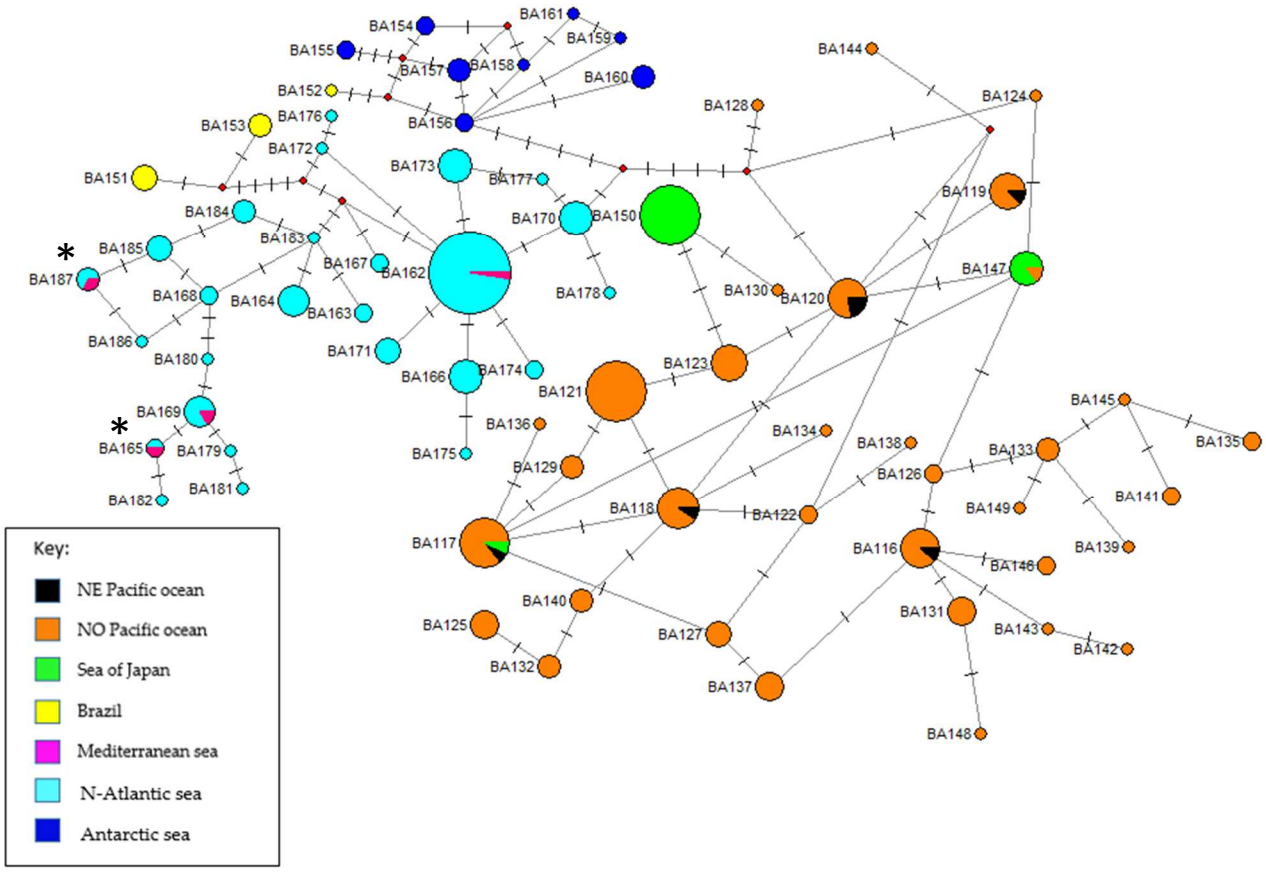


Figure 20 Haplotypic network based on 335 bp fragments of the mtDNA CR. The circles represent the different haplotypes and the size depends on the frequency of the samples for that particular haplotype. The colors are indicative of the sea of origin. The horizontal strokes on the lines represent how much a haplotype is differentiated from that near. The "*" indicates where our samples are collocated.

4. Discussion

The first purpose of this work was to determine the origin of some cetaceans stranded along the italian coasts through the analysis of molecular markers, such as the mitochondrial DNA control region and Cytochrome b gene. Knowing the geographic provenance of highly mobile marine species, like marine mammals, is difficult but it is useful in order to understand the evolution of diversity and essential for effective conservation strategies (Engelhaupt et al., 2009). In this context, the use of molecular analyses allowed us to get more information about the movements and frequency of some species in the Mediterranean Sea.

Cetacean species in the Mediterranean Sea can roughly classify as "regular", "visitors" and "vagrant". Notarbartolo di Sciara and Birkun (2010) defined the minke whale (*Balaenoptera acutorostrata*) as a "visitor" and the Dwarf sperm whale (*Kogia sima*) as a "vagrant", of these species we have analysed some individuals in this work. As for the common minke whale, the alignment of our Mediterranean sequences with those described by Pastene et al., (2007), showed a correspondence of 100% with Atlantic haplotypes, confirming what was reported in the study of Maio et al., (2016). *Balaenoptera acutorostrata* is present throughout the northern hemisphere, but probably with a discontinuous distribution, being rarer in tropical waters than in colder waters. Much of the current knowledge of this species in the Mediterranean Sea is due to data collected by strandings and to data recently recorded thanks to a National Working Protocol (Cagnolaro et al., 2015; Maio et al., 2016) which led to the creation of a comprehensive database. In particular, this species has been recorded 49 times in the Mediterranean basin since 1771 (Cagnolaro et al., 2015; Maio et al., 2016) with 15 specimens stranded along the Italian coasts (Cagnolaro et al., 2012; 2014; Bank strandings data, 2014; 2015). All the 15 specimens are represented by young calves, with a length less than four meters (Table 8; Maio et al., 2016). Both individuals analysed in this work have a total length of ~ 3.00 m, in particular the sample identified with the code ID 416 corresponds to the stranded specimen previously described in the work of Insacco et al., (2016) in which only necropsy tests were conducted. Also, in the work of Maio et al., (2016) the stranded specimen of common minke whale was identified as a calf based on the umbilical

scar not completely healed and by the presence of milk in the stomach. Thirteen of the fifteen individuals mentioned above were recorded in spring, a period immediately after the birthing season that, in this species inhabiting warm waters at low latitudes, occurs in winter. In fact, soon after birth, the calves of this species follow their mothers towards high latitude cold waters, to spend their first summer.

Date	Location	Event	Animals (gender, size)	Source and Notes
1878, 18 February	Villefranche-sur-Mer, Nice, France	Captured	1 (3.00 m)	(Reeves and Notarbartolo di Sciara, 2006)
1916, 26 April	Camogli (Province of Genoa, Italy)	Stranded, possibly by caught	1 (3.35 m)	Skull and stuffed skin in Civic Museum of Natural History "G. Doria" of Genoa (Cagnolaro et al., 2014)
1975, May	Mahdia (Tunisia)		1 (about 3 m)	(Reeves and Notarbartolo di Sciara, 2006)
1977, 9 June	Bandol, France	Captured	1 F (3.75 m)	(Reeves and Notarbartolo di Sciara, 2006)
1982, 20 April	St. Raphael (France)		1 F (3.60 m) dead Stillborn	Umbilical cord and placenta still attached (Duguy, 1983; Van Waerebeek et al., 1999; Bompar, 2000; Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara and Birkun, 2010; Cagnolaro et al., 2015)
1991, 17 May	Turas, Bosa (Province of Nuoro, Italy)	Stranded	1 (3.50 m)	(Reeves and Notarbartolo di Sciara, 2006)
1998, 12 April	Antignano (Province of Livorno, Italy)	Stranded	1 (3.40 m)	Skeleton in Natural History Museum of the Mediterranean of the Livorno Province reported as "newborn" by Roselli et al. (2014)
1998, 24 April	Near Giens peninsula, France	Stranded after caught in a net	1 M (3.40 m)	(Robineau, 2005)
1998, May	Toulon region, France	By caught	1 M (3.65 m)	(Macé et al., 1999; Reeves and Notarbartolo di Sciara, 2006)
2000, 8 May	Akko, Israel	Entangled in gill net	1 M (3.50 m)	(Pastene et al., 2007; Kerem et al., 2012)
2008, 11 August	Anse de Bonnieux (Martigues, France)	Stranded	1 M (3.80 m)	(Dhermain et al., 2009, 2011)
2010, 1 April	Salerno, Italy	Stranded	1 M (3.30 m)	Skeleton in Zoological Museum of the University of Naples Federico II (Maio et al., 2012; Cagnolaro et al., 2014)
2014, 28 April	Santa Pola, Alicante, Spain	Stranded	1 F (3.00 m)	(Fraija-Fernández et al., 2015)
2015, 10 April	Yumurtalik, Turkey	Stranded	1 F (3.55 m)	(Öztürk et al., 2015)
2016, 10 April	Baia S. Antonio, Milazzo (Province of Messina, Italy)	Stranded	1 F (3.27 m)	(Insacco et al., in press)

Table 8 Record of nursing or unweaned calves of common minke whale less than four meter long. F-female, M-male (Maio et al., 2016).

The finding of a very young calves in the study area is compatible with the hypothesis that some females enter the Mediterranean Sea to give birth (Fraija-Fernández et al., 2015). Moreover, Van Waerebeek et al., (1999) and Öztürk et al., (2015) also suggest that common minke whales might give birth in Mediterranean Sea. All these evidences support the hypothesis that the Mediterranean Sea can be used as a potential calving or nursery ground by *Balaenoptera acutorostrata*. Considering the migration pattern of this species, it might be possible that the Mediterranean basin may be also a resting area for mother-calf pairs on the way back further North. This species is currently considered as "visitor" in the Mediterranean Sea but Kerem et al., (2012), taking into account the period 1993–2007, calculated a rate of about four occurrences per year of the common minke whale in the Mediterranean basin. The frequency observed is an order of magnitude

above the "several occurrences in a decade" definition to assign the "visitor" *status* to a species (Maio et al., 2016). So, this may lead us to suppose that this species may be more than a simple "visitor" and to consider the Mediterranean Sea as a breeding ground for the minke whale. All these evidences have important consequences in terms of conservation of the species, in fact it is clear that the Mediterranean has an important role for these rorquals and, as a consequence, conservation measures consistent with the *status* of the species in this basin should be considered. As suggested by Maio et al., (2016) the common minke whale should be considered by the IUCN Red List as a "vulnerable" species in Mediterranean Sea. The *status* of "vulnerable" for this species in the Mediterranean Sea is justified due to the small number of individuals and their confinement in a partially degraded marine environment, as evidenced by Notarbartolo di Sciara and Birkun (2010).

The *Kogia sima* has a worlwide distribution, in warm temperate and tropical oceanic waters of both hemispheres. This species lives over the continental shelf and slope as well as in offshore waters. Its distribution range covers the western Atlantic from southeastern U.S.A. to Brazil, including the Antilles, the eastern Atlantic from Portugal to Cape Province, the Indian Ocean from Cape Province to India and South Australia, the western Pacific from Japan to New Zealand, the eastern Pacific from southern Canada to Chile (Maio et al., 2017). The species is also found in the Sea of Japan and in the Persian Gulf (Taylor et al., 2012) while in the Mediterranean Sea, the only records of *Kogia sima* in the past are limited to two stranded individuals, both from Italian waters (Baccetti et al., 1991, Bortolotto et al., 2003). In 2017 an adult female of *Kogia* spp. stranded on the beach of Trentova (Agropoli). The *Kogia sima* samples analysed in this work correspond to the only three strandings individuals recorded along the Italian coasts but, molecular analyses to identify them were performed only for two samples out of three. The sample described by Baccetti et al. (1991) in fact was a small fragment of a tooth so it was impossible to obtain a sufficient quantity of DNA for PCR amplification. The other two samples allowed us to obtain sequences of both mtDNA control region and Cytochorme b gene demonstrating an Atlantic origin of individuals analysed and suggesting a recent colonization of Mediterranean Sea by this rare and elusive whale species.

Despite being globally distributed, Kogiidae are still among the less known families of marine mammals, and most of our knowledge of their anatomy and life history comes from isolated and rare observations on stranded individuals. Although *Kogia* spp. do not inhabit the present-day Mediterranean Sea (McAlpine, 2017), kogiids are known from the Mediterranean region for a few late Neogene fossil specimens. As a matter of fact a partial Kogiid skeleton from lower Pliocene mudstone was found and it was fully described by Collareta et al., (2019). The analysis showed that the mentioned skeleton belongs to the holotype *Pliokogia apeninica*, which identifies the new genus *Pliokogia* which belongs to the subfamily Kogiinae, together with the current genus *Kogia*. The paleoecology of *Pliokogia* has allowed to hypothesise the reason for the disappearance of the Kogiidae family from the Mediterranean basin. It might be related to the definitive establishment of threshold basin conditions in the Gibraltar area, which possibly resulted in the strong depletion of their putative prey (e.g., deep-sea squids and fish). Santoro et al., (2018) performed parasitological studies on the individual stranded in 2017, their analyses highlighted that some species of *Anisakis* use the dwarf sperm whale as their main host. The identification of anisakid species from a given host provides useful insights into the geographical distribution, definitive host preference and life cycles of species of the genus *Anisakis* (Mattiucci & Nascetti 2008, Mattiucci et al., 2009, 2014). Indeed, the life cycles of *Anisakis* spp. involve crustaceans, fish, and squid as intermediate/paratenic hosts and marine mammals as definitive hosts (Mattiucci & Nascetti 2008, Klimpel & Palm 2011). In the dwarf sperm whale analysed by Santoro et al., (2018) a lot of *Anisakis physeteris* worms were found, this parasite is the same that also infest the Mediterranean sperm whale, probably as both feed on mesopelagic squid. These evidences led Santoro et al., (2018) to hypothesise the existence of a dwarf sperm whale population in the Mediterranean basin, in addition it seems that this particular odontocete has returned to visit Mediterranean waters probably to feed. The reason for the presence of the *Kogia sima* in the Mediterranean Sea is still unknown and this leaves an interesting prerequisite for collecting as much information as possible at the time of stranding individuals of this species.

Among the species defined as "regular" in the Mediterranean Sea (Notarbartolo di Sciara and Birkun, 2010), we analysed samples of *Physeter macrocephalus* and

Balaenoptera physalus. Concerning sperm whales, we found that all 10 samples analysed belong to the haplotype C, confirming the low genetic diversity and the isolation of Mediterranean sperm whale population. Sperm whales have a worldwide distribution and amount to about 360,000 individuals (Whitehead, 2002), nonetheless the genetic divergence at mtDNA CR level is low. This could be due to a recent population expansion following the last glacial maximum (LGM) (~20,000 years ago) or to a slow mutation rate. The latter hypothesis has been refuted by Alexander et al., (2013) since they observed that the mutation rate of mtDNA CR in sperm whales, compared with that of other cetaceans, is not very slow. A subsequent work by Alexander et al., (2016), in addition to mtDNA, also analyses the variability of this species at nuclear level through the use of markers such as microsatellites. A significant variability was obtained at social group/school level, moderate at regional level (Engelhaupt et al. 2009; Mesnick et al. 2011) and not significant at oceanic scale (Lyrholm et al. 1999), even lower than the mitochondrial one. According to Engelhaupt et al., (2009) this is a consequence of a highly female phylopatry and a male-biased dispersal. In conclusion the most probably hypothesis explaining the low variability at mtDNA CR seems to be the recent population expansion and the diffusion of a unique matriline during the LGM ~20,000 years ago (Morin et al., 2018). A similar low variability in mtDNA was found in the dwarf blue whale (*Balaenoptera musculus brevicauda*) by Attard et al., (2015) suggesting that this low diversity is due to the expansion during the LGM of part of the Antarctic blue whale population. The presence of a dwarf blue whale population with very low genetic variability is likely the result of a natural founder event, rather than a recent anthropogenic event. Alexander et al., (2016) related the sperm whales' population expansion to population growth and spread of their favourite prey: the giant squid *Architeuthis* spp. in which extremely low mitogenomic diversity was observed too. So, the presence of sperm whales in Mediterranean Sea seems to be attributed to a "lost tribe" or an extended "lobe" of the huge North Atlantic sperm whales population (Rendell and Frantzis, 2016) that after the population expansion became isolate in this basin. All the Mediterranean samples analysed in this study in fact share an identical mtDNA CR sequence, identified as haplotype "C". This is not a private haplotype of the Mediterranean population and at a global level it is one of

the three most common haplotyopes in sperm whales together with haplotypes "A" and "B" (Drouot et al., 2004; Engelhaupt et al., 2009). More recently, Alexander et al., (2016) found a single mtDNA haplotype among 40 individuals sampled from the Mediterranean basin, this result supports the idea that only a single matriline has colonised this area in the past. Engelhaupt et al., (2009), through microsatellite analysis, showed that only the Mediterranean population was significantly differentiated from any other population in the world. The naturally low genetic diversity of Mediterranean sperm whales implies a likely lower ability of reaction to present environmental changes compared to other sperm whale populations, a significant issue for their conservation in Mediterranean Sea.

Variation in ecological conditions can result in a variation of growth patterns in different populations of the same species: in the Atlantic Oceans sperm whale adult females reach about 11 m in length, while a physically mature male is approximately 16 m long (Rice, 1989). Concerning Mediterranean sperm whales, the size of individuals was extrapolated from the analysis of the inter-pulse interval (IPI) (Gordon et al., 1991). Using this method, but also through the measurement of stranded individuals' size, several works (Drouot et al., 2004; Frantzis et al., 2014; Bearzi et al., 2011; Mazzariol et al., 2011,2018; Fosklos et al., 2020) showed that in the northern basin there are larger individuals (sexually mature adult males) than in the southern area (females with calves). This follows the trend distribution of oceanic sperm whales, at high latitudes we find singleton mature males and at tropical and subtropical latitudes there are adult females, calves and juveniles (Rendell and Frantzis, 2016). They also observed that in Mediterranean Sea the average size of sexually mature males is 11.4 m while in adult females it is 9.1 m, so there is a difference in size between Mediterranean and Atlantic individuals. Also, our analysis clearly highlights that the similar-sized Mediterranean sperm whales are older than the Atlantic ones. Interestingly, also in other species of cetaceans, such as killer whales and bottlenose dolphins, size/age variation was observed among different populations. In the case of killer whales, three different ecotypes have been described in Pacific Ocean, namely "A", "B", "C" differing in morphology, feeding and habitat preferences. In fact, ecotype "A" includes larger individuals with an open ocean distribution and a diet based on marine mammals, such as minke

whale's calves. Ecotype "B" includes individuals with a smaller size that are distributed on the Antarctica loose pack-ice and feed on pinnipeds. Ecotype "C" includes the smallest individuals who inhabit the Antarctica dense pack-ice and eat fish (Pitman et al., 2007). The food availability is therefore the main factor that affects the size of individuals belonging to different populations. Even "Levantine nanism" in bottlenose dolphins could help to understand the causes of the small size of Mediterranean sperm whales. Bottlenose dolphins that are distributed in the Levantine basin show a small size compared to other bottlenose dolphins probably due to the physico-chemical characteristics of the basin: high temperatures, high salinity and low primary production seem to anticipate the sexual maturity time. Therefore, all the animal energies are recruited for reproduction at the expense of the increase in body size (Sharir et al., 2011). So physico-chemical characteristics of the basin and the food availability are key factors that can influence the growth trajectories of individuals. The Mediterranean is an oligotrophic sea and it is a small and isolated basin, so sperm whales feed on preys with a lower energy supply and, according to what we have just highlight, this might have the consequence of a smaller development in terms of size compared to sperm whales inhabiting the Atlantic Ocean. On the other hand, another scenario could be suggested to explain the small size ("dwarfism") of the Mediterranean sperm whale population. Indeed, its low genetic variation would be a consequence of a founder effect linked to the recent colonization of the Mediterranean Sea, about 20,000 years ago (Morin et al., 2018). The limited gene flow through the Strait of Gibraltar (Violi et al., 2020) and the small population size, where most or all mates are closely related, could promote inbreeding and inbreeding depression favouring the segregation of deleterious recessive alleles such as those related to dwarfism (see Kardos et al., 2016). It would be appropriate to investigate this second hypothesis which, if confirmed, could lead to an extinction vortex (*sensu* Gilpin and Soulé, 1986).

Concerning the two samples of *Balaenoptera physalus*, we found that one sample shares the private Mediterranenan haplotype BP46 (previously found in the Ligurian Sea), classified as private of the latter basin (Bérubé et al., 1998) while the other has the haplotype BP03 that is widespread in North Atlantic and Mediterranean Sea. These haplotypes match with those described by Cabrera et al.,

(2019) NATL004 and NATL002 respectively. The results of Cabrera et al., (2019) highlight that the haplotype NATL004 was also observed in three individuals from the Atlantic Ocean. The mismatch in frequency of these haplotypes is related to the different number of individuals analysed in previous studies (Bérubé et al., 1988; Cabrera et al., (2019)). However, the discovery of these haplotypes abundant in Mediterranean basin suggests that our samples belong to the Mediterranean fin whale population. Fin whales are migratory animals and make a seasonal shift between feeding and breeding areas: high-latitude summer feeding grounds and tropical winter breeding grounds (e.g. Evans, 1987). The presence of common haplotypes already observed both in Atlantic and Mediterranean populations, indicates that a connection between the two basins through the Strait of Gibraltar is maintained. Gauffier et al., (2018) analysed 15 yr of direct observations combining vessels and land-based surveys and discovered a seasonal bi-directional migration through the Strait of Gibraltar. It seems that all fin whales travel towards Atlantic Ocean between May and October and towards Meditearranean sea between November and April. Furthermore, the observation of young fin whale exiting from this latter sea, highlights that some of these specimens were born in the Mediterranean basin (Gauffier et al., 2018). The Corso-Ligurian basin, the central Tyrrhenian, the Gulf of Lion and the Catalan waters are the Mediterranean areas where fin whale abundance is the highest (Figure 21). By satellite tracking, Cotté et al., (2009) demonstrated that eight whales spent a period of over 10 months in Mediterranean Sea. Spatial modelling results confirm year-round presence of fin whales in the north-western Mediterranean, their abundance is lower in winter when they migrate to the Strait of Sicily where they have been observed feeding on the euphausiid *Nyctiphanes couchii* (Canese et al., 2006).

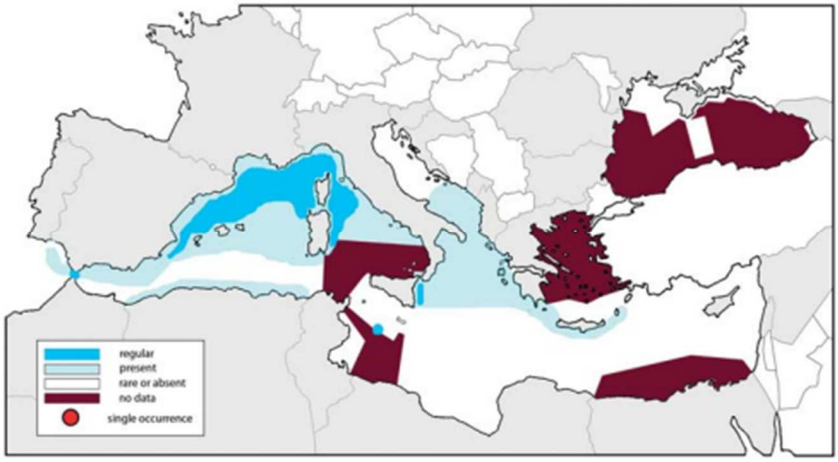


Figure 21 presumed distribution of *Balaenoptera physalus* in Mediterrranean and Black seas (Notarbartolo di Sciara and Birkun, 2010).

The morphological analysis together with the use of mitochondrial and nuclear genetic markers, lead us to discover that the third fin whale analysed (ID 531) is actually a hybrid between a blue and a fin whale. The mtDNA CR analysis shows that this hybrid has a 100% identity with the haplotypes described in the work of Pampoulie et al., (2020) which came from the waters of Iceland. Furthermore, in Pampoulie et al., (2020) is reported the greatest number of hybrids with mother blue whale and father fin whale demonstrating the unidirectional hybridization between these two species. The unidirectional hybridization usually occur when females of a rare species, initially reject allospecific males but then can mate with them as a consequence of the smallest number of conspecific males (Wirtz, 1999). The strong demographic collapse of *B. musculus (*Sears and Perrin, 2018*)* in the North Atlantic compared to *B. physalus (*Aguilar and Garcia-Vernet 2018*)*, is probably the main cause of unidirectional hybridization observed between blue whale and fin whale. The presence of unidirectional hybridization could be also an artefact due to post-zygotic barriers and/or to the generation of offspring with low fitness when a blue whale male crossing with a fin whale female (Wirtz, 1999). Hybridization is a frequent phenomenon in cetaceans occourring both in the suborder of odontocetes and mysticetes. In particular, it is rather common within the same genus, where different species have similar life histories and share similar habitats. Usually, in mammals the hybridization frequency is low, the fitness of the hybrids is low, since

they are sterile and unable to reproduce (Bérubé, 2002). In this case, the phenomenon of hybridization is not a threat for the genetic integrity of the relative parental species. On the other hand, if the fitness of the hybrids is similar to that of parental species and the frequency of hybridization increases, this could reduce the isolating mechanisms between species (Bérubé, 2002). When the hybridization has a positive effect the hybrid speciation could occur, the Clymene dolphin (*Stenella clymene*) is a new species described as a result of interspecific mating between *Stenella coeruleoalba* and *Stenella longirostris* (Amaral et al., 2014). However, the hybridization lead to negative consequences in most cases determining introgression or the complete replacement of the genome of one the two parental species (generally, the demographically weaker one), with its contextual extinction. Until recently, hybridization among cetacean species has been thought to be a "dead-end" because most hybrids were deemed to be infertile (Bérubé & Palsbøll, 2018) but the discovery of a second-generation adult hybrid (Pampoulie et al., 2020) and a pregnant hybrid female (Spilliaert et al., 1991), evidenced that mating between blue and fin whale give rise to fertile offspring. This hypothesis has been confirmed only for hybrid females, while hybrid males appear to be sterile (Arnason et al., 1991). In order to define the real extent of the hybridization phenomenon between *B. musculus* and *B. physalus* and clarify its effects on both species involved, the morphological analysis must always be supported by the genetic analysis of biparentally inherited markers.

5. Conclusion and perspective

Roger Payne said: "There is a message coming from the ocean to us, from the whales directly. What this message says is: it is possible to own a brain as complex as our own without destroying our world. What we have to learn from this message is very simple. If what we do diminish the ability of our planet to support life, then we don't have to do it! Or we have no future. Modern whales, for all their 20 million years, what is 19 million years more than us, have succeeded in living on our planet without destroying it. We could do just the same!"

The present study has been run to analyse some significant biological aspect (ageing, genetic identity, trans-basins movements, interspecific hybridization) concerning cetacean species (both regular and occasional) stranded along the italian coasts. We hope that this work will contribute to a better understanding of the biological cycle traits of these marine mammal species and help to realize efficient conservation strategies.

6. References

Aguilar, A. "Fin Whale *Balaenoptera physalus*". In *ENCYCLOPEDIA OF MARINE MAMMALS*, Academic Press (2002).

Alexander, Alana, et al., "What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)?" *Molecular Ecology* 25.12 (2016): 2754-2772.

Arnason, U., A. N. E. T. T. E. Gullberg, and B. E. N. G. T. Widegren. "Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species." *Molecular biology and evolution* 10.5 (1993): 960-970.

Árnason, Úlfur, et al., "Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow." *Science advances* 4.4 (2018): eaap9873.

Attard, Catherine RM, et al., "Low genetic diversity in pygmy blue whales is due to climate-induced diversification rather than anthropogenic impacts." *Biology letters* 11.5 (2015): 20141037.

Baccetti, N., F. Cancelli, and T. Renieri. "First record of *Kogia simus* (Cetacea, Physeteridae) from the Mediterranean Sea." *Mammalia (Paris)* 55.1 (1991): 152-154.

Bérubé, Martine, and Alex Aguilar. "A new hybrid between a blue whale, *Balaenoptera musculus*, and a fin whale, *B. physalus*: frequency and implications of hybridization." *Marine Mammal Science* 14.1 (1998): 82-98.

Bérubé, M., «Hybridism». In *ENCYCLOPEDIA OF MARINE MAMMALS*. Academic Press, (2002).

Borrell, Asunción, et al. "Stable isotopes provide insight into population structure and segregation in eastern North Atlantic sperm whales." *PLoS One* 8.12 (2013): e82398.

Cagnolaro, L., A. Di Natale, and G. Notarbartolo di Sciara. "Cetacei. Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane." *CNR Roma* (1983).

Cagnolaro L., Cozzi B., Notarbartolo di Sciara G., Podestà M., «Fauna d'Italia». Vol. XLIX. Mammalia IV. Cetacea. Calderini, Bologna (2015).

Canese, Simonepietro, et al., "The first identified winter feeding ground of fin whales (*Balaenoptera physalus*) in the Mediterranean Sea." *Journal of the Marine Biological Association of the United Kingdom* 86.4 (2006): 903-907.

Caputo, Vincenzo, and Massimo Giovannotti. "Haplotype characterization of a stranded *Balenoptera physalus* (Linnaeus, 1758) from Ancona (Adriatic sea, central Italy)." *Hystrix, the Italian Journal of Mammalogy* 20.1 (2009).

Chivers, S. J., et al., "Genetic variation of Kogia spp. with preliminary evidence for two species of Kogia sima." *Marine Mammal Science* 21.4 (2005): 619-634.

Collareta, Alberto, Franco Cigala Fulgosi, and Giovanni Bianucci. "A new kogiid sperm whale from northern Italy supports psychrospheric conditions in the early Pliocene Mediterranean Sea." *Acta Palaeontologica Polonica* 64.3 (2019).

Cotté, Cédric, et al., "Scale-dependent habitat use by a large free-ranging predator, the Mediterranean fin whale." *Deep Sea Research Part I: Oceanographic Research Papers* 56.5 (2009): 801-811.

Crossman, Carla A., Eric B. Taylor, and Lance G. Barrett-Lennard. "Hybridization in the Cetacea: widespread occurrence and associated morphological, behavioral, and ecological factors." *Ecology and Evolution* 6.5 (2016): 1293-1303.

Dale, W. R. "Marine mammals of the World: Systematic and Distribution." *Society of Marine Mammalogy Special Publication* 4 (1998): 231.

Desjardins P., Conklin D. (2010). NanoDrop Microvolume Quantitation of Nucleic Acids. JoVE. 45. http://www.jove.com/index/Details.stp?ID=2565, doi: 10.3791/2565

di Sciara, Giuseppe Notarbartolo, and Jonathan Gordon. "Bioacoustics: a tool for the conservation of cetaceans in the Mediterranean Sea." *Marine & Freshwater Behaviour & Phy* 30.2 (1997): 125-146.

do Nascimento Schaurich, Miriane, Fernando Ricardo Vieira Lopes, and Larissa Rosa de Oliveira. "Revisão sobre o fenômeno da hibridização em cetáceos e pinípedes." *Neotropical Biology and Conservation* 7.3 (2012): 199-209.

Elsner, Robert. «Cetacean Physiology, Overview». In *ENCYCLOPEDIA OF MARINE MAMMALS*. Academic Press (2002).

Evans, K. A. R. E. N., et al. "Factors affecting the precision of age determination of sperm whales (*Physeter macrocephalus*)." *Journal of Cetacean Research and Management* 4.2 (2002): 193-202.

Felsenstein, Joseph. "Confidence limits on phylogenies: an approach using the bootstrap." *evolution* 39.4 (1985): 783-791.

Fortuna C., S. Gaspari, and G. Laurianom, «*Balaenoptera Physalus*». http://www.iucn.it/scheda.php?id=-2094774342 (2013).

Foskolos, Ilias, et al., "A taste for squid: the diet of sperm whales stranded in

Greece, Eastern Mediterranean." *Deep Sea Research Part I: Oceanographic Research Papers* 155 (2020): 103164.

Fraija-Fernández, Natalia, et al. "First stranding event of a common minke whale calf, *Balaenoptera acutorostrata* Lacépède, 1804, reported in Spanish Mediterranean waters." *Mammal Study* 40.2 (2015): 95-100.

Frantzis, Alexandros, Paraskevi Alexiadou, and Kalliopi Charitomeni Gkikopoulou. "Sperm whale occurrence, site fidelity and population structure along the Hellenic Trench (Greece, Mediterranean Sea)." *Aquatic Conservation: Marine and Freshwater Ecosystems* 24. S1 (2014): 83-102.

Gambaiani, D. D., et al., "Potential impacts of climate change and greenhouse gas emissions on Mediterranean marine ecosystems and cetaceans." *Journal of the Marine biological Association of the United Kingdom* 89.1 (2009): 179-201.

Gauffier, Pauline, et al., "Contemporary migration of fin whales through the Strait of Gibraltar." *Marine Ecology Progress Series* 588 (2018): 215-228.

Gilpin, M. E., Soulé, M. E., « Minimum Viable Populations: Processes of Species Extinction». In M. E. Soulé (ed.). Conservation Biology: The Science of Scarcity and Diversity. Sunderland, Mass: Sinauer. pp. 19–34. (1986).

Guarino, F. M., et al., "Age estimation and growth of striped dolphins Stenella coeruleoalba stranded along the coasts of south-western Italy." *The European Zoological Journal* 88.1 (2021): 417-424.

Gordon, Jonathan CD. "Evaluation of a method for determining the length of sperm whales (*Physeter catodon*) from their vocalizations." *Journal of Zoology* 224.2 (1991): 301-314.

IJsseldijk, Lonneke L., et al. "Beached bachelors: An extensive study on the largest recorded sperm whale *Physeter macrocephalus* mortality event in the North Sea." *PLoS One* 13.8 (2018): e0201221.

Jefferson, Thomas A., Stephen Leatherwood, e Marc A. Webber. "FAO species identifications guide". Marine Mammals of the World (1993).

Kardos, Marty, et al. "Genomics advances the study of inbreeding depression in the wild." *Evolutionary applications* 9.10 (2016): 1205-1218.

Klimpel, Sven, and Harry W. Palm. "Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change?" *Progress in parasitology*. Springer, Berlin, Heidelberg, 2011. 201-222.

Maio, Nicola, et al., "Haplotype characterization of a young stranded Common Minke Whale (*Balaenoptera acutorostrata* Lacépède, 1804): is the Mediterranean Sea a potential calving or nursery ground for the species?" *Hystrix, the Italian Journal of Mammalogy* 27.2 (2016): 205-208.

Maio, Nicola, et al., "New record of dwarf sperm whale Kogia sima (Owen, 1866) from the Mediterranean Sea (Cetacea Kogiidae)." *Biodiversity Journal* 8.4 (2017): 947-950.

Mattiucci, Simonetta, and Giuseppe Nascetti. "Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host—parasite co-evolutionary processes." *Advances in parasitology* 66 (2008): 47-148.

Mattiucci, Simonetta, Michela Paoletti, and Stephen C. Webb. "*Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere:

morphological description, genetic relationships between congeners and ecological data." *Systematic parasitology* 74.3 (2009): 199-217.

Mattiucci, Simonetta, et al., "Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae)." *The Journal of Parasitology* 100.2 (2014): 199-214.

Mazzariol, Sandro, et al., "Sometimes sperm whales (*Physeter macrocephalus*) cannot find their way back to the high seas: a multidisciplinary study on a mass stranding." *PLoS One* 6.5 (2011): e19417.

Mazzariol, Sandro, et al., "Multidisciplinary studies on a sick-leader syndrome-associated mass stranding of sperm whales (*Physeter macrocephalus*) along the Adriatic coast of Italy." *Scientific reports* 8.1 (2018): 1-18.

McAlpipne, Donald. "Pygmy and Dwarf Sperm Whales *Kogia breviceps* and *K. sima*". In *ENCYCLOPEDIA OF MARINE MAMMALS*. Academic Press (2002).

McAlpine, D.F., "Pygmy and dwarf sperm whales" in: B. Würsig, J.G.M. Thewissen, and K. Kovacs (eds.), Encyclopedia of Marine Mammals, 3rd Edition (2017).

Milinkovitch, Michel C. and Olivier Lambert, «Cetacea. Whales, dolphins, and porpoises», 2006.

Morin, Phillip A., et al., "Demography or selection on linked cultural traits or genes? Investigating the driver of low mtDNA diversity in the sperm whale using complementary mitochondrial and nuclear genome analyses." *Molecular Ecology* 27.11 (2018): 2604-2619.

Nei, Masatoshi, and Sudhir Kumar. *Molecular evolution and phylogenetics*. Oxford University Press, USA, 2000.

Nicolosi, Paola. "Variabilità intraspecifica in Delfino comune, Delphinus delphis (Linnaeus, 1758). L'utilizzo della morfometria geometrica bi-e tridimensionale come strumento diagnostico e conoscitivo." (2012).

Notarbartolo di Sciara, Giuseppe. "Cetacean species occurring in the Mediterranean and Black Seas." (2002).2002. A report to the ACCOBAMS Secretariat, Monaco, February 2002. Section 3, 17 p.

Notarbartolo di Sciara G., Birkun A.Jr., «Conserving whales, dolphins and porpoises in the Mediterranean and Black Seas: an ACCOBAMS *status* report», 2010.

Pagh, Sussie, et al., "Age determination of sperm whales (*Physeter macrocephalus*) from the west coast of Jutland, Denmark: The 13th Danish Marine Mammal Symposium." *The 13th Danish Marine Mammal Symposium*. 2016.

Pitman, Robert L., et al., "A dwarf form of killer whale in Antarctica." *Journal of Mammalogy* 88.1 (2007): 43-48.

Perrin, W. F., R. L. Brownell. "Minke Whales *Balaenoptera acutorostrata B. bonaerensis*". In *ENCYCLOPEDIA OF MARINE MAMMALS*. Academic Press (2002).

Ponganis Paul J. "Circulatory System". In *ENCYCLOPEDIA OF MARINE MAMMALS*. Academic Press (2002).

Rendell, L., and A. Frantzis. "Mediterranean sperm whales, *Physeter macrocephalus*: the precarious state of a lost tribe." *Advances in marine biology* 75 (2016): 37-74.

Robineau D., «Cétacés de France». Faune de France vol. 89. Fed. Fr. Soc. Sciences Natl., Paris,2015.

Santoro, M., et al. "Helminth parasites of the dwarf sperm whale Kogia sima (Cetacea: Kogiidae) from the Mediterranean Sea, with implications on host ecology." *Diseases of Aquatic Organisms* 129.3 (2018): 175-182.

Sharir, Yuli, Dan Kerem, and Ehud Spanier. "Small size in the common bottlenose dolphin Tursiops truncatus in the eastern Mediterranean: a possible case of Levantine nanism." *Marine Ecology Progress Series* 438 (2011): 241-251.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution.
Taylor B.L., Baird R., Barlow J., et al., "Kogia sima". The IUCN Red List of Threatened Species, Version 2012.

Tomilin A. G., "Cetacea", 1957. In: Heptner V. G. Ed., Mammals of the U.S.S.R. and adjacent countries. Vol. 9. Israel Program for Scientific Translations, 1967, Jerusalem

Van Waerebeek, K., et al., "Spatial and temporal distribution of the minke whale, *Balaenoptera acutorostrata* (Lacépède, 1804), in the southern." *J Cetacean Res Manag* 1 (1999): 223-37.

Violi, Biagio, et al. "Population dynamics and structure of sperm whales (*Physeter macrocephalus*) in the Mediterranean Sea." (2019).

Whitehead, Hal. "SPERM WHALE", 2018.

Wirtz, Peter. "Mother species–father species: unidirectional hybridization in animals with female choice." *Animal behaviour* 58.1 (1999): 1-12.

Yablokov A. V., bel'kovic V. M. & borisov V. I., «Whales and Dolphins», 1972. Israel Program for Scientific Translations. Jerusalem.

7. Appendix

Table S1 Diagnostic sites in the 619 bp CR sperm whale sequences of mtDNA.

	38	53	57	100	102	104	116	145	179	195	202	203	206	230	233	238	255	267	268	278	281	282	283	284	286	290	300	303	314	319	345	569	603	619
A.001.003	T	T	C	C	A	G	C	C	T	T	A	A	C	A	T	G	A	A	C	C	A	A	A	T	A	G	C	A	G	C	C	G	G	G
U.NA.NA	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-
V.NA.NA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	-	-	-
T.NA.NA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-
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Y.NA.NA	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	-	-	-
MM.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.
Z.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	A	.
GG.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.
LL.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	A	.
C.001.002	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.
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PM3	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.
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L.NA.NA	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	-	-	-
K.NA.NA	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	T	.	-	-	-
JJ.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	T	A	.
JJ.002.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	A	.
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Q.001.001	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	A	T	.	.	A	.
S.001.001	.	.	T	T	.	.	.	.	C	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	A	T	.	.	A	.
O.001.001	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	A	.
R.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	A	.
N.001.001	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
DD.001.001	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
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B.001.002	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A
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BB.001.001	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.
FF.001.001	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	A	.
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AA.NA.NA	.	C	.	.	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	-	-	-
NN.001.001	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
G.NA.NA	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	-	-
E.001.001	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.

Table S2 Sequence alignment (288bp) of ID 536 and ID 553 vs. haplotypes *Balaenoptera physalus* from Bèrubè et al., 1998. The haplotypes with an identity percentage of 100% with the samples of this work, are highlighted in the same color.

	DIAGNOSTIC SITES																													
Haplotype	13	18	25	27	56	67	78	86	87	96	103	104	132	140	162	178	180	195	200	213	222	237	253	255	258	263	276	280	282	283
Bp 02	G	A	C	A	T	A	T	C	T	T	T	T	G	C	T	T	T	G	T	T	T	A	A	A	G	T	C	A	C	T
Bp 21	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
Bp 01	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 38	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 31	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 47	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 46	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Bp 553	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Bp 03	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 536	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 15	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
Bp 33	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C

Bp 36	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
Bp 48	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 12	.	.	.	G	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
Bp 25	.	.	.	G	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
Bp 27	.	.	.	G	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
Bp 20	.	.	.	G	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.
Bp 32	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
Bp 26	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bp 13	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.
Bp 41	.	.	.	G	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	T	.
Bp 05	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.
Bp 04	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.
Bp 08	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 16	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	T	G	T	.
Bp 06	.	.	.	G	.	.	.	.	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 11	.	.	.	G	.	.	.	T	C	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	A	.	T	G	T	.
Bp 14	.	.	.	G	.	.	.	T	C	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp	.	.	.	G	.	.	.	T	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.

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Bp 19	.	.	.	G	.	.	.	T	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 23	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 30	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 40	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 44	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	T	.
Bp 42	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	G	T	.
Bp 17	.	.	.	G	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	C
Bp 43	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	T	C
Bp 37	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	T	.
Bp 28	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.
Bp 10	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
Bp 18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
Bp 34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Bp 07	.	.	.	G	.	G	.	.	.	.	C	.	.	.	.	C	.	A	C	.	.	.	G	.	.	.	.	G	T	.
Bp 39	.	.	.	G	.	G	.	T	.	.	C	.	.	.	.	C	.	A	C	.	.	.	G	.	.	.	.	G	T	.
Bp 49	.	.	.	G	.	.	.	T	.	.	.	.	.	T	.	C	.	A	C	.	.	T	.	.	.	.	.	G	T	.
Bp 50	.	.	.	G	.	.	.	T	.	.	.	.	.	T	C	C	.	A	C	.	.	T	.	.	.	.	.	G	T	.

Bp 51	.	.	.	G	.	.	.	T	.	.	.	.	.	T	.	C	.	A	C	C	.	T	.	.	.	.	.	G	T	.
Bp 29	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Bp 22	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	T	.
Bp 35	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	T	.
Bp 09	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	T	.

Table S3 Sequence alignment (285 bp) of ID 536 and ID 553 vs haplotypes *Balaenoptera physalus* taken from Cabrera et al, 2019 including Archer et al, 2013.

	DIAGNOSTIC SITE																																						
Haplotype	10	13	18	25	27	56	67	78	86	87	96	103	104	132	164	178	180	188	195	198	200	202	206	213	222	225	232	252	253	255	257	258	263	264	266	276	280	282	283	285
NA 003	A	G	A	C	G	T	A	T	C	T	C	T	T	G	G	T	C	A	G	G	T	G	T	T	T	A	A	A	A	A	C	G	T	T	T	T	G	T	T	T
NA 036	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
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NA 068	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
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NA 057	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
NA 064	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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NA 032	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.
NA 040	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
NA 028	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
NA 071	.	.	.	.	.	.	.	.	T	C	T	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
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NA 062	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
NA 050	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 079	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 025	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
Bp 553	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 004	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 002	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
Bp 536	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 056	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 063	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.
NA 065	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	A	C	.	.
NA 058	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	A	C	.	.
NA 015	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 044	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	C	.
NA 073	.	.	.	.	.	.	.	.	T	.	T	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	C	A	C	C	.

NA 059	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	C	.
NA 045	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 076	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 009	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 048	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	G	.	T	.	.	.	C	A	C	.	.
NA 046	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	C	C	A	C	.	.
NA 047	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 074	.	.	.	.	.	.	.	.	T	.	T	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 031	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 008	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	C	.	.
NA 066	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 037	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 038	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.
NA 054	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 022	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	A	C	.	.
NA 067	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	C	C	.	.	.	.	.	.	C	A	C	.	.
NA 005	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.

NA 042	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 016	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 017	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 018	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 021	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	C	.	.
NA 014	.	A	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	C	.	.
NA 020	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.
NA 060	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.
NA 019	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.
NA 027	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	.	.	.
NA 053	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	.	.	.
NA 043	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	A	.	.	.
NA 049	.	.	.	.	.	.	.	.	.	.	T	.	.	A	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.
NA 051	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.
NA 013	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.
NA 075	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	C	T	.	.	A	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	C	A	.	.	.
NA 023	.	.	.	.	A	.	.	.	T	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.

NA 011	.	.	.	.	.	.	G	.	.	.	T	C	.	.	.	C	T	.	A	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.
NA 012	.	.	.	.	.	.	G	.	T	.	T	C	.	.	.	C	T	.	A	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.
NA 052	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.

Table S4 Characterization of variable sites in the 334 bp CR rorqual sequences of mtDNA.

	1	2	3	4	5	6	8	9	10	11	18	21	33	36	64	81	83	91	119	120	126	127	133	147	156	174	205	207	209	210	219	220	221	250	252	253	254	268	273	276	290	291	292	299	300	312	333
Ba158	G	A	A	A	A	T	T	A	T	A	G	C	G	C	T	T	C	C	A	G	G	C	C	G	C	G	A	A	C	C	A	A	T	C	T	C	G	G	T	T	A	C	A	A	T	T	A
Ba161	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ba154	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
Ba122	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	.	.
Ba138	-	-	G	.	.	A	.	.	.	T	A	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	.	.
Ba127	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	.
Ba144	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	A	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	.	.
Ba116	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	.
Ba146	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	T	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	.
Ba131	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	G
Ba148	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	G
Ba137	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	.	C	.
Ba142	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	T	T	.	.	.	.
Ba143	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	T	T	.	.	C	.
Ba135	-	G	.	.	.	A	.	.	.	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	G	.	T	.	.	.	C	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba145	-	G	.	.	.	A	.	.	.	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	T	.	.	.	C	.	.	T	.	.	.	.	G	.	T	.	.	C	.

Ba1 41	-	G	.	.	.	A	.	.	.	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	T	.	.	.	C	.	.	T	.	.	C	.	G	.	T	.	.	C	.
Ba1 33	-	G	.	.	.	A	.	.	.	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 49	-	G	.	.	.	A	.	.	.	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	T	.	.	C	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 39	-	G	.	.	.	A	.	.	C	C	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 26	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	C	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 47	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 17	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 36	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	T	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 18	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 34	-	-	G	.	.	A	.	.	C	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 25	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 32	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 40	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 21	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	.	.	.
Ba1 29	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	T	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	.	C	.
Ba1 30	-	G	.	.	.	A	.	.	.	T	A	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	T	G	.	.	.
Ba1 50	-	G	.	.	.	A	.	.	.	T	A	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	G	.	.	.
Ba1 23	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	.	.	.
Ba1 19	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	G
Ba1 20	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	T	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 24	-	-	G	.	.	A	.	.	.	T	.	.	.	T	.	.	.	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	C	.
Ba1 28	-	-	G	.	.	A	.	.	.	T	.	T	.	T	.	.	.	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	G	.	T	.	.	.	.
ID405	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	T	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba1 87	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	T	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.

Ba186	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba185	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba168	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba164	-	-	-	-	G	A	A	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba183	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba184	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba163	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	A	.	.
ID416	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba165	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba182	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	T	T	.	.	.	.
BAC	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba169	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba179	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba181	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	A	.	.	G	.	T	.	C	.	.
Ba180	-	-	-	-	G	A	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	T	T	.	C	.	.
Ba151	-	-	G	.	.	A	A	G	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	T	.	.	.	.	.	T	.	.	.	A	G	.	T	T	C	.	.
Ba153	-	-	-	G	.	A	A	G	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	G	.	T	T	C	.	.
Ba167	-	-	-	G	.	A	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	G
Ba162	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba171	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	G	.	T	.	C	.	.
Ba166	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba175	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba172	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba176	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	C	.	.	T	.	.	.	.	G	.	T	.	C	.	.

Ba1 74	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	G	.	T	.	C	.	.
Ba1 73	-	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	C	.	.
Ba1 57	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ba1 56	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ba1 60	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
Ba1 59	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ba1 52	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.
Ba1 55	-	-	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	.	T	A	.	.	.	.	.	.	.	.	.	.
Ba1 70	-	-	G	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 78	-	-	G	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.
Ba1 77	-	-	G	.	.	A	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	T	.	.	.	.