Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses

Published Version:
Agostino Leone, E.S. (2020). Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses. JOURNAL OF BIOGEOGRAPHY, 47(5), 1119-1129 [10.1111/jbi.13794].

Availability:
This version is available at: https://hdl.handle.net/11585/712445 since: 2024-05-24

Published:

DOI: http://doi.org/10.1111/jbi.13794

Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses

Agostino Leone[1,*], Gregory Neils Puncher[2,3], Francesco Ferretti[4,5], Emilio Sperone[6], Sandro Tripepi[6], Primo Micarelli[7], Andrea Gambarelli[8], Maurizio Sarà[9], Marco Arculeo[9], Giuliano Doria[10], Fulvio Garibaldi[11], Nicola Bressi[12], Andrea Dall'Asta[12], Daniela Minelli[13], Elisabetta Cilli[14], Stefano Vanni[15], Fabrizio Serena[16], Píndaro Díaz-Jaimes[17], Guy Baele[18], Alessia Cariani[1], Fausto Tinti[1]

[1]Dept. Biological, Geological & Environmental Sciences (BiGeA), Laboratory of Genetics & Genomics of Marine Resources and Environment (GenoDREAM), University of Bologna, 48123, Ravenna, Italy

[2]Dept Biological Sciences, Canadian Rivers Institute, University of New Brunswick, Saint John, NB, E2L 4L5, Canada

[3]Genomics Laboratory, Maurice-Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, QC, G5H 3Z4, Canada

[4]Hopkins Marine Station, Department of Biology, Stanford University, 120 Oceanview Boulevard, Pacific Grove, CA 93950, USA

[5]Department of Fish and Wildlife Conservation, Virginia Tech, 310 West Campus Drive, Blacksburg, VA 24060, USA

[6]DiBEST Department of Biology, Ecology and Earth Science, University of Calabria, 87036, Arcavacata di Rende, Italy

[7] Sharks Studies Center (C.S.S.), 58024, Massa Marittima (GR), Italy

[8]Museum of Zoology and Comparative Anatomy of Modena, University of Modena and Reggio Emilia, 41121, Modena, Italy

[9]Dipartmento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), University of Palermo, 90123, Palermo, Italy

[10]Civic Museum of Natural History "Giacomo Doria", 16121, Genova, Italy

[11]Department of Earth Sciences, Environmental and Life, University of Genova, 16132, Genova, Italy

[12]Civic Museum of Natural History of Trieste, 34100, Trieste, Italy

[13]Museum of Comparative Anatomy, University of Bologna, 40126, Bologna, Italy

[14]Department of Cultural Heritage, University of Bologna, 48121, Ravenna, Italy

[15]Museum of Natural History of Firenze "La Specola", 50125, Firenze, Italy

[16]Institute for Biological Resources and Marine Biotechnology (IRBIM), National Research Council – CNR, 91026, Mazara del Vallo (TP), Italy

[17]Laboratorio de Genética de Organismos Acuáticos Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Apdo. Postal 70-305, 04510 Mexico, D.F., Mexico

[18] Department of Microbiology and Immunology, Rega Institute, KU Leuven, 3000 Leuven, Belgium

[*]Corresponding author: agostino.leone@unibo.it

Abstract

Aim

Determine the evolutionary origin of the heretofore poorly characterized contemporary Great White Shark (GWS; *Carcharodon carcharias*) of the Mediterranean Sea, using phylogenetic and dispersal vicariance analyses to trace back its global paleo-migration pattern.

Location Mediterranean Sea

Taxon *Carcharodon carcharias*

Methods We have built the largest mtDNA control region (CR) sequence dataset for the Mediterranean GWS from referenced historical jaws spanning the 19th and 20th centuries. Mediterranean and global GWS CR sequences were analyzed for genetic diversity, phylogenetic relationships and divergence time. A Bayes factor approach was used to assess two scenarios of GWS lineage divergence and emergence of the Mediterranean GWS line using fossil records and paleo-geographical events for calibration of the molecular clock.

Results The results confirmed a closer evolutionary relationship between Mediterranean GWS and populations from Australia-New Zealand and the Northeastern Pacific coast rather than populations from South-African and Northwestern Atlantic. The Mediterranean GWS lineage showed the lowest genetic diversity at the global level, indicating its recent evolutionary origin. An evaluation of various divergence scenarios determined the Mediterranean GWS lineage most likely appeared some 3.23 million years ago by way dispersal/vicariance from Australian/Pacific paleo-populations.

Main conclusion Based on the fossil records, phylogeographic patterns and divergence time, we revealed that the Mediterranean GWS population originated in the Pliocene following the Messinian Salinity Crisis. Colonization of the Mediterranean by GWS likely occurred via an eastward paleo-migration of Australian/eastern Pacific elements through the Central American Seaway, before the complete closure of the Isthmus of Panama. This Pliocene origin scenario contrasts with a previously proposed scenario in which Australian GWS colonized the Mediterranean via antipodean northward migration resulting from navigational errors from South Africa during Quaternary climatic oscillations.

Keywords Great White Shark, Mediterranean, historical DNA, divergence time, phylogeography, *Carcharodon carcharias*

Background

Large predatory shark populations in the Mediterranean Sea have declined dramatically over the last century (Ferretti, Myers, Serena, & Lotze, 2008). The loss of apex predators throughout the world's oceans over the past century is likely to have caused profound ecological alterations and potentially large-scale trophic cascades (Myers, Baum, Shepherd, Powers & Peterson, 2007; Ferretti, Worm, Britten, Heithaus, & Lotze, 2010). Among the species that witnessed the most precipitous declines is the Great White Shark (*Carcharodon carcharias*, L.1758, henceforth GWS; McPherson & Myers. 2009), which is currently listed as Critically Endangered in European seas (Nieto et al., 2015). GWS are widespread throughout the globe, aside from the polar regions, with hotspots of abundance located off the coasts of South Africa, Australia, New Zealand, Japan, North and South America, and in the Mediterranean (Compagno, 1984; Fergusson, 1996). Integrated ecological, genetic and tagging data have revealed natal homing and philopatric behavior of GWS, with extraordinary trans-oceanic migrations of both sexes between geographically distant populations in the Indian (Pardini et al., 2001; Bonfil et al., 2005; Blower, Pandolfi, Bruce, Gomez-Cabrera, & Ovenden, 2012) and North Pacific Ocean (Domeier & Nasby-Lucas, 2008; Jorgensen et al., 2010, 2012). In the Mediterranean, GWS have long been observed and documented by the public, resource users and scientists, who have provided opportunistic occurrence records, from direct sightings, commercial fisheries, records of bite marks found on prey and museum specimens dating back to the early 19th century (Mancusi et al., 2002; De Maddalena, 2006; Sperone et al., 2012). In the past, free-swimming individuals and pairs were frequently observed in areas where large pelagic fisheries were intense (e.g. the Sicilian Channel, the Ligurian and Tyrrhenian seas; Fergusson, 1996; Storai, Vanni, Zuffa, & Biagi, 2005). A few records of GWS pups from Turkey and Tunisia, as well as juveniles in the Sicilian Channel, suggest that the Mediterranean may host GWS nursery areas (Fergusson, 1996; Storai, Mojetta, Zuffa, & Giulian, 2000; Kabasakal, & Gedikoğlu, 2008). However, the natural history of Mediterranean GWS still remains largely uncharacterized.

Efforts have been made in the past to resolve the phylogenetic relationships of the Mediterranean GWS population using mitochondrial DNA (mtDNA) sequences (Gubili et al., 2010; 2015). Using up to five specimens from the Mediterranean, Gubili et al. (2010, 2015) concluded that the population is more closely related to populations in the Pacific Ocean (Australia, New Zealand and Northeastern Pacific) than to those from the western Indian Ocean (South-Africa) and northwestern Atlantic Ocean (Florida). Based on a nucleotide substitution rate between the two major lineages (Northeastern Pacific vs. North West Atlantic and Eastern Indian) calibrated by the formation of the Isthmus of Panama (3.5 Ma) and the Sunda-Sahul Shelves (5 Ma) respectively, Gubili et al. (2010) suggested that Mediterranean GWS are descendants of a few disoriented individuals who immigrated from Australia/New Zealand during the Pleistocene (348-565 ka) by an antipodean route along the western coast of Africa. A scenario of multiple relatively recent colonization events was also considered, based on the haplotype relationships that were generated using a few historical and contemporary Mediterranean specimens (Gubili et al., 2015).

Collection of fresh GWS specimens in the Mediterranean has proven to be difficult in recent decades, due to their precipitous decline in abundance. However, there is a great number of referenced and dry-preserved GWS specimens in several Italian museums and private scientific archives, such as mounted skins, jaws, vertebrae and teeth collected from the Mediterranean during the last two centuries (Mancusi et al., 2002; De Maddalena, 2006). Recently developed ancient DNA (aDNA) techniques present a great opportunity for reconstructing the natural history of marine species using preserved historical materials (Hofreiter, Serre, Poinar, Kuch, & Pääbo, 2001; Riccioni et al., 2010). Unfortunately, most historical GWS specimens have been archived in sub-optimal conditions, thereby compromising their potential for DNA-based applications. Moreover, many collectors are reluctant to loan specimens for molecular study, due to their intrinsic, sentimental and market value.

By analyzing DNA extracted from preserved specimens of GWS caught during the last 195 years, from eight Italian museums and private collections, we have been able to explore the evolutionary history of the Mediterranean GWS. Using effective and affordable aDNA techniques widely used to extract and genotype DNA from historical specimens of marine fish, we have generated a publicly available mtDNA sequence dataset from 18 Mediterranean GWS individuals.

Methods

Full details of the collected historical GWS specimens, sampling procedures, protocols for aDNA extraction, PCR amplification of Control Region (CR), sequencing and sequence analyses are provided in the in the Supporting Information (Supplementary Methods, Appendix S1; Figures S1-S3, Appendix S2; Tables S1-S3, Appendix S3).

Analysis of fossil evidence

The extensive catalogue of taxon-specific GWS fossils featured in the online and open access Paleobiology Database (https://paleobiodb.org/#/), and its associated R package 'paleobioDB' (Varela et al., 2015), was used to create a distribution and stratigraphic map of global GWS fossils. The downloaded database was filtered manually to avoid the use of homonym extinct taxa and dubious records. Only reliable records specifically classified as *Carcharodon carcharias* and relative synonyms were retained (Table S4, Appendix S3). A detailed search of the literature with a focus on *C. carcharias* fossils from the Mediterranean Sea was carried out in order to retain reliable fossil records and exclude potential misidentification of fossil specimens (Table S5, Appendix S3) (Cigala-Fulgosi, 1990; Applegate, & Espinosa-Arrubarrena, 1996; Gottfried & Fordyce, 2001; Bianucci et al., 2002; Marsili, 2006; Marsili, 2008; Adnet, Balbino, Antunes, & Marín-Ferrer, 2009). The fossil records from the paleobioDB database were checked for correctness using the references and fossil collections associated to the paleobioDB codes (Table S4, Appendix S3). Records without descriptions of the fossil or without pictures, especially those from old references, as well as records with misidentified fossils (e.g. *Isurus* spp. or *Carcharodon* spp. identified as *Carcharodon carcharias*), were removed.

Time of the Most Recent Common Ancestor (TMRCA) and estimation of evolutionary rate

The divergence time analysis of the GWS lineages was carried out using Bayesian inference through Markov Chain Monte Carlo (MCMC) sampling as implemented in BEAST v1.10.0 (Suchard et al., 2018). An initial analysis using calibration priors without sequence data was carried out to determine if calibration priors interacted unexpectedly and to assess if the data were informative (Fulton, & Strobeck, 2010). A relaxed molecular clock and a constant population size coalescent model were used to recover time-stamped phylogenies in BEAST. To ensure convergence of the posterior distributions, three independent MCMC analyses were run (20 million steps, sampled every 1k generations, burn-in 50%). Convergence and effective sample sizes (ESS) were verified using Tracer v.1.7.0 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). A Maximum Clade Credibility (MCC) tree was summarized using TREEANNOTATOR V.1.10.0 and visualized in FIGTREE V.1.4.3 (Rambaut, 2009). Since the molecular clock is sensitive to bias when a short fragment with fewer polymorphisms is used, the TMRCA analyses were carried out using the two CR sequence datasets of different length (516bp and 828bp), as previously used in the haplotype network analysis (Supplementary Methods in Appendix S1). The divergence time of the GWS lineages was estimated using two alternative combinations of calibration priors in BEAST v1.10.0 (Suchard et al., 2018). The alternative combinations of calibration priors were built by integrating the estimated age of the earliest GWS fossil records (Applegate & Espinosa-Arrubarrena, 1996) (Mean: 11.0 Ma; SD: 1.0) with two different secondary calibrations: a) the best dated fossil record of GWS in the Mediterranean Sea, dated back to the Pliocene (Cigala-Fulgosi, 1990; Bianucci et al., 2002) (Mean: 3.0 Ma; SD: 0.30 Ma), and b) the molecular divergence previously estimated by Gubili et al. (2010) (Mean: 0.4 Ma; SD: 0.15). Both combinations of calibration priors were implemented as normally distributed. Rather than using debated fossil records from the middle Miocene epoch (Gottfried & Fordyce, 2001), we used the divergence time between the GWS and the outgroup *Lamna nasus* (GenBank Acc. No. GU266755-GU266769) as an alternative first calibration. The divergence time of these two lineages has been estimated at around 46 Ma (Martin, 1996) (Mean: 46.0 Ma; SD: 1.0).

Marginal likelihood estimation and testing divergence time hypotheses

After setting the first calibration for the earliest fossil attributed to the GWS, the two alternative secondary calibrations could be compared by estimating log marginal likelihoods using generalized stepping-stone (GSS) sampling, as implemented in BEAUti v1.10.0 and BEAST v1.10.0 (Suchard et al., 2018; Baele, Lemey, & Suchard, 2015). The log marginal likelihood values for the two different scenarios were first estimated using generalized stepping-stone sampling (100 stepping stones, 1 million iterations, logging every 1,000 iterations). A total of 101 power posteriors, with one million iterations each, were sampled using MCMC for the GSS approach. In order to select the alternative calibrations that fits best with the principal timing information fixed for both scenarios, the log Bayes factor was calculated for both scenarios using the formula logBF = log Pr (D | M1) – log Pr (D | M2), where log Pr (D | M1) is the log marginal likelihood for model 1, and log Pr (D | M2) is the log marginal likelihood for model 2.. This analysis was performed on both sequence datasets (i.e. 516bp and 828bp).

Reconstructing the historical biogeography

Two approaches were implemented in order to reconstruct the historical biogeography of species: the Statistical Dispersal-Vicariance Analysis (S-DIVA; Yu, Harris, & He, 2010), which is a parsimony method of historical biogeography, and Dispersal-Extinction-Cladogenesis analysis (DEC; Ree, & Smith, 2008). An MCC estimated by using BEAST and TreeAnnotator on just haplotype sequences, and the specimen distribution through all biogeographical areas (A: Australia/New Zealand, AUS; B: Northeastern Pacific, NEP; C: Mediterranean, MED; D: South-Africa, SA; E: Northwestern Atlantic, NWA) was used to perform the S-DIVA and DEC analyses implemented in RASP v. 4.0 (Yu, Harris, Blair, & He, 2015).

Results

Partial CR sequences (515bp) from 18 GWS historical specimens were obtained and deposited in GenBank (Acc. No. MN718579-MN718596).

The multiple sequence alignment containing sequences from all 18 historical samples and four Mediterranean homologous modern sequences deposited in GenBank (HQ540294-HQ540296; JF715925; Table S3 in Appendix S3) showed an extremely low nucleotide diversity among the CR fragment (< 0.1%), with only one variable site at position 244, in which the specimen FICC02LI (See Table S1 in Appendix S3), with GenBank Acc. No. MN718587, showed a transition (A > G). A final alignment containing Mediterranean sequences merged with the homologous sequences from the other global populations (N = 99) resulted in a final dataset of 117 CR sequences of 516bp in which an indel at position 60 was revealed.

The global ML haplotype network revealed 31 haplotypes (Figure S4, Appendix S2) that were clustered in two main haplogroups differentiated by 28 mutations. As expected, based on the existing literature, the first haplogroup was formed by the individuals from the MED and the Pacific Ocean (AUS and NEP) and the second was composed of GWS from SA and the NWA. Three haplotypes of GWS collected in AUS (HQ414073, HQ414074 and AY026211; Table S3, Appendix S3) clustered in the latter haplogroup and these individuals are SA−like individuals that likely migrated across the Indian Ocean (Pardini et al., 2001; Blower et al., 2012). The ML haplotype network built with 99 GWS sequences of 828bp revealed 68 haplotypes and a similar topology (Figure S4, Appendix S2) to the one reconstructed using the shorter sequence dataset, with 2 main haplogroups (AUS-NEP-MED vs SA-NWA).

The cross-plot for the haplotype and nucleotide diversity of the Mediterranean and global populations revealed that the MED and AUS populations have the lowest and highest values for both indices, respectively (Figure 1; Table S6, Appendix S3). The NEP and NWA populations showed high haplotype diversity and low nucleotide diversity. The SA population exhibited quite opposite positions in the plot, depending on the reference study used (Pardini et al. (2001) and O'Leary et al. (2015) both offered high values for both indices and Andreotti et al. (2016) provided low values.

No polymorphisms were detected among the four complete contemporary CR sequences isolated from Mediterranean GWS, demonstrating a low haplotype diversity among longer sequences as well, while the genetic diversity (e.g. nucleotide) is proportional to the length of the sequences.

Marginal likelihood estimation and test for divergence time hypotheses

The application of the log Bayes factor formula gave significant support to the Pliocene calibration scenario in every model tested (Table 1). Based on the 828bp dataset, the GWS Mediterranean population diverged from the Pacific populations approximately 3.23 Ma, a time that is congruent with the estimated closure of the Central American Seaway, CAS (about 3.5 Ma), after the formation of the Isthmus of Panama (O'Dea et al., 2016). Considering the "Pleistocene divergence" scenario, we obtained a slight mismatch between the posterior distribution generated from the data and posterior distribution generated from specified calibration using priors only. This may suggest that a second Pleistocene calibration could be conflicting with the data and that a "Pliocene divergence" scenario is preferable.

Time of the Most Recent Common Ancestor (TMRCA) and estimation of evolutionary rate

The analysis of fossil evidence suggested that GWS experienced a long evolutionary history (Tables S4 and S5, Appendix S3). The occurrence of GWS fossils in the Mediterranean area is high in the Pliocene, after the Messinian Salinity Crisis.

The CR relaxed substitution rate range inferred from the TMRCA analysis was estimated at 0.38%-0.72% substitutions/site/my for the best model following the Bayes factor. Based on these estimates, the coalescence of the Mediterranean and Pacific GWS lineages was dated at 3.23 Ma for the alignment containing 828bp (Figure 2) and 1.81 Ma for the alignment of 516bp (Figure S5, Appendix S2).

Historical biogeography reconstruction

The analysis of the ancestral ranges of GWS, using both S-DIVA and DEC approaches, suggested that the Mediterranean population is potentially the result of a dispersal-vicariance scenario. The biogeographical reconstruction of the Mediterranean lineage estimated using S-DIVA resulted in two principal ancestral ranges, AC (AUS-MED) and ABC (AUS-NEP-MED), with probabilities (relative frequencies) for each range of 52.44% and 46.69% respectively. Two minor ancestral ranges were detected with very low probability: AB (AUS-NEP) and BC (NEP-MED), with probabilities of 0.52% and 0.35%, respectively. The DEC analysis resulted in concordant results with two ancestral ranges, AC (AUS-MED); BC (NEP-MED), with probabilities of 50.01% and 49.99% respectively, supporting that the origin of the Mediterranean population is likely Australia and the North Eastern Pacific. Both approaches suggest the following route of dispersal-vicariance: AC>CAB>C|AB. For both S-DIVA and DEC analyses, Australian and North Eastern Pacific lineages originated from a Pacific ancestral range AB (AUS-NEP) with a probability of 100%.

Discussion

Our phylogenetic analysis of contemporary and historical sequences indicates that the existing population of GWS in the Mediterranean Sea could be a relic of the Pacific GWS clade. This scenario would suggest that Pacific-born ancestors migrated eastward across the ocean, before the closure of the Central American Seaway. Following this, these itinerant migrants would have colonized the

North Atlantic Ocean before entering the Mediterranean Sea after the Messinian Salinity Crisis (MSC). They would have entered the Mediterranean during a period of ecological upheaval, following the MSC which caused the extinction of the local marine megafauna due to the total or partial drying up of the nearly landlocked sea (Garcia-Castellanos et al., 2009). This evolutionary pathway (Figure 3) is supported by Bayesian analyses of genetic diversity and divergence time estimates and it is coherent with the age of the main paleo-geographical events, paleo-climatic patterns and fossil records. This pathway represents an alternative to the antipodean dispersal hypothesis (Gubili et al., 2010) which suggested that a few Australian/New Zealand founder females that visited the Good Hope Cape area became confused by Pleistocene climatic oscillations, swam northward and found themselves in the Mediterranean Sea.

Our phylogenetic analysis of the CR sequences as well as the plot analysis of haplotype confirmed that the Mediterranean GWS have a closer evolutionary relationship with the Australian/New Zealand and Northeastern Pacific lineages than with the South African and Northwestern Atlantic lineage. These results are in agreement with previous studies (Gubili et al., 2010; Blower et al., 2012; Andreotti et al., 2016). The Mediterranean GWS exhibited a very shallow mtDNA genetic variation with only two haplotypes and extremely low genetic diversity. Among the modern samples collected from throughout the world that have been analyzed to date, the Mediterranean GWS have provided the lowest estimates of genetic diversity. This is likely an indication that the population is relatively young and has likely originated by a founder event by a limited number of mtDNA lineages or that the population has experienced a recent population bottleneck (Group 1 of Grant, & Bowen, 1998; Grant, & Waples, 2000). Within the Pacific/Mediterranean mitochondrial clade, the Australian/New Zealand GWS population was identified as the most ancestral with a long evolutionary/demographic history and divergent haplotypes that have accumulated over long periods of time (as indicated by the high haplotype and nucleotide diversity values; Group 3 of Grant, & Bowen, 1998; Grant, & Waples, 2000). An intermediate evolutionary position in the clade could be that of the Northeastern Pacific GWS population which possesses high haplotype diversity and low nucleotide diversity. This pattern is indicative of a population that has experienced a bottleneck event followed by rapid demographic growth and accumulation of mutations (Group 2 of Grant, & Bowen 1998; Grant, & Waples, 2000). Both S-DIVA and DEC analyses suggest that the Mediterranean GWS originated from a dispersal-vicariance event via eastward dispersal from a few Australian/Pacific individuals during the Pliocene. The founder event that gave origin to the modern Mediterranean GWS was dated by the TMRCA analysis, with high statistical support, to 3.23 Ma, during the Piacenzian Age (3.6-2.58 Ma).This is much earlier than the estimate provided by the antipodean dispersal hypothesis (348-565 ka; Calabrian, Pleistocene), which is based on a mtDNA substitution rate of 1.19-0.74% of divergence between lineages per million year (Gubili et al., 2010). These rates of evolutionary change were calibrated by Gubili et al. (2010) using estimates of vicariance events that separated GWS populations from the Northeastern Pacific and Northwestern Atlantic oceans (i.e. the rising of the Isthmus of Panama dated at 3.5 Ma; O'Dea et al., 2016) and the Western Pacific and Indian oceans (i.e. the rising of the Sunda-Sahul shelves dated at 5 Ma; Haq, Hardenbol, & Vail, 1987). However, all phylogenetic analyses carried out so far (Gubili et al., 2010; Andreotti et al., 2016; present work) have revealed that the Northwestern Atlantic GWS are phylogenetically linked to the South African population but not to the Northeastern Pacific GWS. Therefore, the use of the vicariance event separating Atlantic and Pacific GWS might have led to an overestimation of the mutation rate and the subsequent time of divergence between Mediterranean and Pacific GWS at 348-565 ka during the Pleistocene.

The origination time of the species is still debated (see dedicated paragraph in Gottfried & Fordyce, 2001). Despite the fossil records from the Miocene are less common, this should not exclude a Miocene origin of the species. Moreover, the molecular clock analysis suggested a divergence between *Carcharodon* and *Isurus* dated back to 43 Ma (Martin, 1996), in contrast with a Pliocene origin of the *C. carcharias* from *I. hastalis*. However, a more dedicated study on the fossil history is needed to better clarify the origination time of the species.

Abundant fossil data suggests that GWS have inhabited the Mediterranean Sea since the early Pliocene, following the Messinian Salinity Crisis, with numerous specimens estimated to be between ~5 and ~2 Ma old, with peaks of abundance occurring during the Pliocene (Cigala-Fulgosi, 1990; Bianucci et al., 2002; Marsili, 2008; Adnet, Balbino, Antunes, & Marín-Ferrer, 2009). After these paleoclimatic phases, GWS could have colonized the Mediterranean Sea occupying the ecological niches left empty by other apex marine predators, such as the giant megatooth shark *Carcharocles megalodon*, which went extinct between 3.5 and 2.6 Ma (Pimiento, & Clements, 2014). Fossil evidence from Central America suggests that many species of sharks and marine mammals that were part of the region's faunal assemblage may have migrated across the CAS continuously before the formation of the Isthmus of Panama (Steeman et al., 2009; Pimiento et al., 2013; Velez-Juarbe, Wood, De Gracia, & Hendy, 2015). Upon the closure of the CAS, the newly formed Gulf Stream current could have facilitated a trans-Atlantic migration by way of the easterly current and subsequent eastward dispersion of nutrients and, consequently, food resources. There is evidence that intense fluctuations in the speed of the Gulf Stream occurred during the formation of the Isthmus of Panama, reaching a height during the late Miocene and early Pliocene (~6.1-4.8 Ma; Kaneps, 1979). The intensification of currents coincides with the end of the Messinian Salinity Crisis (5.33 Ma), which culminated with the Zanclean inflow in the Mediterranean Sea (Garcia-Castellanos et al., 2009). All of these oceanographic phenomena are concordant with our estimated divergence of the Mediterranean GWS population.

A potential earlier formation of the Isthmus of Panama (Bacon et al., 2015; Lessios et al., 2015; Montes et al., 2015; O'Dea et al., 2016) would not affect our results, since it is plausible that the Pacific lineage that gave rise to the MED population may already have been present in the North Atlantic before the closure of the CAS. Moreover, some researchers have suggested that the Caribbean Sea and Pacific Ocean may have remained intermittently connected by shallow waters other than the CAS (Jaramillo et al., 2017). The global phylogeography of contemporary GWS populations reveals a discontinuous distribution of the Pacific/Mediterranean GWS evolutionary lineage in the North Atlantic being interrupted by the recent colonization of the Northwestern Atlantic area by the Indian lineage. The phylogenetic analyses highlighted that the GWS of Northwestern Atlantic population does not exhibit a separated mtDNA cluster like the Pacific and Mediterranean populations, and it form a unique lineage with the South-Africa (Figure S4, Appendix S2). This evidence is suggestive of a recent evolutionary history or a population bottleneck followed by population growth and accumulation of mutations.

A local extinction of the past Pacific GWS lineage in the North Atlantic Ocean and further replacement by components of the Indian lineage could explain such a phylogeographic discontinuity. An extensive analysis of the fossil records, highlighted an exceptional extinction rate of the marine fauna, including sharks, during the Pliocene epoch (Pimiento et al., 2017). Furthermore, the highest extinction rates occurred in the late Pliocene, between 3.8 and 2.4 Ma, which coincides with the Mediterranean population divergence time, and may have led to the local extinction of the ancient Atlantic population of GWS.

Additional genetic and tagging studies are required to determine if the Mediterranean GWS are ecologically and reproductively isolated from the adjacent populations occupying the North Atlantic Ocean. This issue is of high-priority for the development of efficient conservation actions and implementation of management strategies. Despite this knowledge gap, some information can be gleaned from the other populations in the Atlantic, as well as other species. For example, several other epipelagic sharks migrate throughout the temperate waters of the Atlantic and several are suspected of having nursery areas in the mid-Atlantic Ridge (Kohler, Turner, Hoey, Natanson, & Briggs, 2002; Stevens, 2010; Vandeperre et al., 2014; O'Leary et al., 2015). Elsewhere, in the Indian Ocean, GWS have been observed migrating between Australia and South Africa, while in the Pacific, GWS frequently migrate between the western coast of North America and Hawaii (Bonfil et al., 2005; Jorgensen et al., 2010, 2012; Blower et al., 2012). Clearly, the species is not averse to far reaching longitudinal movements. However, antipodean connections appear less frequently and evidence for them is limited to variations in the genetic code (O'Leary et al., 2015). The latitudinal distribution of GWS could be limited by thermal tolerance, prey availability, social structure and fidelity to nursery areas (Cliff, Dudley, & Davis, 1989; Curtis et al., 2014).

Our results suggest that the Mediterranean GWS have a more ancient origin than previously thought, and that this population is genetically disconnected from the adjacent Atlantic population. Due to historical and widespread declines of sharks in the Mediterranean Sea (Colloca et al., 2013; Ferretti, Osio, Jenkins, Rosenberg, & Lotze, 2013, Ferretti et al., 2008) as well as the ongoing over-exploitation of marine resources in the region, the current white shark population is critically endangered (Dulvy, Allen, Ralph, & Walls, 2016). This distinct genetic pool represents a valuable, albeit extremely vulnerable, component of the genetic diversity of a species that is endangered worldwide. Losing the Mediterranean GWS population would thus represent a significant blow to the global conservation of this species. Continued characterization of this population's ecology, spatial dynamics and population structure is paramount for effective management and restoration of this important top predator's ecological role in the region.

Table 1. Best model selection based on Bayes factors of tree topologies reconstructed with the earliest fossil occurrences using 828bp and 516 bp mitochondrial control region sequences. Node constraints and calibration parameters on the phylogeographical tree of *C. carcharias* for the two divergence models are also provided. logML_GSS: log marginal likelihood from generalized stepping stone model, BF_GSS: Bayes factors calculated using the logML_GSS. Models are ranked according to the logML values.

Model	**Dataset**	**Node1**	**Node2**	**logML_GSS**	**logBF_GSS**
MED Pliocene divergence	828bp	Ingroup Mean: 11 Ma, SD: 1.0 Ma	MED/Pacific Mean: 3.0 Ma, SD: 0.30 Ma	-2455.2796	0 (Best)
MED Pleistocene divergence	828bp	Ingroup; Mean: 11 Ma, SD: 1.0 Ma	MED/Pacific Mean: 0.4Ma, SD: 0.15 Ma	-2458.1264	2.8468
MED Pliocene divergence	516bp	Ingroup; Offset: 11 Ma, SD: 1.0 Ma	MED/Pacific Mean: 3.0 Ma, SD: 0.30 Ma	-1440.6171	0 (Best)
MED Pleistocene divergence	516bp	Ingroup; Offset: 11 Ma, SD: 1.0 Ma	MED/Pacific Mean: 0.4Ma, SD: 0.15 Ma	-1443.0069	2.3898

Acknowledgements

We are indebted to Alessandro Velonà, Adriana Villamor and Marco Stagioni for providing valuable technical support and advice. Clemente Ventrone and Gerlando Spagnolo provided samples from Favignana tuna trap.

Funding

AL, GNP, AC and FT were funded by the University of Bologna by RFO 2016-2018 and Canziani grants to FT. AL is also funded by Italian Ministry for University and Research by a PhD grant 2015-2017. All the other authors were funded by respective institutions. GB acknowledges support from the Interne Fondsen KU Leuven / Internal Funds KU Leuven under grant agreement C14/18/094.

Data Availability Statement

The sequence data obtained in the present study have been deposited to the GenBank database of the National Center for Biotechnology Information under accession numbers MN718579-MN718596.

References

Adnet, S., Balbino, A. C., Antunes, M. T., & Marín-Ferrer, J. M. (2009). New fossil teeth of the white shark (*Carcharodon carcharias*) from the early Pliocene of Spain. Implication for its paleoecology in the Mediterranean. *Neues Jahrbuch für Geologie und Paläontologie – Abhandlungen,* 256(1), 7-16. http://dx.doi.org/doi:10.1127/0077-7749/2009/0029

Andreotti, S., von der Heyden, S., Henriques, R., Rutzen, M., Meÿer, M., Oosthuizen, H., & Matthee, C. A. (2016) New insights into the evolutionary history of white sharks, *Carcharodon carcharias*. *Journal of Biogeography*, 43(2), 328-339. https://doi.org/10.1111/jbi.12641

Applegate, S. P., & Espinosa-Arrubarrena, L. (1996). The fossil history of *Carcharodon* and its possible ancestor, *Cretolamna*: a study in tooth identification. In A. P. Klimley & D. G. Ainley (Eds.), *Great white sharks: the biology of Carcharodon carcharias* (pp. 19-36). San Diego, CA: Academic Press.

Bacon, C. D., Silvestro, D., Jaramillo, C., Smith, B. T., Chakrabarty, P., Antonelli, A. (2015). Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences U.S.A*, 112(19), 6110-6115. doi: https://doi.org/10.1073/pnas.1423853112

Baele, G., Lemey, P., & Suchard, M. A. (2015). Genealogical working distributions for Bayesian model testing with phylogenetic uncertainty. *Systematic Biology*, 65(2), 250-264. https://doi.org/10.1093/sysbio/syv083

Bianucci, G., Bisconti, M., Landini, W., Storai, T., Zuffa, M., Giuliani, S., & Mojetta, A. (2002). Trophic interaction between white shark, *Carcharodon carcharias*, and cetaceans: a comparison between Pliocene and recent data from Central Mediterranean Sea. In M. Vacchi, G. Lamesa, F. Serena, & B. Seret (Eds.), *Proceedings of the 4th European Elasmobranch Association Meeting 28-30 November 2000* (pp. 33-48). Livorno, Italy: ICRAM, ARPAT & SFI.

Blower, D. C., Pandolfi, J. M., Bruce, B. D., Gomez-Cabrera, M.D., & Ovenden, J. R. (2012) Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. *Marine Ecology Progress Series*, 455, 229-244. https://doi: 10.3354/meps09659

Bonfil, R., Meÿer, M., Scholl, M. C., Johnson, R., O'Brien, S., Oosthuizen, H., … Paterson, M. (2005). Transoceanic migration, spatial dynamics, and population linkages of white sharks. *Science*, 310(5745), 100-103. https://doi:10.1126/science.1114898

Canese, S. P., Giusti, M., Salvati, E., Angiolillo, M., Cardinali, A., & Consalvo, I. (2011). *Final Report Project ISPRA PR0.3.452 Biodiversity of Sicily Channel*. Rome, Italy: Ministry of the Environment, Territorial and Sea Protection.

Cigala-Fulgosi, F. (1990). Predation (or possible scavenging) by a great white shark on an extinct species of bottlenosed dolphin in the Italian Pliocene. *Tertiary Research*, 12, 17-36.

Cliff, G., Dudley, S. F. J., & Davis, B. (1989). Sharks caught in the protective gill nets off Natal, South Africa. 2. The great white shark *Carcharodon carcharias* (Linnaeus). *South African Journal of Marine Science,* 8, 131-144. https://doi:10.2989/02577618909504556

Colloca, F., Cardinale, M., Maynou, F., Giannoulaki, M., Scarcella, G., Jenko, K., … Fiorentino, F. (2013). Rebuilding Mediterranean fisheries: a new paradigm for ecological sustainability. *Fish and Fisheries*, 14(1), 89-109. https://doi:10.1111/j.1467-2979.2011.00453.x)

Compagno, L. J. V. (1984). FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiformes to Lamniformes. *FAO Fish Synopsis*, 125, 1-249.

Curtis, T. H., McCandless, C. T., Carlson, J. K., Skomal, G. B., Kohler, N. E., Natanson, L. J., ... Pratt Jr., H. L. (2014) Seasonal Distribution and Historic Trends in Abundance of White Sharks, *Carcharodon carcharias*, in the Western North Atlantic Ocean. *PLOS ONE* 9(6), e99240. doi:10.1371/journal.pone.0099240

De Maddalena, A. (2006) A catalogue of great white sharks *Carcharodon carcharias* (Linnaeus, 1758) preserved in European museums. *Journal of the National Museum, Natural History Series*, 175(3-4), 109-125.

Domeier, M. L., Nasby-Lucas, N. (2008). Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. *Marine Ecology Progress Series*, 370, 221–237. doi: https://doi.org/10.3354/meps07628

Dulvy, N. K., Allen, D. J., Ralph, G. M., & Walls, R. H. L. (2016). The conservation status of sharks, rays and Chimaeras in the Mediterranean Sea. IUCN, Malaga, Spain.

Fergusson, I. K. (1996) Distribution and autoecology of the white shark in the Eastern North Atlantic and the Mediterranean Sea. In A. P. Klimley & D. G. Ainley (Eds.), *Great white sharks: the biology of Carcharodon carcharias* (pp. 321-345). San Diego, CA: Academic Press.

Ferretti, F., Worm, B., Britten, G. L., Heithaus, M. R., & Lotze, H. K. (2010). Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters*, 13(8), 1055-1071. https://doi:10.1111/j.1461-0248.2010.01489.xFerretti, F., Myers, R. A., Serena, F., & Lotze, H. K.

(2008). Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology*, 22(4), 952-964. https://doi:10.1111/j.1523 1739.2008.00938.x

Ferretti, F., Osio, G. C., Jenkins, C. J., Rosenberg, A. A., & Lotze, H. K. (2013) Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact. *Scientific Reports*, 3, 1057. https://doi:10.1038/srep01057

Fulton, T. L., & Strobeck, C. (2010). Multiple fossil calibrations, nuclear loci and mitochondrial genomes provide new insight into biogeography and divergence timing for true seals (Phocidae, Pinnipedia). *Journal of Biogeography,* 37(5), 814-829. https://doi: 10.1111/j.1365-2699.2010.02271.x

Garcia-Castellanos, D., Estrada, F., Jiménez-Munt, I., Gorini, C., Fernandéz, M., Vergés, J., & De Vicente, R. (2009). Catastrophic flood of the Mediterranean after the Messinian salinity crisis. *Nature*, 462, 778-781. https://doi:10.1038/nature08555

Gottfried, M. D., & Fordyce, R. E. (2001). An associated specimen of *Carcharodon angustidens* (Chondrichthyes, Lamnidae) from the late Oligocene of New Zealand, with comments on *Carcharodon* interrelationships. *Journal of Vertebrate Paleontology*, 21(4), 730-739. https://doi:10.1671/0272-4634(2001)021[0730:AASOCA]2.0.CO;2

Grant, W. S., & Bowen, B. W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, 89(5), 415–426. https://doi.org/10.1093/jhered/89.5.415

Grant, W. S., & Waples, R. S. (2000). Spatial and temporal scales of genetic variability in marine and anadromous species: implications for fisheries oceanography. In P. J. Harrison & T. R. Parsons (Eds.), *Fisheries oceanography: an integrative approach to fisheries ecology and management* (pp. 63–93). Oxford, UK: Blackwell Science.

Gubili, C., Bilgin, R., Kalkan, E., Karhan, S. Ü., Jones, C. S., Sims, D. W., … Noble, L. R. (2010). Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to genetic discontinuity and an endangered anomalous population. *Proceedings of the Royal Society B: Biological Sciences,* 278(1712), 1679–1686. https://doi:10.1098/rspb.2010.1856

Gubili, G., Robinson, C. E. C., Cliff, G., Wintner, S. P., de Sabata, E., De Innocentiis, S., … Jones, C. S. (2015). DNA from historical and trophy samples provides insights into white shark population origins and genetic diversity. *Endangered Species Research* 27(3), 233-241. https://doi: 10.3354/esr00665

Haq, B. U., Hardenbol, J., & Vail, P. R. (1987). Chronology of fluctuating sea levels since the Triassic. *Science*, 235(4793), 1156-1167. https://doi:10.1126/science.235.4793.1156

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2), 160-174.

Hofreiter, M., Serre, D., Poinar, N. H., Kuch, M., & Pääbo, S. (2001). Ancient DNA. *Nature Reviews Genetics*, 2, 353-359. https://doi:10.1038/35072071

Jaramillo, C., Montes, C., Cardona, A., Silvestro, D., Antonelli, A., Bacon, C. D. (2017). Comment (1) on "Formation of the Isthmus of Panama" by O'Dea et al. *Science Advances*, 3(6), e1602321. doi: 10.1126/sciadv.1602321

Jorgensen, S. J., Arnoldi, N. S., Estess, E. E., Chapple, T. K., Rückert, M., Anderson, S. D., & Block, B. A. (2012). Eating or meeting? Cluster analysis reveals intricacies of white shark (*Carcharodon carcharias*) migration and offshore behavior. *PLOS ONE* 7, e47819. https://doi:10.1371/journal.pone.0047819

Jorgensen, S. J., Reeb, C. A., Chapple, T. K., Anderson, S., Perle, C., Van Sommeran, S.R., … Block, A. B. (2010). Philopatry and migration of Pacific white sharks. *Proceedings of the Royal Society B: Biological Sciences,* 277(1682), 679-688. https://doi:10.1098/rspb.2009.1155

Kabasakal, H., & Gedikoğlu, Ö. S. (2008). Two new-born great white sharks, *Carcharodon carcharias* (Linnaeus, 1758) (Lamniformes; Lamnidae) from Turkish waters of the north Aegean Sea. *Acta Adriatica*, 49, 125–135.

Kaneps, A. G. (1979). Gulf stream: velocity fluctuations during the late Cenozoic. *Science*, 204(4390), 297-301. https://doi:10.1126/science.204.4390.297

Kohler, N. E., Turner, P. A., Hoey, J. J., Natanson, L. J., & Briggs, R. (2002). Tag and recapture data for three pelagic shark species: blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), and porbeagle shark (*Lamna nasus*) in the North Atlantic Ocean. *ICCAT Collective Volumes of Scientific Papers,* 54, 1231-1260.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7 Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Lessios, H. A. (2015). Appearance of an early closure of the Isthmus of Panama is the product of biased inclusion of data in the metaanalysis. *Proceedings of the National Academy of Sciences USA*, 112(43), E5765-E5765. doi: 10.1073/pnas.1514719112

Mancusi, C., Nicolosi, P., Arculeo, M., Barbagli, F., Carlini, R., Costantini, M., … Vacchi, M. (2002). The presence of elasmobranchs in the collections of the main Italian natural history museums. In M. Vacchi, G. Lamesa, F. Serena, & B. Seret (Eds.), *Proceedings of the 4th European Elasmobranch Association Meeting 28-30 November 2000* (pp. 97-108). Livorno, Italy: ICRAM, ARPAT & SFI.

Marsili, S. (2008). Systematic, paleoecologic and paleobiogeographic analysis of the Plio-Pleistocene Mediterranean elasmobranch fauna. *Atti della Società Toscana di Scienze Naturali, Memorie, Serie A*, 113, 81-88.

Marsili, S. (2006). Analisi Sistematica, Paleoecologica e Paleobiogeografica della Selaciofauna plio-pleistocenica del Mediterraneo. University of Pisa. PhD thesis

Martin, A. P. (1996). Systematics of the Lamnidae and the Origination Time of Carcharodon carcharias Inferred from the Comparative Analysis of Mitochondrial DNA Sequences. In A. P. Klimley & D. G. Ainley (Eds.), *Great white sharks: the biology of Carcharodon carcharias* (pp. 49-53). San Diego, CA: Academic Press.

McPherson, J. M., & Myers, R. A. (2009). How to infer population trends in sparse data: examples with opportunistic sighting records for great white sharks. *Diversity and Distributions* 15(5), 880-890. https://doi: 10.1111/j.1472-4642.2009.00596.x

Montes, C., Cardona, A., Jaramillo, C., Pardo, A., Silva, J. C., Valencia, … Niño, H. (2015). Middle Miocene closure of the Central American Seaway. *Science* 348(6231), 226–229. doi: 10.1126/science.aaa2815

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P., Peterson, C. H. (2007). Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean. *Science*, 315(5820), 1846-1850. doi: 10.1126/science.1138657

Nieto, A., Ralph, G. M., Comeros-Raynal, M. T., Kemp, J., García Criado, M., Allen, D. J., … Williams, J. T. (2015) *European Red List of marine fishes*. Luxembourg, EU: Rosseels Printing.

O'Dea, A., Lessios, H. A., Coates, A. G., Eytan, R. I., Restrepo-Moreno, S. A., Cione, A. L., … Jackson, J. B. C. (2016). Formation of the Isthmus of Panama. *Science Advances*, 2(8), e1600883. https://doi:10.1126/sciadv.1600883

O'Leary, S. J., Feldheim, K. A., Fields, A. T., Natanson, L. J., Wintner, S., Hussey, N., … Chapman, D. D. (2015). Genetic diversity of white sharks, *Carcharodon carcharias,* in the Northwest Atlantic and Southern Africa. *Journal of Heredity*, 106(3), 258-265. https://doi:10.1093/jhered/esv001

Pardini, A. T., Jones, C. S., Noble, L. R., Kreiser, B., Malcolm, H., Bruce, B. D., … Martin, A. P. (2001). Sex-biased dispersal of great white sharks. *Nature*, 412(6843), 139-140. http://doi:10.1038/35084125

Pimiento, C., & Clements, C. F. (2014). When did *Carcharocles megalodon* become extinct? A new analysis of the fossil record. *PLOS ONE*, 9(10), e111086. https://doi.org/10.1371/journal.pone.0111086

Pimiento, C., Gonzalez-Barbad, G., Hendy, A. J. W., Jaramillo, C., MacFadden, B. J., Montes, C., … Shippritt, M. (2013). Early Miocene chondrichthyans from the Culebra formation, Panama: a window into marine vertebrate faunas before closure the Central American Seaway. *Journal of South American Earth Sciences*, 42, 159-170. https://doi:10.1016/j.jsames.2012.11.005

Pimiento, C., Griffin, J. N., Clements, C. F., Silvestro, D., Varela, S., Uhen, M. D., & Jaramillo, C. (2017). The Pliocene marine megafauna extinction and its impact on functional diversity. *Nature Ecology & Evolution*, 1, 1100-1106. https://doi:10.1038/s41559-017-0223-6

Rambaut, A. (2009). FigTree v1.4.3. Retrieved from http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5), 901–904. https://doi:10.1093/sysbio/syy032

Ree, R. H., & Smith, S. A. (2008). Maximum Likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57(1), 4–14. https://doi.org/10.1080/10635150701883881

Riccioni, G., Landi, M., Ferrara, G., Milano, I., Cariani, A., Zane, L., … Tinti, F. (2010). Spatio temporal population structuring and genetic diversity retention in depleted Atlantic bluefin tuna of the Mediterranean Sea. *Proceedings of the National Academy of Sciences of the United States of America,* 107(5), 2102–2107. https://doi:10.1073/pnas.0908281107

Sperone, E., Parise, G., Leone, A., Milazzo, C., Circosta, V., Santoro, F., … Tripepi, S. (2012). Spatiotemporal patterns of distribution of large predatory sharks in Calabria (Central Mediterranean, Southern Italy). *Acta Adriatica*, 53, 13-24.

Steeman, M. E., Hebsgaard, M. B., Fordyce, R. E., Ho, S. Y. W., Rabosky, D. L., Nielsen, R., … Willerslev, E. (2009). Radiation of Extant Cetaceans Driven by Restructuring of the Oceans. *Systematic Biology*, 58(6), 573-585. https://doi:10.1093/sysbio/syp060

Stevens, J. D. (2010). Epipelagic Oceanic Elasmobranchs. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation* (pp. 3-37). Boca Raton, FL: CRC Press.

Storai, T., Mojetta, A., Zuffa, M., Giulian, S. (2000). Nuove segnalazioni di *Carcharodon carcharias* (L.) nel Mediterraneo centrale. *Atti Società Toscana di Scienze Naturali*, 107, 139-142.

Storai, T., Vanni, S., Zuffa, M., & Biagi, V. (2005). Presenza di *Carcharodon carcharias* (Linnaeus, 1758) nelle acque toscane (Mar Ligure meridionale e Mar Tirreno settentrionale; Mediterraneo): analisi e revisione delle segnalazioni (1839-2004). *Atti Società Toscana di Scienze Naturali*, 112, 153-166.

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4(1), vey016. https://doi:10.1093/ve/vey016

Tamura, K, & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512-526. https://10.1093/oxfordjournals.molbev.a040023

Vandeperre, F., Aires-da-Silva, A., Fontes, J., Santos, M., Serrão Santos, R., & Afonso, P. (2014). Movements of blue sharks (*Prionace glauca*) across their life history. *PLOS ONE* 9(8), e103538. https://doi:10.1371/journal.pone.0103538

Varela, S., González-Hernández, J., Sgarbi, L. F., Marshall, C., Uhen, M. D., Peters, S., & McClennen, M. (2015). paleobioDB: an R package for downloading, visualizing and processing data from the Paleobiology Database. *Ecography*, 38(4), 419–425. https://doi:10.1111/ecog.01154

Velez-Juarbe, J., Wood, A. R., De Gracia, C., & Hendy, A. J. W. (2015). Evolutionary patterns among living and fossil kogiid sperm whales: evidence from the Neogene of Central America. *PLOS ONE*, 10(4), e0123909. https://doi:10.1371/journal.pone.0123909

Yu, Y., Harris, A. J., & He, X. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, 56(2), 848-850. https://doi.org/10.1016/j.ympev.2010.04.011

Yu, Y., Harris, A. J., Blair, C., & He, X. J. (2015) RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution,* 87, 46-49. https://doi:10.1016/j.ympev.2015.03.008

Biosketch

Agostino Leone is a marine molecular ecologist and population geneticist. Former PhD student at the University of Bologna. He has broad interests in researches related to marine conservation, ranging from biogeography to population genomics, investigating marine populations structure and their response to different pressures. AL, FT conceived the study; AL, GNP, EC, AC carried out molecular work and sequences analysis; AL, GNP, FF, PDJ, GB carried out statistical analyses; ES, ST, PM, AG, MS, MA, GD, FG, ADA, DM, SV, FS collected specimens; AL, GNP, MA, EC, FS, PDJ, GB, AC, FT drafted the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version:

Appendix S1 Supplementary methods for the tissue sampling, historical DNA extraction, species-specific primers design, DNA amplification, sequencing and genetic diversity analysis.

Appendix S2 Supplementary figures

Appendix S3 Supplementary tables

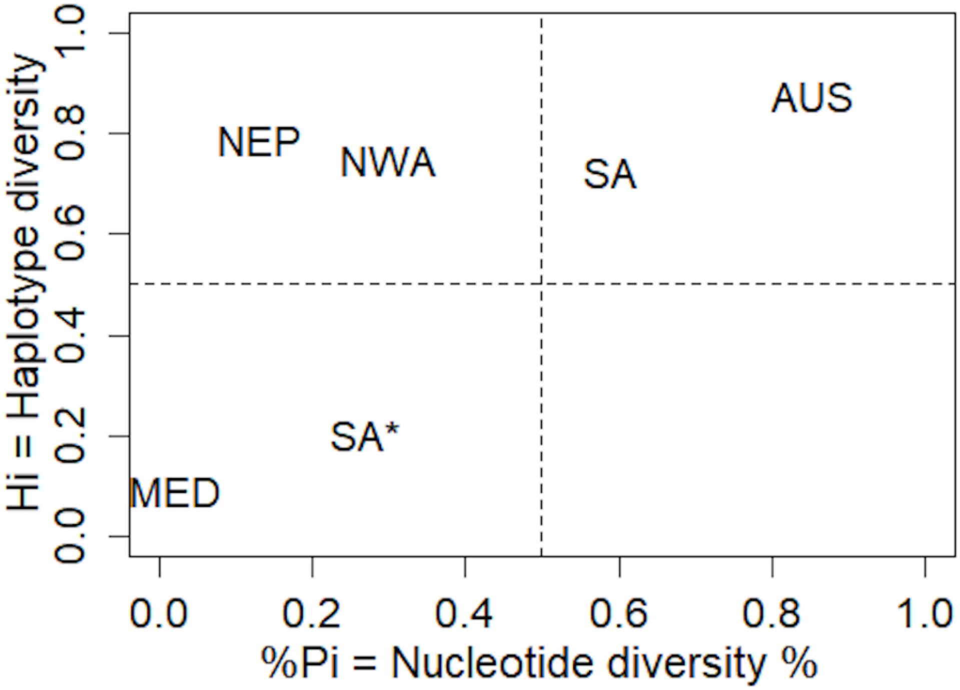


Figure 1. Plot of the haplotype and nucleotide diversity values (expressed as percentage values) of global populations of Carcharadon charcarias inferred using sequences from the mitochondrial control region AUS: Australia/New Zealand; NEP: Northeastern Pacific; MED: Mediterranean (516bp; n = 22); SA: South-Africa; NWA: Northwestern Atlantic. Asterisk indicates values reported by Andreotti et al. [29] for the South African population.

78x56mm (300 x 300 DPI)

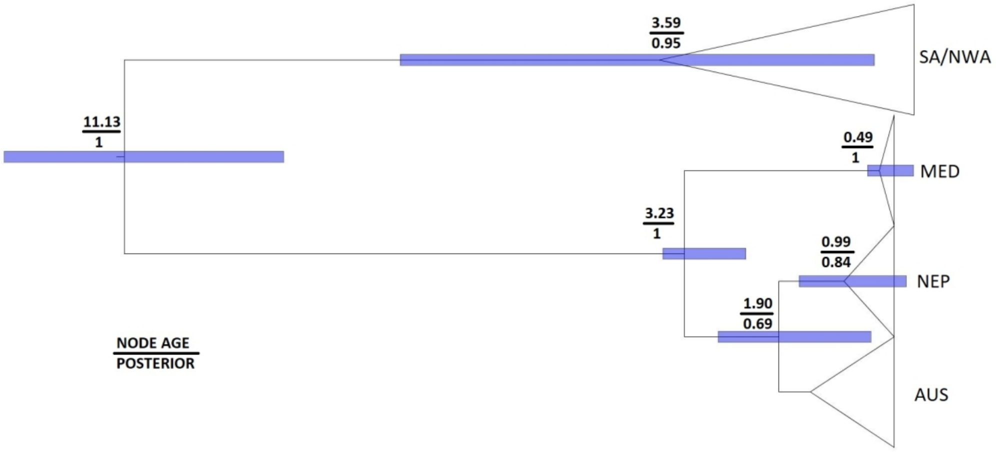


Figure 2. Bayesian divergence time tree of populations of Carcharadon charcarias inferred using sequences from the mitochondrial control region (828bp). High posterior density (HPD 95%) values are featured as blue bars. Nodes with posterior values <0.5 are not shown. Abbreviations are provided in Figure 1.

153x71mm (300 x 300 DPI)

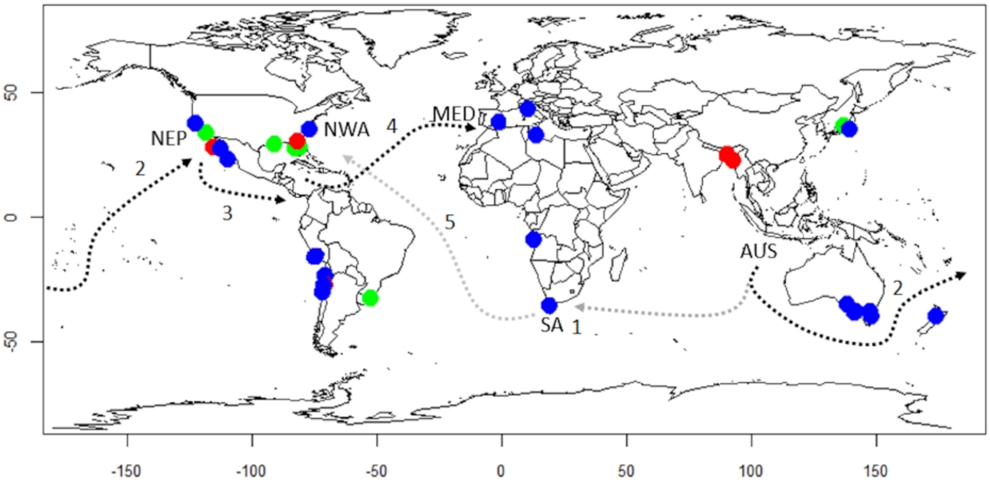


Figure 3. Global dispersal and Pacific/Mediterranean vicariance hypothesis for Carcharadon charcarias. Ancient great White Sharks from the Pacific Ocean, namely Australia, dispersed via two routes: westward to South African coasts (1, light grey dotted line) and eastward to the Northeastern Pacific Ocean (2, black dotted line). The Pacific Great White Sharks were free to move eastward to the Atlantic, and in the Pliocene to an ancient Mediterranean Sea after the Messinian Salinity Crisis (3-4). Past climatic oscillation due to the closure of the Central America Seaway and the formation of the Isthmus of Panama, could have caused a local extinction or an eastward mass migration of white shark from the North Atlantic Ocean, isolating the Mediterranean population from other ancestral populations. The North Atlantic was colonized, then, in relatively recent history, when the climate conditions became more suitable (5). Fossil records extrapolated from the Paleobiology Database are indicated with colored points (red: Miocene, blue: Pliocene, green: Pleistocene). The map was created using ‘paleobioDB’ package in R version 3.5.1 (Varela et al., 2015). Abbreviations are provided in Figure 1.

167x83mm (300 x 300 DPI)

Journal of Biogeography

Supporting Information

Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses

Agostino Leone, Gregory Neils Puncher, Francesco Ferretti, Emilio Sperone, Sandro Tripepi, Primo Micarelli, Andrea Gambarelli, Maurizio Sarà, Marco Arculeo, Giuliano Doria, Fulvio Garibaldi, Nicola Bressi, Andrea Dall'Asta, Daniela Minelli, Elisabetta Cilli, Stefano Vanni, Fabrizio Serena, Píndaro Díaz-Jaimes, Guy Baele, Alessia Cariani, Fausto Tinti

Appendix S1 Supplementary methods for the tissue sampling, historical DNA extraction, species-specific primers design, DNA amplification, sequencing and genetic diversity analysis.

Supplementary methods

Sampling

Tissue samples from 18 historical specimens identified as *Carcharodon carcharias*, captured in the Italian Seas from 1823 to the 1980s, were collected from museums and private archives (Figure S1, Appendix S2; Table S1, Appendix S3). Due to the cultural importance of the GWS museum specimens, sampling operations were carried out with utmost care to avoid extensive and unsightly damage (e.g. collecting tissue samples from the inner surface of jaws, internal dental pulp of teeth and dried skin debris). When jaws were available, the internal point of attachment between the lower hemi-arches was used for sampling, as it is concealed from public view and is the thickest part of the jaw. In this way, it was possible to drill deep into the jaw and avoid the use of surface materials that could act as a source of contaminants (Figure S2, Appendix S2). Exogenous DNA was removed by saturating sample surfaces with a 3.0% v/v sodium hypochlorite solution for approximately 10 minutes according to the protocol of Kemp and Smith (Kemp & Smith, 2005). All instruments were sterilized with bleach and UV irradiation between samplings. Holes of 5 mm in diameter were drilled into each jaw using an electric drill set to minimum speed to avoid damage to DNA due to thermal stress (Gibbon, Penny, Štrkalj, & Ruff, 2009). As the drill bit was removed from the cartilaginous jaws and vertebrae, all dust was carefully collected. The amounts of collected tissue ranged from 16 to 409.2 mg. The powdered tissue was then transported to a sterilized laboratory dedicated to aDNA analysis. The resulting holes left in specimens were filled with a low temperature restorative paste commonly used in anthropological studies (Figure S2, Appendix S2).

Historical DNA extraction

Extraction of DNA and polymerase chain reaction set-up were conducted in the “pre-PCR” clean-room of the Laboratory of Genetics & Genomics of Marine Resources and Environment (GenoDREAM) of the University of Bologna, dedicated to the analysis of degraded and low copy number DNA. The most stringent criteria to minimize and detect exogenous contamination in aDNA analysis (Cooper & Poinar, 2000) were followed (e.g. the use of extraction and amplification blanks as negative controls in each reaction).

DNA was extracted from all samples using a protocol modified from Riccioni et al. (2010), whereby homogenized tissue powder was incubated in EDTA buffer (0.5M, pH 8.0) in a shaker overnight at room temperature. Samples were then precipitated and incubated in an extraction buffer (0.1M EDTA, 0.5% N-laurylsarcosine-Na) and Proteinase K (20 mg/mL) for an additional 24 hours at 44°C. After spinning, 250 µL supernatants were transferred to tubes containing 3.5 µL of 1 µg/µL Dextran Blue, 250 µL of 4M NH_4-acetate, and 500 µL of 96% v/v ethanol. After precipitation, washing and drying, the genomic DNA was re-suspended in 50 µL of distilled sterile water and stored at -20°C.

Design of primer pairs, PCR amplification and sequencing of historical DNA

A fragment of the highly variable fragment of mitochondrial control region (CR) was targeted for analysis, since it is the most commonly used marker for reconstructing white shark phylogeography, and because there are available several sequences and haplotypes deposited in the public repositories (e.g. GenBank). Since historical DNA can be extensively fragmented (Allentoft et al. 2012), species-specific primers suitable for amplifying short fragments (167-221bp) of overlapping and adjacent DNA sequence fragments were developed (Figure S3, Appendix S2). Due to the intrinsic characteristic of ancient genetic material to be highly damaged, mainly for deamination processes, resulting in transitions from C to T and G to A (Hansen, Willerslev, Wiuf, Mourier, & Arctander, 2001), at least two independent amplifications were performed for each sample, in order to improve the detection of the damaged sites. Control region sequences/haplotypes of contemporary GWS specimens were retrieved from GenBank (Table S3, Appendix S3) and aligned with MEGA v.7.0.14 (Kumar, Stecher, & Tamura, 2016). using the ClustalW algorithm (Thompson, Higgins, & Gibson, 1994). From the alignment, five CR primer pairs (Table S2, Appendix S3) were designed with the software PRIMER3 v.4.0.0 (Untergrasser et al. 2012). These were subsequently tested *in silico* (Figure S3, Appendix S2) using AmplifX software, version 1.44 (©Nicolas Jullien 2004-2013; CNRS, Aix-Marseille Université, http://crn2m.univ-mrs.fr/pub/amplifx-dist). PCR conditions for all gene fragments consisted of 3 minutes of denaturation at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 50°C, 30s at 72°C, and a final extension period of 7 minutes at 72°C. All PCR reactions were performed in a volume of 50 µL containing approximately 10-20ng of template DNA, 1X Tris-HCl, 200mM of each dNTP, 3mM $MgCl_2$, 0.5µM of forward and reverse primers, and 1.25 units of *Taq* DNA Polymerase (Invitrogen). PCR amplicons were cycle-sequenced from both strands by a commercial sequence service provider (Macrogen Europe, Amsterdam, Netherlands).

Sequence Analysis

The mitochondrial Control Region (CR) partial sequences obtained from the historical samples were checked and edited using MEGA v.7.0.14 (Kumar, Stecher, & Tamura, 2016) and aligned with homologous sequences deposited in the GenBank (Table S3, Appendix S3) using the ClustalW algorithm (Thompson, Higgins, & Gibson, 1994). The total number of haplotypes and haplotype and nucleotide diversities of the Mediterranean sequences (with associated standard deviations) were estimated using DnaSP v.5.1 (Librado, & Rosaz, 2009). The genetic diversity data of the global populations were taken from literature (see results). The relationship between haplotype and nucleotide diversity of each population was plotted using the R software package (R Core Team, 2008) to investigate GWS population diversity history. Phylogenetic relationships and haplotype genealogies were inferred using HapView (Salzburger, Ewing, & von Haeseler, 2011). Maximum likelihood (ML) clustering was constructed using the DNAML program in PHYLIP v.3.695 (Felsenstein, 2005), run in HapView. The best evolutionary model used in the phylogenetic analyses was inferred with JModelTest 2.1.1 (Darriba, Taboada, Doallo, & Posada, 2012), according to the Akaike Information Criteria (AIC; Akaike, 1974). Subsequently, the Hasegawa-Kishino-Yano model (Hasegawa, Kishino, & Yano, 1985) with the discrete Gamma distribution (0.8) and allowing for a proportion of invariant sites (0.4) to exist (HKY85+G+I) was selected as the best-fit model.

Since the historical Mediterranean sequence alignment (515bp) was shorter than most of the CR sequences deposited in the GenBank, a ML haplotype network was also reconstructed using a longer sequence alignment of 828bp based on haplotypes available in GenBank obtained from global contemporary GWS populations and specimens, including four Mediterranean sequences previously reported (Table S3, Appendix S3). A comparison between the topologies of the two haplotype networks permitted a test of the potential loss of informative sites in the shorter sequence alignment and the possible effects this might have on the reconstructed phylogenetic relationships.

References of the supplementary methods

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6), 716–723. https://doi:10.1109/TAC.1974.1100705

Allentoft, M. E., Collins, M., Harker, D., Haile, J., Oskam, C. L., Hale, M. L., … Bunce, M., (2012). The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings of the Royal Society B: Biological Sciences,* 279(1748), 4724-4733. https://doi:10.1098/rspb.2012.1745

Cooper, A, & Poinar, H. N. (2000). Ancient DNA: do it right or not at all. *Science*, 289(5482), 1139. https://doi:10.1126/science.289.5482.1139b

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772. https://doi:10.1038/nmeth.2109

Felsenstein, J. (2005). *PHYLIP (Phylogeny Inference Package) version 3.6*. University of Washington, Seattle. Retrieved from http://evolution.genetics.washington.edu/phylip.html.

Gibbon, V. E., Penny, C. B., Štrkalj, G., & Ruff, P. (2009). Brief Communication: Minimally Invasive Bone Sampling Method for DNA Analysis. *American Journal of Physical Anthropology*, 139(4), 596-599. https://doi:10.1002/ajpa.21048

Hansen, A. J., Willerslev, E., Wiuf, C., Mourier, T., & Arctander, P. (2001) Statistical Evidence for Miscoding Lesions in Ancient DNA Templates. *Molecular Biology and Evolution,* 18(2), 262–265. https://doi:10.1093/oxfordjournals.molbev.a003800.

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2), 160-174.

Kemp, B. M., & Smith, D. G. (2005). Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. *Forensic Science International*, 154(1), 53-61. https://doi:10.1016/j.forsciint.2004.11.017

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7 Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Librado, P., & Rosaz, J. (2009). DNAsp v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452. https://doi:10.1093/bioinformatics/btp187

R Core Team. (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Riccioni, G., Landi, M., Ferrara, G., Milano, I., Cariani, A., Zane, L., … Tinti, F. (2010). Spatio temporal population structuring and genetic diversity retention in depleted Atlantic bluefin tuna of the Mediterranean Sea. *Proceedings of the National Academy of Sciences of the United States of America,* 107(5), 2102–2107. https://doi:10.1073/pnas.0908281107

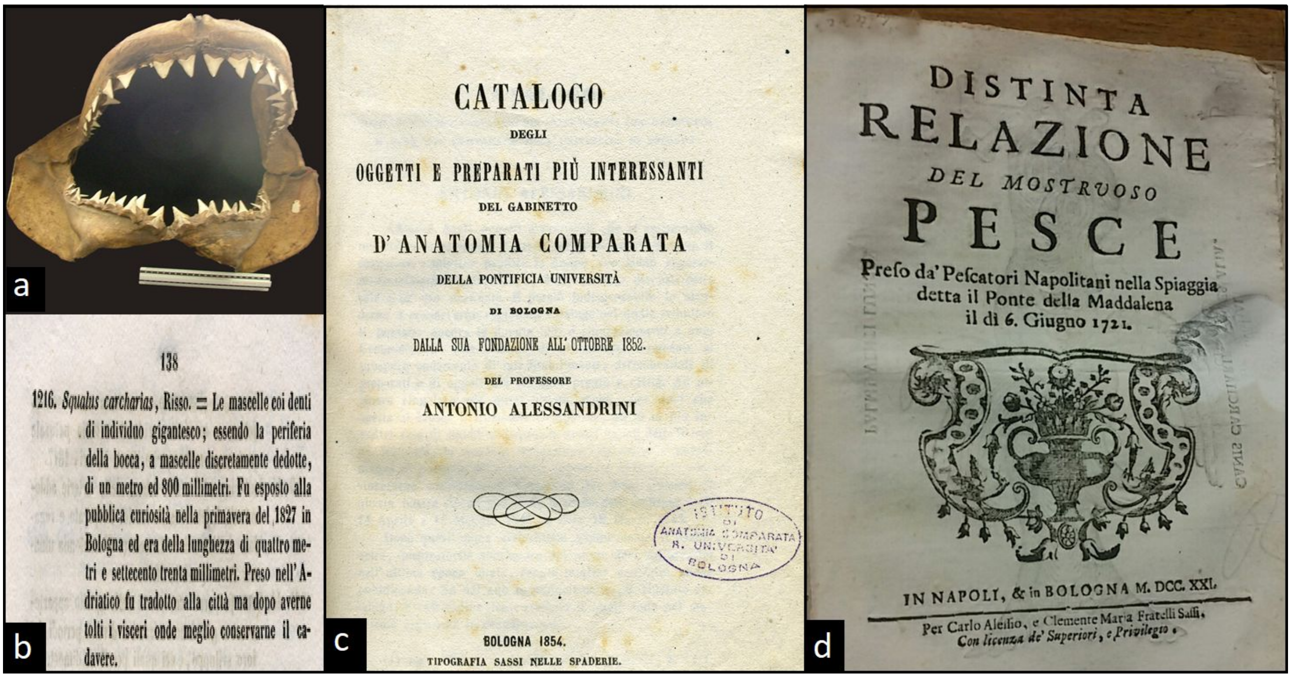
Salzburger, W., Ewing, G. B., & von Haeseler, A. (2011). The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, 20(9), 1952-1963. https://dx.doi.org/10.1111/j.1365-294X.2011.05066.x

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673-4680. https://doi:10.1093/nar/22.22.4673

Untergrasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012 Primer3 - new capabilities and interfaces. *Nucl. Acids Res*. **40**, e115. https://doi:10.1093/nar/gks596)

Appendix S2 Supplementary figures

Figure S1. a) Jaws of the Great White Shark archived in the Museum of Comparative Anatomy of the University of Bologna. b, c) The cover and original illustrative note from Antonio Alessandrini (1854) “Catalogo degli Oggetti e Preparati più interessanti del Gabinetto D’Anatomia Comparata della Pontificia Università di Bologna dalla sua Fondazione all’ Ottobre del 1852”. The jaws were prepared from a GWS individual (TL = 473 cm) collected in the Adriatic Sea in 1827 and displayed to the public at the University of Bologna. d) Original cover from the publication of Ricciardi (1721) Pontificia Università di Bologna, Italy.



138

1216. *Squalus carcharias*, Risso. = Le mascelle coi denti di individuo gigantesco; essendo la periferia della bocca, a mascelle discretamente dedotte, di un metro ed 800 millimetri. Fu esposto alla pubblica curiosità nella primavera del 1827 in Bologna ed era della lunghezza di quattro metri e settecento trenta millimetri. Preso nell’ Adriatico fu tradotto alla città ma dopo averne tolti i visceri onde meglio conservarne il cadavere.

CATALOGO
DEGLI
OGGETTI E PREPARATI PIÙ INTERESSANTI
DEL GABINETTO
D’ ANATOMIA COMPARATA
DELLA PONTIFICIA UNIVERSITÀ
DI BOLOGNA
DALLA SUA FONDAZIONE ALL’ OTTOBRE 1852.
DEL PROFESSORE
ANTONIO ALESSANDRINI

BOLOGNA 1854.
TIPOGRAFIA SASSI NELLE SPADERIE.

DISTINTA
RELAZIONE
DEL MOSTRUOSO
PESCE
Preſo da’ Peſcatori Napolitani nella Spiaggia
detta il Ponte della Maddalena
il dì 6. Giugno 1721.

IN NAPOLI, & in BOLOGNA M. DCC. XXI.
Per Carlo Aleſſio, e Clemente Maria Fratelli Saſſi.
Con licenza de’ Superiori, e Privilegio.

Figure S2. Illustration of the sampling procedures for the museum specimens of Great White Shark. a: Tooth pulp extraction from the crown. b-d: Drill sampling from dried jaw and restoration of the holes.

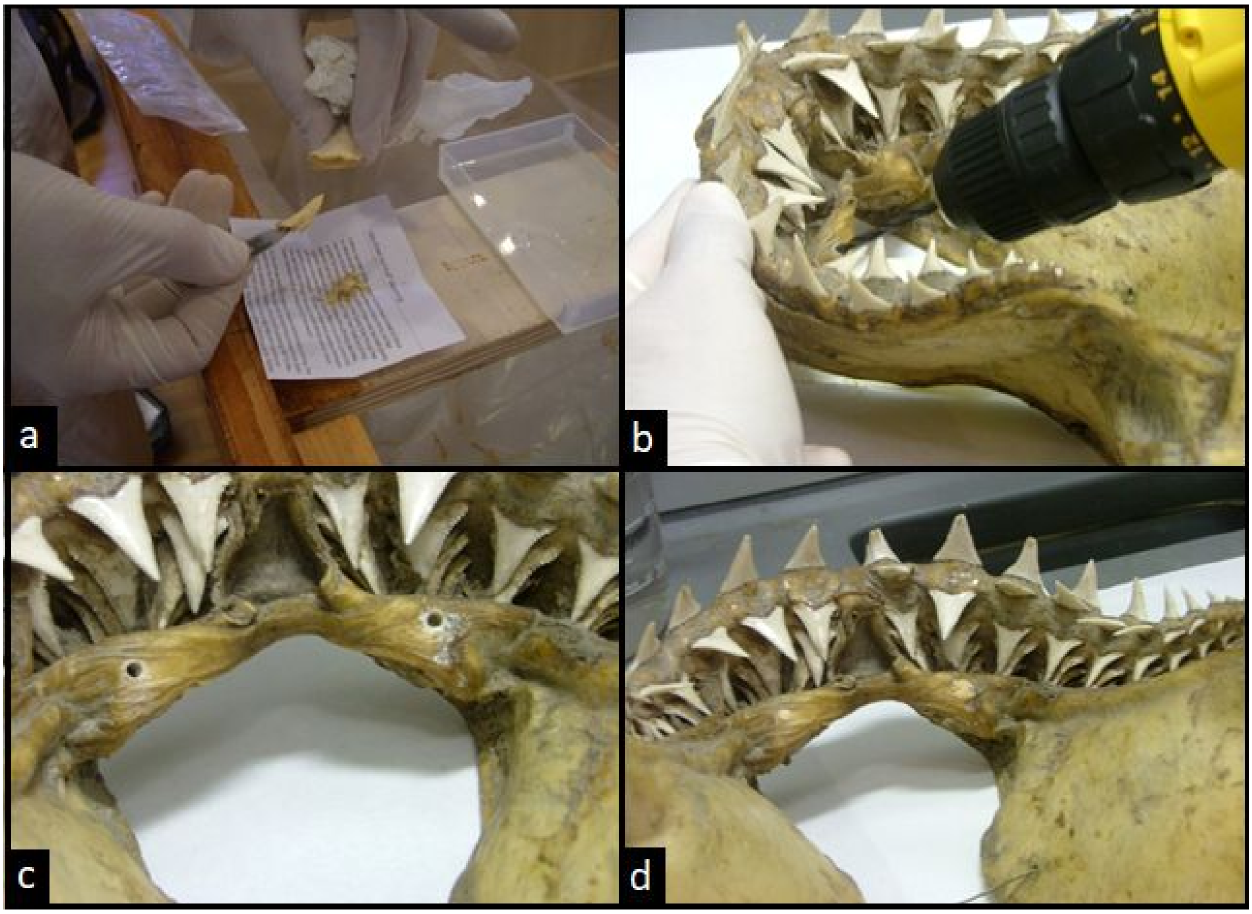


Figure S3. Results of the *in silico* PCR simulation with primer pairs designed for the amplification of the Control Region (CR) of *Carcharadon charcarias* (see Table S2, Appendix S3). The annealing positions of the primers with respect to the starting position of the gene on a reference mitochondrial genome (GenBank accession number NC_022415) was shown in brackets. The length in base pairs (bp) of the amplified fragments are marked in yellow.

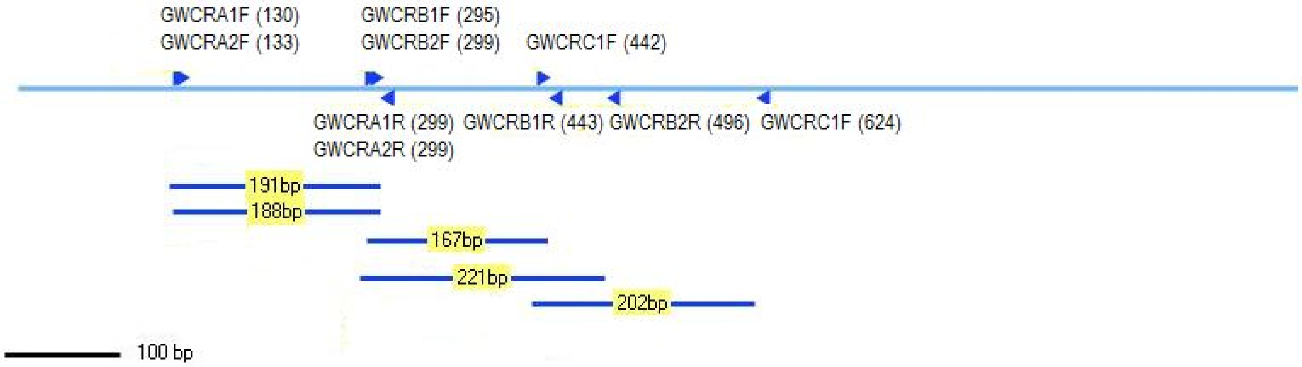


Figure S4. Maximum likelihood haplotype networks of worldwide *Carcharadon charcarias* population samples reconstructed using the 516bp (a) and the 828bp (b) CR sequence alignments, respectively. Acronyms are given in Table S2; Appendix S3. Numbers inside circles indicates the number of GWS individuals bearing the CR haplotype. Small blue dots indicate single nucleotide substitutions. Numbers outside the circles indicates the gross number of nucleotide substitutions separating the two haplogroups.

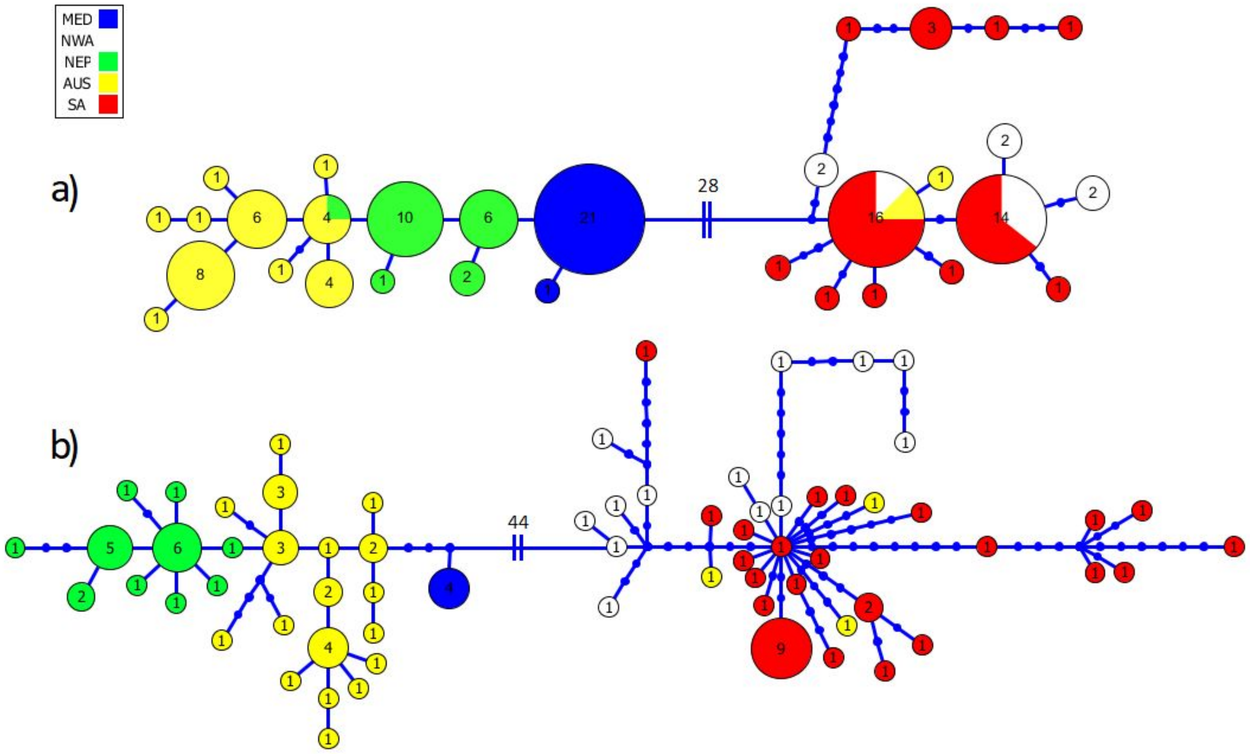
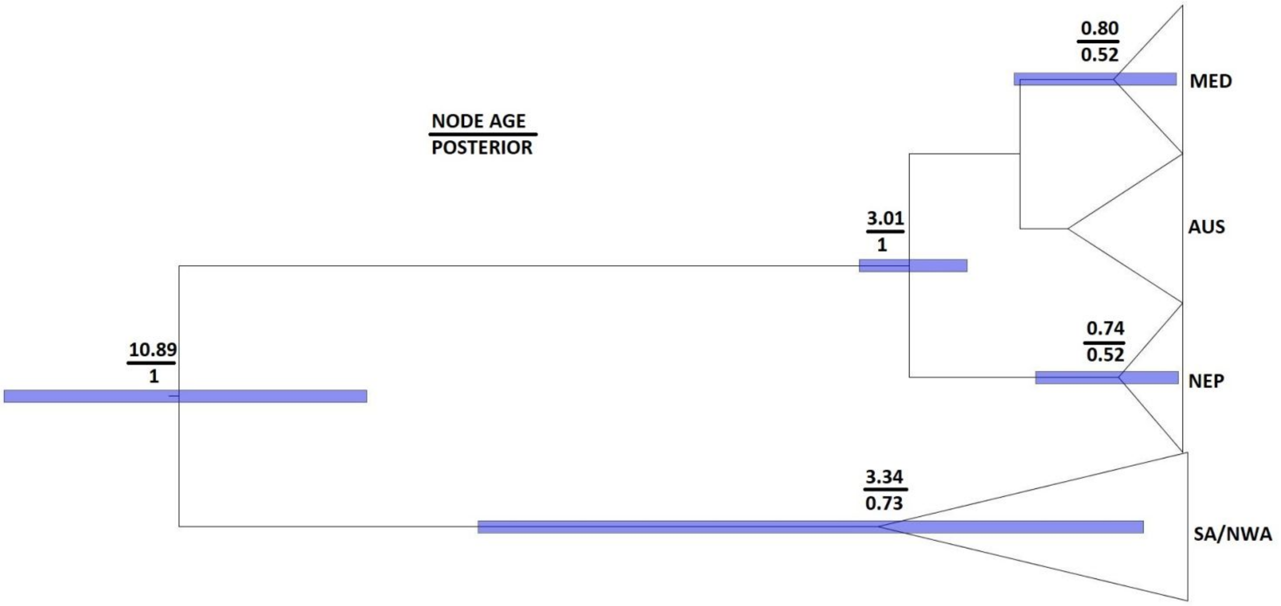


Figure S5. Bayesian divergence time tree of populations of *Carcharadon charcarias* inferred using the 516bp dataset of control region sequences. High posterior density (HPD 95%) in blue bars. Abbreviations are given in Table S2, Appendix S3.



Appendix S3 Supplementary tables

Table S1. List of the 18 historical specimens of Mediterranean Great White Sharks, *Carcharodon carcharias* collected from museum and private archives.

Specimen code	GenBank Acc.No.	Geographic area	Sampling year/period	Tissue type*	Source §	Museum/Archive, Biological and Collecting Data
TRCC01AD	MN718579	Adriatic Sea	1906	V, T	1	Cat. No. 1182. Sex: female. Collecting site: Quarnero Gulf (Istria, Croatia). Collecting date: 29 May 1906. Donor: A. Morin
GECC01LI	MN718580	Ligurian Sea	1935	J	2	Cat. No. C.E. 32695. Sex: unknown. Collecting site: Riva Trigoso, Sestri Levante (Genoa, Italy). Collecting date: 03 July 1935
GECC02LI	MN718581	Ligurian Sea	1930s	J	2	Cat. No. C.E. 31916. Sex: unknown. Collecting site: unknown. Collecting date: 17 March 1933. Donor: E. Olivieri
GECC03LI	MN718582	Ligurian Sea	1958	J, T	2	No detailed data are available
LICC01LI	MN718583	Ligurian Sea	1950s	J	3	No detailed data are available
BOCC01AD	MN718584	Adriatic Sea	1823	J	4	Cat. No. 811, Catalogue Alessandrini. 1823 Sex: unknown. Collecting site: unknown. Collecting date: unknown. Additional info: mouth extension of 1.15 m
BOCC02AD	MN718585	Adriatic Sea	1827	J	4	Cat. No. ACP 114*; 1216 Catalogue Alessandrini. Sex: unknown. Collecting site: unknown. Collecting date: unknown. Additional info: mouth extension of 1.80 m
FICC01LI	MN718586	Ligurian Sea	1891	J	5	Cat. No. 6032; *Carcharodon rondeletii* M.H., 2775. Sex: female. Collecting site: Monterosso (Spezia, Italy). Collecting date: 10 December 1891. Additional info: Total length ~600 cm; Weight ~600 Kg. Donor: S.H. Giglioli.
FICC02LI	MN718587	Ligurian Sea	1879	V, T	5	Cat. No. 5983. Sex: unknown. Collecting site: Viareggio (Lucca, Italy). Collecting date: unknown.
MOCC01LI	MN718588	Ligurian Sea	1883	J, T	6	Cat. No. 50; *Carcharodon rondeletii* M.H. Sex: Male. Collecting site: Portofino (Genoa, Italy). Collecting date: January 1883.
PACC01TI	MN718589	Tyrrhenian Sea	1980s	J	7	No detailed data are available
PACC02TI	MN718590	Tyrrhenian Sea	1980s	J	7	No detailed data are available

PACC03TI	MN718591	Tyrrhenian Sea	1980s	J	7	No detailed data are available
FACC01TI	MN718592	Tyrrhenian Sea	1980s	V, M	8	Sex: unknown. Collecting site: Favignana (Trapani, Italy). Collecting date: unknown.
FACC02TI	MN718593	Tyrrhenian Sea	1980s	M	8	Sex: unknown. Collecting site: Favignana (Trapani, Italy). Collecting date: unknown.
FACC03TI	MN718594	Tyrrhenian Sea	1980s	V	8	Sex: unknown. Collecting site: Favignana (Trapani, Italy). Collecting date: unknown.
FACC04TI	MN718595	Tyrrhenian Sea	1980s	V	8	Sex: unknown. Collecting site: Favignana (Trapani, Italy). Collecting date: unknown.
FACC05TI	MN718596	Tyrrhenian Sea	1980s	M	8	Sex: unknown. Collecting site: Favignana (Trapani, Italy). Collecting date: unknown.

* V: cartilage, vertebrae; T: osteodentine, tooth; J: cartilage, jaws; M; dried skeletal muscle

§ 1: Civic Museum of Natural History of Trieste; 2: Civic Museum of Natural History of Genova "Giacomo Doria"; 3: Regional Agency for Environmental Protection of Tuscany, ARPAT Livorno; 4: Museum of Comparative Anatomy, University of Bologna; 5: Museum of Natural History of Firenze "La Specola"; 6: University Museum of Natural History and Instrumentation of Modena; 7: Museum of Zoology of Palermo "P. Doderlein"; 8: Favignana Tuna Trap

Table S2. List of primer pairs (F: forward primer; R: reverse primer) designed for the PCR amplification and sequencing of the CR gene fragments from the historical DNA of Great White Shark.

Primer	5'>3' sequence
GWCRA1F:	TGACCTTCACCTAATGGTATCACA
GWCRA1R*:	AAGTCTCTGTGAGTGGAAGGAA
GWCRA2F:	CCTTCACCTAATGGTATCACACTC
GWCRA2R*:	AAGTCTCTGTGAGTGGAAGGAA
GWCRB1F:	TTCCTTCCACTCACAGAGACTT
GWCRB1R:	CAAGGACTGAAGTGTTACAAGCA
GWCRB2F:	TTTATTCCTTCCACTCACAGAGAC
GWCRB2R:	GACGGAAATGCTGTTAAAGG

* these two primers have identical sequences

Table S3. The mtDNA control region haplotypes and/or sequences of modern Great White Shark deposited and retrieved from the GenBank.

Geographic Origin	Acronym	N	Reference	GenBank Accession Number
Australia	AUS	14	[9]	HQ414073 - HQ414086
Australia	AUS	12	[7]	AY026196 - AY026224
New Zealand	AUS	4		
South Africa	SA	13		
Northeastern Pacific	NEP	20	[10]	GU002302 - GU002321
Florida	NWA	2	[17]	HQ540294 - HQ540298
Mediterranean	MED	3		
Mediterranean	MED	1	[27]	JF715925
Northwestern Atlantic	NWA	11	[28]	KC511601 - KC511626
South Africa	SA	15		
South Africa*	SA	4*	[29]	KP058665 - KP058902*

*During the analyses, the 238 unique sequences from Andreotti et al. [29], were collapsed in the unique four haplotypes observed and added to the final dataset. AUS: Australia/New Zealand; NEP: Northeastern Pacific; MED: Mediterranean; SA: South-Africa; NWA: Northwestern Atlantic.

Table S4. Fossil data downloaded from PaleoDB (https://paleobiodb.org/#/) using the species-specific taxon "*Carcharodon carcharias*" and synonyms. References downloadable from the paleoDB database. The records, especially those from old references, without description of the fossil and without pictures, and those records with misidentified fossils (e.g. *Isurus* spp. or *Carcharodon* spp. identified as *C. carcharias*), were considered dubious and subsequently removed in order to create a fossil dataset of reliable *C. carcharias* records.

PaleobioDB N°	coll_no	PaleobioDB Epoch	early_age	late_age	Ref_no	longitude	latitude
465463	45458	Pliocene	7.246	5.333	12282	-115.175552	28.11472
465581	13079	Pliocene	7.246	5.333	12182	-74.719719	-15.5808
506112	51328	Pliocene	5.333	3.6	13672	141.603058	-38.3619
506149	51335	Pliocene	5.333	3.6	13672	141.944443	-37.7283
506450	46068	Pliocene	5.333	3.6	13672	147.96666	-37.8547
506468	51405	Pliocene	3.6	1.806	13672	138.609726	-34.8333
506557	51414	Pliocene	5.333	2.588	13672	148.083328	-39.9833
518397	52644	Pleistocene	1.806	0.781	14149	-52.326389	-32.3883
520948	28039	Pliocene	3.6	3	14399	174.28334	-39.5833
533364	20400	Pleistocene	1.8	0.3	1960	-82.5	27.7
558936	58089	Pleistocene	1.8	0.3	15601	-80.811386	27.83028
593653	52582	Pliocene	5.333	3.6	18094	-76.817497	35.35972
634589	68271	Pliocene	5.333	3.6	19640	-71.5	-30.3333
639047	55535	Miocene	7.246	5.333	19852	-70.841667	-27.0808
645725	69730	Miocene	11.62	7.246	23394	-70.87944	-27.1392
645727	69731	Pliocene	5.333	3.6	23394	-70.87944	-27.1392
668275	72085	Miocene	15.97	11.608	24392	90.666664	25.16667
732439	78614	Miocene	15.97	11.608	26436	92.73333	22.88333
789236	20646	Pleistocene	0.126	0.0117	28773	-118.199997	34
807796	88328	Pliocene	3.6	2.588	29650	10.888611	43.67222
899319	100174	Pliocene	3.6	2.588	34371	-70.534447	-23.3575
981089	117471	Pliocene	5.333	3.6	37795	-122.407997	37.288
984829	118104	Pliocene	5.333	3.6	38036	-0.676944	38.085
1087691	136597	Pliocene	5.333	3.6	43697	14.004444	32.7325
1192384	154111	Pliocene	5.333	2.588	49963	13.3	-8.75
1192410	154114	Miocene	15.97	3.6	49966	-70.833336	-27.1333

1192476	154117	Miocene	7.246	3.6	49968	-112.291389	27.36806
1192505	154118	Pliocene	5.333	2.588	49969	-109.616669	23.16667
1221552	159914	Pliocene	3.6	2.588	52184	138.600006	-34.9167
1227809	161451	Pliocene	5.333	2.588	52598	139.645554	35.4475
1228090	161503	Pleistocene	2.588	0.781	52618	136.96666	36.76389
1234295	162457	Miocene	11.608	5.333	52569	-81.650002	30.33
1360649	184953	Pliocene	3.6	2.588	37795	-122.407501	37.29
1360660	184959	Pliocene	3.6	2.588	37795	-122.404999	37.31
1360661	184960	Pliocene	5.333	3.6	37795	-122.407997	37.288
1374190	187584	Pleistocene	0.126	0.0117	62969	-88.183891	30.24965
1374545	55759	Pliocene	3.6	2.588	62984	9.998611	44.82667
1388536	190889	Pleistocene	0.126	0.0117	33088	-117.902222	33.65722
1406990	188304	Pleistocene	0.126	0.0117	66045	-80.31115	33.10401
1431917	100313	Pliocene	5.333	3.6	62247	18.42804	-33.6651

Table S5. Fossil data from the Mediterranean area. For museal fossil teeth catalogued from 1 to 87 see S. Marsili (2006).

n	Location	Age	Reference	Stratigraphic References
1a	Salsomaggiore Terme, Parma, Emilia-Romagna (Italy)	Pliocene	Bianucci et al. 2002	Bianucci et al. 1998
2a	Guardamar del Segura, Alicante (Spain) Pliocene	Pliocene	Adnet et al. 2009	
1	Terreti, Reggio Calabria: Calabria(Italy)	Pliocene sup./Pleistocene ?	Seguenza, 1901	Gaetani et al., 1986.
2	Nasiti e S.Agata, Reggio Calabria: Calabria(Italy)	Pleistocene inf.	Seguenza, 1901	Lombardo
3	Cetona (vicinanze), Siena: Toscana (Italy)	Pliocene	Principi, 1920	
4	Castiglione del Lago (a Ovest di), Perugia: Umbria (Italy)	Pliocene	Principi, 1920	
5	Città della Pieve, Perugia: Umbria (Italy)	Pliocene	Principi, 1920	
6	Imola (varie località): Emilia Romagna (Italy)	Pliocene	Collection Scarabelli (De Stefano, 1911)	
7	Imola (varie località): Emilia Romagna (Italy)	Pliocene	Collection Scarabelli (De Stefano, 1911)	
8	Castell'Arquato, Piacenza: Emilia Romagna (Italy).	Pliocene	De Stefano, 1912	
9	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Landini, 1977	
10	Punta Ristola, Capo di Leuca, Lecce: Puglia (Italy)	Pliocene middle	Menesini, 1968	
11	Torre del Lago, Lucca: Toscana (Italy)	Pleistocene	Ghelardoni, 1956	
12	Calanna, Reggio Calabria: Calabria (Italy)	Pliocene sup.	De Stefano, 1901	Barrier et al., 1986.
13	Castell'Arquato, Piacenza: Emilia Romagna (Italy).	Pliocene	Carraroli, 1897	
14	Rometta, Messina: Sicilia (Italy)	Pliocene sup-Pleistocene inf.	Seguenza, 1900	For Violanti, 1989
15	Milazzo, Messina: Sicilia (Italy)	Pliocene sup ?	Seguenza, 1900	
16	Guardamar del segura, Alicante: Valezia (Spain)	Pliocene inf.	Mora Morote, 1996	
17	Ruvo del Monte, Potenza: Basilicata (Italy)	Pliocene	Bassani, 1901	
18	Taranto: Puglia (Italy)	Pleistocene	Bassani, 1905	
19	Volpedo, Alessandria: Piemonte (Italy)	Pliocene middle-sup	De Alessandri, 1895	Gabba, 82
20	Ruvo del Monte, Potenza: Basilicata (Italy)	Pliocene	Pasquale, 1903	
21	S.Agata , Reggio Calabria: Calabria(Italy)	Pleistocene inf.	Pasquale, 1903	Lombardo
22	Terreti, Reggio Calabria: Calabria (Italy)	Pliocene inf.-middle	Pasquale, 1903	Gaetani et al., 1986.
23	Reggio, Nasiti, Reggio Calabria: Calabria (Italy)	Pliocene	Pasquale, 1903	

24	Capo di Leuca, Lecce: Puglia (Italy)	Pliocene middle	Pasquale, 1903	Bossio et al., 2001
25	San Demetrio Corone, Cosenza: Calabria (Italy)	Pleistocene	Pasquale, 1903	
26	Tabiano, Piacenza: Emilia Romagna (Italy);.	Pliocene inf.	De Stefano, 1912	
27	Calanna, Reggio Calabria: Calabria (Italy)	Pliocene sup.	Pasquale, 1903	Barrier et al., 1986.
28	Matera: Basilicata (Italy)	Pliocene sup.- Pleistocene inf.	Pasquale, 1903	Iannone et al., 1979; Cherubini et al., 1996; Pomar et al., 2001.
29	Sestano e Medano, Siena: Toscana (Italy)	Pliocene inf.	Manganelli & Spadini, 2003	
30	San Quirico d'Orcia, Giustrigona, Terre Rosse e I Sodi, Siena: Toscana (Italy)	Pliocene middle	Manganelli & Spadini, 2003	
31	Allerona, Terni: Umbria (Italy)	Pliocene middle	Bellocchio et al., 1991	
32	Guardamar del segura, Alicante: Valezia (Spain)	Pliocene inf.	Mora Morote, 1996	
33	Colline Toscane (Italy)	Pliocene	De Stefano, 1910: Collection Lawley di Bologna	
34	Colline Toscane (Italy)	Pliocene	De Stefano, 1910: Collection Lawley di Bologna	
35	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection Lawley of Pisa	
36	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
37	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
38	Val d'Orcia: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze	
39	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
40	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
41	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
42	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
43	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
44	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
45		Pliocene	Collection fossil teeth of Firenze	
46	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
47	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
48	San Frediano, Pisa: Toscana (Italy)	Pliocene middle	Collection fossil teeth of Firenze	
49	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	
50	Colline Senesi: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze	
51		Pliocene	Collection fossil teeth of Firenze	
52	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze	

53	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
54	San Vivaldo, Firenze: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
55	San Quirico d'Orcia, Siena: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
56	Colline Senesi: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
57	Chiusi, Siena: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
58		Pliocene	Collection fossil teeth of Firenze
59	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
60	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
61	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
62	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
63	Siena: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
64	San Quirico d'Orcia, Siena: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
65	Bagni di Casciana, Pisa: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
66	Orciano, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
67	San Quirico d'Orcia, Siena: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
68	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
69	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection fossil teeth of Firenze
70	Santa Luce, Pisa: Toscana (Italy)	Pliocene	Collection fossil teeth of Firenze
71	Rione Castellana, Palermo: Sicilia (Italy)	Pleistocene	Gemellaro, 1913
72	Pradalbino (varie loc.), Bologna: Emilia Romagna (Italy)	Pliocene inf.-middle	Vinassa de Regny, 1899
73	Pradalbino (varie loc.), Bologna: Emilia Romagna (Italy)	Pliocene inf.-middle	Vinassa de Regny, 1900
74	Orciano; Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Bassani, 1901
75	San Quirico d'Orcia (dintorni), Siena: Toscana (Italy)	Pliocene inf.-middle	Simonelli, 1880
76	unknown locality	Pliocene	Accademia Fisiocritici of Siena
77	unknown locality	Pliocene	Accademia Fisiocritici of Siena
78	? Monte Follonico(1), Siena: Toscana (Italy)	Pliocene	Accademia Fisiocritici of Siena
79	Monte Follonico, Siena; ? Volterra(1), Pisa: Toscana (Italy)	Pliocene	Accademia Fisiocritici of Siena
80	Pod. Casabianca (Trequanda), Siena: Toscana (Italy)	Pliocene	Accademia Fisiocritici of Siena
81	Medane (Asciano), Siena: Toscana (Italy)	Pliocene	Accademia Fisiocritici of Siena
82	Volterra, Pisa: Toscana (Italy)	Pliocene inf.-middle	Collection Lawley of Pisa
83	Pontedera, Pisa: Toscana (Italy)	Pliocene	Collection Lawley of Pisa

84	Piacentino: Emilia Romagna (Italy)	Pliocene	Collection Lawley of Pisa
85	Terricciola, Pisa: Toscana (Italy)	Pliocene	Collection Lawley of Pisa
86	Val di Cecina: Toscana (Italy)	Pliocene	Collection Lawley of Pisa
87	Peccioli, Pisa: Toscana (Italy)	Pliocene	Collection Lawley of Pisa

Table S6. Control Region diversity of the Mediterranean and global populations of *Carcharodon carcharias.*

Population	Acronym	N	Nh	*h*	±	SD	π	±	SD	Reference
Mediterranean	MED	22*	3	0.091	±	0.124	0.0002	±	0.0007	This work
Australia-New Zealand	AUS	94	14	0.880	±	0.015	0.0085	±	0.0045	[Blower et al., 2012]
Northeastern Pacific	NEP	59	20	0.790		na	0.0013	±	0.0009	[Jorgensen et al., 2010]
South Africa	SA	34	15	0.723		na	0.0059		na	[O'Leary et al. 2015]
South Africa	SA*	238	4	0.205	±	0.033	0.0027	±	0.0005	[Andreotti et al. 2016]
North West Atlantic	NWA	44	12	0.749		na	0.0030		na	[O'Leary et al. 2015]

Acronyms are given in Table S3.

N: number of individuals analysed; Nh: number of haplotypes; *h*: haplotype diversity; π nucleotide diversity; SD: Standard deviation

* this sample included sequence records from GenBank (Acc. Num. JF715925, HQ540294, HQ540295, HQ540296).

Table S6. Best model selection based on Bayes factors using alternative primary calibration (estimated divergence between *Carcharodon carcharias* and *Lamna nasus*). logML_GSS: log marginal likelihood from generalized stepping stone model, BF_GSS: Bayes factors calculated using the logML_GSS.

Model	**Dataset**	**Node1**	**Node2**	**logML_GSS**	**logBF_GSS**
MED Pliocene divergence	828bp	*C.carcharias*/*L.nasus* divergence Mean: 46 Ma, SD: 1.0 Ma	MED/Pacific Mean: 3.0 Ma, SD: 0.3 Ma	-3210.6566	0 (Best)
MED Pleistocene divergence	828bp	*C.carcharias*/*L.nasus* divergence Mean: 46 Ma, SD: 1.0 Ma	MED/Pacific Mean: 0.4Ma, SD: 0.15 Ma	-3215.5172	4.8606
MED Pliocene divergence	516bp	*C.carcharias*/*L.nasus* divergence Offset: 46 Ma, SD: 1.0 Ma	MED/Pacific Mean: 3.0 Ma, SD: 0.3 Ma	-1905,6997	0
MED Pleistocene divergence	516bp	*C.carcharias*/*L.nasus* divergence Offset: 46 Ma, SD: 1.0 Ma	MED/Pacific Mean: 0.4Ma, SD: 0.15 Ma	-1914,4034	8.7037

Great White Shark, Dyer Island, Gansbaai, South Africa, 2011, Photo: Agostino Leone

1286x965mm (72 x 72 DPI)

Lateral view of a Great White Shark historical jaw from Alessandrini Collection of the University of Bologna.
Photo: Mr. Leonardo Piol

Frontal view of a Great White Shark historical jaw from Alessandrini Collection of the University of Bologna.
Photo: Mr. Leonardo Piol