UNIVERSITÀ
DEGLI STUDI
DI BRESCIA

DOTTORATO DI RICERCA IN
GENETICA MOLECOLARE, BIOTECNOLOGIE
E MEDICINA SPERIMENTALE

Settore Scientifico Disciplinare MED/43

XXXV CICLO

ESTIMATING THE BIOGEOGRAPHICAL ORIGIN OF THE UNIDENTIFIED BODIES OF THE SHIPWRECK OF APRIL 18TH, 2015 IN THE MEDITERRANEAN: COMPARISON OF GENETIC AND ANTHROPOLOGICAL ASSESSMENTS.

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Anno Accademico 2021-2022

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ABSTRACT (EN)

The migratory crisis has recently drawn attention to the need to identify the victims of shipwrecks in the Mediterranean Sea. This study is part of a larger project carried out by the Forensic Laboratory of Anthropology and Odontology (LABANOF – Laboratorio di Antropologia e Odontologia Forense) of the University of Milan aimed at identifying the victims of the shipwreck of April 18, 2015.

The present research had the objective of exploring the current methods of geographic origin estimation in forensic genetics and anthropology and contributing to the search in the field of identification.

To this end, the remains of 150 victims were subjected to genetic investigation at the forensic genetics laboratory of the University of Brescia, Turin, Pavia and of the Eurofins Genoma for identification purposes. Also, on 49 cases with good quality profiles biogeographical ancestry estimation was performed using different techniques, according to the protocols used in the different laboratories involved (Brescia and Turin).

Furthermore, having detected differences in terms of recovery of genetic information useful for comparison with the profiles of alleged relatives, the possible correlation between the quality of the results in the analysis of autosomal markers and the taphonomic condition of the cadaveric remains was investigated. The only variable that showed significant variation (p-value <0.01) was the time interval between the shipwreck and the autopsy procedures, during which sampling was performed. Bone samples with optimal analytical results were taken earlier (<200 days) in almost all cases. This finding underlines the importance of early victim recovery and identification procedures.

The genetic samples used for biogeographical estimate were also analyzed by applying morphological methods developed on populations allegedly comparable to those of the origin of the victims. The estimates were then compared with the genetic results obtained.

With regard to geographical ancestry, the predictions of the OSSA method appear to coincide with the group with probability percentages related to the higher estimate resulting from the hefneR software. However, the low number of OSSA findings does not allow for any additional considerations to be made.

As far as the non-metric dental characters are concerned, the results of the analysis with the rASUDAS software show little agreement with the cranial morphological predictions, referring in this sense only to the hefneR method: the OSSA score, in fact, contrary to the first two, does not consider the Asian population among the reference population and is therefore

less expendable in terms of comparison. The assessment resulting from the hefneR software show a greater presence of the African and Asian components while the rASUDAS estimates show a European morphological predominance. These findings appear to be related to the type of reference populations chosen in the different methods and, in particular, to the absence of African traits in the rASUDAS and to the paucity of African populations among the references of the hefneR. This selection bias could also explain the many resulting mixed forms. The comparison with the predictions of biogeographical origin using Y-STR polymorphisms showed a certain pattern of agreement only with the estimates obtained by the hefneR method. The opportunity to confirm what was observed on a larger sample and when the identification process is completed would be an ambitious goal for future research.

In conclusion, the present research made it possible to identify the potentially most suitable methods for geographical origin estimation, opening new perspectives in the identification process of the unknown victims of the Mediterranean. The availability of accurate anthropological and odontological methods can complement genetic analysis investigations.

In this sense, the comparison of suspicious identities can be restricted to individuals from a specific geographical area, just as the search for relatives can be directed to certain African countries and thus suitable reference data can be selected for genetic comparison. In addition, knowing any links between the state of preservation of the remains and the quality of the genetic material stored in it may allow the selection of the most suitable portion of bone and/or protocols for subsequent genetic investigations.

ABSTRACT (IT)

La crisi migratoria ha recentemente posto l'attenzione sulla necessità di identificare le vittime dei naufragi del Mediterraneo. Il presente studio fa parte del progetto del LABANOF (Laboratorio di Antropologia e Odontologia), Dipartimento di Scienze Biomediche dell'Università degli Studi di Milano, che ha come finalità quella di identificare le vittime del naufragio del 18 aprile 2015. Oltre ad occuparsi di tale aspetto identificativo, il presente lavoro di Tesi ha avuto come obiettivo il confronto tra metodiche genetiche e antropologiche al fine della stima dell'origine biogeografica di tali individui. A tal fine, i resti di 150 vittime sono stati sottoposti ad indagine genetica presso il laboratorio di genetica forense dell'Università degli Studi di Pavia, Torino, Brescia and Eurofins Genoma, a scopo identificativo con la tipizzazione dei marcatori autosomici STR. Sui 49 campioni che hanno restituito una qualità analitica ottimale, sono poi state effettuate analisi mirate alla valutazione dell'origine biogeografica: in particolare, sui casi si è proceduto alla tipizzazione dei marcatori autosomici sul cromosoma Y, oltre a marcatori SNP sia autosomici sia sul cromosoma Y.

Essendosi altresì rilevate differenze in termini di resa e recupero dell'informazione genetica tra i diversi individui, utile al confronto con i profili dei presunti parenti, si è inoltre indagata l'eventuale sussistenza di una correlazione fra la qualità del materiale genetico risultante dall'analisi dei marcatori autosomici e le condizioni tafonomiche dei resti cadaverici studiati. L'unica variabile che ha mostrato di variare in modo significativo (p-value <0.05) è stato l'intervallo di tempo intercorso fra il naufragio e il momento dell'espletamento delle procedure autoptiche, nel corso delle quali si è provveduto al prelievo dei campioni ossei: i campioni con risultato analitico ottimale sono stati prelevati nella quasi totalità dei casi più precocemente (<200 giorni).

Parallelamente i 49 campioni sono stati analizzati dal punto di vista antropologico applicando metodi morfologici sviluppati su popolazioni sovrapponibili a quelle di provenienza delle vittime.

Per quanto riguarda l'ancestralità geografica, le predizioni dei due metodi valutati sui caratteri morfometrici del cranio (OSSA e hfeneR) appaiono coincidere tra loro se si considera il gruppo con percentuali di probabilità correlate alla stima. Tuttavia, la bassa numerosità delle risultanze del sistema OSSA non consente ulteriori considerazioni.

Per quanto concerne i caratteri non metrici valutati sui denti, le analisi sono state effettuate mediante software rASUDAS. Le stime risultanti hanno rivelato una scarsa concordanza con le predizioni morfologiche craniche, in particolare con quelle del software hefneR, che è più

paragonabile del sistema OSSA. Nelle previsioni risultanti dal software hefneR risulta una maggior presenza delle componenti africane e asiatiche mentre dalle stime rASUDAS emerge una predominanza morfologica europoide. Tali rilievi risentono chiaramente della tipologia di popolazioni di riferimento prescelte nei diversi metodi e, in particolare, dalla scarsità o totale assenza di tratti africani di riferimento nelle metodiche attualmente a disposizione. Questo *bias* di selezione potrebbe spiegare altresì le molte forme miste risultanti. Il confronto con le predizioni di origine biogeografica mediante polimorfismi Y-STR ha evidenziato un certo pattern di concordanza solamente con le stime ottenute mediante il metodo hefneR. L'opportunità di confermare quanto osservato su un campione maggiore rappresenterebbe un ambizioso obiettivo per ricerche future.

In conclusione, il presente lavoro ha permesso di identificare i metodi potenzialmente più idonei per lo studio del campione in analisi, aprendo nuove prospettive nel processo identificativo delle vittime del Mediterraneo. La possibilità di avere metodi antropologici e odontologici accurati permetterebbe di circoscrivere il confronto a sospetti di identità provenienti da una specifica area geografica, oltre a focalizzare la ricerca dei parenti in alcuni paesi e a consentire la selezione di dati di riferimento idonei per il confronto genetico. In aggiunta, il riscontro di come la qualità dei profili genetici venga significativamente influenzata dal tempo di sommersione sottolinea l'importanza di procedure precoci di recupero e identificazione delle vittime. Conoscere eventuali nessi tra lo stato di conservazione dei resti e la qualità del materiale genetico in esso conservato potrebbe permettere inoltre di selezionare la porzione di osso e/o i protocolli più idonei per le successive indagini genetiche.

INTRODUCTION

Migrant deaths in the Mediterranean

According to the International Organisation for Migration (IOM), since 2014, more than 25,000 men, women, and children have died or disappeared in the Mediterranean Sea during crossings to Europe to escape war and abuse in their countries of origin or simply in the hope of a better life. In most cases the bodies of migrants were never recovered, or their disappearances was never reported, resulting certainly in an underestimation of the real phenomenon (IOM, 2020a)[i,ii,iii].

Within this migratory crisis, the migrants take different routes within the sea, of which the Central Mediterranean represents the busiest as well as the most dangerous seaway (Figure 1).

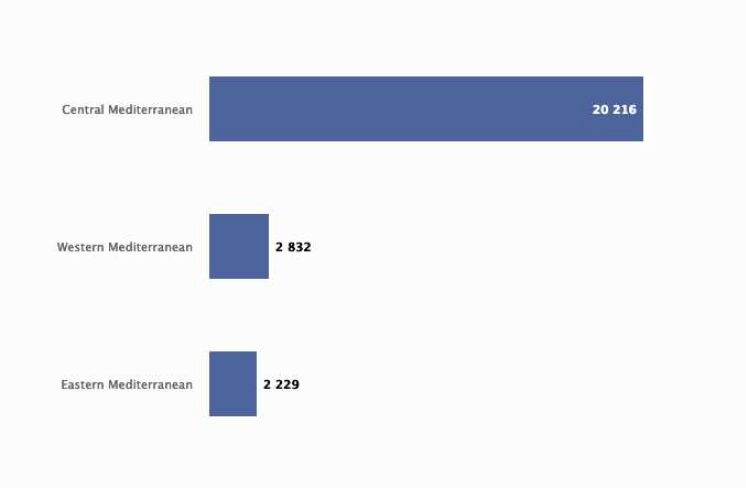

Figure 1. Number of missing migrants based on the different routes (from IOM).

Since 2014, more than 20,000 people are estimated to have disappeared on this route, with a confirmed stability in the large number of victims worldwide along transnational migration routes every year (Figure 2).

[i] https://missingmigrants.iom.int/region/mediterranean
[ii] https://frontex.europa.eu/media-centre/news/news-release/migratory-situation-at-eu-s-borders-in-september-increase-on-the-central-mediterranean-and-western-balkan-routes-RZRnEH
[iii] https://data2.unhcr.org/

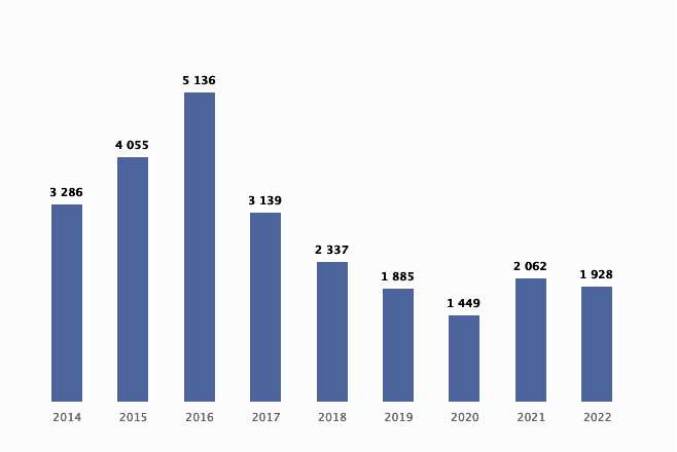

Figure 2. Dead and missing by year (from IOM).

In 2016, on the Central Mediterranean route, more than 181,000 migrants were recorded – the highest number ever observed in the region. Consistent with migration data, 2016 is also the year in which the highest total number of dead and missing along this route was recorded (5,136).

Also in 2015, the Central Mediterranean route was under intense migratory pressure. Even though the total number of arrivals in Italy during 2014 and 2015 was lower (153,946), with a parallel decrease in the number of deaths and missing persons recorded, 2015 holds the record for the year with the highest monthly incidence of victims ever in the Mediterranean[iv]. It is not a coincidence that in April 2015 there was, as it was defined, *"the largest civil maritime accident that occurred in the Mediterranean in the post-war period"*, the shipwreck of April 18th, 2015.

The shipwreck of April 18th 2015

During the night of April 18th-19th, a fishing boat of Eritrean nationality, which left from a port near Zwara (west of Tripoli, Libya), sank off the Libyan coast carrying its almost full "cargo".

According to survivors' testimonies, it is estimated that more than a thousand migrants (about 5 per square metre) were crammed onto the boat, which was about 23 metres long, including about 200 women and 50 children.

[iv]https://frontex.europa.eu/we-know/migratory-routes/central-mediterranean-route/

The shipwreck of the boat, whose dynamics are still to be clarified, caused between 800 and 1,000 missings, making it one of the most tragic maritime events in the Mediterranean Sea since the beginning of the 21st century.

According to the Catania Public Prosecutor's Office, the capsizing of the vessel and consequent sinking were due to two causes: firstly, the wrong maneuvers carried out by the boatman, who, in an attempt to board the Portuguese merchant ship King Jacob, sent to their rescue, caused the collision between the vessels; secondly, the overcrowding of the boat, which was unbalanced by the wrong maneuvers, and the sudden movement of the migrants on board to one side of the boat when they saw the imminent impact with the merchant ship.

At the time of the shipwreck, in addition to the 28 survivors brought to Catania by an Italian Coast Guard vessel, the first 24 bodies were also recovered and transported to Malta.

An unprecedented operation by the Italian Governmental started after the shipwreck. The following summer, a memorandum of understanding was signed on July 23rd 2015, between the Extraordinary Commissioner for Missing Persons and the University of Milan aimed at recovering and identifying the victims of this shipwreck.

The following year, the entire wreck was recovered by the Navy and the remaining bodies were extracted by the Fire Brigade and Military Red Cross personnel, an activity that was completed in June 2016 (15 months after the shipwreck).

The bodies were retrieved from the sea bottom (400 m deep) and put into individual body bags. For all bodies, the Prosecutor ordered full autopsies, which were carried out by a team of forensic pathologists and anthropologists. The recovered remains, mostly mixed and in different states of preservation, up to completely skeletonised material, were collected in 506 body bags and subsequently subjected to medico-legal and anthropological analysis according to protocols drawn up ad hoc in the mission called “Melilli 5”.

To date, although many remains are still being studied, the ascertained victims of the shipwreck are 528, a dramatic number that makes the event a true *mass disaster*.

The challenges of the recovery and the identification of Mediterranean victims

Although the tragedy of April 18th 2015 is the most numerically significant shipwreck, it represents only one of many tragedies that have made the bottom of the Mediterranean a real cemetery for a countless number of men, women, and children who lied without an identity.

The continuous flow of deaths due to an incalculable number of shipwrecks, with even hundreds of victims, has the size of a humanitarian tragedy of such magnitude that it confronts us with the serious issue of the failure to recover the bodies of migrants lost during the Mediterranean crossing and to identify them.

In fact, in the vast majority of cases, with priority given to rescuing and securing the survivors, the victims are not recovered, and thus their bodies lie unidentified on the Mediterranean seabed. Even in the few cases where bodies are recovered, they are buried in anonymous graves (Siccardi, 2018).

The failure to recover and identify the bodies of migrants appears to be linked to the absence of laws establishing the obligation to recover and identify the bodies of migrants and of shared protocols at the national and European level to concretely standardize operations. As a matter of fact, international law imposes the rescue of migrants (still alive) as a priority without specifically addressing the issue of victims.

Although, in fact, international laws, and in particular Articles 32-34 of the Additional Protocols to the Geneva Conventions of August 12th 1949 and the ICRC (2013a), establish the obligation for parties involved in conflicts, wars and enforced disappearances to activate procedures to search for persons reported missing, to recover bodies on their territory, to gather information useful for identification and to cooperate in their repatriation, recognizing on the one hand the rights of missing persons (i.e. the right to recognition of legal status; the right to be sought by appropriate means; the right to be considered alive until proven guilty), and on the other hand those of their family members (i.e. the right to receive information on missing persons and to dispose of their remains; the right to recognition of the specific legal status of the family members of a missing person; the right to social and/or economic support from the competent authorities in case of need), to date there are no specific laws available in peacetime.

The same international legal lacuna is also found at the level of our national legal system, when neither the Criminal Code (art. 116) nor the Mortuary Police Regulations (art. 12) establish an obligation to provide for the identification of corpses, even though they advise that this is necessary.

However, it is not just a matter of naming a corpse, but it is necessary to consider that behind every deceased person there is a family. Indeed, the failure to identify the bodies of migrants risks having a strong negative impact on the lives of their loved ones for several reasons.

Firstly, uncertainty about the fate of a family member can have important psychophysical and psychosocial repercussions on the surviving members of the household in the individual,

social, and community spheres. The term “ambiguous loss” refers to a condition of “unresolved grief” (Boss, 2016) resulting from an experience of permanent uncertainty about the fate of a departed loved one whose death has not been ascertained. This is a situation of unfinished and persistent grief, symptomatically comparable to post-traumatic stress disorder, which can accompany a family for a lifetime. From this point of view, therefore, the identification of a corpse and the return of the remains to the family, with the possibility of providing a dignified burial, make it possible to complete the narrative not only of the individual himself but also, and above all, of the family: on the one hand, this allows the family to find “closure”, thus being able to return to their daily lives after having adequately processed the bereavement, and on the other hand, it guarantees the survivors public and collective recognition of the suffering inflicted on the family by the loss, bringing the surviving unit back into the culture of belonging (Mazzarelli, 2021).

From a legal-administrative point of view, the failure to complete the procedures for identifying the bodies of migrants, an option left to the public prosecutor’s office, precludes family members from enjoying several rights, first and foremost access to justice, since the identification of the bodies depends on the possibility for family members to bring civil actions in the court cases on shipwrecks, thus damaging their right to defense. Moreover, the uncertainty over the fate of the family member and consequently the absence of a death certificate do not allow the performance of all those acts that derive from ascertaining the reality of a person’s death, preventing, for example, relatives from obtaining ownership of property (inheritance, but also just a house), recognition of the civil status of widow/orphan, the missing person’s survivor’s pension, and other reserved economic benefits. In the case of orphaned minors, if the parents who died during the crossing are not identified, the children who remain in the country lose the right to be reunited with any other relatives residing in the European territory, since this right is recognised only for natural children, adopted, fostered or subject to guardianship. It is therefore clear that the enjoyment of a plurality of rights by families presupposes knowledge of the fate of the missing person (Siccardi, 2018).

All this being said, it seems necessary to question the configurability also for the families of missing migrants of an autonomous right to receive news about the fate of their loved ones, the so-called *right to know the truth*, as provided for by international law to protect the families of persons disappeared as a result of conflicts and enforced disappearances. The lack of commitment of the authorities involved in various capacities in the investigation and search for missing migrants in the Mediterranean could, in this sense, constitute a violation of conventional standards (Articles 3 and 13 ECHR).

Another reason linked to the failure to recover and identify victims is linked to the technical criticalities of the procedures, due on the one hand to the concrete possibilities of implementing recovery operations, with their notoriously high costs, as well as to the impossibility of tracing the actual number of missing persons to be recovered, given the absence of an official list of passengers (a mass disaster defined as an "open system") and, on the other hand, to the objective difficulty of finding the *ante* (AM) and *post-mortem* (PM) data of the verified victims, indispensable to proceed to their identification (Figure 3).

Critical issues in the collection of PM data stem from the phenomenon's extreme fragmentation: in fact, countless corpses have been collected over the years in various countries, sometimes as victims of great disasters, and sometimes as individual bodies that surface on Italian coasts as evidence of tragedies consumed in silence. Sometimes, even the victims of the same shipwreck do not come from a specific place. This spatial scattering has repercussions on the possibility of taking charge of the victims, who are often under the jurisdiction of different authorities: at the prosecutors' discretion, autopsies (or only external examinations) may be requested, which may be carried out by the police, universities, or private individuals. PM data from the different disasters are therefore often uneven and incomplete, fragmented in the different prosecution offices (Olivieri, 2018).

Even greater difficulties concern the collection of AM data. In fact, unlike mass disasters involving victims from industrialised countries, where it is possible and relatively easy to retrieve adequate health records, especially dental records, and material for genetic comparison, in the case of Mediterranean tragedies, clinical information (e.g., surgical history, implant prostheses, X-ray and OPT images, dental care, dental implants and treatments...) or even personal effects of the missing are only rarely available. This information is generally provided by families in the country of origin or by relatives of various degrees, scattered all over Europe: not only it is difficult to identify and get in touch with them, but it is even more critical to recover personal effects useful for DNA comparison. Often, in fact, the degree of kinship of the person claiming death is not adequate for genetic identification. Therefore, in these situations where even genetic comparison often does not provide strong support for identification, new strategies must be developed, among which anthropological and dental methods seem to play a key role.

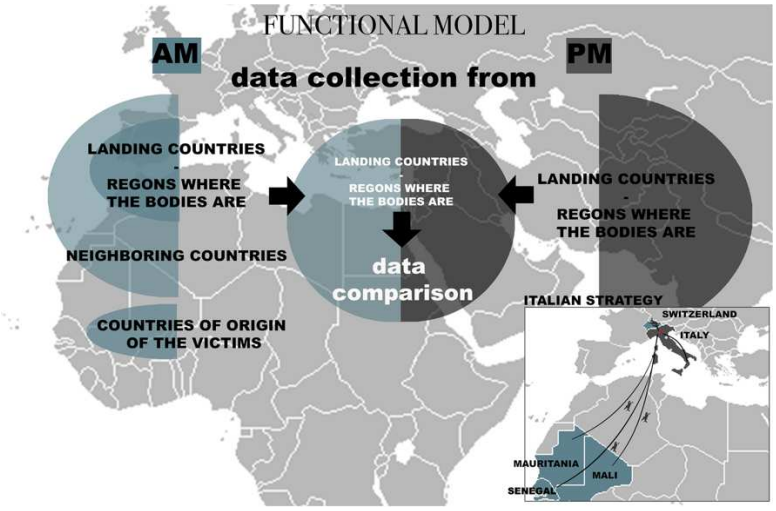

Figure 3. Functional data collection model derived from the recent Italian experience. In light blue, the countries where AM data have been collected; in dark grey, the countries where the PM data have been collected: the red dot indicates the collector of information where the comparison occurred (from Cattaneo et al., 2022)

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In Italy, to compensate for the extreme fragmentation and discretion in the approach to the problem of the recovery and identification of Mediterranean victims, caused by the aforementioned legal vacuum, and to guarantee the migrant population dignified treatment on a par with that reserved for the victims of other mass disasters of greater media resonance (conflicts enforced disappearances, natural disasters and terrorist attacks), a memorandum of understanding was created between the Office of the Extraordinary Commissioner for Missing Persons (UCPS) and the Laboratory of Anthropology and Forensic Odontology (LABANOF) of the University of Milan, aimed at the recovery and identification of the victims of the Mediterranean migratory tragedy[v] (Figure 4).

[v] Memorandum of Understanding. 30 September, 2014; Memorandum of Understanding. 6 March, 2015; Memorandum of Understanding. 23 July, 2015; Memorandum of Understanding, 31 March 2016

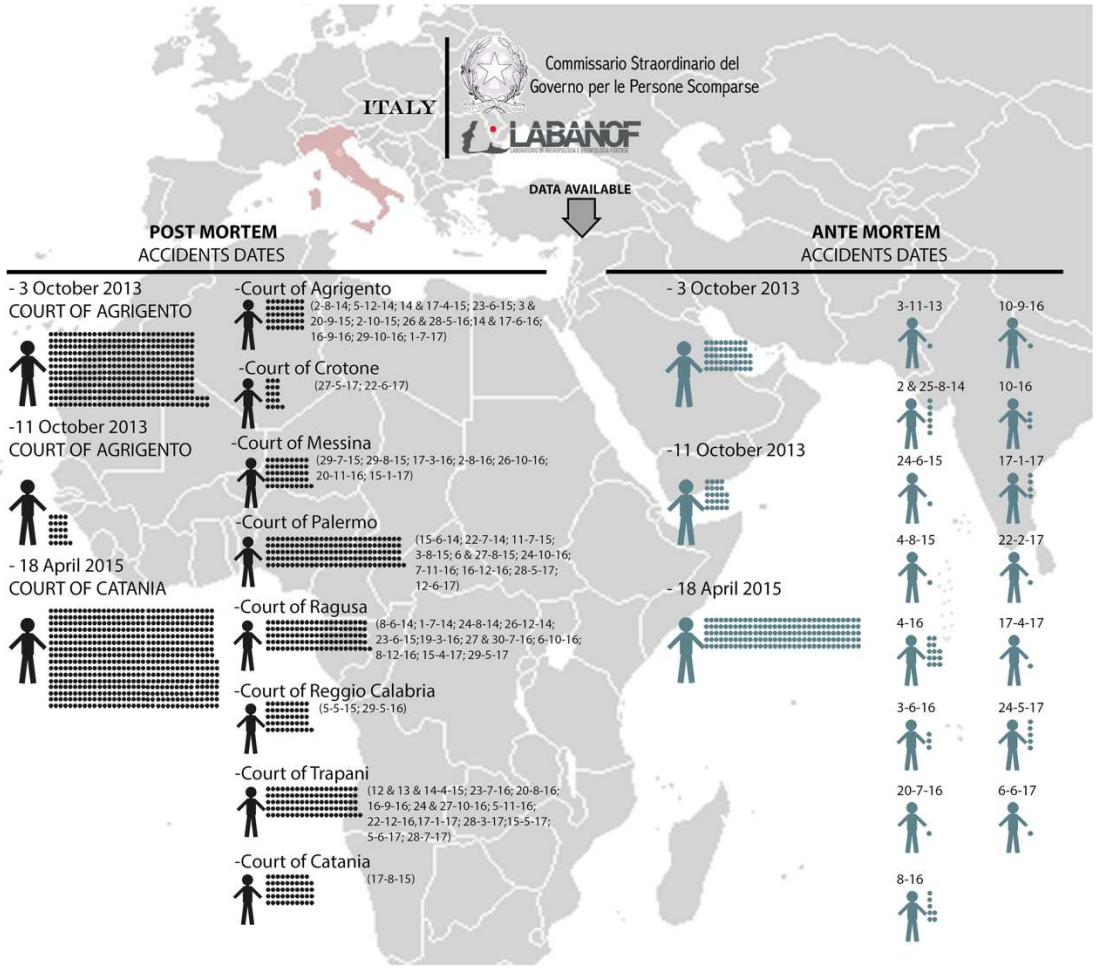

Figure 4. AM and PM data collection concerning unknown dead migrants involved in disasters/shipwrecks which occurred in Italian waters or who arrived in the domestic ports, carried out by the the UCPS Office. Each dot represents the data (anthropological and/or genetic) related to an individual, in black for the PM data and in light blue for the AM data. The dots are grouped by the court that handled the case for PM data and by disaster for AM data. In the left column of PM and AM data, the information concerning the three largest disasters (October 3, 11, 2013, and April 18, 2015) (from Cattaneo et al., 2022)

The 18.04.2015 shipwreck, together with those of 3 and 11 October 2013 in Lampedusa, was one of the three major disasters that received standardised post-mortem treatment involving, in addition to UCPS and LABANOF, several Italian universities (Catania, Messina, Palermo, Pavia, Parma, Ferrara, Milano Bicocca, Ancona, Bari, Torino) and law enforcement agencies. Casualty recovery operations, which began shortly after the tragedy, were conducted by the Italian authorities and the Italian Navy. The subsequent necropsy operations were carried out at the NATO base in Melilli (CT). Each corpse recovered was subjected to an autopsy, at the end of which post-mortem forms were drawn up containing a brief description of sex, age, height, personal characteristics, clothing and personal effects, as well as a photographic documentation of the bodies, with particular attention to faces and markings (tattoos, scars,

piercings and moles), when preserved. At the autopsy, samples were also taken for DNA analysis, as well as skeletal and dental samples useful for delineating a more accurate biological profile. All the PM data thus obtained were collected for comparison with the AM data provided by the families of the disappeared in order to arrive at a positive identification. We are currently proceeding with the anthropological/odontological study of the recovered remains and, in collaboration with Italian universities and the police, we are performing the DNA typing of the samples, in order to obtain the genetic profiles of the victims useful for comparison with those provided by the presumed family members.

At the same time, the genetic results will provide useful information for assessing the results of the methods used in the anthropological field for constructing the biological profile, making it possible to select the most reliable ones for the populations of interest. In the available literature, there is a lack of methods developed and/or evaluated on the African populations from which the migrant victims of shipwrecks come.

In this regard, although *ad hoc* morphological and metric methods have been developed for some African populations, such as in the case of South Africa (morphological methods: Krüger et al., 2015; L'Abbé et al., 2011; and metrics: Dayal et al., 2008; Franklin et al., 2005a, 2005b, 2006, 2008, 2010), few studies are currently available on the sub-Saharan area, despite the fact that the most conspicuous migratory flow observed towards Europe comes from this region. Precisely in order to increase the sub-Saharan representation among the reference populations used in software for estimating geographical origin, in recent years many projects have started to collect metric data related to this specific area (Jantz & Manthey, 2018; Navega et al., 2015).

Currently, in the absence of an adequate reference sample, the available methods for assessments on the migrant population of interest is being evaluated by comparing the anthropological results obtained with those that will be collected from the genetic analysis of the victims. The results of the previous preliminary studies, although relating to a small group of samples, have made it possible to identify, among those already available, the methods potentially most suitable (in terms of greater accuracy than metric methods) for the diagnosis of the sex of the remains under examination and, above all, the importance of collaboration between the various medico-legal disciplines also in the critical evaluation of methods applied to the study of skeletal material.

AIM OF THE THESIS

The need to identify the victims of the Mediterranean and, at the same time, to have accurate methods for constructing the biological profile of the victims motivated this thesis work, the aim of which was the identification of these unknown victims. Also, similarly, the purpose is to compare anthropological/odontological data, in particular those relating to the estimation of the geographical provenance and state of preservation of the remains, with those obtained from the genetic analyses carried out at the forensic genetics laboratories of the Universities of Brescia, Turin, Pavia and Eurofins Genome.

From an anthropological point of view, the possibility of demonstrating the reliability of anthropological and odontological methods would make it possible to limit the comparison to suspicious identities from a specific geographical area, and to focus the search for relatives in certain African countries. Furthermore, it would be possible to create a database in which different DNA profiles could be entered according to their geographical distribution of frequency, and the chances of successful genetic comparison would be increased. Furthermore, knowing any links between the state of preservation of the remains and the quality of the genetic material they contain would allow the identification of the portion of bone and/or the selection of the most suitable protocol for subsequent genetic investigations.

To this end, the remains of the 150 victims, previously subjected to genetic investigations at the forensic genetics laboratory of the Universities of Brescia, Turin, Pavia and Eurofins Genome, were analysed by applying morphological methods commonly used in anthropology to estimate geographical origin. The estimation results were compared with the genetic findings obtained to assess the reliability of the anthropological evaluation.

Furthermore, after highlighting the differences in terms of yield and recovery of genetic information suitable for comparison with the profiles of presumed relatives, the possible correlation between the quality of the results of the analysis of autosomal markers and the taphonomic condition of the cadaveric remains was investigated.

MATERIALS AND METHODS

Sample collection

The sample subject of this study consists of the remains of 150 individuals recovered in Mediterranean waters after the 18 April 2015 shipwreck.

The individuals were located as follows: 88 in the open sea, 62 on the vessel (22 in the hold, 27 below deck, 5 in the engine room, 3 in the forepeak and 5 in the afterpeak).

All 150 corpses were subjected to a medico-legal autopsy examination (between August 2015 and July 2016) at the end of which, for each of them, an anthropological report was compiled accompanied by detailed photographic records and a list of personal effects (objects, documents, clothing) was inventoried.

Each report comprised the following nine sections to be filled in with the available data: 1) state of preservation of the remains and their cataloguing; 2) dental record; 3) anatomical and anthropological diagnosis of sex; 4) estimate of geographical provenance; 5) estimate of chronological age (skeletal and dental age); 6) pathological conditions; 7) traumatic outcomes; 8) taphonomic findings; 9) highly individualising elements (markings such as tattoos, scars, ...).

None of the cadavers under analysis proved complete on preliminary inventory. In particular, in 65/150 cases only the postcranium was found; of the 85/150 cases in which the cephalic extremity was also recovered, in 23/85 the mandible was absent. Given the mixed nature of the remains, in 13/150 cases the assignment of skull (disarticulated)-postcranium and mandible-cranium was established through the objectification of the anatomical connection between the elements on external examination or through anthropological and odontological compatibility assessment.

In 37 cases, examination of the remains revealed signs attributable to previous traumatic and/or pathological events: in 20 cases, these were ante-mortem fractures (remodeled bony calluses and more recent fractures with signs of osteitis/osteomyelitis); in one case, the result of a previous amputation of the forefoot was found; 4 individuals also showed signs of age-related degenerative vertebral conditions (osteoarthritis, Schmörl nodules); in one case, there was a peri-mortem fracture of the right ulna.

As far as the detection of highly individualising elements useful for identification purposes is concerned: in 11 cases an inveterate scarring outcome were detected at the level of persistent dermal dermis shreds, mostly in the lower limbs, despite the advanced putrefactive stage; in 4 cases, monochrome tattoos were detected.

For each body, bone and dental elements useful for describing the biological profile (mandible and maxilla, where present, pubic symphysis, etc.) were taken and preserved, as well as the 'gloves' if available for possible dactyloscopic comparisons.

Lastly, a diaphyseal cross-section from a long bone was taken from all 150 PMs selected, with preference for those of the lower limbs (femur and, failing that, tibia, humerus, radius or clavicle), which was subjected to genetic investigations at the forensic genetics laboratory of the Universities of Brescia, Pavia, Turin and Eurofins Genome aimed at obtaining a genetic profile useful for identification purposes. On 49 samples of superior analytical quality, further investigations were carried out to estimating the biogeographical origin by analyzing both Y-chromosome polymorphisms, SNPs, AIM-Indel polymorphisms and PCA analysis.

Genetic analysis

Since the samples remained in the sea for a prolonged period at about 400 meters below sea level and as the state of conservation of the genetic material were unknown, different methods were used in the four above-mentioned genetics laboratories.

Brescia

The bone specimens (17 femurs and 13 tibias) were submitted to a powder-free protocol for DNA extraction, currently used in the Laboratory of University of Brescia. After an EDTA-based demineralization process (Loreille et al., 2007), superficial external slices of the bones were sampled with a scalpel and submitted to DNA extraction using QIAamp® DNA Investigator Kit (Qiagen, Germany) following the tissues protocol.

DNA quantitation was performed on a StepOne qPCR platform according to the protocol developed by Correa et al., 2020.

The amplification was carried out in a GeneAmp® PCR System 9700 Gold Plate (Applied Biosystems). Autosomal DNA amplification was performed using Identifiler™ Plus, NGM SElect™ (Applied Biosystems), PowerPlex® ESX 17, PowerPlex® Fusion 6C System (Promega) and Investigator® 24plex QS (Qiagen, Germany) kits, while Y haplotype

genotyping was made with the PowerPlex® Y23 (Promega, USA) and Y Filer™ Plus (Applied Biosystems) kits.
The amplified DNA fragments were detected on a 3500 Genetic Analyzer (Applied Biosystems) and the electropherograms were interpreted using the software GeneMapper™ ID-X v3.2.1 software (Applied Biosystems).
Amplification and fragment analysis were carried out according to the instructions of the manufacturers.

Pavia

Bone samples composed of 58 femoral, 21 tibial diaphyses and one clavicle were analysed. Each specimen was cleaned and decontaminated with bleach (2%) before removing bone powder by drilling. Three different DNA extraction kit were used: Promega Bone DNA Extraction kit followed by automated extraction with DNA IQ casework PRO kit for Maxwell 16 (Promega, USA), Prepfiler BTA Forensic DNA Extraction kit (ThermoFisher, USA), QIAmp DNA Blood Maxi kit Qiagen (Qiagen, Germany) with a pre-decalcification step with EDTA 0.5 M before extraction.
Quantification was realized using the Quantifiler Duo DNA Quantification kit (ThermoFisher, USA) on a 7500 Real Time PCR System (ThermoFisher, USA).
Three amplification kits were used to characterize 21 autosomal STR loci: PowerPlex ESX 17 Fast System (Promega, USA), AmpFISTR Identifiler Plus (ThermoFisher, USA) and PowerPlex ESI 17 Fast System (Promega, USA).
DNA eletrophoretic separation was performed on a 310 sequencer (Applied Biosystems) and the genotypes were interpreted using the software GeneMapper™ ID-X v3.2.1 software (Applied Biosystems). Amplification and fragment analysis were carried out according to the instructions of the manufacturers.

Turin

Two methods of DNA extraction were applied for the 30 bone samples (14 tibias, 13 femurs, two humerus and one radius): Prepfiler BTA Forensic DNA Extraction kit (ThermoFisher, USA) and DNA IQ casework PRO kit for Maxwell 16 (Promega, USA).
Quantification results from extracted DNA showed a significantly higher yield difference ($p < 0.001$) for BTA compared to B-IQ. The recurrence of increased co-isolation of the inhibitors with B-IQ (by evaluating the threshold cycles of the internal positive control) was ruled out,

which can determine an underestimation of DNA concentrations obtained by real-time PCR quantification such as to justify the difference in DNA yield observed between the two extraction systems.
Autosomal DNA amplification was performed using PowerPlex® ESX 17 and ESI 17 (Promega) kits, while Y haplotype genotyping was made with the PowerPlex® Y23 (Promega) kit. Amplification and fragment analysis were carried out according to the instructions of the manufacturers.

Eurofins Genoma

Ten bone samples composed of 9 femurs and one tibia were subjected to DNA typing. The bone marrow was sampled and submitted to DNA extraction using QIAamp® DNA Investigator Kit (Qiagen, Germany) following the tissues protocol.
Quantification results from extracted DNA were analysed though Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems).
Autosomal DNA amplification was performed using GlobalFiler™ PCR Amplification Kit (Applied Biosystems). Amplification and fragment analysis were carried out according to the instructions of the manufacturers.

Biogeographical origin estimation

The best quality of results was then subjected to further analyses to define biogeographical origin. At the University of Brescia, autosomal Y-chromosome markers were entered into the Yhrd database. Yhrd is an online database in which there are currently 350,500 profiles with the minimum haplotype. The resulted Y-chromosome profiles were evaluated according to the matching metapopulations available for comparison within the database.
Then, Y-chromosome SNPs typing SNaPshot™ Multiplex Kit (Applied Biosystems, USA) were used. In particular, samples were analysed for 18 SNPs (M170, M172, M35, M9, M45, M173, M89, M267, M282, M304, M214, M52, M201, M96, M181, M174, M91, M216), belonging to the non-recombinant region of Y chromosome, through two multiplexes, arbitrarily called MY1 and MY2, containing 10 and 8 markers respectively (Cortellini et al., 2013). Studying the geographical distribution of each of these lines allows to consider human evolution from a phylogeographical perspective, through the identification of haplogroups and sub-haplogroups, the definition of their geographical distribution and the quantification of their internal variation. In that way, important information on ancient and recent migratory

processes and on demographic events that, having left traces in the genetic structure of modern populations, can be provided.

Also, multivariate techniques were employed to interpret the autosomal STRs profile and provide an estimation of the biogeographical ancestry (BGA) information of the unknown individuals. Specifically, the BGA predictor software developed in the research paper by Alladio et al., 2020 was used performing Principal Component Analysis (PCA).

Meanwhile, at the University of Turin the analysis of 46 AIM-Indel polymorphisms was conducted. The analysis was conducted by calculating the frequency of the observed genotype combinations (expressed as a negative logarithm in the table: -loglik) given the allele frequencies of 3 reference populations (derived from HGDP) representative of 3 well-defined continental/subcontinental areas (Africa, Europe, East Asia); each sample was typed in duplicate and only genotypes repeated in the two PCR replicates were considered.

Taphonomic analysis of the remains

The macroscopic assessment of the state of preservation of the remains was conducted through the scoring system developed by De Donno et al. which includes nine categories (score I-IX) ranging from 'fresh corpse' to partial/complete skeletonisation with final disarticulation (Figure 5).

To this end, the photographic images acquired during the autopsy procedures were analysed, with particular focus on the portion of the lower/upper limbs from which samples were taken for genetic analysis.

For the subsequent comparison of results, the scoring system defined by De Donno et al. (2014) was dichotomised into two categories: complete skeletonisation (score IX) or soft tissue persistence (score below score IX).

Score	
1	Head/neck: no visible or relevant changes. Trunk: no visible or relevant changes. Limbs: no visible or relevant changes.
2	Head/neck: slight pink discoloration, darkened lips, goose pimpling. Trunk: slight pink discoloration, goose pimpling. Limbs: mild wrinkling of skin on hands and/or feet, possible goose pimpling.
3	Head/neck: reddening of face/neck. Marbling visible on face/neck. Possible early signs of animal activity/predation concentrated on the nose, ears, and lips. Trunk: yellow/green discoloration of the abdomen and upper chest. Marbling. Early decomposition/autolysis of internal organs. Limbs: skin on palms of hands and/or soles of feet becoming white, wrinkled, and thickened. Slight pink discoloration of arms and legs.
4	Head/neck: bloating of the face. Green/black discoloration. Skin beginning to slough off. Trunk: dark/green discoloration of abdomen, mild abdominal bloating. Initial skin slippage. Limbs: skin on palms of hands and/or soles of feet becoming soggy and loose. Marbling of the limbs predominantly on upper arms and legs.
5	Head/neck: head hair beginning to slough off mostly at the front. Brain softening and becoming liquefied. Tissue becoming exposed on face and neck. Green/black discoloration. Trunk: green/purple discoloration, extensive abdominal bloating, tense to touch, swollen scrotum in males, exposure of underlying fat and tissues. Limbs: skin on hands/feet starting to slough off. Yellow/green to green/black discoloration on arms and/or legs. Initial skin slippage on arms and/or legs.
6	Head/neck: bone becoming exposed, concentrated over the orbital, frontal, and parietal regions. Some on the mandible and maxilla. Early adipocere formation. Trunk: black discoloration, bloating becoming softer, initial exposure of internal organs and bones. Limbs: degloving of hands and/or feet – exposing large areas of underlying muscles and tendons. Patchy sloughing of skin on arms and/or legs.
7	Head/neck: more extensive skeletonization on the cranium. Disarticulation of the mandible. Trunk: further loss of tissues and organs, more bone exposed, initial adipocere formation. Limbs: exposure of bones of hands and/or feet. Muscle, tendons, and small areas of bone exposed in lower and/or legs.
8	Head/neck: complete disarticulation of the skull from torso. Extensive adipocere formation. Trunk: complete skeletonization and disarticulation. Limbs: bones of hands and/or feet beginning to disarticulate. Bones of upper arms and/or legs becoming exposed.
9	Limbs: complete skeletonization and disarticulation of limbs.

Figure 5. Descriptive phases of decomposition of corpses in an aquatic environment observed in the head/neck, trunk and limbs with associated scoring from 1 to 9 (from De Donno et al., 2014)

Taphonomic analysis of the remains and correlation to the genetic findings

In order to verify whether the observed difference in DNA yield of the different samples analysed, under the same environmental conditions of sampling (within the wreckage, at the same depth), could be attributable to exogenous factors, the following variables were compared using a statistical test (t-score and chi-square):

- the time elapsed between the time of the shipwreck (18.04.2015) and the time of the autopsy, in terms of days (< 200 days / > 200 days);
- macroscopic taphonomic conditions: presence of soft tissue, score VII/VIII, vs. skeletonization, score IX;
- the number of garments covering the anatomical region from which the bone sample was collected: no clothes, one, two, three or four layers of clothes garments;
- the anatomical region from which the bone dowel intended for extraction was taken: femurs, tibiae and other;
- place of recovery of the bodies: open sea or sequestered environment.

Anthropological methods for estimating ancestry

Anthropological analyses were carried out aimed at estimating ancestral origins based on specific morphological traits. For this purpose, the following were evaluated:

o the cranial morphology of the samples of interest, through the direct evaluation of portions of the skull (inspection and by profilometer) and, if not available, the photographic images of the skulls acquired during the autoptic procedures. Specifically, for the estimation of biological ancestry, the eleven cranial *morphoscopic traits* described by Hefner in 2009 (Table 1) were taken into account, each of which was assigned a score in relation to the different degree of morphological expression (Hefner, 2009);

1	Anterior Nasal Spine (ANS) – from 1 to 3
2	Inferior Nasal Aperture (INA) – from 1 to 5
3	Interorbital Breadth (IOB) – from 1 to 3
4	Malar Tubercle (MT) – from 0 to 3
5	Nasal Aperture Windth (NAW) – from 1 to 3
6	Nasal Bone Contour (NBC) – from 0 to 4
7	Nasal Overgrowth (NO) – from 0 to 1
8	Postbregmatic Depression (PBD) – from 0 to 1
9	Supranasal Suture (SPS) – from 0 to 2
10	Transverse Palatine Suture Shape (TPS) – from 1 to 4
11	Zygomaticomaxillary Suture (ZS) – from 0 to 3

Table 1. The cranial traits for ancestry estimation (from Hefner, 2009)

o the recurrence of the 42 non-metric dental characters (CNMd) described by Turner et al. (1991) and by Scott and Irish (2017) and 3 further, namely crenulation of the molars (Pilloud 2018), *dens inaginatus* (Hallett 1953) and *foramen molar*, through the direct evaluation of the morphology of crowns and roots of the dental elements of specific interest. Each trait detected in the sample of interest was then assigned a score based on the extent of morphological manifestation of the CNMd.

The analysis of the different degree of morphological expression detected of the traits considered was carried out by applying statistical and software methods (OSSA score and hefneR software for cranial morphology; rASUDAS software for CNMd) described below.

Morphoscopic characters of the cranium

The analysis of these sections was carried out by applying two different statistical methods:

I. The OSSA method (Hefner and Ousley, 2014)

The OSSA (*Optimized Summed Scored Attributes*) method involves the analysis of only 6 (ANS, INA, IOB, NAW, NBC and PBD) of the 11 morphoscopic traits described by Hefner.

In the present study, the NBC trait was also excluded from the analysis as visual interpretation of the nasal contour from photographic images is not the most effective way of analysis due to the high intra/inter-observer error. The process foresees that the score attributed to each of the six characters of interest according to Hefner classification (2009) is subsequently converted into a binary code (0.1), where the value "0" is assigned to the most frequent scores in the *American Black* population and the value "1" to those of the *American White population*.

Once all six traits have been converted to their new binary variable, the sum of the *scores* obtained from all the traits considered is calculated, resulting in an overall OSSA *score* between 0 and 6: values equal to or less than a score 3 are associated with a greater probability of belonging to the *American Black population,* while values equal to or greater than 4 are more likely to be associated with the *American White* population, with an accuracy of more than 80%.

II. The hefnerR software (Hefner, 2009)

The hefneR Software, available free of charge at the web address https://osteomics.com/hefneR/, considers, instead, all the eleven morphoscopic characters described above. Also, in this case the NBC section was excluded. After each of the 10 characters considered has been assigned a score according to Hefner (2009) morphological classification, the system analyzes them using a Bayesian algorithm returning a probability of belonging to each of the four population groups considered: Africans, American Indians, Asians and Europeans. Below is an example of analysis.

Both applied methods were selected considering that the reference sample included populations of our interest (African and Asian populations, according to IOM 2020 data).

Non-metric characters of teeth with rASUDAS software

The analysis of the ancestry with regard to non-metric dental characters was conducted using the rASUDAS software: it is a free application, developed by David Navega and João Coelho, and available at the link http://apps.osteomics.com/rASUDAS/ .

It takes into account 21 of the non-metric dental characters, described by Turner et al. (1991) and Scott and Irish (2017), 15 of the crown and 6 radicular, grouping the scores into discrete classes based on the extent of morphological manifestation of the trait of interest they represent (Figure 6).

Trait and Tooth	Rank	American Arctic & NE Siberia	Australo-Melanesia & Micronesia	East Asia	American Indian	Southeast Asia & Polynesia	Sub-Saharan Africa	Western Eurasia
Winging UI1	0	0.773	0.860	0.746	0.500	0.773	0.967	0.938
	1+	0.227	0.140	0.254	0.500	0.227	0.033	0.062
Shoveling UI1	0–1	0.027	0.370	0.026	0.005	0.336	0.443	0.817
	2–3	0.811	0.606	0.654	0.542	0.589	0.558	0.181
	4+	0.162	0.024	0.319	0.453	0.074	0.000	0.002
Interruption	0	0.376	0.804	0.587	0.490	0.703	0.916	0.629
grooves UI2	1+	0.624	0.196	0.413	0.510	0.297	0.084	0.371
Hypocone UM2	0–1	0.289	0.059	0.097	0.115	0.018	0.086	0.253
	2–3	0.491	0.210	0.320	0.417	0.200	0.167	0.255
	4+	0.220	0.731	0.583	0.468	0.692	0.747	0.492
Carabelli's trait	0–1	0.845	0.606	0.690	0.620	0.647	0.450	0.450
UM1	2–4	0.134	0.213	0.165	0.325	0.168	0.416	0.288
	5+	0.021	0.182	0.145	0.055	0.185	0.134	0.262
Cusp 5 UM1	0	0.824	0.415	0.809	0.833	0.705	0.725	0.853
	1+	0.176	0.585	0.191	0.167	0.295	0.275	0.147
Enamel extensions	0–1	0.569	0.932	0.585	0.563	0.735	0.993	0.978
UM1	2–3	0.431	0.068	0.415	0.437	0.265	0.007	0.022
Multiple lingual	0–1	0.604	0.253	0.300	0.602	0.191	0.333	0.371
cusps LP2	2–3	0.396	0.747	0.700	0.398	0.809	0.667	0.629
Groove pattern	X and +	0.721	0.666	0.750	0.759	0.703	0.540	0.735
LM2	Y	0.279	0.334	0.250	0.241	0.297	0.460	0.265
4-cusped LM2	5	0.943	0.647	0.697	0.914	0.679	0.744	0.254
	4	0.057	0.363	0.303	0.086	0.321	0.256	0.746
Cusp 6 LM1	0	0.525	0.586	0.633	0.449	0.521	0.890	0.935
	1+	0.475	0.414	0.367	0.551	0.479	0.110	0.065
Cusp 7 LM1	0	0.962	0.931	0.945	0.939	0.945	0.674	0.956
	1+	0.038	0.069	0.055	0.061	0.055	0.326	0.044
Protostylid LM1	0	0.815	0.928	0.758	0.621	0.843	0.891	0.901
	1	0.169	0.061	0.137	0.321	0.231	0.100	0.091
	2+	0.016	0.011	0.106	0.060	0.012	0.009	0.008
Deflecting wrinkle	0–2	0.426	0.737	0.637	0.335	0.641	0.950	0.871
LM1	3	0.574	0.263	0.363	0.665	0.359	0.050	0.129
UP1 root number	1	0.942	0.612	0.744	0.857	0.644	0.359	0.501
	2–3	0.058	0.388	0.256	0.143	0.356	0.641	0.499
UM2 root number	1–2	0.624	0.301	0.355	0.441	0.386	0.188	0.391
	3	0.376	0.699	0.645	0.559	0.614	0.812	0.609
LC root number	1	1.000	0.999	0.988	0.993	0.993	0.991	0.939
	2	0.000	0.001	0.012	0.007	0.007	0.001	0.061
Tomes root LP1	1–3	0.985	0.815	0.842	0.801	0.809	0.823	0.885
	4+	0.015	0.185	0.158	0.199	0.191	0.177	0.115
3-rooted LM1	1–2	0.773	0.967	0.803	0.934	0.899	0.963	0.995
	3	0.227	0.033	0.197	0.066	0.101	0.037	0.005
LM2 root number	2	0.686	0.871	0.700	0.672	0.722	0.943	0.752
	1	0.314	0.129	0.300	0.328	0.278	0.057	0.248
Pegged-reduced-missing UM3	0	0.786	0.936	0.641	0.842	0.792	0.950	0.835
	1	0.232	0.064	0.359	0.158	0.208	0.050	0.165

Figure 6. Frequency of the 21 rASUDAS sections in the seven reference groups.

After assigning each trait a value based on the presence or absence and/or degree of morphological manifestation of the character and selecting the biogeographical reference ancestor clusters, the scores are analysed.

The fact that there is a 'Missing/Unobservable' option for each section allows the application to be used even in cases where some of the teeth of interest are completely absent or lack coronal components and/or highly informative radiation for estimation.

In the present study, only 5 (Western Eurasia, Sub-Saharan Africa, South-East Asia and Polynesia, Arctic American and North-East Asian populations, and East Asia) of the 7 clusters of the reference population groups were selected for analytical purposes, excluding those from which, according to data reported by the IOM in 2020, the migrants who died in the Mediterranean Sea did not allegedly originate.

At the end of data processing, a table called “Predicted Biogeographical Origin” is returned, showing the a posteriori probability of the unknown individual belonging to the different groups studied. This is followed by a second table that gives the significance of the individual traits used in the analysis to estimate the ancestry produced.

Estimation of the ancestry for each anthropological method used

In the present study, for the purpose of attributing the geographical origin, in each anthropological method applied (OSSA score, hefneR software and rASUDAS software) the group with the highest probability was considered in the event that this presented percentage values above 75%; if, on the other hand, the group with the highest probability of belonging does not reach the *cut off* of 75%, all population groups with probabilities greater than 10% have been taken into account in the attribution.

Comparison of genetic findings and anthropological methods

The anthropological results were then compared with the genetic ones. For the estimation of ancestry, cross comparisons were made between the different morphological methods (cranial and dental: OSSA vs hefneR, OSSA vs rASUDAS, hefneR vs rASUDAS) and the results of genetic analyses.

RESULTS

Genetic analysis

Brescia

Genetics results showed a low amount of DNA and high degradation index in all samples. However, the use of a combination of commercial kits allowed good results to be obtained for each sample. In particular, the amelogenin sex test made it possible to determine that all the bones belonged to males; a minimum of 17 and a maximum of 23 autosomal STR loci were successfully typed, while Y haplotyping resulted in a minimum of 23 and a maximum of 29 successfully typed STR loci.

Pavia

After amplification with the PowerPlex ESX 17 Fast System and AmpFISTR Identifiler Plus kits, 20 DNA samples provided genetic profiles with a number of autosomal STR loci ≥ 16 confirmed in different amplifications while the remaining DNAs showed degraded profiles with a variable number of STR markers below 16. These latter 60 bone were then re-extracted using the Prepfiler BTA forensic DNA extraction kit. In 36 samples 16 or more autosomal STRs were found, in 14 a number of markers between 10 and 15 and in 10 samples a number STR of loci below 10. In the 10 challenging cases, bone powder was extracted with QIAmp DNA Blood Maxi kit columns following a 3-days pre-decalcification step with 0.5 M EDTA (pH 8). Only one sample provided a STR profile of 20 autosomal STRs, three other samples delivered genetic profiles characterized by 12-15 STRs, while the remaining specimens yielded no results or low-quality profiles with a number of markers ≤ 8.

Turin

Based on the results of the quantification assays, samples extracted with the BTA protocol were used for genetic identification. The samples were then initially amplified with the PowerPlex ESX 17 Fast kit (Promega) resulting in extreme variability in the analytical quality of the results, with the co-presence of samples with complete genotypes and negligible risk of

stochastic events. 19 samples (63.3%) provided optimal analytical result, while others were completely unsuitable for comparison (11 samples, 36.7%). These latter were samples with critical analytical result, bearing a high percentage of loci below the analytical and/or stochastic threshold.

Eurofins Genoma

After amplification with the kit, 8 DNA samples provided genetic profiles with a number of autosomal STR loci ≥ 16 confirmed in different amplifications, while the two remaining DNAs showed degraded profiles with a variable number of STR markers below 16.

Biogeographical origin estimation

Brescia

All 30 samples with established STR genotypes were further typed for Y-STR and SNPs.
The typing of the resulting Y-STR polymorphisms did not reveal any overlapping genotypic inheritance, indicating that, at least in the male line, the tested subjects do not have a recent common ancestor.
The Y-chromosome haplotypes obtained were analysed using the Yhrd database (yhrd.org) and considering different metapopulations, resulting in 7 cases (23.3%) originating in Africa. In other 3 cases (10%), Afro-Asian metapopulation was found as well as for Eurasian in 3 more cases (10%). In almost half of all cases (46.7% - 14 cases), no metapopulation was matched.
According to the nomenclature defined by the *SNP Consortium (YCC)*, the analysis with 18 SNPs, showed two main different haplogroups, the most represented being E1b1b1 (11 cases, 36.7%) and E (16 cases, 53.3 %). In three cases (10%), the haplogroups were K, J1 and G. According to the haplogroup frequency distribution, the populations can be grouped into the main clade of Africans (Haplogroups E1b1b1 and E). Haplogroup K belongs to populations originating from Southwestern Asia, haplogroup J1 to the populations from Mediterranean area and Middle East, and haplogroup G to Eurasian populations (Figure 7).

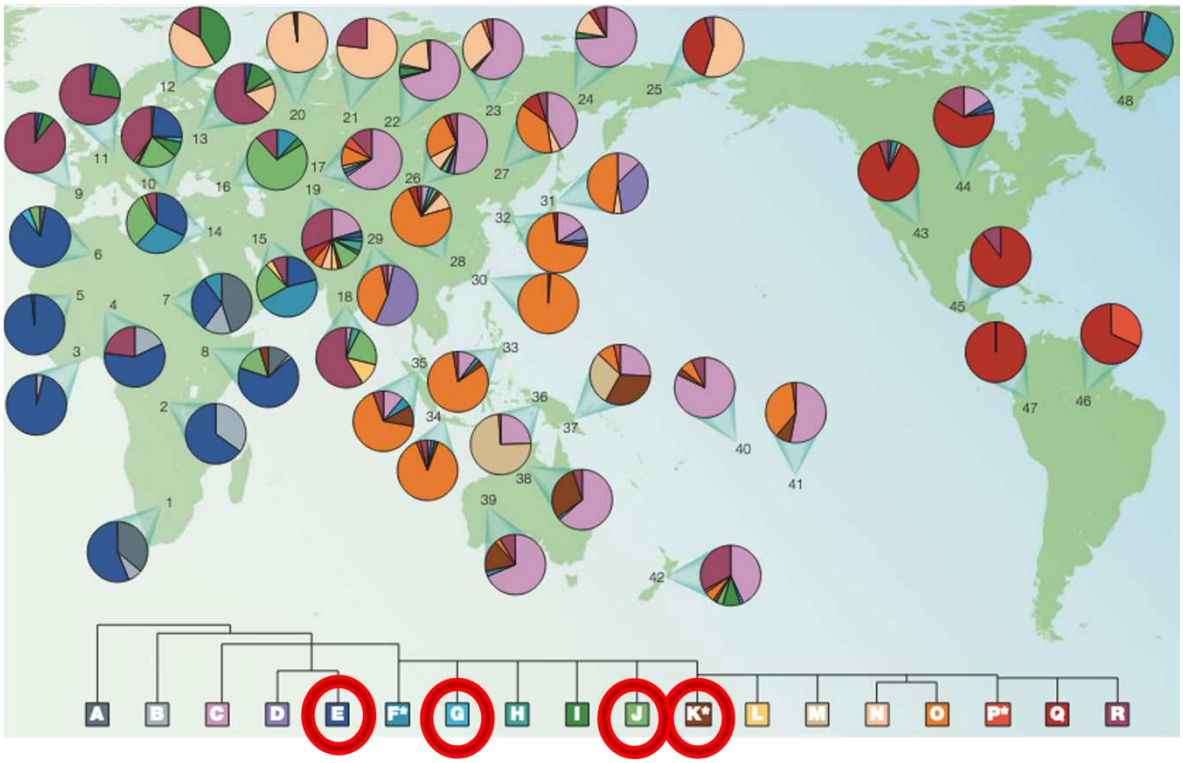

Figure 7. Worldwide distribution of the haplogroups described by Jobling and Tyler-Smith, 2003.

The PCA analysis (Figure 8) using the NIST dataset shows an efficient clustering of the three reference populations. The 30 samples were tested for STRs resulting in 27 cases in the Afr_Am cluster. All these samples showed the most probable population is the African-American one, in the vast majority of cases with strongly indicative LR values $\geq 10^5$. The second most probable population was Caucasian. Two samples provided Caucasian as the most probable population and in one case the population was Asian.

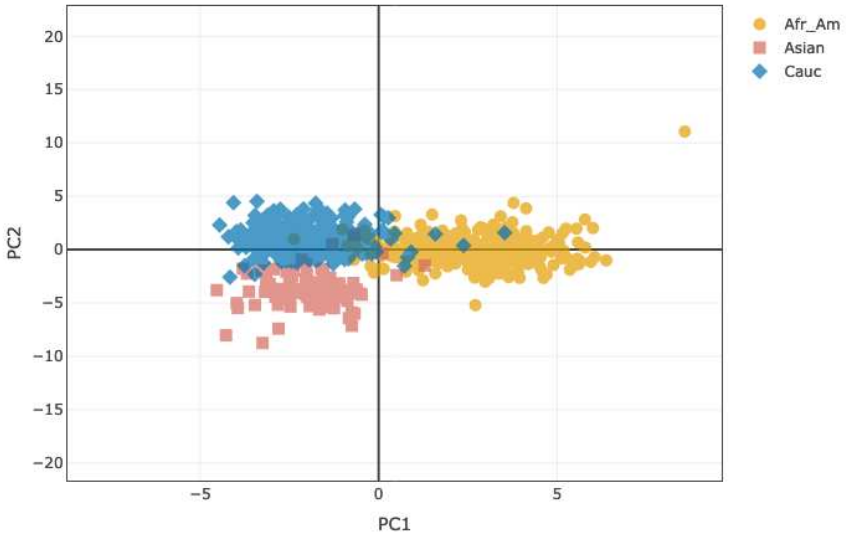

Figure 8. PCA, representation of the distribution of the samples analyzed among the three populations (African_American in orange, Caucasian in blue, and Asian in pink), as described by Alladio et al., 2020.

Turin

A subset of 19 samples of higher analytical quality, i.e. those with at least 10 loci out of the 16 tested with established aSTR genotypes, were further typed for Y-STR and AIM-Indel polymorphisms in order to determine their biogeographical origin.

The typing of the Y-STR polymorphisms of the aforementioned 19 samples did not reveal any overlapping genotypic inheritance, indicating that, at least in the male line, the tested subjects do not have a recent common ancestor.

The Y-chromosome haplotypes obtained were analysed using the Yhrd database (yhrd.org) and considering different metapopulations, resulting in almost 70% of cases (13/19, 68.4%) originating in Africa. In two cases (10.4%) a mix of populations were found. The analyses showed as metapopulations Europeans, Eurasian and Afro-Asian, one case each (5.3%). In one case (5.2%), the database did not provide any result.

The 19 samples with higher analytical quality were further typed for the 46 AIM-InDel polymorphisms (Pereira et al., 2012). All samples showed the most probable ancestral origin in Africa, in the vast majority of cases with strongly indicative LR values (ratio of -loglik most probable population to -loglik second most probable population, which was always European) $\geq 10^6$ [vi]. Lower LR values are observed only for samples 11, 12 and especially 22. The tendency of samples to cluster with African reference samples (orange), compared to Europe (blue) and East Asia (pink), is also evident with the analysis of the main components (PCA) carried out with the Snipper app suite (Figure 9).

[vi] based on the validation carried out by Pereira et al. (2012), African subjects, when compared with Africa - Europe - East Asia reference databases, are all (100%) correctly recognized as African. This attribution is therefore highly reliable even in the presence of not very high LR.

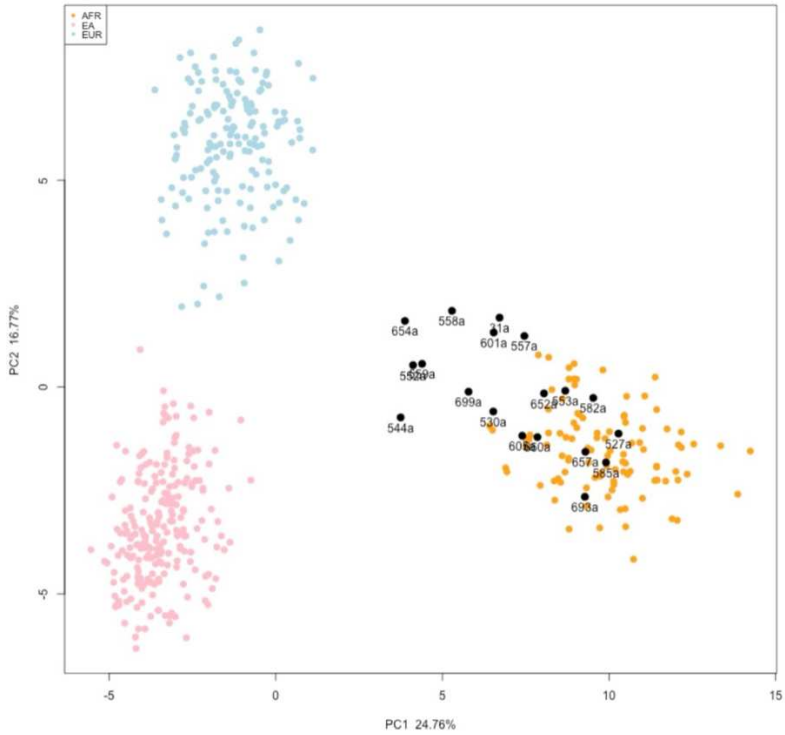

Figure 9. PCA, representation of the distribution of the 19 samples analyzed among the 3 populations (AFRICA in orange, EUROPE in blue, and EST-ASIA in pink).

An analysis of the 19 selected samples was also carried out with the STRUCTURE software, of which a representative graph of the analysis replications conducted is shown below. The 19 unknown samples correspond to the bars of each histogram and are compared, internally, with three different ancestral genetic components: red (Africans); blue (Europeans); green (East Asians). The samples constantly have an exclusive ancestral component or in any case strongly prevalent red (i.e., it is African), with the exclusion of three samples (the first, eighth and fifteenth of the series) that show a strong mixture of European type (blue) (Figure 10). These samples correspond to individuals 1 (32% European component), 11 (38% European component) and 22 (48% European component). Individual 12, who had provided a relatively low LR value for the prediction of African origin, shows in STRUCTURE a completely African ancestral origin.

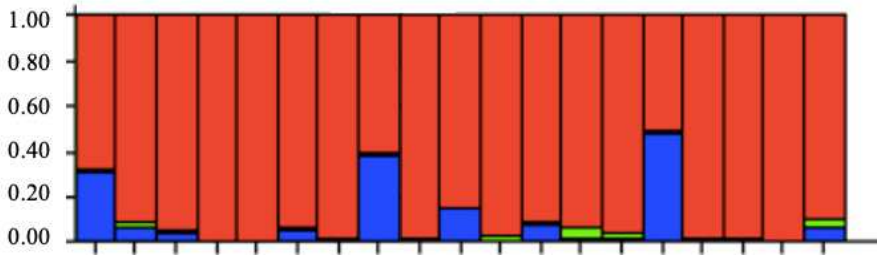

Figure 10: Graphic illustration of the STRUCTURE analysis in the 30 cases of University of Turin.

The calculation of –loglik for AIM-Indel genotypes was, therefore, traced using as a reference database only populations within the African continent: the sample of sub-Saharan Africans HGDP previously used (AFR), one from the Horn of Africa (ETH, Tigray population of Ethiopia described in Kumar et al., 2020) and one from North Africa (ALG, Algerian HGDP). Of the 19 samples analyzed, 16 show more likely ancestral origin in Sub-Saharan Africa. Exceptions are samples 1, 11 and 22 which show a more likely ancestral origin in the Horn of Africa, with an LR between 10^1 and 10^3 (in line with the previous structure analysis). According to preliminary validation of panel 46 AIM-Indel (Kumar et al. 2020), in a "three-way" classification (Africa – Sub-Saharan / Horn of Africa / Middle East including North Africa) no subject not from sub-Saharan Africa was incorrectly classified as sub-Saharan African when attribution to sub-Saharan Africa had LR $\geq 10^1$, as in this case. Therefore, the allocation to sub-Saharan Africa of 16 of the 19 samples examined can be said to be highly reliable. As for the three samples classified as belonging to the Horn of Africa, it must be considered that assignment errors are possible. In the previously cited study, for LRs between 10^1 and 10^2 (as in the case of sample 31), 2% of sub-Saharan African subjects were incorrectly classified as belonging to the Horn of Africa and 20% of Middle Eastern subjects were classified as belonging to the Horn of Africa. For LRs between 10^2 and 10^3 (as in the case of samples 11 and 22), 0.4% of sub-Saharan African subjects were incorrectly classified as belonging to the Horn of Africa and 14% of Middle Eastern subjects were classified as belonging to the Horn of Africa. Discrimination between North Africa and the Horn of Africa therefore appears difficult because of the lack of information of 46 AIM-Indels at this level of sub-classification.

The PCA analysis (figure 11) shows a less efficient clustering of the three reference populations, with substantial overlap of the Algerians (Mediterranean Africa, in pink) to the Tigray population (Horn of Africa, in blue) and a sort of continuity between the Horn of Africa group and sub-Saharan Africa (orange). Samples 1, 11 and, in particular, 22 (identified by numbers 31, 558 and 654, respectively, in the figure) are the innermost in the Horn of Africa cluster.

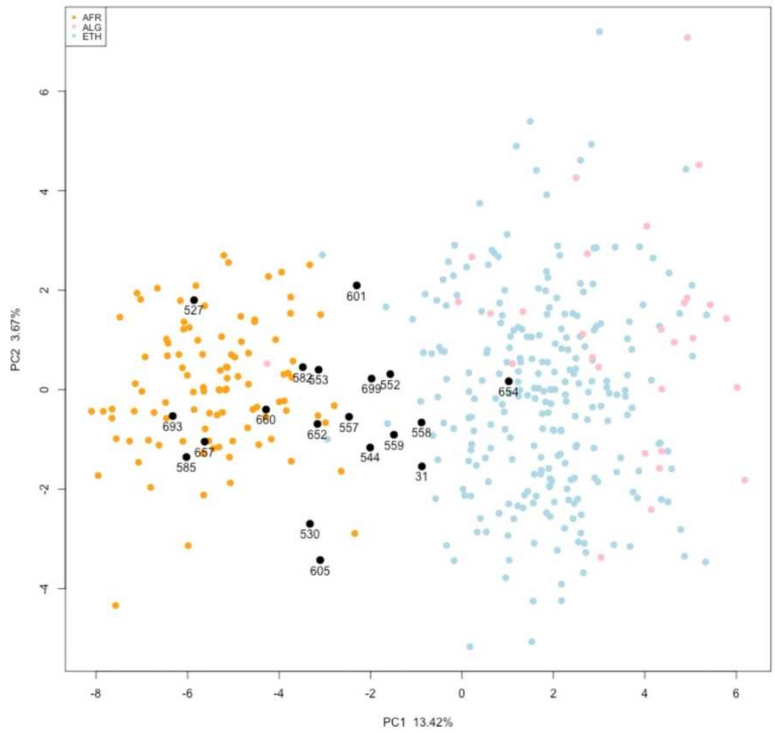

Figure 11. PCA, representation of the distribution of the 19 samples analyzed among the 3 populations (Sub-Saharan Africa in orange, Algerians in pink, Horn of Africa in blue).

The following table summarizes the results of the genetic analysis aimed at estimating the biogeographical origin (Table 2).

N. of case	***Y-STRs***	***InDels***	***PCA***
1	African	African	Horn of Africa
3	African	African	Sub-Saharan Africa
4	African	African	Sub-Saharan Africa
5	ND	African	Sub-Saharan Africa
6	African	African	Sub-Saharan Africa
7	African	African	Sub-Saharan Africa
10	African	African	Sub-Saharan Africa
11	Afro-Asian	African	Horn of Africa
12	African	African	Sub-Saharan Africa
13	Mixed Population	African	Sub-Saharan Africa
14	African	African	Sub-Saharan Africa
19	African	African	Sub-Saharan Africa
20	African	African	Sub-Saharan Africa
21	African	African	Sub-Saharan Africa
22	Eurasian	African	Horn of Africa
25	African	African	Sub-Saharan Africa
26	Mixed Population	African	Sub-Saharan Africa

27	European	African	Sub-Saharan Africa
29	African	African	Sub-Saharan Africa
		SNPs	
31	Afro-Asian	E1b1b1	Afr_Am
32	African	E	Afr_Am
33	Mixed Population	E1b1b1	Afr_Am
34	ND	E1b1b1	Afr_Am
35	African	E	Afr_Am
36	African	E	Afr_Am
37	ND	E1b1b1	Afr_Am
38	ND	E	Afr_Am
39	African	E	Afr_Am
40	ND	E	Afr_Am
41	ND	E1b1b1	Afr_Am
42	ND	E1b1b1	Afr_Am
43	African	E	Afr_Am
44	African	E	Afr_Am
45	ND	E	Afr_Am
46	ND	E1b1b1	Afr_Am
47	ND	E	Afr_Am
48	Mixed population	E1b1b1	Afr_Am
49	ND	E	Afr_Am
50	Eurasian	E	Afr_Am
51	Mixed population	J1	Asian
52	Eurasian	E	Afr_Am
53	ND	E	Afr_Am
54	ND	K basal	Cauc
55	ND	E	Afr_Am
56	ND	E1b1b1	Afr_Am
57	African	E	Afr_Am
58	Afro-Asian	E1b1b1	Afr_Am
59	Eurasian	E1b1b1	Afr_Am
60	Afro-Asian	G	Cauc

Table 2: Understanding of genetic findings from the 49 cases.

Taphonomic analysis of the remains

The following table (Table 3) summarises the characteristics of the 150 individuals in relation to the number of days between shipwreck and autopsy date, place of recovery, bone taken for genetic analysis, state of decomposition of macro remains (TADS scoring according to De Donno et al. 2014) and number of clothes to cover the bone segment subjected to genetic extraction.

N. of case	Day of autopsy	Place of recovery	Days between the shipwreck and the autopsy	TADS	Collected bone	Number of clothes covering the bone sample
31	03-jul-15	Sea	76	VI	Femur	4
61	03-jul-15	Sea	76	VI	Femur	2
62	03-jul-15	Sea	76	VI	Femur	2
63	03-jul-15	Sea	76	VI	Femur	4
64	03-jul-15	Sea	76	VI	Femur	0
71	03-jul-15	Sea	76	VI	Femur	2
65	03-jul-15	Sea	76	VI	Femur	4
72	03-jul-15	Sea	76	VI	Femur	4
32	03-jul-15	Sea	76	VI	Femur	3
66	03-jul-15	Sea	76	VI	Femur	2
33	03-jul-15	Sea	76	VII	Femur	1
67	03-jul-15	Sea	76	VI	Femur	3
34	03-jul-15	Sea	76	VII	Tibia	2
68	05-aug-15	Sea	78	V	Femur	4
1	05-aug-15	Sea	78	VI	Tibia	1
69	06-aug-15	Sea	79	VI	Femur	2
35	06-aug-15	Sea	79	VI	Femur	3
70	06-aug-15	Sea	79	V	Tibia	3
36	06-aug-15	Sea	79	VI	Femur	3
37	06-aug-15	Sea	79	VIII	Tibia	1
38	06-aug-15	Sea	79	VII	Tibia	0
39	06-aug-15	Sea	79	VII	Tibia	2
73	06-aug-15	Sea	79	VI	Femur	3
74	06-aug-15	Sea	79	V	Tibia	3
75	14-sep-15	Sea	149	VII	Tibia	3
40	14-sep-15	Sea	149	VII	Tibia	2
76	14-sep-15	Sea	149	VII	Tibia	1
41	14-sep-15	Sea	149	VII	Tibia	2
42	14-sep-15	Sea	149	VI	Tibia	2
77	14-sep-15	Sea	149	VII	Tibia	2

43	15-sep-15	Sea	149	VIII	Tibia	1
44	15-sep-15	Sea	149	VIII	Femur	1
78	15-sep-15	Sea	149	VII	Tibia	2
79	15-sep-15	Sea	149	VIII	Tibia	1
80	15-sep-15	Sea	149	VII	Tibia	1
45	15-sep-15	Sea	149	VIII	Tibia	1
46	15-sep-15	Sea	149	VII	Tibia	2
81	15-sep-15	Sea	149	VI	Femur	3
82	15-sep-15	Sea	149	VI	Femur	3
83	15-sep-15	Sea	149	VI	Femur	3
84	15-sep-15	Sea	149	VI	Tibia	4
85	15-sep-15	Sea	149	VI	Tibia	3
3	16-sep-15	Sea	150	VII	Tibia	2
47	16-sep-15	Sea	150	IX	Femur	1
4	16-sep-15	Sea	150	VII	Tibia	1
48	16-sep-15	Sea	150	VI	Tibia	3
5	16-sep-15	Sea	150	VI	Femur	3
49	16-sep-15	Sea	150	VII	Tibia	1
50	16-sep-15	Sea	150	VI	Tibia	4
6	05-nov-15	Sea	201	IX	Tibia	0
7	05-nov-15	Sea	201	VII	Tibia	2
86	05-nov-15	Sea	201	VIII	Tibia	3
8	05-nov-15	Sea	201	VIII	Tibia	3
9	05-nov-15	Sea	201	VIII	Tibia	1
10	05-nov-15	Sea	201	VIII	Tibia	1
87	05-nov-15	Sea	201	IX	Femur	0
51	05-nov-15	Sea	201	VIII	Femur	3
88	05-nov-15	Sea	201	IX	Femur	4
89	05-nov-15	Sea	201	VII	Tibia	3
20	05-nov-15	Sea	201	IX	Femur	2
21	05-nov-15	Sea	201	VIII	Tibia	2
22	05-nov-15	Sea	201	VII	Femur	3
90	05-nov-15	Sea	201	IX	Tibia	0
25	05-nov-15	Sea	201	VIII	Femur	0
52	05-nov-15	Sea	201	IX	Femur	0
29	05-nov-15	Sea	201	VII	Tibia	2
23	06-nov-15	Sea	202	IX	Femur	2
11	06-nov-15	Sea	202	VII	Femur	3
12	06-nov-15	Sea	202	VI	Femur	4

91	06-nov-15	Sea	202	VII	Tibia	4
18	06-nov-15	Sea	202	IX	Tibia	1
19	06-nov-15	Sea	202	VIII	Tibia	0
24	06-nov-15	Sea	202	VII	Femur	3
25	06-nov-15	Sea	202	VIII	Femur	3
27	06-nov-15	Sea	202	VIII	Tibia	2
92	06-nov-15	Sea	202	VII	Tibia	4
93	06-nov-15	Sea	202	VI	Tibia	1
28	06-nov-15	Sea	202	VII	Tibia	1
13	28-jan-16	Sea	285	IX	Humerus	0
94	28-jan-16	Sea	285	IX	Femur	0
14	28-jan-16	Sea	285	IX	Femur	2
53	28-jan-16	Sea	285	IX	Femur	0
15	28-jan-16	Sea	285	IX	Radius	1
16	28-jan-16	Sea	285	IX	Femur	1
17	28-jan-16	Sea	285	IX	Femur	2
95	28-jan-16	Sea	285	IX	Femur	2
96	05-jul-16	Deck	444	IX	Femur	3
97	07-jul-16	Deck	446	IX	Femur	3
54	07-jul-16	Hold	446	VII	Femur	3
55	07-jul-16	Deck	446	VIII	Femur	1
56	11-jul-16	Deck	450	VIII	Femur	2
98	11-jul-16	Deck	450	IX	Femur	3
99	11-jul-16	Deck	450	IX	Femur	1
2	13-jul-16	Sea	452	VIII	Femur	2
30	13-jul-16	Deck	452	IX	Homerus	3
100	13-jul-16	Sea	452	VIII	Femur	3
101	13-jul-16	Deck	452	VIII	Femur	2
102	15-jul-16	Deck	454	VIII	Femur	2
103	15-jul-16	Deck	454	IX	Femur	1
104	16-jul-16	Deck	455	VIII	Femur	3
105	18-jul-16	Deck	457	VIII	Femur	3
106	18-jul-16	Deck	457	IX	Femur	3
107	18-jul-16	Deck	457	IX	Femur	2
108	19-jul-16	Forepeak	458	VII	Femur	3
109	19-jul-16	Deck	458	VIII	Clavicle	4
110	19-jul-16	Deck	458	VII	Femur	2
111	19-jul-16	Deck	458	VIII	Femur	3
112	20-jul-16	Deck	459	VIII	Femur	2

113	20-jul-16	Deck	459	IX	Femur	4
114	21-jul-16	Deck	460	IX	Femur	3
57	21-jul-16	Deck	460	IX	Femur	3
115	21-jul-16	Deck	460	IX	Femur	3
116	21-jul-16	Deck	460	IX	Femur	4
117	21-jul-16	Forepeak	460	VIII	Femur	3
118	22-jul-16	Deck	461	VIII	Femur	3
119	22-jul-16	Deck	461	IX	Femur	3
58	22-jul-16	Forepeak	461	IX	Femur	2
59	22-jul-16	Deck	461	IX	Femur	2
60	23-jul-16	Deck	462	VIII	Femur	2
120	26-jul-16	Engine room	465	IX	Femur	2
121	28-jul-16	Engine room	467	VI	Femur	3
122	19-sep-16	Engine room	520	VIII	Tibia	3
123	19-sep-16	Engine room	520	IX	Tibia	3
124	20-sep-16	Engine room	521	IX	Femur	3
125	21-sep-16	Hold	522	IX	Femur	3
126	26-sep-16	Hold	527	VI	Tibia	2
127	27-sep-16	Hold	528	VIII	Tibia	1
128	28-sep-16	Hold	529	VIII	Femur	3
129	28-sep-16	Hold	529	VIII	Tibia	4
130	29-sep-16	Hold	530	IX	Femur	4
131	30-sep-16	Hold	531	VIII	Femur	3
132	30-sep-16	Hold	531	VIII	Tibia	2
133	30-sep-16	Hold	531	IX	Femur	3
134	01-oct-16	Hold	532	IX	Femur	3
135	01-oct-16	Hold	532	VIII	Femur	2
136	05-oct-16	Hold	536	VII	Femur	2
137	06-oct-16	Hold	537	IX	Femur	1
138	06-oct-16	Hold	537	IX	Femur	1
139	07-oct-16	Hold	538	VII	Femur	1
140	10-oct-16	Hold	541	VI	Femur	4
141	11-oct-16	Hold	542	VIII	Femur	3
142	12-oct-16	Hold	543	VII	Femur	3
143	13-oct-16	Afterpeak	544	IX	Femur	3
144	13-oct-16	Afterpeak	544	IX	Femur	3
145	14-oct-16	Afterpeak	545	IX	Femur	3
146	14-oct-16	Hold	545	VIII	Femur	3
147	14-oct-16	Hold	545	VII	Femur	2

148	14-oct-16	Afterpeak	545	IX	Femur	4
149	15-oct-16	Afterpeak	546	IX	Femur	3
150	17-oct-16	Hold	548	VII	Femur	2

Table 3: Description of the 150 individuals. TADS: total aquatic decomposition score; Red color: cases of Brescia; Green color: cases of Pavia; Yellow color: cases of Torino; Light blue color: cases of Eurofins Genoma.

All subjects were autopsied over a period ranging from a minimum of 76 days from the time of the shipwreck to a maximum of 548 days.

In 97.3% of cases (146/150) the samples for DNA extraction were taken from the diaphyses of the long bones of the lower limbs, 97 from the femurs (64.7%) and 49 from the tibias (32.6%). Only in 4/150 cases (2.7%) sampling was taken from other body parts: three diaphyses of long bones of the upper limbs (two humerus and one radius) and one clavicle.

In 31.4% of cases, the bones collected for genetic purposes were completely skeletonised (De Donno score IX: 47/150), while the remaining still had residual soft tissue. Specifically, De Donno score VIII was recorded in 38/150 cases (25.3%), De Donno score VII in 32/150 cases (21.3%), De Donno score VI in 30/150 cases (20%) and De Donno score V in 3/150 cases (2%).

In 92.7% (139/150) of the cases, the skeletal segment from which the bone for extraction was taken was covered by garments. Of these, in 36 of 150 cases (24%) one layer of clothes was found; two layers were present in 37 cases (24.7%), while three garments covered 51 body parts (34%) and in 15 cases (10%) four clothes were described. In 11/150 cases (7.3%) no clothes were found.

Taphonomic analysis of the remains and correlation to the genetic findings

In the following table (Table 4), the descriptive statistics of the distribution of the 150 cases according to the main characteristics of the retrieved bodies and the quality of the DNA profiles.

	STRs loci			***Total***
	>15	***between 10 and 15***	***<10***	
	114	18	18	150
	Days between the shipwreck and the autopsy			
< 200 days	45	1	3	50
> 200 days	69	17	15	100
	Bone samples for genetic analyses			
Femur	71	13	13	97

Tibia	41	5	3	49
Upper limbs	1	0	2	3
Clavicle	1	0	0	1
	TADS Score			
V	2	0	1	3
VI	26	2	2	30
VII	27	3	2	32
VIII	26	8	4	38
IX	33	5	9	47
	Number of clothes covering the bone sample			
No clothes	10	0	1	11
1	30	2	4	36
2	29	4	4	37
3	33	11	7	51
4	12	1	2	15
	Place of recovery			
Open sea	72	8	8	88
Wreck	42	9	10	62

Table 4. Descriptive statistics of the 150 cases according to the quality of the DNA typing.

The correlation and significance between the distribution of samples with optimal (autosomal STR loci ≥ 16), average (autosomal STR loci between 15 and 10) and critical (autosomal STR loci < 10) analytical results was calculated on the basis of the variables considered (Table 5).

T-score	***p-value***
Time elapsed between the recovery and the autopsy	**0,011**
Taphonomic conditions	0,077
Number of garments	>0.05
Chi-square	***p-value***
Bone collected for genetic analysis	>0.05
Place of the recovery of bodies	>0.05

Table 5. Correlation and significance with genetic results.

The only variable that showed a statistically significant difference (p-value <0.05) was the time interval between the shipwreck and the time of completion of the autoptic procedures, during which bone samples were taken: samples with optimal analytical results were collected earlier in almost all cases (< 200 days). Also, the taphonomic variable group showed p-value close to statistically significance (p-value =0.77).

Anthropological estimation of the ancestry

On the 49 samples that returned reliable DNA profiles, the anthropological analysis of the remains was then carried out at the LABANOF of University of Milan.
Among them, 17 bodies showed the cephalic district which was then available to perform the anthropological study of the skull and dental features.

Morphoscopic features of the cranium

The OSSA method

The results of the evaluations showed (Table 6):

- 2/17 individuals (individuals 1 and 10) have an OSSA value of 4;
- 15/17 individuals have an OSSA value of less than 4.

Taking into account the number of traits considered, only in 4 of the 17 cases was an estimate of geographical origin possible (individuals 1 and 11: *American White* population; individuals 7 and 25: *American Black*). In the remaining cases, in fact, the OSSA score weighted for the number of traits analyzed did not allow a sufficiently accurate prediction of geographical origin.

ID	ANS	INA	IOB	MT	NAW	NBC	NO	PBD	SPS	TPS	ZS	OSSA SUM	Considered traits/11	Prediction
1	2	4	nd	0	2		nd	0	nd	3	nd	4	9	WHITE
3	3	2	2	nd	3		0	0	0	3	nd	3	10	
4	2	1	2	nd	2		0	1	0	3	nd	3	10	
5	1	2	nd (3)	nd	2		0	0	2	1 dx; 4 sx	nd	2	9	
6	2	3	2	0	3		0	0	2	3	1	3	10	
7	1	3	3	1	2		0	nd(0)	0	3	1	1	9	BLACK
10	1	2	2	0 dx; 2 sx	2		1	2	3	1	0	2	9	
11	2	3	2	0	2		1	0	0	3	0	4	10	WHITE
12	1/2	3*	3	1	2		nd	0	0	nd	nd	2	8	
13	1	2	2	2	2		nd	0	2	2 dx, 1 sx	1	3	10	
14	1	2/3	2	1	2		nd	0	nd	3	1	3	9	
19	2	2	3	2	2		0	0	0	3	1	3	10	
21	1	2	2	1	2		nd	0	nd	3	0	3	10	
22	1	3	2	1	2		nd	0	nd	2	nd	3	10	
25	1	2	2	0	2		nd	1	2	nd	1 dx, 0 sx	2	10	BLACK
26	2	2	2	1	2		nd	1	2	nd	0	3	10	
29	2	2	2	0	3		0	0	0	3	1	3	10	

Table 6. Results from the OSSA method.

hefneR software

The results of the evaluations showed (Table 7):

Only 3/17 cases showed a probability of attribution greater than 75% (individual 4: 91.7%; individual 10: 80.4%; individual 19: 84.9%): all three cases were attributed to the African population.

In the remaining cases (14/17) more than one population group appears.

However, in 7/14 cases a "dominant" component with a probability of attribution greater than 50% can be found:

- 1/7 (individual 1) has a European majority component (66.9%);
- 3/7 have an African majority component (individual 3: 63.5%; individual 5: 54.2%; individual 29: 73.6%);
- 3/7 have an Asian majority component (individual 13: 59.3%; individual 21: 68.3%; individual 22: 73.2%).

As for the other 7/14, with probabilities below 50%, 5/7 individuals (71.4%) carried mixed African-Asian components (individuals 7, 12, 14, 25 and 26).

Overall, the contribution of the European component is low, except for individual 1 where it is the majority and individuals 3, 6 and 11 where it is between 20% and 25%.

As regards the number of sections considered:

- In the 3/17 cases, in which a probability of attribution of more than 75% was reached (individuals 4, 10 and 19), the number of traits considered in one case was at least 8 out of the 11 which were evaluable: in the remaining cases the number of traits considered was 10/11;
- in the 7/17 cases, in which a "dominant" component with a probability of attribution greater than 50% can be traced, the number of traits considered was at least 6 out of the 11 which were evaluable: in 1/7 (individual 29) 10/11 traits were considered; in 1/7 (individual 13) 9/11 traits were considered; in 3/7 (individuals 3, 5 and 21) were considered 8/11; in 1/7 (individual 22) 7/11 traits were considered; in 1/7 (individual 1) 6/11 traits were considered;
- in the remaining 7/17 mixed component cases the number of traits considered was at least 7 out of 11 evaluable: in 3/7 cases (individuals 6, 7 and 11) 10/11 traits were considered; in 3/7 cases (individuals 14, 25 and 26) 8/11 traits were considered; in 1/7 cases (individual 12) 7/11 traits were considered. 2/3 individuals in which 10/11 traits have been considered (individuals 6 and 11) have a mixture of European, African and Asian origins.

ID	ANS	INA	IOB	MT	NAW	NBC	NO	PBD	SPS	TPS	ZS	Considered traits/11	RESULTS				Prediction
													EU	Af	Aml	As	
1	2	4	nd	0	2		nd	0	nd	3	nd	6	66.86%	12.5%	15.16%	5.48%	EU>Aml>Af
3	3	2	2	nd	3		0	0	0	3	nd	8	21.9%	63.46%	10.62	4.01%	Af>EU>Aml
4	2	1	2	nd	2		0	1	0	3	nd	8	1.7%	91.68%	1.54%	5.07%	Af
5	1	2	nd (3)	nd	2		0	0	2	1 dx, 4 sx	nd	8	00.28	54.15	11.8	33.74	Af>As>Aml
6	2	3	2	0	3		0	0	2	3	1	10	25.35%	19.99%	17.36%	37.3%	mix
7	1	3	3	1	2		0	nd(0)	0	3	1	10	6.53%	49.79%	22.1%	21.58%	Af>Aml>As
10	1	2	2	0 dx, 2 sx	2		1	1	2	1	0	10	00.21%	80.4%	1.56%	17.84%	Af
11	2	3	2	0	2		1	0	0	3	0	10	20.21%	39.93%	12.29%	27.56%	mix
12	1/2	3*	3	1	2		nd	0	0	nd	nd	7	3.79%	43.32%	30.88%	22%	mix Af/As/Aml
13	1	2	2	2	2		nd	0	2	2 dx, 1 sx	1	9	00.73%	10.25%	29.71%	59.31%	As>Aml>Af
14	1	2/3	2	1	2		nd	0	nd	3	1	8	09.27%	18.11%	35.75%	36.86%	mix As/Aml>Af
19	2	2	3	2	2		0	0	0	3	1	10	2.08%	84.85%	9.88%	3.19%	Af
21	1	2	2	1	2		nd	0	nd	3	0	8	00.85%	16.73%	14.09%	68.32%	As>Af>Aml
22	1	3	2	1	2		nd	0	nd	2	nd	7	9.8%	4.56%	12.44%	73.2%	As>Aml
25	1	2	2	0	2		nd	1	2	nd	1 dx, 0 sx	8	1.48%	44.4%	14.2%	39.9%	mix Af/As> Aml
26	2	2	2	1	2		nd	1	2	nd	0	8	1.09%	46.63%	12.67%	39.62%	mix Af/As> Aml
29	2	2	2	0	3		0	0	0	3	1	10	10.64%	73.62%	12.20%	3.53%	Af>Aml>EU

Table 7. Results from hefneR software.

Non-metric characters of teeth with rASUDAS software

In 11 of the 17 cases under analysis (64.7%), the number of traits evaluated was equal to or greater than 12 out of the 21 considered, a threshold that should guarantee greater reliability of the estimates.

In 5 (individuals 12, 13, 19, 21 and 25) of the remaining 6 cases, the impossibility of reaching the reliability threshold of 12/21 traits was due to the failure to find the jaw during the recovery procedures of the remains (Table 8).

ID	Winging	Shoveling	Interruption grooves	Hypocone	Cusp 5	Carabelli's Cusp	Enamel extensions	Upper premolar Root Number	Upper Molar Root Number	Peg/mal-/reduced/missing third molars	LPM cusp Number	LM Groove pattern	LM Cusp number	Deflecting Wrinkle	Protostylid	Cusp 6	Cusp 7	LP1 Root Number	LC Root Number	Three rooted LM	LM Rooth number	Considered traits/21	RESULTS %					prediction
	1	4	6	14	16	18	20	21	22	24	27	30	32	33	35	36	37	38	39	40	41		West Eurasia	Sub-Saharan Africa	Sud-Est Asia	EST Asia	NORD-EST Asia	
1	1		0	5	4	5	0	nd	nd	nd	0	2	nd	nd	0			nd	0		2	12	32,84	57,94	9,09	0,12	0,00	Af>EU
3	nd		nd	nd	0	0	0	nd	nd	nd	0	2	+	+	0	0	0	0	0	nd	nd	14	47,51%	35,27	7,09	8,84	1,29	mix EU/Af
4	0	1	0	4	nd	0	0	1	0	nd	1	1	+	4	nd	3	0	0	1	0	2	18	85,47	3,47	9,98	0,73	0,2	EU
5	0	1	M	2	nd(0)	0	1	0	0	nd	nd	1	+	0	0	nd	nd	0	0	nd	2	17	87,05	0,03	1,62	0,37	0,68	EU
6		0		4	0	0	0	0	0	nd	nd	1	Y	4	nd	nd	0	0	nd	nd	2	14	62,32	9,03	16,19	10,21	2,24	EU>SEAs/Eas
7	0	2	0	0	1	0	0	0	0	2	1	3	0	0	1	nd	+	nd	nd	0	2	19	66,17	8,56	13,27	8,61	3,39	EU>SEAs
10	0	nd	nd	0	4	0	0	0	0	1	1	nd	0	0	1	Y	+	0	0	nd	2	17	72,05	5,21	11,05	9,44	2,25	EU>SEAs
11	1	1	M	0	0	0	1	1	0	2	nd	1	1	nd	+	0	0	nd	0	nd	nd	14	85,35	0,84	3,82	0,15	0,02	EU
12				0	0	1	0	1		nd	nd	0										6	34,73	2,94	14,77	12,2	35,36	mix EU/NEAs>SEAs/Eas
13	0	nd	0	0	0	0	0	0	1	nd	1	0	0	0								9	40,47	1,9	15,66	9,58	32,39	mix EU/NEAs>SEAs
14				5	5	0	0	0	nd	nd	nd	nd	nd	0	X	nd	5	5	1	0	0	0	0	nd	nd	nd	12	17,51	18,83	23,81	24,7	15,15	mix
19				4	4	0	0	0	0		nd	nd	0									5	22,02	27,59	24,01	16,1	10,28	mix AF/SEAs/Eu>NEAs
21	0	1		3	3	0	0	1	2	0	0	nd	2	3	3	0	0					9	57,26	38,64	3,85	0,21	0,04	EU>Af
22		0	1	1	0	0	0	0	0	1	1	1	1	3	3	3	nd	+	+	0	0	0	0	0	0	0	0	17	94,47	0,18	4,39	0,56	0,05	EU
25				0	nd	nd	nd	4	4	0	1	2	2	nd	3		0					6	69,07	18,67	8,07	3,5	0,69	EU>Af
26				6	6	nd	2	0	0	0	0	2	2	nd	nd	0	0	3	Y	+	5	5	0	0	0	1	1	1	16	0,07	96,53	2,96	0,41	0,03	Af
29				nd	nd	0	0	0	0	0	1	1	nd	nd	nd	nd	nd	0	0	nd	nd	0	nd(0)	nd	nd	2	2	10	20,88	3,04	10,06	12,6	53,41	NEAs>Eu>SEAs/EAs

Table 8. Results from rASUDAS software.

As far as the ancestry results are concerned, only in 5/17 cases (29.4%) does the resulting estimate exceed 75% probability (highlighted by the red colour in the table above): for individuals 4, 5, 11 and 22 a western Eurasian origin was estimated, while for individual 26 a sub-Saharan African origin; in all cases the probability relative to the estimate is at least 85%. In all 5 cases mentioned above, the estimate is derived from the analysis of at least 14 non-metric dental characters out of the total 21 traits that can be considered.

In a further 7/17 cases (41.2%), the resulting estimate appeared to be composite (no population group with a percentage >75%), although one component appeared to be in the majority with a probability of more than 50% (highlighted by the orange colour in the table above): 1/7 attributed to the Sub-Saharan African population (individual 1), 1/7 to the North-East Asian population (individual 29) and 5/7 to the Western Eurasian population (individuals 6, 7, 10, 21 and 25). In 3/7 cases (individuals 21, 25, 29) the number of traits considered was less than 12 (1/3 attributed to the Northeast Asian population and 2/3 to the Western Eurasian population).

In the remaining 5/17 cases the resulting percentages do not allow a majority group to be identified, allowing the conclusion to be drawn for mixed forms. In 3/5 cases the number of traits considered was below the threshold of 12 characters; these three cases were retrieved from the shipwreck without the mandible.

Comparison of anthropological methods and genetic findings for ancestry

Summary Table 6 shows the results of the different methods used to estimate the ancestry of the 21 individuals studied.

The results highlighted in red in the table (Table 9) correspond to probability percentages relative to the estimate greater than 75%; those highlighted in bold, on the other hand, represent results with probabilities less than 75% but greater than 50%.

The evaluations on the estimation of geographical ancestry have been carried out considering the populations of each method.

N. of cases	*Anthropological assessments*			*Genetic assessments*	
	OSSA	*hefneR*	*rASUDAS*	*Y-STRs*	*AIMs*
1	**WHITE**	**Eu**>As>Af	**Af**>Eu	African	Horn of Africa
3		**Af**>Eu>As	mix Eu/Af	African	Sub-Saharan Africa
4		**Af**	**Eu**	African	Sub-Saharan Africa
5		**Af**>As	**Eu**	ND	Sub-Saharan Africa
6		mix	**Eu**>As	African	Sub-Saharan Africa
7	**BLACK**	Af>As	**Eu**>As	African	Sub-Saharan Africa

10		**Af**	**Eu**>As	African	Sub-Saharan Africa
11	**WHITE**	mix	**Eu**	Afro-Asian	Horn of Africa
12		mix Af/As	mix Eu/As	African	Sub-Saharan Africa
13		**As**>Af	mix Eu/As	Mixed population	Sub-Saharan Africa
14		As>Af	mix	African	Sub-Saharan Africa
19		**Af**	mix	African	Sub-Saharan Africa
21		**As**>Af	**Eu**>Af	African	Sub-Saharan Africa
22		**As**	**Eu**	Eurasian	Horn of Africa
25	**BLACK**	mix Af/As	**Eu**>Af	African	Sub-Saharan Africa
26		mix Af/As	**Af**	Mixed population	Sub-Saharan Africa
29		**Af**>As>Eu	**As**>Eu	African	Sub-Saharan Africa

Table 9. Comparison between predictions of anthropological and genetic assessments.

Regarding cranial morphology, the predictions of the OSSA method seem to coincide with the group with higher probability percentages related to the highest estimate resulting from the hefneR software, except for a single case (individual 11: mix). However, the low sample size does not allow for further considerations.

As far as the non-metric dental traits are concerned, the results of the analysis with the rASUDAS software show a poor agreement with the cranial morphological predictions, referred in this sense only to the hefneR method: the OSSA score, in fact, contrary to the first two, does not consider the Asian population among the reference ones and is therefore less expendable in terms of comparison.

In the predictions resulting from the hefneR software, there is a greater presence of the African and Asian components, whereas the rASUDAS estimates show a Europoid morphological predominance.

Comparison with the results of genetics is not possible in the case of the OSSA method due to the extremely small number of cases; in the case of the hefneR and rASUDAS methods, the comparison is invalidated by the high prevalence of the resulting mixed forms.

That said, with respect to the predictions of biogeographical origin by means of Y-STR polymorphisms, there is a concordance with the estimates offered by the hefneR software: in fact, the population predicted by the genetic analysis always corresponds to one of the components of the hefner estimate. Sometimes there is a match with the group with the highest Hefner estimate probability percentages, and sometimes a match with minority components. In any case, it is always possible to find a match. In contrast, in the case of rASUDAS a higher percentage of non-match is observed, given the clear predominance of Europoid components found in the dental results.

With respect to predictions of biogeographical origin by means of AIMs polymorphisms, all individuals genetically originated from Africa mainly correspond to an African or mixed African-Asian origin in the hefneR estimates. The same results regarding the PCA analysis, in all cases of sub-Saharan prediction. For the remaining three individuals for which the PCA analysis predicted an African origin outside the sub-Saharan area (individuals 1, 11, 22, origin estimated from the Horn of Africa), the hefneR method returns an Europoid origin for individual 1, a mixed form for 11 and an Asian origin for 22. However, given the low representation in relation to the total of these genetically non-sub-Saharan forms, no further considerations can be made.

Compared to the rASUDAS method, no pattern is recognised, as the Europoid component in this analysis is prevalent in all considered cases.

DISCUSSION

The migratory crisis in the Mediterranean, which has seen a continuous flow of deaths over the years, is a humanitarian tragedy of such magnitude that it confronts our country with the serious issue of identifying the bodies of migrants who have been victims of the countless shipwrecks during the crossing to Europe.

This need to identify the victims of the Mediterranean and, at the same time, to have accurate methods for the genetic typing and construction of the biological profile of the victims motivated the present thesis work, which is part of a broader project carried out by LABANOF of the University of Milan aimed at identifying the victims of the shipwreck of 18 April 2015.

Considering the peculiar context of this study and the problems related to the process of arriving at a positive identification, the possibility of having accurate genetic and anthropological methodologies for ancestry estimation would allow the comparison to be limited to suspicious identities from a specific geographical area, as well as focusing the search for relatives in some African countries for genetic comparison.

To this end, the remains of 150 victims of the shipwreck of 18 April 2015, were genetically investigated at the forensic genetics laboratory of the Universities of Brescia, Pavia, Turin and Eurofins Genome for identificative purpose. Good quality results were then analysed by applying morphological methods commonly used in anthropology to estimate geographical origin. The anthropological estimation results were then compared with the genetic results obtained to assess strengths or weaknesses and points of agreement between the different methods.

The main limitation of this study is the absence of a definite identification, as the population of the study sample is unknown. However, even if we do not know the precise biogeographical origin, the analyses that have been carried out during this study converge towards a common datum. The problem arises when differences are detected and how they can be handled from a practical point of view is an essential issue. It is important to understand how much weight should be given to the individual data and the result as a whole.

Nevertheless, on the basis of the information provided by the various reports on arrivals in Europe via the Mediterranean routes at that time (Frontex) and the information obtained from

a preliminary investigation of the material found during the body recovery operations (personal belongings, documents...), it is possible to hypothesize that the victims of the 18 April 2015 shipwreck came from three different geographical areas:

- sub-Saharan regions of Africa, in particular the Horn of Africa (about 28% from Eritrea, 16 % from Somalia and 12% from Nigeria);
- Mediterranean Africa;
- Asia Minor, to a lesser extent.

These data appear consistent with the results of the genetic analyses carried out on the sample under study, which place all the individuals typified on the African continent in the sub-Saharan area, with the exception of three individuals (1, 11 and 22) for which it was further possible to circumscribe the origin to the Horn of Africa, having demonstrated a strong ancestral component of European type (Pereira, 2012; Kumar, 2020). Also, three other individuals showed a strong ancestral component of Caucasian population, providing haplogroups and PCA analysis suggestive for European component. The population analysis performed confirmed the data in the literature on the association between a specific haplogroup and metapopulations and a specific geographical area of origin.

The profile of the population sample taken into consideration was well evaluated in the general framework of the diversity of the existing populations studied to date. Y-chromosome STR and autosomal STR systems have largely demonstrated a considerable utility in forensic genetics, both in the criminalistic field (identification of perpetrators of male crimes even in mixed traces) and in paternity investigations (especially in situations where it is necessary to trace the relationship of kinship between male individuals), and in areas that go beyond the actual forensic medicine such as the present one of population studies.

The data obtained showed a considerable haplotype variability within the considered population, as all haplotypes were unique.

In the present study, Y chromosome SNPs were also typed in 30 samples in order to identify the haplogroups that characterise the considered samples. The results identified four distinct haplogroups from a total of 30 subjects. These data clearly indicate that SNPs and InDels cannot replace the discriminative ability of STRs in distinguishing subjects.

The association between a specific allele of a given STR locus and a given haplogroup could, however, provide useful elements for the appropriate interpretation of the STR data. Indeed, in light of the better performance of SNPs on “difficult” samples, as the ones affected by advance decomposition or skeletonisation, if the results obtained from the STR tying are not

of clear interpretation, a sort of “internal control” between the autosomal data obtained and the haplogroup related to the same sample could be envisaged.

However, the main utility of biallelic markers in forensic practice lies in the possibility of using them to obtain information on the geographical origin of male subjects. This is particularly important also in the criminal field, if human DNA belonging to an unknown male subject is identified at a crime scene. The possibility of providing indications to the investigators on the ethnic origin of the subject to whom the biological traces analysed belong may be of considerable importance in directing the inquiries.

The possibility of typing even the most distal branches of the phylogenetic tree, through the appropriate preparation of specific primers, will allow an even greater discriminative capacity, as well as the possibility of having additional information, linked for example to the individual’s somatic traits, closely associated with the geographical area of origin of the individual and potentially very useful in the criminal investigation field. Otherwise, in the humanitarian field discussed in the present study, two main problems arise: the first concerns the geographical origin of the sample. If phylogenetic markers with a high discriminatory power are used and the survey area is very large, such as a continent, the risk is to have a very specific result only concerning a particular area and therefore it would be necessary to increase the number of markers to test. The second issue is intertwined with the previous one: to be able to effectively use discriminatory markers in the attempt to trace the victims who are not identified and without antemortem information, it is necessary to rely on population studies or databases available for specific populations or states, which nowadays, however, are scarcely available.

Furthermore, differences in terms of yield and recovery of genetic information useful for comparison with the profiles of alleged relatives were detected, although in the different laboratories involved several techniques were applied. In cases of severely decomposed bodies, skeletonized or human remains, bones and teeth – organs composed of mineralized tissues – are among the body parts most likely to preserve their integrity. In these cases, the extracellular matrix contains a structured assembly of minerals and proteins. The physical and chemical properties of mineralized tissue, which are responsible for the endurance of these tissues, offer major barrier during the extraction of the DNA preserved in it, making bone samples a problematic substrate for forensic analyses. For this reason, there are different protocols and commercial kits that can be used but the results are rather low.

Having detected differences in terms of yield and recovery of genetic information between different individuals also in the present research, the hypothetical existence of a correlation

between the quality of genetic profiles resulting from the analysis of autosomal markers and the taphonomic conditions of the cadaveric remains studied was investigated: knowing any links between the state of preservation of the remains and the quality of the genetic material preserved in them could allow the selection of the most suitable portion of bone and/or protocols for subsequent genetic investigations.

As discussed above, DNA extraction from bone remains overcomplicates the identification process due to the skeletal matrix (Edson, 2019). At the same time, however, the strength of bone, resulting in greater strength and persistence than soft tissue and blood, means that in highly compromised conditions of the remains, such as those found in Mediterranean shipwreck victims, skeletal elements constitute the only biological material available to obtain a genetic profile (Edson, 2005).

For skeletal remains, a close correlation between bone density and probability of DNA recovery has been observed (Johnston, 2016): the highest success rate is in fact for sampling from compact bone of the diaphysis of the long bones of the lower limb, particularly the femur. In fact, the mineralised extracellular matrix of compact bone constitutes an effective barrier to the penetration of microorganisms and water and, therefore, to the enzymatic and non-enzymatic degradation of the DNA contained in osteoblasts/osteocytes, to which the genetic material of the spongiosum is more vulnerable. (Androwski, 2016). Due to the same correlation with higher bone density, the genetic yield of the cortical bone of the long bones of the lower limbs, which support the entire weight of the individual, i.e. the femur and, secondarily, the tibia, is higher than that of the upper limbs. Similarly, as male bones are significantly denser than female bones, a better success rate in genetic extraction from male remains has been shown. Bone density is also correlated with age: indeed, the bones of the young and old are less dense than those of adults (Johnston, 2016).

In the present study, although for almost each of the 150 individuals the genetic extraction was carried out from different parts (cortical bone, internal bone, bone powder, bone marrow) taken from the shaft of a long bone, with a preference for the femur and tibia, the outcome of the analyses revealed extreme variability in the analytical quality of the results with coexistence of samples with complete genotypes and negligible risk of stochastic and other events that are completely unsuitable for comparison, despite the sample appearing rather homogeneous. Indeed, it consists of 150 young adults, all male, recovered from Mediterranean waters following the 18 April 2015 shipwreck and autopsied between August 2015 and July 2016. All individuals therefore died at the same time and were left in the marine environment, at the same depth (400 m), exposed to the same extrinsic factors for an overlapping time. Thus,

the variability found is not attributable to statistically significant differences inherent to extrinsic factors related to the marine environment, nor to statistically significant differences in the study population or the chosen sampling site. Indeed, as described above, although the yield is better from the bone elements of the lower limbs due to their greater density, in cases such as this study where the remains are often incomplete, other bones are used for analysis: given the absence of femurs and tibias, humerus, radius and a clavicle were used without however determining a statistically significant difference in terms of yield compared to those from the tibia or femur.

Furthermore, as the samples may have been exposed to environmental insults, the possible correlation between the observed yield and the presence of potentially protective factors was investigated: neither the existence of soft tissue residues nor that of clothing covering the harvested bone segment proved to be factors significantly correlated with the success rate of genetic extraction, the latter being independent, in the study sample, of the degree of skeletonization of the remains. This evidence further consolidates the inappropriateness of assuming *a priori* that a better genetic yield in terms of extraction relies solely on a better macroscopic condition of the taken bone.

The only variable shown to significantly influence the genetic extraction success rate in the present study ($p\text{-value} < 0.05$) was the time interval between the shipwreck and the completion of the autopsy procedures, during which bone samples were taken: samples with optimal analytical results (number of STRs detected > 16) were taken earlier in almost all cases. This result appears consistent with the well-established notion that degradation of genetic material is time-related (Edson, 2019): after cell death, in fact, DNA undergoes enzymatic degradation by endogenous nucleases (following the alteration of membrane permeability caused by cell apoptosis/necrosis with release and activation of lysosomal proteases which, in turn, activate endonucleases) or bacterial, but also spontaneously by hydrolysis (also favoured by products derived from microbial action, such as polyamine putrescine) or by oxidation (O2 and H2O2 produced by aerobic microorganisms colonising tissues) through the action of putrefactive decay (Ambers, 2013). The DNA present in ancient and forensic samples is often highly fragmented and this fragmentation hinders successful PCR amplification by affecting the size (length) of the target loci that can be examined. For amplification to be successful, both the target region and associated primer binding sites must be intact. It is therefore necessary, especially in the case of identification procedures for shipwreck victims, that the search, the recovery, and the identification of corpses be as rapid and timely as possible: in fact, although putrefactive phenomena are slowed down as long as the bodies remain in water, they

manifestly accelerate after extraction. Therefore, the longer the time elapsed since when the remains were recovered from the sea after the shipwreck, the greater the extent of putrefactive decay and degradation of genetic material and, in parallel, the less chance of obtaining a useful profile for comparison.

Starting from the known genetic data, a comparison was then made between the results of the anthropological analyses conducted on a subset of 21 individuals for whom typing for the Y-STR and AIMs (SNPs and Indel) polymorphisms was possible, in order to assess which of the various selected methods (OSSA, hefneR, rASUDAS) were the most accurate in describing the origin of this unknown sample. Therefore, among the anthropological methods for ancestry estimation available in the literature, those that included populations from geographical areas of interest for the material under study in the reference sample were chosen.

It must be stated at the outset that the comparison of the results obtained through the different morphological analysis methods applied (OSSA, hefneR, rASUDAS) did not allow an unequivocal prediction of ancestry to be made. This is in fact a very complex analysis, in which the estimate was rarely restricted to a single population group since the prevalence of mixed forms is high. This seems to be linked to two types of reasons.

Firstly, the ancestry estimation methods applied in this study either completely lacked reference populations suitable for studying the analysed sample (Hefner and OSSA) or included a rather small representation of them (rASUDAS). Indeed, sub-Saharan populations are still little studied from an anthropological point of view. As there are no adequate reference samples, the resulting predictions are therefore to be considered as the populations that are likely to have the greatest similarities with the study sample, but which will not necessarily coincide with the actual group to which the individuals belong (Fernandes, 2021).

Secondly, these methods require the analysis of a precise number of morphological features to ensure an estimate of sufficient accuracy: therefore, fragmentary skulls lacking the mid-facial skeleton or vault should not be evaluated (Hefner, 2014). In the present case, analyses were conducted on skeletal remains that were often fragmentary, in some cases to the extent that specific morphological areas of interest could not be adequately assessed. Furthermore, the analysis was often linked to the observation of the autopsy photographic dossier, as the skull and mandible were not taken and collected in their entirety: this led, for example, to the necessary exclusion of the NBC trait from the OSSA and hefneR analyses, as a three-dimensional assessment involving the use of a level meter is required to score this character.

This means that the predictions produced are derived from the analysis of fewer than the optimal number of traits.

While the selected morphological methods must be credited with providing a statistical approach to the evaluation of non-metric characters, thus removed from the subjectivity and experience of the operator, it must be emphasised that the limitation of the aforementioned software is that it returns a result in every case, regardless of the existence of an adequate reference population and/or the congruence of the number of characters considered, thus leaving the interpretation of the results to the operator (Hefner, 2009 and 2014).

Although, as mentioned above, it was not possible to arrive at a sufficiently accurate estimate of the biogeographical origin of the different individuals under study, the following highlights emerged from the comparison of the different methods.

The methods of ancestry estimation based on cranial morphology (OSSA and hefneR) seem to be in fairly good agreement in their predictions, however, the scarcity of useful results obtained by the OSSA method and the type of reference populations considered in it precluded the possibility of a real comparison of it with the other methods and/or genetics.

The low number of predictions with the OSSA method (only six results) is due to the fact that in none of the cases were all the traits necessary for the evaluation available: this constituted an important limitation due to the fact that OSSA does not adequately take into account the multivariate relationships between the traits, but assigns equal weight to each trait in the final score, despite the fact that it is now known that some traits are more indicative of certain populations than others (Hefner, 2014).

Another critical issue for comparison with other methods is the fact that, at present, the OSSA method only classifies individuals into “white” and “black”, having a reference sample consisting of African-Americans and Caucasian Americans. Since, however, the individuals in question appear, also considering the results of the other methods, to be a “mosaic” of different population groups, it is necessary to expand the reference populations of the OSSA method to include at least an Asian sample.

On the other hand, as far as the hefneR method is concerned, a comparison with the genetic data, and in particular with the Y-STR typing, shows that individuals genetically located in the sub-Saharan area are in all cases attributed by the software to morphologically African or Afro-Asiatic populations, while individuals originating from the Horn of Africa appear phenotypically more Europoid or Asian. At the moment, however, these are only preliminary results: this is due to the small size of the sample considered in the present study and, in particular, to the low representation of non-sub-Saharan individuals, as well as to the fact that

only 4.3% of the reference sample for the hefneR method is represented by African individuals (East and West Africa), moreover collected in the early 1900s during the Smith African Expedition (1909). Therefore, although the results obtained appear promising given the correlation shown with the genetic data, further studies with larger case series are needed for adequate verification.

In contrast, for the rASUDAS method, there is no pattern of agreement with either the other morphological methods or the genetic data, the Europoid component prevailing in all the cases considered and the high prevalence of mixed forms in this analysis. This evidence seems to be related to the fact that the current software does not take into account more frequent traits in African populations. As far as the source of the dental data is concerned, the reference sample should therefore be improved on the one hand by assessing its applicability to modern populations, since rASUDAS has a predominantly archaeological reference sample, although there are numerous studies attesting that dental morphology has not radically changed over the last millennia (e.g. Scott 1994). On the other hand, it is necessary to add new morphological variables to the already incorporated list, which, with its original emphasis on Native American and Asian populations, has an Asian-centric bias. New traits could include labial convexity, midline diastema, mesial canine crest and molar crenulations, traits that are more common in sub-Saharan Africans than in other populations of the world (Irish, 1993 2013 and 2016; Pilloud, 2017). These improvements would be an important contribution, as incorporating dental morphological analyses to estimate biogeographical origin has undoubted advantages in order to reconstruct the biological profile of an unknown individual. Firstly, it is known that dental morphology is genetically determined, according to a polygenic inheritance, while showing little influence from the environment (Scott, 2018). Secondly, teeth are often well preserved even in contexts where human remains as a whole appear very compromised (fragmented, burnt or otherwise affected by trauma). Finally, the development of tooth morphology is directed by biological and genetic processes distinct from those of skeletal regions (cranial morphoscopic traits) used to estimate ancestry (Scott, 2018). Therefore, in its current form, rASUDAS can complement other methods in ancestry estimation, while it is not advisable to use it as the sole method of ancestry estimation, at least not in a modern context.

Future research, with larger sample numbers, will focus on ultrastructural analysis of the bone tissue of the samples to verify through specific staining (histological and/or immunohistochemical) the recurrence of any subversions or alterations in the composition of the bone matrix or osteocyte density, as well as the quantification of surface salinity through

electron microscopy analysis, which on the one hand can enhance cellular damage and on the other act as a confounder for genetic extraction. In addition, further analyses on multivariate statistical analysis, performed with specific population of African continent, could help the identification of the victims dead in the Mediterranean as well as the implementation of genetic analyses using, for instance, Next-Generation Sequencing technology.

CONCLUSIONS

This study revealed a significant relationship between the timing of the recovery and initiation of identification procedures (autopsies) of migrants' corpses and the yield in terms of analytical quality of genetic material and consequent suitability of the profile for comparative purposes. In fact, the greater the number of days elapsed since the shipwreck, the lower the yield of genetic information and consequently and simultaneously the possibility of obtaining a useful genetic profile for identification.

For this reason, it is essential to proceed with the rapid recovery of shipwreck victims in the Mediterranean and to promptly initiate the procedures for identifying the corpses in order to obtain the best yield of genetic material so that suitable profiles can be obtained for comparison with family members, thus guaranteeing the protection of the victims' right to identity on the one hand, and that of their loved ones to know the fate of their relative and end their mourning on the other.

With regard to the estimation of geographical ancestry, starting from the known genetic data, a comparison was made between the results obtained with the different anthropological methods.

In particular, greater concordance was observed between measurements obtained with the cranial morphological methods, OSSA and HefneR, than those obtained with ASUDAS analyses. Although all methods are limited by the lack of reference populations, rASUDAS seems to be the most affected by this factor, resulting in more discordant results. Also, genetic analysis was affected by a generic biogeographical estimation, limited to all African continent or big area, Horn of Africa, with high population variability within.

In order to be able to use these technical approaches for the biogeographical estimate of migrant populations, it is therefore necessary to implement the international database with morphological traits and DNA data from the African population and in particular from some specific area, like the sub-Saharan one.

Finally, it seems that the hefneR software is the most promising, having shown a certain pattern with the results of typing genetic AIMs. However, it is necessary to bear in mind the

limitations of the analysis conducted in terms of actual uncertain identification and limited sample size.

In the future, it will be necessary to continue the comparison by expanding the case study to verify the validity of the data obtained. In addition, the investigation on which morphological traits are most frequently found in those individuals that genetic typing showed a sub-Saharan area ancestry, could be studied in order to verify the persistence of the observed pattern of concordance and strengthen the validity of morphological ancestry estimation.

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AKNOWLEDGMENTS

A special thank you to the colleagues of the Universities of Palermo, Messina, Catania, Pavia, Parma, Ferrara, Milano Bicocca, Ancona, Bari, Torino, to the Polizia Scientifica, to the Italian Navy, the Sicilian Fire Brigade, the Commissioner for Missing Persons of the Italian Government, to Fondazione Isacchi Samaja for their support in the examination process and ongoing scientific and identification activities.

MODULO DI EMBARGO DELLA TESI
(da compilare solo se si richiede un periodo di segretazione della tesi)

Il sottoscritto……Lorenzo Franceschetti…… Nato il……18/04/1988……
a (indicare anche l'eventuale paese estero)……Ponte dell'Olio……..
provincia di (ovvero sigla del paese estero)……PC……
Dottorato di Ricerca in Genetica molecolare, Biotecnologie e Medicina sperimentale……

DICHIARA

- che il contenuto della tesi **non può essere immediatamente consultabile per il seguente motivo**
  I dati utilizzati per la realizzazione del progetto di Tesi, gentilmente concessi, fanno parte delle indagini inerenti al naufragio del 18 aprile 2015, su disposizione dell'autorità giudiziaria della Procura della Repubblica di Catania e dell'Ufficio Straordinario delle Persone Scomparse del Ministero dell'Interno.

La motivazione deve essere dettagliata e controfirmata obbligatoriamente dal Tutor e/o Relatore
(Brevetto, segreto industriale, motivi di priorità nella ricerca, motivi editoriali, altro)

- che il testo completo della tesi potrà essere reso consultabile dopo:

□ 6 mesi dalla data di conseguimento titolo
□ 12 mesi dalla data di conseguimento titolo
□ 24 mesi dalla data di conseguimento titolo
☒ altro periodo ___indefinito_____

- che sarà comunque consultabile immediatamente l'abstract della tesi, che viene caricato in Esse3, profilo studente.

Luogo e Data
Brescia, 04/11/2022

Firma del Dichiarante

Lorenzo Franceschetti

Controfirma del Tutor e/o Relatore del Dottorato
per la motivazione di embargo e il periodo.