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NEW TRENDS IN FORENSIC ANTHROPOLOGY

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PRESENTATION

NEW TRENDS IN FORENSIC ANTHROPOLOGY

The idea to publish a monograph on forensic anthropology in an anatomical journal arose as result of both the inherent relationship between anatomy and forensic anthropology and the growing importance of this last scientific field. Forensic anthropology refers to the study of living or dead humans, or what remains of the human, for medico-legal or humanitarian purposes. It is for this reason that the first paper of this monograph focuses on the inherent relationship between anatomy and forensic anthropology. In addition, there is currently an increasing role of forensic anthropologists in crime scene investigation, which leads this science to develop new trends. As a result of this, there is a larger emphasis on DNA analysis, forensic archaeology, forensic taphonomy and trauma. The analysis of human remains can certainly be examined directly or remotely through imaging such as CT scans. These developing trends and techniques have led to the creation of new specialisations. In the past, one forensic anthropologist may have analysed all the human remains of a case. Currently, different forensic anthropologists with a variety of specialisations such as histology, trauma, taphonomy, age-at-death estimation, craniofacial reconstruction, etc. can be found working on the same case. With this in mind, the main objective of this monograph is to promote dissemination of forensic anthropology knowledge and its developing new trends.

Following the proposed objective, this monograph is international and multidisciplinary, with participating authors from eight different countries including Canada, Colombia, Italy, Mexico, Portugal, Spain, United Kingdom and the United States, and summing up a wide range of experience and perspectives. This monograph comprises 16 papers devoted to forensic anthropology. The first seven papers are primarily conceptual in their content and introduce the notion of forensic anthropology, its research, its ethical challenges, its methods and techniques, its evolution and the role of donated bodies and documented skeletal collections for the development of the discipline. The following six papers are dedicated to particular fields of research in forensic anthropology such as non-adult or juvenile osteology, chemometrics, facial and craniofacial identification and dermatoglyphics. The last three papers are methodological, focusing on the application of forensic anthropology to the study of mummified remains, the analysis dental variation, and cut mark analysis.

More specifically, in the first paper, Carme Rissech introduces the concept of forensic anthropology and demonstrates its relationship to anatomy based on three streams, which are the following: (i) the role of forensic anthropology, (ii) the ten basic questions to be answered in any physical anthropological (osteological) examination, and (iii) the examples of five forensic anthropological cases. In the second paper, Nicholas Márquez-Grant and Julie Roberts, focus on mass disasters, briefly re-examining the role of forensic anthropologist and the definition of the discipline in the first quarter of the 21st century. The third paper by Nicholas Passalacqua and Marin Pilloud, briefly reviews the current state of forensic anthropology as a profession, focusing on the structural characteristics of education, training, research and practice, covering qualifications, ethical codes, guidelines and standards. The fourth paper by César Sanabria-Medina and coauthors presents an overview of the state of forensic anthropology in

Colombia, examining the cases of enforced disappearances and the institutions and tools that have emerged from the peace agreement between the government and the FARC-EP guerrilla. The fifth paper, by John Albanese, discusses the importance of documented skeletal reference collections as sources of data for the development and validity of methods that are used in forensic anthropology, including estimation of age-at-death, sex, stature, and ancestry. He also presents a theoretical model and some methodological strategies for building reference samples from documented skeletal collections. In the sixth paper, Lorena Valencia discusses the importance of body donation programs for the growth and development of forensic anthropology, in particular highlighting the benefits that these programs bring not only to the medical-surgical and anatomical fields but also to the field of forensic anthropology. The seventh paper written by Douglas Ubelaker and colleagues examines the subject of research of recently published papers in four leading journals featuring forensic anthropology. This paper was published in this journal previously (May 2020, EJA: Vol. 24: 221-227). Turning to the studies dedicated to a particular field in forensic anthropology, in the eighth paper, David Gonçalves and colleagues discuss the potential of chemometric analyses in the examination of burnt human skeletal remains; in particular the potential of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and X-ray fluorescence (XRF). Following this, in the ninth paper, Laure Spake and collaborators explore bias in age estimation in forensic cases when applying two different types of ageing methods, those that use living non-adults as reference and those that use non-adults skeletal remains as reference. The tenth paper, by Rebeca García-González and collaborators, tests the accuracy of several morphological traits for sex estimation in non-adults based on methods proposed by previous authors. The following chapter, authored by Rubén Martos and colleagues, highlights the importance of craniofacial superimposition when DNA, dental comparison or fingerprints are not available for human identification. Subsequently, in the twelfth paper, Joan Viciano and Luigi Capasso retrospectively evaluate the contributions of anthropological reports (dated between 1996 and 2019) to the resolution of personal identification cases at the Operative Unit of Anthropology of the 'G. d'Annunzio' University of Chieti–Pescara, Italy. This is followed by a thirteenth paper, where Esperanza Gutiérrez-Redomero and Noemí Rivaldería-Moreno thoroughly review the state of the art of dermatoglyphics in the field of forensic science in the 21st century. Because of the increasing importance of statistical models for supporting forensic identification, these authors discus the need to exhaustively analyse the variability of dermatoglyphic characteristics in order to have robust models of analysis. Turning to the fourteenth paper, Adriana Clausell and collaborators, presents the application of three-dimensional confocal microscopy to assess the origin of markings on bone surface in two historical cases. In the penultimate paper, Francisco Pastor and colleagues present an anthropological study based on 12 naturally mummified bodies found in the Spanish region of Castile and Leon. Lastly, in the sixteenth paper, María del Socorro Barraza and collaborators develop logistic regression equations to estimate the biological sex, based on measurements of maxillary and mandibular canines, using a modern sample from Colombia.

We are grateful to the Scientific Committee, which was in charge of scientifically reviewing the papers for this monograph. This Scientific Committee was constituted by the following: Inma Alemán, Maria-Eleni Chovalopoulou, Derek Congram, Francisco Etxeberria, Rebeca García-González, Sara Getz, David Gonçalves, Jennifer Grant, Kelly Kamnikar, Lluís Lloveras, Nicholas Márquez-Grant, Nicholas Passalacqua, Aniol Pujol, Marta San-Millán, Tim Thompson, Douglas Ubelaker, Ceferino Varón-González, Joan Viciano and Allysha Winburn. We are also grateful to the editorial board of the European Journal of Anatomy for accepting to publish this monograph in their journal.

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The importance of Human Anatomy in Forensic Anthropology

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SUMMARY

How important is anatomy in forensic anthropological analysis and caseworks? This basic question needs to be addressed in a monograph on forensic anthropology, especially if it is published in an anatomical journal, as is this case. Therefore, the main objective of this study is to analyse the importance of human anatomy in forensic anthropology. This is done by a literature review taking into account two perspectives: a historical perspective and a practical perspective. The historical perspective refers to the evaluation of the common history of human anatomy and forensic anthropology. The practical perspective refers to the evaluation of three practical points: (i) the role of forensic anthropology, (ii) the ten basic questions to be answered in any osteological study firstly defined by Ubelaker (1988), and (iii) five practical forensic anthropological cases. Results showed an intrinsic historical relationship between human anatomy and forensic anthropology. This inherent relationship between forensic anthropology and human anatomy is evident in the analytical procedures and the development of any forensic anthropological case. In fact, the relationship between both scientific fields is indubitable. Human anatomy is basic in forensic anthropology. A forensic anthropologist must have a wide and sound understanding of human anatomy, embracing foetus, new-born, infantile, juvenile and adult individuals. This is extremely important to resolve each case. Therefore, universities, which offer a degree in forensic anthropology, whether medical, biological, humanities and social sciences, should provide students with an extensive foundation in human anatomy.

Key words: Human morphology – Osteology – Non-adults – Adults – Forensic anthropology

INTRODUCTION

How important is anatomy for forensic anthropologists? This is a basic question that needs to be addressed in a monograph on forensic anthropology, especially when the monograph is published in an anatomical journal, as is this case. This question points directly to the current discussion of how a forensic anthropologist should be educated academically, which has been formulated by Sue Black and Soren Blau (Blau, 2016a). This paper starts posing the aims and scopes of both disciplines as a basis to discuss the relationship between forensic anthropology and anatomy.

Strictly speaking, forensic anthropology is the application of biological anthropology knowledge to legal cases (Black, 2008). Biological

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anthropology is the new term for physical anthropology. Currently, textbooks and academic departments mostly use the term biological anthropology instead of physical anthropology. The term of physical anthropology evokes antiquated focuses of the discipline, as for example racial typology. This is what caused the American Association of Physical Anthropologists to vote in favour of changing its name to American Association of Biological Anthropologists, in 2019. Therefore, biological anthropology is the scientific field that studies the human being from a biological perspective. Biological anthropology includes: the study of human and non-human primate morphology and evolution, human morphological development, human morphological variability and adaptation to different environments, human genetics and the biological bases of human behaviour (Valls, 1985; Ellison, 2018; Sanabria, 2019). The study of past populations of fossil hominins and non-human primates are basic to understanding: (i) the diversity within and between human populations; (ii) the course that human evolution has taken; and (iii) the processes that have brought about human evolution (Washburn, 1951; Valls, 1985; Ellison, 2018; Sanabria, 2019). Consequently, forensic anthropology refers to the study of living or dead humans, or what remains of the human, for medico-legal purposes (Black, 2008; Black et al., 2010; Wilkinson and Rynn, 2012; Cummaudo et al., 2014). In 2004, Red Cross developed the concept of humanitarian forensic actions (forensic humanitarianism) to guarantee that those who die in war, disaster and other complex emergencies are treated with dignity and do not become missing persons (Cordner and Tidball-Binz, 2017, Parra et al., 2020). Humanitarian cases (humanitarian forensic actions) are also included within the term of forensic anthropology (Cordner and Tidball-Binz, 2017, Parra et al., 2020). The terms of forensic osteology and forensic anthropology are often confused and interchanged (Black, 2008). Forensic osteology, as its name indicates, refers to that information recovered from the skeleton (Black, 2008). Forensic osteology is a sub-area of forensic anthropology, which in turn is a sub-specialty of biological anthropology.

Anatomy is a branch of biology. Anatomy focuses on the study of animal morphology and their variations (Rosse and Gaddum-Rosse, 1997). It provides a universal language for understanding how and why form supports function. Anatomy is intrinsically tied to developmental biology, embryology, comparative anatomy, evolutionary biology, and phylogeny, as these are the processes by which anatomy is generated over immediate (embryology) and long (evolution) timescales (Rosse and Gaddum-Rosse, 1997). The discipline of anatomy is divided into macroscopic and microscopic anatomy. Macroscopic anatomy, or gross anatomy, is the examination of animal body parts with the naked eye. Gross anatomy also includes the branch of surface anatomy (the study of the external features of the body of an animal). Microscopic anatomy or histology involves the study of the tissues of various structures and cells by optical instruments (Rosse and Gaddum-Rosse, 1997). Gross anatomy can be divided in systematic anatomy and regional anatomy. Systematic anatomy examines the body taking into account systems separately such as skeletal, vascular, digestive, lymphatic, etc. Regional anatomy examines the different systems as a whole located in a particular region of the body such as the head, chest, upper limbs and foot, etc. Human anatomy is of special interest within human biology, corresponding to a great conceptual framework that furnishes information to understand the human living or dead body. A general understanding of the systems of the body aids in an appreciation of a more detailed interrelationship between parts and regions. Consideration of the functional and clinical aspects of the human anatomy provides a better background on which to build human body knowledge. In current anatomical journals, there can be seen different published papers on cell biology, tissue architecture, neuroanatomy, evolutionary developmental biology, comparative functional morphology, evolutionary morphology, forensic anthropology, integrative vertebrate palaeontology, and palaeoanthropology, among others (see the web pages of different anatomical journals such as the European Journal of Anatomy, Journal of Anatomy, Anatomical Record, and Journal of Anatomical Science and Research, among others).

The objective of this paper is to evaluate the importance of anatomy in forensic anthropology using two different perspectives: a historical perspective and a practical perspective. The historical perspective refers to the common history of both sciences. The practical perspective refers to the analysis of three practical points: (i) the forensic anthropologist role; (ii) the 10 basic questions to be answered in any skeletal study firstly described by Ubelaker (1988); and (iii) the analysis of some forensic anthropological cases.

MATERIALS AND METHODS

A literature review of the history of human anatomy and physical (biological) anthropology, of the role and techniques of forensic anthropologists and of all published forensic anthropological cases was undertaken in bioanthropological, anatomical and forensic publications such as anthropological reference books as those of Brothwell (1962), Scheuer and Black (2000), Klepinger (2006), Ubelaker (1988), Komar and Buikstra (2008), Drikmaat and Cabo (2012), and Barone and Graen (2018), among others, and scientific journals such as American Journal of Physical Anthropology, American Anthropologist, Journal of Forensic Sciences, International Journal of Legal Medicine, Clinical Anatomy and Journal of Anatomy, among others. The search was structured from two perspectives: a historical perspective and a practical perspective. The historical perspective consisted of a review of the common history between forensic anthropology and human anatomy. The practical perspective included a search of forensic anthropology methodological/research papers. In these last papers, three types of assessment were done, which are: (1) the analysis of the role of forensic anthropologists; (2) the analysis of the 10 basic questions to be answered in any skeletal study firstly defined by Ubelaker (1988); and (3) the analysis of five forensic anthropological cases in which the importance of human anatomy in their resolution is evidenced. In fact, every forensic anthropological case is useful to evaluate the importance of human anatomy in their resolution. However, because of the great quantity of cases found in the anthropological literature, only five of the most engaging cases were chosen.

RESULTS

For a better comprehension of the text, the findings of this study have been split into four different sections, according to the different areas of search and discussed topics, and these are set up below.

Historical relationship between forensic anthropology and human anatomy

Physical (biological) anthropology had its origin in the 18th century with George-Louis Leclerc (Buffon) in France and Johann Friedrich Blumenbach in Germany, who created, respectively, the French and German Schools of physical anthropology (Varéa and Tomás, 2014). Buffon introduced the concept of "race" in the study of human variability and was the author of the *Histoire Naturelle, générale et particulièr,* which had a great influence among Naturalists. Blumenbach used the term anthropology in a non-philosophical sense, but as a naturalistic study of the human being for first time. He applied his comparative anatomical methods to his classification of human "races", of which he claimed there were five: Caucasian, Mongolian, Malayan, Ethiopian, and American. At this point, it is necessary to remark that, currently, the term "race" is not biologically accepted, because genetic differences between both sexes are greater than the genetic differences between different human populations (Lewontin, 1972; AAPA, statement on race, 1996; Relethford, 2003). Nowadays, to refer to different human groups, we use the terms populations or ancestry. After this clarification, and continuing with the historical relationship between forensic anthropology and anatomy, in the 19th century the French anatomist Paul Broca was awarded the first Chair in Physical Anthropology in the University of Paris. In the same century, a Spanish disciple of Broca, Federico Olóriz, was awarded the Chair in Human Anatomy in the Complutense University of Madrid and was responsible for introducing physical anthropology research in Spain (Valls, 1985; Reverte-Coma, 1991). Olóriz is well known

for his studies on the Spanish cephalic index and the creation of a Spanish cranium collection consisting of 1900 individuals. He is also famous for introducing fingerprint analysis as a human identification technique in Spain. At the end of the 19th century, in Europe, physical anthropology had crystalized enough to be recognized as a scientific field of research, and because of this, in this period, different publications and associations in physical anthropology were created. In 1865, the Spanish Anthropological Society was founded by the Spanish anatomist Pedro González; and in 1874, he also created the first Spanish anthropological journal called *Revista de Antropología* (Arquiola, 1981). Other names that are renowned in this period in Spain are Ángel Pulido Fernández and Manuel Antón Fernández, both from the Central University of Madrid (Márquez-Grant et al., 2011). In the rest of Europe, important names are Retzius, from the Karolinska Institutet (Sweden), de Quatreforges, from the Museum National d'Histoire Naturelle in Paris, and Topinard, from l'École d'Anthropologie also in Paris. In America, Hrdlička, educated in medicine and disciple of Topinard, created in 1918 the American Journal of Physical Anthropology and, in 1920, the American Association of Physical Anthropology (Shapiro, 1959, Brickley and Ferllini, 2007).

Current forensic anthropology originates from these first USA physical anthropologists such as Hrdlička, Dorsey and Tood (among others), who were intermittently asked to provided human identification services for the police (Kerley, 1978). In addition, the Second World War and Korean War gave the opportunity for trained physical anthropologists and anatomists to contribute to the recovery, identification, and repatriation of USA soldiers' remains (Dirkmaat and Cabo, 2012). In this period, research on different identification indicators were also developed by physical anthropologists such as Troter and Gleser (1952), McKern and Stewart (1957), and others, increasing skeletal knowledge (Dirkmaat and Cabo, 2012). In 1970, forensic anthropology was recognised as a new scientific field in the USA. This was influenced by Krogman's series of papers published in the *FBI Law enforcement Bulletin* (Dirkmaat and Cabo, 2012). In 1972, the American Academy of Forensic Sciences opened the Physical Anthropology Section (Dirkmaat and Cabo, 2012). In the eighties, forensic anthropology was increased with caseworks of human rights and abuses. Two examples of this are El Equipo Argentino de Antropología Forense (Argentine Forensic Anthropology Team) and forensic anthropologists international missions involved in the international criminal tribunal for the former Yugoslavia war crimes. At the end of the 20th century, forensic anthropology was well known in the world. Educational and training courses in forensic anthropology were asked in the different universities of the different countries, and the forensic anthropologist's skills were increased from bones to decomposed, burned, and fully fleshed remains, and even occasionally the living (Dirkmaat and Cabo, 2012). At this point, it is necessary to say that, sometimes, forensic anthropology is also called forensic physical anthropology to avoid confusing it with other anthropological actuations related to social anthropology and that sometimes are also called forensic anthropology (Márquez-Grant, 2018).

Nowadays, in Europe, physical (biological) anthropology, including forensic anthropology, is mostly located in Biological Sciences Faculties such as in the University of Barcelona (Spain), Pompeu Fabra University (Spain), the Autonomous University of Barcelona (Spain), University of Athens (Greece), University of Liverpool (UK), Durham University (UK), Bournemouth University (UK), Universität Hildesheim (Germany), and Masaryk University (Czech Republic), among others. There are also some groups focused on forensic anthropology (and human osteoarchaeology) in archaeological or anthropology schools such as the University of Leicester (UK) and Kent University (UK). There is also the Cranfield Forensic Institute in Cranfield University, which is a Defence Academy of the United Kingdom. In Canada, USA and Australia, departments of biological anthropology are in the anthropology school along with cultural anthropology (Blau 2016b), such as Simon Fraser University (Canada), University of Toronto (Canada), University of Nevada (US), University of

Illinois (US) and Australian National University (Australia), among others. In South America, the forensic anthropological groups are also in social anthropology departments; however, in these last cases, the objective is usually the study of archaeological human remains, except in some universities where forensic anthropology is closely related to victim identification (Sanabria, 2019). In all these countries, most forensic anthropologists are academically educated in social anthropology, and for this reason they are asked to be accredited by Forensic Anthropological institutions (Blau, 2016b; Sanabria, 2019). In the world, there are also some departments or units of forensic anthropology located at medical or health sciences schools such as the University of the Basque Country (Spain), the University of Granada (Spain), Loughborough University (UK), Centre Universitaire Romand (Switzerland), Boston University (US) and Antonio Nariño University (Colombia), among others. According to the American Association of Physical Anthropology, currently, around one in eight physical anthropologists have some professional affiliation with a medical school, usually in a department of anatomy. Some examples of current anatomists working on forensic anthropology or osteology in Europe are Sue Black from Lancaster University (UK), Francisco Pastor from the University of Valladolid (Spain) and myself from the Rovira i Virgily University (Spain), among others. Nowadays, there is the discussion about what discipline offers the best basis to form forensic anthropologists. The question is whether forensic anthropologists should be educated in medical or biological schools with a focus on anatomy or in humanity schools, which can offer relevant technical and social skills (Blau, 2016a).

It is clear that there is an intimate historical relationship between biological anthropology (and forensic anthropology) and human anatomy, and that the discipline of biological anthropology in part originates from human anatomy. However, biological anthropology also has the historical influence of zoology through naturalists. This last relationship is evidenced by both (1) the importance of human variability in biological anthropology and (2) the location of biological anthropological units in zoology departments when it is in a biology school, in Europe. On the other hand, zoology is in part animal anatomy. Zoology studies the animal form and structures, a part of their physiology, and this is included in the definition of anatomy, as it has been seen in the introduction.

The role of a forensic anthropologist

While a forensic pathologist applies his/her medical knowledge to establish the manner and cause of death through necropsies and the analysis of the corpse at the crime scene, the task of the forensic anthropologist mainly focuses on searching, recovering and identifying human remains. The objectives of a forensic pathologist are to know the types and cause of wounds, and causes of unexplained death, whether suicide, accidental, natural or violent. The objectives of the forensic anthropologist are to: (i) assist in the location and recovery of human remains, (ii) seek to provide an opinion on post-mortem interval, (iii) evidence of any foul play and (iv) analyse the human remains. They look for the morphological particularities of the individual, dead or alive, intact or fragmented, to determine the biological profile (anatomical parts which are present; age, sex, ancestry, and height, etc.), as well as to contribute to the reconstruction of the facts by means of the taphonomic principles and techniques, to answer legal questions (Velho et al., 2012). Taphonomy is the science that reconstructs past events or processes that lead to the formation of associated biological remains (Lloveras et al., 2016; Pokines and Baker, 2013). The contribution of taphonomy in forensic anthropological cases is seen in the third (Cultural factors influencing the identification) and fourth (Córdoba kids) cases exposed in the Forensic Cases as Evidence section in this paper. Currently, new trends in forensic anthropology are developing, due to the increasing role of forensic anthropologists in crime scenes. There is an increasing role of: DNA analysis, forensic archaeology, forensic taphonomy and trauma; and the human remain analysis can be done directly on bone or indirectly through imaging and remotely. These developing trends and techniques led to the creation of

new specialisations. In past times, one forensic anthropologist may have analysed all the human remains of a case. Currently, different forensic anthropologists with a variety of specialisations such as histology, trauma, taphonomy, age at death, craniofacial reconstruction, etc. can be found working on the same case.

In live individuals, forensic anthropology can be applied in complex cases, as for example in ambiguous genitalia (França, 2011) and in cases where there are doubts on the age of criminal responsibility of the criminal subject, when it is necessary to estimate the age of the individual (Silva et al., 2008). These methods of age estimation can also be applied in cases of border control, migration and human trafficking (Scheuer and Black, 2000; Doyle et al., 2019). This is necessary, because the law is different for minors and adults. For forensic cases, as in our social structure, a minor is one who is younger than 18 years of age. However, from a biological point of view a sub-adult individual (a non-adult) is one who is younger than 21 years of age (Rissech, 2016).

In dead individuals, and depending on the police necessities, forensic anthropologists can be asked to estimate the biological profile of skeletonized, mummified, decomposed, mutilated or burned bodies with the objective to identify the individual and to contribute to the determination of the cause of death, identification of the circumstances that caused the death (Silva et al., 2013). However, they can also only be asked to perform trauma analysis, craniofacial reconstruction, etc. or just DNA sampling. Corpses can be classified as fresh (or recent) and complex corpses (Muñoz-Silva, 2019). A fresh corpse is one that has all its soft tissues, and its biological profile is relatively easier to obtain. A complex corpse is one that shows alterations that hinder its identification as a body in advanced decomposition, skeletonization, carbonization or a dismembered body (Muñoz-Silva, 2019). The process of identification of complex corpses is different from that performed in fresh corpses, since complex corpses have conservation states in which there is neither external nor internal genitalia, no face, and no fingerprint. Examples of this are the cases of disrupted human remains of recently deceased individuals derived from a massive disaster or fatal accident, intentional dismemberment or fragmentation following a homicide, or, for example, coming from the results of scavenging activities of predators or showing extreme stages of decomposition or preservation. In these cases, previous information on the individual identity does not usually exist, which complicates the resolution of the case. In some cases, when the search is focused on a particular individual because he or she disappeared or can be the victim of a crime, the remains found have a potential identity (this particular individual), which facilitates the investigation. This allows comparing both DNA obtained from the remains and that from the family members of this particular individual (potential identity). The existence of this potential identity also allows comparing the dental pattern of the unidentified remains (if there is a complete mandible or maxillary) with the previous dental records of the individual that corresponds to the potential identity. Both DNA and dental records will indicate whether the remains correspond or not to this possible individual (potential identity). In the cases where there is not potential identity (a possible individual), bone and dental diagnoses are used in order to construct the biological profile of the corpse/remains (sex, ancestry, age, stature, etc.), which will be cross checked with databases of missing persons. If a possible identity is not found, a facial reconstruction can be done to be shown in the media, just in case it is recognised by someone. In the case that, during the search, a possible identity is got and their possible family members exist, a DNA test will be done between possible family members and the remains.

The ten basic questions to be answered in any skeletal study

Usually, the process of identification of a complex body is based on its bones and teeth because of the lack of soft tissues or because of the advanced state of decomposition. In these anthropological analyses, forensic anthropologists must respond to the 10 basic questions related to those bones and teeth, which are the following (Ubelaker, 1988): (1) Is it a bone? (2) Is it human? (3) Is it modern? (4) How many bones are there? (5)

How many people are there? (6) What biological population does it belong to? (7) Which sex is it? (8) How old is it? (9) How tall is it? (10) What are the individual characteristics of the remains? What pathologies, traumatisms, stress markers and enthesopaties does it have?

The identification of isolated, fragmented or commingled remains, which are habitual in mass disasters and violent conflicts (Mundorff and Vidoly, 2018), can be difficult even for a specialist. Burned plastics and small stones can look like a bone and they can be confused with a bone. The damage to remains can be great, thus making them small and hard to identify. Sometimes, human remains may be commingled with faunal remains and they may have been sufficiently altered by weather elements, cultural practices, or exposed to extreme temperatures, so that anatomical features are not readily appreciated.

Usually, one of the first difficulties to resolve is the differentiation between human and non-human bones. The faunal bones that more frequently are wrongly identified as human are those from deer, bear, pig and dog, and young cow and horse (Ubelaker, 1988). Particularly, and as examples, bear carpal and tarsal bones can be confused with human carpal and tarsal bones. Pig teeth (Fig. 1) can also be confused with human teeth, particularly molars, and some chicken bones can be confused with human perinatal bones (Fig. 2) - A perinatal individual is one who is around birth, between 30 and 42 weeks of gestation. For recognizing human bones, anthropologists use gross bone morphology; however, in these cases of human and non-human bone mixture it is useful to remember that the long bones of most adult animals, which are a similar size to humans, have different bone architecture and thicker and more compact cortical bone (Ubelaker, 1988). In those extremely difficult cases, in which bones are fragmented and also morphologically modified due to some disease, surgery or outdoor exposure, bone microscopic morphology (Histology) can be used. In humans, osteons are dispersed and uniformly distributed in the circumferential laminar bone (layer aspect), while in many animals they tend to line up in rows called osteon bands (Andronowski et al., 2017).

Forensic anthropologists usually do not have to deal with very old material because they usually work on modern individuals belonging to forensic cases. Although sometimes the question about the modernity of a bone is usually reduced to distinguishing whether the remains are modern

Fig. 1.- Some molars and a canine from a common pig.

or archaeological, in these cases the forensic anthropologist has to look around the remains observing the context and the stratigraphy of the site. However, the limit between modern/forensic and archaeological can be a small boundary, which varies between different countries in a range of 30 to 100 years, and because of this some remains such as those of the Spanish Civil War are between forensic and archaeological (Márquez-Grant et al., 2011).

When the skeletal remains are indeterminate in that they cannot be associated with any particular skeleton, the forensic anthropologist calculates the minimum number of individuals (MNI). This is done by taking into account the number of repeated bone elements. That is to say, forensic anthropologists classify the bones by taking into account their morphology (joint congruence, same pathological set and symmetry of even bones), colour, sex and degree of maturation. This classification allows one to estimate the MNI present in the sample (Brothwell, 1987; Bradley and Byrd, 2014).

The estimation of sex and age-at-death is based on the morphological differences observed between men and women at different ages (sex differences) and on the morphological differences in each maturational stage (age differences) for each sex. In adults, where secondary sexual features have developed, sex estimation is relatively easy. However, in sub-adults, particularly in children, foetuses and perinatal

Fig. 2.- Perinatal human clavicle (at left) and chicken humerus (at right).

individuals, sex estimation is difficult, because in these immature individuals the secondary sexual features (robustness of the bones, muscular insertions and the features that give amplitude to the pelvis) have not developed yet (Scheuer and Black, 2000; Rissech, 2016). Contrarily, in sub-adult individuals age estimation is more precise, because they are growing, and changes occur in predicted times (Scheuer and Black, 2000; Rissech, 2016). In adults, growth (quantitative process expressed by an increase in body size and number of cells) has finished and the maturation process (qualitative process expressed by changes in the structure, composition and functioning of cells, organs and body in their conjunct) continues; however, this maturational process is less genetically controlled than in sub-adults. This contributes to an increase in the variability of the morphological changes due to ageing, which in turn contributes to the increase in the age estimation error (Rissech, 2016). To help to diminish the error of estimation, in 2019 the IDADE2 web page was created. This is a free web, which allows estimating the age at death based on probabilities and Bayesian inference (Rissech et al., 2019). In fact, adult age estimation is a difficulty to be resolved, because our population lives long and the older a person is, the bigger the estimation error. In addition, the best-known adult age estimation methods such as Todd (Todd, 1921), Lovejoy (Lovejoy et al., 1985), Suchey-Brooks (Brooks and Suchey, 1990), Hartnett (Hartnett 2010) and other, are up to 60 or 70 years of age, depending of the methods. This limit of age makes difficult to estimate age of the most elderly individuals, increasing the error of estimation (Rissech, 2016). Therefore, to have an accurate adult age estimation, morphological age changes need to be precisely and meticulously evaluated by the forensic anthropologist. Usually, she/he needs specific training in adult age estimation to be competent in this technique (Rissech, 2016).

The estimation of stature and ancestry is part of the identification process of a non-identified skeleton or of dismembered body parts. Even though measurements of body parts such as a vertebra or a metacarpal bone can be used to estimate the stature, the use of long bones is preferable, because they are intrinsically related to stature, particularly femur (Rissech et al., 2008). Estimation of the stature is done by the application of equations in which the length of the bone is introduced as variable and the equation gives you a result, corresponding to the stature of the individual. There are several equations to be used. The best-known are those of the Trotter and Gieser (1952) based on USA samples. However, these equations systematically overestimate stature in both female and male skeletons from the Spanish and Italian populations (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). In these Mediterranean populations, the equations of Pearson (1899), based on a French sample, perform better. This is probably due to the intertwined biological population history of French, Spanish and Italian populations (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). Ancestry assessment represents an important part of forensic anthropological analysis. Its interpretation, together with the other aspects of the biological profile, can be clues to narrow the search for missing persons and facilitate positive identification of the remains. The skull, particularly the mid region, is universally accepted to be the most informative region in relation to ancestry (Cunha and Hubelaker, 2019). However, there is not a single trait that can be found only in a single population. This is due to human variability. Forensic anthropologists use multiple traits as a guide to the most probable biological origin. The list of skull non-metric traits is particularly large: they can be found in Hauser and De Stefano (1989) and Hefner (2009). In addition, Hefner and Ousley (2014) created OSSA a statistical programme which quantifies the probability of belonging to a given population for a particular individual. Cranial and postcranial metric traits can also be used for ancestry assessment. FORDISC software based on a maximum of 34 cranial and 39 postcranial measurements and through discriminant functions is able to classify individuals according to their ancestral group (Ousley and Jantz (2013).

Pathologies, traumatisms, stress markers and enthesopaties are useful to help to individualise

individuals (Ortner, 2003). They can be like a mark, which differentiates a particular individual from a group of individuals that share similar characteristics such as sex, age, ancestor, stature and skeletal constitution.

In spite of all of these anthropological techniques and methods, sometimes the answer to these ten questions can be obstructed by the influences of several factors such as humans, animals, plants and weather elements. This is the field study of taphonomy.

The influence of taphonomic agents on bones

There are several factors that can morphologically modify the bones such as human (cultural or criminal), animal, plant and environmental influences. Cultural modifications can be intentionally or non-intentionally done in live individuals or after death. The intentional modifications are usually due to cultural or aesthetic reasons such as the cranial deformations (Reina et al., 2016), and incisions and inlays in teeth (Ubelaker, 1988; Ferré, 2013). However, they can be also criminal modifications such as bone cuts and cut marks which can be related to dismemberment, skinning, disarticulation, defleshing or scraping (Botella et al., 1999; Lloveras et al., 2016). The non-intentional modifications are usually related to non-intentional behaviours, as for example the custom of squatting which causes the squatting facets in the distal epiphysis of the tibia (Ubelaker, 1988). The human modifications that originated after death are usually related to the burial process or other rituals. However, they can also be of criminal origin, for example to hide identification of the victim. Particularly, in this last case, lack of knowledge of the morphological results of these processes can lead the forensic anthropologist to an inaccurate interpretation of the bones observations (and thus the sequences of facts that occurred in the crime) and leave, for example, the victim unidentified, favouring the criminal.

Animals, plants, and weather elements can also modify the bones. Animals can usually fragment and move the bones, and crush the epiphyses. They can also leave beak, tooth and digestion marks. Plant activity can importantly affect human remains, causing different types of chemical and mechanical alterations, and affecting the survival of the remains (Lloveras et al., 2016; Pokines and Baker, 2013). Exposure of skeletal remains to weather elements can lead to the gradual decomposition of bone materials as a result of chemical and mechanical transformations supported by different agents such as sun, wind or rain, as well as temperature and humidity variations (Lloveras et al., 2016). The main feature of the actuation of these factors is the whitening of the bone. The appearance of longitudinal and parallel fissures/cracks, the exfoliation of the bone surface (Fig. 3), and the appearance of a fibrous and rough texture are also the result of weather elements (Lloveras et al., 2016; Pokines and Baker, 2013). In addition, pathologic influences can modify the morphology of the bones, hindering the identification of bone type and the species to which it belongs. To know how to interpret all these types of morphological modifications suffered by bones is absolutely necessary to resolve forensic anthropological cases (or to interpret archaeological bone remains). This taphonomic knowledge is necessary to identify correctly the remains and to reconstruct possible sequences of the facts, as will be shown below in the Forensic Cases as Evidence section in this paper.

Forensic cases as evidence

Five forensic cases illustrate the application of these previously exposed concepts and demonstrate the significance of human anatomical knowledge for forensic anthropologists

Case 1 – The bone recognition and inconclusive identification (Modified from Ubelaker 1988)

In a street in Chicago, some small fragments of bone were found in a human blood puddle. Circumstantial evidences suggested that a man had been shot in the head. In addition, the microscopic structure of those small pieces of bones was compatible with human cranial bone microscopic structure. However, the observation of osteons distribution with a similar pattern to other animals made the identification inconclusive.

Fig. 3.- Frontal of adult horse showing the effects of weathering. Whitening and flaking of outer bone layers are observed.

This case evidences the importance of human anatomy (both gross and microscopic anatomy) in forensic anthropological cases. In addition, this case also evidences the difficulty that can exist in the assessment of small pieces of bones and their importance in resolving forensic cases. However, as in this case, sometimes, the difficulty of recognising bones can lead us to an inconclusive identification.

Case 2 – Cultural factors influencing the identification (Modified from Ubelaker 1988)

In Alaska, a bone fragment without epiphyses, displaying a non-consolidated fracture, was found. The fracture had been surgically treated by a metallic plaque. Bone growth around the plaque indicated that the surgery had taken place a long time previously. Therefore, and because of the surgery, it was assessed as human. However, efforts to find the surgeon were fruitless. In the end, a microscopic analysis of the osteon pattern was carried out. Results revealed a band pattern like that of a big dog, indicating that the surgeon was a veterinary.

The conclusion of this case is that we cannot get carried away with the appearances of the case. The most important thing is the anatomy and the anatomical observation, in this case the morphology of the osteons.

Case 3 – Comparative anatomy, documented faunal collection complementing the identification (Modified from Ferreira da Silva et al., 2013)

In 2005, some skeletal remains were found in a desert area of North America. These remains were initially attributed to a missing person who possibly was the victim of a homicide. The skeletal remains consisted of four bones and one tooth. Three of the bones were long bones with fused epiphyses, compatible with upper or lower limbs. These three long bones did not display any point of fracture. The fourth bone was the smallest bone. It was fractured irregularly and was compatible with a vertebra fragment. The

tooth had one root with a curve, compatible with a canine tooth. Through the morphological analysis of these four bones, mainly epiphyses and mussels origin and insertions areas, it was concluded that it was a non-human origin, belonging to an adult animal of medium size. The analysis of the tooth indicated that it had different size and shape from a human adult canine tooth, which reaffirmed the non-human origin.

To determine the animal specimen from which the skeletal elements came from and in order to be sure of their faunal origin, these bones were morphologically compared with a documented faunal collection. Results indicated the compatibility of the forensic material with the tibia, femur and canine tooth of a domestic dog of medium size (*Canis lupus familiars*).

This case, apart from indicating the importance of human anatomy in any forensic anthropological study, also indicates the importance of the comparative anatomy and the documented faunal collections to conclude some forensic cases.

Case 4 – Córdoba kids (Modified from Alvarez (2018) and Bravo-Cuiñas (2012)

The case of the Córdoba kids is well known in Spain. On 8 October 2011, a father reported the disappearance of his two kids in a Park in Córdoba city in Spain. He stated that someone took them in an oversight when he went to his car for a moment. The kids were siblings a 6 year old girl, and a 2 year old boy. The father had taken the two kids to spend the weekend with them at his family farm. He had agreed this with his wife with whom he was in the process of separation. That day, whilst the children were eating lunch in the father's family farm, he took the opportunity to burn memories of his coexistence with his wife. Pursuant to the father's declaration, when the kids woke up from the nap, he took them to play in the park. At 18.40 hours on this day, he reported the disappearance of the children by phone to the police. However, according to the police's investigations, this call was made from the farm, not from the park. In addition, the security cameras surrounding the park recorded the father alone this day. There was not any evidence that the children had ever been in the park in the afternoon of that day.

On 10 October, the Scientific Police made the first search for the kids in the farm. They found the remains of a large bonfire, where the father had allegedly burned memories and photographs of his marriage. In this bonfire, there were also some skeletal remains. The forensic anthropologist of the Scientific Police reported that these bones corresponded to a dog. On October 11 and 12, the search of the kids was intensified in the farm and surroundings. Results were null.

After eleven months of no results, the mother's family asked for a counter-report on the bones found in the bonfire of the farm by an independent forensic anthropologist. This new forensic anthropologist was well known as a judicial expert in many judicial proceedings, inside and outside Spain, including cases of torture by the Spanish law enforcement agencies. In the Córdoba kids case, this new anthropologist analysed about two hundred pieces of bone and nine teeth (Fig. 4). He concluded that they were the remains of two children between two and six years of age. This report resulted in the Police Commission asking for a third anthropological analysis of the farm's bones by another well-known independent anthropologist. This last anthropologist was expert in children's teeth and bones. The conclusion of this third anthropologist was the same as that done by the anthropologist appointed by the wife's family: the farm's bones involve bones of two different infantile individuals, particularly teeth and talus (Fig. 4).

According to the report of the forensic anthropologist hired by the wife's family, the father did not make a simple fire, but practically built an oven with bricks and a metal plate to achieve greater combustion. Both the bone colour and the cracks morphology in the bones were evidence of the fire's very high temperature. When the bones burn at high temperatures, they whiten and the cracks are transversal to the longitudinal axes of the bones (Fig. 5). These higher temperatures usually destroy DNA, because of this, this forensic anthropologist asserted that it was impossible to find DNA in these bone remains, which indicated the intentionality of impeding to check DNA between bones and kid's family and therefore the objective of hiding the victims' identity.

Fig. 4.- Teeth and two tali of different size found in the bonfire of Las Quemadillas made by Bretón. Modified from Bravo-Cuiñas (2012) published in "El Mundo, Andalucia" in *August 28th*.

If the case had been resolved from the first bone analysis on October 10, the family's suffering, the money spent on almost 11 months of searching for the children and subsequent analyses would have been reduced to some days.

Case 5 – Minimal expression of a zygomatic bone (Modified from Black, 2008)

A man announced the disappearance of his wife. According to him, he had not seen his wife for two days. He explained to the police that at 4 pm on 30

Fig. 5.- Example of bones burned at high temperatures. They show white colour. The cracks are transversal to the longitudinal axes of the bones.

November she went shopping at Sainsbury's local, which was located 12 minutes from their house. This couple lived in an apartment building with a door attendant. The police interrogated neighbours and the door attendant. None of them had seen her on 30 November and much less at 4pm of that day. The door attendant said with certainty that she had not left her home that day. The husband responded to this assertion, that perhaps at 4pm on 30 November the door attendant was busy with things of the building, which probably did not allow him to see how and when she left the building. However, apartment building video surveillance cameras showed that she had never gone out of the building that day. In addition, supermarket video surveillance cameras indicated that she never was shopping in the Sainsbury's local on 30 November. The husband insisted that she went shopping at 4 pm on 30 November. He suggested that the apartment building cameras perhaps had some problems that time that day. He also suggested that perhaps she got disoriented and lost, and maybe she never arrived at the supermarket. The police searched in the couple's house with no success. They again asked friends and neighbors about her. There was no evidence of her escaping from home or kidnapped. Later, the police decided to search the couple's flat more thoroughly. They looked for blood or some human remains in each small corner of the flat, as for example the sink, bath, laundry and bathtub drain. Particularly, the police found the wife's blood in the bathtub drain. The husband explained that she got cut when she was having a bath 3 days previously. The police thought that this was logical and possible. Therefore, they did not consider this blood evidence. However, later, they looked into the washing machine filter and found a small piece of bone. After an intense analysis, the forensic anthropologist arrived at the conclusion it was a human bone, concretely it was a small part of a human zygomatic bone. In this case, the husband could not argue that she hit the washing machine by chance, living a piece of a cheekbone in the filter of the washing machine.

This case illustrates again the importance of human anatomy in forensic anthropological cases and the necessity of having a deep knowledge of this scientific field.

DISCUSSION

Throughout this paper, the importance of human anatomical knowledge in forensic anthropology and therefore in forensic osteology has been evidenced. This paper aims to illustrate the inseparable bond between forensic anthropology/osteology and human anatomy thereby justifying the necessity of increasing anatomical knowledge in the academic education of physical (biological) anthropologists, particularly forensic anthropologists/osteologists (and human osteoarchaeologists). In addition, it is crucial to highlight the importance of forensic anthropology along with eliminating the common belief that forensic anthropology is just about a pile of bones.

Although, traditionally, forensic anthropologists worked with dry, complete, and fragmentary bone remains, nowadays they also face a wide range of differentially preserved human remains that include fleshy, decomposed, burned, dismembered and very disturbed remains (Blau, 2016a). In addition, as is shown in this monograph on forensic anthropology, current anthropologists are also asked to assess identification questions in: (i) clinical cases, as for example, the age estimation of an individual (see Black et al., 2010; Doyle et al., 2019); (ii) cases of living individuals, which can involve facial and body measures, craniofacial identification and fingerprint recognition (judicial anthropology); and (iii) cases involving missing persons and victims of crimes, accidents, mass disasters, and crimes coming from periods of violence because of ethnic, political and religious reasons (Blau, 2016a).

The common history between physical (biological) anthropology and anatomy evidences the intrinsic relationship between both disciplines. Most of the first physical anthropologists were human anatomists. This also evidences the inherently close relationship between forensic anthropology/osteology (and human osteoarchaeology) and human anatomy. Each forensic anthropology/osteology (and human osteoarchaeology) study runs within the field of the human anatomy. The forensic anthropologist and osteologist analyse the form, aspects and metrics of every bone to facilitate

the identification of the individuals to whom the remains belongs. Forensic anthropologists and osteologists need a knowledge of human biology in general, together with some chemistry, physics and mathematics, and a sound understanding of human anatomy (Klepinger, 2006). This is observed in each anthropological technique and the detailed knowledge of every bone of the human body that forensic anthropologists must have. This anatomical knowledge is necessary to recognise each tiny part of a bone (as in the zygomatic bone case), avoiding identification being inconclusive. In addition, forensic anthropologists must be able to work indiscriminately on remains of different maturation stages such as foetuses, new-borns, children, juveniles and adults, implying a great and wide knowledge of human anatomy. It is important to recognise human skeletal material and not confuse it with faunal remains (as in the Córdoba kids' case). The definitive and early resolution of the case is of extreme importance (Holobinco, 2012) to avoid the suffering of the victim's family and not waste time, effort and money unnecessarily. The quick resolution of the case mostly depends on the anatomical knowledge of the forensic anthropologist/osteologist. The lack of anatomical knowledge, and particularly the ignorance of the childhood anatomy, was the cause the eleven months delay to the resolution of the Córdoba kids' case. In contrast, the extensive human anatomical knowledge of the forensic anthropologist resulted in the resolution of the extremely difficult case of the tiny fragment of a zygomatic bone which could have slipped past during the search. In addition, histology (microanatomy) helped to overcome the appearances of the third case (cultural factors), in which the presence of a surgery (cultural factors) was influencing the opinion of the anthropologist, who was dubious about the biological origin (species) of the remains. He/she thought that they belonged to a human when in reality the bones belonged to a big dog with a surgery. Therefore, there is no doubt of the importance of anatomy in the full sense and human anatomy in particular, in forensic anthropology.

This unquestionable importance of human anatomy (and human biology) in forensic anthropology points to the current discussion about the academic education of forensic anthropologists. Over the last 20 years the media has contributed to interest in the term "forensic", leading to an increase in the number of courses that start or finishes with this term (Black, 2000; Blau, 2016a, 2016b). Many of the courses on forensic anthropology and osteology offer a superficial introduction to osteology and technical principles of the discipline, but very few provide a thorough education in practical and technical abilities, and much less in human anatomy (Blau, 2016a). It is of vital importance that forensic anthropology students be prepared in anatomy (at least in musculoskeletal system), making them able to interpret every tiny crease or wrinkle of the bones, and preparing them to deal with a wide range of human remains of different ages, sexes and different states of preservation. In addition, they should be prepared to deal with unpleasantness, for example bodies in advanced states of decomposition, semi eaten by animals or remains resulting from the actions committed by criminals to their victims to hide evidences (Wright et al., 2005).

In America, Australia and some European countries, social anthropology and physical (biological) anthropology (and thus forensic anthropology) are in the same school or department of anthropology together with archaeology. In light of the growing role of anthropologists in dealing with life (Black, 2010) and managing the identification of victims of large-scale mass disasters (Blau, 2016a), what discipline offers the best basis for forensic anthropology is under discussion. Should forensic anthropologists be academically educated in medical or biological schools with a focus in human anatomy or should they be educated in humanities schools, which can offer technical and socially relevant abilities? (Blau, 2016a). However, although human anatomy is basic for forensic anthropologists, knowing the different beliefs and behaviours in relation to death in different cultures and peoples and how they deal with it is also important. For example, in Western countries the physical contact with the dead and the dying is avoided (Blaauwn and Lahteenmaki, 2002). In contrast, in some Muslim cultures, prior to burial, the body of the deceased

is washed by a relative. While some cultures do not have any problem to display human remains (Blau, 2016b), as in the case of Capela dos Ossos (Bone Chapel) in Evora (Portugal) and All Saints Cemetery Chapel in Sedlec (Czech Republic), other cultures, such as the indigenous of Australia and Maori communities, are very offended if the remains of their ancestors are displayed (Blau, 2016b). As we have seen in the Results section, the academic education received by forensic anthropologists in different countries varies greatly (Márquez-Grant et al., 2016). In addition, most of the university degrees in anthropology, particularly those of US do not highlight training in physical (biological) anthropology and osteology (Klepinger, 2006). It is true that whatever training one chooses to undertake, the important thing is the anthropological experience that one acquires along one's career in forensic anthropology, the experience in forensic and osteological caseworks and internships (Klepinger, 2006) and different types of remains and different states of maturation. However, as we have also seen along with this paper, human anatomy is basic in any forensic anthropological case. A forensic anthropologist must have a sound anatomical (and biological) basis of the human body (Klepinger, 2006). This is extremely important to resolve each case and help to get a conclusive identification of the analysed remains. Therefore, schools, whether medical, biological or humanities/social, should provide forensic anthropology students with a great foundations in human anatomy, together with some taphonomy, chemical, physics and mathematical knowledge.

CONCLUSION

The information provided by this study furnishes information on the state of current forensic anthropology and demonstrate the close relationship between forensic anthropology and human anatomy. It is clear that historically, there is an intimate relationship between forensic anthropology and human anatomy. Forensic anthropology developed from physical anthropology, and this last science originated, in great part, from human anatomy. Not only is the history of forensic anthropology related to human anatomy, but the role and techniques of forensic anthropologists are also within the human anatomy field. Each forensic anthropology (and human osteoarchaeology) study runs within human anatomy. The forensic anthropologist's tasks include in part focusing on searching, recovering and analysing human remains; they also focus on a detailed and exhaustive analysis of the form, aspects and metrics of the remains to facilitate the identification of the individual to whom they belonged, or the bone, if it is one tiny bone, as we have seen in the Results section of this paper. Forensic anthropologists must be able to work indiscriminately on remains of different maturation stages such as foetuses, new-borns, children, juveniles and adults, implying a great and wide knowledge on human anatomy. In addition, currently, they can also be asked to assess: clinical cases, as for example age estimation; cases of living individuals, which can involve facial and body measures, craniofacial identification and fingerprint recognition; and cases related to missing persons and victims of crimes, accidents, mass disasters, and crimes coming from periods of violence because of ethnic, political or religious reasons. The roles and techniques (DNA analysis, forensic archaeology, forensic taphonomy and trauma) of forensic anthropologists are increasing, which generate new forensic anthropology specialisations. Formerly, one forensic anthropologist may have analysed all the human remains of a case. Currently, different forensic anthropologists with a variety of specialisations such as histology, trauma, taphonomy, age at death, craniofacial reconstruction, etc. can be found working on the same case. Therefore, human anatomy is basic in forensic anthropology. The forensic anthropologist and osteologist must have a sound anatomical and biological basis of the human body to resolve each anthropological case. Consequently, schools, which offer a degree in forensic anthropology, whether medical, biological or humanities/social, should provide forensic anthropology students with powerful foundations in anatomy.

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Redefining forensic anthropology in the 21st century and its role in mass fatality investigations

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SUMMARY

Forensic anthropology is a dynamic field, with its definition having to reshape itself and expand continuously. The challenges faced as well as its potential have grown in synergy with other areas in forensic science. In academia, as well as in practice, the discipline has seen much specialisation in different areas. In particular in recent years, the importance of the forensic anthropologist has led to an increase volume of casework for these scientists, and an important role in disaster victim identification worldwide. This paper sees the need for a revised definition which reflects the nature of the work, both with the living as well as with the dead, in individual domestic cases or international human rights' investigations. A review of the work undertaken by forensic anthropologists is provided with special emphasis on mass fatality incidents. This paper also explores the discipline's contribution to DNA analysis and positive identification. It is important to highlight the forensic anthropologists' role and their potential in any deployment of a National or International Disaster Victim Identification team.

Key words: Forensic Anthropology – Mass disasters – Disaster Victim Identification – Human remains – Fire scenes – DNA

INTRODUCTION

Definitions of forensic anthropology have reflected the evolution of the discipline over the past 20 years. A commonly accepted definition is currently "the application of the science of physical or biological anthropology to the legal process" (e.g., see ABFA, 2018), although this has now expanded to include human rights cases, as part of what some have called 'Humanitarian Forensic Action' or 'Forensic Humanitarianism' (Guyomarc'h and Congram, 2015; Cordner and Tidball-Binz, 2017; Moon, 2013; Parra et al., 2020). More recently, historical cases involving the recovery, identification and repatriation of casualties from the First and Second World Wars and subsequent conflicts have also been categorised as forensic anthropology (Loe et al., 2014; Cox et al., 2016; Emanovsky and Belcher, 2012). The expansion of the definition in these contexts recognises the need for the

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anthropologist to conform to the standards required by forensic genetic laboratories. He or she is required to work closely with DNA scientists and be cognisant of forensic protocols relating to chain of custody, integrity of evidence and how to minimise the risks of cross contamination and misidentifications.

The original remit of the forensic anthropologist was to help with the identification of the deceased and he or she was based in the laboratory or mortuary. The role has now expanded to crime scene attendance, starting with understanding the context of human remains and assisting with recovery (Dirkmaat et al., 2008; Blau, 2018). Today, forensic anthropology has evolved into different specialities and stronger ties have developed with forensic pathology, odontology, radiology, genetics and crime scene investigation. In many countries, the interrelation with archaeology is also particularly strong (Blau and Ubelaker, 2016; Hunter et al., 2013; Groen et al., 2015). From an initial focus on skeletonised remains it has now been recognised that the skills of the forensic anthropologist can also be applied to the examination of fresh, decomposed, mummified and burnt bodies from a variety of contexts. Previously working as assistants to forensic pathologists primarily, forensic anthropologists now often take the lead in certain types of humanitarian and mass fatality scenarios, collaborating with and advising the pathologist, although it must be emphasised that it is still not the role of the forensic anthropologist to determine cause of death in a forensic setting. In addition, some management of the unidentified decedents and other roles have been undertaken by forensic anthropologists within a Medical Examiner or Coroner's office (Crowder et al., 2016).

Although much of the work of the forensic (physical) anthropologist deals with the dead, not all definitions of forensic anthropology have encompassed the fact that some also study the living. This skill-set is sometimes "country specific", depending on the educational background of the forensic anthropologist and the organisation they work from which varies between nations (e.g., see Kranioti and Paine, 2011; Obertová et al., 2019; Christensen et al., 2015). Thus, the forensic anthropologist can assist with gait analysis, age estimation in the living or identification of a suspect (Black et al., 2010; Beh and Payne-James, 2010; Cattaneo, 2007; Schmeling et al., 2008; Black et al., 2010). In some cases, there has been also an awareness of the value of forensic anthropologists with assisting in the identification of living individuals left unconscious from a mass disaster (Quatrehomme et al., 2019; De Boer et al., 2019).

Further specialisation in the field has been increasing too, as methods become more niche, and what was an examination of human remains by one anthropologist may now require several, with different areas of expertise, on a single case. Finally, in previous and current definitions of forensic anthropology it is important to consider whether the term should be limited to just those examining the body, as anthropology in its broadest sense also encompasses social and cultural anthropology. There has been some mention of including these branches of anthropology in the definition of forensic anthropology, or conversely, defining the discipline as we currently understand it more narrowly as 'forensic physical anthropology' (Maples, 1980; Lovis, 1992). It is known that a number of social and cultural anthropologists have worked in forensic or judicial cases (e.g., Rosen, 1977; Turner, 1992; Burke, 2011; Trigger et al., 2013; Holden, 2019). These experts may provide opinion regarding cultural practices around the dead, provide information on cultural and bio-cultural indicators or modifications on a body. They may also work closely with families in mass fatality incidents or human rights cases. In these instances, it is true that some of the anthropologists who undertake the examination of remains have also been trained in social and cultural anthropology, so in this context it could indeed be classified as 'forensic anthropology'.

The history and current status of forensic anthropology and the methods used within the discipline have been covered extensively in the literature (e.g., Klepinger, 2006; Komar and Buikstra, 2008; İşcan and Steyn, 2013; Tersigni-Tarrant and Langley, 2017; Blau, 2018; Ubelaker, 2018; Ubelaker et al., 2019a). A number of specialised books have been written on trauma,

ethics, dismemberment, and burnt remains (e.g. Schmidt and Symes, 2015; Ross and Cunha, 2019; Thompson, 2001; Walsh-Haney and Lieberman, 2005; France, 2012; Blau, 2016a; 2016b; Passalacqua and Pilloud, 2018; Márquez-Grant et al., 2019), bias (Nakhaeizadeh et al., 2014; Passalacqua and Pilloud, 2018), and specific methods for estimating age, sex, etc., as well as reviews on research trends worldwide (Lei et al., 2019). The importance of forensic anthropology as well as archaeology has increased primarily since the Human Rights Investigations of mass graves in Latin America in the 1980s and subsequently the Balkans in the 1990s. Although initially much work concentrated in the identification of the deceased in the laboratory or mortuary, as Dirkmaat et al. (2008) indicated, this has recently expanded to attending the crime scene to understand the context in which the remains were found, and encompassing the field of forensic taphonomy. Moreover, this now extends to participating not only in the recovery of human remains in the field but also actively participating in the search for missing persons (Márquez-Grant, 2018). The forensic anthropologists works closely alongside the forensic archaeologist or they may use their own skills depending on their background and training (e.g., Hunter and Cox, 2005; Ferllini, 2007; Dupras et al., 2012; Hunter et al., 2013; Groen et al., 2015; Barone and Groen, 2018). With this in mind, we consider that a revised definition which encompasses the contribution forensic anthropologists provide in the search, recovery (whether applying archaeological knowledge or working alongside a forensic archaeologist), identification and management of the deceased, the interpretation of circumstances surrounding death in medico-legal and humanitarian contexts, and the identification of the living, could be proposed. Of course, social and cultural anthropology may well be used in casework and this may also be part of the physical anthropologist's training. Likewise, in some countries forensic anthropologists may already work as forensic pathologists. The suggested definition below, therefore, reflects how the role of the forensic physical anthropologist, who may work alongside others experts in the field of forensic medicine, forensic odontology, social and cultural anthropology, etc., has evolved in recent times:

"Forensic Physical Anthropology can be defined as the analysis of the human body to assist with the identification of the living and the dead, the recovery and repatriation of the deceased, and the interpretation of circumstances surrounding death in medico-legal and humanitarian contexts".

In addition to domestic casework, human rights missions and other operations, and increasing specialisations in the fields of imaging, histology, trauma analysis, and other methods used to produce biological profiles; the role of the forensic anthropologist in mass fatality incidents has become more prominent and its contribution continues to be recognised (e.g., Hinkes, 1989; Kahana and Hiss, 2009; Mundorff, 2008, 2011; Dirkmaat, 2012; de Boer et al., 2019).

This paper considers the role of forensic anthropologists in mass fatality incidents, highlighting the value and the information that forensic anthropology can provide. A review of the work undertaken by forensic anthropologists is followed by an exploration into some of the different scenarios encountered in mass disasters and the process of disaster victim identification (DVI). The specific role of the anthropologist in these incidents and the relationship with DNA analysis and positive identification is also explored (see also Cattaneo et al., 2010; Goodwin, 2017; Alonso et al., 2005; Mundorff et al., 2014).

THE ROLE OF THE FORENSIC ANTHROPOLOGIST

The participation of a forensic anthropologist in post-mortem examinations has varied historically and geographically depending on the country it is practised in, legislation, police structure and a number of other factors (Brickley and Ferllini, 2007; Márquez-Grant and Fibiger, 2011; Kranioti and Paine, 2011; Groen et al., 2015; Ubelaker, 2015; Márquez-Grant et al., 2016; Obertová et al., 2019). It is worth remembering that in some countries forensic anthropologists will have a medical and a forensic pathology background, whilst in others they will come from a biology, anatomy and anthropological (social, cultural,

biological, etc.) and/or archaeological background. Some practicing forensic anthropologists may also have undertaken police training and become police officers and/or crime scene investigators. This range in forensic anthropology practice can be observed throughout Europe (e.g. Kranioti and Paine, 2011; Obertová et al., 2019). The relationship between archaeology and anthropology and how they overlap is also another matter than can be discussed (e.g., see also Blau and Ubelaker, 2016), especially if the anthropologist has an archaeological background; and, likewise, the boundary between anthropology and forensic pathology when the practicing anthropologist also has this medical background.

If for the purpose of this paper, we focus on the remains of the recently deceased (different time frames depending on the country), and leave aside the examination of historical remains or images of living individuals, the forensic (physical) anthropologist will work with human cadavers in various stages of decomposition. These could range from relatively fresh bodies (requiring access to bone via imaging or invasive autopsy, see, e.g., Leo et al., 2013; Brough et al., 2015), through to varying degrees of decomposition, mummification, burnt and charred remains, complete skeletons and small bone fragments (see forensic taphonomy literature, e.g., Boddington et al., 1987; Haglund and Sorg, 1996, 2001; Pokines and Symes, 2013; Schotsmans et al., 2017; Schmidt and Symes, 2015). Indeed, recently, casework undertaken by forensic anthropologists for age estimation (e.g., Rainwater et al., 2012) and trauma analysis (e.g., García Ruiz and Gutiérrez Buitrago, 2020) in particular, has been undertaken on fresh cadavers.

Following the literature (Komar and Buisktra, 2008; Roberts and Márquez-Grant, 2012; Márquez-Grant, 2015), and with some additions and modifications, forensic anthropologists may be requested to undertake the following tasks (excluding training, education, research, validation, and proficiency testing), depending on their remit, the case and scenario:

1. Participate in and advise on the search, location and recovery of human remains, including large sieving operations.
2. Distinguish bone from any other material.
3. Identify bone as human or non-human.
4. Provide an inventory of the bone(s) and any missing elements.
5. Comment on antiquity of the bone if possible or on post-mortem interval; or if contemporary whether the bone(s) is an anatomical specimen, a museum exhibit, a trophy skull or from any other context.
6. Comment on minimum number of individuals (MNI) or maximum likelihood number of individuals (MLNI).
7. Provide information on the biological profile of the remains that may assist in the identity of the deceased: age-at-death, sex, ancestry, stature and any unique identifying traits where possible. These features could be anatomical variations, cultural deformation of the bones or pathological conditions. In addition, anthropologists in some countries may examine any medical implants or devices (e.g., Ubelaker et al., 2019b), whilst in others this is done by the pathologist or police.
8. Examine any aspects around the factors which have influenced the body after death (forensic taphonomy), or preservation of the remains, which may provide information on the deposition of the body, including for example differentiating between dismemberment by sharp force trauma and disarticulation of human remains by scavengers.
9. Assess trauma and provide opinion on whether it is peri-mortem injury or post-mortem damage or even anatomical variation (e.g., sternal aperture, large parietal foramen). With regard to trauma analysis, it should be noted that in most countries, whilst the forensic anthropologist may comment on the mechanism of injury or the damage on the bone, although it is the duty of the forensic pathologist or medical examiner to certify cause and manner of death.
10. Craniofacial reconstruction to assist in identification.
11. Sampling of bone and providing information on other possible analysis such as stable isotope analysis for provenance or radiocarbon dating.

12. Reconstruct the remains if fragmented in order to assess trauma, manage commingling, etc.
13. Attribute bones to a particular individual in cases of commingling.

In addition, the anthropologist will often assist with formulating strategies to maximise recovery of evidence from the bones, particularly environmental evidence, such as moss, barnacles, etc. that may assist with further information (e.g., Márquez-Grant and Roberts, 2012; Pokines and Symes, 2013). These requests are in addition to report and statement writing, providing advice on which related specialist areas might benefit an investigation, peer review, and expert witness testimony where applicable (e.g., Galloway et al., 1990). This may be within a criminal justice or humanitarian context and the anthropologist may be working with coroners, a team of law enforcement personnel, or lawyers acting on the behalf of a defendant. Of course, the questions that anthropologists can answer are dependent on the condition of the remains and not all are necessarily addressed in a single case. It may be that the medico-legal team has already identified the deceased and the only request for the anthropologist is to examine an area of damage to the skeleton to provide an opinion as to whether it is a fresh (peri-mortem) trauma or a dry (post-mortem) break.

Although achieving positive identification by using forensic anthropology alone is rare, it is true that in some cases the deceased have been positively identified from the assessment of non-metric traits on the skeleton and previous ante-mortem radiographs, or where frontal sinuses have been used in a similar way (Ubelaker et al., 2019; Steadman et al., 2006; Baraybar, 2008; Ross et al., 2016; Christensen, 2005). This, however, should be qualified by acknowledging that this is generally only accepted when other primary methods of identification have not been available (e.g., Olivieri et al., 2018). Examples of this might include absence of DNA reference samples or ante-mortem dental records (or indeed dentition), or simply lack of resources including funding. In such situations the forensic anthropologist can assist in the identification of the deceased by liaising with families to obtain ante-mortem data (asking the right questions in view of the physical evidence) and assist relatives with viewing the deceased and interacting with relatives to explain the process, in particular in Human Rights cases (e.g., Mladina, 2016; see also Devisser et al., 2014; Anderson and Spradley, 2016; Wade, 2016). Ideally, however, this should only ever be done by experienced anthropologists who have had appropriate training in working with the bereaved. On occasions, sometimes the identification is provided by ID tags or markers or personal information within the graves and the anthropologist aims at checking whether the biological profile is consistent with that of the material, cultural and other documentation provided. One example of this is the dating of 'cemetery of the bottles' to the Spanish Civil War (Etxeberria et al., 2014).

The types of cases that forensic anthropologists may be engaged in are many and varied, and include the following:

- Search operations for missing persons.
- Fatal fires (domestic, workplace, vehicle, wildfire).
- Clandestine graves as a result of homicide.
- Burials undertaken by families following war crimes.
- Exhumations of unidentified bodies from cemeteries.
- Surface deposition sites (natural, accidental, suspicious, suicidal or unexplained deaths).
- Transportation accidents involving one individual or more.
- Industrial accidents (e.g., explosions, building collapse) involving one individual or more.
- Mass disasters from natural phenomena.
- Terrorist attacks.
- Cold case reviews.
- Age estimation in the living.
- Identification of the living.

The above (as seen similarly for forensic archaeology in Groen et al., 2015), would encompass cases for domestic police or law enforcement agencies, humanitarian cases with no judicial process, humanitarian cases with a

judicial process, and mass fatality incidents with criminal and no criminal involvement.

FORENSIC ANTHROPOLOGY WORK IN MASS FATALITY INCIDENTS

Mass disasters resulting in mass fatalities have been present throughout human history (Ziętkiewicz et al., 2012). Tragically too in recent years, these incidents have resulted in a few individuals to thousands of dead and missing presumed dead (Watherston et al., 2018; Alonso et al., 2005; de Boer et al., 2019). The definition of a mass fatality incident will vary between countries and between states or regions within each country, but it is widely accepted that it should not be based on the number of fatalities alone. The process of identifying the remains from mass fatality incidents tends to be in English, Disaster Victim Identification or DVI (e.g., Black et al., 2011). Factors such as resources of the host nation, experience and training of responders and the condition of the remains, including levels of fragmentation, commingling and burning, must be taken into account as they provide their own challenges. It is now well documented that an incident with a relatively small number of victims who are severely disrupted may raise more challenges than an incident with larger numbers of whole, unburnt bodies (e.g., Byard and Winskog, 2010; Winskog, 2012; Gunawardena et al., 2018; Ellis, 2019; de Boer et al., 2020).

Mass fatality incidents may be categorised as Closed or Open, although in reality they are most often a combination of the two types (e.g., Black et al., 2011). Closed incidents are where the identities of the deceased are known, for example in a military air crash where the names of the personnel on board would have been logged. An open incident is one in which the deceased could include anyone who might have been in the area at the time, for example, a bomb in an open market place. An example of a combined incident might be a civilian air crash, which landed on a busy motorway. It is clearly far easier to positively identify the deceased quickly in a closed incident, as DVI teams will know where to go and who to approach for DNA reference samples, ante-mortem dental records and/or fingerprint data. It could be argued that the role of the forensic anthropologist is far greater in open incidents, where there is a need to collate information relating to the biological profiles of the deceased in order to provide a "starting point" for the identification process. This work would of course go hand in hand with police investigations to ascertain who may have been at the disaster site at the time of the incident. Even where the identity of the deceased is presumed, the forensic anthropologist can still play a vital role in the identification and repatriation process if remains are burnt and / or highly fragmented.

Many types of disasters, whether natural, terrorist attacks, industrial accidents, transportation incidents, arson, etc. have often led to charred, burnt and highly fragmented and commingled human skeletal remains. Where previously these body parts might have remained unidentified, the involvement of forensic anthropologists has shown that in some such cases, positive identifications can still be achieved. The work of the Interpol DVI Pathology and Anthropology Sub Working Group (PASWG), previously led by Dr Peter Ellis (e.g. Ellis, 2019) and now by Hans de Boer, has vastly improved the collaboration between forensic pathologists and anthropologists in the arena of DVI. Most recently this has led to the production of a series of policy documents, guidelines and recording forms designed specifically for use in incidents where there is a high volume of disrupted, commingled, fragmented and burnt remains, for example those developed by INTERPOL (https://www.interpol.int/How-we-work/Forensics/Disaster-Victim-Identification-DVI). Each country and regions within that country may have their own emergency response and DVI protocols. In the UK some guidelines have been produced by the Home Office (2004). As part of National DVI teams (such as UK DVI, or DMORT in the USA) whether civilian, police and / or military (such as Guardia Civil DVI Team in Spain), or as employees of independent forensic science providers or emergency response teams (private, governmental or other organisations such as United Nations or ICRC), forensic anthropologists have been involved in assisting with the management of the dead, their identification and providing information to loved

ones. They have been involved in training and capacity building in war torn areas, liaising with families for ante-mortem information on the deceased, scene and mortuary attendance, participating in identification commissions and finally providing further information to families (e.g., Carroll et al., 2017).

Examples of forensic anthropology participation in the DVI process worldwide in the last decades have included terrorist attacks (e.g. Prieto et al., 2007; Kahana et al., 1997; Hiss and Kahana, 2000; MacKinnon and Mundorff, 2006; Buck and Briggs, 2016; Quatrehomme et al., 2019) natural disasters including tsunamis, wildfires or earthquakes (Cordner et al., 2011; Blau and Briggs, 2011; Black, 2016; Beauthier et al., 2009; Leditschke et al., 2009; Gin et al., 2020), transportation accidents such as air crashes, road traffic collisions, cable car and rail accidents (e.g. Hinkes, 1989; Saul and Saul, 2003; Dirkmaat, 2012; Cattaneo et al., 2015; Barbería et al., 2015), industrial accidents such as gas explosions, building collapse and fatal fires caused by accident or arson (e.g. Park et al., 2009; Rutty et al., 2020), amongst others (e.g. Ubelaker et al., 1995). In addition, it has been reported that more than 20,000 migrants have died trying to cross the Mediterranean since 2014 and many remain unidentified and buried in unmarked graves (Olivieri et al., 2018; Dearden et al., 2020). In other areas such as the Mexico-US borders, anthropologists have a significant role in assisting with the identification of the deceased (e.g., Fleischman et al., 2017). Violence on a mass scale such as genocide and burial of victims in mass graves (e.g., Groen et al., 2015; Ferllini and Croft, 2009; Mohd Noor et al., 2017) also constitute mass fatality incidents but they are not the focus of this paper.

Finally, in light of the recent COVID-19 pandemic, a number of forensic (physical) anthropologists have also been working on protocols for the management of the dead during this pandemic, aiming to provide as much dignity and respect as possible to both the dead and their relatives (e.g., Finegan et al., 2020, and also the Argentine Forensic Anthropology Team or EAAF in its Spanish acronym, eaaf.org).

Below we have summarised the most frequent tasks in which the forensic anthropologist might be involved at the mass disaster site and in the mortuary. These may vary according to the scope and nature of the incident and the resources of the host nation. It must be emphasised that this work is collaborative and would be undertaken within a team of pathologists, odontologists, fingerprints experts, archaeologists, radiographers, DVI trained police officers and emergency response teams. In England and Wales for example, the way in which these teams will be organised and the order in which the specialist examinations will take place, should be decided by Coroner (or other judicial authority leading the operation), the Senior Identification Manager (SIM), the Scene Evidence Recovery Manager (SERM) and the Police Mortuary Coordinator (PMOC) in consultation with the relevant experts (Interpol, 2018). All the tasks described below would be carried out within the framework of the Interpol DVI Guide (2018).

THE FORENSIC ANTHROPOLOGIST AT THE MASS FATALITY SCENE

Anthropology can assist with multiple lines of inquiry (Figura, 2018; Olivieri et al., 2018) in both large and small incidents, (Wiersema and Woody, 2016).

Search and Recovery of Human Remains

- Working within the multidisciplinary team described above and utilising Interpol DVI protocols (Interpol, 2018), the forensic anthropologist can assist by:
- Advising on how to set up the search grid, including reaching a decision on the most appropriate size of grid squares. This would depend on the level of fragmentation and dispersal of remains, which in turn might vary in different parts of the scene. He or she may also advise on the likely survival of remains and produce a forensic taphonomy report if required. This may be in relation to preservation in mass graves, or the likelihood that body parts will have survived intense heat and/or a large explosion.
- Assisting with strategies for sieving debris, supervising or conducting sieving operations and examining recovered items to determine whether they are human bone. If it is not possible

to do this directly, the anthropologist can provide awareness training for police officers and CSIs who may be undertaking sieving operations (Nannetti, 2020), and later review any material that has been recovered.

- Assisting with the recognition of smaller body parts and bone fragments at the scene, particularly where they are modified, for example small fragments of calcined (completely burnt) bone. It is important to consider here that in some scenarios, only one or very few small fragments of bone might be all that is left (or recovered) from an individual, therefore it is essential that they are identified, recorded and recovered correctly.

Correct recording of the remains *in situ* at the scene is particularly important in a mass fatality incident for re-assignation of body parts and fragments where it is apparent that it will not be possible to obtain a DNA profile. Accurate recording of distribution patterns at the scene may also be vital for subsequent accident or criminal investigations, coroners' inquests, public and private inquiries (de Boer et al., 2020).

In terms of the collection and packaging of small fragments, the anthropologist can advise on use of Unique Reference Numbers (URN) at the scene, also referred to as post-mortem (PM) numbers. There is often uncertainty surrounding whether to group very small fragments together and collect them under a single URN or whether to still attempt to assign each tiny fragment its own number. An informed judgement can be made in relation to this by the anthropologist, who will consider which elements are represented and whether there are any genuine associations between the fragments (Interpol, 2018).

In addition to assisting with the completion of the Interpol Victim Recovery Booklets, the forensic anthropologist / archaeologist might record the remains at a mass fatality scene in a number of ways, depending on the resources available and the type of scene being recorded.

Methods include:

- Tape measurements within grids and zones and production of scale drawings.
- Survey using a Total Station or Theodolite.
- Survey-grade GPS.
- Georectified photography and photogrammetry.
- UAV survey.

Often a number of the above methods will be combined, for example photogrammetry and Total Station survey is particularly useful for recording complex fatal fire scenes, where they can illustrate the location of body parts within different levels of building collapse and debris (Dirkmaat, 2012; Schmitt et al., 2015). This is not only useful for post-incident reconstruction of the site and presentation of evidence in court or at inquests, it can assist with the interpretation of events including the whereabouts of the deceased at the time they died.

Special consideration needs to be given to burnt remains, as they are often highly fragmented and fragile. They may be dispersed over a wide area or mixed in with other material such as building debris or the burnt interior of an aeroplane. This together with the fact that intense heat can alter the size, colour and shape of body parts and bones means that recognition of burnt remains is often difficult (de Boer et al., 2020).

If remains are burnt to the point where they become calcined (white, brittle and with no organic material surviving) then it will not be possible to extract DNA from them (Symes et al., 2015; Devlin and Herrman, 2015). In these situations, observations at the scene become very important, particularly with regard to the position of fragments and correct identification, as this information can assist with the reconciliation process, and this is where forensic anthropology specialism is important.

Quality assurance and triage are vitally important at the scene. The forensic anthropologist can assist by reviewing the victim recovery forms accompanying body parts before body bags are sealed and they leave the scene, in order to ensure that the descriptions of remains are correct and accurate sketch plans of locations have been completed.

The experienced forensic anthropologist is also qualified to make a judgement on prioritization of body parts for examination and collection of DNA samples in the mortuary, based on many

factors including the type and size of part, level of disruption, contamination and degradation, and presence of identifying features. This information can be passed to the mortuary team prior to the arrival of the part, which can assist with decisions relating to triage and the order in which DNA samples are submitted to the forensic laboratory. Forensic anthropologists and DNA scientists have adopted an increasingly collaborative approach in recent years and this has had a positive impact on DNA success rates and positive identifications (e.g., Mundorff et al., 2014; de Boer et al., 2019; Gin et al., 2020).

The forensic anthropologist may provide preliminary information about the estimated (minimum) number of individuals involved in the mass fatality incident, based on counting repeated skeletal elements (bones) or body parts and the identification of individuals of different sex and age. Caution must be exercised with regard to these preliminary observations, however, and they should only be regarded as an initial guide, subject to confirmation in the mortuary and where possible the DNA laboratory.

The forensic anthropologist in the mortuary

Post mortem examinations may be conducted in a DVI-designated permanent mortuary or a temporary mortuary set up specifically for the mass fatality incident (Anderson et al., 2017; Interpol, 2018). Figure 1 shows a standard workflow pattern through the mortuary during a mass fatality incident. From this, it can be seen that the anthropologist may be engaged at several stages in the process, and in particular during the image analysis prior to opening the body bag and during the examination of the remains.

In some cases, a separate mortuary specifically for anthropological cases may be designed, or areas within an existing mortuary may be adapted for anthropological examination. Some mortuaries will also have a triage area where the anthropologist may separate human from non-human bone or non-bone material, before it enters the post mortem examination room. As such, the forensic anthropologist must have a good working knowledge of mortuary procedures, including the measures required to minimise contamination. Specific tasks that the forensic anthropologist can assist with in the mortuary include the following:

- Identification of body parts, particularly where they are small, incomplete and / or burnt. This can be done by examination of CT scans and radiographs as well as by examination of the remains themselves (Rutty et al., 2020; Brough et al., 2015). In a mass fatality situation where there is a high throughput of badly disrupted remains, CT scanning can reduce the amount

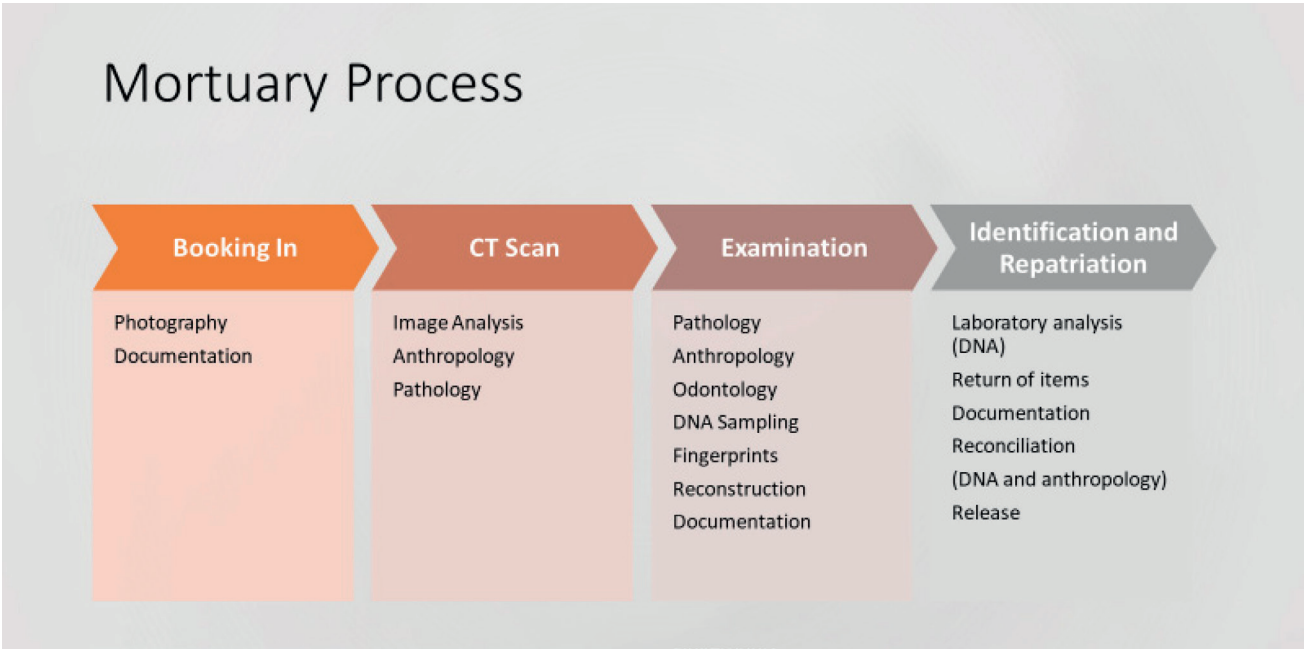

Fig. 1.- Workflow through the mortuary in a mass fatality incident.

of dissection required. Layers of tissue can be virtually removed so that bones can be visualised within masses of soft tissue, enabling the anthropologist to establish what is present or absent. This can also be useful for working out which side the body part is from, therefore assisted with calculation of numbers of individuals. It is possible to rotate the images if a different view is required and to recall images at a later date for further clarification or use in statement writing.

- Completion of Interpol or DVI post-mortem forms plus production of supplementary detailed records, including an inventory of smaller / burnt fragments. This information can later be used in summary victim reports and body maps (see below).
- Provision of a "running total" of minimum numbers of individuals based on count of repeated bones and taking into account age and sex differences.
- Provision of advice on best samples for DNA analysis, particularly in burnt, fragmented and / or degraded remains.
- Collection of DNA samples in the absence of the pathologist, or in addition to the pathologist depending on resources available, number of workstations in operation and examinations in progress.
- Recording of DNA samples prior to destruction.
- Provision of biological data which can assist with identification of the deceased
- Physical reconstruction of remains for the purpose of identification, e.g., re-joining burnt fragments which are not suitable for DNA with unburnt fragments which can be tested, from the same individual, thus also minimising the amount of samples required for DNA analysis.
- Re-association of remains to one particular individual or element identified by either DNA or odontology. This may be done via articulation, visual pair-matching, physical fits, biological profile consistency and thus minimising any 'common tissue' and providing more remains to the family.
- Physical reconstruction of remains to assess and interpret defects, e.g., gunshot wounds or trauma caused by explosive devices.

Following the post-mortem examination, the forensic anthropologist can liaise with the DNA scientists to reconcile DNA results with sample type taken and the source of the sample. If an accurate description of the body part or fragment from which the sample was derived has been made, then the forensic anthropologist can produce a "body map" of all the parts tested from each individual. This can be presented in a 2D or 3D paper or digital format depending on the requirement of the investigation or inquiry. The forensic anthropologist can also produce summary reports for each victim, which include the body map, plus a table of body parts identified presented in scientific and non-scientific terminology. If they wish, families of the deceased can be shown this information by specially trained officers in preference to viewing the remains themselves or photographs of the remains.

DNA AND FORENSIC ANTHROPOLOGY

Although DNA is now the primary means of identification in mass fatality incidents (e.g. Watherston et al., 2018), and this has caused a paradigm shift in the field (Dirkmaat et al., 2008), there is still much value in having an anthropologist in the DVI team (see also Mundorff, 2009, 2012; De Boer et al., 2019). Here is a final summary of the contribution it can make:

a. Identification of whether material is bone and, if bone, whether it is human. This can certainly save a lot of time, resources and money. This may be better in the sieving stage or triage stage where the anthropologist, skilled in bone identification and in bone in different states of decomposition, can identify the bone as human. This is such an important skill, as in some cases what is left of one person is only one fragment of bone which has been retrieved.

b. Assistance with establishing a minimum number of individuals present. At times, in open incidents or even in closed incidents, there may have been someone unaccounted for. It is possible that forensic anthropologists may identify an additional person by repeated bone counts and/or different biological profile.

c. Production of biological profiles where there are no suitable DNA or dental reference samples available for comparison.
d. Production of biological profiles of the deceased when DNA cannot be retrieved from the remains and where there is no other evidence. For example, in calcined remains of an infant with no dental work or an adult with no dental or fingerprint records.
e. Reconstruct bones from several fragments in order to minimise DNA sampling and submit only one fragment for testing.
f. Recording the bone prior to DNA sampling and destruction.
g. In rare cases of identical twins, if skeletal pathologies or unique features were present and ante-mortem medical records were available.
h. Other information beyond identification, such as skeletal trauma analysis, including interpretation of injury patterns in blast trauma. This can provide additional physical evidence relating to the detonation of explosive devices in terrorist investigations.
i. With regard to ethics, ensuring as much bone reassociation as possibly has been achieved in order to minimise the amount of 'common tissue' remaining at the end of an operation.
j. Liaison with relatives in some humanitarian contexts and management of their expectations, especially with regard to DNA analysis and repatriation of remains (Aronson, 2016; Wade, 2016; Etxeberria Gabilondo, 2007, 2012; Ritter, 2007; Byrd and Adams, 2016, Sledzik and Mundorff, 2016; Wagner, 2014).

Table 1 summarises the tasks which the forensic anthropologist might undertake, divided in categories: i) specialist examinations ii) supervision of police / CSI teams or military officers during large search and recovery operations, iii) documentation of work, iv) acting in a managerial capacity. This has been taken from the literature cited above and the authors´ casework experience.

ENGAGING THE SERVICES OF A FORENSIC ANTHROPOLOGIST

In the UK, there are no longer any government owned and funded forensic laboratories, so a request to engage the services of a forensic anthropologist might come via a number of routes. In domestic casework this includes:

1. A direct request from the police (the Senior Investigating Officer, Crime Scene Manager, Forensic Services Manager, or Submissions Officer), to a Forensic Service Provider (laboratory) or individual Forensic Anthropologist.
2. Referral or request from a Forensic Pathologist or Odontologist.
3. Referral through the National Crime Agency (NCA) Special Operations Help Desk.
4. Request from a solicitor acting on behalf of a client.

In the case of a mass fatality incident, again, there are several routes:

1. Direct request by a UK police force to a contracted forensic service provider.
2. Request from a UK police force via another agency such as the NCA.
3. UK DVI (the national capability of the UK police service to respond to mass fatality incidents in the UK and overseas when requested by HM Government), particularly if it is an international incident in which British citizens have died.
4. Other agencies, e.g., repatriation companies such as Blake Emergency Services or Kenyon International Emergency Services.

If the request comes from UK DVI, this will go to the UK DVI anthropology point of contact who will coordinate the response.

It is the responsibility of the authority engaging the services of the forensic anthropologist to ensure that they are able to demonstrate their competence, and comply with the requirements of the UK Criminal Justice System (CJS) if the request is to assist with a criminal investigation.

Table 1. Forensic Anthropology Tasks

Specialist Examinations	Supervision	Documentation	Management and Coordination
Species and fragment identification	Line or fingertip searches	Recording remains at the scene: location, position, elements present, damage	Liaison and collaboration with other experts at scene and mortuary
Biological profile (estimation of age, sex, ancestry, stature and individuating features)	Sieving operations- sifting large amounts of material for human tissue	Creation of a skeletal inventory	Liaison with police, judiciary, solicitors, government agencies and NGOs
Estimation of postmortem interval/ time since burial or deposition	Directing CSI/police photographers to capture images required at scene and in mortuary	Documentation of examinations	Case file management; compliance with requirements of CJS and UKAS (in the UK)
Interpretation of traumatic injuries	Supervision of technical assistants and junior colleagues in the laboratory, e.g. exhibit handling, photography, preparation of slides and samples, documenting procedures	Production of specialist report or witness statement	Liaison with forensic laboratories, in particular DNA scientists
Interpretation of thermal damage		Production of presentations for court	Ensuring compliance with quality management systems at all stages of involvement in a case
Classification of postmortem modification, e.g. scavenger activity		Maintenance of portfolio for proof of competency and, if applicable, professional certification	
Microscopy: LPM, HPM, SEM, histology			
Selection of DNA samples			
Physical or virtual reconstruction of fragmented remains			
Specialist image analysis in the living or deceased: age at death, growth and development studies, skeletal indicators of stress			

CONCLUSIONS

This paper has briefly re-examined the role of forensic anthropology and its definition in the first quarter of the 21st century. It can be seen that forensic anthropology can often play a pivotal role, particularly when mass disasters tragically occur, and this has been recognised by its inclusion as a key discipline in Annexure 17 of the Interpol DVI Guide 2018. The annexure states that *"As the complexity of a disaster scene increases, so does the need to employ a broader range of expertise. DVI investigations benefit from the involvement of FAs because disasters are often characterized by compromised or highly fragmented human remains and decreased states of preservation"* (Interpol, 2018). It also emphasises that whilst the forensic anthropologist is *"an integral part of the multidisciplinary DVI team, in scenarios resulting in fragmented and/or compromised remains"* it is vital that the FA operates in collaboration with the rest of the DVI team and liaises closely with the DVI-operation manager (*ibid*). The latter is an important point, as working within a multidisciplinary team on a DVI operation requires a very different approach to undertaking domestic forensic casework. In this sense, mentoring and training for forensic anthropologists who wish to become involved in DVI work is vitally important, and this perhaps needs to be reviewed in the near future.

Many mass fatality incidents will unfortunately result in multiple commingled body parts and burnt fragmented remains. Recovery of these

remains and selection of samples for DNA is entirely different from dealing with whole unburnt bodies or body parts. The forensic anthropologist is specifically trained in the recognition and recovery of these body parts, especially if too degraded and fragmented, providing a representation of the number of individuals, assistance with reconstruction and repatriation, obtaining samples for DNA analysis if no other specialist is present.

The importance of continual professional development (CPD) must also be recognised by forensic anthropologists who regularly provide assistance to the courts in criminal investigations. It is vital that the practitioner is familiar with the latest research and developments in the field in order to provide the best service possible, and so CPD should be seen as a necessity and not an afterthought. This should include exercises regarding temporary mortuary set ups, and completing INTERPOL forms, etc. Moving forward, closer collaborations between forensic laboratories and academic institutions providing research and teaching in forensic anthropology, can only serve to strengthen the discipline and further augment its position alongside the more traditional branches of forensic medicine and science.

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The need to professionalize forensic anthropology

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SUMMARY

As the discipline of forensic anthropology continues to grow and expand in scope and depth, it is necessary to define the discipline within the greater framework for a forensic science profession. This paper aims to review the current state of forensic anthropology as a profession, focusing on the structural characteristics of education and training as well as the practice of forensic anthropology through the concepts of qualifications, ethical codes, guidelines, and standards. As a profession, the concepts of expertise, ethical practice, and societal need of forensic anthropology are discussed. The current system for certification of forensic anthropologists is outlined in addition to the need to define qualifications and to create standards for forensic anthropological practice. Additionally, the need for professional organizations to lead the charge in ethical practice, defining qualifications and adopting and creating standards, is discussed.

Finally, the societal needs fulfilled by forensic anthropology are reviewed.

Key words: Forensic Anthropology – Professionalism – Ethics – Scientific integrity – Conflicts of interest – Expertise – Certification – Best practice

INTRODUCTION

The scope and breadth of forensic anthropology have continued to increase since its introduction over 80 years ago, stemming from a publication by Krogman (1939), which focused on identification efforts of unknown human remains. The discipline has since grown to include the analysis of trauma and taphonomy, as well as the recognition of the importance of forensic archaeology and the inclusion of forensic anthropologists into leadership roles in large-scale investigations of missing and disappeared individuals (e.g., Christensen et al., 2019; Dirkmaat, 2012; İşcan, 1988; Snow, 1982). More recently, practitioners have begun to recognize the importance of ethics in forensic anthropology. This ethical consideration extends to not only the proper treatment of human remains, but also the professionalization of the discipline, in addition to the conduct of forensic anthropologists themselves (Christensen et al., 2015; France, 2012; Márquez-Grant et al., 2019; Passalacqua et al., 2019; Passalacqua and Pilloud, 2018; Passalacqua and Pilloud, 2020; Pilloud and Passalacqua, in press; Walsh-Haney and Lieberman, 2005).

These important ethical considerations have led to larger discussions about the need for certification of individuals, accreditation of laboratories, formalized education/training

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programs (Passalacqua and Pilloud, 2020), the further development of standards and guidelines in the practice of forensic anthropology, and possible licensure in order to practice (Langley and Tersigni-Tarrant, 2020). Further, it has been argued that the ethical codes presently applicable to most forensic anthropologists are lacking, if present at all (Passalacqua and Pilloud, 2018). Taken together, these issues demonstrate that, while forensic anthropology is more important and widespread than ever (Boyd and Boyd, 2018; Cattaneo, 2007; Christensen et al., 2015; Dirkmaat and Cabo, 2012; Dirkmaat et al., 2008), it is also lacking a foundation for its practitioners, both present and future.

The goal of this paper is to briefly review the current state of forensic anthropology as a profession, focusing on the structural characteristics of education and training as well as the practice of forensic anthropology through the concepts of qualifications, ethical codes and guidelines, and standards. To avoid confusion, we have included a short glossary at the end of this article with the definitions of terms used (many also used in Passalacqua and Pilloud, 2018 and Passalacqua and Pilloud, 2020).

NEED FOR PROFESSIONAL ETHICS

Generally, a professional is someone who (1) possesses a body of special knowledge (i.e., expertise); (2) practices within an ethical framework (i.e., adheres to a code of ethics and avoids conflicts of interest); and (3) fulfills a societal need (Passalacqua and Pilloud, 2018; Pellegrino, 2002). Based on these criteria, we can consider forensic anthropologists to be professionals working within the broader profession (i.e., paid occupation) of forensic anthropology. Below we will discuss the concepts of expertise, ethical practice, and societal need in regard to forensic anthropology in more detail.

Expertise

Forensic anthropology is "the application of anthropological method and theory to matters of legal concern, particularly those that relate to the recovery and analysis of the human skeleton" (Christensen et al., 2014b). While education in broad anthropological concepts is crucial for the practice of any form of anthropology, forensic anthropologists are unique experts in the analysis of contemporary human skeletal remains in order to contribute to establishing an individual's identity. Additionally, forensic anthropologists contribute to determination of cause and manner of death through the reconstruction of the death-event via the analysis of skeletal trauma and archaeological/taphonomic evidence (Passalacqua and Pilloud, 2018). In the United States, many forensic anthropologists also have expertise in forensic archaeology, or the application of archaeological method and theory to matters of legal concern (Christensen et al., 2014b). However, outside of the United States, individuals are often experts in *either* forensic anthropology *or* forensic archaeology, as anthropology and archaeology educational programs are often separate, thus producing independent experts.

Unfortunately, in the current paradigm, the ability to demonstrate expertise in forensic anthropology (and/or forensic archaeology) is not straightforward. Expertise is typically generated and evaluated via qualifications, or the education and/or training an individual has received in a particular discipline/subject-matter area. Education and training are documented via credentials (e.g., transcripts, certificates). While there are a number of best practice guidelines or other documents that discuss education, training, and/or qualifications in forensic anthropology (discussed more below), none of these are currently widely accepted or required in order to develop qualifications for forensic anthropology expertise in the United States or abroad (Passalacqua and Pilloud, 2020).

Recently, Passalacqua and Pilloud (2020) surveyed practicing forensic anthropologists in the United States and found wide variability in terms of education and training experiences. Individuals reported taking many different courses while they were in graduate school. However, when asked about what knowledge areas should be required to practice forensic anthropology, a number of areas were supported by general consensus (in this case meaning greater than 50% of individuals) (Table

1). Similarly, when asked about education level and training experiences that should be required to practice forensic anthropology, consensus was reached for few items (Table 2).

Certification was only considered to be required to practice forensic anthropology by 50% of respondents; thus, it did not quite achieve consensus support. Similarly, having a doctoral degree as a requisite to practice forensic anthropology was only supported by 45% of respondents. When asked, "what makes you qualified to be a forensic anthropologist?", experience and education were by far the most common responses. Similarly, when asked what would make someone *unqualified* to practice forensic anthropology, responses focused on a lack of experience, training, and/or education; this finding is noteworthy as it suggests individuals believe their personal education/training experiences make *them* qualified to practice forensic anthropology. At the same time, there is no consensus on how to demonstrate that an individual has the proper qualifications to practice forensic anthropology, which would be achieved through certification. Nor are there any accepted best practice guidelines or standards

Table 1. Knowledge areas respondents think should be required to practice forensic anthropology

Topic	Frequency	Percent
Human osteology	169	97%
Forensic anthropology methods	164	94%
Skeletal trauma	164	94%
Archaeological methods	161	93%
Taphonomy	155	89%
Human variation	151	87%
Human decomposition	145	83%
Bone biomechanics	140	80%
Gross anatomy	138	79%
Statistics	138	79%
Introduction to forensic anthropology	137	79%
Dental anthropology	134	77%
Growth and development	133	76%
Paleopathology	127	73%
Ethics	125	72%
Research methods	117	67%
Radiology	113	65%
Zooarchaeology	112	64%
Bioarchaeology	108	62%
Laboratory management	92	53%

Table 2. Education/training experiences respondents think should be required for an individual to practice forensic anthropology.

Experience	Frequency	Percent
Some period of work supervised by a certified forensic anthropologist	128	74%
Master's degree in anthropology	104	60%
Graduate education supervised by certified forensic anthropologist	100	58%
Some amount of continuing education	98	57%
Membership in relevant professional organization	95	55%
Advanced applied training	92	53%

for qualifications in forensic anthropology. When asked about developing standards for education and training in forensic anthropology, and about developing accreditation for forensic anthropology educational programs, 98% and 96%, respectively supported these concepts (Passalacqua and Pilloud, 2020). In fact, a recent report by the National Institute of Justice (within the United States) identified forensic anthropology as one area of academic study that could be subject to accreditation by the Forensic Science Education Programs Accreditation Commission (National Institute of Justice, 2019).

Standardization

The reason to pursue the development of best practice guidelines and standards documents is outlined in the findings of the National Research Council report (2009). In which the authors state:

Standards and best practices create a professional environment that allows organizations and professions to create quality systems, policies, and procedures and maintain autonomy from vested interest groups. Standards ensure desirable characteristics of services and techniques such as quality, reliability, efficiency, and consistency among practitioners. Typically, standards are enforced through systems of accreditation and certification, wherein independent examiners and auditors test and audit the performance, policies, and procedures of both laboratories and service providers (National Research Council, 2009).

The need to follow available standards is also part of the Daubert criteria for courtroom admissibility (Christensen et al., 2014a; Daubert, 1993). Further, while the publication of standards is important because it sets a minimum for how various tasks are to be performed by a disciplinary professional, published standards are also used to inform outside certifications and accreditations, meaning that the criteria used to certify individuals or accredit laboratories are (in theory) based on published standards.

Efforts to develop standards for many forensic sciences predate the 2009 report from the National Academy of Sciences (NAS) (National Research Council, 2009), which were largely being pursued by various Scientific Working Groups (SWGs). These SWGs were all operating independently, following different procedures, and without oversight. In response to the publication of the NAS report in 2009, the United States National Institute of Standards and Technology (NIST) created the Organization of Scientific Area Committees (OSAC) for Forensic Science. The OSAC was originally organized into five scientific area committees (Biology/DNA, chemistry/instrumental analysis, crime scene/death investigation, digital/multimedia, and physics/pattern interpretation). These committees were further divided into 25 subcommittees. Anthropology is currently a subcommittee within the committee of crime scene/death investigation. In 2020, OSAC 2.0 was announced (with planned implementation in late 2020) as a reorganization of the OSAC structure. In OSAC 2.0, the upper-level OSAC resource committees would be directly integrated into the subcommittees, which would theoretically eliminate an internal level of bureaucracy and a bottleneck for documents. Additionally, in OSAC 2.0, the SACs and their subcommittees are also being reorganized, with forensic anthropology falling under the "forensic medicine" SAC (essentially a renaming of "crime scene/death investigation").

The OSAC forensic anthropology subcommittee replaced the pre-existing SWG for Forensic Anthropology (SWGAnth), which went on hiatus in 2014. The SWGAnth was established in 2008 with the goal of developing standards for the practice of forensic anthropology in the United States. The SWGAnth generated 21 guidelines covering topics ranging from sex assessment, resolving commingled human remains, laboratory management and quality assurance, and a code of ethics and conduct. These documents are currently available on the NIST website at: https://www.nist.gov/topics/organization-scientific-area-committees- forensic-science/anthropology-subcommittee (Passalacqua and Pilloud, 2018).

The SWGAnth created consensus-based documents via committee, and then posted draft versions of these documents online for public

comment. After resolving public comments, the SWGAnth posted final versions of these documents online. Like other SWGs the SWGAnth operated independently from the larger standards community, which was in part why it was replaced by the OSAC. After the creation of the anthropology subcommittee of the OSAC, this organization took the pre-existing SWGAnth documents and began revising and formatting them into either best practice or standard documents. However, unlike the SWGs, the OSAC subcommittees submit their finalized documents to accredited Standard Development Organizations (SDOs), which act as third-party peer review and an independent publishing organization.

In the case of the Anthropology subcommittee of the OSAC, the SDO used is the American Academy of Forensic Sciences (AAFS), Academy Standards Board (ASB), which is accredited by the American National Standards Institute (ANSI). The ASB has its own anthropology consensus body made up of individuals each representing an interest category. These interest categories exist in order to ensure that the membership of the consensus body is diverse (i.e., not all academics), as well as to ensure that various stakeholders have input into the consensus body documents, which is also why documents are circulated for public comment. The current interest categories of the ASB are: Academics and researchers; Organizations; General Interest; Producer; User – Government; User – Non-Government; and Jurisprudence and Criminal Justice.

Once a document is submitted to the ASB from the OSAC, it is presented to the ASB's anthropology consensus body (ACB), who then works to create their own working group for each document. The ACB's working group revises the draft document from the OSAC (as needed), which is then posted online for a 30-day period for public comment. After public comments are received, the working group within the ACB works to resolve the public comments. The updated documents are then recirculated for additional public comments, or published depending on the amount of substantive changes made to the document during the public comment resolution process (Passalacqua and Pilloud, 2018). At the time of writing, the ASB has published two standards in forensic anthropology: *Stature estimation in forensic anthropology* and *Sex estimation in forensic anthropology*. Future standards will further outline the practice of forensic anthropology to include the documentation of other aspects of the biological profile (i.e., ancestry, age); as well as pathological conditions and anomalies; scene detection and recovery; trauma analysis, etc. While there are currently no published standards for qualifications in forensic anthropology, the OSAC has revised the SWGAnth's "Qualifications" best practice document, into a standard and this revised document is working its way through the OSAC and ASB processes. Assuming this document is published, it has implications for education, certification, and the tasks individuals can perform at various qualification levels within forensic anthropology.

Outside of the United States, various other codes of practice exist; however, there are no published best practice or standard documents. A code of practice is essentially a best practice document, used by an individual organization, which only applies to its members. The Royal Anthropological Institute (RAI) has a published "Code of Practice for Forensic Anthropology" in conjunction with the Forensic Science Regulator. This 20-page document provides guidelines on how to "establish the professional standard of performance within the field of forensic anthropology in the UK" (Royal Anthropological Institute of Great Britain and Ireland, 2018). The document generally focuses on response and documentation at the scene and at the morgue, as well as report writing. Methods of analysis are not generally discussed. Further, the document specifically states that it does not address education or research in forensic anthropology. This document is not published by an accredited SDO, and is to be considered a stand-alone document that is not aligned with International Standards (thus only applying to members of the RAI). As such, it is separate from the Forensic Science Regulator's Codes of Practice and Conduct, which follows ISO/IEC 17025 and is a standard for testing and calibration laboratories. However, the RAI's code of practice is in general alignment with International Laboratory

Accreditation Cooperation (ILAC) G19:08/2014, Modules in a Forensic Science Process, which is a general guideline for laboratories that perform forensic analysis and examination according to ISO/IEC 17025 published by the ILAC.

Certification

Certifications are credentials that exist to demonstrate expertise in a knowledge area and/or skill. Within forensic anthropology, there are currently four organizations that provide some form of certification: the American Board of Forensic Anthropology (ABFA), the RAI, the Forensic Anthropology Society of Europe (FASE), and the Asociación Latinoamericana de Antropología Forense (ALAF). While these certifications exist relatively regionally (United States, United Kingdom, Europe, and Central/South America, respectively), all of these certifications are open to anyone who meets their application requirements. However, currently the ABFA is the only certifying body that is accredited (in their case, by the Forensic Specialties Accreditation Board [FSAB]). Independent accreditation of a certifying body is important, as it demonstrates that an organization's certification procedures follow a standard and are transparent, fair, and consistent. Without accreditation, there is no assurance that an organization is operating in an unbiased or systematic manner.

Presently, in order to apply for certification by the ABFA, an individual must have: 1) completed a PhD in anthropology or a closely related field (demonstrated via academic transcripts), 2) experience in forensic anthropology (demonstrated by submitted case reports authored by the applicant in addition to letters of recommendation), and 3) knowledge of forensic anthropology (demonstrated through the successful completion of the ABFA certification exam). If an individual's application is accepted, they are allowed to sit for the two-part ABFA certification exam. This exam consists of a written and a practical portion and is offered once a year. Examinees much achieve an 80% or greater on both portions of the exam to pass. If one or both portions are not passed, they may be retaken the following year. Once an individual passes both portions of the exam, they have met all requirements of the ABFA and are a certified Diplomate of the ABFA. In order to maintain certification, ABFA Diplomates must submit a re-certification document every three years which documents their continuing education. Since its inception in 1977 to 2020, 146 individuals have been certified by the ABFA (Boyd et al. 2020).

In the United Kingdom (UK), there is a certification process overseen by the RAI. This organization has a certification procedure that recognizes three different levels of practitioner: Forensic Anthropologist III, Forensic Anthropologist II, and Forensic Anthropologist I. Forensic Anthropologist III is the entry-level practitioner. For this level, applicants must have an Honors degree, have attended two professionally relevant conferences, be a fellow or student fellow of the RAI, and have demonstrated professional experience for two years after graduation. In addition to documenting these qualifications, applicants must also include two letters of reference, at least one of which must be from a Forensic Anthropologist I. Two examiners, as determined by the RAI, then review this application and determine if the applicant is eligible for certification. A Forensic Anthropologist III is expected to work under the supervision of a Forensic Anthropologist I or II.

To apply to become a Forensic Anthropologist II, applicants must demonstrate participation in a minimum of five cases; an understanding of health and safety protocols; knowledge of osteology/anatomy, skeletal biology, population variation, human identification, and statistics; continuing education or training; two years of experience after attaining Forensic Anthropologist III; attendance at two additional relevant professional conferences; and membership in a professional organization beyond the RAI. Additionally, two letters of reference must be submitted, at least one of which must be from a Forensic Anthropologist I. The applicant then must demonstrate theoretical knowledge, either via documentation through casework or other documents, or a written examination. Additionally, there is a practical portion that involves writing a case report on a mixed skeletal assemblage. Candidates have

three hours to take appropriate notes and photographs. They then have two additional weeks to complete and submit a final report with all relevant accompanying documentation. A Forensic Anthropologist II is meant to work under the direction of a Forensic Anthropologist I.

Finally, to become a Forensic Anthropologist I, an individual must have completed at least an MSc, but ideally a PhD. Applicants are to have been a Forensic Anthropologist II for at least three years and submit their application with two letters of reference, at least one of which should be from a mentor and the other from a relevant professional. The applicant is to supply five case reports, which demonstrate a range of knowledge and conform to the standards of the UK criminal justice system. The examination then consists of an expert witness oral examination based on these reports. It is also possible to apply for direct entry if an applicant is able to demonstrate: a minimum of five co-authored/sole-authored peer-reviewed case reports demonstrating a range of knowledge, participation in continuing education or training, evidence of expert witness testimony, an understanding of health and safety protocols, attendance at two relevant conferences, and membership in the RAI and one other professional organization. All information regarding examination of the RAI is from the following website: https://www.therai.org.uk/images/stories/Forensic/Practitioner_Levels.pdf. According to the RAI website (at the time of writing), eight individuals are certified as Forensic Anthropologist I, there are currently five individuals at the level of Forensic Anthropologist II, and 21 at the level of Forensic Anthropologist III.

The FASE also recognizes tiered certification with two levels – Level II and Level I. For FASE certification Level II, a candidate must have a master's degree and be a member of FASE. They need to provide documentation of training and continuing education in forensic anthropology, proof of assisting with casework, and a letter from a supervisor. Once materials are reviewed, applicants are allowed to sit for both a practical and written examination. Examinees must achieve at least an 80% on both portions of the exam to pass. If one section of the exam is failed, examinees are allowed to retake only the failed portion.

To achieve FASE certification Level I, applicants must have a PhD or MD. They then must demonstrate training and continuing education in forensic anthropology, submit two case reports, provide a list of at least 20 forensic anthropology case reports, and provide evidence of at least five years of experience. They then take the same written and practical examination as the examinees at FASE certification Level II; however, they must also complete an oral examination that focuses on two mock cases. It is also possible for practitioners with over 15 years of experience to obtain certification via *honoris causa* based on a review of the curriculum vitae, qualifications, professional experience, or academic status. All information regarding FASE certification obtained from the FASE website at: http://forensicanthropology.eu/.

Certification in Latin America is overseen by the ALAF and the Directorio Latinoamericano de Antropología Forense (DLAF). The first certification exam was conducted in 2012 at the AAFS annual scientific meeting and was overseen by Douglas Ubelaker, Jose Luis Prieto, and Steve Symes. To apply to become certified by the DLAF, one must be a current member of the ALAF and demonstrate at least five years of experience within forensic anthropology. If accepted to sit for the exam, it now takes place during the ALAF annual meeting and consists of two days of theoretical and practical exams. The theoretical portion is multiple choice and the practical portion consists of analysis and report writing of two cases in addition to multiple stations with additional questions (pers. comm. Derek Congram, 2020). Recertification occurs every three years through documentation of activity in the discipline. According to the ALAF website (at the time of writing), thus far, 20 individuals have been certified by the ALAF.

Accreditation

In forensic anthropology, accreditation currently exists for certifying bodies (such as the ABFA) and laboratories (such as the Laboratory of the Defense POW/MIA Accounting Agency [DPAA]). Organizations seek accreditation as a formal recognition that they are following

a set of standards and are thus competent to carry out specific tasks (Taverniers et al., 2004). Accreditation then, is an effort to demonstrate that an organization meets minimum standards of quality. Accreditation must be conferred by an independent accrediting body, such as the FSAB. These accrediting organizations essentially enforce particular protocols that must be followed by the organizations they accredit. These protocols are theoretically based on published standards, if applicable.

As discussed above, there are no accreditations for forensic anthropology education/training programs, and the only certifying body for forensic anthropology that is currently accredited is the ABFA. For forensic laboratory accreditation, the largely accepted international standards are those overseen by the International Organization for Standardization (ISO). This non-governmental agency housed in Switzerland produces international consensus-based standards. Accrediting bodies then administer accreditation according to these published standards. The largest accreditation body in North America is the American National Standards Institute (ANSI) National Accreditation Board (ANAB). The ANAB has accredited three forensic anthropology laboratories under one of two ISO standards. At the time of writing, these are the only accredited forensic anthropological laboratories of which we are aware. These include: the DPAA forensic anthropology laboratories in both Hawaii and Nebraska (ISO/IEC 17025 - effective through 3/31/2020), the Harris County Institute of Forensic Sciences Forensic Anthropology Division (ISO/IEC 17020:2012 - effective through 10/31/2022), and the New York City Office of Chief Medical Examiner – Forensic Anthropology Unit (ISO/IEC 17020 - effective through 3/31/2023). This information was found at the ANAB website (http://search.anab.org).

According to the ANAB, both ISO/IEC 17025 (requirements for the competence to perform tests or calibrations) and 17020 (requirements for the operation of inspecting bodies) are standards for forensic accreditation (https://anab.ansi.org/en/forensic-accreditation). Based on ANAB documents for requirements for accreditation, both ISO/IEC 17025 and 17020 outline guidelines for laboratory management, competency and proficiency testing, method validation, evaluation of measurement uncertainty, technical review, reporting of results, equipment calibration, maintaining chain of custody, and quality assurance (ANAB, 2019a; ANAB, 2019b). Of note is that in the more recent revisions of these ANAB documents (ANAB, 2019a; ANAB, 2019b) there is also a requirement for a code of ethics, which was not in an older version of the ANAB requirements for ISO/IEC 17025 (ANAB, 2017). According to Bencivenga (2015), both standards are equally respected internationally and both have very similar management requirements (largely based on ISO 9001 for quality management systems). Where the two standards differ is where most focus is placed. In ISO/IEC 17025 more weight is given to validation, traceability, and measurement uncertainty; whereas, ISO/IEC 17020 focuses more on limiting bias and confidentiality (Bencivenga, 2015).

The lack of accredited forensic anthropology laboratories is due to a variety of factors. For example, many forensic anthropology laboratories often fall under the accreditation of the medical examiner office in which they are housed. Other labs may find that accreditation is either too expensive, and/or that accreditation is not possible due to the small size/caseload of the laboratory. Further, many accreditations are a poor fit for small university-based forensic anthropology laboratories due to the required quality assurance protocols, which are unfeasible to implement due to staffing/time restrictions, as these labs are typically overseen by faculty who have teaching, research, service and advising responsibilities in addition to overseeing forensic anthropological casework. Further, these laboratories tend to be underfunded without the resources to hire full-time laboratory managers.

Ethics

Ethics are formalized (i.e., written/codified) guidelines for the behavior (i.e., conduct) of individuals within a specific profession or discipline (Bowen, 2009). Generally, ethical codes are a set of rules regarding the behavior

of individuals, which have been agreed upon by members of a professional organization (e.g., the ABFA). Different professional organizations' ethical codes may vary in terms of the types of behaviors discussed, but all are used in order to ensure that members avoid unprofessional behaviors and thus maintain the credibility of the profession and professional organization.

There are different forms of ethical codes: aspirational and preventative (Harris, 2013). Aspirational ethical codes are meant to promote human wellbeing and present a number of guiding and/or motivational behaviors that an organization *would like* its members to follow/achieve. Typically, aspirational codes are longer than preventative ethical codes, but also more general/vague and lack enforcement. Within the context of forensic anthropology, the American Association of Physical Anthropologists (AAPA) and the Society for American Archaeology (SAA), are examples of organizations with aspirational ethical codes. Preventative ethical codes, sometimes called ethics statements, are rules for professional behavior that *must* be followed. Preventative ethical codes are enforced by an adjudicating committee within the organization that performs an investigation when a complaint is filed alleging that an individual acted unethically (i.e., performed professional misconduct). Individuals who are found to have committed an act of misconduct may be subject to various actions, such as warnings, sanctions, censure, or loss of membership/certification. Unfortunately, while most professional organizations have their own ethical codes, one issue with having ethical codes attached to specific organizations is that individuals can simply withdraw from the organization rather than face repercussions for their actions. There is currently no oversight body to track or manage ethical complaints or unethical behavior (Murphy, 2000), which makes all organizational ethical codes more or less unenforceable.

Current Ethical Guidelines

At present, the discipline of forensic anthropology has no overarching ethical code that is accepted by all practicing forensic anthropologists (within the United States or abroad). Many relevant professional organizations have their own ethical codes to which members are expected to adhere. Such organizations with ethical codes include the AAPA, the AAFS, the ABFA, the Society of Forensic Anthropologists, the British Association for Forensic Anthropology, the RAI, and the British Association for Biological Anthropology and Osteoarchaeology. Some of these organizations have aspirational codes. For example, the AAPA provides aspirational guidelines for ethical conduct in biological anthropology, but the ethics committee of the AAPA is not an adjudicating body, meaning that the organization does not enforce these ethical guidelines. Rather, the AAPA's ethics committee is primarily used to educate and promote ethics in biological anthropology generally.

The ABFA and AAFS on the other hand have preventative ethics codes, and adjudicating ethical bodies to enforce these codes on their memberships. For both organizations, breaches of ethical conduct are largely related to misrepresentation of self and ones' analytical findings. These codes are summarized on each organizations' website and in Passalacqua and Pilloud (2018), so they will be not discussed in detail here. However, as noted in Passalacqua and Pilloud (2018), both ethical codes fail to mention/enforce many unprofessional forms of misconduct, including discrimination and harassment.

It is worth noting, that of the four certifying bodies discussed here (ABFA, RAI, FASE and ALAF), only the ABFA and RAI have published ethical codes, whereas FASE and ALAF have not. Rather, according to the FASE website, one of their missions is to "encourage and promote adherence to high standards of ethics, conduct and professional practice in forensic anthropology" (http://forensicanthropology.eu/about-fase/); without a provided document. Further, ALAF discusses in their published statutes that one of their goals is to "establish ethical and professional criteria for the practice of forensic anthropology to guarantee the quality of practice" (https://alafforense.org/es/quienes-somos/estatutos); however, again no document is provided.

Recently, Passalacqua and Pilloud (2018) presented an aspirational ethical code for forensic anthropologists, stating that all forensic anthropologists should: "(1) follow all forensic, legal, medical, and evidentiary requirements (i.e., *truth-telling*), (2) not misrepresent themselves or their findings (i.e., *truth-telling*), (3) treat remains, casework, colleagues, and invested parties with professional and cultural respect (i.e., *respect for persons*), (4) only perform tasks they are competent to do, and avoid conflicts of interest (not misrepresenting themselves or their qualifications), (5) do no harm (i.e., *non-malfeasance*), (6) follow the scientific method (*maintain scientific integrity*), (7) document actions in a traceable manner (*maintain transparency*), (8) avoid and report misconduct (act in an ethical manner), (9) maintain confidentiality of materials (i.e., *respect for persons*), (10) avoid intellectual, personal, and/or emotional biases (i.e., *avoid conflicts of interest*), and (11) conduct research in an ethical manner." As part of this aspirational code, it is important to highlight that scientific integrity and transparency are essential to the practice of all professional science. Identifying and avoiding misconduct and conflicts of interest are key to maintaining scientific integrity (discussed in detail in Passalacqua et al., 2019). Further, Passalacqua and Pilloud (2018) noted that a national or international preventative ethical code for forensic anthropologists, such as the National Code of Ethics and Professional Responsibility for the Forensic Sciences (NCEPRFS) generated by the National Commission on Forensic Science (2017b), should be established to provide universal guidance, enforcement, and monitoring.

Societal Need

As medicolegal professionals, forensic anthropologists fulfill a societal need in their assistance-resolving medicolegal investigations. Additionally, as humanitarian professionals, forensic anthropologists fulfill a societal need in identifying unknown individuals and bringing closure to families and communities. In the current model in the United States, there are very few full-time staff forensic anthropologists working on recent medicolegal cases. Rather, much of this work is done by consulting forensic anthropologists in various capacities. While the number of full-time forensic anthropologists employed at medical examiner/coroner (ME/C) offices is not tracked anywhere, various estimates indicate that the number in the United States is less than 40 (Tersigni-Tarrant and Shirley, 2013; theabfa.org). This estimate does not attempt to take into account that there are a number of individuals trained as forensic anthropologists at the master's degree level, working in ME/C offices as autopsy technicians or death investigators who will also perform forensic anthropological analyses as needed.

Not only is there a growing need for forensic pathologists (National Commission on Forensic Science, 2017a; Swgmdi, 2012), particularly in the United States; we also argue that there is also a growing need for full-time employment of forensic anthropologists at ME/C offices throughout the United States and abroad. Forensic anthropologists have a unique expertise, which improves or may be required for many death investigations. However, few jurisdictions have access to a forensic anthropologist on staff. While more jurisdictions have access to case-by-case contracted forensic anthropologists, this gig-economy model is not appropriate for a death investigation, nor does it attract the most qualified individuals. As we move to professionalize, standardize, and improve the forensic sciences, we must push to expand the range of services offered by forensic anthropologists, and include forensic anthropologists as full-time professionals staffed in ME/C offices alongside forensic pathologists, as our skillsets are complementary and essential for the scientifically valid resolution of death investigations (Christensen et al. 2015).

Recent work by Passalacqua and colleagues (2020) found that, forensic anthropologists in the United States earn around 1/3 the annual salary of forensic pathologists. This finding is in spite of the fact that many of these forensic anthropologists also have extensive and advanced training holding a doctorate in anthropology. This disparity in salaries hints at the undervaluation of forensic anthropological work. More work is needed in the regulation and oversight of how this professional

service is provided to the community. Much of this oversight can be achieved through the further ethical practice of professionalization of forensic anthropology. Professional organizations can, and should, be at the forefront of this process.

CONCLUSIONS

Depending on how one views the discipline of forensic anthropology, it could be considered new and developing, or older and well-established. The science of forensic anthropology has been applied for almost 100 years (or more depending on when you want to mark the first instance of forensic anthropology; be it by casework or official publications). However, the actual practice of forensic anthropology is not well regulated or standardized. We consider forensic anthropology a profession, and its practitioners, professionals; however, when it comes to the three criteria of a professional (i.e., expertise, ethical practice, and societal need), forensic anthropology is lacking in structure.

The subject-matter areas of forensic anthropology have consensus (Passalacqua and Pilloud, 2020), but are not officially recognized in any way, and the practice of forensic anthropology currently lacks minimum published standards (although, more should be on their way to publication via the OSAC and ASB). Considering the ethical practice of forensic anthropology, preventative ethical codes are lacking and there are no overarching official ethical documents or databases to track misconduct. Finally, while the utility and societal need of forensic anthropology is apparent, forensic anthropologists are rarely found as full-time staff in ME/C offices, and based on salaries, their work is undervalued.

There is a need to professionalize forensic anthropology. Creating standards and structure for the qualification and practice of forensic anthropology, can provide a framework for how to be a forensic anthropologist, who to hire as a forensic anthropologist, when to use a forensic anthropologist, and what to expect from a forensic anthropological analysis. Wherever an individual practices, they should meet (when possible, accredited) consensus-based minimum standards to ensure that they are competent and qualified to perform their work. However, these standards may not be universally applicable based on local legal/court requirements, and professionals should consider the varying requirement of different regions. Creating detailed preventative ethical codes with extra-organizational oversight/tracking will ensure that individuals practice forensic anthropology ethically and avoid misconduct. Demonstrating the societal value of forensic anthropology will increase the use and availability of forensic anthropological analysis (and staff positions).

GLOSSARY

Accreditation – A credential used to demonstrate that an organization (e.g., a university, medical examiner's office, forensic anthropology laboratory) meets a set of published standards.

Beneficence – to do something to benefit others (Appelbaum, 1997).

Certification – A credential provided by a professional organization demonstrating that an individual has met the knowledge and/or skills required to pass their certification process.

Competency - the application of knowledge, skills, and abilities to correctly complete specific *tasks.*

Conflict of interest – a violation of practitioner independence and are considered a type of professional misconduct.

Credential - verifiable document used to demonstrate completion of education and/or training (e.g., transcripts, licenses). Frequently used to "acknowledge, restrict, or protect the use of a title, and/or activities" (Pryzwansky, 1993).

Education - formal academic coursework from an accredited school, college, or university, resulting in a degree (Passalacqua and Pilloud, 2018; Swgtox, 2015).

Expert – an individual possessing authoritative knowledge or skill in a particular area (note that expertise is demonstrated via credentials such as certification) (Nichols, 2017).

Guidelines (i.e., best practice documents) – published documents providing recommendations

for how to perform a particular action or process. Guidelines are typically published and vetted by accredited organizations. Their content must be based on consensus of practitioners and stakeholders. Guidelines are typically more detailed/descriptive than standards, but are also open to interpretation.

IEC - International Electrotechnical Commission.

ISO – International Organization for Standardization.

Licensure – restricting practices and/or the use of a title without a license. Licenses are typically enforced via state or federal laws (e.g., practice and/or title acts) requiring an individual to have a license to perform a particular act or use a particular title.

Non-malfeasance – to avoid doing harm if at all possible (Appelbaum, 1997).

Professional misconduct – behavior that is contrary to a professional code of ethics.

Proficiency - refers to levels of requisite competency to effectively complete work; a means to measure mastery of a subject area.

Qualifications – the education and/or training of an individual.

Scientific integrity – the "commitment to truthfulness, to personal accountability, and to vigorous adherence to standards of professional conduct" within the context of the scientific method (Warner and Weiss, 2004).

Standard (i.e., formal standards) – published documents providing mandatory rules for how to perform a particular action or process. Standards are typically published and vetted by accredited organizations. Their content must be based on consensus of practitioners and stakeholders.

Training – formal structured process of teaching and assessment at a laboratory or other non-educational institution, often resulting in a certificate (Passalacqua and Pilloud, 2018; Swgtox, 2015).

Transparency – the perceived quality of intentionally shared information (Schnackenberg and Tomlinson, 2016).

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Forensic Anthropology in the Colombian post-conflict era: technical and scientific challenges in the face of the postmortem examination of forcibly disappeared persons

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SUMMARY

This paper presents an overview of the state of the art of forensic anthropology in Colombia, and examines the crime of forced disappearance and the institutions and tools that emerged from the peace agreement between the government of Juan Manuel Santos and the FARC-EP guerrilla. In addition, it explores some of the technical and scientific challenges to forensic anthropologists in the postmortem examination of human remains, particularly those related to forensic identification in the context of forced disappearance. Three main challenges are identified: 1) to obtain proper training for local practitioners given the lack of graduate programs in forensic anthropology; 2) to continue working on the establishment of bone and dental standards for the Colombian population and 3) to design and promote the use of standard operating procedures in forensic anthropology by all forensic agencies and to stimulate an inter-institutional dialogue to generate standards in the analysis of human remains.

Key words: Colombian post-conflict – Forensic anthropology – Forced disappearance – Postmortem examination – Methodological standards

INTRODUCTION

The aim of this article is to present an overview of the state of the art of forensic anthropology in Colombia and the challenges that this field will have to face in the following decades in relation to the search, recovery and identification of approximately 120,000 disappeared persons. This enormous task is only one of those posed for the post-conflict scenario following the Peace Process signed between the government and the FARC-EP guerrillas in 2016. This paper will provide an examination of the crime of forced disappearance from international regulations as well as a brief description of the institutions and tools that emerged from the peace agreement with the FARC. In particular, this article focuses the analysis of two main ideas: 1) the current

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issues and technical challenges for local forensic agencies in post-conflict times and 2) the scientific difficulties and challenges to forensic anthropologists in the postmortem examination of human remains, particularly those related to forensic identification.

The crime of Forced Disappearance

The Disappearance of persons is a complex phenomenon that occurs in different modalities, i) voluntary; ii) fortuitous; and iii) forced. The latter has been the subject of extensive research in the social sciences as well as in the legal and forensic fields.

The crime of forced disappearance is synonymous with the involuntary disappearance of people, and it is a conduct that, in the first instance, is presumed to be the perpetration of actions that violate human rights. Additionally, when committed in certain contexts, it automatically acquires attributes framed by the International Humanitarian Law (IHL), to become a crime against humanity according to the Rome Statute (International Criminal Court, 2011). Various international organizations have formulated different and relatively consensual definitions of the crime of forced disappearance, namely:

In the 1980s, in response to the complaints of countless cases by the civilian population, the international community, in particular the Inter-American Commission on Human Rights (IACHR, 1980), typified the forced disappearance of people and proposed the creation of tools to prevent such crime, and raise awareness about the seriousness of this practice. The IACHR defines forced disappearance as a multi-offensive crime that violates multiple human rights and various rules of IHL.

Through its Declaration on the Protection of All Persons from Enforced Disappearances, the United Nations (UN, 1992), defines forced disappearance as: "arresting, detaining or transferring people against their will, or deprive persons of their freedom in any other way by government agents of any level, organized groups or individuals acting on behalf of the government or with its direct or indirect support, authorization or consent; and refusing to reveal the fate or whereabouts of people or acknowledge that they are deprived of freedom, thereby removing them from the protection of the law."

The Rome Statute (1998) defines the forced disappearance of persons as "the arrest, detention or abduction of persons by, or with the authorization, support or acquiescence of a State or a political organization, followed by a refusal to acknowledge such deprivation of freedom or to give information on the fate or whereabouts of those persons, with the intention of removing them from the protection of the law for a prolonged period of time."

Based on the definitions provided by various international regulations, the three central elements of forced disappearance are: 1) The deprivation of freedom against the will of the person concerned; 2) The participation of government agents, at least indirectly by acquiescence; 3) The refusal to reveal the fate or whereabouts of the person concerned.

Although not exclusive of the internal armed conflicts and military and civil dictatorships of the 20th century, forced disappearance has been a characteristic crime in these types of contexts. Some scenarios in which this phenomenon has occurred are, for example, Guatemala, 1962, Chile, 1973; Cyprus, 1974; Argentina, 1976; El Salvador, 1980; the countries that made up the former Yugoslavia, 1991; Rwanda, 1994; East Timor, 1999 and, of course, Colombia, where an internal armed conflict characterized by various actors in confrontation has persisted for more than sixty years (Sanabria-Medina et al., 2018).

Forced disappearance -International context-

I had long understood that this anguish was the most desperate torture (Junod, 1951). The quote by Dr. Marcel Junod, referring to the suffering of the families of the disappeared he met during his work as delegate of the International Committee of the Red Cross (ICRC) in the Spanish civil war, anticipated the current universal recognition of deep suffering by those who have lost a loved one as a result of armed violence and are unaware

of their fate and whereabouts. The obligation contained in International Humanitarian Law (IHL), to ensure the correct and dignified management of the remains of people who died as a result of an armed conflict and the need to provide information about their whereabouts to their loved ones, in addition to the prohibition of forced disappearance, derives of such universal recognition (TidBall-Binz, 2016).

By the beginning of the twentieth century, forced disappearance had not been defined yet as a crime but its perpetration is considered related to practices against humanity developed during the World Wars (Centro Nacional de Memoria Histórica -CNMH-, 2016). In fact, one of the first reports of the phenomenon of forced disappearance dates back to 1941, when General Keitel received an order from Hitler, known by the name of *"Night and Fog,"* which started the clandestine disappearance of thousands of people by the German authorities (Huhle, 2014). The Nuremberg trials that took place between 1945 and 1946 were the first scenario where a Nazi officer, (Oberkommando der Wehrmacht) was ruled for his responsibility in the execution of forced disappearances.

The first cases of forced disappearance in Latin America occurred in countries such as Guatemala, El Salvador, Honduras, Cuba, Panama and the Dominican Republic. These took place in the first half of the 20th century, where forced disappearance was part of the modus operandi of Latin American dictatorships. Likewise, between 1970 and 1980, different dictatorial regimes in the Southern Cone such as Uruguay, Paraguay, Brazil, Argentina, Bolivia and Chile left hundreds of victims via the implementation of joint plans such as the *Operación Cóndor* (Condor Operation), where people were thrown from planes in flight into the ocean. Operación Cóndor was the name of the plan for the coordination of actions and mutual support between the leaders of the dictatorial regimes of South America and the United States. On the other hand, the problem of forced disappearance has only become visible in recent years in Mexico, despite the fact that it has had decades of evolution. This problem has been mainly related to the growing power of drug trafficking, the strengthening of criminal groups and the state corruption (Ramírez, 2016).

In the forensic and social literature, the problem of the disappearance of persons is almost exclusively addressed when the focus is on forced disappearances. Yet, the concept of people considered or reported disappeared has a common element: relatives do not know the fate and whereabouts of their loved ones. Thus, people may go missing in the framework of different situations, for example: victims who were forcibly disappeared; victims who were kidnapped and were never released; victims (minors or adults) who were illegally recruited by different actors in armed conflicts; or civilians who disappeared during the hostilities without belonging to one of the groups in conflict, e.g., a citizen who dies during an aerial bombing.

International instruments and international human rights jurisprudence are uniform and convergent in defining the constituent elements of forced disappearance. Thus, based on the doctrine of the *Working Group on Enforced or Involuntary Disappearances* as well as the I*nter-American Convention on Forced Disappearance* and the *International Convention for the Protection of All Persons from Enforced Disappearance*, the IACHR stated that the deprivation of freedom and the refusal to recognize such deprivation, and to hide the fate or whereabouts of the disappeared with direct or indirect intervention of state agents, are the elements that characterize the crime of forced disappearance (Inter-American court of Human Rights, 2005). However, it should be noted that, in accordance to the evolution of international law, non-state actors who act without the authorization, support or acquiescence of the State could also perpetrate this crime according to the Rome Statute (International Criminal Court, 2011).

Colombian armed conflict and peace dialogues between the Colombian Government and the FARC- EP guerilla

After being permeated by an armed conflict that lasted for more than five decades, peace conversations began during the first half of 2012 between the government in office and the

Revolutionary Armed Forces of Colombia (FARC-EP). Conversations culminated with the signing of the document that marked the beginning of official dialogues between the two parties in the city of Havana, Cuba, on August 26, 2012. These dialogues are better known as the "Peace Process" named officially the *Acuerdo Final para la Terminación del Conflicto y la Construcción de una Paz Estable y Duradera* (Agreement to the End the Conflict and to Construct a Stable and Lasting Peace), whose aim was to reach the end of a long-lasting internal armed conflict. The parties involved were accompanied at its different stages, pre and post negotiation, by recognized members of the international community, where Norway and Cuba were the "guarantor" states of the negotiation.

During peace conversations, various commitments were made, all of which would be of tremendous impact for Colombian society. One of them is numeral 5, or the *"Acuerdo sobre las Víctimas del Conflicto"* (Agreement on the Victims of the Conflict), which included the creation of the Comprehensive System of Truth, Justice, Reparation and Non-Repetition (*Sistema Integral de Verdad, Justicia, Reparación y No Repetición* -SIVJRNR- in Spanish), whose main purpose was to place the victims at the center of the peace agreement in response to their demands and expectations.

As established in transitory article 1 of the Legislative Act 01 of 2017, the SIVJRNR is made up of judicial and extrajudicial mechanisms such as the *Comisión para el Esclarecimiento de la Verdad, la Convivencia y la No Repetición* (CEV) (Commission for the Clarification of Truth, Coexistence and Non-repetition); *the Jurisdicción Especial para la Paz* (Special Jurisdiction for Peace); the *Unidad de Búsqueda de Personas dadas por Desaparecidas en el contexto y en razón del conflicto* (Search Unit to find Disappeared Persons) hereinafter UBPD, and the specific measures of integral reparation necessary for the construction of peace and guarantees of non-repetition.

One of the results of the aforementioned peace dialogues was the commitment made by the parties, in the sense that the FARC-EP promised to provide information on the whereabouts of those who died in the context of armed confrontations against state forces, but also in contexts such as kidnappings. In response, the government would begin the establishment of a humanitarian and extrajudicial unit, whose exclusive function would be the search for disappeared persons in the context of the armed conflict, that is, the UBPD, institution that came into operation in 2018.

Despite the fact that there are no single official figures on the universe of people reported disappeared, the Centro Nacional de Memoria Histórica (National Center for Historical Memory-CNMH) conducted a study that indicated that there are at least 82,998 people who were forcibly disappeared between 1958 and 2017 (CNMH, 2016). Of those cases with known perpetrator (52% total), the distribution of disappeared persons is as follows: by paramilitary groups. 26,475 (62.3%); by guerrillas, 10,360 (24.3%); by post-demobilization groups; 2,764 (6.5%); by state agents, 2,484 (5.8%), and by state agents and paramilitary groups: 388 (0.9%). The total number of cases with available information is 42,471 (CNMH, 2016).

The figure of 82,998 disappeared will undoubtedly rise and reach figures reaching 120,000, mainly due to the fact that there is a marked under-reporting of missing people in Colombia. Unfortunately, the armed conflict continues due to the coexistence of other armed actors different from the FARC-EP, such as the National Liberation Army (ELN) guerrilla and other criminal groups associated with drug trafficking activities. Additionally, other groups such as the emerging criminal bands (BACRIM) and the international organized crime, for example Clan del Golfo, keep perpetrating crimes, including forced disappearances.

Forensic identifications of forcibly disappeared persons

Homicide, torture and other cruel and inhuman treatments, among others, are usually collateral crimes perpetrated after forcibly disappearing persons. The crimes that are often committed along with forced disappearance by one or more perpetrators are also investigated during the judicial proceedings in any case of forced

disappearance. In such investigations, the forensic sciences become the field that provides technical and scientific answers to support the judicial inquiries.

In the context of the judicial investigation of deaths, the forensic identification process responds to one of the requirements of the criminal process that investigates unnatural deaths. The deceased must be identified so that the judicial investigators can complete their investigative tasks, since the probability of having successful criminal investigations without identifying the body is very low, as investigators require interviewing the victim's next of kin, and the people who were part of their social life.

Technically, the forensic identification process is a comparative exercise between two sets of variables: (1) physical antemortem information vs. (2) postmortem information obtained from the examination of the remains (i.e., biological profile, dental information, individualizing features on skin (tattoos, scars, etc.) and antemortem anomalies or pathologies found on the remains) (Sanabria-Medina et al., 2018). This comparison, in addition to results obtained from the genetic profile comparison between relatives' samples and deceased samples, has two purposes: 1) to achieve the positive identification of a set of human remains and 2) to return the remains to family members with the aim of assisting their mourning process. In the case of Colombia, returning the remains of forcibly disappeared persons to their families is part of the families' right to *'integral reparations'* which include individual, collective and symbolic reparations. The first two types correspond to monetary compensations, whereas symbolic reparations include a variety of measures adopted by the state in favor of victims, such as psychosocial care workshops, or forensic activities focused on the search and identification of missing persons (López-Cerquera, 2018).

The successful identification of a set of remains depends on multiple variables, for example: (1) the state of decomposition of the body, (2) the reliability and quality of the physical information provided by relatives or close friends of the disappeared person, (3) the reliability of the qualitative and quantitative method(s) applied to obtain information from the remains, and (4) the expert´s interpretation in the process of comparing ante-mortem information with post-mortem findings.

It is in the group of unidentified remains (which are often found skeletonized, in advanced decomposition, dismembered or burned) where victims of homicide and/or forced disappearance are usually found. The work of experts and the resources invested in solving the difficulties inherent to the process of identifying human remains of complex cases, are more than rewarded when local regulations and international law are applied to the investigation and punishment of possible violations of human rights (Morales, 2009). According to the Instituto Nacional de Medicina Legal y Ciencias Forenses (National Institute of Legal Medicine and Forensic Sciences-INMLCF), a complex case is one where the body presents intentional alterations aimed to obstruct its identification, to hinder or mask injuries, or where the perpetrator has had the intention to hinder the body or discard it in conditions that delay, impede or complicate its discovery and therefore the judicial investigation.

Likewise, complex cases involve those deaths in which torture, associated sexual activity and/or child abuse are suspected. Deaths in custody and deaths in combat or armed confrontation also constitute a complex case. This definition does not intend to decrease the technical complexities of the examination of remains subject to any violent death, but constitutes an alert during the forensic analysis of cases that present additional variables in comparison to other deaths; variables that must be examined with priority resorting to the use of all the available tools provided by the forensic sciences.

MATERIALS AND METHODS

In order to examine the state of the art of the field of forensic anthropology, as well as the technical issues and upcoming tasks to forensic agencies in post-conflict times in Colombia, the authors turned to their personal experiences as forensic experts at two of the four forensic agencies that currently exist in Colombia, namely the INMLCF

and the UBPD). With the aim of exploring the scientific difficulties and challenges to forensic anthropologists in the postmortem analysis of human remains, besides relying on their own experience as local forensic practitioners for several years, the authors also made an examination of official data and statistics and the existing literature that investigates these and other similar questions.

RESULTS AND DISCUSSION

Below we will describe the scope, limitations as well as the technical and scientific challenges in the postmortem analysis of human remains in Colombia.

Forensic agencies: administrative and technical tasks and challenges in the post-conflict scenario

In Colombia, two state entities search for missing persons in the framework of criminal investigations. These are: i) the Fiscalía General de la Nación (Office of the Attorney General- FGN) through its Technical Investigative Body (Cuerpo Técnico de Investigación- CTI) and ii) the National Police with an area called the Dirección de Investigación Criminal (Office of Criminal Investigation and INTERPOL- DIJIN). In addition, these institutions have identification laboratories where experts examine the bodies they recover. At these labs only skeletonized remains are examined.

On the other hand, the mission of the INMLCF is to conduct medico-legal autopsies of all the bodies exhumed (regardless their state of decomposition) by the two judicial entities mentioned previously. This institution does not participate in the search of remains, although their support is occasionally requested. Autopsies performed by the INMLCF include the examination of cadavers from all contexts of violent deaths, including of course, those remains that are found years later in clandestine graves or other complex settings such as water environments, illegal crematoriums, dumpsters, etc.

As already mentioned, the UBPD is a state entity of humanitarian and extrajudicial nature, whose main mission is to search for people considered to be disappeared in the context of and due to the armed conflict. However, the UBPD does not anticipate the design and implementation of infrastructure for identification purposes in the short run, as forensic analyses related to identification are a task of the INMLCF. In spite of this setback, the UBPD will support the search and identification of remains in different ways, for example, it will establish or unify the figures of people reported missing and will support the process of identification of remains that have been recovered but have not been identified. According to the Ministry of Interior, these remains go up to 25,000 and correspond to those that were initially autopsied but given their state of "unidentified" were handed over in custody to public cemeteries and medical departments of different national universities.

Another future task for the UBPD is related to the bodies that were subjected to a medico-legal autopsy, which despite being identified, could not be delivered to their relatives because these families were never located. Thus, relatives were never informed about the death of their family member(s) or the whereabouts of their remains. These remains, according to the law, must always be handed over in custody by the INMLCF to the cemeteries, and, in the face of the humanitarian actions that characterize the UBPD, this institution will have to carry out actions to search for the next of kin to proceed with the return of those remains. The number of remains identified and not claimed by their relatives amounts to approximately 18,000, and thus the task of returning them to their families will involve enormous administrative and technical efforts.

In addition to the aforementioned, all forensic agencies will have to face a variety of challenges in the context of the search for disappeared persons and the identification process, namely: the lack of human capacity and infrastructure to search for the 82,998 forcibly disappeared reported by the CNMH in 2018; the absence of enough forensic experts, mainly forensic anthropologists who can assist the medico-legal autopsies of remains that will be recovered by the CTI, DIJIN and UBPD. This will inevitably occur, as the recovery of remains will be exacerbated in response to the intervention of the UBPD. The latter problem

worsens as none of the forensic agencies that exist in Colombia has officially spoken regarding the possibility of expanding the number of jobs for forensic anthropologists, whose work is crucial both for the search and for the identification of disappeared persons.

Scientific issues and challenges in the postmortem examination of human remains: the role of forensic anthropologists

The creation of the UBPD and the hiring of a small number of forensic anthropologists by this institution have been gradual ways of recognizing, on the part of the government and civil society, that in the search and identification of the remains of the forcibly disappeared, the forensic anthropologist plays a fundamental role due to her/his knowledge in bio-archeology, human anatomy and biostatistics, among other fields. However, as has already been recognized, Colombia lacks graduate programs that train forensic anthropologists, hence the expertise of most local professionals is based on the experience they have acquired at national forensic agencies over the years (Guatame et al., 2015; López-Cerquera 2018; Sanabria-Medina and Osorio-Restrepo, 2015) and the training in forensic issues they have received from international agencies such as the International Criminal Investigative Training Assistance Program (ICITAP) (Fig. 1) or the International Red Cross (ICRC). Currently, of the three agencies conducting lab analyses in forensic anthropology, only the INMLCF has its own certification process aimed at evaluating their own anthropology experts, although this evaluation is only valid at this institution. At the international level, only six local forensic anthropologists have been board certified by the Latin American Association of Forensic Anthropology (ALAF), of which only one still works at one of the three forensic agencies mentioned before. However, in Colombia it is not a requirement to be board certified by an international organization to be hired and considered an expert in the field of forensic anthropology, which makes sense considering the lack of formal graduate education in the field. In addition, none of the forensic anthropology services offered by national labs have been accredited according to ISO/IEC 17020. However, some of the forensic anthropology labs

Fig. 1.- Forensic anthropologists belonging to the three state forensic agencies during a training course in scientific research methods taught by anthropologist from International Criminal Investigative Training Assistance Program (ICITAP) in Bogota, Colombia.

of the INMLCF are currently in the process of being accredited by experts in the Quality Management System of the INMLCF and the ONAC (Organismo Nacional de Acreditación de Colombia - National Accreditation Body of Colombia).

The current state of forensic anthropology in the country reveals that one of the main challenges that current local anthropologists will continue to face is obtaining proper training, even if outside academia. The amount of casework that anthropologists will have to handle in the post-conflict scenario requires that forensic agencies organize trainings with national or international experts to update their professionals in current methods and research not only in forensic anthropology but other forensic sciences as well. In the long term this could result in a higher number of certified anthropologists and probably the accreditation of national laboratories different from those of the INMLCF.

A second challenge to the field of forensic anthropology has to do with the use of methodologies that serve the process of forensic identification. Most of the bodies exhumed in the context of Colombia´s internal armed conflict are found in advanced states of decomposition or skeletonized; therefore, various methods and techniques have to be employed by forensic anthropologists to estimate the biological profile (i.e., the estimation of sex, ancestry, age-at-death and stature) of the remains. The estimation of these variables is key, as these are entered in forensic state databases of people reported missing and are open for public consultation. However, often times, the populations' biological variability has led to biased results or wide error margins when those methods are applied to forensic cases of populations different from the original populations from which the studies were designed.

The validity of the application of various scientific methods has been discussed for several years in various scientific scenarios. The consensus is that techniques should come from studies carried out in the same population to which the remains belong in order to have confident statistical results (Kimmerle and Jantz, 2008; Bass, 2005; Dayal et al., 2008; Howells, 1996).

However, despite this consensus, the trend in Latin America has been the recurrent use of methods derived from population studies conducted in North America and Europe. One of the reasons that explains why Latin American researchers have not produced enough population studies to create skeletal and dental standards lies mainly in the absence of documented human bone collections that are chronologically recent and representative of the local population, particularly from different geographical regions and sub-regions.

Most of the studies published since 2009 based on Colombian populations have been carried out thanks to the curation of the Modern Human Skeletal Collection housed at the INMLCF in Bogotá. This fully documented skeletal collection has 600 individuals that correspond to males and females who died between 2005 and 2008 (Sanabria et al., 2016). The 600 skeletons correspond to people who were born in different cities and the number of individuals per city is variable. For example, there are 150 individuals from Bogotá; 70 from Medellín; 30 from Cartagena, and 20 from Cali. These figures mean that this collection does not have a number of individuals representative of the 1,118 cities or municipalities of the country. Yet, the chances of achieving a bone collection with these characteristics are very low due to the required infrastructure and resources as well as the countless bureaucratic procedures to overcome.

The absence of skeletal collections is not the only reason for not having skeletal standards in a given country, since these can be obtained through other types of cross-sectional studies, i.e., with living populations or using non-invasive technologies such as radiographic techniques or computed axial tomography, among others. The absence of these types of studies in Colombia might be related to a lack of initiative on the part of local researchers and/or due to little financial support provided by academic institutions and forensic agencies.

When examining the forensic and biological anthropology literature, a small number of published population studies that seek to establish skeletal standards in Colombia is encountered

(Rosique et al., 2004, Mantilla et al., 2009, Sanabria-Medina, 2011a, 2011b; Isaza et al., 2014; González-Colmenares et al., 2016; Guerrero et al., 2015; Moore et al., 2016; Monsalve and Hefner 2016; Lopez-Cerquera and Casallas, 2018; Rivera-Sandoval et al., 2018; González-Colmenares et al., 2019). Although these studies constitute a great scientific contribution for the country, these are insufficient due to the high population miscegenation and the existence of population subgroups located in various geographic regions across the country.

Another problem related to the absence of population studies is the growing global phenomenon of migration, which can be voluntary or involuntary—the latter being frequently associated with forced displacement. In the case of Colombia, Venezuelan citizens have migrated massively to Colombia due to the current socio-political situation in Venezuela. This migratory phenomenon means that the recipient country, already genetically and phenotypically admixed, ends up with an additional admixture of sub-populations, making forensic identification of immigrants an enormous challenge.

The need to use new or validated methods from the population with which the forensic expert works triggers a new problem that has been rarely addressed and is, if the bio-demographic bone standards (equations, functions, etc.) of a country are valid, to be applied to remains found in any area of its territory. This inquiry is based on the understanding that such standards arose from studies that complied with all the regulations and rigor required by the scientific method. However, the specific problem has to do with the size of the sample and whether or not it statistically represents the population from which the samples were drawn.

In studies carried out in North America, South Africa, Spain, Egypt, India, Greece or anywhere in the world to estimate sex, age, ancestry or stature, when researchers generate the mathematical formulae to estimate bio-profile variables, it is generally concluded, for example, that data were taken from "whites", "blacks", "Hispanic", "Asian", "Greek" or "Spanish" populations (Trotter and Gleser, 1952; Vance et al., 2011; Trancho, 1997; Steyn et al., 1998, 1999; San Millán et al., 2013; Sahar et al., 2016; Rissech et al., 2012; Raghavendra et al. 2012; Peckmann et al., 2015; Otong et al., 2016). With this information, the examiner assumes that if, after estimating the ancestry, the remains' skeletal features are compatible with frequent features of "African," "European" or "Asian" populations, he/she should use the function or equation as appropriate. This exercise leads to question whether the original study meets the sufficient criteria to be used in any skeletonized set of remains found in North American territory or in the country where the formula was generated. In other words, are all "white" or "black" American citizens similar at the osteometric or osteomorphological level? Are all the remains with "European" or "African" morphology found in North American soil, actually North American? It is not necessary to conduct any population study to answer these questions. The intra- and inter-population biological diversity of the world, as well as the global phenomenon of migrations, makes the answer to these concerns a resounding no. This problem, which is real both for Colombia and other contexts, indicates that the probability of finding a morphologically homogeneous population within a country is practically inexistent. Conversely, we find ample population diversity, which means that the skeletal and dental standards arising from academic studies designed for a single country are not applicable to all of its inhabitants.

Returning to the problematic in Colombia, population studies carried out to date in this country are insufficient. In addition to the studies mentioned before, other studies have been conducted by professionals from the forensic anthropology laboratory at the INMLCF with the participation of senior students from the University of Los Andes, the University Javeriana and the National University (Correa, 2002; Chavarro, 2000; Díaz, 2002; Gómez and Sanabria-Medina, 2002; Guerrero and Sanabria-Medina, 2013; Jiménez, 2015; Villalobos and Sanabria-Medina, 2013; Rodríguez et al., 2002; Puerto, 2001). Others have been part of Master´s and Doctoral theses from national and foreign universities (Brian De La Cruz et al., 2007; Ruiz,

2018; Sánchez et al., 2019; Osorio-Restrepo et al., 2019; Muñoz, 2019). Yet, none of these studies have been published in peer-review journals.

One of the factors that validate a method, both in the scientific and legal fields, is that they be published in academic journals, in order to be subjected to scientific scrutiny by academic peers. In this context, and despite the fact that some efforts have been made to carry out population-specific investigations to create techniques that can be used in the forensic identification process in Colombia, the vast majority of studies have not been published, which significantly reduces such efforts. At the moment, most of these academic investigations are stored on the shelves of university libraries and are not available to the forensic agencies that require them. Thus, the challenge to forensic practitioners is twofold: 1) to publish existent research in peer-reviewed journals and 2) to make efforts to expand the current modern skeletal collections to include individuals from peripheral regions in order to enrich the geographical and hopefully the genetic and phenotypic diversity of the samples.

The third challenge related to the forensic identification of the disappeared presented herein is the need to have Standard Operating Procedures (SOPs) for anthropological postmortem examinations at the different forensic agencies. This practice is closely related to the use of new or validated methods to estimate biological profile as it standardizes forensic anthropological practice but also institutionalizes best practices in the application of forensic sciences.

As of today, the INMLCF has made good advances in the design of institutional SOPs to examine and identify complex cases. For example, in 2015 this institution published a guide titled "Minimum Forensic Standards for the Search of Missing Persons and the Recovery and Identification of Human Remains." However, neither the Attorney General, nor the DIJIN have designed or published minimum standards for the post-mortem examination of human remains. To date, all forensic agencies follow the recommendations of the Minnesota Protocol for lab analyses, however, there seems to be little-to-none inter-institutional dialogue between local forensic anthropologists with regards to the criteria used to conduct bio-profile estimations or skeletal trauma analyses (López-Cerquera, 2018).

Particularly worrisome is the use of foreign, but especially old, methodologies at some of these agencies which reflects the pressing need to update the academic knowledge of both experienced anthropologists as well as new generations of anthropologists in modern methodologies and technologies used in forensic human identification.

CONCLUSIONS

The technical difficulties and the associated challenges exposed in this paper require the political will and public financial resources to carry out the search and identification of the disappeared in the next decades. On the other hand, in the face of scientific challenges, forensic agencies must commit to periodically train their forensic practitioners so that they can apply novel methodologies and technologies to their laboratory analyses. Additionally, it is suggested that there be an inter-institutional dialogue between the different forensic agencies, in order that SOPs be designed and/or standardized to follow best practices in the application of forensic anthropology to human identification analyses.

Finally, the problem of methodologies explored in the previous section results in a reflexive exercise on the limitations of not having a wide range of methods tested in Colombian populations to estimate the biological profile, as well as the questionable practice of using foreign techniques that, in addition to generating possible biases in the results, may lead to legal implications in the context of the validity of the evidence presented by the forensic expert. In the particular case of forensic anthropology, the application of methodologies that use foreign populations, opens the possibility that the evidence may be contested in court, especially in cases where Colombian war criminals could be tried, either by national or international authorities, such as the International Criminal Court. This situation is well described by Kimmerle and Jantz (2008), who report objections by the defense

to the demographic evidence presented by the prosecution in cases that tried persons accused of having perpetrated war crimes during the armed conflict in the former Yugoslavia.

In addition to this problem, another issue follows. That is, not having enough modern human skeletal collections with specimens of all regions of Colombia and population studies that incorporate samples that are sufficiently representative of the total population of the country. In relation to the latter, in the early research design stages, scholars or local forensic practitioners need to ask what would the ideal or statistically representative sample number be for a given population study in order to generate confident results for a method to be used in the analysis of human remains found, for example, in a cosmopolitan city like Bogotá, which has almost nine million people. An additional inquiry may complicate the previous picture: What is the characteristic bone or dental morphology of a citizen of Bogotá? Does the immigrant population (black, white and indigenous citizens) who has entered into Bogota's population in the last thirty years allow the estimation of such bone or dental morphology? Given the exorbitant number (82,998) of people reported disappeared, which hypothetically equates to a similar number of unidentified bodies to be recovered, the forensic disciplines that support the process of identifying remains of complex cases (including forensic anthropology) have been recognizing the need to update the existing methodologies, including those that aid in the estimation of the biological profile. In addition, forensic anthropology and other forensic fields will have to face complex challenges, especially those related to the analysis of a population characterized by a wide phenotypic variability of a remarkably mestizo population.

Likewise, a final big challenge that the country must solve is the encouragement of academia to create (even if in the long run) graduate programs to train new generations of forensic anthropologists. This will increase the number of well-trained forensic practitioners in the country, an achievement that will be consistent with the demands of thousands of families who expect not only successful searches of disappeared persons, but also the positive identification of the remains of their loved ones.

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Approaches for constructing and using reference samples to maximize the utility of new forensic methods

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SUMMARY

Identified reference collections (IRCs) are essential sources of data for the development and testing of key methods that are used in forensic anthropology, including estimation of age, sex, stature, and in some jurisdictions race or ancestry. For decades, various key collections, whether anatomically derived (for example, the Terry Collection) or cemetery-derived (for example, the Coimbra Collection), have been described as essentially obsolete for forensic applications. However, regardless of the age of the collection, both recently amassed collections and older collections share several fundamental problems.

First, while there has been some discussion of how representative collections are of a hypothetical population, in a great deal of forensic research IRCs have been treated as biological or statistical *populations* rather than highly biased *samples*. All skeletal assemblages, regardless of how they were amassed, are biased samples and need to be treated as such. Second, the value of IRCs stems from the documentary data that are available for each individual in the collection, and also for the collection as a whole. However, the documentary data have not been too often critically assessed for their accuracy, precision, and relevance. Newer data are not necessarily better data.

Although additional sources of data available through new collections are clearly an asset for research, the newer collections do not solve any of these underlying problems with IRCs and the approaches that are commonly used to study these collections. In this chapter I argue that what is needed are new ideas for assessing and quantifying patterns of human variation. I present a theoretical model and some methodological strategies for constructing reference *samples* from IRCs for maximizing the research potential of IRCs in a forensic context. Specific examples are provided throughout the chapter to illustrate these concepts.

Key words: Forensic Anthropology – Theory and Methods – Identified skeletal reference collections – Sex estimation – Stature estimation – Age at death – Skeletal variation

INTRODUCTION

Identified reference collections (IRCs) are essential sources of data for the development and testing of methods that are used in forensic anthropology, including estimation of age, sex,

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stature, and in some jurisdictions race or ancestry (Hunt and Albanese, 2005; Tobias, 1992). These collections have been the foundation for forensic anthropology since the beginning of the 20th century, but have not been the focus of attention until relatively recently (Hunt and Albanese, 2005).

For decades, various key anatomical-derived collections, such as the Terry Collection (Hunt and Albanese, 2005) and cemetery-derived collections, such as the Coimbra Collection (Rocha, 1995), have been described as essentially obsolete for forensic applications (see Fig. 1 for pictures of the Terry Collection at the Smithsonian Institution as previously curated at the National Museum of Natural History and currently at the Museum Support Center). This idea that a collection can be obsolete has been proven categorically false by the use of these "old" collections to develop methods that have been shown to be highly effective and reliable when tested on forensic cases in the 21st century (for example, Albanese, 2003a; Albanese, 2013; Albanese et al., 2016a). However, the myth persists that "new" collections will solve all the problems when in fact the real issue is bias, and all collections, regardless of when they were amassed and what format they take, are biased in some way due to the collection process which is politically, socially and legally situated. The timing of the collection period is only relevant in how it is associated with these factors.

Regardless of the age of the collection, recently amassed collections and older collections share several fundamental problems. First, while there has been some discussion of how representative collections are of a hypothetical population (for example, Komar and Grivas, 2008), in a great deal of forensic research IRCs have been treated as biological or statistical *populations* rather than *samples*. All skeletal assemblages regardless of how they were amassed are biased samples and need to be treated as such. Second, although obvious, it is worth stating that the value of IRCs stems from the documentary data that are available for each individual in the collection, and for the collection as a whole. There could be no development and testing of forensic anthropology methods without this documentation. This issue goes beyond whether any data, such as age at death, are accurate. The documentary data have typically not been critically assessed for their

Fig. 1.- The Terry Collection curated at the Smithsonian Institution. The current state-of-the-art storage facilities for the Terry Collection at the Museum Support Center in 2019 (**left**). The author retrieving specimens in 2008 when the collection was curated at the National Museum of Natural History (**right**).

relevance, or how they have been curated and presented, regardless of the age of the collection.

There is no one single solution to solve the complex problems with IRCs. Although additional data available through new collections are clearly an asset for research, the newer collections do not solve any of the underlying problems with all IRCs and the commonly used methodologies that are tightly linked to these collections. These problems that affect all collections can be identified and, through careful research design, many of these problems can be overcome or even exploited when addressing specific research questions. In this chapter, I argue that what is needed are new approaches for identifying and assessing patterns of human variation using IRCs. I present a theoretical model and some methodological strategies for constructing reference samples from IRCs for maximizing the research potential of IRCs in a forensic context.

Several notes at the outset will help the reader with this chapter. First, the reader should note the difference between reference *collections* and reference *samples*. Reference collections are described in detail in the next section and can take many forms derived from various sources. A reference sample is a sub-set of one or more reference collections and is the sample analysed to address specific research questions such as developing or testing a method for estimating sex. Second, many collections are named after the principal person responsible for amassing the collection. Throughout this chapter, I often refer to the collector and the collection. I will use "Dr" as a title to help emphasize when I am referring to the collect*or* (person) rather than the collect*ion*. Third, as I discuss at length below, an exhaustive list of IRCs with only superficial information is not a useful tool for research involving IRC. By design, there has been no attempt in this chapter to provide such a list of collections or to follow the usual approach of presenting only superficial information about IRCs without context. Specific examples using various traditional and newer IRCs are provided to illustrate key theoretical and methodological issues. Readers are encouraged to review the references cited for detailed descriptions of collections mentioned throughout the text.

VARIOUS TYPES OF IDENTIFIED REFERENCE COLLECTIONS

IRCs are essential for forensic anthropology, because premortem records are available for each individual and the collection as a whole. In many cases, IRCs were specifically amassed to be reference collections and not to be the source of data for reconstructing any specific living population in time or space. Traditionally, IRCs could be classified into two general types based on their source: anatomical sources and cemetery sources.

The Terry Collection (Hunt and Albanese, 2005) and the Dart Collection (Dayal et al., 2009) are two of the major extensively used anatomical collections, while other smaller collections include the Grant Collection (Albanese, 2018a) and the Cobb Collection (Cobb, 1936; see also Watkins, 2018). Many of these anatomical collections, which are still widely used because they are useful sources of data, were started in the first half of the 20th Century. At that time, most physical anthropologists were anatomists who established systematic protocols for amassing reference collections derived from the skeletons of the cadavers used for anatomical instruction in medical schools (Hunt and Albanese, 2005).

For various historical and legal reasons, several of the more widely used cemetery-derived collections are found in Great Britain and Portugal—see Cardoso (2006) for details; see also Eliopoulos et al. (2007) for an example from Greece, and Rissech and Steadman (2010) for an example from Spain. These cemetery collections can be subdivided into two types. First, several collections such as the older Coimbra Collection (Rocha, 1995), newer Coimbra Collection (Ferreira et al., 2014), and to a lesser extent the new Lisbon Collection (Cardoso, 2006) were amassed from the start, as with the anatomical-derived collections, to be IRCs. Second, in other cases such as the collections from the crypts at St Bride's (Scheuer and Bowman, 1995) and Spitalfields (Molleson, 1995; Molleson and Cox, 1993), the collections came to be valued as potential reference collections due to the positive identification of individuals in each collection and contextual information for each of the collections.

These collections can be thought of as "incidental" or "accidentally" IRCs.

In the last 30 years, various collections have emerged by design or by accident that do not fit neatly into the two categories of anatomical-derived or cemetery-derived. The Bass Collection of real skeletons and the Forensic Anthropology Database (FDB) of skeletal data (Ousley and Jantz, 1998) are examples of various IRCs that were started and continue to grow in an effort to amass large collections to replace older anatomical collections, such as the Terry Collection, that have been *erroneously* discounted as no longer forensically relevant (see below). Although the source is different, the purpose of these efforts is similar to the older anatomical-derived collections: to compile a reference collection. In other cases, new more-widely available technologies and a lack of available skeletal collections, particularly of juveniles, have led to the use of alternative sources of data such as 3D models derived from CTs and MRIs. In most cases, these databases of scans can be considered "accidental" or "incidental" with respect to forensic anthropology because they, like the crypt collections, were not compiled specifically for research in forensic anthropology, but have been used for this research because of the lack of suitable real-bone specimens for analysis (for example, Spake and Cardoso (2018), see also their chapter in this volume) or perceived lack of real-bone specimens for analysis (for example, Noble et al. (2019), see below for details). Any source of data with pre-mortem records that is used to develop or test methods that are applicable to forensic anthropology is in all but name an IRC and should be considered inherently biased.

BIAS AND REPRESENTATIVENESS OF REFERENCE COLLECTIONS

Questioning the representativeness of IRCs is nothing new. For example, while the Terry Collection was being amassed, Dr Terry (1940) noted some of these types of issues about his own collection. He identified the source of the skeletons as one of the primary issues (see below where bequeathed and unbequeathed remains, and the timing of death during the Great Depression are discussed). Giles (1964) reinforced this view that the source of the skeletons was the principal reason why most collections in the USA were not representative of populations in that country. However, by the end of the 20th century, others argued that these collections were not useful for the development of forensic methods, because of the age of the collections and the impacts of secular change (for example, Ousley and Jantz, 1998). Secular changes or secular trends are non-genetic changes that occur across generations and that have been correlated with changes in living conditions. Secular changes are on-going and have been identified in stature, sitting height, limb lengths and proportions, head size and shape, etc.—see Albanese (2010) for a detailed review of the methodology for assessing secular changes specifically using skeletal data from IRCs). Generally, more individuals grow to their full potential with the improvement in living conditions. One of the proposed solutions to deal with secular changes in the collections was to compile a database collection with an arbitrary cut off for year of birth, initially at 1900, to control for secular changes (Ousley and Jantz, 1998). Despite Ousley and Jantz's (1998) critique of the "old" collections, a major sample from the Terry Collection is the foundational sample for the FDB. While year of birth may be relevant as a coarse indicator of secular changes, the arbitrary cut-off of 1900 for year of birth, or any other arbitrary year, provides a false sense of controlling for variation without controlling for or addressing any real problems, because the fundamental problems are ignored (see Albanese et al., 2016a; Albanese et al., 2016b; Albanese et al., 2012, for a detailed exploration of these issues when dealing with IRC and stature estimation).

Regardless of the type or source of IRC, the major issue that affects all collections is bias stemming from the collection process, which is politically, socially, and legally situated (for example, see Blakey, 1986). None of these collections are free of bias regardless of whether they are real bone or virtual, recently amassed or older, cemetery or anatomical. None are an "osteological census" or a random sampling of human variation. However, bias is not necessarily a problem unless it goes

unidentified. Failing to identify the biases inherent in these collections will necessarily produce erroneous results. For example, the unique historical circumstances related to both Dr Terry's and Dr Mildred Trotter's contributions to amassing the Terry Collection have resulted in a situation where mortality bias is easily misunderstood as racial difference (Albanese and Saunders, 2006). When Dr Trotter took over from Dr Terry and continued to add skeletons to the Terry Collection, but in a much more limited way, she focused on including white females (Hunt and Albanese, 2005; Trotter, 1981; Overholser et al., 1956). Because of this historical context for adding to the Terry Collection, a disproportionate number of females described as black are younger adults, whereas a disproportionate number of females described as white are older adults (Albanese and Saunders, 2006). Any comparison of black females to white females in the Terry Collection is a comparison of younger adult females who died prematurely to older females that survived into older age. The comparison of black females to white females reveals the impacts of extreme poverty and rac*ism* in the second half of the 19th and beginning of the 20th century if the context for collecting skeletons, the history of the USA (segregationist period after the American civil war), as well as age at death data are considered (Albanese, 2018b; see also de la Cova, 2012, 2011, 2010 for examples that include other anatomical IRCs form the USA). In addition to the highly problematic nature of a typological approach (see below), an ancestry method developed using a reference sample from the Terry Collection is impossible to apply with confidence in any forensic context. In contrast, considering the context for the variation and the biases in the collection will result in a better understanding of human variation and the underlying causes of that variation.

Determining when the collection was amassed is important for context, but the elapsed time since the collection was amassed is only superficially related to bias. With the Terry collection, for example, the fact that collecting ceased about 60 years ago is not the issue. Some of the key issues are historically relevant and include a changing racial paradigm in the discipline, progress with the fight for civil rights, views of anatomical instruction, changing laws on the bequeathment of human remains, and that most of the collection was added during the Great Depression, when many individuals were very poor at the time of death, but not all were necessarily poor at the time of their growth and development (Hunt and Albanese, 2005; Trotter, 1981).

A THEORETICAL FRAMEWORK FOR UNDERSTANDING HUMAN VARIATION SAMPLED IN IDENTIFIED REFERENCE COLLECTION

The focus of this section is the presentation of a theoretical framework or model that has been used extensively for critiquing typological approaches to assessing human variation (i.e., race and ancestry), and developing sex and stature estimation forensic methods using the Terry, Coimbra, Grant and Lisbon Collections and the FDB (Albanese, 2003; Albanese and Saunders, 2006; Albanese, 2013; Albanese et al., 2016a). An earlier version of this model was developed for doctoral research (Albanese, 2003b) that focused on sampling through ICRs to investigate patterns of human variation in *Homo sapiens*. It is worth stating clearly that this is *one* continually evolving approach which has been modified with every application over 20 years, and not the one and only model that can or should be used when dealing with IRCs (see Albanese (2018b) for a detailed presentation in English, and Albanese (2019) for an overview in Portuguese). The main point of this section and this chapter is to illustrate the importance of a model like this one to select a reference sample to address specific research questions using IRCs, and to illustrate the problems that may arise when bias is not considered. This approach is in contrast to the reductionist approach of sampling of IRCs based on racial/ancestry categories, continental origin, nationality or region.

This model was developed by combining and modifying cemetery studies theory (Hoppa, 1996, 1999; Saunders and Herring, 1995) and the New Biocultural Synthesis (Goodman and Leatherman, 1998a, specifically, the chapters by Armelagos and Goodman, 1998; Blakey, 1998; Goodman and Leatherman, 1998b; Levins and Lewontin, 1998;

Roseberry, 1998; Singer, 1998) for the study of IRCs (Albanese, 2018b). Initially it was developed for collections that were designed specifically to be reference collections such as the Terry Collection and the Coimbra Collection. However, the model has been expanded and is applicable with some modifications to "accidental" collections and database collections. The approach is directed at addressing specific research questions using data from IRCs, whatever form they take. Biases may be different in their origin, scope and magnitude, but they are still present regardless of whether the reference collection was created by design or the collection's potential as an IRC was recognized after the fact.

A necessary approach to understanding the impacts of bias on the variation sampled in IRCs is to begin to think of them as specialized mortuary assemblages, or in other words biased samples. Burial in a crypt at St Bride's and Spitalfields was reserved for those with the economic means for this type of burial, and as a result may not reflect health and variation in the greater cemeteries (which no longer exist), the mortality sample for the entire community or the living population at the time in the immediate parish or greater municipality (Molleson, 1995, Fig. 2). All IRCs including those derived from anatomical sources such as the Terry Collection should be thought of in a very similar way as a highly specialized interment. The criteria for inclusion in a church crypt or anatomical collection are likely very different and even diametrically opposite, but the point is that inclusion in any of these collections was not random. Inclusion was based on very specific socioeconomic factors related to living conditions before death, which had a complex impact on the skeletons of those included in a collection. These issues are complex because having the means for crypt burial at the time of death does not mean that individuals were of higher status for their entire life, and having been included in the Terry Collection during the Great Depression may reflect extreme poverty only at the time of death in some cases. Additionally, "better" and "worse" living conditions are relative concepts (Halliday and Albanese, 2019). Poverty or a chronic lack of access to resources will mean different things for late-17th Century London (Spitalfields Collection) versus late-19th Century St Louis (Terry Collection). Lastly, an acute crisis

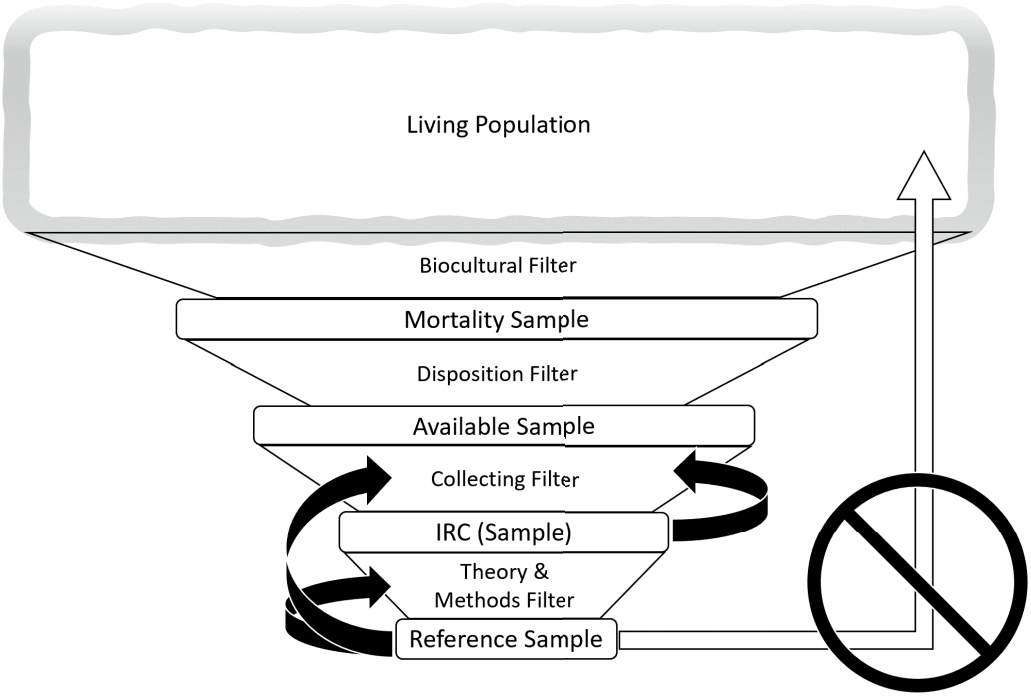

Fig. 2.- Graphical illustration of the impacts of filters and feedback loops on various levels of *samples* that may result in a biased reference sample (after Albanese, 2018b, and based on Albanese, 2003b; Hoppa, 1996, 1999; Saunders and Herring, 1995; Goodman and Leatherman, 1998). The curved solid black arrows represent the feedback loops that shape research practices and the composition of the collection as it is being amassed. The unshaded arrow highlights the issue that reference collections are poor sources of data for reconstructing a living population that is almost impossible to define. The fuzzy line around the living population highlights the problems of defining such a population.

resulting in a temporary lack of resources will have complex impacts on variation because of the timing of the physiological stress (Brauer, 1982).

Expanding this issue to more-recent sources of data, as Spake and Cardoso (2018) have demonstrated using data derived from scans, there is a tendency for children who are the victims of homicide in some areas of the USA to be small for their age (see also chapter by Cardoso and Spake in this volume for details). They found that similar datasets of scans (i.e., IRCs in all but name) acquired from similar sources, but from other countries did not have the same biases: juvenile victims of homicide were small for their age only in the sample from the USA. The collection process for IRCs is politically, socially and legally situated and applies to all compilations of skeletons or datasets in a unique fashion specific to that collection.

There have been various attempts to investigate the representativeness of IRCs with respect to a greater local or regional population (for example, Komar and Grivas, 2008; Rissech and Steadman, 2010). While some of these approaches have been systematic and have identified some biases, they have not gone far enough in addressing issues that affect research involving IRCs. There is no simple relationship between the collection and the population from which it is derived, and typically a reference sample, a subsample of one or more IRCs, is used for research. A number of stratified filters separate this reference sample used in research from the pattern of variation in any one biocultural and historically situated population. The parameters of the living population are almost impossible to define in many cases because of various factors such as temporal parameters (19th versus 20th century for years of birth and/or life span?), geographic parameters (parish, neighbourhood, municipality or region?), social parameters (those considered full legal citizens versus those excluded based on gender, religion, race, ethnicity or caste?), etc. Thus, the only statistical population is a hypothetical living population, which is an almost irrelevant abstraction with no clear parameters (see Fig. 2). The IRCs are poor sources of data for understanding the greater populations from which they were derived. The crypt sample from Spitalfields would provide a very narrow understanding of the entire city of London, England from the mid-17th to mid-19th centuries. Similarly, it would not be advisable to use the Terry Collection to try and reconstruct the population from the mid-19th to the mid-20th century in and around St Louis or the entire state of Missouri, where the collection was amassed. By analogy, these collections can be considered partial dictionaries. All the words found in this chapter can be found in *The Oxford English Dictionary*, but random sampling of words from the dictionary will not inform the reader about the contents of this chapter. Furthermore, in a forensic context we are dealing with an unknown in a non-population when unidentified remains are analysed. Spake and Cardoso (2018) have demonstrated that a failure to consider bias will present problems when estimating age, even when using a new collection that should "fix" the problems with the old collections. In order to develop methods that can be reliably and confidently applied in this context, we need to understand the filters that have led to the construction of the reference sample used to develop and test those methods.

An important, but rarely considered, publication that began to address some of these issues is Ericksen's (1982) comparison of two sub-samples of the Terry Collection: bequeathed versus unbequeathed individuals. Due to major changes in the greater society in the USA after World War II, it became legally possible to bequeath one's remains for anatomical instruction or other medical uses such as organ donation (Hunt and Albanese, 2005; Trotter, 1981; Pregaldin, 1958; Blake, 1955). Ericksen surmised that there were likely socio-economic differences between these two groups, and that these differences had had an impact on growth, development and aging, which of course would manifest differently in the skeletons in each group. Ericksen's approach highlighted the importance of considering greater socio-economic and legal context to guide sampling to construct a research sample. A failure to consider these issues amounts to a reductionist typological approach that assumes variation exists outside of all context.

Regardless of the nature of the living population, the point of the model presented here is that the collection that is available for potential analysis has been severely filtered on a number of nested levels that often have feedback loops (see Fig. 2). The first two levels and filters are similar for reference collections and bioarchaeological collections and borrow heavily from relevant theoretical frameworks (specifically Hoppa, 1996, 1999; Saunders and Herring, 1995). First, the living population is filtered by a biocultural filter (who dies at what age) which leads to a mortality sample. At this point and for the next several levels and filters, factors that include processing, handling, errors in transcription or curation, and taphonomy all play a role in the final composition of the collection regardless of its form (real bone or virtual), and may render all or parts of the collection unusable. Second, this mortality sample is affected by the disposition filter that reflects how remains are processed and disposed of (for example, inclusion in a crypt or used as a cadaver for anatomical instruction).

At this point, there is a divergence from strictly bioarchaeological collections as IRCs undergo more and unique filters. Third, the sample that is available to be included in the collection is affected by the collecting filter. All reference collections are constructed. This issue is most obvious for some cemetery and anatomical collections when the historical context is considered (see Albanese, 2018b, for details about the Coimbra and Terry Collections and Albanese, 2018a, for details about the Grant Collection.) As mentioned above, when Dr Trotter was forced to limit the number of skeletons that could be included in the Terry Collection, she made conscious choices about whose skeletons to retain. The choices that are made reflect the research interests of the collector, the parameters of the discipline, and the world view derived from the greater society of the collector. However, regardless of the sources of the collection, choices were made by specific researchers or curators as to which individual skeletons (or scans) are retained, processed, and made available for research.

Through the collecting filter, the skeletal collection in its entirety, which is a sample and not a population, is established. In many cases, IRCs were constructed over extended periods or in several waves and individuals were added to a collection, only to be removed and occasionally to be intentionally or accidently re-added to the skeletal collection. Persistent handling of widely used collections, no matter how careful, will necessarily degrade bone quality, and make individual bones or entire individuals no longer useful for some research. At the same time, new techniques and research designs can mean that the previously unusable specimens can be included in research again. These factors and many others have a direct impact on the observed sample. For example, throughout the 20th Century, it was considered essential that any sampling of the Terry Collection and other similar collections had to be based on racial criteria (more recently referred to as ancestry but see below for details). However, as already noted, what seem to be apparent differences between white females and black females from the Terry Collection are the result of mortality bias accentuated by Dr Trotter's attempts to balance the sex ratio of the collection.

The last issue that needs to be addressed in this section is the feedback loops. The apparent racial differences between black and whites in the Terry Collection reinforced the racial approach in physical anthropology in the mid-20th century (see Washburn, 1951, 1963, for a contemporaneous critique), and the racialism and outright racism in the greater American society. Dr Trotter's awareness of the sex bias in all anatomical-derived collections had a huge impact on her choice to focus on including females in the Terry Collection. Dr Trotter's very positive impact of balancing the sex ratio in the Terry Collection resulted in a biased collection if the observed sample is selected based on racial criteria. These feedback loops for the construction of collections had an impact on the methodologies used to conduct research (sample size, statistical approaches, sampling techniques) and the construction of the observed samples for future research. Trotter and Gleser's (1951, 1952, 1958) work established the persistent trend for stature estimation with group/sex-specific equations calculated using linear regression (Albanese et

al., 2016a). A similar pattern was established by the 1950s for metric approaches for estimating sex (Albanese, 2003b). The persistent erroneous assumptions have been that metric methods must be population-specific, and discriminant function analysis is the best approach for developing multivariate methods (Albanese, 2003a). These "standards" were set while Dr Trotter was still amassing the collection and had an impact on Dr Trotter's choices for including individuals in the collection. The standard methodology for using the collections, both shaped and were shaped by the IRCs. The removal of individuals, the re-adding of individuals, and the selective nature of additions were based on conscious choices by collectors to address real and perceived problems that were historically situated. The collectors corrected some biases and accentuated others based on their personal research interests, the parameters for acceptable research in the discipline, and the greater society at the time of collecting.

When approached from this perspective there are two issues that seem to make IRCs very problematic. First, all collections are biased in complex ways. Second, an IRC cannot be representative of the population from which it was drawn. However, representativeness is a non-issue in forensic anthropology because the parameters are never clear or discrete for group membership in "the population", and we need methods that work for an unknown individual for whom we have no *a priori* knowledge. Bias is only a problem if it is not considered. Once the relevant biases are identified, it becomes possible to address them or even exploit them. Spake and Cardoso's (2018) research illustrates this issue. They were able to identify that poverty, reflected in compromised growth, is correlated to homicide of juveniles in USA (see Albanese, 2018b for additional examples). The approach should not be to sample variation in any one collection, but rather to sample through the collections to construct a reference sample to address specific research questions. For example, when developing methods for sex estimation, multiple collections may be sampled through a stratified process to create a reference sample that includes a range of variation found in *Homo sapiens* (Albanese, 2003). When the test sample is also selected in a way to exploit biases, it is possible to test the parameters of applicability of a method, and then the resulting method can be confidently applied in almost any context where there is no *a priori* knowledge of the unknown individual (Albanese, 2013).

CRITICALLY ASSESSING THE ACCURACY, RELEVANCE AND MEANING OF DOCUMENTARY DATA

The research potential of IRCs is directly attributable to the quality of the documentary data that are available. Many researchers have taken these data at face value without considering what this information meant when it was compiled, whether it was verified, why it was retained, and what it means now. The focus in the previous section was on how bias can affect which skeletons are included in a reference sample, and thus the patterns of variation in a collection. However, it is important to note that bias has had an impact on what documentary data were recorded, verified, retained, readily curated with the skeletons, and widely circulated, and not just on which skeletons are available for analysis. These concepts can be illustrated with an example contrasting two contemporaneous collections, the Grant Collection (Albanese, 2018a) and the Terry Collection (Hunt and Albanese, 2005), regarding age at death data and race/ancestry designations.

Within the context of the theoretical framework summarized above, age at death data have been essential criteria for constructing reference samples to deal with or control for biases in IRCs in research that focused on developing and testing sex and stature estimation methods and critiquing typological approaches to understanding human skeletal variation. In other words, even when age at death was not the focus of the research, age data were critical to conducting the research. A review of the age data has served two important purposes. First, an assessment of age at death data is one of the most direct ways to begin assessing the overall quality of documentary data for an IRC. Second, age at death data are good criteria for sampling a wide range of variation related to sexual dimorphism, growth and development, body proportions, etc.

After reviewing the archival and documentary data available for both collections, it becomes very clear that age at death data were of the utmost importance to both Dr Grant and Dr Terry (see Hunt and Albanese, 2005 and Albanese, 2018a, for details on the review of documents). Neither Dr Terry nor Dr Grant assumed that death certificate data were correct and both collectors developed a protocol for reviewing, assessing and independently verifying these data for their respective collections. They used information from third parties such as hospitals, and people who knew the deceased such as friends and landlords to verify the accuracy of the age data for each individual in the collection. Furthermore, they clearly indicated when the age data had not been or could not be verified. By reviewing the data collection and verification protocol and the age data for the collection as whole for both the collections, it becomes possible to then have certainty in the age data for any one individual in the collection.

As a result of the comprehensive review of all relevant documents within the social and legal context, it is possible to conclude that the age at death data in both the Terry and Grant Collections can be considered more accurate than death certificate data for most individuals. Internal documents that accompanied each cadaver/skeleton were amended as new information became available from third parties. In some cases, a failure to verify the age data was one of the filters between the available sample (all the cadavers used for anatomical instruction in the respective medical school) and the IRC (all the skeletons that were retained and still currently part of the respective collections). However, original death certificates or similar official documents were not changed and had to be retained in perpetuity in accordance with the respective anatomy acts in the State of Missouri (Terry Collection) and the Province of Ontario (Grant Collection). Although highly unlikely, the next of kin still have legal rights to claim the remains of an individual who was used for anatomical instruction including individuals in either of these collections, and the unmodified death certificate or similar documentation is literally the key for identification.

Despite Dr Terry's and Dr Grant's systemic approach to verifying age at death, there are still some notable problems with age data for each of the respective collections. These problems are well-illustrated when the age at death data are presented in a histogram for the Terry Collection (Hunt and Albanese, 2005). There are clear spikes at ages that are multiples of five (particularly at ages 30, 40, 50, 60, 65 and 75). The age heaping is still present even when only considering those individuals whose age was verified. The heaping is present also for those with age verified in the Grant Collection, but the problems are less apparent, because the collection is much smaller than the Terry Collection (Albanese, 2018a).

Based on the review of the cases from both collections where age data were corrected, the initial documented ages versus verified ages could be off by many years. If the age is designated as unverified, then nothing is certain except that the person would have been considered an adult and thus suitable for anatomical dissection and instruction. Potential problems may arise with those individuals who fall into the heaped categories of multiples of five and whose age was verified. These problems are not limited to demographic data from the middle of the 20th century. These problems persist, particularly with older adults well into the 21st century due to a variety of factors (He and Muenchrath, 2011). One strategy is to avoid individuals with these documented ages regardless of whether they are verified or not. Any difference in skeletal age between an individual who was 64 years old and an individual who was 65 years old at the time of death will be eclipsed by a number of other factors. However, it is much more likely that the first individual was actually 64 years old at the time of death whereas the second individual may have actually been 65 years old or may have been much younger or much older than 65 years at the time of death (see Sharman and Albanese, 2018 for a detailed review and systematic approach to addressing potential problems using age data in IRCs). Age at death data should be thoroughly assessed for all IRCs regardless of when they were amassed.

The divergence in the documentary data between the Terry Collection and the Grant Collection occurs

with data on race (Albanese, 2018a). Dr Terry and Dr Trotter on the one hand, and Dr Grant on the other hand were contemporaneous. They made additions to their respective collections throughout the middle of the 20th century in major North American cities. There are clear parallels in how they collected, verified and curated documentary data. There were many similarities in the respective Anatomy Acts that allowed for the creation of their respective collections. The difference is in the emphasis on racial classification, and this focus can be attributed to the different historical and geopolitical context. Dr Grant was working in Canada and Drs Terry and Trotter were working in the USA.

Borrowing from Armelagos and Goodman (1998:259), it is important to make a clear and unambiguous statement that race "...has failed to work as a core anthropological concept; it fails to describe and explain variation. What race does do and does well, is type individuals, and this typing supports the existing structures of power." Human variation does not cluster by race or continental origin, and racial categories are not useful units of analysis for assessing human variation (Albanese, 2010; Albanese and Saunders, 2006; Molnar, 2005; Duster, 2004; Tishkoff and Kid, 2004; Keita et al., 2004; Armelagos and Van Gerven, 2003; Excoffier and Hamilton, 2003; Duster, 2003; Brown and Armelagos, 2001; Keita and Boyce, 2001; Lieberman, 2001; Relethford, 2001; Smay and Armelagos, 2000; Kittles and Keita, 1999; Armelagos and Goodman, 1998; Blakey, 1998; Templeton, 1998; Goodman, 1997; Keita and Kittles, 1997; Gould, 1996; Brace, 1995; Corcos, 1997; Blakey, 1987; Brace, 1982; Armelagos et al., 1982; Latter, 1980; Ossenberg, 1976; Lewontin, 1972; Livingstone, 1962; Cobb, 1936). Racial data were not recorded or retained for any individuals in the Grant Collection. Most of the individuals in the Terry Collection are designated as "negro" or white. Although the term is at best passé and at worst derogatory in some 21st Century contexts, before the mid-1960s the term "negro" was considered to be socially, politically and scientifically neutral (see Martin, 1991 for the sociopolitical context for the change in terminology in the USA, and Albanese and Saunders, 2006 for a description of the change in forensic anthropology).

From a strictly anthropological perspective it is valuable to use a 21st Century lens to look back and be critical of racialized approaches to research in the middle of the last century, but this perspective has been outside the scope of most research of forensic anthropologists developing and testing methods in the 21st Century. Although the prevailing view is that forensic anthropologists deal with ancestry and not race, the trend in the discipline since the 1990s has been to substitute racial terms with ancestry terms without addressing any of the underlying conceptual issues or problems with this typological approach to human variation (Albanese and Saunders, 2006). The concept of ancestry can be useful when used correctly. A good example to illustrate this point is the elevated levels of the sickle cell allele in groups whose ancestors lived in a malarial environment. The link is between an allele frequency and an ecological context and not a specific geographical location. There is no direct link of the sickle cell allele to melanin production (skin colour) or continental origin (see Molnar, 2005 for a summary of key issues regarding the distribution of the sickle allele, other hemoglobin variants and the correlation with malaria environments). However, ancestry as a substitute for race, as it has been used and continues to be used in forensic anthropology, fails to explain or account for human variation, because it is a 19th century concept of human variation that predates the adoption of theory of evolution and genetics by physical anthropologists (Washburn, 1951). The racial classifications that Drs Trotter and Terry made are based on those views of human variation that Washburn criticized in the 1950s when he advocated for the "New Physical Anthropology" to move away from a descriptive approach to an evidence-based approach to understanding human variation within evolutionary and genetic theoretical frameworks. Within this context, changing "Negro" to "Black" or "African-American" addresses none of the inherent problems in the original classifications. Those terms were arbitrary and typological in nature then and they still are now.

The racial data were and continue to be prominently curated with each individual in the Terry Collection, because race was and still is a significant issue in the greater American society. These issues are historical in origin but persist in the discipline and are reflected in how all documentary data are presented. Various compilations or lists of identified collections are available. In some cases, these lists were compiled and presented in an official capacity by professional organizations such as the Forensic Anthropology Society of Europe (FASE, http://forensicanthropology.eu/osteological-collections/) and in other cases these lists are compiled in an unofficial capacity by interested non-professional third parties such as the "Highfantastical" website (http://highfantastical.com/skeletal-collections/). At the time of writing this chapter in January 2020, "Highfantastical's" dead links to "Interactive Fiction" and a database of "Knitting Stiches" are red flags suggesting a healthy dose of skepticism about the content of the site, but all of these over-simplified lists should be considered as only a very preliminary starting point for finding potential samples for forensic research. The FASE site provides useful information such as links to institutional websites with the contact information of the curator and/or information for gaining access to the collections for research. However, caution should be exercised when reviewing the information that is listed for each collection, which is provided without any scholarly or peer-reviewed citations. In some cases on the FASE list, information may be ambiguous or erroneous. For example, for the Terry Collection term "ethnic origin" is erroneously used.

The entry for the Grant Collection is even more problematic. FASE describes the Grant Collection as having been "founded" in 1928, several years before Dr Grant arrived at the University of Toronto (Albanese, 2018a). While a few of the cases in the Grant Collection have death years from the 1920s (the earliest being 1927 and not 1928), collecting of skeletons was under the careful direction of Dr Grant after he took up the chair of Anatomy at the University of Toronto in 1930. The key issue is Dr Grant's personal influence on the collection. Even when the information is correct about collections, only the most superficial information about collections is provided. Thus, while the FASE site notes correctly that there are 202 individuals in the collection, the limiting issue with respect to research potential which is not mentioned is that only 26 are female and age was verified for only 16 of those females. Furthermore, there is no information about the source, accuracy and precision of documentary data. Although likely unintentionally, the FASE website reinforces the idea that because something was written down it must be accurate or correct and that documentary data do not need to be critically assessed. In the case with the Grant Collection, the data have been extensively reviewed and the documentary data can be considered more accurate than death certificate data except in a few cases where Dr Grant clearly indicated that the data should be considered suspect (Albanese, 2018a). The same conclusion cannot be assumed for other IRCs.

The final issue with these compiled lists that is relevant to the discussion in this chapter is categorizations of collection based on arbitrary temporal criteria. The FASE site places collections into one of three categories: 1) "Modern identified collections," 2) "Non-modern identified collections," and 3) "Identified collections of uncertain temporal status". The third category is obviously a catch-all for collections where not enough was known for categorization. The criteria for distinguishing between the first two categories is: "Modern identified collections are defined as being comprised of individuals born after 1920, for whom age at death and sex are known." It is not clear from the FASE website why 1920 was selected as the cut-off for birth year, or why any given year should be selected as a cut-off. Certainly, secular changes are a source of variation and/or bias, but as noted above there is no simple threshold effect at any given year, and certainly not on a world-wide basis. While not solving any problems, arbitrary cut-offs for year of birth cause other potential problems with mortality bias. Given the narrow window for the inclusion of individuals in any given collection, year of birth is highly correlated to age at death with bias pulling in opposite directions (Albanese, 2003b). Those individuals with the most recent years of birth and likely to be affected by a positive secular change are the youngest individuals who manifest the greatest

impact of mortality bias—i.e., premature death related to physiological stress during growth and development. This confounding effect of mortality bias and secular changes is a potential issue for both the Grant and Terry Collections, but on the FASE website the Terry Collection is assigned to the first category "modern identified collections", because some of the individuals included in the collection were born after 1920, and the Grant Collection is assigned to the second category "non-modern identified collections."

The point here is not to be critical of FASE for this list, but rather to highlight the problematic nature of this approach in the entire discipline of forensic anthropology with respect to summarizing and presenting demographic information available for IRCs. These lists of collections do not provide enough data and context to make informed decisions about their utility in forensically relevant research. Systematic, detailed reviews that place identified collections in context are essential to understanding the bias and thus the variation sampled by these collections. One of the first of these was Rocha's (1995) work with the Coimbra Collection. Unfortunately, its greater importance has never been recognized, possibly because it was published in French and the Coimbra Collection was a relatively obscure collection at the time of publication. After Hunt and Albanese's (2005) detailed description of the Terry Collection and its historical context in a major widely read English language journal, a series of similar papers were published describing various collections in similar detail (for example, Albanese, 2018a; Rissech and Steadman, 2010; Dayal et al., 2009; Eliopoulos et al., 2007; Cardoso, 2006, which are discussed in this chapter). Only through this type of systematic assessment of the reference collections and their placement in the relevant context when collecting happened can their full research potential be realized.

CASE STUDY: THE IMPLICATIONS OF CONSTRUCTING A REFERENCE SAMPLE VERSUS HAVING IT CONSTRUCTED FOR YOU

In a recent publication, Noble et al. (2019) used a sample of 400 MDCT scans from Toronto (Canada) and followed a pattern of research that is common in forensic anthropology where country-specific standards are tested or developed. In countries with a long racial history the race/ancestry approach persists (Albanese, 2003b), but outside of nations such as the USA and South Africa, this country-specific approach has become very common for developing and testing sex estimation methods. The approach is the same and typological in nature, and fails to capture or control for variation, regardless of whether the racial/ancestry approach is used, or a country-specific approach is used. The point here is to illustrate that the framework presented in this chapter reveals that this country-specific approach is highly problematic on multiple levels. It is worth stating emphatically that the paper by Noble and colleagues is used as a case study to illustrate the greater problems in the discipline of forensic anthropology. The publication drew my attention at two levels: personal and professional. The variation in Canada in general and Toronto in particular as presented by Noble and colleagues is inconsistent with my personal experience of having lived in Toronto for decades, and it is inconsistent with my 25 years of research experience developing and testing sex estimation methods using skeletal and digital data from IRCs. This article by Noble and colleagues is in no way unique and is at the intersection of the problems with reference sample construction, definitions of a population, accidental collections, digital models, a recent publication date, and other major themes and issues presented in this chapter. This common approach is widespread throughout the discipline and is highly problematic. The same critique is applicable to any number of publications (some examples include but are not limited to Cappella et al., 2020; Liu et al., 2020; Čechová et al., 2019; Tallman and Blanton, 2019; Colman et al., 2018; Hora and Sladek, 2018; Nikita and Michopoulou, 2018; Peckmann and Fisher, 2018; Kotěrová et al., 2017; Scott et al., 2017; Peckmann et al., 2016; Moore et al., 2016; Torimitsu et al., 2016; Kim et al., 2013; Tise et al., 2013; Abd-elaleem et al., 2012; Papaioannou et al., 2012; Zhenget al., 2012; Charisi et al., 2011; Akhlaghi, 2010; Nagesh et al., 2007).

As with any group-specific approach, the rationale is that methods developed for a specific group should perform better on a member of that group. The main issue with these approaches is that the parameters for group membership are poorly defined. The country-specific approach is arbitrary and fails to account for human variation. The research is formulaic and follows a typical pattern. An existing method for a specific skeletal element (cranium, radius, femur, etc.) is tested on a sample that is described based on geopolitical boundaries that have no bearing on patterns of human variation. The only other information typically provided are age data that are presented as a range or mean by sex, when what matters is the *distribution* of ages by sex (Albanese, 2003a; Albanese, 2013). The method typically performs poorly or seems to perform poorly. Performance is based on arbitrary criteria and the poor performance is attributed to the need for group-specific standards without adequately excluding other possible issues. A new group-specific method is recommended and/or presented in the same paper and/or a companion paper. In this case study, Noble and colleagues tested three cranial sex estimation methods including a metric approach by Giles and Elliot (1963), a morphoscopic approach by Walker (2008), and a scan-based approach by Franklin et al. (2013) on a "Canadian population".

Methodologically, this country-specific approach presents an insurmountable problem. It is impossible to assess group membership of an unknown individual based on skeletal morphology, and the location where an unidentified skeleton was found is not helpful considering the amount of movement of people across international or regional boarders. For example, the author of this chapter was presented with a forensic case where the remains of an individual had been found on the shoreline near the mouth of the Detroit River, the international border between Canada and the USA. The unknown could have fallen in river on either side of border and ended up in the same location in Canada because of the currents where the Detroit River empties into Lake Erie. In this case, what should be used, American or Canadian standards, and how could it be possible to assess if the unknown individual was an American or a Canadian?

Theoretically, these geopolitical categories are arbitrary and do not account for or control for the diversity of human variation. As described above, Spake and Cardoso (2018) demonstrate the problematic nature of this assumption. The murdered children from their American sample are on average small for their age and would not be a suitable reference sample for an American standard. Similarly, a test sample consisting of 400 MDCT scans from the Sunnybrook Health Science Centre in Toronto is a very biased sample, and it is not a "Canadian population" or even representative of a Canadian population. The sample is a subset of individuals who required a MDCT scan as part of a diagnostic procedure at one facility in Toronto, which is the most diverse city in the world (Ryan, 2019). Canada has a universal single-payer healthcare system that is administered at the provincial level, and there is equal access to health care for all legal residents of the Province of Ontario. The MDCT scan sample likely reflects some of this diversity present in the most diverse city in the world. It includes dozens of overlapping secular trends that reflect local changes in living standards in a major North American city and the waves of immigration of people from all over the world, which is not a recent trend or unique to Canada.

These 400 MDCT scans are a modern sample, by all accounts an IRC, and as biased as any other IRC. Not accounting for these biases means that it is difficult to assess why the three methods that were tested performed the way they did. Allocation accuracies will be affected by fundamental statistical and methodological limitations in the original methods being tested that have nothing to do with group-specificity, and problems with the application of methods from real bone to virtual models. It is essential that the impacts of the reproducibility of data collection on real bone versus digital models be thoroughly assessed (Stull et al., 2014) and the more complex issues associated with using data collected from 3D models in methods designed for real bone is considered (Mullins and Albanese, 2018). It is likely not a coincidence if the best

overall allocation accuracy can be achieved for a method designed using 3D models and tested on 3D models. Other sources of variation may be due to subtle difference in the age distribution by sex between the reference samples used to develop the original methods and those in the test samples since a clear age-related bias has been identified in the cranium (Walker, 1995). As with other similar studies Noble and colleagues present the range of age data but not the distribution of ages for of their test sample. With their test sample, more females are misallocated, which is consistent with a sample that has disproportionate number of older post-menopausal females (Walker, 1995).

The last issue worth considering is how to measure the performance of a method. In most cases cited above, arbitrary and unsubstantiated statements are made about how to measure performance which undermine the conclusion that reduction in allocation accuracy is do to the lack of group-specific standards. Overall allocation accuracy, and sex-bias in allocation are obviously important indicators. However, statistically speaking, a reduction in allocation accuracy should be expected when applying any method to a new sample not used to develop the method, which is why it is essential to test the method on truly independent samples that were not used to develop the method in the first place (Albanese, 2003a). Furthermore, unless *post hoc* statistics such as typicality and posterior probability are calculated, discriminant function analysis does not provide a probability statement for certainty in any one estimate of sex. The linear distance of the unknown's discriminant function score from the cut off for male and female (for example, as depicted in Noble et al., 2019) is not a probability statement. These issues are well-known problems with discriminant function analysis and make it a less robust option when compared to logistic regression when estimating sex (Albanese, 2003a). Certainty in an allocation is essential and an important measure of the utility of a method in any investigation, but most methods cited above continue to use discriminant function analysis despite its limitations when compared to a probabilistic approach. In a forensic case, wrong information is worse than no information. No information means that the investigation continues in pursuit of answers to fundamental questions of identification. Wrong information becomes an impediment to identification and ends the pursuit for more information. An answer of "unknown" or "uncertain" is much more useful to an investigation than a wrong estimation of sex. When assessing a method, it is best to avoid a simple dichotomous approach, and it is necessary to consider probabilities of a correct allocation.

A country-specific approach amounts to little more than anthropometry as nationalism and results in methods that cannot be applied in forensic cases. In the few cases where these concepts were questioned (for example, O'Bright et al., 2018; Králík et al., 2014), no attention is given to how the respective reference samples were constructed, and thus there is no consideration of any inherent biases in the variation sampled or the sources of that variation. In advance of some recent calls for world-wide standards for sex estimation (Garvin and Klales, 2020), the approach presented in this chapter for considering IRCs has been used for 20 years to develop universally applicable metric methods for sex estimation and also to layout an entire alternative approach for developing methods (Albanese, 2003a). Sex estimation methods that are designed from the start to be probabilistic and widely applicable will provide useful information when tested on a wide range of cases regardless of whether the data are metric or morphology (see Albanese, 2003a for a detailed summary of the issues). Methods do not work when they are based on poorly selected reference samples. The "debate" over metric versus morphological approaches is a non-issue (for example, Mays' 2010 description of metric methods as "schemes"). Variation such as femur head diameter that can be easily measured is measured, and variation that cannot be easily measured is scored as present/absent or on a multipoint scale. Similarly, an accurate and reproducible measurement of the length of the pubic bone captures the same variation assessed through the scoring of the ventral arc (Albanese, 2003a). Both the metric and the morphological approach are two ways of assessing the differential growth of the symphyseal end of the pubic

bone in females (Anderson, 1990; Budinoff and Tague, 1990). The Albanese (2003a) method was extensively tested in the original paper and the best equation, requiring only five measurements of the *os coxa* and femur, consistently resulted in an allocation accuracy of over 98% with zero sex bias even when tested on a large sample not used in any way to develop the method. The probability scores for this hold-out test sample were then presented to give a better sense of how often a method would provide information that would be useful in a forensic investigation: i.e., certainty in any one allocation outside of the reference sample used to develop the method (Albanese, 2003a). Furthermore, one of the equations could be tested using data from the FDB. The best equation could not be tested because one of the measurements used for the method was not available in the FDB. Thus, the length of the pubic bone, one of the most important indicators of sex, was not included in this test. The sample from the FDB (n = 213) was selected to test the forensic applicability of the method and is the equivalent of testing this method developed using the "old" Terry and Coimbra Collections on 213 forensic cases. As expected with any statistical model used for prediction, allocation accuracy and sex bias varied from the initial allocation accuracy of 93% overall (females = 93.7%, males = 92.3%) when compared to the allocation accuracy 93.3% overall (females = 95.5, males = 91.0) for the hold out test sample (n = 125) from the Terry and Coimbra Collections, and 94% overall (females = 89%, males = 96%) for the FDB sample. Regardless of the fluctuations, a probabilistic metric method that was designed to be widely applicable was consistent in allocating individuals correctly even when tested on several hundred forensic cases.

The theoretical model presented in this chapter has been applied to the development of other methods. The first method using the pelvis and femur (Albanese, 2003a) was followed up with a method using the upper limb for sex estimation (Albanese, 2013) and a series of equations for estimating stature (Albanese et al., 2016a). The sex estimation method using the upper limb (Albanese, 2013) was developed and tested using almost the same sample as the pelvis and femur method and is not reviewed here. The development of the stature estimation method varied because there are very few sources of data where skeletal measurement can be collected from a diverse sample with reliable documented stature (Cardoso et al., 2016). The approach outlined in this chapter was applied to develop a series of univariate and multivariate equations using long bones to estimate stature that are not-group specific. Testing with large, independent samples demonstrated that the methods could be consistently and reliably applied with confidence in a range of cases where, sex, age, year of birth, nationality, ancestry, and race did not need to be assessed first. The equations were developed using a carefully constructed sample from the "old" Terry Collection that included "negro" females. In a direct test of two methods, these equations performed as well as equations developed specifically for Portuguese males (Mendonça, 2000) when tested using a sample of Portuguese males.

Country-specific standards provide the illusion of controlling for variation but are harder to apply because group membership cannot be assessed from skeletal remains, and this added difficulty in choosing the "right" equation does nothing to increase the accuracy or reliability of the method. In some cases, investigators may suspect where the unknown may have originated or even who the unknown might be. However, the forensic anthropologist should not be presupposing anything about the unknown and should be addressing questions of sex, age and stature, blind. Portuguese, like Canadian described above, is not a meaningful group when dealing with human skeletal variation. Ancestry when used as substitute in name only for race is equally problematic. A better approach is to avoid topological perspective based on preconceived poorly defined categories when using IRCs.

CONCLUSION

In this chapter I presented an evolving theoretical framework that has been highly effective for sampling IRCs to develop methods that are widely applicable in forensic anthropology. There are some key themes and

issues that are fundamental to this framework. The skeletal variation present in all IRCs is biased by the collection process. Skeletal collections are not populations, but rather highly biased samples of human variation. Bias is also an issue in what demographic information was collected, verified, and prominently curated with the collection. Newer is not necessarily better and does not solve underlying problems inherent with all IRCs. The timing of the collection period is only relevant in how it is associated with the political, social and legal context for the collecting of skeletons. The conclusion is clear that all IRCs are inherently biased. However, we use reference *samples* and not reference *collections* for research, and bias is only a problem if it is ignored when constructing these reference samples. Researchers who use IRCs have a choice: the reference samples can be constructed for you through a series of historical accidents (filters) or you can construct a reference sample to develop methods that can be applied with confidence in most forensic contexts almost anywhere in the world.

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Importance of donors body programs for forensic anthropological research

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SUMMARY

This article discusses the importance of body donation programs for the growth and development of forensic anthropology in order to highlight the benefits that these programs bring not only to the medical-surgical and anatomical fields but also to the field of forensic Anthropology. The benefits are manifest both in the teaching and research fields, since contemporary and reference osteological collections can be created from the Donation Programs. To emphasize the aforementioned importance, we will approach the Mexican experience, which from the Body Donation Program (PDC) of the National Autonomous University of Mexico (UNAM) has managed to form the first contemporary osteological collection, in which each of the skeletons is associated with donor's medical records containing data on age, weight, sex, origin, diseases, ante-mortem fractures, surgical interventions, imaging studies, among many other information. Therefore, the Mexican osteological collection has been of great interest, because it can be used for teaching students interested in osteology and particularly in forensic anthropology, but also in the development of different research projects that allow innovating in the techniques of human identification according to the biological characteristics of the Mexican population. Also, because both undergraduate and graduate students have been incorporated into the research team, that is, human resources are being trained in forensic anthropology. However, in order to understand the importance of body donation programs to forensic anthropology research it is pertinent that next we will address some of the antecedents of the use of the corpse, the first body donation programs and the osteological collections that are kept in various parts of the world. We conclude by presenting research projects and some results obtained in the Department of Innovation in Human Biological Material of the Faculty of Medicine of the National Autonomous University of Mexico (UNAM).

Key words: Anatomy – Dissection – Body donation – Human identification – Forensic anthropology

INTRODUCTION

The direct study of the human body is undoubtedly and will continue to be the best means to access its knowledge and enable the development of new research topics that allow the development of the scientific bases of various disciplines (e.g., physical anthropology, odontology, entomology, genetics, criminalistics, forensic archaeology, medicine). Throughout history it could be said that medicine and anatomy have been those disciplines that have had the greatest interest in knowing in detail the

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functioning and constitution of the human body. Proof of this are the great historical events and figures of medicine and human anatomy, such as: the Alexandrians, Herophilus of Chalcedon, Erasistratus of Ceos, Ptolemy Soter, Praxágoras of Cos, who cultivated the practice of dissection, or the Ptolemies, who allowed and encouraged the dissection of human bodies or the vivisection of criminals sentenced to death. Anatomy rapidly progressed as a pure science and as an auxiliary to medicine (Campohermoso et al., 2009).

During the Renaissance, anatomical studies were promoted and medical knowledge progressed from the second half of the 15th century, reviving the knowledge of the human body as a basic reality of medicine. At that time, teaching and innovation in the development of dissection techniques and autopsy practices should be highlighted, which were recognized as indispensable tools for teaching and research in these disciplines (Romero et al., 2005). But it was not until the 18th century, with the approval of the Murder Act (1752), that the use of corpses for the practice of dissection of bodies who belonged to prisoners executed for their criminal acts was formally allowed in England.

In the 19th century, the Anatomy Act was approved in the United States of America, specifically in Massachusetts in the year 1831. In the United Kingdom, it was approved a year later, allowing deceased bodies to be donated by their relatives (Rokade et al., 2013). Subsequently, the first donation programs created in 1967 were the Willed Body Program at The University of Arizona, College of Medicine, Department of Cellular & Molecular Medicine (College of Medicine Tucson, 2019) and the John A. Burns University School of Medicine, from Hawaii, created the Body Donation Program and received his first donation in 1968 (John A. Burns School of Medicine, 2020).

Brief history of skeletal collections

In the 18th and 19th centuries, in the field of natural history, there was a growing interest in the diversity of existing populations on the different continents, which led to the analysis of collections and studies of human skulls and skeletons in more systematic ways (Spencer, 1997). Since then, numerous collections have been built in museums and universities around the world (Quigley, 2001). Some collections were built for medical teaching or to document and show anomalies that science could not explain. Other collections of skulls were created in Europe and the United States by phrenologists, who believed that the shape of the brain and skull was related to the moral characteristics and intellectual abilities of individuals (Quigley, 2001; Spencer, 1997). Also, in museums there were collections of mummies and skeletons considered representative of the "inferior races" or of the customs of "primitive peoples.

The collections of skulls and skeletons contributed to the study of human evolution, past populations, paleopathology, the history of medicine, physical or biological anthropology, among others. In other words, until the mid-20th century the osteological collections identified were used mainly to make anatomical comparisons between peoples from different regions of the world and with the fossil specimens, and knowledge about human evolution was strengthened (Santos, 2018; Spencer, 1997). In particular, physical or biological anthropology began to analyze collections to establish methods to estimate sex, age, height, or biological affinity. Thus, at present there is a need to increase the number of individuals and expand the geographical distribution of the samples leading to the continuous development of these collections.

Santos (2019) mentions that there are currently about fifty collections in countries of North and South America, Africa, Europe and Asia. In the field of physical anthropology, the best-known collections—those from which parameters were obtained to estimate age, sex or height—are the Terry and Hamman-Todd, both of which were formed in the United States.

In Europe and America, in both medical and biological schools, there are some documented skeletal collections constituted by modern individuals. Since the beginning of the nineties, the need for a modern human skeletal collection has been expressed by many authors, and this has led to the creation of new collections constituted by modern (contemporary) individuals such as

the Colombian Collection in Bogota, the UAB collection, Valladolid collection and the Granada Collection in Spain, the Athens Collection in the Department of Animal and Human Physiology at the University of Athens, the Lisbon Collection in the Department of Zoology and Anthropology in the University of Lisbon and Pretoria Collection in the Department of Anatomy in the University of Pretoria. These collections come from both body donation programs and cemeteries (Rissech and Steadman, 2011).

The aims of this paper are two: (1) to present the new creation of the National Autonomous University of Mexico (UNAM) documented osteological collection and (2) to demonstrate the importance of body donation programs of the anatomy units to create documented osteological collections.

Contemporary Osteological Collections

According to the European Society for Forensic Anthropology, there are 28 contemporary osteological collections, which are made up of individuals born after 1920 and their age of death and sex are known (Forensic Anthropology Society of Europe, 2018). Depending on the collection, demographic information, biological affinity and even the profession may be available. In other collections, medical data, including cause of death, and antemortem photographs are also accessible. Collections may not necessarily include complete skeletons; some are made up solely of skulls. For example, among some of the countries that protect some collections are: South Africa, Portugal, Spain, Italy, the Czech Republic, Romania, Greece, Thailand, the Philippines, Argentina, Chile, Brazil, Colombia, the United States and Mexico. The latter only has two collections, that of San Nicolás Tolentino, made up of 102 individuals and the Xoclán Cemetery Collection made up of 84 individuals (Forensic Anthropology Society of Europe, 2018). Of all the collections mentioned, there is no certainty of having all the antemortem information of the skeletons: at most, one can have the death certificate, name, age and sex of the person.

According to Rissech and Steadman (2011), there are three types of documented human skeletal collections:

a) Individuals recovered from modern cemeteries, as in the UAB collection (Spain), where identifications are reached through obituary records, and it is possible to get clinical and social information from the individuals looking at the corresponding archives.

b) Individuals recovered from more ancient cemeteries, as in the Spitalfields and Coventry collections in UK, where identification was accomplished by means of plaque inscriptions on the coffins.

c) Individuals derived from forensic material recovered from autopsies or anatomy courses. These collection types are created mainly by forensic or medical professionals.

Each type of reference sample is replete with inherent biases and limitations, including demographic and regional representation (Wilson et al., 2006; Komar et al., 2008, Winburn et al., 2020).

It is the position of this paper that physical and forensic anthropologists should either supplement existing contemporary collections by creating new ones or, failing that, augment the number of individuals in those that are already formed. The purpose is to have contemporary osteological material that enables research in the different techniques used for human identification, and to generate scientific knowledge according to the population in which it will be applied.

In Mexico, the forced disappearance of persons and the disappearance committed by individuals, or the discovery of clandestine graves, have become recurring facts during the last ten years, within a context of violence and human rights violations that has spread throughout the country. It is vitally important to have a contemporary Mexican documented osteological collection because it can help to test anthropological methods in order to evaluate the human remains of missing persons found in the country, or to carry out specific research or investigations that allow the implementation of new human identification techniques in bone remains burned or exposed to different chemical substances.

By the figures issued by different media, it is presumed that there are approximately 32,604 corpses, bone pieces and other human remains that have been found in 26 states and have not been fully identified (Chávez, 2020). On the other hand, there is another disturbing figure that has been issued by a government agency: this corresponds to the National Registry of Missing and Missing Persons. At a press conference, headed by the Secretary of the Interior, Olga Sánchez Cordero, the undersecretary of Human Rights, Population and Migration, Alejandro Encinas Rodríguez, the Report on clandestine graves and the national registry of people who were disappeared or not located so far was presented by the current administration. The information provided was that until December 31, 2019 there would be 61,637 people missing or not located in the country, and 85,396 located, which gives a total of 147,033 missing persons. Likewise, they pointed out that search actions in 519 sites have been carried out, 873 clandestine graves have been identified and 1,124 bodies have been exhumed, of which 395 have been identified and 243 of them have been handed over to their families (Gobierno de México, 2020).

Unfortunately, the figures cannot be considered definitive or accurate, because not all the complaints, findings nor a database that brings together all the information from all the states that make up Mexican territory are available. On the other hand, it is worrying that in Mexico there is not a sufficient number of forensic anthropologists who can attend to the different cases of identification of bodies as unknown, and that the few in the country do not have adequate and updated scientific information to the study population, and therefore have to adapt the methodology and anthropological data generated in other countries and populations that are biologically distant from ours.

To address the above issues, we present some advances in researches developed in the line entitled: Human identification techniques for the Mexican population and established from the creation of the new osteological collection of contemporary reference of the Department of Innovation in Human Biological Material of the Faculty of Medicine of the UNAM. This in turn will demonstrate the importance and benefits of body donation programs for science.

MATERIALS AND METHODS

The osteological collection of the Department of Innovation in Human Biological Material (DIMBIH) of the Faculty of Medicine, UNAM is made up of complete skeletons and specific bone structures, such as: pubic symphyses, fourth ribs, teeth and sterna. This biological material comes from two sources. The first corresponds to the UNAM Body Donation Program (PDC), that is, people who in life and in their full mental faculties decide to donate their body to science. The second is through an agreement for the use of unclaimed corpses, signed with the government of the then Federal District, so that, through the Centers for Attention and Social Integration (CAIS), people who die in the aforesaid center and are not claimed by any family member can be used for teaching and research.

It is important to mention that the bodies that enter the DIMBIH are fully registered in a database to maintain control of their entry at all times, from their registration and of course the documentation and legal permits by the Health Authorities and the Civil Registry.

Of the bodies coming from the PDC there are official documents such as: the donation letter signed by the donor and by his witnesses, the authorizations by the Sanitary Protection Agency and the Civil Registry of Mexico City and in a database of the DIMBIH, which serves as a controlled procedure with the following data: the body's file number, date of entry to DIMBIH, donor name, age (voluntary donation in Mexico can be made from 18 years old), the sex, place of birth, name and address of the donor and of the relatives who have served as witnesses, causes of death, medical history (provided by the donor himself) and sometimes images from imaging studies or diagnoses of diseases, surgeries or trauma that occurred during the life of the donor. Additionally, fingerprints, facial photographs and dental molds are taken.

Each of the bodies from the CAIS have the necessary authorizations to protect the bone structure fragment for investigation and demographic data such as: origin, age, sex, cause

of death and sometimes the name of the person. Additionally, fingerprints, facial photographs and dental molds are taken.

The osteological reference sample is constructed once the bodies have been used in the teaching environment and this objective has been completed. Subsequently, some of the bodies become part of the research aimed at improving human identification techniques for the Mexican population. Osteotechnic technique is applied to all bodies and bone elements, which consists of removing the soft tissue to have exclusively the skeletal remains. Each of the elements that make up the skeleton is labeled with the corresponding registration number, for which an indelible ink is used. Subsequently, it is placed in its respective cardboard box to keep it individually protected. Additionally, each of the skeletons and bone fragments have an anthropological registration card, in which the registration number, file, demographic data, osteological measurements and morphological characteristics are recorded. Finally, this information complements the research for which certain bone elements are used.

Currently, the collection consists of 25 complete bones, 123 sternocostal ends of the 4th right and left rib, 129 pubic symphyses, 62 sterna. 150 fingerprints, 30 dental plaster casts, 33 dental pieces, 57 tooth samples and bone structures recovered from the cremation process. All individuals are of Mexican origin, of both sexes and of legal age (as mentioned above, the age of majority in Mexico is 18 years old).

RESULTS

From the creation of the osteological collection, it was possible to establish a research line entitled: Human identification techniques for the Mexican population. This line of research has different research topics, including:

- DNA degradation in bone remains due to the action of fire and other chemical substances.
- Techniques for human identification: Comparison of fingerprints.
- Analysis and characterization of dental structures.
- Biodiversity of callihoridae and sarcophagidae (Diptera) from Mexico, families of forensic importance.
- Immunochemistry and metaproteomics of mineralized matrix of teeth and bones and their use in supporting forensic diagnosis.
- Age estimation by means of the fourth rib and the pubic symphysis for the Mexican population.
- Differentiation of biological affinity through the metric and non-metric morpho-anatomical characteristics of the sternum in a Mexican population.

Some of these research topics started in 2017 and have already been completed and others started later and are still in development. It is worth mentioning that each and every one of the research topics has been reviewed and approved by the respective research and ethics committees of the Research Division of the UNAM Faculty of Medicine. Likewise, there are the respective procedures and legal permits managed by the lawyers of our legal area before the Ministry of Health and the Civil Registry. Given that there are many research topics mentioned in this article, we will cite in general the data regarding the sample number, the research objective, the methodology used and the progress or results obtained.

- Degradation of DNA in bone remains due to the action of fire and other chemical substances. This research consists of 33 dental samples. The objective is to determine if it is possible to obtain DNA from teeth that were subjected to the action of fire during the cremation process. The work is being carried out in collaboration with the Institute of Cardiology of Mexico City. DNA extraction and analysis are carried out at this institution. It should be noted that the dental samples have been exposed to a temperature ranging between 300° and 800° C for a period of 15, 30 or 45 minutes. The final results will be presented in a Bachelor Thesis (Fig. 1).
- A sample of 57 dental structures exposed to fire and during the same times and at the same temperatures mentioned above has also been analyzed. The difference is that the analysis is microscopic and the structure and color changes of the human biological material exposed to fire are determined (Fig. 2).

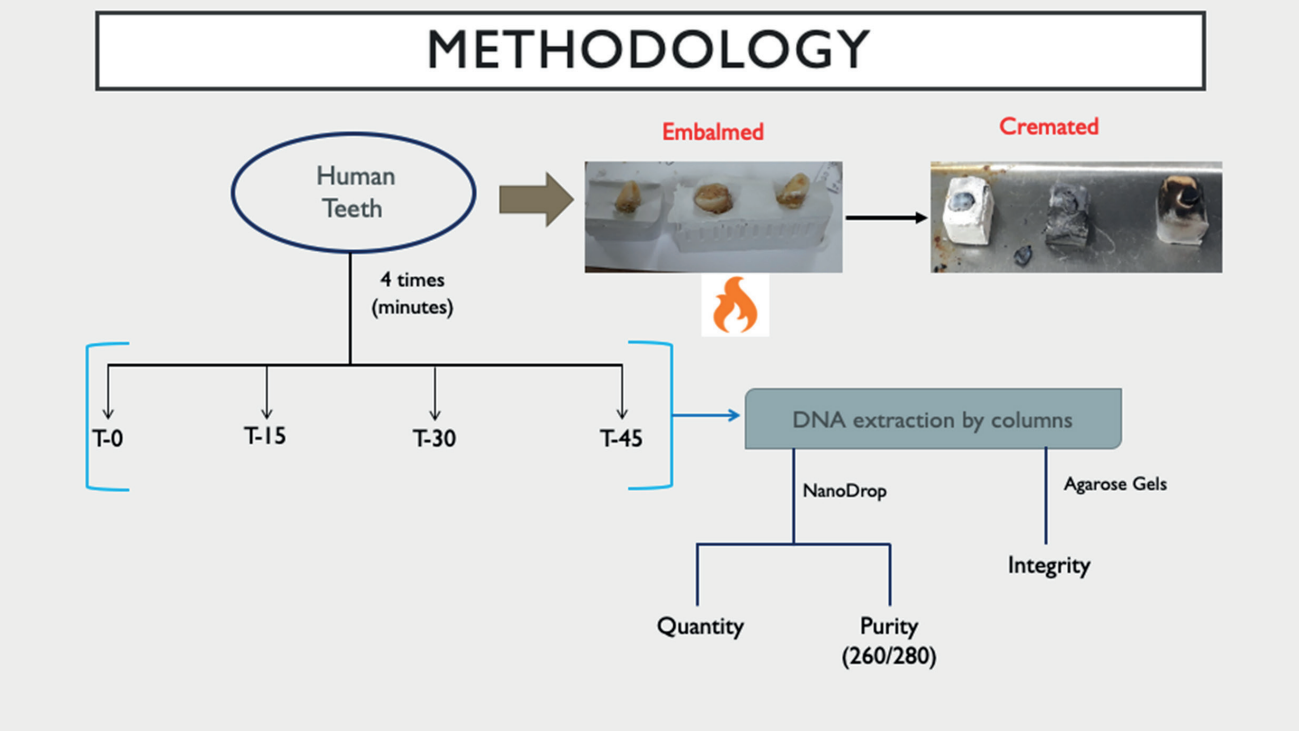

Fig. 1.- Methodology used to determine Degradation of DNA in bone remains due to the action of fire and other chemical substances. Four dental structures are extracted from the corpse. One piece is kept as a control sample and the other three are placed in a plaster structure and inserted back into the mouth of the corpse. The dental structures are exposed to fire for 15, 30 and 45 minutes respectively. Finally, the DNA of each of the dental structures is extracted.

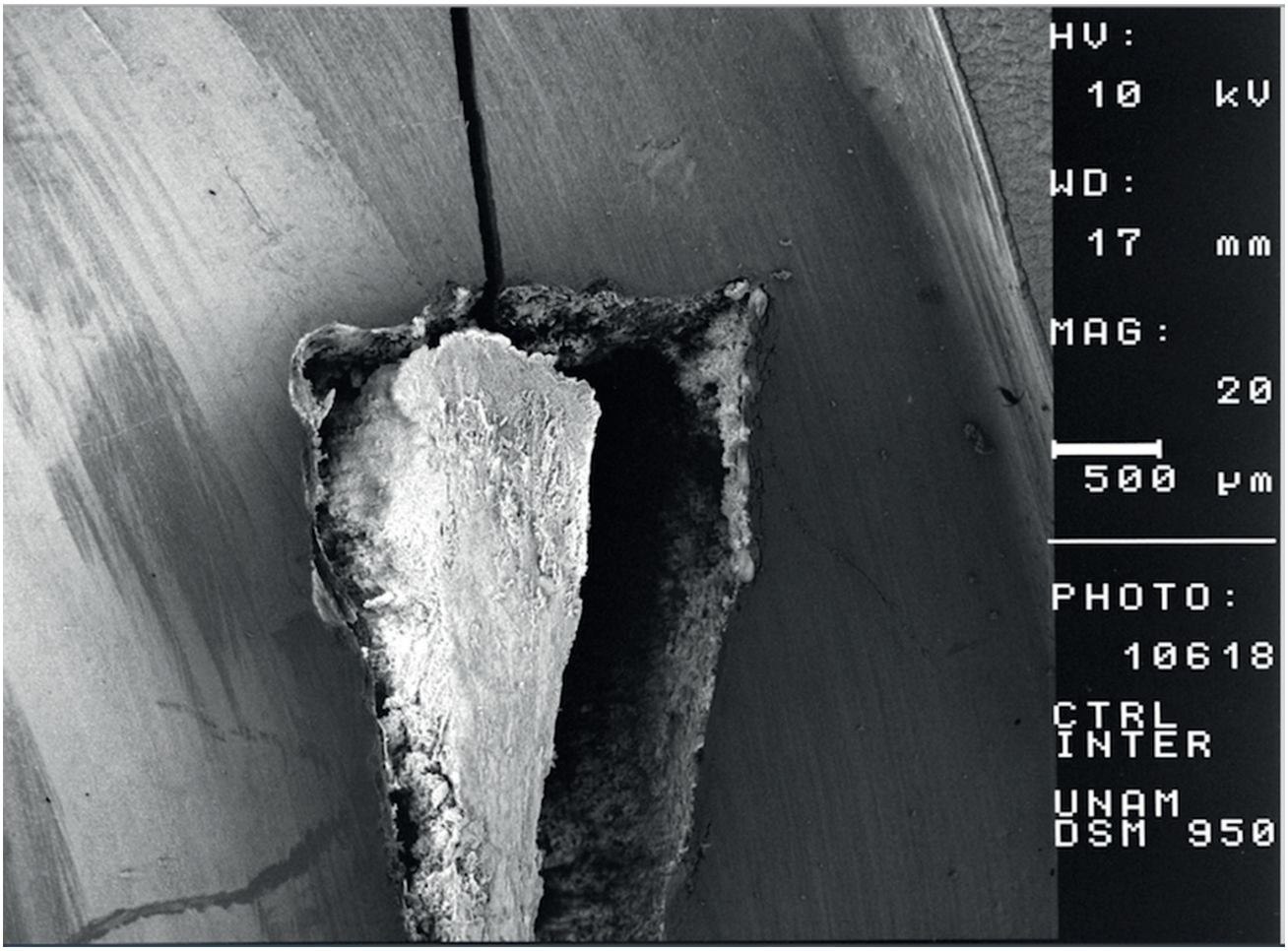

Fig. 2.- Dental structure exposed to fire and analyzed whit scanning electron microscopy. Mandibular Right Canine. Mid sagittal plane, Position: Buccal (right) – Lingual (left). Portion: Crown. Structures: Pulp and Dentine.

- Techniques for human identification: comparison of finger images. To date, there are more than 150 finger cards of bodies from both the PDC, CAIS and records prior to the start of the investigation. The purpose of these is to analyze the characteristics of the fingerprints from Mexican individuals and then compare the results with other populations. Likewise, it is intended to contribute to the creation of the fingerprint database of missing persons, so that in the future some family member can claim them.
- Analysis and characterization of dental structures. Currently, there are 30 dental molds that were obtained from the bodies that enter the DIMBIH. This sample, like that of fingerprints, is very easy to obtain since it does not involve invasive techniques and only one mold is obtained from the corpse's teeth or the fingerprint form. The analysis carried out in dental molds is morphological and morphometric.
- Immunochemistry and metaproteomics of mineralized matrix of teeth and bones and their use in the forensic diagnostic support. This work already has two publications, which can be consulted in the Spanish Journal of Legal Medicine (Díaz et al., 2019a) and in Forensic Science International (Díaz et al., 2019b). These articles address the issue of obtaining proteins in osteological material exposed to fire.
- Age estimation by means of the fourth rib and the pubic symphyses for the Mexican population. This investigation has a sample of 112 sternocostal ends of the right and left fourth rib and 129 pubic symphyses. The objective is to establish the parameters to estimate age by means of said bone elements in individuals of Mexican origin (Figs. 3 and 4).
- Differentiation of biological affinity through the metric and non-metric morpho-anatomical characteristics of the sternum in a Mexican population. Currently, the sample is made up of 62 sterna. The morphological and metric characteristics are being analyzed to establish the characteristics of the Mexican population, that is, to establish the biological affinity from the sternum.

It should be noted that, in addition, a three-dimensional database of the contemporary osteological sample is being created. The purpose is that, if at any given moment a family member requests the remains of their loved ones, they can be returned immediately and in accordance with all legal guidelines. On the other hand, it is the intention that this three-dimensional sample can be consulted by other researchers interested in bone material of Mexican origin, and especially of contemporary origin. In addition, different anatomical techniques have been perfected, such as the osteotechnical technique, rehydration of mummified tissues, or restoration of poorly preserved bones.

The UNAM Body Donation Program (PDC), without a doubt, has had important results, both for medicine, as for forensic physical anthropology, even for forensic genetics and dentistry. Proof of this is that as between October 10, 2016, the date on which the PDC was institutionalized, and the date of this writing, two Research Lines specific to Anatomy and Physical Anthropology have been established in the Department of Innovation in Human Biological Material. The topics studied are anatomical variants, surgical techniques and human identification techniques. The aforementioned topics have been developed in order to innovate and promote new human identification techniques that are undoubtedly necessary for the Mexican anthropological discipline.

On the other hand, students have been trained in the field of research, both undergraduate and postgraduate; postdoctoral research stays have been received; collaborations have been initiated with the Cytoskeleton Biology Laboratory and the Microscopy Department, both from the Medical School, and also with the Institute of Applied Sciences and Technology (ICAT) of UNAM and with the National Institute of Cardiology of Mexico City. Likewise, other collaborations with international universities include: the Faculty of Medicine of the University of Costa Rica, where a contemporary osteological collection is also being formed, and the University of Tennessee by receiving one of its doctoral students to analyze the sample of ribs and pubic symphyses of our department.

Fig. 3.- Sternocostal ends of the right and left fourth ribs.

Fig. 4.- Scanning of pubic symphysis.

It should not be forgotten that the program has also participated in national and international conferences, where researchers and students have been able to show research work and some case reports, such as anatomical variants in the skeleton or some osteopathologies (Fig. 5). In these latest investigations, there was an opportunity to include students of the Degree in Physical Anthropology of the National School of Anthropology and History (ENAH). While some researches continue to develop, other have already been completed and the results have already been published in scientific articles.

DISCUSSION

Obtaining contemporary osteological reference material that has extensive antemortem information to form representative samples and conducting research in the forensic anthropological field is definitely not an easy task. However, we consider that it is possible thanks to the Body for Science Donation Programs because, since it is a voluntary and anticipated donation, reliable personal information can be obtained. For example, the donors of the UNAM Body Donation Program provide personal information regarding their age, origin, diseases, treatments, fractures, surgical interventions, among many other valuable information such as computerized axial tomography, radiographs or dental treatments. Thus researchers can have a detailed medical history that does not allow speculation. In this way, the bias when applying the parameters to identify contemporary individuals involved in forensic cases could be considerably reduced.

When a person decides in life to donate their body, initially they have no idea of the great contribution they make with that action, because they may think that their body will only provide knowledge of medicine and that only soft tissues, organs, muscles or skin can be useful. However, when they receive all the information about the PDC, donors know that the skeleton also provides endless information relevant to disciplines such as physical, genetic, chemical, entomological or forensic dentistry.

Additionally, various research topics can be promoted that contribute to strengthening the human identification techniques applied in specific populations, because among human groups there will always be biological, environmental, health-disease variability, very particular eating and cultural habits. Discussion of the benefits that body donation programs represent to the discipline of forensic anthropology and many others is endless, and we now leave the topic open for future discussions, reflections and proposals.

Fig. 5.- Pathological condition in a Mexican individual.

CONCLUSIONS

The formation of the osteological collection of the Department of Innovation in Human Biological Material (DIMBIH) of the Faculty of Medicine, UNAM, based on the UNAM Body Donation Program, has confirmed the importance and contribution that it can bring to the promotion and development of new investigations in the field of forensic anthropology in Mexico. The information obtained from each of the skeletons has reliable and highly reliable antemortem information, because the donors were the ones who provided it in life.

In this way, it is considered that, even when there are other types of means such as civil registration, death certificates, medical or dental records to obtain demographic and clinical information of some skeletons from medical schools (mainly from anatomy departments), pantheons, institutes of forensic sciences, historical or archaeological sites, there will be no better source than the personal interview of the donors, who will collaborate in the construction of their own biological life history.

Additionally, the provision of imaging analyses, such as X-rays, tomographies, ultrasounds, MRIs or even clinical studies in which a health problem could be determined, have an invaluable contribution, since any researcher will be able to access the cited information without fear of bias or inaccuracy. Finally, it is expected that not only forensic physical anthropology will benefit from the Body Donation Programs for science but also all those disciplines directly linked and whose subject of study is the human body.

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Recent research in Forensic Anthropology

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SUMMARY

Examination of recently published articles in four leading journals featuring forensic anthropology reveal strong research interest in the estimation of age at death and sex, trauma analysis and various other techniques of analysis. The 782 articles examined document the dynamic and evolving nature of forensic anthropology and strong research interest in all of the major components of the field.

Key words: Forensic Anthropology – Publications – Age at death – Methods

INTRODUCTION

Forensic anthropology represents a mature science with a solid scholarly foundation. The academic roots are anchored in the fields of human anatomy and biological anthropology, and extend back into the nineteenth century. As early pioneers became involved in high profile casework, the need for new research, documented comparative collections and improved techniques became both recognized and addressed. In the twentieth century, forensic anthropology became a recognized subdiscipline of forensic science and biological anthropology. Key textbooks emerged (e.g. Krogman, 1962; Stewart, 1979), and a physical anthropology section was formed at the American Academy of Forensic Sciences along with the first certification program. Currently, the field is global in scope with certification programs in place in Europe, Latin America and the UK, in addition to North America.

While these historical developments are impressive as forensic anthropology has matured, the field remains dynamic and evolving. Increased involvement in global humanitarian and human rights projects, as well as participation in recovery and identification efforts after mass disasters and acts of terrorism, present new challenges. New technology, especially expanded computer and related statistical capability have ushered in new methods. Concern about cognitive bias and probabilities associated with case results have triggered new research. Questions about population variation in many of the variables addressed by forensic anthropologists have led to the formation of new global collections and research in many world areas. With growth in student and professional interest in forensic anthropology, funding sources have opened up and publication outlets have expanded. This chapter focuses on the recent advances of this dynamic field.

MATERIALS AND METHODS

To capture recent professional interest in key research areas, publications within four professional journals featuring forensic anthropology were examined. The following

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journals were selected due to their prominence in the fields of forensic anthropology and forensic science: *Journal of Forensic Sciences*, *International Journal of Legal Medicine*, *Forensic Science International* and *Forensic Sciences Research*.

Twelve methodological subfields of forensic anthropology were identified: "age at death"; "miscellaneous"; "trauma"; "sex"; "time since death (post-mortem interval)"; "taphonomy"; "stature"; "detection and recovery"; "ancestry"; "facial imagery"; "animal vs human"; and "stable isotope research". These subfields are listed in the order of frequency of article occurrence. The four journals listed above published from 2016 to 2019 (four years) were examined for articles focusing on these topics. Of course, many articles addressed more than one of these topics, but an effort was made to discern the primary topical focus of the article. Only one topic was scored for each article. In this study, 782 articles published between 2016 and 2019 in the four journals were examined.

Table 3.1 Topical breakdown of Anthropology contributions published in the *Journal of Forensic Sciences* from January 2000 to July 2018.

	Frequency	Percent
Biological profile	300	39.5
Taphonomic studies/Postmortem interval estimation	104	13.7
Skeletal trauma analysis	73	9.6
Facial approximation	61	8
Positive identification	54	7.1
Histology/Bone anatomy/Bone identification	21	2.8
Human rights/Humanitarian issues	21	2.8
Field methods/Forensic archaeology	19	2.5
Isotopes/Elemental composition	17	2.2
DNA analyses	16	2.1
History	16	2.1
Osteometrics/Laboratory procedures	15	2
Skeletal pathology	11	1.4
Commingling	10	1.3
Processing/Maceration	7	0.9
Legal issues	7	0.9
Ethics/Theory	6	0.8
Secular change	2	0.3
Total	760	100

Table 3.2 Breakdown of topics focused on the biological profile published in the *Journal of Forensic Sciences* from January 2000 to July 2018.

	Frequency	Percent
Age	119	39.7
Sex	112	37.3
Ancestry	38	12.7
Stature	22	7.3
Parturition	3	1
Body mass	4	1.3
Handedness	2	0.7
Total	300	100

Relevant tables from: Bethard, JD and EA DiGangi (2019) From the laboratory to the witness stand: Research trends and method validation in forensic anthropology. In: Fulginiti LC, Hartnett-McCann K, Galloway A (eds.). Forensic Anthropology and the United States Judicial System, pp 41-52.

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RESULTS AND DISCUSSION

Table 1 lists the numbers of articles on each of the 12 topics in each of the four journals. With 331 publications, *Forensic Science International* is the primary publication outlet, followed by the *Journal of Forensic Sciences* with 218, *the International Journal of Legal Medicine* with 209 and 24 for the comparatively new journal *Forensic Sciences Research*. The topics are listed in Table 1 in the order of their total occurrence in the four journals. The table reveals that 201 articles focus on issues of "age at death", while only 9 feature "stable isotope research". These results are similar to those revealed by Bethard and Digangi (2019), who found contributions on the biological profile to have the highest frequency of articles in their survey of *the Journal of Forensic Sciences* from 2000 to 2018. The table also reveals considerable variation among the four journals in the topics featured. While articles focusing on "age at death" are most popular in the *International Journal of Legal Medicine*, *Forensic Science International* and *Forensic Sciences Research*, other areas of analysis are mostly featured in the *Journal of Forensic Sciences*. The following provides a discussion of recent research for each of the 12 topics in the order of their popularity in the journals. In addition, one article for each category is featured to illustrate current trends in research.

Age at Death (201 articles)

Different techniques are involved in assessing age at death at different life stages (e.g. fetal, child, adolescent, young adult, old adult). For this reason, many publications and research orientations are involved. In addition, new research in estimating age at death recognizes some population variation in the aging process that must be considered in casework. New technology and recently developed documented collections have fueled research leading to significant advances. Research also has focused on different areas of the skeleton in recognition that many cases involve fragments or isolated bones.

Winburn (2019) investigated the changes of the acetabulum as a valid method of age estimation and its susceptibility to be impacted by factors such as physical activity and demographic qualities. Variables of the acetabulum were examined in 409 modern European-Americans. Variables were analyzed for association with osteoarthritis, Body Mass Index (BMI), age and metabolic intensity of physical activity. Results highlight a significant positive correlation among acetabulum changes, osteoarthritis and age. Correlation tests also illustrate no associations between acetabular changes and BMI or metabolic levels. This study demonstrates that the degenerative changes of the

Table 1. 2016-2019 Trends in Forensic Anthropology: Analysis of article topics from four major forensic science journals.

	Journal of Forensic Sciences	*International Journal of Legal Medicine*	*Forensic Science International*	*Forensic Sciences Research*	*Total*
Age at Death	34	82	84	1	**201**
Miscellaneous	57	13	67	13	**150**
Trauma	31	49	48	1	**129**
Sex	26	28	48	0	**102**
Time Since Death (Post Mortem Interval)	3	22	25	2	**52**
Taphonomy	22	4	16	3	**45**
Stature	6	2	17	0	**25**
Detection and Recovery	9	0	13	1	**23**
Ancestry	12	3	4	0	**19**
Facial Imagery	12	0	1	2	**15**
Animal v. Human	3	5	4	0	**12**
Stable Isotope Research	3	1	4	1	**9**

acetabulum are valid markers for age that remain unaffected by variations in demographic traits.

Miscellaneous (150 articles)

The "miscellaneous" category includes areas of analysis not featured by the others. Sub-topics within the "miscellaneous" category include the following: identification techniques using specific bones and features; DNA extraction techniques from bone; alternative measurement techniques; region specific status of forensic anthropology; hazards and risks; historical trends; victim identification in mass disasters, bone pathology; accuracy of three dimensional reproduction of bones; commingling; osteometric pairing; the relationship between hard and soft tissue, prior information bias in analysis; observer reliability; reanalysis of anthropological records; skeletal collections; site survey techniques; legal and public health issues in the management of human remains; fragment reconstruction and variation in specific bone features.

Bertsatos and Chovalopoulou (2019) introduced a toolkit that features a new approach to long bone shape analysis as an alternative to computed tomography and the latex cast method. Known as the long-bone-diaphyseal-CSG-Toolkit, this procedure employs 3D laser scanning and 3D photogrammetry software to create 3D models of the diaphyseal cross-sectional geometric properties of long bones. The toolkit allows for the graphical analysis of diaphyseal cross section geometric properties and reduces inter- and intra-observer error by creating well-defined and accurate orientation of the bone and its contours. This new approach can facilitate osteometric sorting to address commingling issues.

Trauma (129 articles)

Trauma research has focused on issues related to recognition of antemortem trauma and distinguishing it from perimortem trauma and post-mortem alterations. Experimental research has resulted in key new information on the biomechanics of bone fracture and the factors involved. New microscopic and imaging techniques improve capability to recognize trauma in decomposed and burned cases and to clarify the timing of injuries sustained. Advances are registered in understanding the relationship between soft and hard tissue alterations.

Rickman and Shackel (2019) tested two hypotheses on the formation of conoidal wounds from projectile impact on scapulae; the plug and spall hypothesis, and the cone crack hypothesis. Twenty-eight pig scapulae were impacted with 6-mm carbon-steel spheres at varying velocities. This study delves deep into the morphological features of conoidal fractures and presents an extensive analysis of specific fracture formations due to varying speeds of a projectile. They found that numerous features of conoidal fractures were consistent with the cone cracking method. However, a central component of the plug and spall hypothesis was rejected. Beveling occurred in the absence of a projectile exit, which was stated otherwise in the aforementioned hypothesis. Projectile trajectory and its impact on bones is a critical aspect of medicolegal interpretation.

Sex Estimation (102 articles)

Estimation of sex continues to be a vital component for the establishment of the biological profile of recovered human remains. Research focuses on exploration of new areas of the anatomy offering information, testing of previous methods to clarify error rates and the probabilities involved, use of new technology and documenting population variation. Estimating sex of the immature from skeletal remains continues to be problematic, but methods for adults are both accurate and useful, especially if the pelvis is available for examination.

Oner et al. (2019) introduced a new sexing technique using artificial neural networks (ANNs) focusing on lengths of manubrium sterni (MSL), processus xiphoideus (XPL), corpus sterni (CSL) and sternal angle (SA), using computerized tomography (CT) images on an orthogonal plane. Measurements of the MSL, XPL, CSL, and SA were analyzed with a multilayer ANN that used a stochastic gradient descent (SGD) and two hidden layers. They discovered that measurements of the MSL, XPL, SA and CSL were all larger in males than females. The approach offers improved accuracy over linear models.

Time Since Death (Post-Mortem Interval) (52 articles)

Research in this area of forensic anthropology focuses on experimental studies of soft tissue decomposition and skeletal alterations, and use of the radiocarbon bomb-curve and other new approaches. The former efforts involve decay facilities that utilize donated human remains or non-human animals to document the diverse factors that influence the post-mortem changes. The latter methods focus on absolute methods to assess time since death using markers preserved in human tissues.

Ishikawa et al. (2019) presented a new method to assess time since death in individuals immersed in seawater. In their study of dental enamel, they found that, with increasing immersion time, the quantity of diatoms and the elements O, Si, Mg, K, Al and S increased along with a decrease in Ca and P. The documented changes allowed them to develop a new regression equation that can be useful in estimating time since death in remains recovered from seawater.

Taphonomy (45 articles)

Observations on taphonomic changes are useful to assist in estimating the post-mortem interval and to examine the after-death history of remains. Studies on human taphonomy are diverse, ranging from research on scavenging and scattering of remains to the effects of burial, sunlight and water exposure.

Ross and Hale (2018) focused on the decomposition of juvenile and infant remains with a strong emphasis on examining the environmental variables most influential in decomposition, as well as testing if the adult methodology of quantifying decomposition was applicable to sub-adult remains. Thirty-five pigs were used as proxies for human infant and juvenile remains, and were deposited in five depositional styles (bagged, blanket wrapped, and surface control fetal remains, surface juvenile remains, and buried juvenile remains). Soft tissue decomposition was examined and scored, and after completion and skeletonization, femora from the remains were collected and analyzed using the Oxford Histological Index (OHI). Results illustrate that standardized time variables employed in quantifying decomposition such as accumulated degree days (ADD) can be impacted by seasonal variation, especially by soil moisture. When examining quantification methods, the study also found that the total body score (TBS) was not as highly correlated to ADD and time in days, revealing it to be inapplicable to juvenile remains. The study emphasizes that juvenile decomposition is heavily driven by body mass, style of burial, and seasonal variations.

Stature (25 articles)

Estimates of living stature add to the biological profile, but have limited value in the identification process. Progress involves noting errors in the reported statures of missing persons, especially those involving self-reporting and estimates of family members. New research involves formulae for individual bones and incomplete skeletal remains, as well as approaches derived from new documented collections from previously understudied world regions.

Spies et al. (2019) addressed the issue of determining stature from incomplete remains through the formulation of sex- and population-specific regression equations. The study focused on creating equations to assist in calculating the total skeletal height (TSH) of a black South African population when examining fragmentary tibial remains. The total skeletal heights for 99 female and 99 male complete skeletons were calculated using the anatomical method. Additionally, eleven measurements of different areas of the tibia were recorded from the intact tibia and used to form uni-variate and multi-variate equations to establish TSH. Both types of equations created using the fragmentary measurements were found to significantly positively correlate with TSH. The study highlights the importance of formulating sex- and population-specific equations to address the issue of estimating stature from incomplete remains.

Detection and Recovery (23 articles)

The location and proper recovery of human remains represents a key feature of the activity

of forensic anthropology. Errors made during recovery can negatively impact later analysis and interpretation. Progress is marked by the inclusion of forensic anthropologists and archeologists in search and recovery teams and recognition of their positive contributions. Activity in global humanitarian and human rights initiatives, often focusing on the location of mass graves, identifies new research directions for detection and excavation. New geophysical testing technology stimulates new research in subsurface detection.

A study by Wisniewski et al. (2019) exemplifies the numerous methods and technologies that can be applied to assist in detection and recovery. The study utilized a multiphase geophysical approach in searching for a clandestine grave, focusing specifically on a reported 30-year-old vertical burial in a wooded area. Beginning with bulk ground conductivity and metal detectors, magnetics and ground penetrating radar (GPR) surveys were then administered as they were deemed best for the specific soil type. They utilized a tight grid pattern and investigated any electromagnetic anomalies. The study emphasizes the use of multiphase geophysical surveys as the best methodological approach to execute thorough and careful cold case searches.

Ancestry (19 articles)

Progress in the estimation of ancestry involves increased recognition of the social dimensions of ancestral categories, as locally defined. Recent research also presents enlarged and more representative databases using both metric and non-metric observations on teeth and different elements of the skeleton. Use of proper, carefully defined terminology has emerged as an important issue, as well as advanced technology, advanced statistical approaches and comprehensive approaches that combine different types of biological data.

Kranioti et al. (2019) examined metric variation of the tibia as a method for estimating ancestry. Multinomial regression models were created from a reference sample and then applied to a validation sample in an attempt to classify individuals into one of six Mediterranean populations. With the first model examining three variables of the tibia and the second model examining six variables, they found that the second model featured higher classification accuracy. The Greek sample and Turkish sample were two of the groups with the highest classification accuracy in both the reference and validation samples, while the greater morphological overlap shared by the Spanish, Portuguese and Italian groups led to a decrease in accuracy. This research highlights the high variation of the tibia among populations, but emphasizes avoidance in using it as the sole feature to estimate ancestry. This study also draws attention to the field's expanding focus from cranial and dental features to the postcranial skeleton as a means of estimating ancestry.

Facial Imagery (15 articles)

Forensic applications of facial imagery involve features for positive identification, techniques of craniofacial photographic superimposition and facial approximation. Facial approximation is used in studying a recovered skull to estimate what a missing person looked like to seek leads from the public. Techniques of craniofacial photographic superimposition are employed to compare a recovered skull with facial images of a missing person, usually to either exclude or recognize the possibility of inclusion. Recent research involves providing new data on the relationship of soft and hard tissues of the head, development of standards, recognition of the probabilities and errors involved in the process and use of new technology and computerized approaches.

Flores et al. (2019) introduced a novel landmark-positioning approach for facial photo-anthropometry (FPA). They suggested that the classic methods of positioning landmarks in FPA led to inconsistencies in reproducibility due to unclear definitions. The novel method presented provides additional marking procedures that reference visible facial features. This addition was successful as research illustrated that both examiner groups, with and without professional anatomical knowledge, were able to have lower landmark dispersion levels and higher reproducibility compared to classic methods.

This new approach emphasizes the importance of refining traditional methods to increase accuracy and reduce variation of interpretation.

Animal vs Human (12 articles)

While distinguishing human bones and teeth from those of other animals is usually an easy task based on comparative morphology, the process can become challenging with fragmentary or burned remains. With such difficult cases, methods involving histology, molecular analysis or protein radioimmunoassay can provide insights. Recent research focuses on histological variation recorded in different bones and parts of bones of various species.

The Wang et al. (2019) article is an example of the new technologies being explored and employed in the field. This study examined the use of Fourier Transform Infrared (FTIR) spectroscopy and chemometrics methods to survey the spectral variances of human and non-human bones with principal component analysis (PCA), and utilized partial least squares discriminant analysis (PLS-DA) to analyze the data. Results illustrate that pig bones were the most sensitive to external factors, such as boiling and decomposition, compared to the other non-human bones used. This is an important discovery, as it calls into question the accuracy of using pig bone as proxy for human bone in forensic studies. The technology is reliable, as the spectral variance is mainly caused by stable inorganic portions of the bones. The study highlights the movement from a narrow focus on morphological features to the use of molecular examination when differentiating between human and non-human bones in an attempt to increase accuracy and save time and resources.

Stable Isotope Research (9 articles)

Stable isotopes, especially those of carbon and nitrogen have been used extensively in the past to examine dietary issues. More recently, forensic anthropologists have used isotopes of those elements and others to determine the geographical origin of recovered remains. This approach is especially useful in cases related to immigration and terrorism. Detailed isotopic analysis can determine if recovered remains are from a local individual or a person from another locality. New research using multiple elements aims at formulating isoscapes that suggest the geographic region of origin.

Warner et al. (2018) utilized isoscape refinement to predict the region of origin. Isoscapes, which are isotopic ratios specific to certain geographical areas, were determined through the isotopic analysis of 57 tap water samples collected across Mississippi. After performing isotopic analysis on the third molars of individuals with a known residential history, results demonstrate the high accuracy of region-of-origin assignment for Mississippi residents. Using this isoscape distribution, a linear regression equation was then formulated in an attempt to create a conversion factor for the southeastern US, with lower success rates for Georgian residents. The further refinement of isoscape equations and the use of various isotopes can lead to increased accuracy of the method.

CONCLUSIONS

Clearly, the field of forensic anthropology is expanding and evolving. A majority of the articles examine research on new approaches to traditional methods in the hope of improving accuracy, reducing error and utilizing new technology. While current research continues to focus on core issues such as age estimation, scope has expanded dramatically to include interpretations of trauma and region of origin. Methodological approaches include traditional measurement and observation but also involve increased controlled experimentation. Progress is also marked by formation of large databases, advanced statistical analysis and the involvement of the growing number of students and highly qualified professionals.

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The potential of chemometric analyses in the examination of burnt human skeletal remains

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SUMMARY

Recent decades have seen a major increase in bone studies involving advanced physical-chemical techniques. A review of both the confirmed and prospective contribution of some of these techniques for the investigation of burned skeletal remains found in forensic settings is carried out here. In particular, an evaluation about the potential of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and X-ray fluorescence (XRF) is presented based on previous research. The use of these techniques brings about several advantages, namely thanks to their minimally destructive and non-invasive application, low cost, rapidity in data collection, and reliability. Although with a variable degree of success, their combined application allows valuable inferences about: i) the composition of skeletal remains; ii) the preservation of bones, namely regarding their feasibility for biomolecular studies; iii) the chronological recentness of human remains; iv) the minimum number of individuals present in an assemblage; v) the heat intensity to which skeletal remains have been exposed; vi) and the probable impact of heat-induced changes on anthropological analyses. The major hindrance to the use of FTIR, XRD and XRF is related to the confounding effect of diagenesis, which must always be taken into account during the interpretation of each case.

Key words: Chemical Anthropology – FTIR – XRD – XRF – Heat-induced changes

INTRODUCTION

Burnt remains are a somewhat common find during forensic investigations. Generally, the

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level of complexity that involves the examination of this kind of remains finds no parallel in any other cases worked by forensic anthropologists. The amount of fragmentation added to the usual meddlesome heat-induced changes often turn burnt skeletal remains into very challengeable study targets (Fig. 1).

Most often, macroscopic analysis is not enough to gather relevant information about the remains. For this reason, anthropologists felt the need to explore other kinds of approaches, namely those involving analyses performed at smaller scales of observation. As a result, chemometric analyses have been especially under the limelight in recent decades (Shipman et al., 1984; Hiller et al., 2003; Etok et al., 2007; Munro et al., 2007; Piga et al., 2008, 2009; Thompson et al., 2009; Hüls et al., 2010; Lebon et al., 2010; Wang et al., 2010; Squires et al., 2011; Thompson et al., 2013; Snoeck et al., 2014; Gilpin et al., 2015; Marques et al., 2016; Reidsma et al., 2016; Cascant et al., 2017; Gonçalves et al., 2018; Greiner et al., 2019; Vargas-Becerril et al., 2019; Lemmers et al., 2020). Although it can be argued that the exploration of this chemometric research avenue is still under development and the field is much in need of solid procedures based on experimental reference work, a review of the potentialities and limitations that it entails is in order.

The present paper addresses infrared spectroscopy, X-ray diffraction and X-ray fluorescence, which have been among the most popular advanced techniques used in the analysis of burnt skeletal remains. Although others could be included in this review, we find that their approach here is not as relevant as for those three approaches. For example, power analysis constraints can be appointed to Raman spectroscopy, since fluorescence interferes dramatically with the resulting spectra in non-calcined bones (Carden and Morris, 2000; Chikhani et al., 2018; Mamede et al., 2018). Other techniques, such as inelastic neutron scattering or neutron-diffraction analyses are not easily accessible, thus limiting their actual prospective value for the analysis of skeletal remains (Marques et al., 2016, 2018; Mamede et al., 2019). Finally, techniques based on both stable and radiogenic isotopes have already been broadly discussed in previous literature (e.g., DeNiro et al., 1985; Harbeck et al., 2011; Snoeck et al., 2014, 2016) and a review of their potential is not as timely as is the case for the techniques addressed here.

As mentioned earlier, heat exposure encompasses a myriad of changes in skeletal remains. At the macroscopic level, such alterations affect the colour, shape, size, mass and integrity of teeth and bones (for a more comprehensive description of such changes, see Thompson et al., 2004, 2005; Symes et al., 2008, 2014; Walker et al., 2008; Ubelaker, 2009; Gonçalves et al., 2011; Ellingham et al., 2015). It also affects their composition, which means that chemometrics allows us to obtain information regarding the skeleton and the circumstances of death by comparing it with pre-established references. Here, an overview of approaches used to address recurrent questions raised within the forensic arena is carried out. Benefits and shortcomings of chemometric analyses are discussed along with prospective developments. Ultimately, with this paper, we aim to contribute for an increase in critical thinking and discussion regarding chemometric analysis and to help setting an agenda for future work that enables the research community to further develop, ameliorate and validate procedures based on infrared spectroscopy, X-ray diffraction and X-ray fluorescence.

Bone composition

Bone is a heterogeneous biphasic material comprising proteins (mostly collagen I) and lipids woven into an inorganic matrix of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp), the hydroxyl and phosphate groups being partly substituted by carbonate (Weiner and Price, 1986; Wagner and Weiner, 1992; Weiner and Traub, 1992; D'Elia et al., 2007; Etok et al., 2007; Mkukuma et al., 2004; Wang et al., 2010). In vivo, carbonates may substitute phosphate (type B substitution) and/or hydroxyl groups (type A substitution) (Peters et al., 2000; Wang et al., 2010). Taking into account the total mass of the bone, a femur for example, consists of ~55 wt% mineral, ~35 wt% collagen, and ~15 wt% water (Rogers and Zioupos, 1999).

Whereas hydroxyapatite has a Ca:P ratio of 5:3 (1.67), bone mineral itself has Ca:P ratios ranging from 1.37–1.87. This occurrence is due to the composition of biological apatite, which is chemically more complex than the stoichiometric hydroxyapatite and, according to several authors, and the following substitutions can occur: i) some Ca^{2+} for Mg^{2+}, Sr^{2+}, K^{+} or Na^{+}; II) some PO_4^{3-} for HPO_4^{2-} or CO_3^{2-}; and iii) OH^{-} for CO_3^{2-}, F^{-}, Cl^{-} or H_2O (Montel et al., 1981; Elliott, 1994; Wilson et al., 1999; Elliott, 2002; Pan and Fleet, 2002).

After death, diagenetic alterations—post-mortem processes triggering physical and chemical alterations—lead to loss of carbonates (mainly type B) and ion uptake (such as fluoride from the surroundings) as a consequence of collagen hydrolysis. Although type A carbonate substitution is much less common than type B (Rey et al., 1989), the hydroxyl content in bone has been controversial for many years. Some authors state that the bioapatite lattice contains little or no hydroxyl groups (Rey et al., 1989; Loong et al., 2000; Pasteris et al., 2004), while others have detected hydroxyls in defatted and deproteinized bone (Taylor et al., 2001; Taylor et al., 2003), as well as in intact bone (Cho et al., 2003) and in bone subjected to heat (Marques et al., 2016; Marques et al., 2018; Mamede et al., 2018b). Upon diagenesis, bone also undergoes changes in molecular structure, namely recrystallization (associated with collagen loss) usually leading to an increase in crystal size and often associated with warping and dimensional variations (Stathopoulou et al., 2008; Thompson et al., 2009; Thompson et al., 2011; King et al., 2011; Hollund et al., 2013; Beasley et al., 2014; Marques et al., 2016; Vassalo et al., 2016; Mamede et al., 2018a; Gonçalves et al., 2018; Marques et al., 2018; Mamede et al., 2019).

Most spectroscopic studies have been focused on the analysis of 'normal' non-human bone, usually from sheep, deer, bovines and porcines (Shipman et al., 1984; Munro et al., 2007; Thompson et al., 2009; Lebon et al., 2010; Snoeck et al., 2014). Results are then extrapolated for the human species, but little research has been done so far to determine how alike those proxies really are at the molecular level. Additionally, differential heat exposure may complicate inferences (Wang et al., 2018). Some work suggests that such procedure may carry some problems. Compact bone architecture is often not the same between humans and their other animal counterparts. The first usually display lamellar bone, while the latter present plexiform bone (Cuijpers, 2006; Crescimano and Stout, 2012). The impact of such differences on, for example, infrared spectroscopic profiles has not been extensively debated, but some differences have indeed been reported at the nanoscale among different types of bone (Reznikov et al., 2015; Gourrier et al., 2017), which could be related to variable mineralization states of the tissue (Paschalis et al., 1997; Gourrier et al., 2017). This effect seems patent also at the intra-bone level, since different bone regions (epiphyses, metaphyses and diaphyses) tend also to present some variability in their infrared profiles (Thompson et al., 2009; Gonçalves et al., 2018).

Bone remodelling may also have an effect on bone infrared profile, since bone mineralization gradually increases with time (Paschalis et al., 1997). Therefore, choosing the sampling location is apparently not an innocuous decision. Areas more prone to remodelling such as muscle and tendon insertions, as well as areas displaying lesions, probably provide chemometric values that are somewhat different from neighbouring regions. That may partly explain why Gonçalves et al. (2018) found important differences among bone regions.

Moreover, chemical composition is influenced by diet, physical activity and age (Biltz and Pellegrino, 1983; Driessens and Verbeeck, 1990; Rodríguez-Navarro et al., 2018). A number of different factors can therefore alter this mineral composition. One of the more significant changes to the microstructure of bone (i.e., to its spatial extension) occurs when this material is heated. Heated bone is one of the most challenging osteological materials to study, since the heating produces a range of complicated changes within the material, which is already complex by itself.

Vibrational spectroscopy

The potential of vibrational spectroscopic techniques for the analysis of skeletal remains

has been demonstrated since the 1990's, allowing the identification of major chemical and structural alterations, contaminants, and new inorganic phases. Fourier transform infrared (FTIR) is nowadays the most commonly applied vibrational spectroscopic method for the analysis of bone, including burnt skeletal remains, with a clear increase in recent years regarding its application especially within the archaeological sciences (Wright and Schwarcz, 1996; Trueman et al., 2004; Arroyo et al., 2008; Snoeck et al., 2014; Beasley et al., 2014; Lachowicz et al., 2017; Festa et al., 2019), although less so in forensic settings (e.g. Cascant et al., 2017). Diagenesis-induced chemical alterations in bone—such as fluorination, carbonate substitution or mineral uptake, as well as crystallinity variations—can be accurately detected by vibrational spectroscopy, namely by infrared spectroscopy (Weiner and Bar-Yosef, 1990; Stiner et al., 1995, 2001). Its potential for discriminating between fossilised and burnt bone (Mamede et al., 2018) and between burnt archaeological bone and burnt modern bones has been proposed (Snoeck et al., 2014).

On the other hand, research focused on burnt teeth has been rarer than research on bone. Some work has been done, but usually in unburnt teeth or teeth burnt at low temperatures (e.g., Bachmann et al. 2003; Reyes-Gasga et al., 2013). Teeth components have compositions that are somewhat different from bone. Although the dentin and the cementum are not as dissimilar to bone in terms of inorganic and organic fractions, the enamel is by far composed of larger inorganic fractions (~95%) (Scott and Turner, 2000; Hillson, 2005). In terms of heat-induced behaviour, teeth seem to differentiate themselves from bones regarding the heat intensity required to promote the metamorphosis into the β-tricalcium phosphate phase (β-TCP). This occurs much more frequently in teeth and at lower intensities than the one pinpointed for bone at around 1100 °C (Piga et al., 2016a, 2016b). Temperatures as low as 400-800 °C have been reported for teeth (Piga et al., 2016a, 2016b; Vargas-Becerril et al., 2018, 2019). Phase conversion appears to be dependent on the type of tooth component being studied (Vargas-Becerril et al., 2018, 2019).

Several spectral relationships have been developed to attempt to quantify heat-induced variations in infrared profiles (Thompson et al., 2009; Lebon et al., 2010; Thompson et al., 2013; Snoeck et al., 2014; Mamede et al., 2018c; Marques et al., 2018) (Table 1). However, devising a method capable of quantifying exact diagenetic changes in bones subjected to high temperatures is still an

Fig. 1.- Example of heat-induced colour changes in bone (photo courtesy of Dr. Ignasi Galtès).

unmet challenge, although it is being continuously investigated by infrared spectroscopy (coupled to complementary spectroscopic and diffraction techniques) (Thompson et al., 2009, 2011; Marques et al., 2016; Vassalo et al., 2016; Gonçalves et al., 2018; Mamede et al., 2018c, 2019). It can be argued that infrared studies of human skeletal remains are still in a developmental stage. Research teams are still assessing the potential of several chemometric indices. This groundwork is critical to make sure that future inferences based on infrared profiles are even more solid. This was how the power of the crystallinity index to evaluate crystallinity, how the potential of hydroxyl groups to pinpoint high intensity burns, and how the capacity of Amide I as a proxy of organic matter have been determined (Bellamy, 1975; Weiner and Bar-Yosef, 1990; Piga et al., 2016b; Gonçalves et al., 2018).

Gainfully, FTIR, mainly in attenuated total reflectance (ATR) mode, is a low-cost, non-destructive and rapid method for this type of analysis, which requires very small amounts of sample and yields very reliable data providing information on the chemical and structural characteristics of the bone, namely its organic components (protein and lipids), possible mineral contaminants (e.g., including fluorapatite [francolite], chloroapatite, cinnabar or gypsum) or pathology-induced changes. Any modification in the structure of bone is reflected in the corresponding vibrational pattern with high sensitivity—both regarding the inorganic framework of bone (hydroxyapatite) and its organic constituents (lipids and proteins).

Table 1. Spectral relationships for the calculation of infrared-based indices (adapted from Mamede et al. (2018a).

Parameter	Description	Spectral Relationship	Reference
CI	Cristallinity Index	Abs603 cm^{-1}+Abs(565 cm^{-1}) / Abs(595 cm^{-1})	Weiner and Bar-Yosef (1990)
	1060/1075	Abs(1060 cm^{-1}) / Abs(1075 cm^{-1})	Lebon et al. (2010)
	1030/1020	Abs(1030 cm^{-1}) / Abs(1020 cm^{-1})	Lebon et al. (2010)
C/P	Carbonate to Phosphate	Abs(1415 cm^{-1}) / Abs(1035 cm^{-1})	Rey et al. (1989)
API	Type A carbonate	Abs(1540 cm^{-1}) / Abs(603 cm^{-1})	Rey et al. (1989) Sponheimer and Lee-Thorp (1999) Snoeck et al. (2014)
BPI	Type B carbonate	Abs(1415 cm^{-1}) / Abs(603 cm^{-1})	Rey et al. (1989) Sponheimer and Lee-Thorp (1999) Snoeck et al. (2014)
C/C	Carbonate (A+B) to carbonate B	Abs(1450 cm^{-1}) / Abs(1415 cm^{-1})	Thompson et al. (2009) Snoeck et al. (2014)
Am/P	Amide I to Phosphate	Abs(1660 cm^{-1}) / Abs(1035 cm^{-1})	Bellamy et al. (1975)
OH/P 1	Amount of OH^- groups	Abs(630 cm^{-1}) / Abs(603 cm^{-1})	Thompson et al. (2013) Snoeck et al. (2014)
OH/P 2	Amount of OH^- groups	Abs(3572 cm^{-1}) / Abs(603 cm^{-1})	Mamede et al. (2018c)
OH/P 3	Amount of OH^- groups	Abs(3572 cm^{-1}) / Abs(1035 cm^{-1})	Mamede et al. (2018c)
CN/P	Cyanamide content	Abs(2010 cm^{-1}) / Abs(1035 cm^{-1})	Snoeck et al. (2014)
CO/P	Protein to Phosphate	Abs(1650 cm^{-1}) / Abs(1035 cm^{-1})	Thompson et al. (2013)
CO/CO_3	Protein to Carbonate	Abs(1650 cm^{-1}) / Abs(1415 cm^{-1})	Thompson et al. (2013)
CO_3/P	Carbonate to Phosphate	Abs(900 cm^{-1}) / Abs(1035 cm^{-1})	Thompson et al. (2013)
PHT	Phosphate High Temperature	Abs(625 cm^{-1}) / Abs(610 cm^{-1})	Thompson et al. (2013)
Full width at half maximum		FWHM (1035 cm^{-1})	Thompson et al. (2013)

X-ray diffraction

Nowadays, XRD is one of the most widely used techniques to characterize the state of condensed, inorganic, organic materials, and sometimes biological molecules. It is a useful tool in different fields such as: archaeometry, archaeology, biological and forensic sciences. In all these areas, most of the crystallized matter that can be analysed is in fact in polycrystalline state; each crystal is the size of a few microns or even just a few nanometres. The major breakthrough of the powder diffraction as a quantitative tool in material sciences happened in 1967 with the introduction of the so-called Rietveld method (Rietveld, 1967), or whole pattern fitting profile refinement, a technique for crystal structure refinement which, for the first time, made use of the entire powder pattern instead of analysing individual non-overlapped Bragg reflections. This method permitted an extension of the diffraction technique on polycrystalline compounds, from structural refinement to quantitative and structural and microstructural characterization.

In the Rietveld's approach, the experimental powder diffraction data are utilized without extraction of the individual integrated intensities, and all structural, microstructural and instrumental parameters are optimized by a least-squared refinements method to carry out the best fit between the experimental data and the calculated pattern. Full profile refinement is computationally intense and requires a reasonable starting structural model and a good set of experimental data for the analysis success (Rietveld, 1969; Young, 1993). An important application of the Rietveld's method in powder material field is on multiphase samples (see Fig. 2).

The knowledge of the spatial arrangement of atoms and molecule inside the unit cell and the fraction composition of different phases present in the polycrystalline compound under investigation, are complemented with the possibility to determine the microstructural information, such as dimension, orientation of crystallites and lattice disorder (Young, 1993). The knowledge of these details can allow determining or to hypothesizing possible properties of bone material, with a particular view onto the hydroxyapatite mineral phase.

Pioneered by Bonucci and Graziani (1975) and Shipman et al. (1984), who observed a sharpening in diffraction peaks of bones subjected to heating, XRD has become one of the main methodologies for the examination of changes in the matrix of bone to obtain primary material information in forensic fields, such as the accuracy of maximum

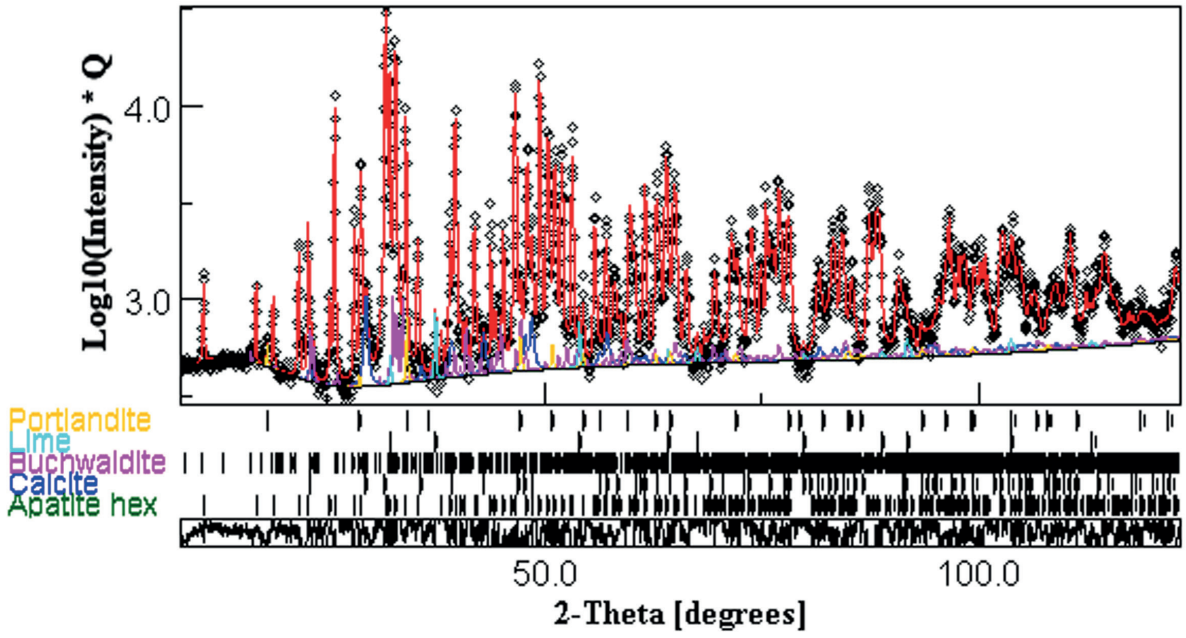

Fig. 2.- The XRD pattern (data points) and Rietveld fit (full line) of a burnt human bone specimen shows a rather complex phase constitution of five phases, with the notable presence of portlandite [$Ca(OH)_2$], buchwaldite [$NaCa(PO_4)$], calcite ($CaCO_3$) and lime (CaO).

temperature determination, mineral composition and the study of crystallinity (Newesely, 1988; Holden et al., 1995; Stiner, 1995; Rogers and Daniels, 2002; Piga et al., 2008, 2009; Galeano and García-Lorenzo, 2014). Later, Bartsiokas and Middleton (1992) introduced the measurement of the so-called Crystallinity Index (CI), which is defined as the intensity ratio of the (300)/ (200) line profiles of the hexagonal apatite when using Cu Kα radiation in an angular range of 15-120° in 2θ (Piga et al., 2008).

X-ray fluorescence

Bones and teeth are often the only biological remains of animals and humans, and hence represent valuable archives for archaeology and forensic sciences. Among the different physico-chemical and spectroscopic techniques that can be applied to characterize and quantify their structural and chemical integrity of bones, (Piga, 2012), X-ray fluorescence spectrometry (XRF) has several advantages. It is a useful, rapid and cost-efficient method that has been recently employed in both archaeological and forensic contexts (González-Rodriguez and Fowler, 2013; Finlayson et al., 2017).

The XRF is an X-ray emission spectroscopy technique that allows the identification of the chemical elements that are present in the examined sample. This technique therefore allows to establish the presence of a certain element and, using an appropriate method of measurement and data analysis, to establish the concentration of it in the sample. Portable XRF (pXRF) is particularly useful to examine *in situ* samples which are often rare or cannot be transported to the laboratory for benchtop analyses (e.g., mummies or samples obtained from museological materials); it directs high-energy X-ray photons at a sample to induce X-ray emission and build a spectrum (see Fig. 3). The energy of characteristic X-rays is represented in the latter through peaks, thus providing the elemental composition of the sample (Byrnes and Bush, 2016). This equipment has the capability of detecting very low concentrations of elements, but has some limitations with lighter elements,

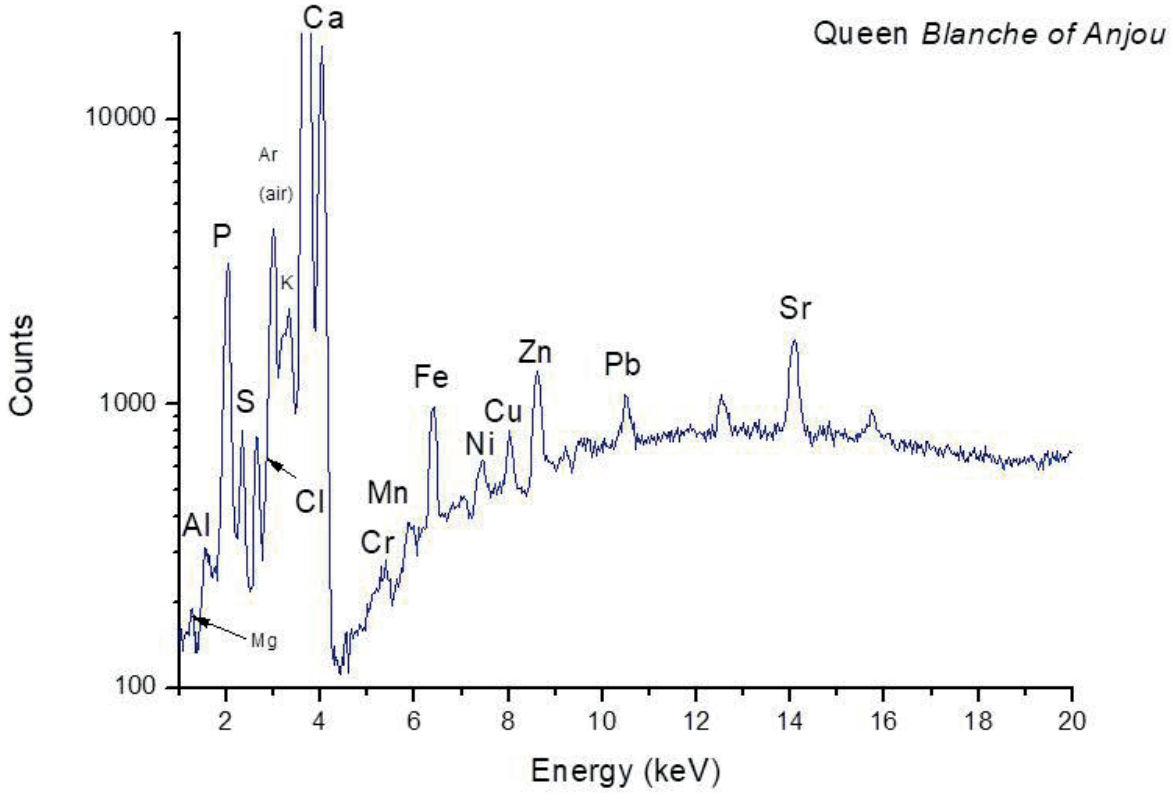

Fig. 3.- X-ray fluorescence spectra taken in situ on the metacarpus of Queen Blanche of Anjou (1280–1310 A.D.) with a portable XRF instrument.

such as phosphorus, meaning that not all bone constituents are within analytical reach using this technique (Byrnes and Bush, 2016).

In archaeology, XRF is useful to study elemental concentrations in bones of different chronologies and conditions (Carvalho et al., 1998, 2000, 2004, 2007; Anjos et al., 2005; Bush et al., 2007; González-Rodriguez and Fowler, 2013; Piga et al., 2014), and to evaluate diagenesis or fossilization processes (Carvalho and Marques, 2008; Piga et al., 2009, 2011, 2014, 2016; Thomas and Chinsamy, 2011), even though this technique is barely used for this latter scope. It has also been introduced into the forensic domain: i) for the search of trace evidence at crime scenes (Schweitzer et al., 2005) including gunshot residue and bodily fluids (Trombka et al., 2002); ii) to differentiate human from non-human material in cases of fragmented degraded samples or skeletal materials (Christensen et al., 2012; Buddhachat et al., 2016; Nganvongpanit et al. 2017); and iii) to discriminate bone from other materials (Christensen et al., 2012).

In addition, XRF has been proposed as a potential solution for re-associating disarticulated remains in a mass grave context (González-Rodriguez and Fowler, 2013)—see section 2.4— having already been used in other forensic contexts to identify bone from other non-osseous material (Christensen et al., 2012; Perrone et al., 2014; Finlayson et al., 2017). Therefore, it can be used as a support to the osteometric approach, and as an alternative method to DNA profiling, which is much more complex and expensive.

RECURRENT RESEARCH QUESTIONS

How preserved is the bone?

One of the first ever questions addressed by researchers working on bone chemometry regards the preservation of bone or, better yet, the preservation of the different usual components of bone composition. Initially, researchers attempted to screen the presence of the organic content on burnt bone to determine the feasibility of examinations focused on collagen such as radiocarbon dating, ancient DNA or stable isotopes analyses (DeNiro and Weiner, 1988; Weiner and Bar-Yosef, 1990; Stiner et al., 1995). Such attempts were mostly framed within archaeological research, as would be expected, since preservation problems are a more pressing issue for ancient materials. Nonetheless, the findings obtained from these endeavours are extensive to forensic contexts.

The questions answered by this kind of approach are usually more relevant for unburnt bones, since they focus on organic matter. However, it may still be important to assess its content in bones and teeth, namely for the purpose of radiocarbon dating. Nowadays, dating burnt skeletal materials is carried out routinely (Lanting et al., 2001; Olsen et al., 2008; Zazzo et al., 2009; Fitzpatrick et al., 2017). However, studies show that dating procedures must be adapted according to how intensively those materials have been burnt. Given that bone collagen is usually not preserved in skeletal material burnt at high temperatures, Lanting and Brindley (1998) proposed that structural carbonates be used instead for radiocarbon dating. Van Strydonck et al. (2005) argued that this is possible thanks to bone mineral recrystallization, which impedes exchanges with the environment. Bone compaction, as well as the very low concentration of structural carbonate remaining in bone, causes environmental reactive agents to have trouble reaching the reactive part of bone. In turn, no carbonate ion exchange or precipitation of secondary carbonate occurs. On the other hand, bones burnt at lower temperatures do not meet these conditions and thus constitute a different kind of material that must be excluded from radiocarbon dating based on structural carbonates.

Burnt bones do not always contain organic matter, which in general, will be entirely absent in calcined skeletal material. However, forensic settings involving burnt remains do not exclusively comprise completely calcined skeletons. Thanks to the shielding provided by soft tissues and heat-related pugilistic posture (Symes et al., 2008, 2015), a more variable pattern of heat-affected bones is often present and organic matter, namely good-quality collagen, is sometimes found. Therefore, DNA analyses are sometimes carried out (Clayton et al., 1995; Staiti et al., 2004; Mundorff et al., 2009).

The contribution of infrared spectroscopy as a pre-screening method of collagen availability has been investigated by Trueman et al., (2008) and Lebon et al. (2016), who even calculated regression equations to estimate the weight percent of bone collagen content from the Amide I/PO_4 ratio. The latter also proposed an equation to estimate the weight percent of bone nitrogen content from this very same index. The promising results of Lebon et al. (2016) on bones with ~4 ± 1.2 wt% of collagen were later confirmed during a multi-approach investigation by Kontopoulos et al. (2019) who concluded that the regression model reliably predicted collagen yields. Additionally, the crystallinity index and the C/P ratio obtained through FTIR-ATR were deemed useful for the identification of bones with high endogenous DNA content. Using a larger sample, Leskovar et al. (2019) found weaker correlations among those indices and DNA preservation. However, they concluded nonetheless that chemometric indices as well as some spectral regions have clear potential after further data exploration, namely through machine learning statistics. Bouchard et al. (2019) further confirmed the potential of the Amide I/PO_4 ratio in this matter, but argued that the method must be tested on a site-by-site basis, and that several measurement methods should be explored (peak height; average height; peak area). It should be noted that collagen quality cannot be inferred directly from the mere presence of collagen in bone (Bouchard et al., 2019). However, the replacement of bone unsuitable for DNA and other collagen-based analyses by bones offering better conditions appears to be quite feasible using FTIR as a pre-screening method. Of course, burnt bones involve an additional challenge regarding DNA analyses since, even when it is present, heat is known to degrade it (Brown et al., 1995; Cattaneo et al., 1999; Ye et al., 2004; Fredericks et al., 2015).

As mentioned above, bone preservation studies also intend to determine the presence of extraneous material in bone due to diagenesis (Sponheimer and Lee-Thorp, 1999). In this regard, Snoeck et al. (2014) investigated the usefulness of sodium hypochlorite and acetic acid to remove diagenetic apatites and exogenous carbonates from both archaeological and modern samples. They concluded that calcined bones, which tend to have large crystallites, are less reactive to environmental carbonates. Even if smaller crystallites are present, these will in time become larger due to diagenesis, and form a more stable and less reactive structure in cases of prolonged inhumation. Therefore, Snoeck et al. (2014) argue that archaeological burnt bone will usually contain small amounts of exogenous carbonates, and that a pre-treatment is safe and recommendable, since endogenous material will not be removed. In contrast, the authors state that such a procedure is not advisable for recently burnt bone, because pre-treatment may eliminate non-exogenous carbonates due to the higher bone reactivity—this greater reactivity being the result of the potential presence of less crystallized crystallites. Calcined bones also proved to be quite impervious to strontium contamination, a capacity provided by bone recrystallization and enhanced crystallinity (Snoeck et al., 2014). Potential exogenous strontium, if present, can be removed by pre-treating calcined bone with acetic acid and ultrasonication, so results will reflect the *in vivo* strontium isotopic ratio, which is useful to assess the provenance and mobility of individuals. Regrettably, the same cannot be said for the carbon and oxygen isotopes. Although the isotopic composition of calcined bone appears not to be substantially affected by burial, the burning itself leads to major changes which therefore do not reflect the *in vivo* composition (Snoeck et al. 2016a).

Crystallinity is frequently quantified by X–ray diffraction and infrared spectroscopy, as these methods are sensitive to structural order (Rogers et al., 2010). The bioapatite crystallinity is used to differentiate the degree of diagenesis in fossil specimens. Compared with “fresh” modern bone, fossil bone shows increased crystallinity (Schoeninger et al., 1989; Sillen, 1996; Hedges and Millard, 1995; Sillen and Parkington, 1996; Elorza et al., 1999; Lee-Thorp, 2002; Reiche et al., 2002; Farlow and Argast, 2006), reflecting changes in apatite crystallite size and strain, as well as incorporation of F^- into the apatite crystal structure while the latter loses CO_3^{2-}. Crystallinity can increase in bones exposed on the surface

of the ground for several years (Tuross et al., 1989), but a timescale of millennia is generally necessary for major crystallinity change in buried bone (Sillen and Morris, 1996). Piga et al. (2009) have estimated that fluorination of bone apatite takes about 4-5 Ma to occur, by examining the unit cell volume change of biogenic apatite vs. geological age with XRD. In addition, warmer temperatures affecting the bone deposit probably result in increased crystallinity, as do other diagenetic modifications of bone (Hedges, 2002). Tooth enamel shows less crystallinity changes than bone between modern and fossil specimens (Michel et al.,1996).

Is the bone human?

The separation of faunal bone from human bone is an important component of any archaeological or forensic analysis (Cattaneo et al., 1999; Cuijpers, 2006; McKinley, 1994; Whyte, 2001). It is important for a range of reasons, from determining the minimum number of individuals present to understanding funerary behaviour and to comprehending human-faunal relations. This is also true for burnt skeletal material, but this work is greatly complicated by the range of heat-induced changes that bone undergoes when burnt (Thompson, 2005). Thus, studies which focus on the discrimination of bones according to different species, especially if fragmented, are extremely valuable.

Recently, some papers (Beckett et al., 2011; Piga et al., 2013; Greenwood et al., 2013) addressed the use of XRD to distinguish non-human animal bones from human bones. Beckett et al. (2011) reported the possibility of determining the human rather than animal origin of bone from the lattice parameters of the inorganic bioapatite phase from the diffraction patterns of bones subjected to a high-temperature heating treatment.

For the case of bioapatite crystals found in bones, a space group $P6_3/m$ is generally attributed with a hexagonal unit cell where two lattice parameters (a- and c-axis respectively) need to be determined. According to Beckett et al. (2011), the plot of *a*- vs *c*-axis data points from human bone occurs in a typical and distinct area from that observed on non-human animal bone.

The paper by Beckett et al. (2011) has employed a simplified approach for lattice parameter determination, starting from the peak positions which are calculated by the automatic location of the maxima of diffraction patterns (which may not be completely satisfactory). The determination of lattice parameters depends upon the precision of locating the peak profiles in XRD diagrams (Masciocchi and Artioli, 1996), but in bioapatite this is difficult to do because of large peak broadening resulting from the small crystallite size of the phase combined with the high amount of lattice strain (Danilchenko et al., 2002). To alleviate this problem, Beckett et al. (2011) suggested that lattice parameter determination be performed on highly crystallized single-phase materials following thermal treatment of the bone. However, this in turn creates a potential problem in the identification of the most appropriate heating temperature for differentiating faunal from human bone. Another potential issue with the approach in Beckett et al. (2011) stems from the fact that their analysis was limited to a sample of just 8 human specimens vs. 65 non-human samples from 12 different species.

Piga et al. (2013) applied the XRD technique to 95 specimens (42 animals and 53 humans) burnt at 1100°C to address the problem of whether the monoclinic $P2_1/b$ vs hexagonal $P6_3/m$ space group can make a substantial difference in terms of lattice parameter values for the bioapatite of bones between species. The study addresses a number of important points:

a) Bones typically give broad XRD lines unless heated, so it is only possible to compare human and faunal bones that have had substantial heat treatment.

b) The a-axis and c-axis values of bioapatite are not biased by the use of a $P2_1/b$ monoclinic rather than $P6_3/m$ hexagonal unit cell.

c) Lattice parameters data has been carried out according to the Rietveld method.

d) The chemistry of bone material changes significantly due to heating.

e) The apatite unit cell axis can be affected by *post-mortem* ion exchange reactions such as

fluorination during diagenesis or chlorination during boiling of bones in salted water for cleaning.

Results from Piga et al. (2013) reject the possibility to distinguish human from animal bones through XRD. Figure 4 clearly shows that, in terms of lattice parameters, the variability of human specimens is completely overlapped by the non-human variability, making the use of XRD in order to distinguish faunal from human bones very questionable.

Until very recently, species differentiation using FTIR had not been attempted. However, given the differences observed between humans and other animals (see section 1.1), those may potentially be reflected by infrared spectra. To our knowledge, Wang et al. (2018) were the first to investigate this issue, using fresh bone samples from several species besides humans: caprines, bovines and porcines. By using principal component analysis and partial least squares discriminant analysis, they were able to discriminate between them. However, results were not as encouraging for boiled bone samples as well as for bones which had been buried for one year. Therefore, expectations regarding the potential of infrared spectra to help distinguishing species are not high. Hopefully, this seminal paper from Wang et al. (2018) will be followed by other investigations able to provide further enlightenment on this issue.

Like the other techniques, little has been done in the matter of species discrimination with XRF. A seminal paper was published by Nganvongpanit et al. (2017), in which the authors state having been able to differentiate human teeth from teeth of 20 other species in a large sample with a handheld XRF. Although human teeth are usually easily recognizable, fragmented ones may be more challenging. This study gives good prospects about the feasibility of XRF to achieve species discrimination although validation studies must be produced in the future. Similar work in bone must also be encouraged.

Is the bone recent?

Determining whether skeletal remains are recent or not is of paramount importance for forensic science since, at least for prescriptive law systems, it is one of the criteria to establish forensic and penal relevance. Regrettably, methods to estimate the post-mortem interval (PMI) in burnt bones are scarce and not entirely enlightening. Actually, the only verified osteological method able to do this is by radiocarbon dating calcined bone which has been previously addressed in section 2.1. No other osteological methods allow

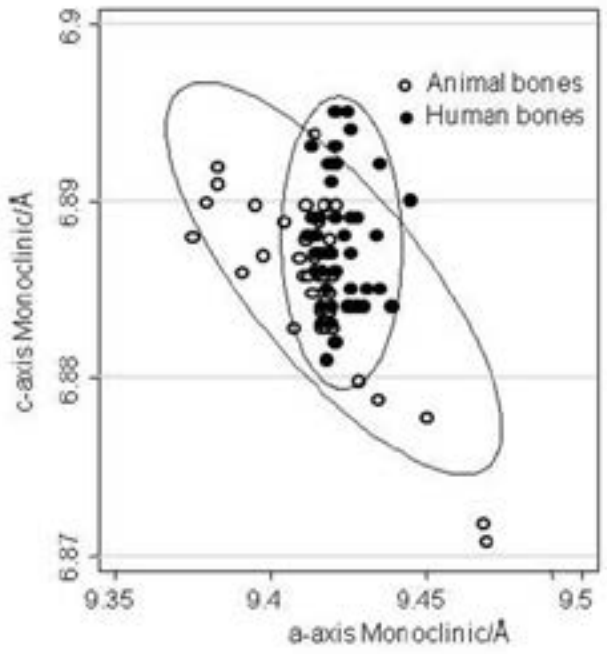

Fig. 4.- Plot of c- vs a-axis for the two groups of bones treated at high-temperature; the two groups of humans and animal bones are overlapped so as to identify a single cluster (reproduced from Piga et al., 2013).

for estimations and only entomology regarding charred bodies and forensic botany can indirectly provide an approximate PMI (Introna et al., 1998; Pai et al., 2007; Fairgrieve, 2008).

The contribution of infrared spectroscopy to address this question is quite limited. Snoeck et al. (2014) proposed that acetic acid may potentially be used to distinguish between archaeological burnt bone and recent burnt bone. As mentioned above, bones burnt at low to medium temperatures will tend to display smaller and less crystallized crystallites which will therefore be more reactive (Snoeck et al., 2014). However, given enough inhumation time, such crystallites will become larger making them less reactive so acetic acid treatment should not lead to a major decrease of the carbonates content. In recent non-calcined bone, recrystallization has not taken place yet, so acetic acid treatment leads to a considerable decrease of carbonates which is shown in a comparison of pre- and post-treatment infrared spectra. Therefore, the absence of a major decrease in carbonates should be indicative of somewhat ancient bone. The rationale behind this claim is sound, but further work is required to determine the precise potential of this approach. Whatever it is, it will predictably be relevant only for non-calcined bone, because these are already recrystallized, showing larger crystallites, and therefore less reactive to the depositional environment.

Lately, several researches have been carried out about the potential of spectroscopy to assess the post-mortem interval of unburnt skeletal remains (Howes et al., 2012; Patonai et al., 2012; Wang et al, 2017). Although some value has been found, possible problems related to the effect of diagenesis on the composition of bone and, in turn, on how it can affect further PMI inferences still seem patent. Predictably, the confounding effect of diagenesis will be complicated by the additional effect of heat on bone composition. Clearly, more research is needed to determine whether this kind of approach is promising or not. For the time being, estimating relatively precise PMI directly on burnt bones appears to be impossible, and this is one of the major research lines that most need addressing.

Bartsiokas and Middleton (1992) suggested that quantitative indices of bone crystallinity could be used to date archaeological and paleontological bone samples up to chronologies of about one million years. Sillen and Parkington (1996) likewise found a relationship between bone crystallinity and chronology, but only for bones up to about 20,000 years old. Over a timescale of thousands or millions of years, Person et al. (1995, 1996), however, found no relationship between bone crystallinity and age (also see: Hedges and Millard, 1995), and argued that crystallinity changes occur in the earliest phases of inorganic diagenesis.

Piga et al. (2009) observed a positive correlation between average bone apatite crystal size and specimen chronology in a set of fossils ranging in age from the Middle Triassic (around 245 Ma) to the present day (see Fig. 5).

In another work, Piga et al. (2011) reported that the average crystallite size for apatite of a dinosaur samples collection as a function of age in the range 150-65 Ma – ordered according to their geological age – showed no correlation between the crystallization process and chronology. From these data, it would seem that the crystallization induced by time alone is overlapped by other factors depending on the geological formation that may inhibit or enhance the process. The unpredictable change of the average crystallite size values thus suggests that correlation between crystallisation indices and bone chronology has to be regarded with obvious caution. All the outcomes described above indicate that XRD analyses have little use to assist in the assessment of the PMI in forensic settings.

How many individuals are present in the assemblage?

Skeletons are not all the same. Instead, they are the outcome of several factors such as age, sex, cultural behaviours, diets, metabolic function, breathing environment, and drug intake (Richards et al., 2003; Bentley, 2006; Fleming and Blom, 2007; Hedges et al., 2007; Swanston et al., 2012; Perrone et al., 2014; Daar et al., 2015; Emslie et al., 2015). Theoretically speaking, differences resulting from this multivariable effect should

create individual signatures that are specific enough to allow successful individualization of most bones from different skeletons in assemblages with multiple individuals. To achieve that, XRF appears to be one of the methods of choice since it is a useful, rapid and cost-efficient technique to determine trace element ratio.

Indeed, successful sorting of bones from specific skeletons has been achieved in the study of a small number of individuals by Gonzalez-Rodriguez and Fowler (2013). Following a previous attempt from Fulton et al. (1986), who used XRF as a complementary method to other sorting methods, those authors combined portable XRF data with Principal Component (PCA) analysis to successfully discriminate five medieval human skeletons. The research indicated that trace elemental composition differs between individuals and can be used through chemometric analysis to identify which bone belongs to a specific individual. In the first instance, individual elements were chosen, which are considered to be associated with diet or physiological importance. Individual elements were 100% successful in separating two individual skeletons; but, when further skeletons were included, it became increasingly more difficult to differentiate between individuals, although correct allocation was still quite high. Therefore, bone element ratios have been used to differentiate between skeletons in an attempt to increase separation and reduce chemical noise. Various ratios were used: Pb/C, K/Fe, Zn/Fe, Sr/Ca, and Sr/Pb, with the most effective being Pb/Ca, Zn/Fe, and Sr/Pb (González-Rodriguez and Fowler, 2013).

Regrettably, there has been little follow-up to those papers. Perrone et al. (2014) and Finlayson et al. (2017) have nonetheless demonstrated the usefulness of this kind of technique. Perrone et al. (2014) found that the intra-skeleton elemental concentration of Si, P, K, Ca, Mn, Fe, and Co did not vary a lot in a collection of identified skeletons. On

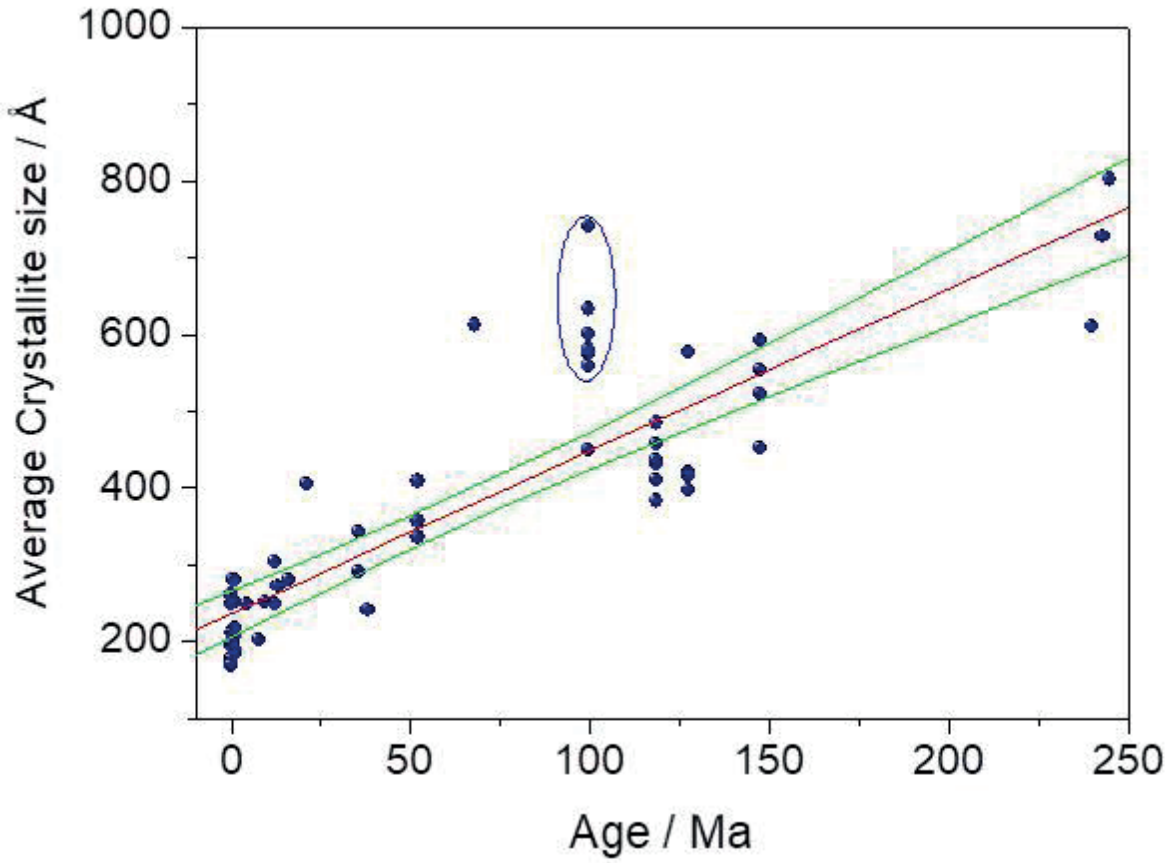

Fig. 5.- Correlation between average crystallite size values and age from stratigraphic chart analysis of the bone specimens analysed by Piga et al. (2009). The data inside the ellipse refer to specimens from Upper Cretaceous not conforming to the linear trend. (reproduced from Piga et al., 2009).

the other hand, those concentrations presented important differences among skeletons. Finlayson et al. (2017) preferred to use portable XRF as one of several methods—DNA analysis, taphonomic analysis and osteometric sorting—to help discriminating between two different individuals, and found phosphorus to be quite helpful.

The potential of the elemental composition of bone for individual discrimination lies on the premise that the uptake of skeleton trace elements is made through the diagenetic process. In fact, the length of time that bones are in contact with the soil affects the separation of the bone element ratios. The different behaviours regarding this process lead to differential chemical signatures that can be valuable for individual discrimination. In modern mass grave contexts, however, diagenesis may be less of an issue and all skeletal elements are likely to be subjected to the same elements and processes within the soil. Therefore, it could be possible that any variation in elemental analysis is related to the diet of each individual. Perrone et al. (2014) found very little intra-skeletal variation in pXRF measurements, while finding greatest differences between individuals, especially for Si, P, K, Ca, Mn, Fe, and Co. This explains the success that was obtained in theirs and other researches aiming at individual discrimination. Such successful outcomes obtained on the researches mentioned above suggest that diagenetic changes are not entirely impeditive of XRF application. Besides, the impact of diagenesis in the use of XRF is probably much larger for archaeological materials than it is for forensic remains.

Is the bone burnt and to which temperature?

Burning events are known to prompt chemical and structural changes in bones and teeth, depending on the temperature and duration of the heating process (Fig. 6), which interfere with the reliability of analyses performed by forensic anthropologists usually based in parameters from unburnt bones (Binford, 1963; Herrmann, 1976; van Vark et al., 1996; Wahl, 1996; Thompson, 2004, 2005; Ubelaker, 2009; Symes et al., 2015; Gonçalves et al., 2015; Vassalo et al., 2019). That is why it may be important to determine to which temperature bones have been exposed, so that an assessment of possible harming changes may be established.

Although heat exposure often leaves unambiguous trademarks, recognizing heat marks in skeletal remains is sometimes challenging. Heat-induced changes can be mistaken for other taphonomic processes such as oxide and soil staining, fungal activity, contact with metals or water, and sun exposure (Piepenbrink, 1986; Buikstra and Ubelaker, 1994; Shahack-Gross et al., 1997; López-González et al., 2006; Arroyo et al., 2008; Schmidt, 2008; Dupras and Schultz, 2014; Evans, 2014; Higgs and Pokines, 2014; Junod and Pokines, 2014; Pokines and Baker, 2014). Some processes may even replicate chemometric profiles typical of burnt bones and teeth. That is the case of fossilisation and weathering mechanisms, which may also be present and cause changes in the composition of bones that replicate those from burnt bones (Stiner et al., 1995). Detailed studies in paleontological, archaeological and modern bones and teeth are therefore required with a view to comprehensively document taphonomic changes.

The estimation of the maximum temperature affecting skeletal remains has been previously attempted *via* infrared-based methods (Munro et al., 2007; Thompson et al., 2009). Thompson et al. (2013) investigated the usability of principal components analysis and linear discriminant analysis to separate burnt bones according to burn intensity (low, medium, high). Although this approach appears lacking in terms of precision, it is not exempt of potential. Ellingham et al. (2016) further explored the infrared approach by using linear regression to estimate temperature, and obtained results with a mean estimate standard error of 70 °C. The effect of soft tissue was also highlighted by slowing down combustion in low temperature and by accelerating it in high temperature exposures. The review carried out by Ellingham et al. (2015) supports the notion that a multi-approach based on several techniques, infrared spectroscopy included, is the soundest choice to estimate maximum temperature.

The infrared crystallinity index (CI) or splitting factor (IRSF) appears to be a reliable indicator of burn intensity due to its little intra-bone variation

(Person et al., 1996; Gonçalves et al., 2018). Its usefulness is greater when combined with other indices (Thompson et al., 2013; Ellingham et al., 2016). It has been reported by several authors that CI values increase considerably up to temperatures around 800 ºC followed by a decrease at higher temperatures (Stiner and Surovell, 2001; Munro et al., 2007; Thompson et al., 2009, 2011, 2013; Snoeck et al., 2014). However, this trend has not been observed in other works (Piga et al., 2016a; Piga et al., 2016b; Gonçalves et al., 2018) which, instead of using non-human bone, used human bone. It is not clear if the use of animal proxies can lead to this difference or if it was related with, for example, different sampling procedures (Surovell and Stiner, 2001; Munro et al., 2007; Kontopoulos et al., 2018). Nonetheless, it serves to show that additional research is required to clarify this issue. Moreover, the CI does not show considerable change at temperatures lower than 600 ºC thus being of little value to identify bones and teeth burnt at low and medium intensities including boiled bones (Koon et al., 2003; Lebon et al., 2010; Thompson et al., 2009, 2013; Snoeck et al., 2014). Indeed, identification of skeletal remains burnt at lower intensities appears to be more problematic than those burnt at higher intensities. This is, at least in part, due to the fact that most heat-related changes in the infrared profile are easily replicated by other taphonomic agents.

The potential of the FTIR hydroxyl vibrational bands from bone samples to identify burnt bone has been investigated by Mamede et al. (2018). The OH/P spectroscopic index (intensity ratio of characteristic infrared bands for OH and phosphate groups, respectively) has been proposed as a valuable parameter to pinpoint heat-induced compositional and structural changes in bone heated at high temperatures (Piga et al., 2016a). The OH signals became visible in infrared spectra from bones burnt for two hours until they reached temperatures between 600 ºC and 700 ºC (Gonçalves et al.,

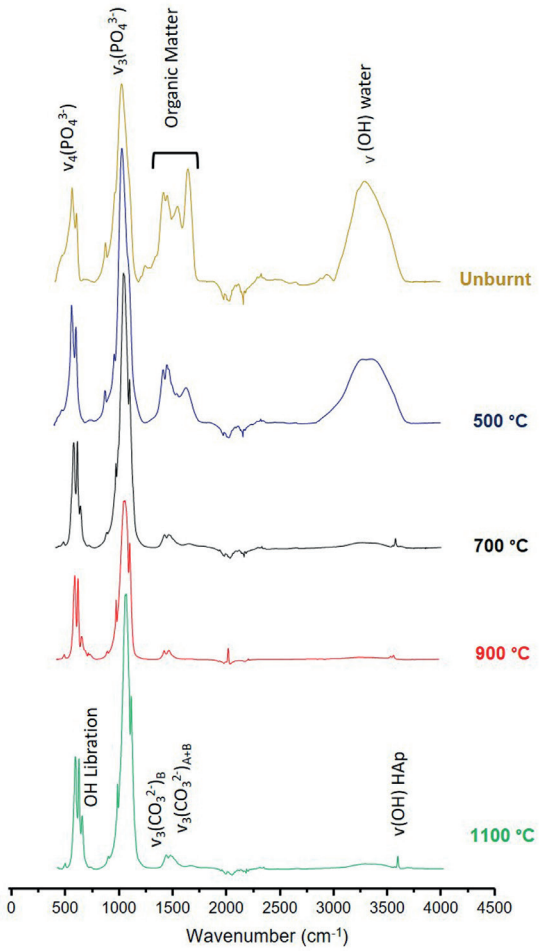

Fig. 6.- Heat-induced spectral alterations observed in human bone.

2018). Although stretching OH signals appear to be rare in fossil bones, their presence can bias the use of this feature as a fingerprint of burn events. Actually, fossilisation mechanisms and long exposure to diagenesis typical of archaeological bones can mimic the chemical rearrangements of burnt bones. Of course, those are usually not primary concerns regarding skeletal remains from forensic settings (Thompson et al., 2013). The authors concluded that high values of the CI are usually associated to visible hydroxyl signals in burnt bone, so the presence of the two features are strongly indicative of the latter.

The potential of other IR based indices to determine if a bone or tooth is burnt or not appears to be more challenging. Most other indices popularly used in research involving skeletal material are based on carbonates and organic matter—e.g., carbonates to phosphate ratio (C/P), type-A carbonates to phosphate ratio (API), type-b carbonates to phosphate ratio (BPI), amide to phosphate ratio (Am/P) (Bellamy, 1975; Trueman et al., 2008; Lebon et al., 2010; Thompson et al., 2013; Snoeck et al., 2014). Carbonates contamination of skeletal material (already discussed in section 2.1) is quite frequent, and organic matter decomposition unrelated to heat exposure can replicate its loss by heat-induced events (Stiner et al., 1995; Snoeck et al., 2014). Therefore, interpretations must take this into account.

Maximum temperature estimation of skeletal remains also benefits from XRD analyses. As noted by Piga et al. (2008), an increase in crystal size when using XRD is only noticeable at burning temperatures above 700 °C. From this temperature onwards, they additionally observed the disappearance of the calcide peak at 36° and the simultaneous appearance of a calcium oxide peak at 37.5° in 2θ at around 775 °C. These observations, as well as the fact that it is able to detect crystallographic direction (Greenwood et al., 2013), make XRD useful for the determination of temperatures in the high ranges above 700 °C, but it is not very diagnostic for low temperature estimations. Another shortcoming of the XRD technique is that it only allows a crystal size determination up to 1500 Å (Piga et al., 2009). This possibly damages any attempts to estimate maximum temperature estimation since it is clearly correlated to crystal size (Piga et al., 2011, 2018).

The transformation of bioapatite into other mineralogical phases (β–tricalcium phosphate, lime, portlandite, calcite and buchwaldite) can help estimate the temperature to which the bone has been exposed (Piga et al., 2016b; 2018). Particularly, the presence of β–TCP appears to be sporadic and seems to occur only at high temperatures, around 1100 °C (Piga et al., 2013). On the other hand, Piga et al. (2016a) observed a more systematic occurrence of β–TCP in teeth specimens at temperatures as low as 750 °C. The reason why β–TCP appears in teeth at relatively moderate temperature in comparison to bones is still obscure.

What was the impact of burning on skeletal remains?

The impact of heat on the microstructure and composition of skeletal materials has already been addressed in previous sections. Here, a review of its impact for other kinds of analyses is carried out. Besides the usual fragmentation, the major predicament involved in the forensic anthropological analysis of burnt skeletal remains is related to uncertainty. Hesitation becomes a commonplace for examiners due to their inability to determine to which extent have bones and teeth been transformed by heat. Although some practical pointers have been proposed over the years, the truth is that a lot of them have been proven unreliable or at least not able to completely tackle the breath-taking variability in heat-induced changes that skeletal remains may encompass. For example, that is the case for the use of correction factors to counteract the typical heat-induced shrinkage calcined bones experience, inspired by works such as the ones from Shipman et al. (1984) and Buikstra and Swegle (1989). This approach attempts to return bone features to their pre-burnt size. However, it applies inflexible correction factors (e.g. 10%) to all cases, even though large variation in metric changes has been reported for bones burnt under the same conditions. Therefore, this procedure

is bound to result in large errors when used in a case-by-case basis (Gonçalves, 2016). So how can forensic anthropologists determine the precise extent of heat-induced metric change? This realisation is paramount to establishing how reliable inferences based on metric features are.

Almost nothing has been done so far to solve this problem with more reliable approaches, except for some exploratory work by Thompson (2005), using mercury-intrusion porosimetry and small-angle X-ray scattering techniques. None has been done with infrared spectroscopy, XRD or XRF though. In a recent paper, Gonçalves et al. (2020) explored the potential of FTIR-ATR to calculate the amount of metric change experienced by bones. The outcome comprised a series of regression models including chemometric data that provided heat-induced metric change predictions with smaller associated errors than the ones obtained by using the correction factor approach. Results suggested that the regression models have little, if any, room for improvement in terms of infrared spectroscopy data so the search for other influent variables is probably better allocated to other techniques.

Bone mass is another variable drastically affected by heat events, which is unfortunate, because, given the usual incapability of building skeletal inventories of highly fragmented remains, it is often used to assess about skeletal completeness. For the same reason, it can provide clues about the minimum number of individuals comprised in a skeletal assemblage (Gonçalves et al., 2015b). Mass-based inferences are obtained after comparisons with reference values (e.g. Lowrance and Latimer, 1957; Silva et al., 2009; Gonçalves et al., 2013). However, these refer only to skeletons that are either unburnt or entirely calcined. Therefore, they can only act as suitable references in these two cases, which are only two ends of a much larger spectrum of possibilities regarding how intensively has bone been affected. Most mass loss occurs at low to medium temperatures and are caused by dehydration and decomposition of organic matter (Thompson, 2004). Therefore, assessing for their presence in infrared profiles is useful in order to determine if both those components, which have specific wavelengths attributions (Mamede et al., 2018a) are present in bone. If absent, calcined-specific skeletal mass references can be used (e.g. Warren and Maples, 1997; McKinley et al., 1993; Bass and Jantz, 2004).

Taphonomic analysis of marks left in bones by other than heat-related agents can also be affected by our inability to discriminate between the two as was mentioned in section 2.5. A lot of experimental work is still needed to determine the actual potential of infrared spectroscopy to distinguish between heat and other taphonomic events as responsible for colour changes. Nonetheless, the endeavours of Shahack-Gross et al. (1997), with the aim of pinpointing charred bones from manganese oxidized bones, demonstrate that infrared spectroscopy can make relevant contributions to this issue, although little follow-up has been raised so far.

Finally, X-ray diffraction can provide useful indications regarding the thermal impact on bones, particularly those that can potentially affect inferences obtained from chemometric analyses. That is the case of the already mentioned β–TCP which visibly affects $\nu_4(PO_4^{3-})$ bands, thus interfering with the correct calculation of the CI (Figs. 7 and 8).

FUTURE RESEARCH

One of the upcoming challenges regarding chemometric analyses with FTIR, XRD and XRF techniques in the future refers to transferability issues. An important research increment has been achieved in the last few decades, which has paved the way onto the feasibility of using such approaches to obtain a better understanding of how bones and teeth react to heat exposure. It is now necessary to increase our trust on inferences based on the information that chemometry can provide us. In order to do that, clear demonstration that invariant results on the same bone and teeth samples are obtained on different equipment is required.

Another concern for the future regards the increment of research focused on teeth. So far, little has been done in this department, as can be inferred from the contents of this very same

paper. Teeth are very resilient and are often found in assemblages involving burnt skeletal remains. In addition, teeth are frequently used for positive identification (Barsley et al., 1985; Hutt et al., 1995; Nambiar et al., 1997; Krompecher et al., 2000; Mayne Correia and Beattie, 2002; Schmidt, 2008; Burkes, 2011; Lain et al., 2011; James and Taylor, 2011), and are therefore very useful in forensic investigations. It is therefore of paramount importance to increase our understanding of heat-induced changes in teeth and potentiate our capacity to draw inferences from this kind of skeletal material. The multicomponent dimension of teeth may potentially bring advantages that bones do not provide. The differential behaviour that enamel, dentin and cementum manifest regarding heat exposure can eventually correspond to multiple and combinable sources of information about the heat event. These may help achieving more solid inferences. This has not been investigated yet, so no one knows whether that is the case.

Investigation carried out so far has already demonstrated that FTIR, XRD and XRF have a lot to offer to the examination of skeletal remains. However, most practical applications appear to have been done within the archaeological arena, since not many forensic anthropology papers report work based on such techniques. Therefore, it seems that their full potential has not been explored yet by forensic anthropologists. Probably, this scenario will change as further validation studies are accomplished and the confidence and reliability criteria required in forensic science are achieved.

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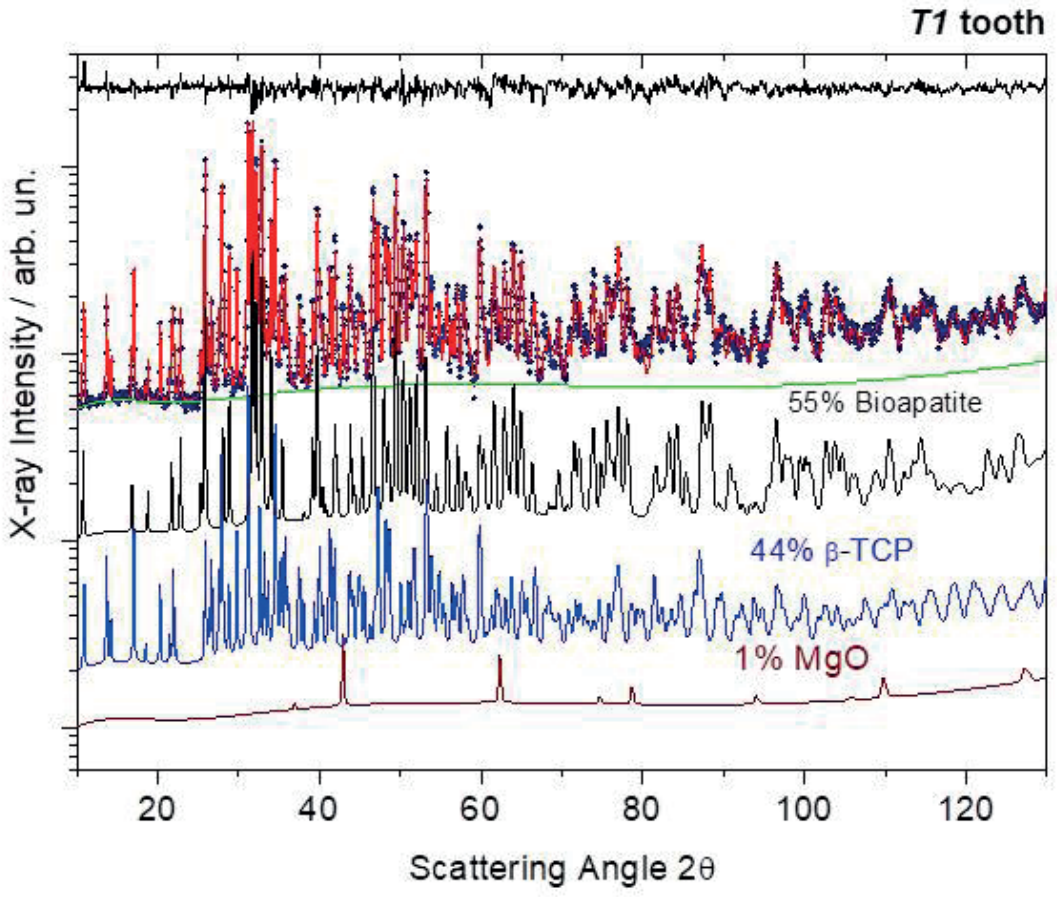

Fig. 7.- The XRD pattern of a human tooth (T1) heated up to 1050 °C for 1 hour. The phase analysis suggest that the material is a mixture of bioapatite (55wt. %) and β–TCP [$Ca_3(PO_4)_2$] (44.0 wt.%), with a minor contribution of MgO (reproduced from Piga et al., 2016a).

and physico–chemical investigation using a multi–technique approach on archaeological, anthropological and paleontological materials from the Mediterranean area and Sardinia".

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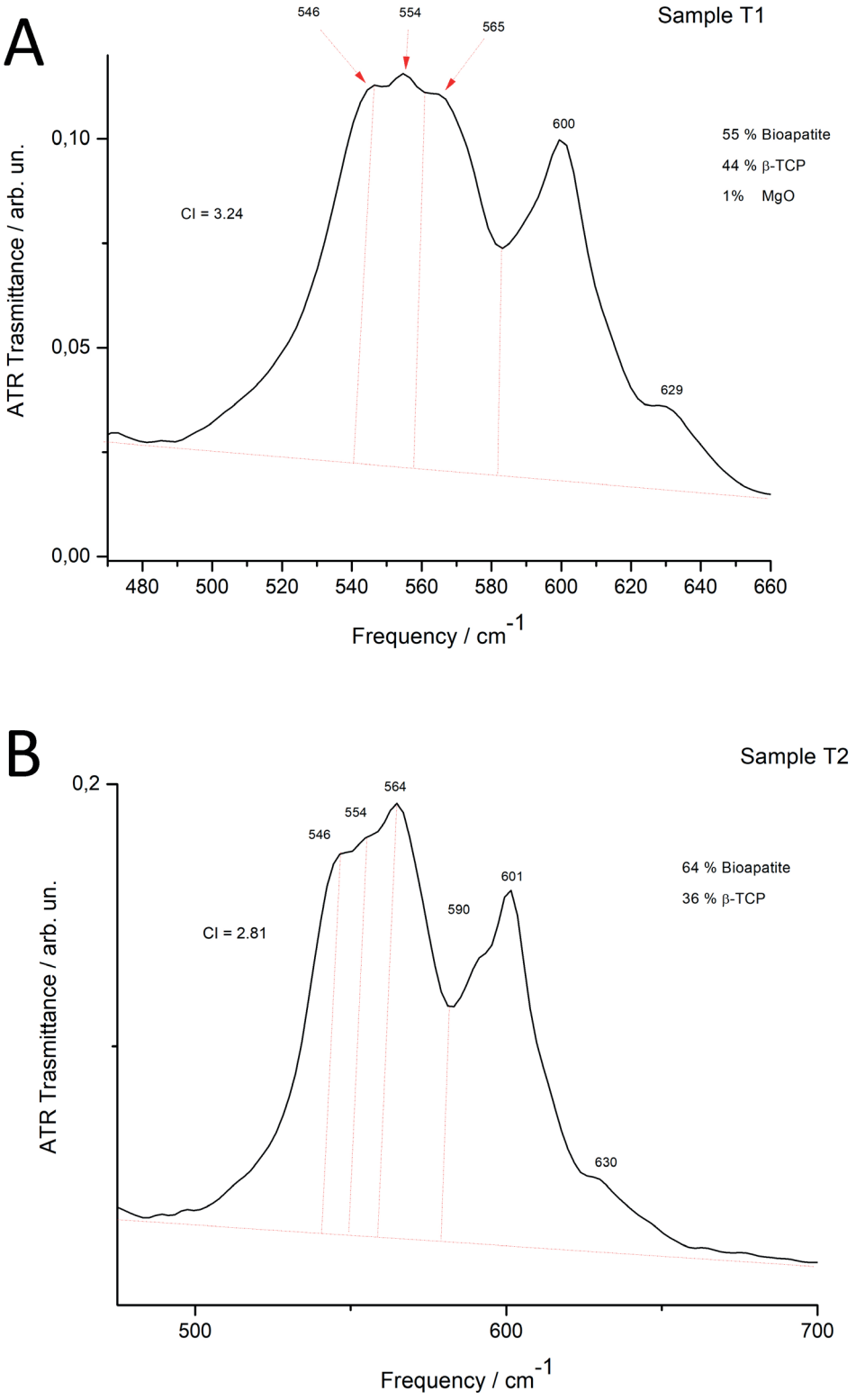

Fig. 8.- Two different distortions of ATR-IR 4 phospate band in T1 and T2 human tooth sample with the corresponding XRD Rietveld phase and ATR crystallinity index evaluations. Particularly, when the presence of β–TCP is massive (≥ 20%) the ν_4 band appears strongly deformed due to the overlap with the ν_4 band of bioapatite (see the deformations of the peak at 563 cm_{-1} or the shoulder at 590 cm_{-1} in sample T2). Also, the CI calculation becomes problematic (adapted from Piga et al., 2016a).

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The impact of forensic mortality bias in juvenile age estimation

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SUMMARY

Forensic mortality bias is the totality of biological, social, and random filters that select forensic anthropology casework individuals from the larger living population. Although forensic mortality bias affects all age groups, it is particularly important for children because age estimation relies on growth indicators which are sensitive to environmental influences associated with social and biological selecting filters. Using post-mortem computed tomography scans of 188 children from the USA and Australia, we compare long bone lengths between children dying of accidents and homicides as proxies for children in the living population and forensic sample, respectively. Growth differences are quantified using ANCOVA analysis and z-scores. Forensic mortality bias is especially detectable in children under 2 years of age, although it is also present but less easily detected by this analysis in older children. This analysis is supplemented by visual exploration of the very few available forensic anthropology cases of juveniles in the USA and Australia. Altogether, we suggest that forensic mortality bias is linked to socioeconomic inequality. When selecting and developing methods for forensic use, practitioners should consider how forensic mortality bias affects their casework and make attempts to select growth-appropriate rather than population-specific methods.

Key words: Forensic anthropology – Methods – Growth – Bone development – Accuracy – Prediction bias

INTRODUCTION

When choosing skeletal estimation methods, forensic anthropologists rely on selecting a reference population that most resembles the sample of individuals that make up their casework. That assessment of resemblance traditionally relies on socially, geographically or politically defined groups, such as countries, nationalities and "races," with some noting cross-population applications of methods can yield poor results (Ross and Koningsberg, 2002; Komar, 2003). A long-recognized source of error in this process is that the identified skeletal reference collections used to develop many classic skeletal estimation methods are biased samples of the living population they are drawn from (Ericksen, 1982; Hunt and Albanese, 2005; Komar and Grivas, 2008). However, the same critical consideration has not been extended to the sample of individuals that actually constitute the forensic anthropology

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casework. They too represent a biased sample of the living population, albeit not in the same ways as the skeletal reference collections used to develop forensic methods. This is important because it may create hard to recognized error in the estimation of the biological profile.

Several layers of sampling "filters" act on a living population to produce a non- random forensic anthropology sample. The diagram in Figure 1 provides a hierarchical schematic illustrating these layers of filters. The diagram was inspired by a similar formulation of the sampling process that occurs in archaeological samples of human skeletons carried out by Hoppa (1996). The first layer of filters summarizes risks (biological, social, and stochastic filters) and express the notion that not everyone in the living population experiences the same risk of death. The biological filter includes factors that are intrinsic to the individual and increase their susceptibility to death from illnesses. They primarily affect individuals dying of natural deaths but can also said to have a role in deaths of a suicidal aetiology. The social filter is perhaps the most wide-ranging of the filters and includes all risks that are extrinsic to the individual but are also socially or culturally mediated. Certain cultural behaviours or social contexts can increase the probability of individuals dying of non-natural or violent causes, for example risk-seeking, criminal behaviours or living in unsafe environments. The social filter does not affect all deaths evenly: it is particularly strong where an individual's death is least likely to be affected by the remaining two filters, i.e., for the homicide manner of death. The stochastic or random filter includes all factors that are extrinsic to the individual but not socially or culturally mediated. These factors are in effect related to chance, and primarily affect accidental deaths but can also play a role in some homicide-related deaths.

The forensic anthropologist may deal with all four manners of death (natural, suicide, homicide, and accident), and the transition between death and forensic anthropology examinations is influenced by a taphonomic filter (Fig. 1). Here a natural or non-natural death becomes a forensic anthropology case by factors, both human and environmental, that cause the decomposition and degradation of the body so that the individual is no longer visually unidentifiable. Once the body of an individual is putrefied, burned, decomposed,

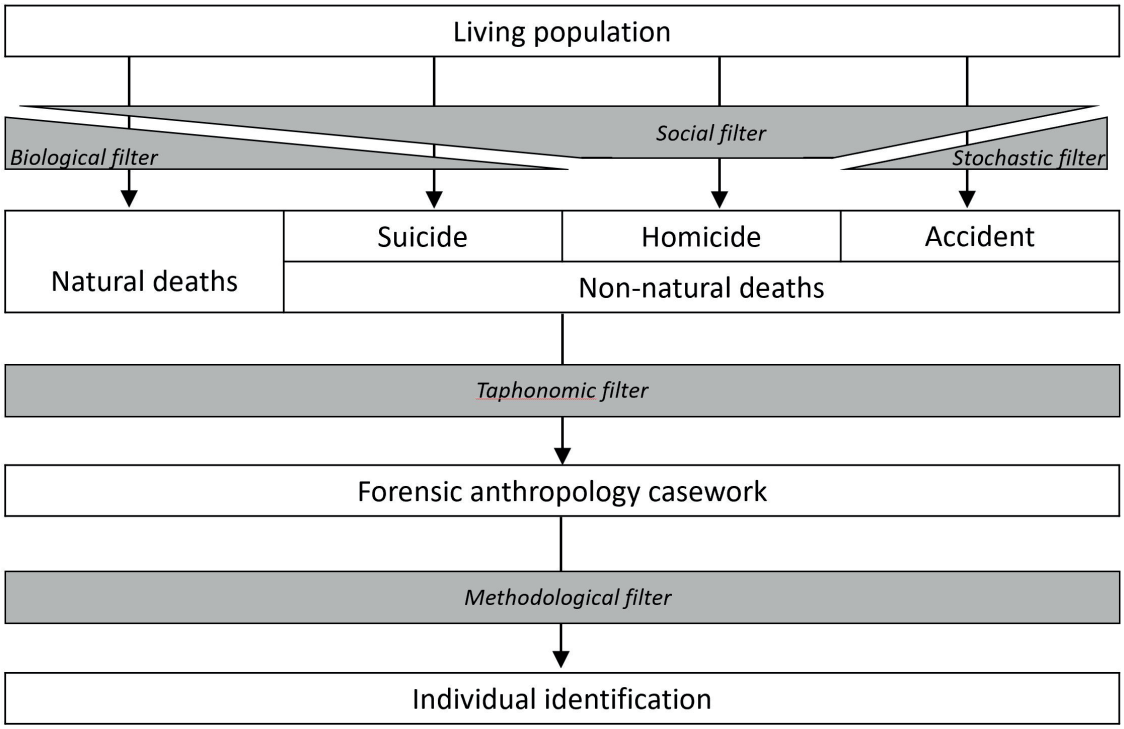

Fig. 1.- Schematic representation of the various filters acting on the living population to produce the forensic anthropology sample.

dismembered, fragmented and/or skeletonized, analysis and identification proceed with the assistance of the forensic anthropologist. The methods chosen to carry out the analysis influence the reconstruction of the biological profile, thereby influencing whether the individual is identified at the end of the process. We use the term *forensic mortality bias* to denote the layers of filters reviewed above and their selection of the living population into the forensic anthropology casework individuals.

The forensic mortality bias outlined above represents the process by which the forensic anthropology casework results in a biased, distorted or non-random sample of the living population. This bias can be particularly important in children whose deaths are under medico-legal investigation. For children, the forensic anthropology sample is typically comprised of those who died of non-natural causes (accidents, suicides, and homicides), and often more specifically those who died of criminal violent causes, particularly homicides. This is supported by our review of forensic anthropology cases involving child deaths, which suggest that homicides represent the bulk of these cases (Table 1). This has a significant impact on the nature of forensic samples, because homicide disproportionally impacts individuals of lower socioeconomic status (Christoffel, 1990; Finkelhor, 1997; Finklehor and Omrod, 2001; Hjern and Bremberg, 2002; Bennett et al., 2006; Douglas and Vanderminden, 2014; Shields et al., 2014), and thus individuals with a greater experience of environmental stress are disproportionately represented in the forensic anthropology sample. In addition to greater homicide rates associated with poverty and inequality, in many parts of the developing world civil conflict and human rights violations have been responsible for mass graves of victims, which can comprise of significant numbers of

Table 1. Reported cases of forensic anthropology casework involving child identification. This review excludes cases consisting only of trauma analysis as well as disaster victim identification and mass grave exhumations where sufficient detail regarding identification of children was not available.

Source	Country	Manner of Death	Type of Case
Snow and Luke, 1970	USA	Homicide	Suspected identity; examination to exclude other possible identity
Skinner and Anderson, 1991	Canada	Homicide	Cranium, identified with help of linear enamel hypoplasias
Houck et al., 1996	USA	NA	Mass fatality event (fire in a compound), 17+ juveniles identified
Hunter, 1996	UK	Homicide	Suspected identity
W alker et al., 1997	USA	Homicide	Case 1. Suspected identity; survey for abuse
W alker et al., 1997	USA	Homicide	Case 2. Suspected identity; survey for abuse
W alker et al., 1997	USA	Homicide	Case 3. Suspected identity; survey for abuse
W alker et al., 1997	USA	Homicide	Case 4. Suspected identity; survey for abuse
Morton and Lord, 2002	USA	Homicide	Suspected identities in the context of multiple abductions and recovery of skeletonized remains
Lewis and Flavel, 2006	Guatemala	Homicide	Differentiation between three children in a multiple inhumation
Cattaneo, 2007	Italy	Homicide	Suspected identity
Cunha et al., 2007	Angola	Homicide	Suspected identity
Bassed and Hill, 2011	Australia	Accident	Differentiation between two children in a fire
Introna et al., 2011	Italy	Accident	Found human remains; suspected identity; examination to determine manner of death
Ross, 2011	USA	NA	Suspected identity; survey for abuse and neglect
Blau and Hill, 2014	Australia	Natural	Case 1. Stillbirth concealed by parents
Blau and Hill, 2014	Australia	Homicide	Suspected identity
Ross and Juarez, 2016	USA	Homicide	Suspected identity; survey for abuse and neglect
Nase, 2019	USA	Accident	Differentiation between three children in a house fire

children, in some instances representing as much as 95% of buried individuals (Lewis and Flavel, 2006; Simmons, 2007; Ansom and Trimble, 2008; Doretti and Snow, 2009; Roberts, 2011). Often these mass killings involve indigenous or other segregated and marginalized communities who are already experiencing the most adverse living conditions (Lewis and Flavel, 2006; Doretti and Snow, 2009; Roberts, 2011). Similar impacts on the forensic anthropology casework can and do exist in developed nations. In Canada, the problem of missing children and unmarked child graves at the sites of former Indian Residential Schools exemplify the systematic oppression, marginalization, and killing of Indigenous groups typically thought to occur mainly in developing nations (Nichols, 2021; Nichols et al., in press).

Because low socioeconomic status children or children from marginalized communities make up the majority of homicide victims, and because globally, child homicides primarily occur in lower and mid income countries (LMICs), forensic anthropology cases are individuals selected by social and economic circumstances. Therefore, not only is the forensic anthropology sample of children not a cross-sectional sample of the living, but it is also not a random sample of deceased children. Even in wealthy countries, children from lower socioeconomic status backgrounds are in poorer health than their better-off peers and show compromised or retarded physical growth, often expressed in terms of stunting and wasting (Garn and Clark, 1975; Bielicki et al., 1981; Garn et al., 1984; Lasker and Mascie-Taylor, 1989; Lasker and Mascie-Taylor, 2005; Crooks, 1999; Moffat et al., 2005; Moffat and Galloway, 2007; Ehouxou et al., 2009; Grimberg et al., 2009). Because juvenile age estimation methods rely on size, maturation and development, age estimation methods developed on samples of normal children are likely or can misrepresent the growth of low socioeconomic status and homicide victims, providing biased age estimates (Spake and Cardoso, 2018a, b).

Generally, juvenile age estimation methods used in forensic casework are based largely on "healthy" living children (Maresh, 1970; Stull et al., 2014; Tsai et al., 2016). Several methods explicitly exclude individuals with diseases that could impact growth, or where children suffered from abuse and neglect that could have delayed their growth (Tsai et al., 2016). Exceptions to this are the methods provided by Pfau and Sciulli (1994), which rely on a sample of radiographs from deceased children collected at a medico-legal institution. While these authors did not explicitly address the notion of forensic mortality bias discussed here, the unintended consequence of their data collection process is that it provided one of the few age estimation standards that may more closely represent the exact subset of the population actually studied by forensic anthropologists in their daily work. Another set of exceptions to the use of "healthy" children are the methods using historical identified skeletal collections (e.g., Facchini and Veschi, 2004; Rissech et al., 2012; Cardoso et al., 2014). These methods rely on samples of known-sex-and-age children who lived in Western Europe from the 18th to early 20th century. While some have argued that these methods are inappropriate for modern forensic casework (Stull et al., 2014), others have contended that they represent more appropriate methods for child forensic anthropology casework in this part of the world due to the geographic proximity of the reference sample (Rissech et al., 2012). However, the skeletal collections represent children living under adverse environmental conditions from the 18th to 20th centuries, predating major secular increases in body size (Cole, 2003) and including children who were likely chronically malnourished and exposed to high rates of infectious disease (Cardoso et al., 2014), when compared to living European populations, and perhaps also to modern European forensic samples. As a consequence, they may be more appropriate to archaeological samples and forensic case work in developing nations (Cardoso et al., 2014).

This paper further explores the problem that forensic anthropology case work is not a random representation of the living population, and which we have termed forensic mortality bias. Our examination of the nature and magnitude of forensic mortality bias is done by comparing diaphyseal length for age between children dying of accidental deaths and those victims

of homicides. Homicide victims were taken as proxies for the forensic anthropology casework sample and children dying of accidental deaths are taken as proxies for the living population. While this is not a perfect comparison, it relies on two assumptions. The first is that homicides are the most frequent manner of death involving child death investigations and thus homicide victims comprise the bulk of forensic anthropology examinations. The second is that accidental deaths are less frequently examined by forensic anthropologists, and they are independent from the social factors that impact growth and development and increase the chance of dying from homicide (they are more dependent on random and unexpected events). As such they are a good proxy for the living population, in the absence of an actual sample of surviving peers. The forensic biological mortality bias revealed in this paper by comparing the growth status of the homicide and accident victims represents a measure of the expected bias in the estimation of age of children whose deaths are under medicolegal investigation, when they are estimated from methods using reference samples extracted from the general living population.

MATERIALS AND METHODS

Full-body post mortem computed tomography (PMCT) scans of children aged 12 and under at death were obtained from two institutions. The first was the Office of the Medical Investigator (OMI), New Mexico, USA and the second was the Victorian Institute of Forensic Medicine (VIFM), Victoria, Australia. For both data sources, scan reconstruction settings were 1.0 mm slice thickness with 0.5 slice spacing or better.

Deaths occurring from both accidental sources and homicides were targeted. These were taken as proxies for a cross section of the population (accidents) and for a forensic sample (homicides). The final sample was comprised of 188 individuals. Of these, 101 were from the OMI (70 accidents and 31 homicides) and 87 were from the VIFM (61 accidents and 26 homicides). The composition of the sample by source institution, manner of death, sex, and age is available in Fig. 2. Maximum diaphyseal lengths were collected from each of the long bones (humerus, radius, ulna, femur, tibia, and fibula). Measurement was performed according to a previously published protocol which has been shown to provide reliable measurements that accurately replicate standard anthropological techniques (Spake et al., 2020).

The samples were first tested for differences in long bone length for age between them. This was performed using an ANCOVA for diaphyseal length, taking into consideration source institution, sex, and the interaction between these two factors. The ANCOVA analysis was performed separately for the accidental death group and for the homicide group in order to ensure that the source samples were consistent in their growth patterns in each of the manner of death groups. ANCOVA analysis requires a linear relationship between the predictor and response variables. However, growth does not proceed as a linear process across the age range of interest here. Growth shows a greater velocity until around 2 years of age, after which it slows down and remains approximately constant till puberty (Humphrey, 2003). Thus, the sample was split into two age groups, under 2 years at death and over 2 years at death, and ANCOVA analysis was performed for these groups separately. ANCOVA assumptions were checked using Levene's tests for homogeneity of variances and by building a second model including interactions between the predictors as a test of equality of slopes.

Based on the results of this analysis, it was decided to conduct the remainder of the analysis for the source institutions separately. To compare growth between the manner of death groups, another set of ANCOVA analysis were run, this time comparing diaphyseal lengths while taking into consideration manner of death, sex, and the interaction between these two factors. The ANCOVAs were again conducted for the under and over 2-year-old groups separately, and assumptions were checked as outlined above.

To quantify and illustrate the differences revealed in the ANCOVA analysis, the manner of death groups was also compared through z-scores. These were calculated using modified values of the Maresh (1943; 1955; 1970) reference data. The sex-specific mean and mean-plus-one

standard deviation of femur length for age were first corrected for radiographic magnification (Cardoso, 2005). Then, fourth order polynomials were fit to the data and used to calculate sex-specific mean and standard deviation values in one-month intervals from birth through 12 years of age (Spake and Cardoso, 2021). The resulting values were used to calculate z-scores for femur length for each child, rounding subject age to the last completed month.

Lastly, in previous analyses, we have found that children who die of accidental causes tend to be short for age relative to the reference mean height (Spake and Cardoso, 2018a, b), and thus may not be the best proxy for the general non-forensic population. To test whether this was the case here, we converted cadaver length into estimated stature for accidental death victims aged 2 and over using a regression formula (Krishan and Sharma, 2002). Children under 2 years of age are typically measured in a recumbent position, and reference values for this age group are given in recumbent lengths rather than standing height (stature). Z-scores for recumbent length or estimated stature were calculated using the CDC-2000 (Kuczmarski et al., 2000) reference value, which is the standard international growth reference in use in Western nations today. Resulting z-scores more than ±6 z-score units from the mean were excluded, as these extreme values are considered likely data entry errors (WHO Multicenter Growth Reference Study Group, 2006). Z-scores were compared to the reference mean using a one sample t-test supplemented by Cohen's d for effect size (Smith, 2018).

RESULTS

In the ANCOVA used to pool the samples, the accidental death group appeared to be similar in long bone length for age across the OMI and VIFM samples. The source institutions differed in long bone length for age in only one bone,

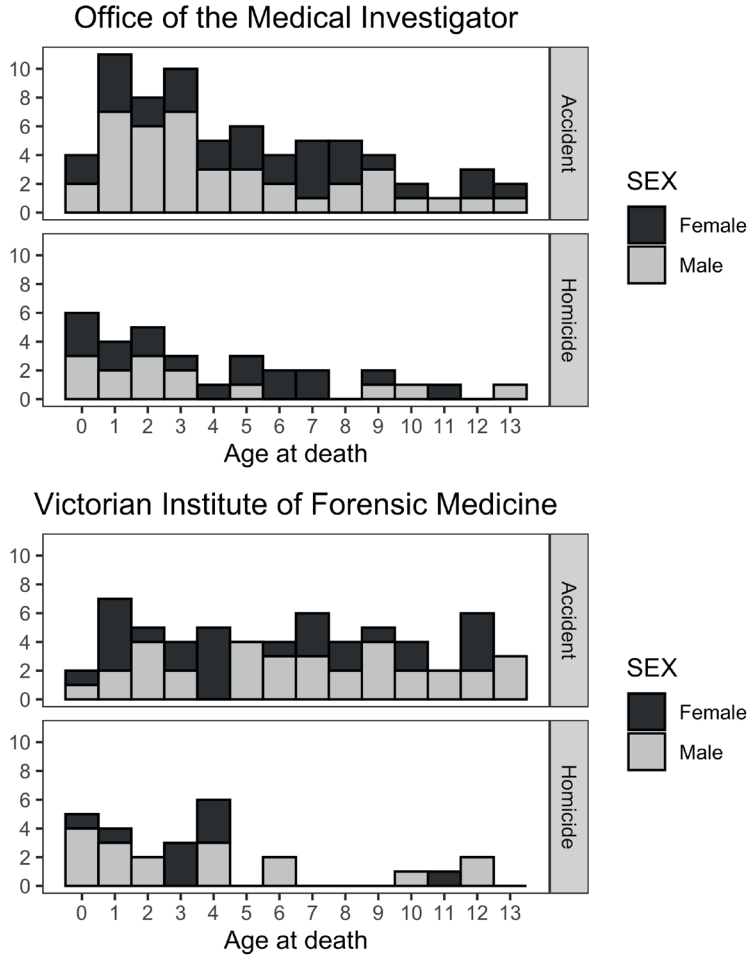

Fig. 2.- Age distribution of the sample by each source institution, manner of death, and sex.

namely the femur, and only in the 2 years and over group (Table 2). However, in the homicide group, differences between the source institutions were apparent. In the under 2 years at death group, there were differences between the OMI and VIFM in length for age of the humerus, femur, tibia, and fibula (Table 3). These differences did not extend into the 2 years at death and older category, where the source institutions did not differ in any bone length for age. However, because of the difference in the samples noted in the under 2 years of age group in the homicide deaths, it was decided to keep the source institutions separate for the remainder of the analysis.

In the ANCOVA used to compare the manner of death groups, differences between accident and homicide victims were noted in the OMI children, but not in the VIFM children. For children under 2 years at death, the OMI group showed significant differences between the manner of death groups for every bone (Table 4). However, in three of the six bones (humerus, femur, and tibia), Levene's tests were also significant, indicating heterogeneity in the variances of the groups. In this age group, the VIFM sample showed no difference between the manner of death groups. For children above 2 years at death, neither the OMI nor the VIFM samples showed differences between the manner of death groups (Table 5). This result was consistent with the results of the first set of ANCOVAs, where differences between the source institutions were detected in the under 2 years at death homicide victims.

Mean and standard deviations of z-scores for the femur, for the manner of death groups in each of the source institutions and the sources pooled are available in Table 6. These values clarify the direction of differences revealed in the ANCOVA analysis. Generally speaking, homicide victims have shorter bone length for age than accident victims. This analysis also revealed small bone length for age in accident victims at the OMI. While accident victims in the under 2-year age group in the OMI had a mean z-score of 1.07 (compared to 1.07 for the same group in the VIFM sample), accident victims over the age

Table 2. Results of the ANCOVA analysis to pool the two samples for the accidental deaths.

	<2 years					>= 2 years				
Bone	**N OMI**	**N VIFM**	**Effect**	**F**	**p**	**N OMI**	**N VIFM**	**Effect**	**F**	**p**
Humerus	19	10	Sample	0.02	0.86	48	42	Sample	1.53	0.22
			Sex	1.37	0.25			Sex	0.02	0.88
			Int.	3.24	0.08			Int.	2.6	0.11
Radius	19	10	Sample	0.63	0.44	50	46	Sample	0.40	0.53
			Sex	0.29	0.60			Sex	5.84	0.02
			Int.	3.63	0.07			Int.	2.78	0.10
Ulna	19	10	Sample	0.32	0.58	50	45	Sample	0.42	0.52
			Sex	1.03	0.32			Sex	2.38	0.13
			Int.	6.33	0.02			Int.	2.39	0.13
Femur	18	10	Sample	0.11	0.75	47	45	Sample	5.18	0.03
			Sex	2.84	0.11			Sex	0.05	0.82
			Int.	2.18	0.15			Int.	2.00	0.16
Tibia	19	10	Sample	0.32	0.58	48	49	Sample	1.22	0.27
			Sex	1.27	0.27			Sex	0.09	0.77
			Int.	4.03	0.06			Int.	3.05	0.08
Fibula	19	10	Sample	0.92	0.35	48	49	Sample	1.62	0.21
			Sex	1.47	0.24			Sex	0.00	0.99
			Int.	2.84	0.11			Int.	1.56	0.21

of 2 years had a mean z-score of 0.36 (compared to 0.96 for the same group in the VIFM sample). This suggested a potential sampling bias towards smaller individuals in children older than 2 years of age in the OMI sample.

When investigating the representativeness of the accidental death sample, age dependent trends once again emerged. For the OMI, children under 2 years were somewhat larger than the reference population mean, while children from VIFM were smaller than expected (Table 7). In this age category, mean cadaver length for age for the VIFM children was well below expected (mean z-score of -1.26). However, this was likely due to a small number of individuals in this group, and therefore the large influence of a single particularly small for age individual (see Fig. 3). Children over 2 years at death were quite similar between VIFM and the OMI, and were significantly smaller than the reference sample (mean z-score of -0.34). This suggests that the accidental death victims in the over-2-year age group were not ideal proxies for the general population of healthy children.

DISCUSSION

This study reveals that there is a forensic mortality bias in long bone length for age whereby homicide victims are delayed relative to accidental death victims. This difference is strong in children under 2 years of age, and smaller in children over 2 years of age. However, this study potentially underestimates the mortality bias effect in children over 2 years of age, as the accidental death victims taken to represent a healthy cross section of the population is significantly smaller for age than expected as measured by growth standards developed on healthy US children. Thus, although the forensic mortality bias effect was most strongly detectable in younger children, it may also exist in older children, and is likely to be greater than that shown here.

The magnitude of forensic mortality bias is

Table 3. Results of the ANCOVA analysis to pool the two samples, for the homicides.

	<2 years					>= 2 years				
Bone	**N OMI**	**N VIFM**	**Effect**	**F**	**p**	**N OMI**	**N VIFM**	**Effect**	**F**	**p**
Humerus	13	11	Sample	5.64	0.03	17	11	Sample	5.77	0.02
			Sex	0.02	0.89			Sex	0.02	0.90
			Int.	0.58	0.46			Int.	0.12	0.74
Radius	13	10	Sample	4.14	0.06	18	11	Sample	1.88	0.18
			Sex	1.27	0.28			Sex	0.01	0.91
			Int.	0.59	0.45			Int.	2.09	0.16
Ulna	13	10	Sample	3.32	0.08	18	10	Sample	1.82	0.19
			Sex	1.04	0.32			Sex	0.03	0.87
			Int.	0.74	0.40			Int.	2.01	0.17
Femur	13	10	Sample	5.52	0.03	16	13	Sample	3.67	0.07
			Sex	0.04	0.84			Sex	1.99	0.17
			Int.	0.26	0.62			Int.	1.96	0.17
Tibia	13	10	Sample	5.86	0.03	17	11	Sample	0.16	0.69
			Sex	0.12	0.73			Sex	0.29	0.60
			Int.	0.36	0.55			Int.	3.17	0.09
Fibula	13	10	Sample	6.07	0.02	17	11	Sample	1.03	0.32
			Sex	0.10	0.75			Sex*	1.13	0.30
			Int.	0.35	0.56			Int.	0.00	0.97

*For this predictor, the groups showed significantly different slopes ($p<0.05$) when themodel was built with interaction between this term and age

Table 4. Results of the ANCOVA analysis comparing the manner of deaths FOR THE <2 YEARS CATEGORY.

		OMI				VIFM			
Bone	**N Accident**	**N**	**Effect**	**F**	**p**	**N**	**Effect**	**F**	**p**
Humerus	29	10	MOD !	13.47	<0.01	11	MOD	0.34	0.56
			Sex	1.35	0.25		Sex	0.20	0.66
			Int.	0.04	0.84		Int.	1.01	0.32
Radius	29	13	MOD	5.11	0.03	10	MOD	0.56	0.46
			Sex	0.05	0.82		Sex	0.08	0.78
			Int.	1.09	0.30		Int.	1.73	0.20
Ulna	29	13	MOD	6.77	0.01	10	MOD	0.48	0.49
			Sex	0.04	0.84		Sex	0.01	0.94
			Int.	1.52	0.23		Int.	2.57	0.12
Femur	28	13	MOD !	9.22	0.00	10	MOD	0.23	0.64
			Sex	0.04	0.84		Sex	1.12	0.30
			Int.	0.26	0.62		Int.	0.79	0.38
Tibia	29	13	MOD !	14.00	<0.01	10	MOD	0.17	0.69
			Sex	0.46	0.50		Sex	0.12	0.73
			Int.	0.65	0.42		Int.	1.05	0.31
Fibula	29	13	MOD	15.74	<0.00	10	MOD	0.19	0.67
			Sex	0.58	0.45		Sex	0.13	0.73
			Int.	0.53	0.47		Int.	0.98	0.33

*For this predictor, the groups showed significantly different slopes ($p<0.05$) when themodel was built with interaction between this term and age ! Levene's tests showed heterogeneity in variances ($p<0.05$)

Table 5. Results of the ANCOVA analysis comparing the manner of deaths FOR THE >= 2 YEARS CATEGORY.

		OMI				VIFM			
Bone	**N Accident**	**N**	**Effect**	**F**	**p**	**N**	**Effect**	**F**	**p**
Humerus	90	17	MOD	2.37	0.13	11	MOD	1.56	0.22
			Sex	0.04	0.85		Sex	0.00	0.99
			Int.	0.05	0.83		Int.	0.03	0.86
Radius	96	18	MOD	0.51	0.48	11	MOD	1.62	0.21
			Sex	3.34	0.07		Sex	7.21	0.01
			Int.	3.36	0.07		Int.	0.47	0.49
Ulna	95	18	MOD	0.51	0.48	10	MOD	1.59	0.21
			Sex	1.18	0.28		Sex	3.47	0.07
			Int.	2.09	0.15		Int.	0.77	0.38
Femur	92	16	MOD	0.77	0.38	13	MOD	1.06	0.31
			Sex	0.42	0.52		Sex	0.01	0.92
			Int.	1.99	0.16		Int.	0.24	0.62
Tibia	97	17	MOD	0.43	0.51	11	MOD	0.00	0.97
			Sex	0.07	0.80		Sex	0.49	0.48
			Int.	2.24	0.14		Int.	1.09	0.30
Fibula	97	17	MOD	3.30	0.07	11	MOD	0.04	0.84
			Sex	0.00	0.97		Sex	0.14	0.71
			Int.	0.07	0.79		Int.	0.79	0.38

Table 6. Number of individuals (N) and mean and standard deviation (SD) for femur length z-scores for each of the manner of deaths. Values are calculated for the age categories separately, and for each collection and for the total sample.

	< 2 years			>= 2 years		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Samples combined						
Accident	28	1.07	1.12	87	0.66	1.44
Homicide	23	0.51	1.46	28	0.54	1.36
OMI only						
Accident	18	1.07	1.12	44	0.36	1.13
Homicide	13	-0.04	0.98	15	0.15	1.06
VIFM only						
Accident	10	1.07	1.16	43	0.96	1.66
Homicide	10	1.22	1.72	13	0.99	1.56

also dependent on the socioeconomic context under which the forensic anthropology expert is working, as illustrated in the difference of forensic mortality bias between the samples. In the US sample (from the OMI), children showed greater mortality bias effect than the children in the Australian sample (from VIFM). This was not entirely surprising, as previous analyses have also documented this difference in forensic mortality bias between Australian and US children (Spake

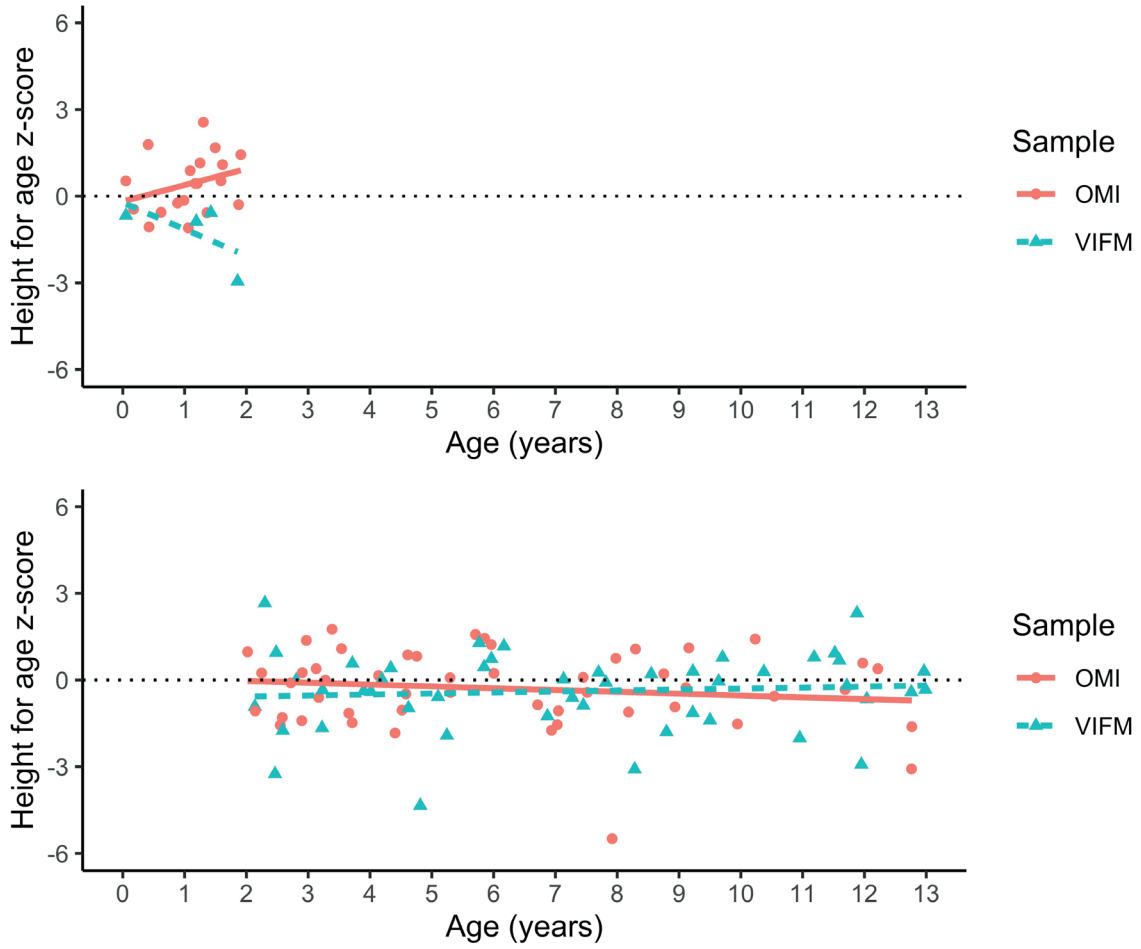

Fig. 3.- Comparison of cadaver length/stature for age z-scores of the accidental death victims in each of the samples, shown separately for children under and above 2 years of age.

and Cardoso, 2018a, b). This difference between the two settings is likely due to a difference in socioeconomic inequality between the settings. Australia is a more economically equal country than the United States as measured by the Gini index (0.33 for Australia 0.39 for the US, varying between 0.22 and 0.62 for the countries listed [OECD 2020]). Additionally, health care is more accessible to lower socioeconomic status groups in Australia as compared to the United States (Khanam et al., 2009; Larson and Halfon, 2010). The wider socio-economic difference between children in the United States means that there is greater potential for health and growth inequalities to develop between children. The greater potential for differences in child health and growth to develop means that there is also a greater potential for a mortality bias effect to be reflected in the forensic anthropology sample.

While there is a strong link between socioeconomic status and death due to homicide, accidental death in childhood is also not free of links to socioeconomic status, and therefore to the factors impacting growth outlined above. Accidental death is also known to disproportionately impact individuals from lower socioeconomic status groups (Singh and Kogan, 2007; Hjern and Bremberg, 2002; Cubbin and Smith, 2002; UCL Institute of Health Equity, 2015). This possibility is supported by the finding that the accidental death victims over 2 years of age included in this analysis were significantly smaller than the US standard (Table 6). This confirms that accidental deaths are not truly representative of a healthy cross section of the population. The forensic mortality bias relative to the living population is therefore likely to be greater than this study reports. If this is the case, this analysis underestimates the true impact of developing age estimation methods based on healthy children for use on a forensic anthropology sample.

Because of the "racial" diversity of the samples, particularly the one from the US, some concerns may be raised about whether the socioeconomically disadvantaged group represented by the homicide sub-sample is comprised largely of individuals of a specific "race", and thus be more representative of "race"-specific growth and development patterns. However, racial classifications are unreliable genetic markers at best, and environmental factors related to poverty or environmental stress have been shown to account for almost all of the differences in growth between populations of the world identified as "races" (Frongillo and Hanson, 1995; Graitcer and Gentry, 1981; Habicht et al., 1974; Martorell, 2010; Ulijaszek, 1994). As a consequence, the findings obtained here are likely to be observed in other populations and contexts, regardless of their "racial" composition. If the homicide sub-sample is indeed comprised largely of individuals of a specific "race", then this results in the homicide sub-sample being different due to the socioeconomic impacts of segregation and

Table 7. Comparison of survivor cadaver lengths to the CDC-2000 reference.

	N	Mean	SD	T	P	d
Samples combined						
< 2 years	23	0.13	1.21	0.53	0.60	0.11
>= 2 years	97	-0.34	1.35	-2.50	0.01	0.25
Total	**120**	**-0.25**	**1.33**	**-207**	**0.04**	**0.19**
OMI only						
< 2 years	19	0.43	1.02	1.82	0.08	0.42
>= 2 years	50	-0.30	1.33	-1.59	0.12	0.22
Total	**69**	**-0.10**	**1.29**	**-0.64**	**0.53**	**0.08**
VIFM only						
< 2 years	4	-1.26	1.13	-2.24	0.11	1.12
>= 2 years	47	-0.39	1.38	-1.95	0.06	0.28
Total	**51**	**-0.46**	**1.37**	**-2.38**	**0.02**	**0.33**

discrimination by "race", and not because of any presumed biological differences due to "race".

To illustrate the impact of forensic mortality bias on developing age estimation methods we use of the difference in bone length between the two manner of death groups. The difference in the z-scores can be manipulated to provide estimates of mean differences in age estimates between the two groups. The OMI children were used to do this as they revealed the greatest differences between the manner of death groups. The difference between the mean z-scores of each manner of death group were calculated for the <2 year olds (-1.01 z-units) and for the >2 year olds (-0.21 z-units). The z-unit differences were then used with the Maresh reference data (Spake and Cardoso in prep) to calculate the months of growth represented by the difference in mean z-score at various points of the growth trajectory: 1 year, 2 years, 7 years and 12 years. This was done by calculating the delay in femur length represented by the mean z-unit at each of those ages, subtracting this difference from the mean length for that age, and then matching the resulting bone length to the reference to find the age at which it most closely matched the mean bone length. The difference between the original and resulting ages therefore represented an estimate of the impact of forensic mortality bias on skeletal age estimation of the forensic anthropology sample.

Throughout the entirety of the growth period, the impact was estimated to range from 1 to 4 months of growth. Although the z-score difference was larger in children <2 years of age, the high growth velocity in this period and the smaller standard deviations at those ages means that the larger difference in z-score translates to a similar difference in months of growth. This is a relatively small age difference when considering that age estimation methods have 95% prediction intervals that can range up to ± 40 months at 12 years of age (Stull et al., 2014; Cardoso et al., 2014). On the other hand, at birth the same methods have 95% prediction intervals of about ± 1 month, which results is a much larger difference in age. Once again, this difference should be considered a conservative estimate of the forensic biological mortality bias effect as the accident victims were noted to be smaller for age than expected in a healthy cross-section of the population. Additionally, while 4 months might not seem important for one particular older individual, the difference represents a shift of the population mean for that group.

Any shift of the population mean results in more individuals being mis-aged than the 5% allowed by the 95% prediction interval. Specifically, it would result in a greater chance of individuals falling below the lower bound of the 95% prediction interval because their age has been underestimated. And the younger the individual the greater the chance of underestimating the age, and the true age falling outside of the 95% prediction interval. Thus, the difference of 4 months is forensically relevant in that it could impact confidence in forensic methods, which is important in the context of the Daubert standards (Christensen and Crowder, 2009).

A previous similar analysis of growth differences in linear growth measured by cadaver length revealed a difference between the general population and the forensic anthropology sample of 3 to 8 months of growth in children aged between 3 and 7 years at death (Spake and Cardoso, 2018a, b). Similar to this analysis, the difference was important in children from the US, but was inconsistent and unimportant in children from Australia and New Zealand. The smaller impact on age estimation reflected in this analysis could stem either from the use of long bones, whereby the differences in cadaver lengths were distributed across the skeleton, or by the fact that smaller sample sizes were available for this study.

Another interesting outcome of this study was that the comparison between the Australian and US children revealed that overall, accident victims and homicide victims over the age of 2 years were quite similar in their growth for age patterns. The similarity between the samples could be considered somewhat surprising, because growth is known to vary considerably across populations. In fact, the variability of populations in terms of their growth has prompted an explosion of calls to create population-specific age estimation methods, particularly in forensic settings (Ubelaker and Khosrowshahi, 2019). The

similarity of these two samples suggests that what is needed is not necessarily population-specific references, but rather growth-appropriate ones. The results of the ANCOVA analysis used here to compare the samples, for example, suggests that Australian and U.S. children display similar growth trajectories. In turn, this means that age estimation methods intended for use in Australia could be appropriate for use in the U.S. and vice versa. If forensic anthropologists continue to rely on population-specific methods as they have traditionally been defined, methods may fail more often than expected because of forensic mortality bias. Instead, forensic anthropologists should rely on growth-appropriate methods, selecting or developing methods based on the knowledge of their casework, keeping in mind the filters detailed in Fig. 1.

In order to develop better age estimation methods and decide which methods are appropriate for a specific context it is paramount to answer the question of who exactly is represented in the forensic anthropology sample? While this is a relatively poorly understood question, particularly for juvenile individuals, and deserves further research, we can provide some insights based on our data. In the VIFM sample included here, several individuals were examined by a forensic anthropologist or odontologist in order to identify the individuals. These cases included homicides (64%) and accidents (36%), and often included multiple child fatalities where there was a need to differentiate between children. Visual analysis of these cases suggested that that VIFM, juvenile forensic anthropological casework is not affected by forensic mortality bias, consistent with the analysis above (Fig. 4). The Forensic Anthropology Data Bank (FDB) curated at the University of Tennessee, Knoxville, has five forensic anthropology cases where the manner of death is indicated. Of these, 4 are homicides (80%) and 1 is a natural death (20%). A review of cases brought to the Smithsonian for forensic anthropological examination found that nearly 25% of cases involved a subject who was under 18 years at death (Grisbaum and Ubelaker, 2001). When comparing the OMI children versus those incorporated in the FDB and several individuals housed at the Maxwell Museum (all considered forensic cases in the US), there is a greater forensic mortality bias exhibited than in the Australian children (Fig. 4). The few published case studies of forensic anthropological casework involving juveniles suggests that these are predominantly cases of homicides of which a large proportion involve abuse and neglect of the child prior to death (Table 1). Additionally, forensic anthropologists encounter children in their casework when contributing to humanitarian work including both disaster victim identification (Bassed and Hill, 2011) and mass graves in the context of human rights violations (Lewis and Flavel, 2006; Simmons, 2007; Ansom and Trimble, 2008; Doretti and Snow, 2009; Roberts, 2011). If forensic anthropology case work includes a large proportion of homicides, but also children from marginalized groups as happens in situations of human rights violations, the development and application of age estimation methods which rely on samples of normal healthy children, will result in biases because of the link between homicide and socioeconomic status.

Forensic mortality bias likely exists in children because of complex links between socioeconomic factors, risk of certain types of mortality, and growth. In high-income nations such as Australia and the United States, violence against children and overall child mortality disproportionately affect lower socioeconomic status individuals (Singh and Kogan, 2007; Sidebotham et al., 2014; Ross and Juarez, 2014). More specifically, homicides are known to disproportionately impact children from socially marginalized or economically disadvantaged groups (Nixon, 1981; Christoffel, 1990; Finkelhor, 1997; Finklehor and Omrod, 2001; Hjern and Bremberg, 2002; Bennett et al., 2006; Douglas and Vanderminden, 2014; Shields et al., 2014). Low socioeconomic status is linked with factors that negatively impact growth, which include: inadequate or insecure nutrition, greater exposure to disease, inconsistent access to health care, poor housing quality, chronic psychosocial stress, and of course poverty (Bradley and Corwyn, 2002; Fernald and Granthan-McGregor, 2002; Block and Krebs, 2005; Chilton et al., 2007; Gilbert et al., 2009; Schell et al., 2009; Norgan

et al., 2012). Plainly speaking, the children that are most likely to become victims of homicide are also children who have experienced negative health and growth experiences throughout their childhood. It is also likely that some portion of the homicide cases include children who have been abused and neglected (Nixon et al., 1981; Christoffel et al., 1990; Bennett et al., 2006), which is widely known to impact or even halt growth (Adelson, 1963; King and Taitz, 1988; Karp et al., 1989; Skuse, 1989; Wyatt et al., 1997; Ellis, 1997; Berkowitz, 2001; Oliván, 2003; Van Ijzendoorn et al., 2007). These experiences are expressed in the delayed growth relative to accidental death victims also found in this study.

While our analysis focused on samples from two high-income countries, both our results and the link between socioeconomic status and homicide rates, suggest that in high income countries the magnitude of forensic mortality bias will increase with the increase in social inequality as exemplified by Fig. 4. However, globally the majority of child homicide victims (90%) live in LMICs, with the highest child homicide rates found in Latin America (UNICEF, 2014). Social inequalities are typically also greatest in these countries, such that there is a strong association between income inequality and international differences in homicide rates in a group of high-and middle-income countries (Elgar and Aitken, 2011). As a consequence, we suggest that forensic mortality bias is small in the most socially equal, high-income countries, such as Australia, but potentially large in LMICs, particularly the most socially unequal of them.

The nature of forensic anthropology casework varies across jurisdictions based on local factors such as social inequality and crime levels (increasing or decreasing the potential

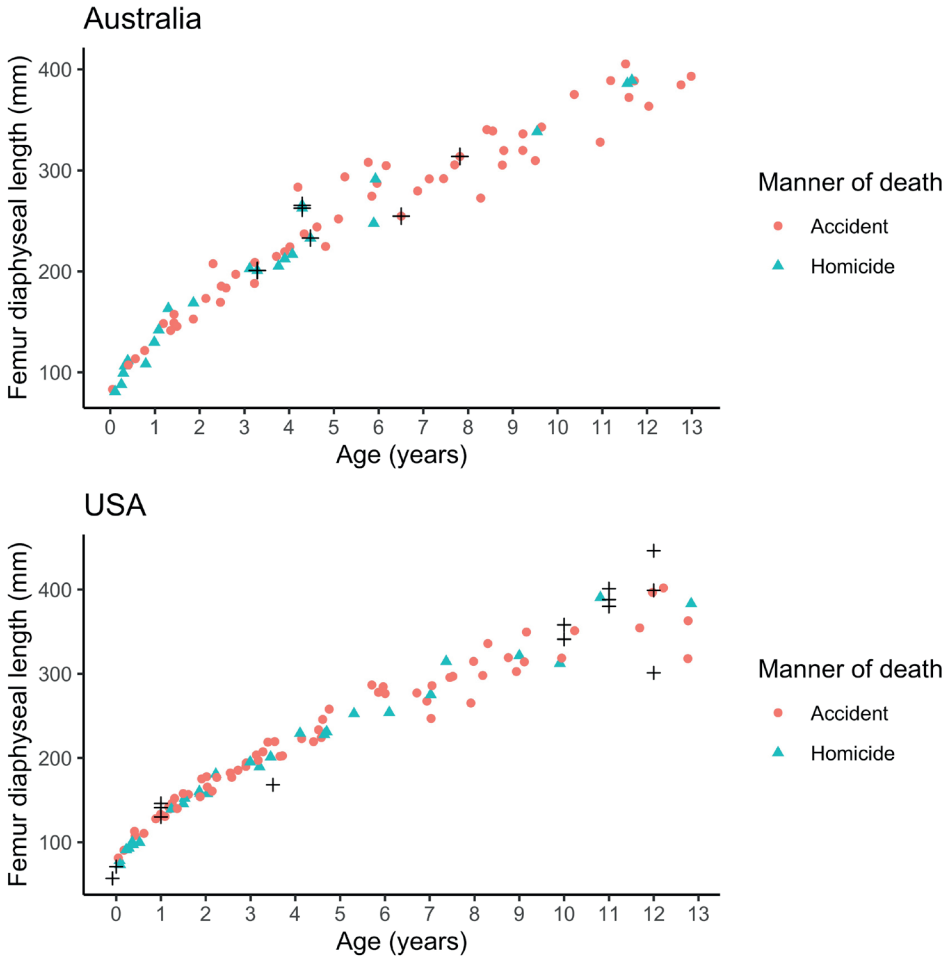

Fig. 4.- Visual exploration of the growth status of children subjects of forensic anthropological assessment (denoted with a cross) versus all children used in this analysis. For the Australian sample, forensic anthropology cases consist of VIFM cases. For the USA sample, forensic anthropology cases are collated from the FDB and the Maxwell Museum.

for forensic anthropological casework to exist), medicolegal procedures (for example, protocols mandating trauma analysis for certain deaths), or funding availability (choosing between no consult versus forensic anthropology or DNA consult, for example). The variation in the type of casework assigned to a forensic anthropologist may correlate with the level of forensic mortality bias that exists in their casework. For example, if a forensic anthropologist's caseload includes a greater proportion of accidents than either homicides or natural deaths, there might be less forensic biological mortality bias present in their casework sample than if the reverse was true. This is because there is less selective filtering of accidental deaths than homicide (stronger social filtering) or natural (stronger biological filtering) deaths. However, the reality is that in many cases it may also be impossible to determine whether a child's death was due to an accident, a homicide or a natural cause. In any circumstance, it is the context in which the expert is working that will inform whether a significant forensic mortality bias is expected. Furthermore, that context will also provide some approximate measure of whether the chosen age estimation methods is appropriate or adequate. Since several methods and approaches rely on sample of normal and healthy children from high income countries, the impact of forensic mortality bias will be exacerbated in forensic casework in LMICs. While there have now been age estimation methods adapted for local growth patterns, the impact of forensic mortality bias may remain, because these methods also tend to rely on samples of normal and healthy children belonging to higher social classes. Other methods, however, rely on samples of deceased children who grew under a variety of environmental stressors and may be appropriate for forensic casework in both high- (Pfau and Sciulli, 1994), low- and middle-income countries (Cardoso et al., 2014).

One way to assess the growth-appropriateness of an age estimation methods is to examine the social background and, if possible, the growth status of the children used to develop the method. For example, the Maresh (1970) data may not provide any reliable or accurate results in casework in higher income countries because of secular trends in growth having occurred over the last 100 years (Duren et al., 2015). We have shown elsewhere that children included in the FDB and Maxwell Museum samples, who represent forensic casework from the 1970s to the 1990s, largely fall within the Maresh 5th and 95th percentiles (Spake and Cardoso, 2018b), but there is no information as to more recent forensic anthropology casework. Ideally, methods should be carefully evaluated against past casework in order to be sure that the methods are growth- appropriate, rather than population or group-specific.

CONCLUSION

This study shows that using samples of healthy individuals is not necessarily the best way to develop juvenile age estimation methods for use in forensic contexts due to forensic mortality bias. This is particularly true for use in low- and middle-income countries, where error is compounded not only by forensic mortality bias, but also by increased socioeconomic inequalities and inter-population differences in growth. This analysis focused on long bone length, and may not extend to other age indicators (especially dental formation and to a lesser extent skeletal maturation) in the same ways that long bone length is affected by forensic mortality bias. However, when developing population-specific methods, practitioners should carefully consider the filters that affect the composition of their forensic casework samples as conceptualized. It is possible that population-specific methods are not necessary for every context, so long as growth-appropriate methods are available and selected. Interpretations of estimated ages based on whatever available methods should also consider the effects of forensic mortality bias that links growth trajectories to socioeconomic factors.

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Sexing subadult individuals: what morphological method is the most appropriate?

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SUMMARY

While a variety of metric and morphological methods have been successfully developed to determine the sex of adult skeletons, the accurate assessment of sex to subadult skeletal material is generally considered problematic. This could be due to different factors such as a possible lack of association between the expression of the traits and sex, subjectivity in the assessment of the stages of each trait, sample-related factors or inter-populational variability in the pattern and degree of sexual dimorphism. Thus, the main goal of this paper is to assess the effect of these factors in the accuracy of the eleven nonmetric subadult sex-related skeletal traits observed in the ilium and in the mandible. Overall, our best results for sex estimations were obtained with mandibular non-metric traits (with the exception of eversion of gonion) up to five years old.

Our findings suggest that besides population differences, sample characteristics such as sex ratio and age coverage differences between reference and target samples have an important effect in the success of sex estimates. This effect is greater in accuracies calculated as rate of success than in weighted probabilities of being a male or a female when a particular trait is observed. Thus, we recommended use weighted probabilities to assess the validity of non- metric traits for sexing subadult individuals.

Keywords: Sex estimation – Subadults – Ilium – Mandible – Non-metric traits

INTRODUCTION

Sex determination is a crucial point of both bioarchaeological and forensic fields, even more when subjects under study are subadults. The most important implication of an accurate and reliable assignment of sex in immature individuals is that it generally increases the precision in the estimation of age at death. Moreover, in bioarchaeological research, the reconstruction of sex-specific child mortality and morbidity rates, sex-specific growth patterns and the construction of demographic profile depend on the ability of sex determination in subadult individuals (Cardoso, 2008).

Because sex estimations in adults using skull and pelvis yield accuracies of 98-100% (Acsádi et al., 1970; Phenice, 1969; Washburn, 1948), physical anthropologists investigated these two skeletal regions for a reliable method for sexing subadult skeletons. These can be sorted as metric and non-

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metric, or morphological, methodologies. Several metric methods have been developed with high rates of success in sex classification of subadult individuals (Campo et al., 2018; Coquerelle et al., 2011; O'Donnell et al., 2017; Rissech et al., 2003; Rissech and Malgosa, 2007, 2005; Stull et al., 2017; Stull and Godde, 2013; Wilson et al., 2011, 2008).

These metric methods possess benefits in comparison with their morphological counterparts, such as their alleged objectivity, because they rely on standard landmarks and result in lower levels of intra- or inter-observer error (Rogers, 2005). However, they also suffer from two major limitations: a) they are difficult to apply on fragmentary material, and b) population differences in bone measurements can alter the results. For these reasons, non-metric or morphological methods have traditionally enjoyed greater acceptance than metric methods in bioarcheology and forensic anthropology.

Weaver (1980) was the first to introduce a non-metric criterion for diagnosis of the sex of immature skeletons. He studied 153 foetal ilia from the Smithsonian Institute, collected by Hrdlicka from numerous sources between 1902 and 1917. Age and sex were recorded for each skeleton. Weaver (1980) proposed a raised versus non-raised auricular surface nonmetric trait. He found that this nonmetric trait sexed his foetal sample at a 75% (female) and 92% (male) accuracy. Later, Schutkowski (1993) proposed seven non-metric traits of the ilia and, for the first time, traits of mandible for sexing 61 known age and sex subadult remains from the Christ Church Cemetery of Spitalfields (London). The seven characteristics proposed by Schutkowski (1993) include the angle of the greater sciatic notch, depth of the greater sciatic notch, curvature of the ilia when observed superiorly and the arch criteria for the ilia and gonial eversion, protrusion of the chin and anterior dental arcade shape as traits that could be used to the mandible. All these traits were classified in two states to distinguish between female and male subadults remains. Schutkowski (1993) did no test the accuracy of each trait, but he reported on the association of each of two possible character states with females and males in the Spitalfields sample. Calculations based on Schutkowski's published data revealed that these traits ranged in accuracy between 54.2 and 95.2% (Sutter, 2003), with traits related to morphology of the greater sciatic notch being the most accurate (Vlak et al., 2008).

Considering evidence of sexual dimorphism in some mandibular traits of juveniles (Schutkowski, 1993) and in some skull features of adults (Acsádi et al., 1970), Molleson et al. (1998) explored the subadult sex-estimation potential of the morphology of the orbit, mandibular angle, and mentum. Based on results obtained in the Spitalfields and the Wharram Percy collections, the authors claimed that sexual dimorphism exits in the facial skeleton of juveniles and, thus, their morphological traits could be useful in sex assignment.

Subsequently, Loth and Henneberg (2001) reported an accuracy of 82% in sex determination of subadult skeletons from the Dart Collection based on shape differences in the anterior body and symphyseal region of mandibles. Following these authors, while female mandibles show a rounded contour with an uninterrupted curve from the lateral to anterior region, in males the lateral body has nearly straight sides which meet the anterior symphyseal region at a sharp angle.

Unfortunately, when these methods have been tested on other known sex subadult samples, it has been shown that they perform poorly overall (Cardoso and Saunders, 2008; Coqueugniot et al., 2002; Mittler and Sheridan, 1992; Scheuer, 2002; Sutter, 2003; Vlak et al., 2008). However, findings about reasons underlying this low performance are inconsistent. While in some cases the poor performance is due to skewed results where accuracy is higher in one sex than in the other, in others it has been showed that the level of accuracy is age-dependent. For instance, Sutter (2003) tested the non-metric traits previously proposed by Weaver (1980) and Schutkowski (1993), showing a clear age-related trend in the accuracy.

Despite of this, he concluded that the arch criteria, sciatic notch angle, depth of the sciatic notch, and mandibular arcade shape reached overall levels of accuracy greater than 75%, which has generally been considered the minimum acceptable level for subadult sex classification, since it is 50% better

than chance (De Vito and Saunders, 1990; DiGangi and Moore, 2012). Sutter's findings on accuracy were largely in agreement with those reported by Schutkowski (1993), indicating a good reliability of these traits for sex determination of sub- adults. Nonetheless, Cardoso and Saunders (2008) and Vlak et al. (2008) did not recommend the application of the arch criteria, greater sciatic notch and, greater sciatic depth for sexing subadult individuals, at least up to 11 years.

These inconsistent findings have been related to inter-populational differences in the pattern and degree of sexual dimorphism along differences in the level of sexual dimorphism in different parts of the skeleton. Yet, besides the population and individual variability as explanations, other factors related to test samples such as age range, an uneven sex distribution, geographical origin and chronology of the test samples may result in the different levels of accuracy obtained. For example, Scheuer (2002) highlighted that sex differences in the sex correct allocation accuracies when Spitalfields juvenile sample is used as test sample may be because it contains a low proportion of females. Thus, it seems that the effect on the levels of accuracy of these sample-related factors is not negligible.

Therefore, the main goal of this study is related to the assessment of the effect of sample-related factors and populational differences in the accuracy obtained when different non-metric traits are applied. Based on this, we tested the 11 non-metric traits previously proposed by Weaver (1980), Schutkowski (1993), Molleson et al. (1998) and Loth and Henneberg (2001) for sexing non-adult individuals using a collection of identified sub-adult skeletons.

MATERIAL AND METHODS

The sample used in this study consisted of 38 ilia (21 males and 17 females) and 56 mandibles (30 males and 26 females) belonging to subadult individuals of known sex and age at death from the Lisbon identified skeletal collection housed at the Bocage Museum, National Museum of Natural History, Lisbon, Portugal. This collection was derived from modern cemetery sources and is formed by Portuguese people who lived in the nineteenth and twentieth centuries representing the middle-to low social class of the city of Lisbon (Cardoso, 2006). In this study, we used only mandibles in which the third molar was unerupted. For ilia, individuals were excluded in those cases in which the ilium had already begun to fuse with the ischium and pubis. The age and sex distribution is shown in Table 1.

The eleven non-metric sex-related traits for mandible and ilium previously proposed by Weaver (1980), Schutkowski (1993), Molleson et al. (1998) and Loth and Henneberg (2001) were evaluated. In Figs. 1 and 2 are depicted the non-metric traits evaluated in the ilium and mandible, respectively. Table 2 show the association of each of the two states of these non-metric traits to one of each sex.

Each scoreable skeletal trait was independently seriated in order of progressive expression for the trait in question (*i.e*, from the more female-like individual to the more male-like) by the first author. Following the scoring of these skeletal nonmetric traits, we tested whether the traits studied are actually related to the sex of the individuals using chi-square analysis and, in cases in which frequencies were lower than five, using Fisher's exact test.

Accuracies were calculated as the success rate in the estimate for each of the sexes and for two sexes together. These accuracies were compared to those obtained in previous studies. In this sense, it is important to note that Loth and Henneberg (2001), Scheuer (2002) and Cardoso and Saunders (2008) reported accuracies obtained by different authors or by the same author in different times. Thus, we only exposed the minimum and maximum values.

Moreover, some of these studies broke down the total sample into four age groups (Schutkowki, 1993; Sutter, 2003; Cardoso and Saunders, 2008; Vlak et al., 2008). For comparative purposes we also established the same age groups.

Although this approach (comparing accuracies calculated as the success rate) has been used by several authors, it has a number of shortcomings for evaluating a method. Many of these limitations were highlighted by Irurita-Olivares and Alemán-Aguilera (2016). For these authors, the most

important caveat of this approach is that these accuracies refer to the probability that an individual of known sex presents a particular trait. Nonetheless, in a forensic context, we must express the probability of being a male or a female when a particular nonmetric trait is observed. Furthermore, this probability must be representative of the actual population or, in other words, the test sample should show a similar sex distribution. In order to take this factor into account, we have also calculated weighted probability using the formulae proposed by Irurita-Olivares and Alemán-Aguilera (2016), which are exposed below:

Table 1. Distribution of age and sex for juvenile skeletons from the Lisbon Collection examined in this study.

Age	Ilium			Mandible		
	Males	Females	Total	Males	Females	Total
0-0.9	2	3	5	0	1	1
1-1.9	4	2	6	6	3	9
2-2.9	2	1	3	2	1	3
3-3.9	0	1	1	0	2	2
4-4.9	2	3	5	2	3	5
5-5.9	1	2	3	1	2	3
6-6.9	1	1	2	1	1	2
7-7.9	1	0	1	1	0	1
8-8.9	0	0	0	1	0	1
9-9.9	1	2	3	1	2	3
10-10.9	1	2	3	0	2	2
11-11.9	2	0	2	2	3	5
12-12.9	1	0	1	1	1	2
13-13.9	1	0	1	1	1	2
14-14.9	2	0	2	1	0	1
15-15.9	0	0	0	2	2	4
16-16.9	0	0	0	4	2	6
17-17.9	0	0	0	4	0	4
Total	**21**	**17**	**38**	**30**	**26**	**56**

Table 2. Nonmetric sex-related traits of the ilium and mandible proposed by Weaver (1980), Schutkowski (1993), Molleson et al. (1998) and Loth and Henneberg (2001).

Source	Trait	Associated expression	
		Males	**Females**
Weaver (1980)	Auricular elevation	Flat	Elevated
Schutkoski (1993)	Sciatic notch angle	≈ 90º	>90º
	Depth of sciatic notch	Deep	Shallow
	Arch criteria	Boardering	Crosses
	Iliac crest curvature	Slight	Marked
	Mandibular protrusion	Protrudes	Smooth
	Mandibular arcade shape	Rectangular	Rounded
	Gonial eversion	Present	Absente
Molleson et al. (1998)	Mandibular angle	Open	Moderated
	Mentum	Outline squared	Outline smooth
Loth and Henneberg (2001)	Sympheal shape	Squared	Rounded

Weighted probability of correctly estimating sex with male features: $A^1/ (A^1+B^1)$ Weighted probability of correctly estimating sex with a female trait: $B^1/ (A^1+B^1)$

Where:

A^1 is the equivalent number of males that would show this trait if the sample had a 50% of each sex. It is calculated as the number of males showing

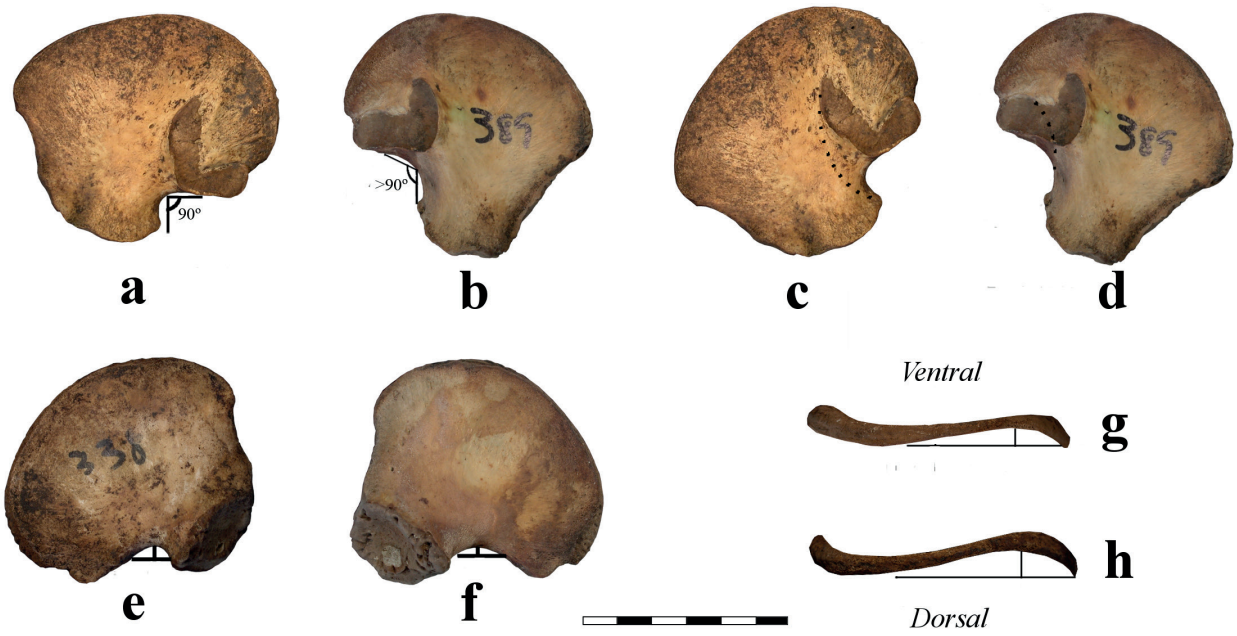

Fig. 1.- Non-metric traits evaluated in the ilium. **a:** angle of greater sciatic notch around 90º, **b:** angle of sciatic notch >90º, **c:** male pattern of arch criterion (line leads into lateral rim of auricular surface), **d:** female pattern of arch criterion (line crosses auricular surface), a and c also show a non-elevated auricular surface, b and d also show an elevated auricular surface, **e:** deep greater sciatic notch, **f:** shallow greater sciatic notch, **g:** marked S-shape of iliac crest, **h:** faint S-shape of iliac crest.

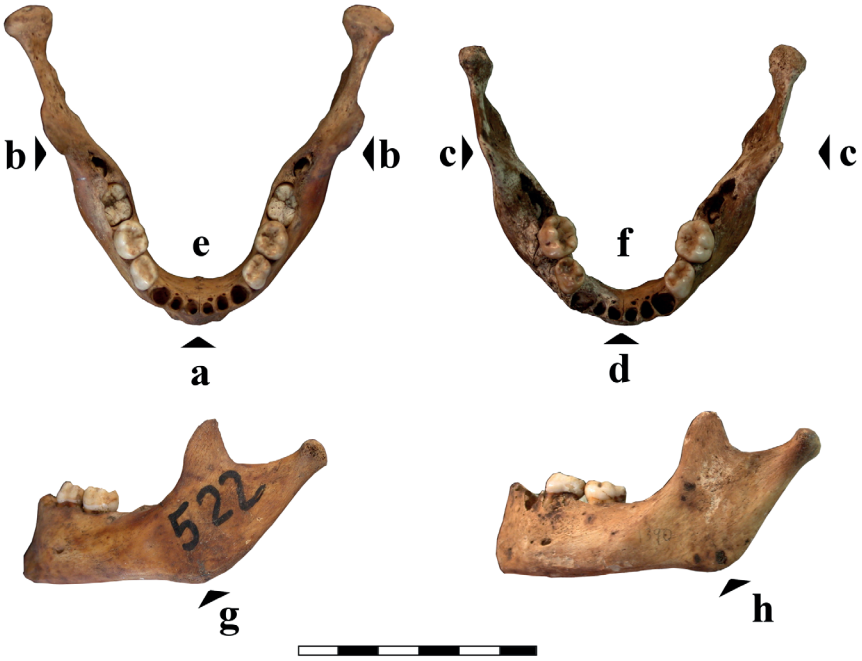

Fig. 2.- Non-metric traits evaluated in the mandible. Top: superior view. Bottom: lateral view. **a:** chin protrudes and the symphyseal region is squared, **b:** eversion of gonion, **c:** non-eversion of gonion, **d:** chin is smooth and the symphyseal region is rounded, **e:** shape of mandibular arcade is rectangular, **f:** shape of mandibular arcade is rounded, **g:** mandibular angle is open, **h:** mandibular angle is moderated.

this trait divide into the percentage of males in the sample and multiplied by 50.

B^1 is the equivalent number of females that would show this trait if the sample had 50% of each sex. It is calculated as the number of females showing this trait divided into the percentage of females in the sample and multiplied by 50.

For comparative purposes, data from studies by Molleson et al. (1998), Loth and Henneberg (2001), Coqueugniot et al. (2002) and Cardoso and Saunders (2008) were weighted according to the sex distribution in the sample. For the same reasons exposed above, we also calculated minimum and maximum weighted probabilities from the data derived from Loth and Henneberg (2001), Coqueugniot et al. (2002) and Cardoso and Saunders (2008).

Data from Schutkowski (1993), Sutter (2003) and Vlack et al. (2008) weighted by Irurita-Olivares and Alemán-Aguilera (2016) and those from the latter authors were also included in this comparison.

RESULTS

In Table 3 are depicted the results obtained for the sexual dimorphism of the non-metric traits studied using chi-square analysis and Fisher's exact test.

When all age groups are considered, none of the non-metric traits of the ilium show significant relationship with sex except the curvature of iliac crest.

In contrast, non-metric traits of the mandible show statistically significant relationship with sex when all age groups are considered. In general, these mandibular traits conformed to the predicted sex-related association in younger age groups.

Table 4 lists the accuracies calculates as success rate of the 11 nonmetric traits evaluated by this study. For comparative purposes, the accuracies previously established for these traits by Weaver (1980), Schutkowski (1993), Molleson et al. (1998), Loth and Henneberg (2001), Coqueugniot et al. (2002), Scheuer (2002), Sutter (2003), Cardoso and Saunders (2008) and Vlak et al. (2008) are also provided.

Table 3. Chi-square and Fisher's exact test results for sexual dimorphism of the nonmetric traits studied. χ2: chi-squared value, OR: odds ratio value in those cases in which we have performed the Fisher's exact test. In bold statistically significant sexual dimorphism.

Trait	0-1			2-5			6-10			>11			All		
	N	χ^2/ *OR*	*p*	*N*	χ^2/ *OR*	*p*	*N*	χ^2/ *OR*	*p*	*N*	χ^2/ *OR*	*p*	*N*	χ^2/ *OR*	*p*
Auricular elevation	4♀+5♂	0.77	1.00	8♀+6♂	1.18	1.00	5♀+4♂	2.60	1.00	---	---	---	17♀+21♂	0.38[2]	0.53
Depth of sciatic notch	4♀+5♂	0.90	0.34	8♀+6♂	1.75	0.18	5♀+4♂	2.68	0.57	---	---	---	17♀+21♂	0.73	0.29
Angle of sciatic notch	4♀+5♂	0.90	0.34	8♀+6♂	1.37	1.00	5♀+4♂	1.00	1.00	---	---	---	17♀+21♂	2.76	0.09
Arch criterium	4♀+5♂	0.90	0.34	8♀+6♂	0.36	0.58	5♀+4♂	1.00	1.00	---	---	---	17♀+21♂	0.12	0.72
Curvature of iliac crest	4♀+3♂	2.10	0.15	6♀+3♂	0.00	1.00	5♀+4♂	8.35	0.21	---	---	---	14♀+15♂	4.21	**0.04**
Protrusion of chin	2♀+6♂	3.43	0.06	10♀+5♂	12.50	0.09	5♀+4♂	8.35	0.21	9♀+15♂	3.70	**0.05**	26♀+30♂	15.88	**0.00**
Shape of dental arcade	2♀+6♂	8.00	**0.00**	10♀+5♂	7.50	**0.00**	5♀+4♂	3.39	0.52	9♀+15♂	3.77	0.20	26♀+30♂	15.67	**0.00**
Eversion of gonion region	2♀+6♂	0.00	1.00	10♀+5♂	5.19	0.25	5♀+4♂	3.21	0.07	9♀+15♂	2.88	0.09	26♀+30♂	4.40	**0.04**
Mandibular angle	2♀+6♂	8.00	**0.00**	10♀+5♂	4.61	**0.03**	5♀+4♂	3.38	0.52	9♀+15♂	3.79	**0.05**	26♀+30♂	12.28	**0.00**
Mentum	2♀+6♂	8.00	**0.00**	10♀+5♂	7.35	**0.00**	5♀+4♂	3.39	0.20	9♀+15♂	6.99	**0.00**	26♀+30♂	16.33	**0.00**
Symphyseal region	2♀+6♂	0.97	**0.03**	10♀+5♂	5.00	**0.02**	5♀+4♂	3.76	0.52	9♀+15♂	1.14	0.28	26♀+30♂	11.88	**0.00**

Table 4. Comparisons of total (%T), female (%F) and male (%M) accuracies for each of the non-metric traits, broken down by age groups. Accuracies were calculated as success rate.

Trait	Study	Age and sex groups														
		0-1			2-5			6-10			11-15			Overall		
		%F	%M	%T	%F	%M	%T	%F	%M	%T	%F	%M	%T	%F	%M	%T
Auricular	This study	60	17	36	29	80	50	60	75	67	...	67	67	47	57	53
elevation	Weaver (1980)	58	87	73	...	...	...	...	...	...	...	...	...	58	87	73
	Sutter (2003)	43	67	54	80	100	92	50	100	82	71	59	62	61	78	72
	Mittler and Sheridan (1992)	...	...	...	...	...	...	...	...	...	...	...	...	58	85	74
Greater	This study	100	17	82	71	0	42	40	75	55	...	83	83	71	43	55
sciatic notch	Schutkowski (1993)	55	82	68	88	89	87	...	...	...	...	...	...	68	89	80
depth	Sutter (2003)	86	50	69	100	85	93	25	100	75	29	100	79	64	89	79
	Vlack et al. (2008)	100	44	69	25	50	39	20	100	67	...	100	70	43	70	59
Greater	This study	100	17	54	86	20	58	60	50	55		83	83	82	43	60
sciatic notch	Schutkowski (1993)	100	55	77	90	82	85	...	...	...	...	...	...	95	71	82
angle	Sutter (2003)	71	50	62	86	86	86	25	100	79	71	100	91	68	89	81
	Vlack et al. (2008)	100	22	56	38	40	39	20	71	50	33	100	80	52	54	54
Arch	This study	100	17	54	57	20	42	80	25	55	...	50	50	82	29	50
criteria	Schutkowski (1993)	70	82	76	50	81	69	...	...	...	...	...	...	60	81	72
	Sutter (2003)	71	50	61	100	100	100	25	100	79	25	100	84	68	92	82
	Cardoso and Saunders (2008)	23/85	18/35	32/47	40/70	25/44	42/47	20/64	22/78	36/55	0/50	22/100	33/67	40/70	26/51	39/46
Iliac	This study	60	75	67	80	0	57	75	100	86	...	67	67	71	67	80
crest	Schutkowski (1993)	80	40	60	91	64	76	...	...	...	...	...	...	86	54	69
curvature	Sutter (2003)	86	0	46	100	71	86	33	80	69	29	87	69	68	70	69
Protrusion	This study	75	100	90	75	80	77	71	75	73	45	73	62	63	80	72
of chin	Schutkowski (1993)	100	61	74	86	57	67	...	...	...	...	...	...	92	59	80
	Sutter (2003)	22	100	72	28	100	74	60	100	82	43	80	68	36	96	74
Shape of dental	This study	100	100	100	100	60	85	71	50	64	55	53	54	77	63	70
arcade	Schutkowski (1993)	50	67	61	86	79	81	...	...	...	...	...	...	69	73	72
	Sutter (2003)	11	100	68	57	92	79	80	83	82	86	80	82	54	92	78
Eversion	This study	100	0	40	75	60	69	100	50	82	64	27	42	80	30	55
gonion region	Schutkowski (1993)	71	45	56	56	86	74	...	...	...	...	...	...	62	68	66
	Sutter (2003)	33	75	60	86	42	58	60	50	54	86	67	76	64	60	62
Angle	This study	100	100	100	100	40	77	86	50	73	64	33	46	83	50	67
	Molleson et al. (1998)	...	...	...	...	...	...	...	...	...	...	...	...	50	78	70
Mentum	This study	25	100	70	100	80	92	86	50	73	55	67	62	70	73	72
	Molleson et al. (1998)	...	...	...	...	...	...	...	...	...	...	...	...	17	77	58
Symphyseal	This study	100	100	100	75	80	77	57	75	64	36	67	54	60	77	68
anterior	Loth and Henneberg (2001)	...	...	...	...	...	...	...	...	...	...	...	...	86/100	67/83	79/89
region	Scheuer (2002)	...	...	...	...	...	...	...	...	...	...	...	...	44/67	78/85	75
	Coqueugniot et al. (2002)													62	42	53

Although accuracies obtained by this study in several sex and age groups for some nonmetric traits achieved the minimum acceptable level for sexing non-adult skeletons in forensic contexts (De Vito and Saunders, 1990), this level of accuracy in the overall sample was reached only for the curvature of iliac crest. In general, while non-metric traits of the mandible perform better in younger age groups, those of the ilium do it in older age groups. The exceptions for the traits of mandible are the shape of the mentum and the eversion of gonion region. For the age group ranging from newborn to one year, these non-metric traits show different accuracies in both sexes. In the case of eversion of gonion region, none of the males are correctly assigned in these age groups. In contrast, when the shape of the mentum is applied, the accuracy is lower in females than in males.

Regarding to sexual differences, sex estimation accuracies are generally higher in females than in males. The exceptions are the auricular elevation in the ilium and the shape of mentum and the symphyseal anterior region in the mandible. Compared to previous studies, our results with the auricular elevation show the lowest accuracy. For instance, in the current study, this trait accuracy was around 37% lower than the accuracy obtained by Weaver (1980), and this trend is exacerbated for males (70% reduction in accuracy).

Although total accuracies for both the greater sciatic notch and depth correspond closely to the study by Vlak et al. (2008), accuracies obtained for our study are higher for females than for males. In contrast, Vlak et al. (2008) obtained higher accuracies for males with greater sciatic notch depth. For the arch criteria, the level of accuracy in the overall sample is lower than that achieved by Schutkowski (1993) and Sutter (2003), but slightly higher than those reported by Cardoso and Saunders (2008).

The total accuracies obtained for non-metric traits of the mandible are slightly lower than those previously published. The only exception is the shape of the mentum, since the accuracy is much higher in our study than in the Molleson et al. (1998).

Table 5 shows the number of males and females showing the two sates of each of non-metric trait of the ilium, as well as the weighted probability of correctly estimating sex. Our results showed that in our sample the weighted probabilities for females were lower than for males. These results were in contrast to those derived from Weaver (1980) and Sutter (2003) for the auricular elevation and to those from Schutkowski (1993), Cardoso and Saunders (2008) and Irurita-Olivares and Alemán-Aguilera (2016) for the arch criteria, since in those cases higher probabilities were obtained if the female trait was observed.

The best results obtained in our reference sample were with the angle of the sciatic notch and the iliac crest curvature, with 0.71 and 0.70 probability of correct assignment respectively if the male trait was observed. Compared to those values obtained from data by Schutkowski (1993) and Sutter (2003), values obtained in this study were generally lower, even more in the case of females. The exception is with the curvature of the iliac crest, when the female trait was observed. In contrast, our results show higher probabilities of correct assignment in both sexes than those obtained from data by Vlak et al. (2008) and Cardoso and Saunders (2008). Very similar probabilities to this study have also been obtained by Irurita-Olivares and Alemán-Aguilera (2016). With regard to these probabilities by age group, the weighted probabilities of correct assignment were not uniform across all age groups and differed between traits. The best results in our reference sample were generally obtained in the youngest age group if the male traits were observed (Fig. 3 d-f-j). However, if the female traits were observed, the probabilities obtained for the estimate were lower for any criteria and age group, except for the curvature of the iliac crest in the age group ranged from six to ten years old (Fig. 3 a-c-e-g-i). Moreover, when the female traits were considered, most of probabilities were lower than 0.60, values which can be considered unacceptable in forensic contexts. These results clearly contrast to those obtained from data by Weaver (1980), Schutkowski (1993) and Sutter (2003), since values obtained in our reference sample were much lower than those provided

by these authors, regardless of age group and of whether a male or female trait were observed (Figure 3). Interestingly, positive predictive value from Vlak et al. (2008) and Cardoso and Saunders (2008) were higher than those in our reference sample in some age groups.

In Table 6 is depicted the number of males and females showing the two sates of each of non- metric trait of the mandible, as well as the weighted probability of correctly estimating sex.

Most of the weighted probabilities were higher than 0.75, suggesting their utility in forensic contexts. However, only with the protrusion of the chin and the morphology of the mentum this minimum value was attained for both sexes. The other indicators (with the exception of symphyseal region) showed probabilities higher than 0.75 only when a male trait was observed. The positive predictive values obtained in this study were generally higher than those obtained by other authors, excluding those from Sutter (2003). In this last case, these values were only obtained when male traits were observed. The comparisons by age group with other studies were only possible with mandibular non-metric traits proposed by Schutkowski (1993). The results published by Molleson et al. (1998), Loth and Henneberg (2001), Coqueugniot et al. (2002) and Scheuer (2002) were not able to be compared, as these authors did not divide the sample into the same age groups.

In our sample, the highest probabilities in the youngest age group are attained with the shape of dental arcade and the protrusion of the chin, but in the last case only when a female trait is

Table 5. Comparisons of weighted positive predictive values when a particular trait of ilium is observed by sex. PPV: positive predictive value.

		Auricular elevation		**Depth of sciatic notch**		**Angle of Sciatic notch**		**Arch criterium**		**Iliac crest curvature**	
	State	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
This study	PPV	0.45	0.55	0.55	0.59	0.59	0.71	0.52	0.55	0.68	0.70
	N♀	9	12	12	5	14	3	13	4	10	4
	N♂	9	9	12	9	12	9	15	6	5	10
Weaver (1980)	PPV	0.79	0.67	---	---	---	---	---	---	---	---
	N♀	41	12	---	---	---	---	---	---	---	---
	N♂	30	70	---	---	---	---	---	---	---	---
Schutkowski (1993)	PPV	---	---	0.84	0.73	0.77	0.94	0.76	0.67	0.65	0.79
	N♀	---	---	13	4	20	8	12	5	18	11
	N♂	---	---	6	26	1	20	5	22	3	13
Sutter (2003)	PPV	0.74	0.67	0.75	0.91	0.72	0.76	0.16	0.21	0.60	0.84
	N♀	14	9	16	4	17	5	14	3	16	12
	N♂	9	28	9	34	9	29	8	35	8	27
Cardoso and	PPV	---	---	---	---	---	---	0.53/0.74	0.27/0.48	---	---
Saunders (2008)	N♀	---	---	---	---	---	---	13/10	30/27	---	---
	N♂	---	---	---	---	---	---	21/41	16/36	---	---
Vlak et al (2008)	PPV	---	---	0.53	0.54	0.49	0.49	---	---	---	---
	N♀	---	---	13	10	12	15	---	---	---	---
	N♂	---	---	13	23	11	18	---	---	---	---
Irurita-Olivares and	PPV	---	---	0.68	0.80	0.59	0.73	0.63	0.59	0.54	0.54
Alemán-Aguilera (2016)	N♀	---	---	46	41	59	67	25	33	46	51
	N♂	---	---	26	64	13	38	47	71	25	53

observed (Fig. 4). These results are very similar to those derived from Sutter (2003). In contrast, in the older age groups the highest probabilities of correct sex estimate are obtained with the eversion of gonion when a male trait is observed.

DISCUSSION

In this study, we evaluated the eleven non-metric traits for the ilium and the mandible previously proposed by Weaver (1980), Schutkowski (1993), Molleson et al. (1998) and Loth and Henneberg (2001) for the estimation of sex in subadults. This evaluation was performed taking into account different aspects related to the value of any non-metric trait for determining the sex of subadult skeletons. First, we considered the association of the presence of each non-metric trait with each sex. Second, we calculated the accuracies as the success rate in the estimate for each of the sexes and for both sexes together. Since these results may be affected for sample characteristics, we also calculated the weighted probabilities of being male or female when a feature is observed.

As far as we know, this is the first time in which all of these non-metric traits are tested in the same sample, and in which all of these methodological approaches are used. Thus, although we are aware of our sample's shortcomings (e.g., small size, uneven age and sex coverage), we think that our results can contribute to understanding the mechanisms underlying the sexual dimorphism of these traits in subadult skeletons

Table 6. Comparisons of weighted positive predictive values when a particular trait of mandible is observed by sex. PPV: positive predictive value.

		Protrusion of chin		Dental arcade		Eversion gonion		Angle		Mentum		Symphyseal region	
	State	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
This study	PPV	0.79	0.75	0.71	0.85	0.57	0.80	0.66	0.93	0.75	0.79	0.75	0.71
	N♀	19	7	23	3	24	2	25	1	21	5	18	8
	N♂	6	24	11	19	21	9	15	15	8	22	7	23
Schutkowski (1993)	PPV	0.69	0.89	0.72	0.70	0.65	0.63	---	---	---	---	---	---
	N♀	12	11	9	7	10	8	---	---	---	---	---	---
	N♂	1	16	4	19	7	17	---	---	---	---	---	---
Molleson et al. (1998)	PPV	---	---	---	---	---	---	0.70	0.61	0.42	0.48	---	---
	N♀	---	---	---	---	---	---	3	3	1	3	---	---
	N♂	---	---	---	---	---	---	3	11	5	10	---	---
Loth and Henneberg	PPV	---	---	---	---	---	---	---	---	---	---	0.54/0.74	0.91/1.00
(2001)	N♀	---	---	---	---	---	---	---	---	---	---	8/10	1/0
	N♂	---	---	---	---	---	---	---	---	---	---	4/2	6/7
Coqueugniot et al.	PPV	---	---	---	---	---	---	---	---	---	---	0.52	0.53
(2002)	N♀	---	---	---	---	---	---	---	---	---	---	25	21
	N♂	---	---	---	---	---	---	---	---	---	---	15	15
Scheuer (2002)	PPV	---	---	---	---	---	---	---	---	---	---	0.71/0.86	0.54/0.66
	N♀	---	---	---	---	---	---	---	---	---	---	4/6	6/4
	N♂	---	---	---	---	---	---	---	---	---	---	5/	21/23
Sutter (2003)	PPV	1.00	0.57	0.94	0.69	0.59	0.58	---	---	---	---	---	---
	N♀	10	18	17	13	18	10	---	---	---	---	---	---
	N♂	3	46	5	44	19	30	---	---	---	---	---	---
Irurita-Olivares and	PPV	0.69	0.67	0.58	0.55	0.56	0.57	---	---	---	---	---	---
Alemán-Aguilera	N♀	48	25	26	45	33	40	---	---	---	---	---	---
(2016)	N♂	39	61	31	69	40	63	---	---	---	---	---	---

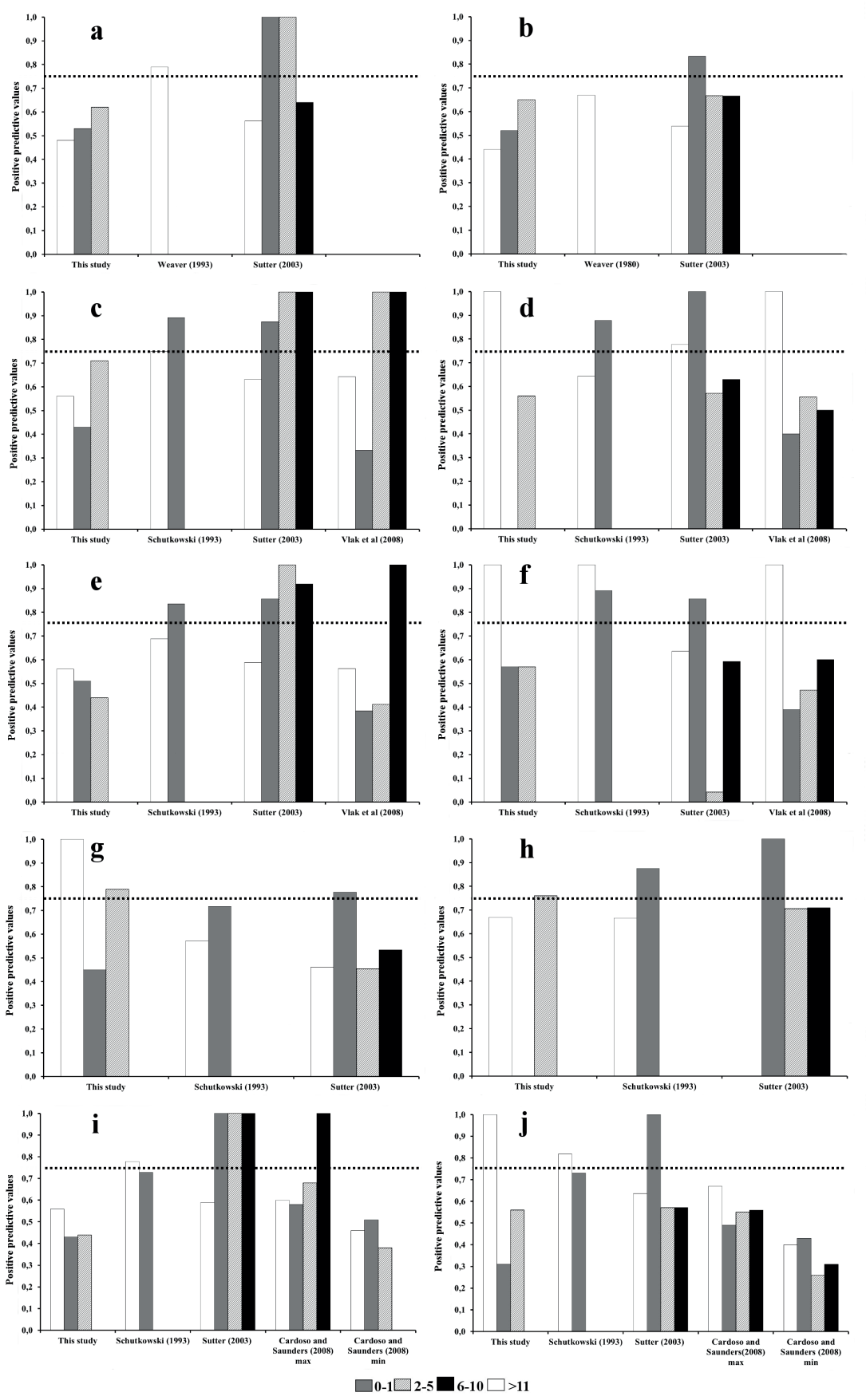

Fig. 3.- Weighted positive predictive values for each of two states of non-metric traits evaluated in the ilium by age groups. Left side: weighted positive predictive values when the female pattern is observed in females; right side: weighted positive predictive values when the male pattern is observed in males. **a** and **b**: elevation of auricular surface, **c** and **d**: depth of sciatic notch, **e** and **f**: angle of sciatic notch, **g** and **h**: iliac crest curvature, **i** and **j**: arch criterion. Dashed horizontal line marked 0.75 probability of correct assignment. White bars: 0-1 age group, gray bars: 2-5 age group, line bars: 6-10 age group, black bars: >11 age group.

The analysis of the association of individual sex-related traits of the ilium and mandible by their respective character states in our reference sample indicate that only traits of mandible and the curvature of the iliac crest are sex-related. Based on these results, it seems that the mandible

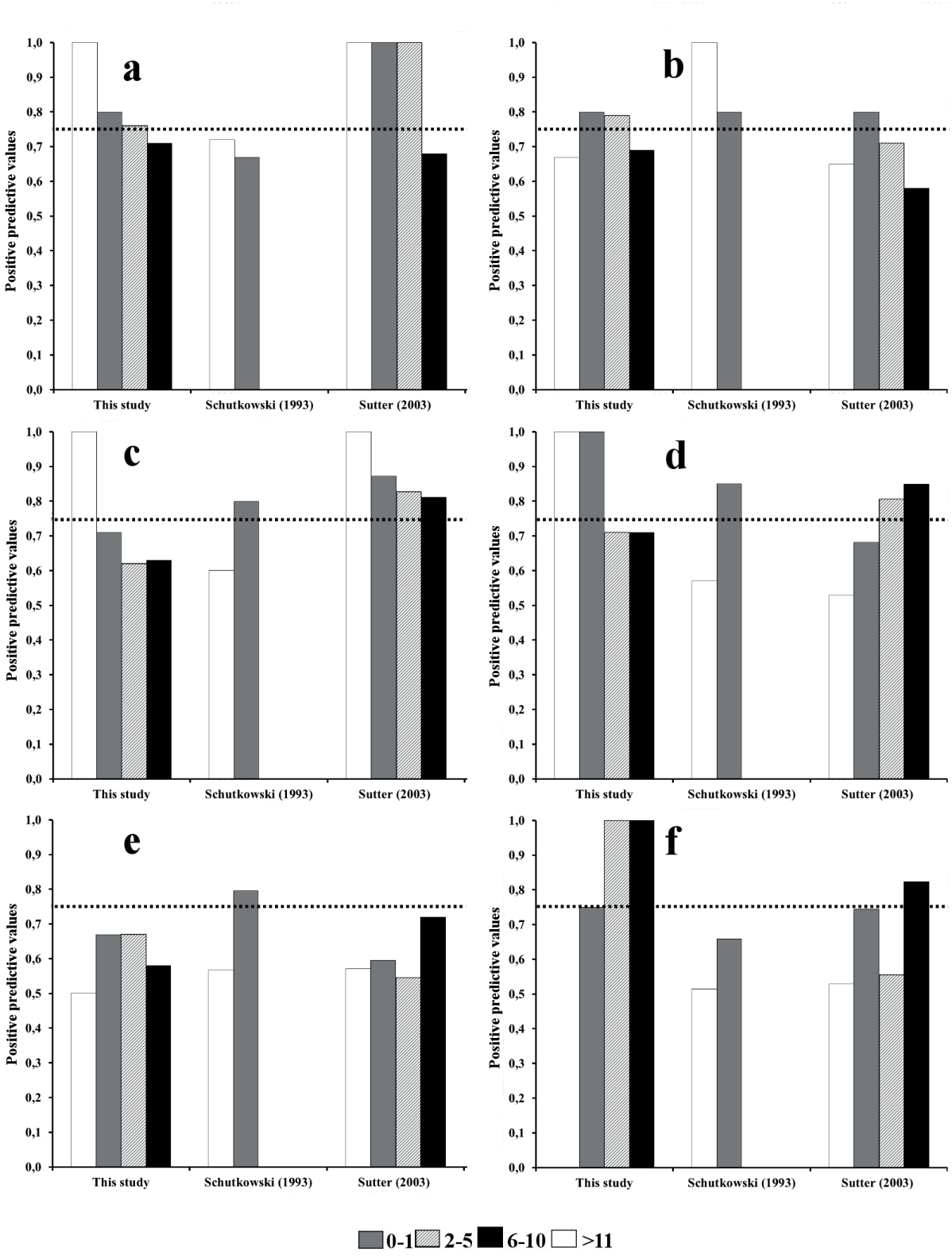

Fig. 4.- Weighted positive predictive values for each of two states of non-metric traits proposed by Schutkowski (1993) evaluated in the mandible by age groups. Left side: weighted positive predictive values when the female pattern is observed in females; rigth side: weighted positive predictive values when the male pattern is observed in males. **a** and **b**: protursion of chin, **c** and **d**: shape of dentla arcade, **e** and **f**: eversion of gonion. Discontinuos horizontal line marked 0.75 probability of correct assignment. White bars: 0-1 age group, gray bars: 2-5 age group, line bars: 6-10 age group, black bars: >11 age group.

is more sexually dimorphic than the ilium, even in the younger age groups. These findings are clearly in contrast with previous studies, in which traits of the ilium showed a closer sex relationship than mandibular traits (Schutkowski, 1993; Sutter, 2003, Irurita-Olivares and Alemán-Aguilera, 2016). Sutter's findings indicate that greater sciatic notch and depth, the arch criterion and chin prominence are sex-related and become even more so with increasing age. Similar sex-related character state tendencies also characterize the known-sex subadult remains from Granada, Spain (Irurita-Olivares and Alemán-Aguilera, 2016). The only exception is that in subadults from Granada, while the two states of the arch criterion did not show a statistically significant relationship with sex, the curvature of the iliac crest did.

These differences in the association of sex-related traits among studies may be related to population differences in the pattern and degree of sexual dimorphism of the different traits, which arise during growth and development due to a complex combination of genetic and environmental factors (Coleman, 1969; Wilson et al., 2015, 2011). Several studies have already showed that generally the impact of these combined factors in archaeological populations resulted in a delayed skeletal growth and development (Conceição and Cardoso, 2011; García-González et al., 2019; Humphrey, 2000; Mays et al., 2009). Because there is a strong relationship between skeletal, sexual, and somatic maturation (Demirjian et al., 1985; Fishman, 1979; Grave and Brown, 1976; Green, 1961), a delay in skeletal development could influence the manifestation of sexual dimorphism of different traits and its interpretation.

Sutter (2003) asserted that the value of any given trait for determining the sex of subadult individuals could be assessed in a different way in bioarchaeological and forensic contexts. In bioarchaeological contexts, it could be enough to know whether the presence of any given trait is more commonly associated with either of the two sexes. Following that, he proposed several non-metric traits for their use in bioarchaeological research. However, our results suggest some caution with this approach, since there is inter-populational variability in the sex association of the eleven non-metric traits evaluated by this study.

Besides the strength of the association of different traits with sex, in forensic contexts, the accuracy in sex estimation of a given trait is very important. Accuracies calculated as a success rate showed that most of traits did not reach the minimum acceptable level of accuracy for sexing non-adult skeletons in the overall sample (De Vito and Saunders, 1990). Our results clearly contrast with those obtained by the original studies and are more similar to those that posteriorly tested these methods. There are several possible explanations for the low performance of the different methods when they are tested on other known sex subadult samples. As it has been mentioned above, one of them is the populational variability in the pattern and degree of sexual dimorphism. This would explain why the accuracies obtained by this study are more similar to those reported by Cardoso and Saunders (2008) and Vlak et al. (2008) than to the other studies. Although the sample size is different in these three works, the reference sample is the same. But, in addition to these population differences, there are other multiple issues that may cause discrepancies between accuracies obtained by different studies, such as subjectivity to score the different non-metric traits and the sex and age distribution of the different samples. Regarding the subjectivity to score non-metric traits, in this study we did not examine intra- and inter-observer agreement in assessing these traits.

Fortunately, and as this problem is inherent in all qualitative studies, several studies have already addressed this issue: for instance, Cardoso and Saunders (2008) found extremely low intra-and inter-observer agreement when the arch criterion was applied. Moreover, these authors pointed out a possible error in the application of the arch criterion by Sutter (2003). This could explain differences in the association of the two states of this trait among studies. In contrast, Valk et al. (2008) and Irurita-Olivares and Alemán- Aguilera (2016) reported high intra- and inter-observer agreement with the angle and depth of the sciatic notch.

Thus, if these results can be extrapolated to our study, the reliability for the assignment of the different traits is not the only responsible for the low performance of these traits.

Another possible explanation for the low performance of one method when it is applied on other samples is the sex ratio of the reference samples used by the original studies. If the reference sample of one method is sex-unbalanced, it will lead to sex estimation in other samples that may be subject to "sex mimicry". Indeed, previous works testing the non-metric traits proposed by Schutkowki (1993) found that these traits are less successful in identifiying females (Table 4), which has been attributed to the possibility that the sex ratio of the Schutkowki's sample is biased towards males. A similar bias for identifying males has been also claimed for mandibular traits previously proposed by Loth and Henneberg (2001) (Scheuer, 2002).

However, our findings showed that females, generally, were identified with a higher level of success (Table 4). Based on this, it seems unlikely that all non-metric traits be biased to identifying males in all samples. This assertion is in disagreement to results obtained by Cardoso and Saunders (2008) and Vlak et al, (2008) using the same reference sample.

However, the size and sex-composition of the sample differ between these three studies. In our study the percentage of females is the highest and the sex ratio is less biased towards males than in the other two studies. Thus, not only the sex ratio of the reference sample used in the original studies can have an effect on the levels of accuracy, but also the sex ratio of the target sample may influence these levels of accuracy. This shortcoming could be solved with samples with more evenly balanced sex distribution but, unfortunately, the availability of subadult samples of known filiation is limited. Yet, we can increase the importance of the data obtained for the under-represented sex if the results are weighted, namely, to consider an equivalent number of individuals of both sexes. Even following this approach, accuracies calculated as rate of success show the probability that an individual of known sex presents a particular non-metric trait and that they depend on the distribution of these non-metric traits in the sample studied (Irurita-Olivares and Alemán-Aguilera, 2016). Therefore, and in order to eliminate these sample effects to evaluate the reliability of one method, it is much better to consider the weighted probabilities of being female or male when one feature is observed. Collectively, our results following this approach indicate that mandibular traits are more successful for juvenile sex determination than non-metric traits of the ilium (Table 5 and Table 6). The weighted probabilities of being male or female were not uniform across all age groups and, generally, are higher in younger age groups (Fig. 4). The shape of dental arcade, the mandibular angle, the mentum and the symphyseal region are the most sexually dimorphic traits up to six years old. These sexual differences in mandibular morphology in younger age groups could be related to differences in developing anterior tooth size (Loth and Henneberg, 2001). However, Cardoso (2010) showed that there were very few sex differences in crown dimensions of deciduous teeth in the Lisbon collection. Moreover, Coquerelle et al. (2011) found that sexual dimorphism in the mandible is not associated with the development of the teeth and it is manifested in the mental region during the earliest ontogenetic stages and again at adulthood. Thus, our results along those provided by Coquerelle et al. (2011) suggest that mandibular non-metric traits (with the exception of the eversion of gonion) are reliable for sex estimation in forensic contexts up to 4-5 years.

Regarding non-metric traits of the ilium, we detected differences among studies and among traits in the weighted probabilities of correct identification for one sex relative to the other.

For example, while, in our sample the chance of correct identification for females is lower than for males when non-metric traits of ilium are considered, the weighted probabilities for females calculated from data by Schutkowski (1993), Sutter (2003) and Irurita-Olivares and Alemán-Aguilera (2016) are higher than for males (Table 5). In contrast, these values are similar to those obtained from data by Cardoso and Saunders (2008) and Vlak et al. (2008). As it has been already mentioned, these three studies used the

Lisbon sample. Wilson et al. (2011) pointed out the variability in the success of sex determination across the Lisbon and London sample using the greater sciatic notch. The London sample comprised juveniles from different crypts, among which is the Spitafields sample used by Schutkowki (1993). Males belonging to the London sample have a shallower greater sciatic notch than Lisbon males (Wilson et al., 2011). The consequence of this result may be that males are typically misidentified more commonly than females in the London sample than in the Lisbon sample. Thus, our findings matched previous observations of population-based differences in the pattern and degree of sexual dimorphism.

However, the most surprising finding of our study is that in our sample the best results are obtained in the younger age groups when non-metric traits of ilium are applied. It has been commonly accepted that shape differences between the sexes increased during ontogeny, due to divergence in the shape trajectories (Bilfeld et al., 2013; Wilson et al., 2011, 2015). For this reason, several scholars did not recommend the use of non-metric traits of ilium up to puberty (Cardoso and Saunders, 2008; Valk et al., 2008). Again, population- specific patterning in the attainment of sexual dimorphism could be responsible of differences in the weighted probabilities across age groups among different studies. However, it is important to note that our reference sample is biased towards younger individuals. Indeed, there are not females in the older age group. It would be preferable to have a more evenly balanced sex distribution to capture the majority of sexual variation, yet narrow enough for the sex estimates to be meaningful. Thus, the low weighted probabilities obtained at older ages in our reference sample may reflect small sample sizes and uneven sex distribution. This assertion can be extended to all age groups.

At this point, it is important to make a consideration on the effect of age groups in the results. First, because the majority of documented samples are small and sex coverage uneven, the researchers were forced to divide the sample into groups with wide age ranges. This adds great uncertainty to elucidate what differences are due to the sex of individuals and what of them are due to age (Stull et al., 2017). Another important issue about age groups is that they may not make sense in the biological level. Ontogeny of sexual dimorphism is related to differences in hormone levels between males and females during development (Irurita-Olivares and Alemán-Aguilera, 2016). The hormonal secretion is affected by environmental and genetic factors and, thus, individuals with the same chronological age can have different hormonal levels. This may affect to the accuracy of the different traits when age-groups are considered. Therefore, it could be better to classify the individuals according to other criteria, such as skeletal age. Skeletal age refers to the degree of maturation of the bones of one sub-adult individual (Roche et al., 1971). Generally, skeletal age is assessed based on maturity indicators that describe the sequence of the onset of ossification in the epiphyses of long bones, the changes in shape and size of the epiphyses and the chronology of the epiphyseal union (Eveleth and Tanner, 1976; Roche, 1992). Since all these events are closely related to hormonal secretion, skeletal age may be a good marked to evaluate the ontogeny of sexual dimorphism of these non-metric traits. Assessment of skeletal age from epiphyseal union is limited to pre-adolescents and adolescents. In younger individuals, assessing skeletal age via the onset of the ossification of the epiphyses and their subsequent changes in size and shape is preferable. Nonetheless, these latter methods for assessing skeletal ages are defined based on radiographic changes in the epiphyses, which are usually difficult to duplicate using direct dry bone observations (Krogman and Iscan, 1987). Fortunately, this issue has been recently solved with the adaptation of several method developed to establish skeletal maturity for their use with skeletal remains (Conceição and Cardoso, 2011; García-González et al., 2019; Shapland and Lewis, 2014, 2013). Thus, as a plausible future line of research, we recommend establishing groups on the basis of skeletal ages.

CONCLUSIONS

Our results show that, in our reference sample, the mandible shows more sexual dimorphism in

subadults than the ilium, overall in the youngest individuals. However, most of the non-metric traits revealed a low level of sexual dimorphism, which leads to low success of correct sexual estimates. These low levels of success may be due to inter-populational differences in the pattern, degree and attainment of sexual dimorphism. But, besides these reasons, sample characteristics also have an important effect in these low levels of correct classification. We showed that the sex ratio of both reference and target samples may affect the results when accuracies calculated as rate of success are considered. For this reason, we recommended the use of weighted probabilities in order to evaluate the applicability of a non-metric method.

Nonetheless, this approach may be affected for the age ranges used by the original studies and those in which these methods are being tested.

In sum, although the significance of our results is limited by small sample size, they have highlighted some interesting findings. We showed that sample-related factors may affect the levels of accuracy of one method in the same way that other factors traditionally considered (such as inter-populational and ontogenetic variability in the pattern and degree of sexual dimorphism). While the effect of sex ratio can be solved with the appropriate statistical analysis, the effect of age ranges seems more problematic.

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Review of the existing artificial intelligence approaches for craniofacial superimposition

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SUMMARY

When usual methods of identification, such as DNA, dental comparison or fingerprints, are not possible or practical, skull-face comparison is utilized for human identification. Craniofacial Superimposition (CFS) is a forensic technique, which is based on the anatomical and morphological correspondences between the skull and the face. This technique involves the process of overlaying a variable number of facial images corresponding to the candidate with an unidentified skull. This technique has a great potential, since nowadays a wide majority of people have photographs where their faces are clearly visible. In addition, the skull is a bone that hardly degrades under the effect of the inclement weather such as fire, humidity, temperature changes, etc. In this paper, an overview of the history and the different technological approaches developed over time is offered in conjunction with the classification of the methodologies based on the technology employed through the diverse phases of the process. The state of the art of CFS supported by Artificial Intelligence is reviewed, with special emphasis on the approaches developed by the Physical Anthropology Lab and the SOCCER group of the University of Granada over the last decade. Finally, the future challenges that could be addressed by using the automated approaches developed for this technique in scenarios of disaster victims such as the deaths of migrants in the Mediterranean are discussed.

Key words: Forensic anthropology – Human identification – Craniofacial superimposition – Artificial intelligence

INTRODUCTION

Nowadays, the untraceable number of unidentified people worldwide is growing due to mass disasters, war conflicts, forced disappearances and criminal investigations of dead and missing people. Additionally, the actual crisis of the deaths of migrants in the Mediterranean leave thousands of bodies each year on the coasts of Europe. Just between 2014 and 2018 alone, there were close to 15.000 dead or missing while crossing the sea, according to the United Nations Refugee Agency (UNHCR, n.d.). All these factors contribute to the increasing need for accurate and efficient tools that allow for establishing and verifying human identity.

Different techniques like DNA or fingerprints have been employed in many human identification (ID) scenarios. However, the application of these methods fails when there is not enough antemortem (AM) or postmortem (PM) information available

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due to the lack of data (second DNA sample) or to the state of preservation of the corpse. While the skeleton usually survives both natural and non-natural decomposition processes (fire, salt, water, etc.), the soft tissue progressively degrades and is lost. This is a very frequent case in disaster victim ID scenarios. In fact, the experience of several practitioners in these scenarios indicates the poorer effectiveness of DNA analysis (around 3% of IDs) and dactyloscopy (15-25%) against skeleton-based forensic identification (SFI) techniques (70-80%) (Beauthier et al., 2009), which are the daily practice of Forensic Anthropology experts. These techniques involve the assessment of human osseous remains to identify the deceased person's identity and cause of death (Bethard, 2014). SFI methods employed by forensic anthropologists, odontologists, and pathologists are crucial in AM data collection and biological profiling. Even more important, they represent the victim's last chance for ID using techniques such as Comparative Radiography (including dental comparison), Craniofacial Superimposition (CFS) or Facial Approximation. When skeletal remains are discovered, the evaluation of the skull is a crucial part of the identification process. The skull can provide information about the biological profile (age, sex, ancestry) of the deceased person and it can be compared with AM dental records (Webster et al., 1986; Whittaker and Mac Donald, 1989), radiographs records or available photographs relating to the suspect to indicate the definitive identification of the skull (Sekharan, 1985; Jayaprakash, 1997). When AM images are present, CFS (Yoshino, 2012)matching of ante-mortem radiographs with various skeletal parts, matching of facial photographs with the skull by craniofacial superimposition and DNA analysis of bones or teeth and reference samples. Ante-mortem dental records and radiographs have been generally accepted to suggest positive identification of skeletal remains. However, the availability of these ante-mortem records is relatively rare in criminal cases. Molecular approaches such as mitochondrial DNA (mtDNA, one of the approaches in craniofacial identification (Aulsebrook et al., 1995; Stephan et al., 2009), may be effective. The CFS technique consists of comparing and superimposing an unknown skull (or a skull 3D model) with a number of AM images of a missing person. It tries to correlate their morphological correspondence in order to determine, with a reasonable degree of confidence, if they belong to the same person.

ANATOMICAL AND TECHNOLOGICAL BACKGROUND OF CRANIOFACIAL SUPERIMPOSITION

The first documented cases on the application of the CFS technique to compare AM images with a skull, date from the early decades of the 20th century. In these instances, the technique served as supporting evidence to be presented in court, rather than with identification purposes. The most important and well-known case of this early era is the Ruxton case in 1937 (Glaister and Brash, 1937), in which CFS was used in order to compare two female skulls to photographs of missing women. A few years later, an X-ray of a skull was used to make the comparison and supporting identification of the victim in the Baptist Church Cellar Murder (Simpson and Dobkin, 1943). In South Africa, in the 50s, a case was reported in which photographic superimposition was accepted as a part of evidence for identification (Prinsloo, 1953).

The first positive identification case based on CFS was in India, in 1962, and it was accepted in a court of law (Sen, 1962). In the next 50 years, the technique became more extended and several CFS identification cases were performed (Sekharan, 1971; Bastiaan et al., 1986; Ubelaker, 2000). In fact, the CFS procedure was highly helpful in the identification of war criminals as Adolf Hitler's chief medical officer Dr. Josef Mengele at Sao Paulo, Brazil, in 1985 (Helmer, 1987). Besides, CFS is currently used in different scenarios where other identification techniques are not available, such as the Indian Ocean tsunami in 2004 (Al-Amad et al., 2006).

All those first-generation methods of CFS were solved using a procedure called photographic superimposition, since only photographs of both the skull and the face were used. In the following years, a new modality of CFS technique based on video technology made their appearance. Helmer

and Gruner (1977) were the first researchers who introduced the video superimposition technique. They took advantage of the live image of the skull and photograph instead of taking photographs. The computer-aided CFS techniques represent the next generation of CFS system and one of the first documented cases of a superimposition performed using a "computer-enhanced" method was developed by FBI, led by Ubelaker in 1992. Since then, computer-aided superimposition has become a popular identification method among experts.

The importance of the face – skull relation

It must be taken into account that one of the main factors on which the CFS technique is based is that: "each skull has a unique face". The structure of the human skull is one of the most complex elements of the human being, making it one of the main identification features. Besides, the face is the main component of social interaction (Hennessy et al., 2005) and recognition or identification to each other.

The evaluation of a superimposition is a significant issue that is dependent on the consistency of the anatomical link between the location of the soft tissue surfaces relative to the underlying bone (Taylor, 2000). For this reason, a full comprehension of the anatomy of the skull and the relationship between it and the face are required.

Paul Broca (1895) was the one who laid the foundations for the creation of a new science, called craniometry, which exhaustively studies the human head with a new methodology of terms and measurements. Later, Martin and Saller (1957) created a treatise in which the fundamental pillars of this discipline were established. They defined an important set of craniometric and somatometric landmarks that has been crucial for all the following anthropological studies. A correlation between a few of these landmarks have been identified in both the skull (craniometric) and the face (cephalometric) (Caple and Stephan, 2016) and several studies measuring soft tissue depth between corresponding landmarks have been published using data from different populations and different measuring techniques (Stephan et al., 2008; Guyomarc´h et al., 2013; Parks et al., 2014; Stephan, 2014; Munn and Stephan, 2018; Stephan, 2018; Stephan and Preisler, 2018; Ayoub et al., 2019) (Fig. 1).

In addition, there are some standards for relating the shape of certain regions (morphological correspondence) to carry out an assessment of face and skull correspondence for the anatomical structures as the nasal bones and the nose (Prokopec and Ubelaker, 2002), the dentition and the mouth (Stephan, 2003; Stephan and Murphy, 2008), the orbits and the eyes (Wilkinson and Mautner, 2003), and others, i.e., the general face shape is studied focusing on the shapes of the transverse arch of the skull and the contour of the jaw (Fedosyutkin and Nainys, 1993).

Fig 1.- Most commonly employed cephalometric and homologous craniometrics landmarks in CFS.

The quality of the superimposition is assessed by the anatomical consistency between the face and the skull following a number of different criteria. The most representative proposals can be found in: Chai et al. (1989), Austin and Maples (1994), Yoshino et al. (1995; 2012), Lan (1995), Jayaprakash et al. (2001), Ricci and Apostol (2006), among others.

Technological development of craniofacial superimposition

Since the first documented use of CFS for identification purposes (Glaister and Brash, 1937), the technique has been under continuous improvement and the associated procedures evolved as new technology was available. Although several authors have proposed various classifications for CFS methods, most of them recognize three main different approaches: photographic, video, and computer-aided superimposition (Ubelaker et al., 1992; Damas et al., 2011; Yoshino, 2012; Huete et al., 2015).

Review articles that exhaustively summarize the history of the CFS technique development can be found in Aulsebrook et al. (1995), Damas et al. (2011), Yoshino (2012), Huete et al. (2015) and Ubelaker (2015), so in this subsection only a brief tour through the technological development will be made.

Photographic Superimposition. The photographic superimposition technique (developed in the mid-1930s) consists of marking contours and/or facial landmarks on the original facial photograph. The same task was done with a photograph of the skull. Then, both images were overlapped and compared (Dorion, 1983; Brocklebank and Holmgren, 1989; Maat, 1989; Yoshino, 2012). Many authors further developed photographic superimposition techniques to improve the scale and the orientation of the skull and the facial images (Dorion, 1983; Brocklebank and Holmgren, 1989; Maat, 1989). A review of the latest developments in photographic superimposition since 2015 can be found in Ubelaker et al. (2019).

Video Superimposition. Craniofacial video superimposition was introduced in 1976 and it presented an enormous advantage over the conventional photographic procedure (Lan and Cai, 1993; Solla and Iscan, 2001; Fenton et al., 2008) by minimizing several problems associated with the photographic systems. Measurable images using anatomical landmarks and anthropometrical measurements of the skull and the face are used in order to ascertain the object-subject distance, to know all technical data of the equipment and filter the image to produce as much useful image information as possible. Skull orientation can be performed in the same manner as in photographic superimposition; however, the correct size of the skull is easier to achieve by adjusting the size of the skull using the zoom mechanism of the video camera (Yoshino, 2012). However, photographic and video superimposition techniques suffer from the same problems; forensic anthropologists follow a trial-and-error method to achieve the skull-face overlay (SFO). The adjustment of the skull is a difficult and time-consuming task until the pose fit with the facial photograph (Ubelaker, 2000; Fenton et al., 2008), and the inherent uncertainty source due to the overlaying of two different "objects" (a skull and a face) and the absence of an objective evaluation of the resulting overlap.

Computer-aided Superimposition. With the availability of new digital equipment, such as computers and 3D scanners, computer-aided approaches to CFS have been gaining ground over video superimposition methods for the last 30 years. General purpose image processing software packages, like Photoshop, and 3D modeling programs, like 3D Studio or Free Form Modeling, are currently employed for manually overlaying a 3D skull model over a digital photograph and posterior morphological analysis. The large majority of computer-aided approaches are those classified by Damas et al. (2011) as non-automatic methods. The methods within this category use the computer for storing, visualizing and/or manually interacting with the data (Ubelaker et al., 1992; Ricci and Apostol, 2006; Al-Amad et al., 2006). A second category, automatic computer-aided methods, has been followed by a really small number of pioneering researchers (Nickerson et al., 1991; Ghosh and Sinha, 2001) with no impact yet on the daily practice of human identification.

These computer programs, based on Artificial Intelligence (AI) techniques, are employed to accomplish any CFS sub-task automatically.

However, the use of computers does not seem to have led to an improvement in the reliability of the CFS method, quite the opposite. Within the framework of the "New Methodologies and Protocols of Forensic Identification by Craniofacial Superimposition" (MEPROCS) project (Damas et al., 2020), a multiple-lab study to analyze the performance of a diverse set of video- and computer-aided CFS methodologies was carried out by 26 participants from 17 forensic and academic institutions from 13 countries (Ibáñez et al., 2015). The results obtained in this study indicate that the computer-aided methods do not necessarily improve the application of the technique and suffer from the same common problems that affected all the CFS methodologies, like the orientation/projection of the skull or the subjective evaluation of the resulting SFO. Indeed, the performance of video superimposition methods was superior in average to those that employed computer programs. The inferior accuracy of computer-aided approaches was related to the limitations of the software employed (general purpose image processing packages) and the less intuitive and controlled environment regard camera parameter settings. The most recent reliability studies (Gordon and Steyn, 2012; Wilkinson and Lofthouse, 2015; Gaudio et al., 2016) corroborate the low reliability of the manual computer-aided methods.

Although the CFS technique has been applied for a century in several countries, there is a lot of controversy within the scientific community since the application of computer-aided paradigms. While some authors classify CFS as a "useful" and "successful" method for positive identification (Furuhata, 1967; Kamijo, 1970; Iten, 1987), others agree that this technique should be used only for excluding purposes (Fenton et al., 2008; Gaudio et al., 2016). A third group of authors contend that CFS cannot claim to qualify for the definite identification of an individual, but it can be used in combination with other methods to reduce mismatch and eliminate the chance of false identification (Jayaprakash et al., 2001; Ricci and Apostol, 2006). The MEPROCS consortium recognized that procedures for CFS varied greatly among practitioners and lacked protocols and standards. In an early phase of this project, the procedures employed by 26 participants in 17 different institutions from 13 countries were surveyed. The participants judged 60 superimposition problems using their own methodology and technology. The group then met in Israel and later in Italy to review results and discuss issues encountered. Multiple problematic issues, 17 items of best practice and 4 practices to be avoided, as a result of the discussion, can be found in Damas et al. (2015). The publication was presented as the first standard of CFS and set the stage for new research.

STATE OF THE ART IN ARTIFICIAL INTELLIGENCE APPROACHES FOR CRANIOFACIAL SUPERIMPOSITION

It seems nowadays quite realistic to think that the real revolution in CFS and Forensic Anthropology probably comes with the application of AI methods that automate certain data-driven processes which are less subjective and easily reproducible. In this sense, the Physical Anthropology lab and the "Soft Computing Applications for Complex Environments Research Group" (SOCCER) of the University of Granada have been working in close collaboration within them and with other international forensic labs over the last decade (Ibáñez et al., 2009; 2011; 2012; Campomanes-Álvarez et al., 2012; 2015; 2016; 2018a; 2018b; Valsecchi et al., 2018), with the aim of automating the whole CFS identification process while increasing its reliability by the application of AI techniques.

2D-3D Computer-aided approaches for Craniofacial Superimposition

The most recent comprehensive surveys of the CFS field differentiate three consecutive stages for the whole computer-based CFS process (Fig. 2) (Damas et al., 2011; Huete et al., 2015):

1) The acquisition and processing of the materials, i.e., skull (or skull 3D model) and the AM facial photographs with the corresponding location of landmarks on both.

2) The skull-face overlay, which deals with accomplishing the best possible superimposition of the skull on a single AM photograph of a missing person. This process is repeated for each available photograph, obtaining different overlays.

3) The decision making, which evaluates the degree of support of being the same person or not (exclusion) based on the previous SFOs. This decision is influenced by the morphological correlation between the skull and the face, the matching between the corresponding landmarks according to the soft tissue depth, and the consistency between asymmetries. These criteria can vary depending on the anatomical region and the pose (Damas et al. 2015).

The following subsections review the main existing computer-aided CFS approaches for each of the three stages, specially emphasizing the automatic methods developed over the last ten years by the Physical Anthropology Lab in collaboration with SOCCER group at the University of Granada.

Acquisition and Processing of the Materials

The most recent approaches for the first stage of CFS are related to the use of advanced scanning devices like laser range scanners (Damas et al., 2011; Huete et al., 2015).

The computerized systems developed for the first stage of CFS are related to face enhancement and skull modelling procedures. Skull 2D images, skull live images (video superimposition), and more frequently nowadays, skull 3D models, can be used in CFS. With the use of current 3D scanning devices, the forensic anthropologist can get a skull 3D model with a precision lower than one millimeter in a reasonable time (Park et al., 2006). The use of a 3D model instead of a skull 2D image is more recommended, because it is a more accurate representation of the real skull. It has already been shown that 3D models are more informative in other forensic identification tasks (De Angelis et al., 2009). In the biomedical field, CT scanning images are the starting data to reconstruct the skull (Fantini et al., 2008; Singare et al., 2009). In the Comparative Radiography field, there are just a few automatic approaches

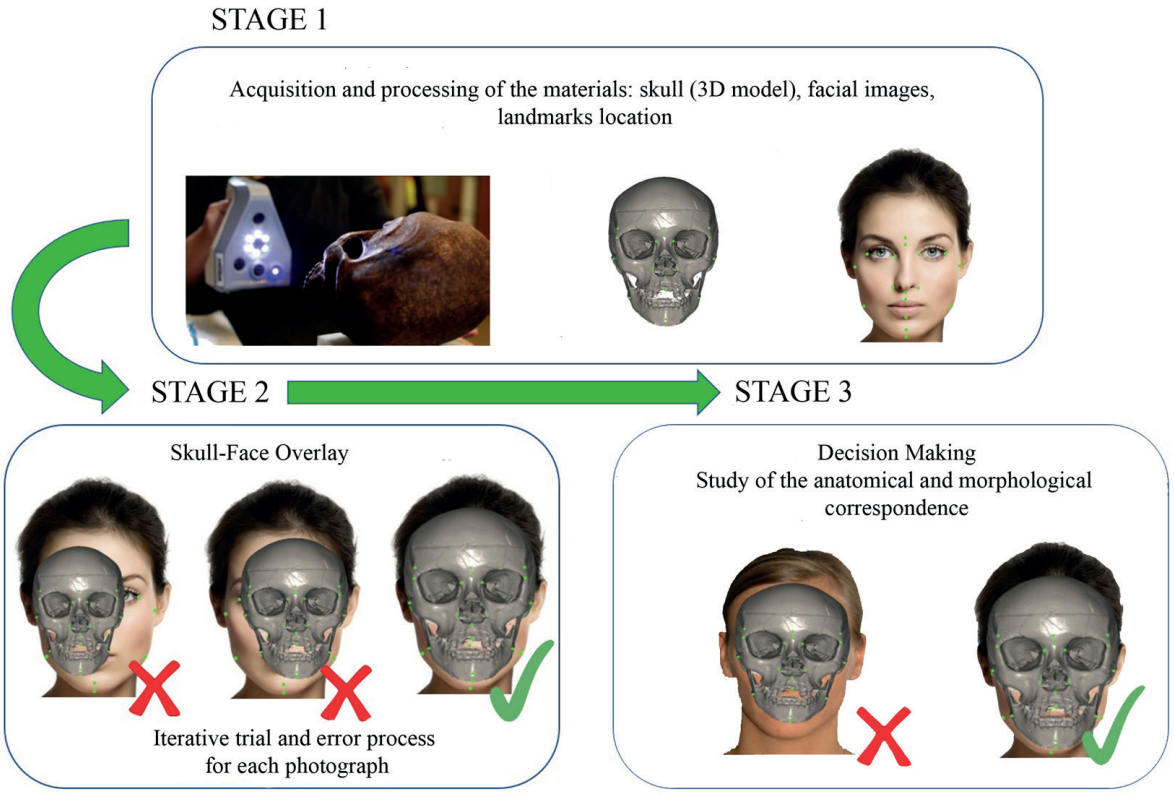

Fig 2.- Stages for the whole computer-based CFS process (Damas et al., 2011).

for the comparison of AM radiographs and PM 3D images, using the silhouette of the target skeletal structures, clavicles in (Stephan et al., 2014; D'Alonzo et al., 2017; Gómez et al., 2018) patellae in (Stephan et al., 2014; Gómez et al., 2018), and frontal sinuses in (Gómez et al., 2018).

Since the first proposal to use a skull 3D model to tackle the CFS problem (Nickerson et al., 1991), 3D image reconstruction software is a real need to construct the 3D model by aligning the views in a common coordinate frame. Such process is usually referred to as image registration (Ikeuchi and Sato, 2001; Zitová and Flusser, 2003), and it consists of finding the best 3D rigid transformation (composed of a rotation and a translation) to align the acquired views of the object. Today's technology, i.e., current 3D acquisition devices and corresponding software, automatically solve the alignment of the different acquisition views without the need of turn-tables or any additional device.

Santamaria et al. (2007a; 2007b; 2009) proposed a method, based on Evolutionary Algorithms (EA), for the automatic alignment of skull range images. Different views of the skull to be modeled were acquired by using a laser range scanner. A two-step pair-wise Image Registration technique was successfully applied to such images. The method is very robust, since it reconstructs the skull 3D model even if there is no turn-table and the views are wrongly scanned.

Ballerini et al. (2009) proposed the automatic reduction of the data provided by the laser range scanner used in the skull 3D model reconstruction task. The dense point cloud corresponding to every skull view is synthesized by considering heuristic features that are based on the curvature values of the skull surface. Those features guide the automatic skull 3D model reconstruction by means of an EA.

Finally, a different related task was presented in Campomanes et al. (2012). The authors proposed an algorithm to deal with the 3D open model mesh simplification problem from an evolutionary multi-objective point of view. An open model refers to a surface with open ends. The problem is based on the location of a certain number of points in order to approximate a mesh as accurately as possible to the initial surface. The algorithm considers two conflicting objectives, the accuracy and the simplicity of a simplified 3D mesh.

Skull-Face Overlay

Several proposals have been presented in the literature to perform the SFO task (Nickerson et al., 1991; Ibáñez et al., 2009; 2011; 2012; Campomanes-Álvarez et al., 2015; Valsecchi et al., 2018). The most natural way to deal with the SFO problem is to replicate the original scenario of the AM photograph in which the living person was in a given pose somewhere inside the camera's field. Regarding computer-aided automatic methods, the task of replicating on a skull the pose and the remaining acquisition parameters of a given facial photograph is the main goal of the SFO process. From a computational point of view, there is a clear relation between the superimposition task and the image registration problem (Zitová and Flusser, 2003). Technically, given "*n*" points having 3D positions $a_1,...,a_n$ and target 2D positions $b_1,...,b_n$. The goal is to find a projection P so that every $P(a_i)$ is as close as possible to b_i. Computer Vision techniques are used to make the SFO operation automatic.

The first computer-aided approach for the SFO task was proposed by Nickerson et al. (1991). The landmarks of the 3D skull mesh were selected to calculate the affine and perspective transformation (rotation, scaling and translation) in order to map with the landmarks of the 2D face image using real-coded genetic algorithms. Another automatic approach was presented in Ghosh and Sinha (2001), in which they considered two different Neural Networks for implement an objective assessment of the symmetry between two nearly frontal 2D images (skull and facial image).

In the last decade, the SFO automation was tackle using EAs and Fuzzy Sets (Ibáñez et al., 2009; 2011; 2012; Campomanes-Álvarez et al., 2015). These approaches are based on overlaying a 3D model of the skull over a facial photograph by minimizing the distance among pairs of landmarks while handling the imprecision introduced by the facial landmark location (Cummaudo et al., 2013;

Damas et al., 2015). The minimization process involves the search for a specific projection of the skull model leading to the best possible matching between corresponding landmarks.

Recently, Valsecchi et al. this requires superimposing the skull over each photo, matching the pose of the skull with that of the face, a problem known as skull-face overlay (SFO(2018) proposed a novel automatic SFO algorithm called "POSEST-SFO" (Fig. 3). The previous methods (Ibáñez et al., 2012; Campomanes-Álvarez et al., 2015) solved the projection problem by an iterative optimization process, where multiple solutions are evaluated at every step, eventually converging to a high quality solution. Unlike these methods, "POSEST-SFO" algorithm solves a system of polynomial equations relating the distances between the points before and after the projection. This latter algorithm was tested on a synthetic data set comprised of 9 CBCTs from 9 different subject and 60 simulated photographs, 30 lateral and 30 frontal images, from each of them, i.e., 540 SFOs. This method is extremely fast, since it lasts 78 milliseconds to automatically perform a single SFO. In the most realistic closed scenario, considering soft tissue thickness (the mean distance was employed) and ±5 pixels of error in facial landmarks, the mean back-projection error was 2.0mm and 3.2mm in frontal and lateral photos, respectively. However, this algorithm, contrary to previous publications (Campomanes-Álvarez et al., 2015; Bermejo et al., 2017) does not address the sources of uncertainty, i.e., the articulation of the mandible, the estimation of the soft tissue thickness, and the intra- and inter-error in landmark location. It is clear then that there is still a large margin for improvement.

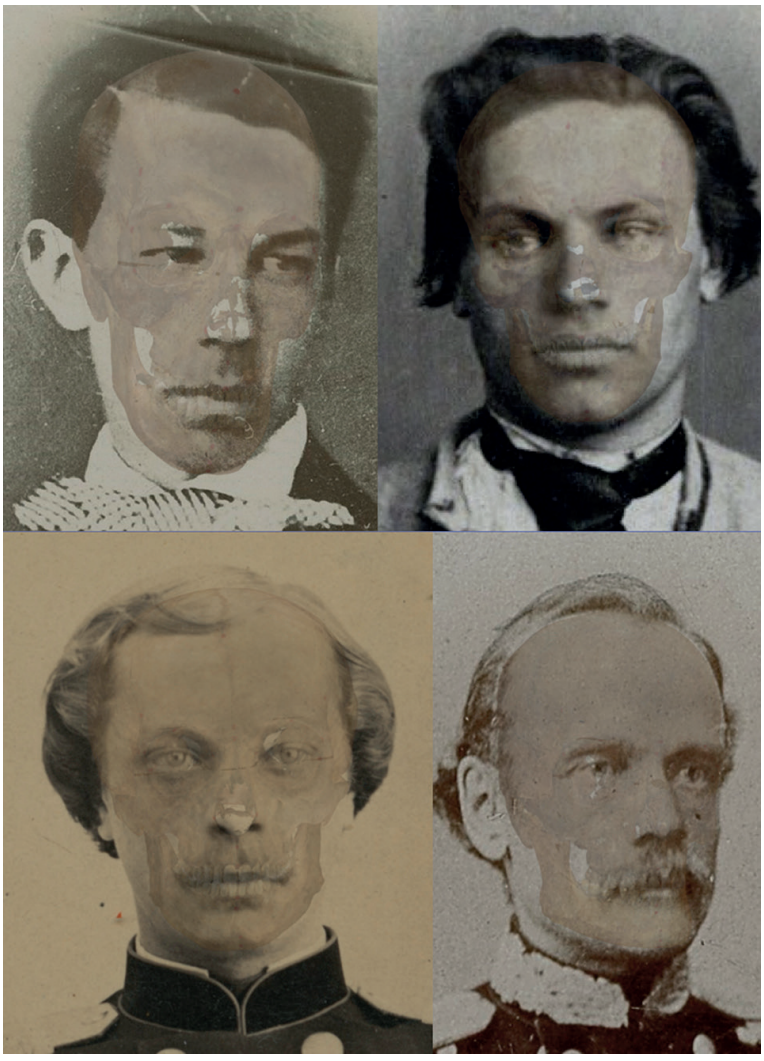

Fig 3.- Automatic SFOs achieved by POSEST-SFO algorithm (Valsecchi et al., 2018) over three different photographs in different views.

Skull-Face Overlay Assessment and Decision Making

In the stage 3 of the CFS process, the forensic expert has to make a decision to determine the degree of support that the skull and facial photographs belong to the same person or not. This decision is made through the analysis of the previous SFOs, and is influenced by several criteria assessing the skull-face anatomical correspondence. Different authors have defined and classified those criteria in four different families (Chai et al., 1989; Austin-Smith and Maples, 1994; Yoshino et al., 1995; Jayaprakash et al., 2001; Ricci and Apostol, 2006); (1) Analysis of the consistency of the bony and facial outlines/ morphological curves; (2) Assessment of the anatomical consistency by positional relationship; (3) Line location and comparison to analyze anatomical consistency; and (4) Evaluation of the consistency of the soft tissue thickness between corresponding cranial and facial landmarks. This is a subjective process that relies on the forensic expert's skills and the quantity and quality of the materials used.

There are just a few works tackling the automation of the analysis of craniofacial correspondences within the framework of CFS identification (Pesce et al., 1993; Yoshino et al., 1997; Ricci et al., 2006). Most of the existing literature was published more than 20 years ago and the works are very basic and limited. In addition, they do not consider the use of either skull 3D models or computer techniques to perform the skull-face overlay. Besides, the technique employed for the shape analysis implies manual interaction. They provide a value that does not take into account the actual spatial relation between skull and face, since the methods employed are invariant to translation, scale and rotation. Finally, these systems only implement a single group of the criteria to assess the craniofacial correspondence.

Recently, Campomanes-Alvarez et al. (2016, 2018a; 2018b) presented a hierarchical system for the decision-making stage and computer-vision algorithms to evaluate the anatomical consistence of morphological criteria between the face and the skull. This means that, from a series of SFOs of the same individual, the Decision Support System (DSS) provides to the forensic expert a quantitative output value indicative of the morphological matching consistency of a given CFS problem. This quantitative value is based on the use of several skull-face anatomical criteria combined at different levels by means of fuzzy aggregation functions, considering the evaluation of the anatomical correspondence between the skull and the face at three different levels: criterion evaluation (level 3), SFO evaluation (level 2), and CFS evaluation (level 1).

The sources of uncertainty and degrees of confidence involved in the process (bone preservation and 3D model quality, discriminatory power of each individual criterion, biological profile influence) were modeled and taken into account at each level of the system (Campomanes-Alvarez et al., 2018a). With the aim of comparing the performance of real forensic practitioners with CFS DSS, the system was applied to the same experimental dataset of Ibáñez et al. (2015). In their study, 26 participants from 17 different institutions were asked to deal with 14 identification scenarios, some of them involving the comparison of multiple candidates and unknown skulls. A total number of 60 SFO problems were tackled. The designed DSS can be considered the first automatic tool for classifying couples of unknown faces and skulls, as positive or negative cases with accuracy similar to the best performing forensic expert (Campomanes-Álvarez et al., 2018b). However, one of the conclusions reached was that the identification results based on the performance of the DSS could be strongly influenced by the poor quality of some SFOs. In this particular system, all SFOs were automatically obtained by the method developed by Campomanes-Álvarez et al. (2015).

A completely different approach was developed by Nagpal et al. (2017). The authors proposed a model for automated skull recognition without the need of superimposition. They claimed the use of a publicly available dataset, IdentifyMe, consisting of 464 skull images, along with semi-supervised transform learning and unsupervised transform learning models for skull to digital face image matching. In order to automate this process, Singh et al. (2018), proposed a Shared

Transform Model for learning discriminative representations. The model learns robust features while reducing the intra-class variations between skulls and digital face images. Experimental evaluation on the IdentifyMe dataset showcases the efficacy of the proposed model by achieving improved performance for the two protocols given with the dataset.

3D-3D Computer-aided approaches for Craniofacial Superimposition

In recent years, some authors have proposed a 3D skull-3D face approach for CFS unlike the existing traditional (image and video) and computer-aided 2D-3D approaches mentioned above.

Duan et al. (2014) proposed a novel ID method based on the correlation measure between 3D skull and 3D face in morphology. The mapping between skull and face is obtained using canonical correlation analysis. Unlike existing techniques, this method does not need the accurate relationship between skull and face, and only measures the correlation between them. In order to measure the correlation between skull and face more reliably and improve the identification capability of the correlation analysis model, a region fusion strategy is adopted. Experimental results validate the proposed method, and show that the region-based method significantly boosts the matching accuracy. The correct recognition rate reaches 100% using a CT dataset.

Tan et al. (2019) presented another 3D-3D superimposition approach in an effort to contribute to computer-aided CFS. The proposed method emphasizes adherence to two important parameters: (1) maintaining the life-size of the face image in relation to the size of the skull, and (2) orienting the skull on an anthropological basis using selected feature points. The proposed method commences by reconstructing the 3D face model from a given 2D face image using a mean simplified generic elastic model, followed by registering the face model to a 3D skull along the jaw line using the analytical curvature B-spline (AC B-spline). The accuracy index of the registration is then evaluated to suggest the degree to which the face image corresponds to a skull. The superimpositions of positive and negative cases were conducted on a set of 3D skulls versus a set of 2D face images. The accuracy indices of the registration results suggest that the AC B-spline is more robust in 3D-3D superimposition compared to the other existing methods. The full experimental results have demonstrated the potential of the proposed method as an assistive tool to the forensic scientists for craniofacial identification.

DISCUSSION AND FUTURE CHALLENGES

Some efforts have been made to standardize the CFS technique with a major outcome of the MEPROCS project (Damas et al., 2020). However, there is still no consensus on the accuracy of the methods used (Gordon and Steyn, 2012; Jayaprakash, 2017), due to the non-standardized nature of the technique and their lack of associated validation (Stephan et al., 2018).

On the other hand, it has been demonstrated that new technologies are a fundamental pillar on which the rest of the sciences, including Forensic Anthropology, can be based. The computing power and automatic algorithms based on AI will allow forensic experts to address and resolve a greater number of cases in less time, allowing the multiple comparisons of cases in databases of missing people, unidentified skeletal remains, mass disaster victims, terrorism, etc. They also facilitate the numerical analysis of subjective tasks by making the ID methods more objective, supporting the expert in decision making and serving as a better legal guarantee in judicial proceedings. The path taken to date shows that CFS is a useful, fast and reliable methodology that can be used for the identification of subjects when other techniques cannot reach. However, although Computer Science approaches promises significant progress, care must be taken when verifying the outcome of any craniofacial superimposition. It is recommended to use an independent line of biological evidence to mitigate risks of error in the identification when they are available.

Nowadays, the crisis of the deaths of migrants in the Mediterranean is a shocking reality as a consequence of the shipwrecks that leave

thousands of bodies each year on the coasts of Europe. The identification of the majority of these people has not been possible to date and their bodies rest in mass graves, thousands of kilometers far from their families who may suffer the psychological drama of the impossibility of closing the mourning. The right of human beings not to lose their identity after death is a universally recognized right. However, this fundamental right remains unfulfilled for the thousands of migrant men, women and children who die each year in their attempt to move to Europe through the Mediterranean. Although DNA, fingerprints and dental comparison are the primary identification methods, they are not applicable in settings where the deceased are migrants from countries with which there are not collaboration agreements that allow access to dental records, fingerprints or taking DNA samples from close relatives. Among the limited AM material available, there are recent digital photographs provided by families, which open the door to the application of image comparison methods. In particular, the Physical Anthropology Lab of the University of Granada and the Legal Medicine Institutes of Andalusia are starting a mobilization with the aim of promoting the use of the CFS technique in this type of scenarios. CFS is a low cost and fast application technique which will guide the investigations in the identification processes when more AM resources are not available.

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Contributions of facial imaging to human identification in forensic cases: a retrospective study (1996-2019) at the Operative Unit of Anthropology of the 'G. d'Annunzio' University of Chieti-Pescara (Italy)

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SUMMARY

Personal identification in legal proceedings and social matters consists of the description, comparison and correct attribution of some relevant biological individualising characteristics. Determination of an identity is generally achieved through construction of the biological profile (i.e. ancestry, sex, age, stature) and comparison of the individualising characteristics, to obtain a positive match. Although present techniques are mainly focused on identification of unknown decedents, due to more recent proliferation of personal, public and commercial installation of video surveillance systems and to image capture on mobile phones, the identification of living persons through video and images has become a major source of evidence in criminal investigations and at trials. This paper retrospectively evaluates the contributions of anthropological reports to the resolution of personal identification cases at the Operative Unit of Anthropology of the 'G. d'Annunzio' University of Chieti–Pescara, Italy, between 1996 and 2019. Of the 476 forensic facial comparisons carried out, for 151 of them (31.7%) it was not possible to carry out any facial imaging analysis due to diverse factors that affected the images and facial features of the subjects being analysed and compared. Of the remaining comparisons (325; 68.3%), the facial imaging techniques used for identification from video recordings and images were: morphological analysis (174; 53.5%); metric analysis (1; 0.3%); combination of morphological and metric analysis (143; 44.0%); photographic superimposition in combination with morphological and metric analysis (4; 1.2%); and facial approximation (3; 0.9%). The aim of this retrospective analysis was a critical evaluation of the advantages and limitations of the different methods used for personal identification in casework. Despite the challenges of facial imaging for human identification, these techniques represent a very important tool in forensic investigations.

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Key words: Forensic anthropology – Personal identification – Facial morphology – Photo-anthropometry – Facial image comparison

INTRODUCTION

Personal identification represents an essential step in legal proceedings and social matters, and it consists of the description, comparison and correct attribution of relevant biological characteristics that can be used to define any specific individual (Gibelli et al., 2016). In the search for a positive match, determination of an identity can generally be divided into two steps: (i) construction of the biological profile, which will include ancestry, sex, age and stature; and (ii) comparison of the individualising characteristics. Present-day techniques mainly focus on the identification of unknown decedents (Ubelaker et al., 2019). However, due to the proliferation of personal, public and commercial installation of video surveillance systems and to image capture technology that comes as an accessory to mobile phones, the use of video recordings and images for the identification of living persons now provides investigative opportunities that can be used as sources of evidence in criminal investigations and at trials (Evans, 2014; Valentine and Davis, 2015). Thus, forensic facial image comparison is defined as the identification or exclusion of a subject depicted within the imagery via analysis of their facial features.

The Facial Identification Scientific Working Group (FISWG; https://fiswg.org) and the European Network of Forensic Science Institutes (ENFSI; http://enfsi.eu/) are active working groups in which the aim is to develop standards, guidelines and best practices for facial image comparison. However, the details for the standardisation of the processes involved in forensic image comparison of these working groups are vague, and the need for specific guidelines that are agreed upon by the forensic community remains an unsolved problem. Currently, the three main techniques used for forensic facial image comparison are morphological analysis, photo-anthropometry and superimposition (Oxlee, 2007; Ritz-Timme et al., 2011; Evans, 2014; Gibelli, et al., 2016; ENFSI, 2018; FISWG, 2018).

Morphological analysis is a subjective process that is based on the observation and comparison of the shape, appearance, presence and/or location of facial features, to define apparent differences and similarities between subjects depicted in the different images used for the comparison. These features include global (i.e. overall face), local (e.g. anatomical structures, such as eyes, nose, mouth, and their components, such as eyeball prominence, nasal root, nostrils, philtrum) and discriminating characteristic facial marks (e.g. scars, moles, wrinkles) (Fig. 1). Morphological analysis is undertaken in a systematic manner and includes a pre-determined list of the features to be compared for every examination, thereby facilitating the structuration and documentation of comparisons, as well the replication of the identification process. Although a variety of facial feature lists are available (e.g. Vanezis et al., 1996; Ohlrogge et al., 2008, 2009), FISWG and ENFSI do not currently endorse any specific list. Morphological comparison is usually sensitive to loss of image quality (e.g. blurring, reduction in spatial resolution), reducing the visibility of gross details (e.g. specific shape of the eyes, mouth and nose), and reducing or eliminating the visibility of fine details (e.g. freckles, creases on the face). Photo-anthropometry is an approach that is based on the measurement of a range of dimensions (e.g. spatial distances, angles) of anthropological landmarks and other facial features, to quantify their characteristics and proportions (Fig. 2). The measurements collected are then compared between two facial images to determine the level of similarity or dissimilarity. Absolute measurements by themselves are a very inaccurate means of comparison, while normalized proportionality indices calculated as a proportion between two absolute measurements are more suitable, but also have limitations. The use of indices does not overcome problems arising from extrinsic factors such as subject distance to camera, focal length of lens, camera angle or orientation of the head. Thus, indices and angles must be used when exact camera positions and subject-to-camera distances are known (Fig. 3). Moreover, photo-anthropometry is extremely sensitive to loss of image quality (e.g. blurring, reduction in spatial resolution, lens or perspective distortion), which

reduces the ability to determine the specific location of facial landmarks, and consequently reduces the accuracy of all measurements. Superimposition is the process of combining two facial images to highlight potential similarities or dissimilarities. Using video techniques and digital image processing, images can be combined using either reduced opacity overlays, to merge certain parts of the face within another facial image, or by rapidly alternating between two images (Fig. 4). In addition, various fading mechanisms can be used to combine images, such as visual flicker and vertical, horizontal or diagonal wiping, so that a line erasing part of one image reveals part of the second. Superimposition is also sensitive to loss of image quality, reducing the ability to determine the specific location of individual features, which subsequently reduces the ability to generate an accurate superimposition (Oxlee, 2007; Ritz-Timme et al., 2011; Evans, 2014; Gibelli, et al., 2016; ENFSI, 2018; FISWG, 2018). Choice of the specific technique to be used is directly related to the quality of the images to be compared, and generally a fusion of these different techniques is applied in real-life analysis in forensic settings.

This retrospective study presents data from a variety of anthropological reports that were evaluated with a focus on their investigative findings, to better understand the advantages and limitations of the different techniques used in personal identification in forensic cases.

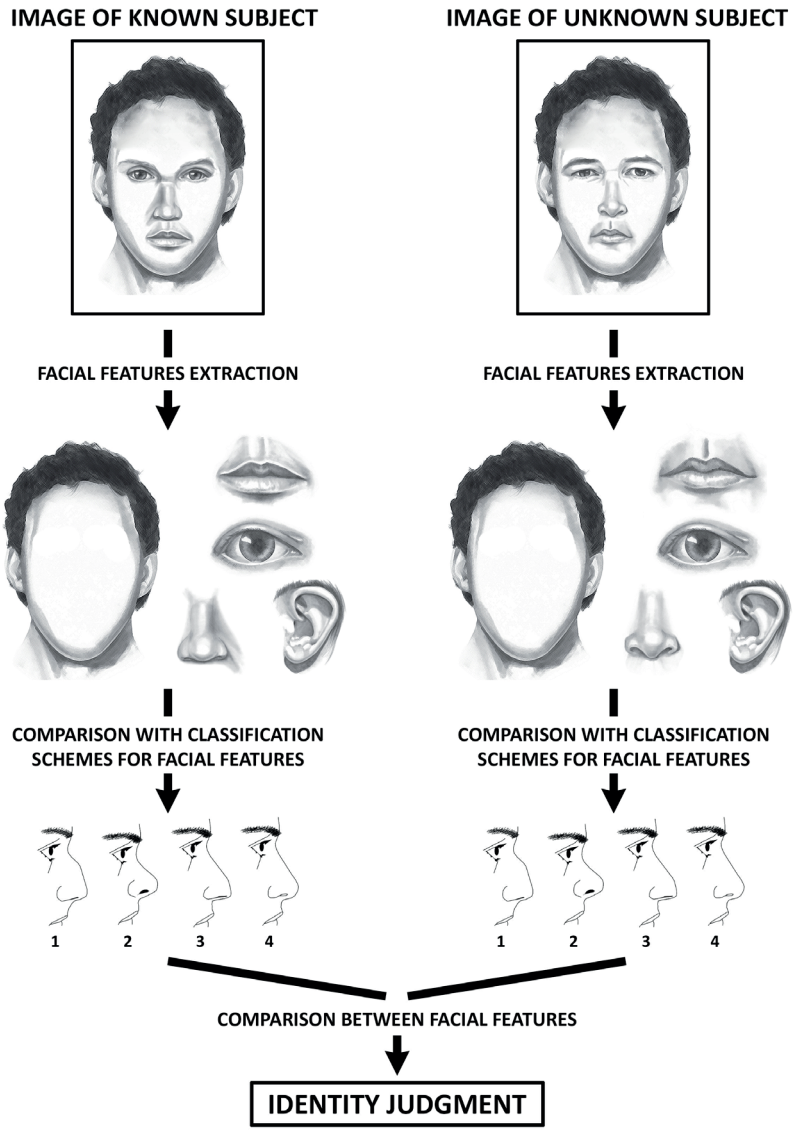


Fig. 1.- Flow diagram illustrating the identification procedure through the characterization of global and local facial features and their components. Morphological analysis is a facial comparison technique in which the features of the face of known and unknown subjects are described and compared.

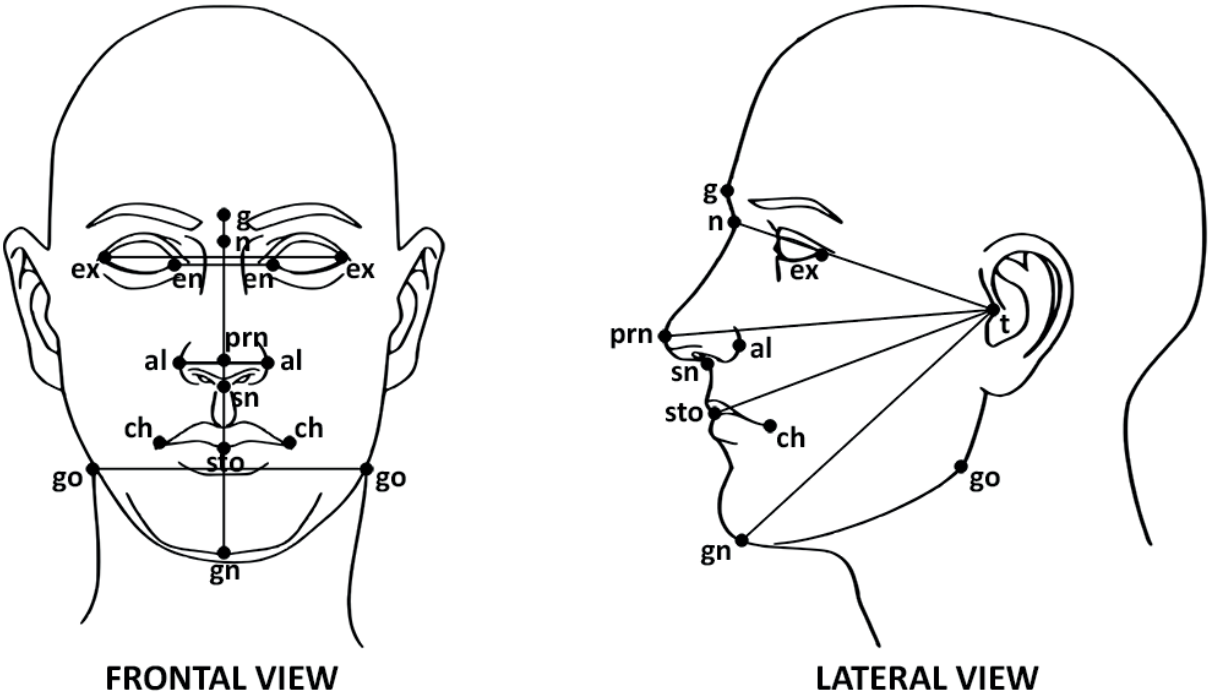


Fig. 2.- Location of some facial landmarks. Frontal and lateral views also show the location of some linear distances and angles. Abbreviations: al, alare; ch, cheilion; en, endocanthion; ex, ectocanthion; g, glabella; gn, gnathion; prn, pronasale; n, nasion; sn, subnasale; sto, stomion; t, tragion.

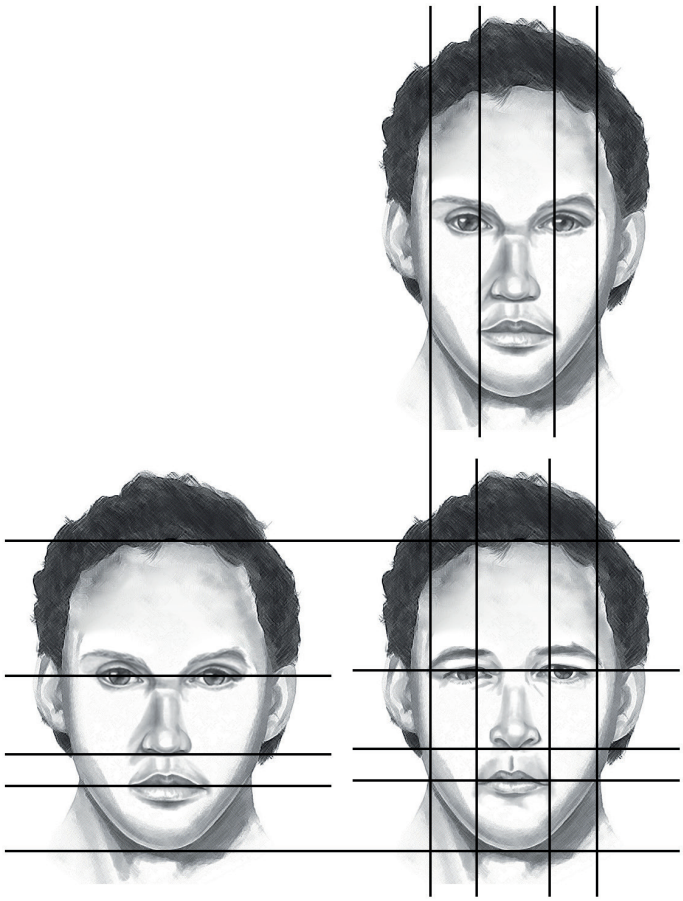


Fig. 3.- Facial landmark alignment. Various facial landmarks are identified on the face and lines are overlaid onto the images at the location of the determined landmarks. The distances between the various landmarks are then compared between the two facial images to determine the level of similarity or dissimilarity, through comparison of the proportions of the distances between landmarks. This Figure illustrates how the results of photo-anthropometrical analysis could be presented by a forensic anthropologist in Court. Accompanying the figure would be a table detailing the measurements in terms of (i) horizontal distances —e.g. expressed as proportions of the distance between the bigonial width (mandibular width)—, and (ii) vertical distances —e.g. expressed as proportions of the distance from the gnathion (chin) to the line crossing the centre of the two pupils (eyes).

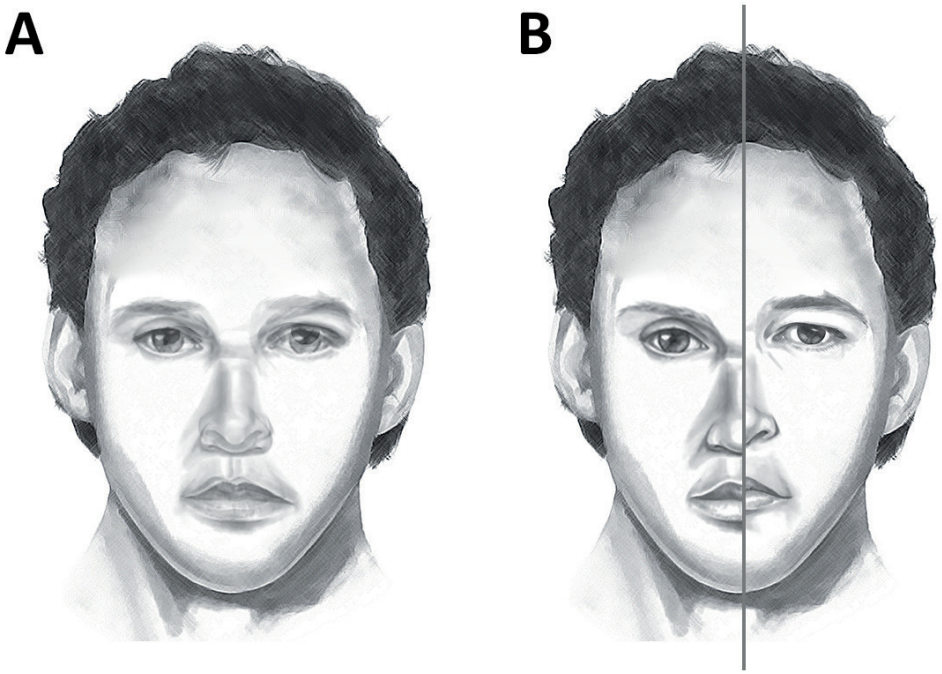


Fig. 4.- Photo-photo superimposition. (A) In this comparison, the face of the unknown subject has been superimposed on the face of the known subject using a reduced opacity overlay. As can be seen there is no difference in the overall face shape and the hairline between the two subjects. However, there are differences in the overall shape, size and position of the eyebrows, eyes, nose and mouth. These differences provide potential indications for exclusion of identity. (B) In this comparison, the right half of the face of the known subject is placed against the left half of the face of the unknown subject (resulting in a chimeric image) to determine if they match. In this case, the differences between the two subjects is more evident.

METHODS

Forensic anthropological reports performed at the Operative Unit of Anthropology of the Department of Medicine and Ageing Sciences of the 'G. d'Annunzio' University of Chieti–Pescara (Italy) were retrospectively evaluated, from 1996 to 2019. These forensic anthropological reports had been performed either at the behest of the *Procura della Repubblica* (Public Prosecutor's Office) or the *Giudice per le Indagini Preliminari* (Preliminary Investigations Judge), or at the request of a lawyer of a victim, the Institute of Legal Medicine, or the law enforcement agencies from national regions or from abroad, all assigned under the responsibility of one of us (L.C.).

For the purpose of the data analysis, the following parameters were considered: geographic location from which a forensic anthropological report was requested; year where the forensic anthropological report was made; requesting Institution for the forensic anthropological report; type of crime/felony committed; number and type of personal identification techniques performed; source of reference images; limiting factors that affected the personal identification process; qualitative scale of level of confidence in the determination of the identification process.

RESULTS

The initial evaluation of the forensic anthropological reports revealed that of the 103 reports included, 84 (81.6%) were reports related to the resolution of personal identification cases. The rest (19; 18.4%) referred to medico-legal death investigations on decomposed cadavers and human remains that were not specifically related to personal identification (e.g. for determination of cause and manner of death, date of death). Thus, only the data for the 84 forensic anthropological reports related to personal identification cases were included in the following analysis.

Geographic location

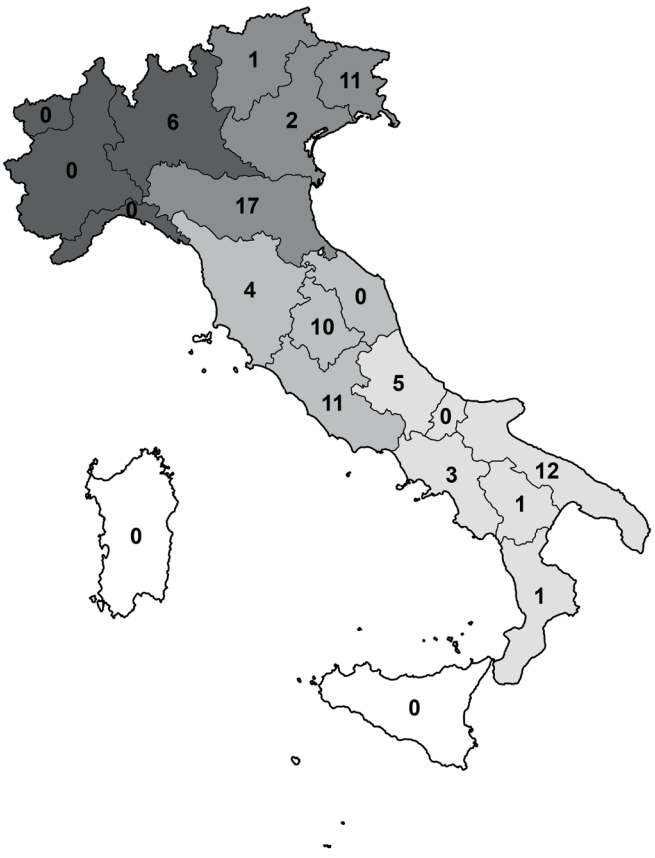
The geographic location is the geographic region from which a forensic anthropological report was requested. For data evaluation, the Nomenclature

of Territorial Units for Statistics (NUTS) standard was adopted. The NUTS standard was developed and is regulated by the European Statistical Office (Eurostat), to provide a single uniform breakdown of territorial units for the production of regional statistics for the European Union (for more information on NUTS, see Eurostat webpage: https://ec.europa.eu/eurostat/web/nuts/background).

Figure 5 summarises the geographic locations for all of the 84 forensic anthropological reports related to personal identification cases performed by the Operative Unit of Anthropology at the request of different Institutions or Agencies. According to the second-level NUTS, of the 20 regions into which the Italian territory is divided, the Operative Unit of Anthropology performed personal identification reports requested by different Institutions in 13 regions (65.0% of all regions). According to the first-level NUTS, of the five macroregions evaluated, the macroregion with the highest number of personal identification reports corresponded to north-eastern Italy (31; 36.9%), followed by central Italy (25; 29.8%), southern Italy (22; 26.2%) and north-western Italy (6; 7.1%). No reports were requested from the island territories around Italy.

Time period

Figure 6 shows that the total number of personal identification reports investigated fluctuated throughout these recent decades, with no cases in 2010 and 2011, and a maximum of nine cases in both 1998 and 2004. Between 1996 and 2006, the number of reports requested followed a fluctuating trend, successively decreasing and increasing, in alternating periods (with a mean of six personal identification reports per year over this period). The number of reports decreased a little between 2007 and 2009 (mean of three reports per year over this period). Finally, the number of reports decreased strongly between 2010 and 2019, with a mean of one report per year related to personal identification.



North-West	
Aosta Valley	0
Liguria	0
Lombardy	6
Piedmont	0
North-East	
Emilia-Romagna	17
Friuli-Venezia Giulia	11
Trentino-South Tyrol	1
Veneto	2
Centre	
Lazio	11
Marche	0
Tuscany	4
Umbria	10
South	
Abruzzo	5
Apulia	12
Basilicata	1
Calabria	1
Campania	3
Molise	0
Islands	
Sardinia	0
Sicily	0

Fig. 5.- Geographical location for all 84 forensic anthropological reports related to the resolution of personal identification cases performed by the Operative Unit of Anthropology of Chieti.

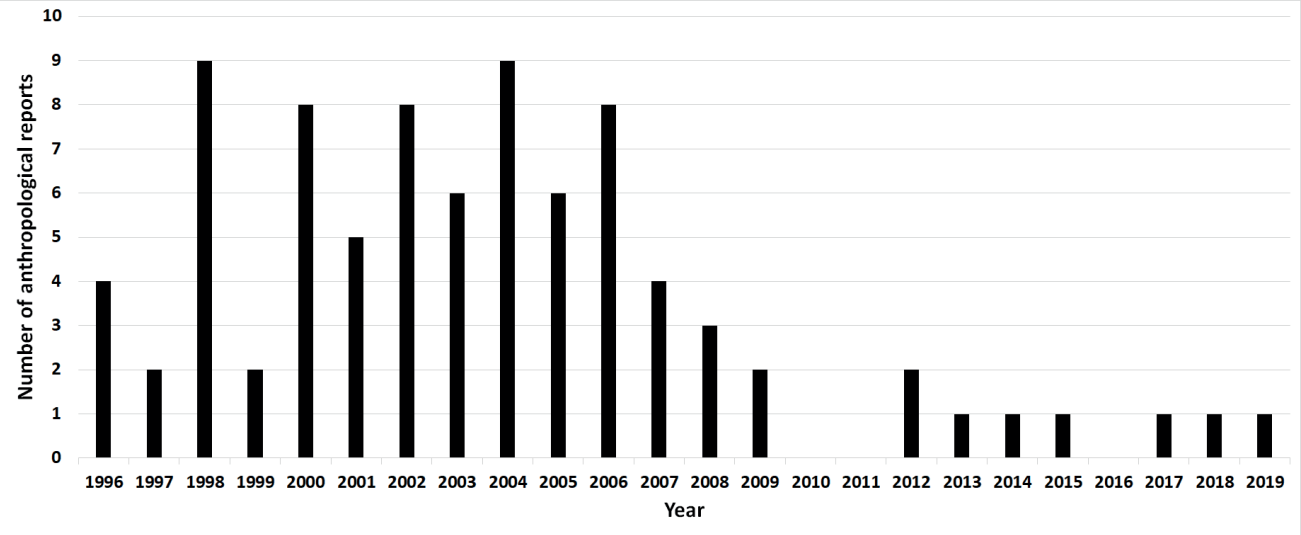


Fig. 6.- Number of personal identification reports performed by the Operative Unit of Anthropology of Chieti per year.

Requesting Institutions

In all, 96.4% of the requests of forensic reports related to personal identification came from the Public Prosecutor's Office (55; 65.5%) and Preliminary Investigations Judges (26; 30.9%), while 3.6% of the requests came from the Institute of Legal Medicine (1; 1.2%) and the lawyers of a victim (2; 2.4%). No requests came from law enforcement agencies (Table 1).

Table 1. Personal identification reports per requesting Institution.

Institution	Number	Proportion (%)
Public Prosecutor's Office	55	65.5
Preliminary Investigations Judge	26	30.9
Law enforcement agencies	0	0
Institute of Legal Medicine	1	1.2
Lawyer of a victim	2	2.4
Total	**84**	**100**

Type of crime/ felony committed

Robberies (including mugging, snatch theft) represented 85.7% (72) of the forensic reports that the different Institutions requested for the resolution of personal identification cases. The other crimes and felonies represented a relatively small number of cases (12; 14.3%) (Table 2).

Table 2. Personal identification reports per crime or felony.

Crime/ felony	Number	Proportion (%)
Bank robbery	64	76.2
Retail store robbery	7	8.3
Motor vehicle theft	1	1.2
Terrorist act	5	5.9
Homicide	3	3.6
Agression	1	1.2
Extortion	1	1.2
Unknown subject near a crime scene	2	2.4
Total	**84**	**100**

On the other hand, 96.4% (81) of the forensic reports represented cases of personal identification of living subjects who were suspected of participating in the different crimes evaluated, while only 3.6% (3) related to the identification of cadavers. In the latter, these three cases referred to were criminal homicides in which the victim to be identified was in an advanced state of decomposition or was completely skeletonised.

Number and type of personal identification techniques performed

Of the 84 forensic anthropological reports related to personal identification processes, 26 (31.0%) were related to the identification

of a single subject at a single crime scene (e.g. robbery at a retail store by a single subject, identification of a cadaver at the scene of a crime). The remaining 58 reports (69.0%) corresponded to multiple identifications, which ranged from simpler investigative situations (e.g. bank robbery by two unknown subjects, and with two suspects to be identified) to more complex situations (e.g. robberies in eight different banks by eight unknown subjects, with unequal participation of each in the robberies, and with six suspects to identify). In this way, the level complexity of each of the forensic anthropological reports led to a total of 476 personal identification processes.

From these 476 forensic facial comparisons (Table 3), for 151 (31.7%) it was not possible to carry out facial imaging analysis due to diverse factors that affected the images and facial features of the subjects being analysed and compared (see below). Of the remaining comparisons (325; 68.3%), the facial imaging comparison techniques used for identification from the video recordings and photographic images were: morphological analysis (174/325; 53.5%); metric analysis (1/325; 0.3%); combination of morphological and metric analysis (143/325; 44.0%); photographic superimposition in combination with morphological and metric analysis (4/325; 1.2%); and facial approximation (3/325; 0.9%).

Table 3. Comparisons performed per facial imaging technique applied.

Facial imaging technique	Number	Proportion (%)
Facial imaging analysis not performed	151	31.7
Morphological analysis	174	36.6
Photo-anthropometry analysis	1	0.2
Facial superimposition	0	0
Morphological analysis + photo-anthropometry analysis	143	30.1
Morphological analysis + photo-anthropometry analysis + facial superimposition	4	0.8
Facial approximation	3	0.6
Total	**476**	**100**

Source of reference images

Excluding the three cases of facial approximation, of the 473 forensic facial comparisons carried out using images, photographic images were provided for the forensic analysis in only 10 cases (2.1%) (Table 4). These images corresponded to analogue images (photographs) printed on photographic paper. Most of the images analysed (330; 69.8%) corresponded to video recordings, which were mainly from closed-circuit television systems (CCTV). Of these, 296 (89.7%) originated from images from consumer-level analogue video recordings on magnetic tape cassettes, in either VHS or Betamax format, while for 34 (10.3%), they were recordings obtained with digital video cameras, as .mov or .mpeg files. These videos corresponded to reliable analogue and digital copies of the original videos that recorded the different crimes or felonies.

Table 4. Source of reference images for forensic facial image comparisons.

Image source	Image form	Number	Proportion (%)
Video	Analogue	296	62.6
	Digital	34	7.2
Photography	Analogue	10	2.1
	Digital	0	0
Film frames	Extracted (analogue video)	113	23.9
	Extracted (digital video)	20	4.2
Total		**473***	**100**

*The three cases related to facial approximation were excluded from this analysis.

In a relatively large number of these forensic facial comparisons carried out using images (133/473; 28.1%), the requesting institutions did not provide copies of the originals, but provided film frames extracted from the original video. These film frames were provided on different media: printed on coloured or black-and-white photographic paper (56; 42.1%), printed on inkjet paper (13; 9.8%); and film frames converted to digital images as .bmp, .jpg or .tiff files (64; 48.1%).

Limiting factors that affect the personal identification process

As indicated above, the imagery came from a wide range of sources. To accurately interpret

the content of an image for facial image comparison, it is imperative to recognize the limiting factors that can occur during the image capture. Table 5 shows the main factors that affected the facial appearances of the subjects in the images for the different forensic facial comparisons performed.

In 31.9% of the cases (151/473, excluding the three cases related to facial approximation; Table 3), the process of facial image comparison was not completed due to critical factors that affected the images and the facial features of the subjects being analysed and compared. The impact of one or the combination of some of these factors precluded the process of facial image comparison, such as facial expressions, occlusion of facial features and aging, as well as extrinsic factors, such as image quality and illumination, camera viewpoint and geometry of the scene (Table 5). In the rest of the cases (322; 68.1%), despite the combined impact of some of the factors on the facial appearance of the subjects, it was possible to proceed with the facial image comparisons.

As a whole (473), the main limiting factors that affected the images and the facial features of the subjects were: occlusion (266; 56.2%); image resolution/ distance from camera (221; 46.7%); image compression (167; 35.3%); camera/ angle pose (104; 22.0%); and number of available images for analysis (61; 12.9%) (Table 5). The other factors involved to a lesser extent (<10%) were: facial expression, growth and aging of the subject, and exposure and illumination of the crime scene.

Identity determination

The evidence in forensic science must be presented in a way that can be accommodated within the proof process used by judges and juries (Ligertwood and Edmond, 2012). This is a non-mathematical inductive process that seeks "the inference to the best explanation" to a test standard of "beyond reasonable doubt". In the field of facial imaging comparisons, to express the identification value of the comparisons, we used the four-point qualitative scale of support of confidence used by both the *Polizia Scientifica* of the *Polizia di Stato* (Scientific Police, of the State Police Force) and the *Ragruppamento Carabinieri Investigazioni Scientifiche* (Scientific Investigations Department of the Military Police Force) (Table 6).

Of the 322 facial image comparisons performed, 204 (63.4%) excluded the subject under investigation as the one who committed the crime or felony (Table 7). Only 53 (16.5%) cases allowed positive identification of the subject investigated, which was mainly due to distinctive facial marks, dental features and ear morphometrics. In 52 (16.1%) cases, the facial comparisons only provided presumptive or tentative (13; 4.0%) identification.

Table 5. Factors affecting facial appearance.

Facial image comparison						
	Not performed		Performed		Total	
Factor	**Number**	**Proportion (%)**	**Number**	**Proportion (%)**	**Number**	**Proportion (%)**
Image resolution/distance from camera	97	64.2	124	38.5	221	46.7
Image compression	94	62.3	73	22.7	167	35.3
Exposure	8	5.3	8	2.5	16	3.4
Lighting	5	0.4	0	0	5	1.1
Occlusion	121	80.1	145	45.0	266	56.2
Camera angle/pose	70	46.4	34	10.6	104	22.0
Number of available images	37	24.5	24	7.6	61	12.9
Expression	2	1.3	5	1.6	7	1.5
Growth and Ageing	0	0	18	5.6	18	3.8

*The three cases related to facial approximation were excluded from this analysis.

Table 6. Qualitative scale of support of confidence used for personal identification.

Scale	Definition
Non-compatibility (negative identity)	In the two images depicting the subjects in the comparison there is at least one feature (not artificial or alterable through time) that allows it to be excluded that the two subjects in the analysis portray the same subject.
Affinity	The poor definition and/or visibility of at least one of the two images in the comparison does not allow detection of facial features to allow a positive judgment of comparison to be reached; however, there are some similar facial features in both of the subjects in the comparison.
Compatibility	The facial components of the two subjects in the comparison make it possible to detect numerous similar facial features for both of the subjects. However, given the poor definition of at least one of the images in the comparison, it is not possible to highlight distinctive marks (e.g., scars, freckles, moles, acne, birth marks, bruises, abrasions, characteristic folds) in the two subjects compared that would lead to a judgment of non-compatibility or, conversely, of total compatibility.
Total compatibility (positive identity)	The two subjects in the comparison images are similar in shape and proportions for all of the facial features visible. There are also singular anatomical features and distinctive marks seen for both of the subjects in the comparison.

Table 7. Strength of the forensic facial image identifications performed.

Scale of support of confidence	Number	Proportion (%)
Non-compatibility	204	63.4
Affinity	13	4.0
Compatibility	52	16.1
Total compatibility (positive identity)	53	16.5
Total	**322***	**100**

* This number corresponds to the number of facial image comparisons performed, excluding the three cases of facial approximation and the 151 cases where none of the facial identification techniques could be applied.

DISCUSSION

According to the criminologist Paul Tappan, "crime is an intentional act or omission in violation of criminal law, committed without defence or justification, and sanctioned by the state as a felony or misdemeanour" (Tappan, 1960). Thus, crime is a deviation from the social norms administered by law that adversely affects the society in which we live, and there is the need to accurately identify the perpetrators of such criminal acts. Personal identification of the living from images is becoming highly relevant due to the increasing number of crimes recorded as videos or photographs (Porter, 2011; Evans, 2014). Thus, forensic investigations require rigorous, accurate and validated techniques to evaluate facial features from images, which can consequently allow comparisons between living subjects and subjects depicted in images, to verify whether a subject seen on a specific image is the same subject as the suspect of the crime. Regardless of the technique chosen for forensic facial image comparison, the reproducibility and accuracy of the conclusions that can be drawn from comparisons of images are directly related to the quality of the images (FISWG, 2019a).

An image can often be difficult, and sometimes almost impossible, to interpret due to various factors that have a negative effect, such as technical factors (e.g. camera resolution and angle, image compression, lens distortion), environmental factors (e.g. illumination of the scenario, occlusion of facial features by objects or clothing) and the facial expression, pose, growth and ageing of the subject, among other aspects (Wilkinson and Evans, 2009; Kaur et al., 2015; ENFSI, 2018; FISWG, 2019b). All of the images analysed by the Operative Unit of Anthropology included one or a combination of some of these factors that affected their quality. Approximately one third of the comparisons performed (31.9%) were far from ideal, and were not useful for the application of any of forensic facial identification techniques because of the poor quality of the imagery. However, as Bromby (2003) asserted, the face of an offender can be identifiable in images if

the quality of the video recording or photograph is sufficient to appreciate the details of the face. In our forensic casework, approximately two thirds of the comparisons performed (68.1%) were based on sub-optimal images, although these still allowed the application of forensic facial comparison techniques.

Although this retrospective analysis extended to the year 2019, the vast majority of the analysed images (88.6%) were obtained from analogue photographs, CCTV cameras or small-scale security systems based on magnetic tape cassettes, like VHS and Betamax tapes. The quality of the images from these systems was low, but their massive spread into the security of our everyday life has resulted in the wide use of such images in forensic contexts for personal identification purposes (Oxlee, 2007; Caplova et al., 2018). In all, 11.5% of the images came from digital sources; however, these systems of video surveillance had also often been installed with little attention to optimisation of the illumination conditions or the viewing angle. All of these situations mean that when a video recording or an image is needed for evidence, such as after a crime, it is not always useful for these personal identification processes.

The different facial image comparison techniques that can be used not only require a minimum of training for the competency of the forensic examiner, but also an understanding of the strengths and weaknesses of the various techniques available. As a consequence, the accuracy of any conclusions that can be drawn from forensic facial image comparison are directly related to the level of training achieved by the forensic examiner (Oxlee, 2007; FISWG, 2010; Evans, 2014). Active working groups such as FISWG and ENFSI recommend morphological analysis as the primary method of comparison, and the use of superimposition techniques only in conjunction with morphological analysis (FISWG, 2012; ENFSI, 2018). However, both working groups and other studies (e.g. Kleinberg et al., 2007; Moreton and Morley, 2011) do not recommend the use of photo-anthropometry for forensic facial comparison.

In the evaluation of our forensic casework, morphological analysis was used as the only technique for personal identification in 53.5% of the facial comparisons, with only photo-anthropometry used in 0.3% of comparisons, with the superimposition technique not used in these cases. However, photo-anthropometry and superimposition were used in conjunction with morphological analysis in 44.0% and 1.2% of the comparisons, respectively. Although photo-anthropometry is not considered reliable for positive identifications, if combined with morphological analysis, it can potentially provide indications for exclusion of identity (Moreton and Morley, 2011; Arbab-Zavar et al., 2015; Gibelli, et al., 2016). For example, in one of our forensic anthropological reports, both the offender of a bank robbery and the suspect of this criminal act shared similar facial features. In addition, both had a scar on the right parietal of the head. However, although these subjects shared similar facial features, the size and topographic location of the scar with respect to the right ear allowed the suspect to be excluded as the perpetrator of the bank robbery.

According to Ali et al. (2010), even though there is a consensus in the categorisation of the different forensic facial image comparison techniques to be used, there are currently no defined standard procedures or specific guidelines within the forensic community. Conclusions based on such comparison processes can be very subjective, and the opinion of one forensic examiner can be different from that of another (Evans, 2014).

Christensen and Anderson (2013) stated that personal identification usually includes tentative, circumstantial, presumptive and positive types. The first three of these indicate that the actual identification cannot be excluded, and therefore image-based evidence might represent a particular individual. Positive identification, however, represents a higher standard of probability, and indicates that the facial features being examined and that are shared by both the offender and the suspect of a criminal act are sufficiently exclusive or unique to enable an identification. Current terms used to qualify an identification (e.g. “circumstantial”, “positive”, “consistent with”, “presumptive”, “possible”, “probable”) are also relatively problematic and

can create numerous inconsistencies between forensic disciplines and in the law courts. Thus, no consensus exists within the forensic community as to the use of these terms (Anderson, 2007; Evans, 2014). Furthermore, there needs to be continual awareness of any situations where misidentification might occur. Great caution is needed in the interpretation of forensic facial image comparisons, as misidentification can produce dire consequences, not only from a societal and legal standpoint, but also through the substantial impact on the families and friends of a suspect (Prahlow, 2010).

We use a simple four-point qualitative scale of support of confidence that is used by the Italian law enforcement agencies to quantify the strength of any image-based identification. Following this scale, we reached positive or negative identifications in 79.9% of the cases here with a reasonable degree of scientific certainty. The facial comparisons provided tentative or presumptive identifications in only 20.1% of these cases. Note that the determination of positive or negative identification was mainly based on the value of exclusive, unique or highly variable anatomical variables, such as distinctive facial marks (e.g. scars, skin marks), dental features (e.g. loss of teeth, patterns of displaced teeth, unusual rotation) and morphological and metric characteristics of the ears.

On the other hand, a related field in the context of forensic identification is forensic facial approximation. Starting with the skull of a body, this technique aims to reproduce the loss of the unknown face of the subject for recognition or identification purposes (Wilkinson and Neave, 2001; Wilkinson, 2007). Three cases referred to in this retrospective analysis were criminal homicides, where the victim was in an advanced state of decomposition or was completely skeletonised. Despite the advances in the techniques of facial approximation, this technique cannot be used directly for positive identification itself, although it is used to communicate with the public in an effort to collect information about missing persons who shared particular visual and demographic characteristics with the recovered remains (Ubelaker et al., 2019).

From the results of this retrospective analysis, it is evident that the Operative Unit of Anthropology is a reference laboratory for the different regions in which the Republic of Italy is structured and for the different Institutions and Agencies that need investigators in the field of forensic anthropology. From 1996 to 2009 there was a fluctuating trend, with small increases and decreases across alternating time periods for the number of personal identification cases investigated (76; mean of five reports per year). However, there was a clear trend for fewer cases in the first decade of the 2010s (8; mean of one report per year in this period). It is not surprising that this analysis reveals that robberies represented 85.7% of the forensic reports for the resolution of personal identification cases, where the majority of cases analysed were bank robberies (76.2%).

According to Dugato (2014), although there has been a decrease in the number of attacks and in the incidence of crimes committed by professional criminals, bank robbery remains a relevant problem in most European cities. Indeed, within Europe, Italy consistently records the highest number of bank robberies. In Italy, the average bank has a 7% risk of attempted robbery in any given year (Maheshri and Mastrobuoni, 2018). Some Italian studies have argued that unemployment can lead to crime, as a result of the feelings of deprivation, rejection and personal failure (Marselli and Vannini, 1997; Masciandaro, 1999). It is also claimed that unemployment can generate mental stress, apathy and illness, factors that could indeed pave the way to criminal behaviour.

Despite the period of general economic decline observed in the world markets during the late 2000s and the early 2010s, the decreasing trend for forensic anthropological reports of bank robberies to date might also be due to an increase in the joint work of banks and law enforcement agencies in the development of crime prevention and security systems. Indeed, the main results of a survey conducted by the *Centro di Ricerca dell'Associazione Bancaria Italiana sulla Sicurezza Anticrimine* (Ossif-ABI; Research Centre of the Italian Banking Association on Anti-Crime Security; https://www.ossif.it) showed that

between 2007 (first year of data collection) and 2018, bank robberies decreased by 92% in Italy, from 3,364 in 2007, to 264 in 2018. According to Ossif-ABI, Italian banks invest over 600 million euros every year to protect their banks better and to make them safer, through the adoption of increasingly modern and effective protection measures. This includes anti-theft systems and helping with the investigative activities of the law enforcement agencies. This argument is also in line with the results of the present study.

When a criminal action is recorded by a video surveillance system, the first operation performed by the investigator who deals with facial imaging comparisons is to scientifically analyse the videos seized at the crime scene in an attempt to identify the faces of the perpetrators or the interactions between them and the environment. The use of images of the suspected perpetrator of the crime/felony who will be investigated and then accused is provided for in Article 361 no. 2 of the Italian Code of Criminal Procedure, which reads: "People, things and objects are presented [...] in image to those who must carry out the identification".

When the subject of a dispute involves issues that cannot be resolved based on the notions of common sense, the Judge can be assisted by Experts or Technical Consultants with particular technical competence in facial identification through imagery. The figure of the Expert or Technical Consultant in the criminal justice system is regulated by Articles 220 to 232 and 508 of the Italian Code of Criminal Procedure.

From a jurisprudential point of view, the growing availability of recordings of images relating to the dynamics of criminal events, and the urgent applications from the Judicial Authority to ascertain the identity of the perpetrators of the crime/felony, testify to the admissibility of this means of proof in judicial practice giving rise to copious jurisprudence.

The probative validity of facial imaging comparisons, in the absence of a specific rule, is governed by the orientation provided by a sentence of the Italian Supreme Court of Cassation: "The so-called anthropological report is based on a method tested over time and now acquired by the patrimony of the scientific community: so that its results —where the relative technical operations have been correctly performed— can constitute evidences against to the suspects and accused persons. Of course, the "anthropological compatibility" cannot alone constitute serious evidence of guilt; and however such judgment is certainly suitable to reinforce the evidence constituted by the recognition made by a witness; as well as a judgment of 'incompatibility' decreases or even nullifies the scope of that element of proof" (Corte di Cassazione, sentence no. 83, 20 January2004).

Therefore, as regards the probative validity of facial imaging comparisons, the most modern doctrine supports full admissibility in accordance with the principle of freedom of proof, although framing anthropological comparisons as a means of investigation not endowed with autonomous efficacy but to support the personal recognition and of photographic recognition as possible sources of evidence. The Expert's reasoning will determine to what degree the outcome of the process of facial comparison influences the opinion of the Judge: belief in the evidence be high if the Expert's reasoning is convincing, while, if the process is of low reliability, room remains for doubt.

To date, the authors have no knowledge that a video recording has been used successfully, and a recent European directive explicitly states that it is not possible to convict an individual on the sole basis of a video recording. On the other hand, it can certainly be used as a subsidiary fact-finding tool.

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Dermatoglyphics in forensic identification in the XXI Century

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SUMMARY

Dermatoglyphics or configurations of the dermopapillary ridges on the ventral surface of fingers, palms and soles have an early formation during foetal life, and they are determined by a polygenic multifactorial inheritance that gives them a great morphological variability. These biological characteristics have made them useful tools for human identification. The primary objective of this paper is to thoroughly review the state of the art of dermatoglyphics in the field of forensic science. Identification by means of fingerprint comparison, obtained from dermatoglyphics skin configurations, has been accepted by all courts worldwide, as probative evidence of identity. However, some mistakes made in the field of fingerprint in particular, and in forensic sciences in general, combined with increasingly rigorous scrutiny of forensic evidence, have prompted the need to reconsider many of the scientific principles on which these disciplines are supposedly based. Therefore, at the present time it is primarily necessary to conduct basic research, in order to endow these disciplines with the scientific foundation necessary to ensure their quality and robustness. In this way, during the last decade, the development of statistical models for supporting forensic identification with fingerprints has been the subject of increasing attention by the scientific community. Nevertheless, we must consider that any attempt to establish solid scientific bases and develop valid statistical models must be based on an exhaustive analysis of the variability of the different dermatoglyphics characteristics; in this respect, the minutiae are especially important, since they are one of the backbones of fingerprint identification, whether they are used quantitatively (numerical standard) or qualitative.

Key words: Fingerprint – Dermatoglyphics – Identification – Minutiae – Ridge density

INTRODUCTION

In all primate species, including humans, the skin covering the volar surface of the digits, palms of the hands and soles of the feet is not smooth, as on the rest of the body, but is instead characterised by the presence of epidermal ridges separated by furrows. These form a wide variety of patterns, constituting what is known as friction ridge skin.

To facilitate the analysis of these patterns, in 1926 Cummins and Midlo coined the term "dermatoglyphs", etymologically derived from the Greek "*derma*", skin, and "*glyphe*", drawing

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or engraving. Subsequently, use of this term has become widespread in studies of these patterns in various scientific fields, including human biology, physical anthropology, human genetics and medicine. Although they do not strictly form part of a dermatoglyph, flexion creases on the digits, palms and soles, as well as other secondary creases, have also generally been studied under this term (Cummins and Midlo, 1943). Meanwhile, in the field of criminology, the term "fingerprints" refers to the scientific study, detection, classification and comparison of digit, palm and sole prints and marks for the purposes of personal identification in the forensic sciences. The equivalent Spanish term, "*lofoscopia*" (derived from the Greek "*lophos*", ridge, and "*skopein*", study) was coined by the Spanish police inspector Santamaría Beltrán in the mid 20th century. However, the most widely used term is "dactyloscopy", etymologically derived from the Greek roots "*dactylos*", finger, and "*skopein*", study, which refers to fingerprint and finger-mark identification, the most widely studied aspect worldwide. This term was coined in 1894 by the Spanish reporter Francisco Latzina (Gutiérrez-Redomero and Hernández-Hurtado, 2011). In the international literature, the most widely used term in the field is "fingerprints".

For the study of these characteristics, Galton (1892) classified the dermatoglyphic patterns into three main types: arch, loops and whorl (Fig. 1). Arches are the figures formed by parallel ridges, slightly curved, without definitive design, which do not have a *triradius* o delta (point of confluence of three ridge flows from different directions). Loops are figures formed by a field of parallel ridges, whose direction exhibits a 180º turn due to the presence of a *triradius*. Loops can be radial or ulnar, depending on the side of the palm to which the loop opens. Whorls are formed by closed designs, where the field of parallel ridges follows a circular or elliptical path, with 360º turns due to the presence of two *triradii*, although occasionally they can appear with three *triradii*.

In addition, Galton (1892) also proposed the term *minutiae* to refer to the small distinguishing features found along each individual ridge (see Table 1).

BIOLOGICAL CHARACTERISTICS OF FINGERPRINTS

Friction ridge skin is rippled and poorly pigmented, has numerous large sweat glands and does not have hair follicles or sebaceous glands (Fig. 2). Pores of the sweat glands open on the surfaces of the epidermal ridges, and both the morphology of the ridges and the furrows and the pores show variability in size and shape.

The external morphology of this ridged skin is a direct reflection of its function. Primarily, this has traditionally been associated with the prehensile function of the hands and feet, to improve grip during locomotion and when handling objects. Epidermal ridges and sweat gland pores, which are distributed evenly over the surface, endow the hands and feet with a strong grip on a variety of surfaces, while the furrows enable the skin to flex (Cummins and Midlo, 1943). More recently, however, it has been shown that ridge morphology and relief enhance the sense of touch, because these areas have abundant nerve endings that relay the vibrations generated by epidermal ridges when moving over a surface, thus allowing tactile perceptions of texture at very small spatial scales (< 200 micrometres) (Scheibert et al., 2009; Adams et al., 2013).

Epidermal ridge formation begins at a very early stage of embryonic development. At around the 7th week of gestation, volar pads appear on the palms of the hands as accumulations of mesenchymal tissue located beneath the epidermis. Subsequently, they appear on the fingers, from the thumb to the little finger, and later on the toes and the soles of the feet. Around the 10th week of gestation, the volar pads start to retreat, first on the fingers and then on the palms of the hands. Between one and two weeks later, the same occurs on the toes and the soles of the feet. In conjunction, this process produces a proliferation of basal cells that penetrate the dermis, giving rise to primary ridges as a consequence of the interaction between the dermis and epidermis (Fig. 3). Primary ridges are associated with sweat glands, the secretory parts of which are located in the deep dermis while the pores open in the epidermal ridges, constituting glandular folds.

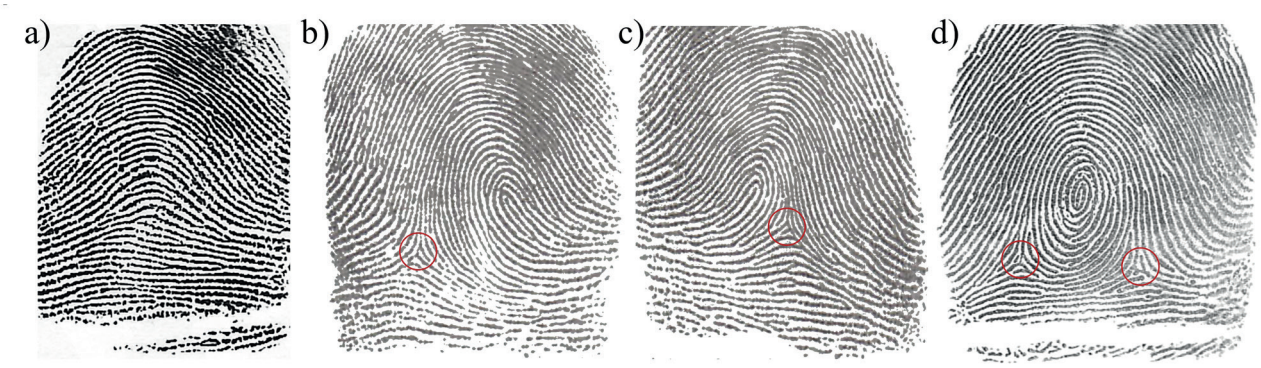

Fig. 1.- Graphical representation of the main types of pattern. Fingerprint of the right hand taken with the ink method. a) arch; b) ulnar loop; c) radial loop; d) whorl. *Triradii* are shown in a circle.

Table 1. Morphology, name, abbreviation and definition of minutiae or characteristic points.

Morphology	Name and abbreviation	Definition of minutiae or characteristic points
	END (E)	Epidermal ridge located between two others, and ending without reappearing.
	BIFURCATION (B)	In the basilar or marginal systems, it is a ridge that is divided in two starting from the left side of the fingerprint; and in the nuclear system when a ridge is divided in two following the clockwise direction.
	CONVERGENCE (C)	In the basilar or marginal systems, two ridges join in one starting from the left side of the fingerprint; and in the nuclear system when two ridges join in one following the clockwise direction.
	FRAGMENT (F)	Variable length ridge with abrupt ends. These minutiae are considered small (F-SM) when their length is less than five times the width of the ridge; they are considered large (F-BG) when their length is five–ten times the width of the dermal ridge.
	BREAK (BR)	Point where a ridge is interrupted, as long as this discontinuity is not less than the width of the ridge either larger than double its width The ends of the ridges that delimit the break will be rounded.
	ENCLOSURE (EN)	Where the ridge path divides and then comes together again. These minutiae are considered small when their length is less than five times the width of the ridge; they are considered large when their length is five–ten times the width of the dermal ridge.
	POINT BETWEEN RIDGE (P-BW)	A small ridge fragment, as short as it is wide, which is situated between two ridges.
	POINT IN RIDGE (P-IN)	A small ridge fragment, as short as it is wide, which is situated in the center of a break.
	OVERLAP (O)	Where two ridges opposite ends meet and overlap on a bias.
	CROSSBAR (CR)	Ridge that separates from its direction crossing between two others.
	BRIDGE (BD)	Where a oblique direction short ridge crosses from one ridge to join the next.
	OPOSSITED BIFURCATION (OP)	Contact point between two ridges that immediately separate forming a cross.
	DOCK (D)	Point formed by three endings, where one of them ends at the beginning of the interpapillary groove formed by the other two ridges that run in the opposite direction.
	TRIFURCATION (TF)	Point where one ridge divides into three ridges. To determine whether it has a bifurcating (TF-B) or convergent (TF-C) direction, the same criteria described in the minutiae bifurcations and convergences must be taken into account.
	M (M)	A merging point of two convergences and one bifurcation (M-C) or viceversa (M-B), which presents a pattern similar to the letter M.
	RETURN (R)	180º turning around of a ridge without being part of the core.

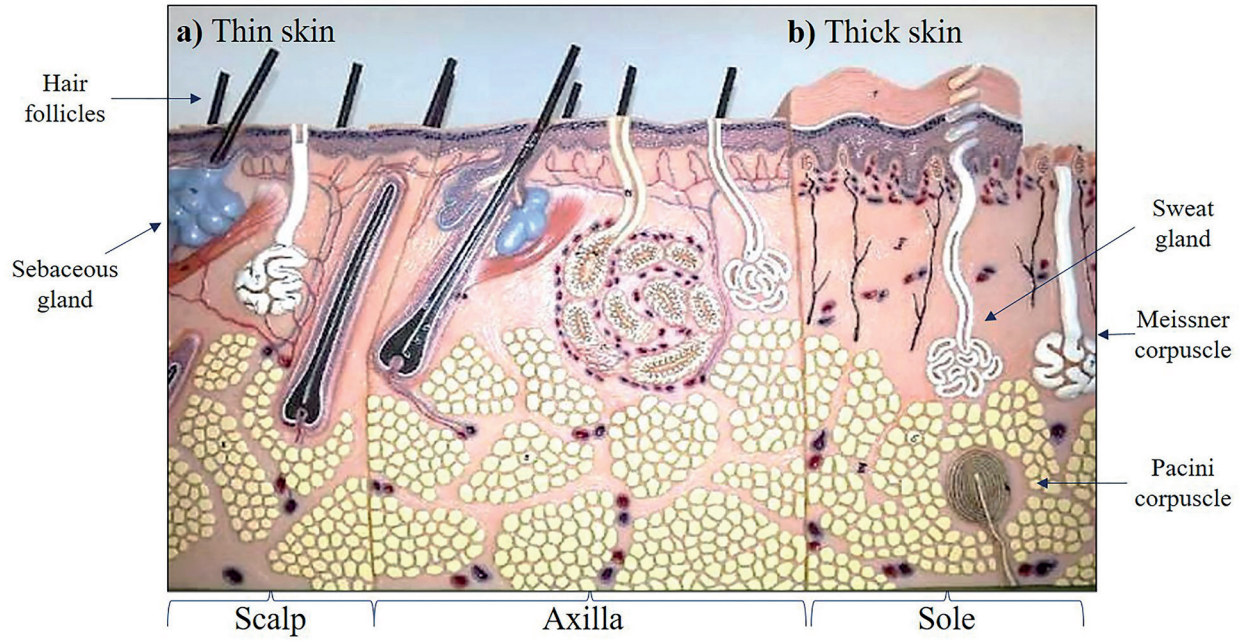

Fig. 2.- Photograph of the comparative diagram SOMSO model between thin skin (a) and thick skin (b), located in the Physical Anthropology laboratory of the University of Alcalá (UAH).

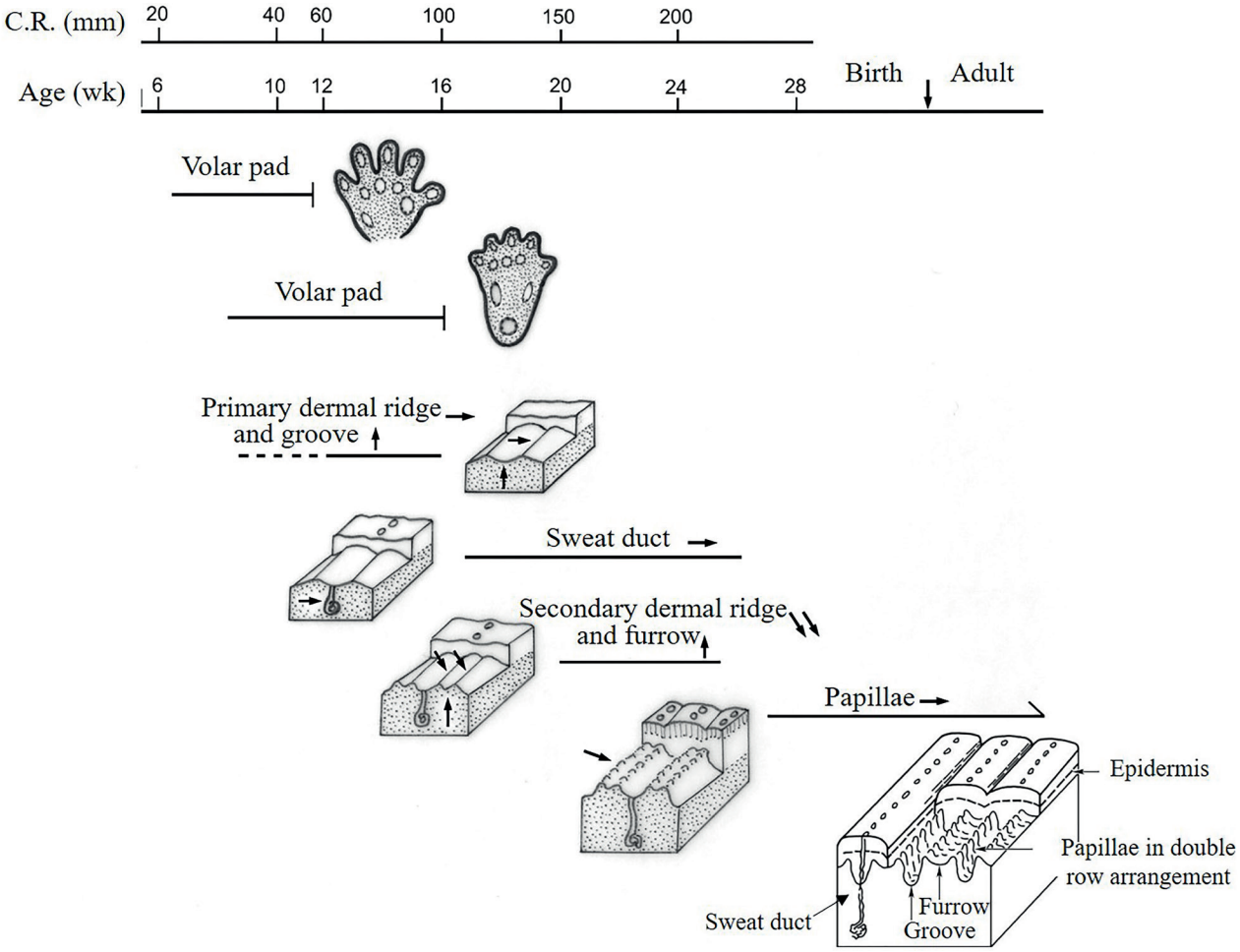

Fig. 3.- Diagram of the process of epidermal ridge formation, modified of the reproduction of Okajima (1975).

In parallel with the formation of primary ridges, the minutiae also begin to develop. These are characteristics that appear along the path of the ridges, forming patterns that can be classified into different types such as bifurcations, ridge endings, dots and islands. The minutiae are also known as Galton details (Okajima, 1975).

The primary ridges continue developing until around the 16th week of gestation. At this point, secondary ridges begin to form, a process that concludes around the 24th week of gestation, coinciding with the rapid development of furrows between the epidermal ridges. Besides time of embryonic development, the main difference between primary and secondary ridges is that the latter are not associated with sweat glands. Dermal papillae form between primary and secondary ridges, increasing the surface area between the dermis and epidermis. The dermal papillae may undergo changes during gestation and even after birth, but such changes do not affect the structure of the epidermal patterns. In the absence of injury, once the epidermal ridges have been formed, they remain substantially unchanged throughout the life cycle until destroyed by decomposition after death. However, although the number of ridges does not change with age, ridge width increases to adapt to the growth and development of the body in general and the hands in particular (Okajima, 1975; Seidenberg-Kajabova et al., 2010; Wertheim, 2011).

GENETIC AND ENVIRONMENTAL DETERMINATION

Although many aspects of the heritability of fingerprint patterns remain poorly understood, family and twin studies have shown that heredity plays an important role in the phenotypic expression of all their characteristics. As discussed by Sara Holt in the text entitled *The Genetics of Dermal Ridges*, fingerprints are polygenic traits with multifactorial inheritance, where the influence of the environment is limited to the early months of gestation when they are formed and developed (Holt, 1968). Later studies have established strong evidence for the heritability of fingerprint patterns, distribution and ridge density on the digits, palms and soles. Once formed at the end of the second trimester of gestation, fingerprints remain substantially unchanged throughout the course of the individual's life. This multifactorial inheritance shows that even monozygotic twins, who share identical genetic information, have different fingerprint characteristics. Still, it is important to emphasise that the dermatoglyphic characteristics of the monozygotic twins are more similar to each other than the dermatoglyphic characteristics of the dizygotic twins or unrelated individuals (Medland et al., 2007; Karmakar et al., 2011; 2012; Tao et al., 2012; Ho et al., 2016, among others).

FINGERPRINTS IN FORENSIC IDENTIFICATION

Due to the biological characteristics described above, the structural details of epidermal ridges on digits, palms and soles are extremely variable. However, it has been possible to classify this variability within a few main types, enabling the scientific study of fingerprints in various fields. For example, in physical anthropology, fingerprints have traditionally been analysed to determine the genetic structure and evolution of human populations (Arrieta et al., 1987; Blangero, 1990; Crawford and Duggirala, 1992; Jantz et al., 1993). In medicine, they have been used to characterise mental syndromes and disorders, among other pathologies (Schaumann and Alter, 1976; Fañanás et al., 1990; 1996; Rosa et al., 2005), while in forensic science fingerprints have been used for over a century for individual identification, and such identification by means of fingerprint comparison has been accepted by all courts worldwide as probative evidence of identity (Jamieson and Moenssens, 2009).

Fingerprint identification requires comparison, at three levels, between a mark (an impression of unknown origin) and a print (an impression of known origin). A level-one comparison of two impressions is based on determining the presence or absence of concurrence in main pattern type (arch, loop and whorl) formed by the flow of ridges. A match at this level does not provide conclusive evidence of identity, but the absence of one excludes identity. A level-two comparison seeks to determine concurrence in the location

and morphology of the minutiae, or characteristic points (Table 1). In this case, a match is evidence of the identity of the source of both impressions, and thus constitutes the basis of fingerprint identification. A level-three comparison analyses concurrence in ridge, furrow and pore in shape and size. This level is not always applied, due to the difficulty of obtaining a clear impression of these characteristics.

The problem of fingerprint individuality was first addressed by Galton in 1892, who estimated that the probability of two individuals' fingerprints presenting 12 matching minutiae was 9.54 $x10^{-7}$ (for 36 minutiae 1.45 x 10^{-11}). Since then, various authors have proposed models to establish fingerprint individuality, for example Henry, Balthazard, Roxburgh, Amy, Trauring, Kingston, Osterburg et al., Stoney and Thornton, Champod, and Meagher et al. Subsequent analyses and criticisms of these models have highlighted their lack of validity as a basis for establishing identity (Stoney and Thornton, 1986; Stoney, 2001). Each of these models has used the probability of the presence of minutiae in fingerprint impressions, but without conducting in-depth analyses of their variability, and thus the problem has not been resolved.

From the standpoint of identification, the minimum number of minutiae to establish an individual's identity (the numeric standard) is founded on the tripartite rule established by Locard in 1914. Based on statistical calculations performed at the time by Galton and Balthazard, Locard's first rule was to use the numeric standard of 12 concurring minutiae to establish the identity of the source of the fingerprints in question. In the early 20th century, this identification technique was quickly adopted in all forensic laboratories worldwide and was accepted as probative evidence. However, over time, the numeric standard employed to establish identity began to vary considerably between forensic laboratories, with some countries such as Russia, South Africa or Turkey using a numeric standard of 7 or 8 points whereas others such as Italy and France use 16 or 17. Countries using a standard of 8 to 12 points form the largest group and include Germany, Sweden, Holland, Switzerland, Belgium, Finland, Israel, Ireland, Greece, Poland, Portugal, Romania, Slovenia, Spain, Japan and the countries of South America (Champod et al., 2016). This divergence is due to the fact that the minimum number of minutiae necessary to determine an identity has not yet been rigorously established. Consequently, following a three-year study conducted by an assessment committee, the International Association for Identification (IAI) concluded in 1973 that "No valid basis exists to require a predetermined number of characteristics to exist between two fingerprint impressions in order to establish positive identity". Subsequently, the United States and then Canada rejected the notion of a numeric standard and stressed the importance of considering qualitative and quantitative elements alike when comparing two fingerprint impressions (Ashbaugh, 1999). In 1995, at a conference on fingerprint detection and identification techniques hosted by the Israeli National Police in Ne'urim, 28 fingerprint experts from 11 countries unanimously approved the IAI resolution (Houck, 2016; Champod et al., 2016). The first European country to adopt this guideline was Norway, followed by Sweden and Finland. Subsequently, Australia and then New Zealand adopted the resolution in 1999, and the United Kingdom, one of the countries most resistant to change, finally abandoned its use of a 16-point numeric standard on 11 June 2001. The resolution of the IAI has been reaffirmed more recently by the work of the Standardisation II Committee (Polski et al., 2011).

Therefore, identification by means of comparing a mark and a print should not rely solely on a detailed count of a set number of points since there is no scientific justification for this. The individual and unique characteristics that serve as probative evidence will depend on their quality and amount of variability. Thus, identification may involve a comprehensive assessment of quantitative aspects (number of minutiae) and qualitative aspects (e.g., general pattern, type of minutiae, pores, ridge endings, scars, etc.) that are clearly visible in the mark. This combined qualitative and quantitative approach constitutes the cornerstone of identification in countries that have ceased to use the numeric standard (Ashbaugh, 1999; Champod et al., 2016).

Identification by fingerprint comparison has been widely used throughout the world for forensic purposes. Forensic experts have adopted the premise that the characteristics observed in fingerprints are highly discriminatory and immutable within the general population. For most of the 20th century, forensic identification based on fingerprints has enjoyed almost universal acceptance as solid forensic evidence, and the scientific bases of the testimonies given by fingerprint experts have rarely been challenged or questioned. However, a series of questions have recently been posed as regards the scientific validity of forensic identification based on fingerprints (Cole, 2008; Haber and Haber, 2008; Koehler and Saks, 2010; Saks, 2010; Spinney, 2010).

To a large extent, the current wave of scrutiny was prompted by a decision taken by the United States Supreme Court in 1993, in the case of *Daubert v Merrell Dow Pharmaceuticals*. This well-known decision refers to the scientific criteria necessary for expert testimony to be admitted as probative evidence. These criteria establish that the technique must be scientifically demonstrated and accepted by the scientific community; it should be subject to peer review through publication in scientifically recognised journals in the field of forensic science; its use should be subject to standardisation and regulation; and the potential error rate should be known and acceptable within the standards corresponding to the technique employed. Consequently, although the *Daubert* criteria were recommendations of a more general nature, it became clear that fingerprint identification could no longer be considered an exact science with no error rate, as some experts had hitherto maintained (Grieve, 1996; Margot and German, 1996; Saks and Koehler, 2005; Dror et al., 2006; Schiffer and Champod, 2007).

Subsequently, mistakes committed within the forensic sciences in general and in fingerprint identification in particular definitively invalidated the notion of a zero rate of error when applying the technique (see Innocence Project; Giannelli, 2002). One of these mistakes, and the one with greatest international impact, was that known as the "Brandon Mayfield case", which occurred in relation to the attacks in Madrid on 11 March 2004. In this case, FBI agents misidentified a finger-mark obtained by the Spanish National Police Force from a plastic bag found inside a van containing explosive material similar to that used in the attacks. The FBI attributed this mark to an American lawyer, Brandon Mayfield, who was arrested. Shortly afterwards, however, the Spanish police identified the fingerprint found on the bag as belonging to an Algerian, Ouhnane Daoud (Office of the Inspector General, 2006). These errors, combined with increasingly rigorous scrutiny of forensic evidence, have prompted the need to reconsider many of the scientific principles on which these disciplines are supposedly based (Grieve, 1996; Champod and Margot, 1996; Saks and Koehler, 2005; Neufeld and Scheck, 2010; Spinney, 2010; Houck, 2016; Champod et al. 2016).

In March 2009, the National Academy of Sciences (NAS) of the United States of America issued a report that has exerted substantial international influence, *«Strengthening Forensic Science in the United States: a path forward»* (NRC, 2009; Giannelli, 2012; Champod et al., 2016). This report highlighted the deficiencies of some of the disciplines applied in this field, and stressed the need for continuous training for experts and standardisation and regulation of the techniques employed; furthermore, it underlined the need to conduct basic research in order to endow these disciplines with the scientific foundation necessary to ensure their quality and robustness. Both the Daubert guidelines and the NRC report have influenced jurisdictions worldwide, as evidenced by the consultation document on expert testimony drawn up by the UK Law Commission (2011), which establishes similar rules, or the extensive reports drawn up by a group of experts convened by the National Institute of Standards and Technology (NIST) and the National Institute of Justice (NIJ) (Expert Working Group on Human Factors in Latent Print Analysis, 2012). Forensic scientists have responded by conducting more research, improving adherence to guidelines, applying quality control and increasing the transparency of their testimonies. Groups such as the Interpol European Expert Group on

Fingerprint Identification (IEEGFI), the European Network of Forensic Science (ENFSI) and the International Association for the Identification (IAI) are internationally recognised for their work on developing and promoting guidelines to standardise the technique (Polski et al., 2011).

Bearing in mind that, within forensics, DNA and fingerprints are considered the primary sources of bioinformation, investment is required to develop probabilistic models that quantify the weight of evidence. Paradoxically, while DNA experts are required to indicate the weight of evidence using statistics calculated according to genetic principles and an appropriate reference database, the courts allow fingerprint experts to express their opinions categorically. As a result, the last decade has witnessed increasing research interest in the design of statistical models to support forensic fingerprint identification. Historically, a probabilistic framework for fingerprint identification has not been popular, and even used to be forbidden by professional bodies such as the International Association for Identification (IAI, 1980). Recently, however, these models are increasingly viewed as useful tools to support the identification process within the established protocol to determine whether two impressions come from the same individual. This protocol, known as the ACE-V (analysis, comparison, evaluation and verification), establishes that more than one expert must be independently involved in the identification process (Huber, 1972; Ashbaugh, 1999). Consequently, in 2010, the IAI invalidate prohibition of issuing their conclusions as possible or probable to its experts (Polski et al., 2011) and instead supported the future use of valid statistical models (provided that these were accepted by the scientific community) to help professionals with the identification procedure. Similarly, the *European Fingerprint Working Group* of the European Network of Forensic Science Institutes (ENFSI) has also advocated the development of statistical models through research to improve the methods employed to calculate the scientific value of fingerprint evidence (Meuwly, 2011). This implies a substantial change in approach on the part of key forensic institutions such as the IAI or ENFSI, and unquestionably adds considerable weight to calls for the research investment necessary to develop satisfactory statistical models for its future implementation in the ACE-V identification protocol.

Most forensic services apply the ACE-V protocol, and as a result, experts can emit a categorical opinion on the common source of a mark (excluded, conclusive or inconclusive). However, there are currently two different ways to reach this conclusion; either using the numeric standard or applying the holistic method.

Individual countries have established their own minimum number of minutiae required for a positive identification when applying the numeric standard method. In contrast, when applying the holistic method, experts issue an opinion on individualisation when personally satisfied that there are sufficient concurring details between the two impressions. This requires three levels of detail that assess the quantity, quality and specificity of epidermal ridge characteristics (Vanderkolk, 2011; Champod, et al., 2016). This method is not without its critics either, since it relies on a totally subjective opinion based on the expert's experience. Although such experience is unquestionably of vital importance in both methods, it can vary widely between experts and should therefore be subject to quality control when applying the technique.

Besides the two identification methods already mentioned (numeric standard and holistic approach), in the document entitled *Best Practice Manual for Fingerprint Examination* published in November 2015, ENFSI described a third approach, based on calculating likelihood ratios. This probabilistic method consists of reporting on the probative value of a comparison of impressions, under two mutually exclusive hypotheses as regards their origin. Probative value is calculated by assigning a subjective probability and/or using software based on a probabilistic model (ENFSI, 2015).

Use of the likelihood ratio (LR) is becoming widespread in forensic science, because it provides a probabilistic assessment of the weight of evidence, thus avoiding deterministic expert

opinion (identification or exclusion) based solely on experience and knowledge. The probabilities obtained by this method can be interpreted as degrees of certainty about attribution of the origin, leaving the role of deciding on the guilt or innocence of the accused to the court hearing the case. Currently, a considerable number of forensic scientists recommend using a LR because of its universality and simplicity (Abraham et al., 2013; Morrison and Stole, 2013; Martire et al., 2014; Tang and Srihari, 2014; Champod et al., 2016). The only requirement for use of a LR is a working knowledge of the characteristics to assess in the population to which it is applied. In addition, given that DNA identification is obtained using a LR, unifying all procedures within the forensic sciences as far as possible would facilitate the court's task of reaching a final decision (Champod et al., 2016).

The LR is calculated based on the evidence and the potential information obtained from the population, where the ratio distinguishes between two probabilities: that the mark and print come from the same source or that the mark and print come from a different source. The calculated LR is multiplied by the probability *a priori*, to obtain the probability *a posteriori*. This latter constitutes the information on which the court must base its final decision. In any event, it should be noted that conclusions based on the LR and the probability *a posteriori*, obtained by applying the Bayes Theorem, are no more than inferences based on the population studied and the assumption of prior probabilities.

Within the field of statistics, significant advances have been made in statistical modelling of minutiae variability (Abraham et al., 2013; Neumann et al., 2015;). Validated statistical models include the one published in the Journal of the Royal Statistical Society, developed by the Forensic Science Service in England and Wales to compute LRs as an indication of the probative value of a comparison (Neummann et al., 2015). Other types of model, based on the scores obtained by the Automated Fingerprint Identification System (AFIS), have also been proposed (Alberink et al., 2014; Egli and Champod, 2014).

The foregoing indicates the need to study and analyse the scientific bases for individualisation in personal identification, taking into account that any attempt to establish such bases requires an exhaustive analysis of variability in dermatoglyphics characteristics, especially the minutiae, since these form one of the cornerstones of fingerprint identification, whether using quantitative (numeric) or qualitative approaches.

RESEARCH ON DERMATOGLYPHIC VARIABILITY APPLIED TO IDENTIFICATION

At present, knowledge about variability in fingerprint characteristics in human populations differs considerably according to the population in question. There is abundant information on variability in the main types of pattern worldwide (see bibliographic reviews of Mavalwala, 1977; Figueras, 1993), but despite constituting the basis of fingerprint identification, variability in the minutiae (comparison of level two detail) has received little research attention, and even fewer studies have examined variability in the characteristics used for a level three comparison, namely ridge and pore morphology.

Minutiae variability

Although the need for systematic studies of the minutiae has long been recognised, current knowledge about these epidermal characteristics unfortunately remains very limited because few studies have been conducted to assess their variability. Hence, important aspects of their biology and genetics are still unknown. Furthermore, the results obtained in the few studies that have been conducted cannot be directly compared due to the diversity of classifications used to assess the frequency with which the different types of minutiae appear in fingerprints, as well as differences in the finger, areas and types of pattern in which these frequencies have been assessed (Rivalderia, 2016). Since Galton first described four types of minutiae (endings, bifurcations, fragments and enclosures) (Galton, 1892), several attempts have been made to classify the minutiae, ranging from very simple distinctions with two types (endings and bifurcations) to others with 95 types. However, the majority of classifications range from 4 to 20 types (Rivaldería, 2016). Penrose (1968) suggested a classification based on the six most common

types of minutiae proposed by Cummins and Midlo (1961) (endings, bifurcations, islands, fragments, enclosures and incipient ridges), to which he added a new type called "combined". At the *Conference on Dermatoglyphic Nomenclature* held in London in 1967, Penrose's proposed classification of the seven types was recommended for clinical use. Subsequently, other classifications were proposed, including one with nine main types (Champod, 1996; Ashbaugh, 1999) and the one employed by the Servicio de Identificación de Policía Científica or the Servicio of the Criminalística de la Guardia Civil in Spain, based on the 20-type classification proposed by Santamaría (1955). With minor variations, it is this latter classification that our research group has used to study variability (Table 1) (Gutiérrez el al., 2007; Gutiérrez-Redomero et al., 2011a; 2012; Rivaldería et al., 2017).

Despite the paucity of studies and their methodological differences, some common patterns can be observed. For example, the most frequent type is the ending, accounting for between 45% and 60% of minutiae, followed by bifurcations, which globally account for around 15-30%, while the remaining minutiae present considerably lower frequencies in all studies (Gutiérrez et al., 2007; Expert Working Group on Human Factors in Latent Print Analysis, 2012; Rivaldería, 2016; Champod et al., 2016). These results confirm the non-equiprobability of frequency for the different types of minutiae assessed in fingerprint comparisons, and thus their different discriminatory value in identification.

Sarkar conducted one of the most comprehensive studies in this field (Sarkar, 2004), assessing a sample of 2010 fingerprints from the Indian population (201 males and 202 females), to determine variability in the frequency of the different types of minutiae, and confirming the patterns found in smaller samples. The study also found that thumbs followed by ring fingers present the highest number of minutiae, whereas little and index fingers present the lowest number, and that the mean number of minutiae is higher in men than in women and in whorls than in loops.

The most comprehensive standardised results on minutiae variability in different human populations were obtained from population samples in Argentina and Spain (Gutiérrez-Redomero et al., 2011a, 2012; Rivaldería et al., 2017). The Spanish sample consisted of tenprints from 200 individuals (100 males and 100 females) and the Argentinian sample of tenprints from 608 individuals (296 males and 312 females), enabling an analysis of minutiae distribution in 8008 fingerprints and four types of pattern (arch, ulnar loop, radial loop and whorl). The frequency distribution results obtained for the different types of minutiae revealed a common finger distribution pattern in both populations, albeit with significant differences in frequency values between the two. This common pattern indicates the existence of topological, digital and sex differences that can be summarised as follows:

Topological: endings are more frequent in peripheral fingerprint regions whereas bifurcations and convergences are more frequent in the central area.

Digital: bifurcations and convergences are significantly associated with the middle, ring and little fingers. In contrast, endings are associated with the thumbs and index fingers on both hands. The vast majority of the remaining types of minutiae are associated with the right-hand thumb and index finger, which present the greatest variability in low frequency minutiae types.

Sex: the distribution of endings, bifurcations and convergences present differences by sex which vary according to the areas studied.

Differences by sex in the distribution of endings, bifurcations and convergences in the Argentinian sample (Gutierrez-Redomero et al., 2012; Rivaldería et al., 2017), coincide with those identified in the study by Gutiérrez et al. (2007) of the index finger in a Spanish population sample. However, in a subsequent study, the mean obtained for these three types of minutiae on the ten fingers showed no significant differences, although an analysis of correspondence did reveal a significant association between the different types of minutiae and sex (Gutiérrez-Redomero et al., 2011a). In a study of samples from the North American population of European, Asian, African, Native American and Hispanic origin,

Reinart (2014) observed differences by sex in samples of European and African origin, but not in those from the other populations. More recently, Fournier and Ross (2016) observed no differences by sex in mean values for five types of minutiae on the index finger (the only finger assessed) in a North American population sample. Consequently, further research is required in different populations in order to elucidate possible differences by sex in the frequency of the different types of minutiae.

As regards differences by digit, in both the Spanish and Argentinian population samples (Gutiérrez-Redomero et al., 2011a; 2012; Rivaldería et al., 2017), the minutiae analysed according to orientation in the fingerprint (bifurcating or convergent) revealed different hand associations. Thus, bifurcations are significantly associated with the middle, ring and little fingers of the left hand, whereas convergences are associated with these same fingers on the right hand. Since bifurcations and convergences present the same morphology and are classified according to their orientation (always on the left-hand side of the fingerprint), this different hand association corresponds to a mirror effect. However, regardless of this effect, the association between these types of minutiae with the middle, ring and little fingers has been demonstrated. Champod (1996) and Champod and Margot (1996) also assessed these minutiae according to orientation and concluded that distribution differences in the fingerprint are sufficient reason to consider them separately rather than as a single type of minutiae, as had been the case in the majority of studies.

Nonetheless, it is important to note that although these results showed the common pattern described above, they also revealed, for the first time, the existence of statistically significant population differences in minutiae frequencies, which were not determined by frequency differences in the main types of pattern (Gutiérrez-Redomero et al., 2011a; 2012; Rivaldería et al., 2017). Thus, the Spanish sample presented a significantly higher frequency of endings, whereas the Argentinian sample presented a higher frequency of bifurcations and convergences (Figure 4). These results indicate an ancestry effect in minutiae variability in human populations which requires further exploration.

All these results appear to provide further support for the notion suggested in earlier studies that the development of minutiae on epidermal ridges is also influenced by genetic factors (Holt, 1968; Dankmeijer et al., 1980; Okajima and Ushukura, 1984; Sarkar, 2004). Consequently, further methodologically comparable research is required to elucidate several aspects of minutiae variability.

In any event, topological, digital, sex and population differences in the distribution of minutiae types should be taken into account as a scientific basis to improve estimations of the weight of evidence in fingerprint identification. In this respect, the Best practice manual for fingerprint examination (ENFSI, 2015), recommends one of the studies published by our research team for use as a reference when conducting a level two comparison of characteristics (Gutiérrez-Redomero et al., 2012). This study provides data on the frequencies of these characteristics in different human populations, assessed using a standardised method.

Variability in ridge density: topological, digital and sex differences

Studies of human populations have frequently used ridge counts in fingerprint patterns as the main quantitative variable analysed. Traditionally, this variable has been assessed based on ridge counts between the *triradius* and the core of the pattern (count of the Galton line or quantitative value), and in palmprints, basically between the *triradii* in interdigital areas. The results obtained from these counts show digital, radio-ulnar, hand, sex and population differences (see bibliographic reviews of Figueras, 1993; Kumbnani, 2007). Although this type of ridge count is of great interest, it can only be conducted in patterns with at least one *triradius*, as in the case of loops and whorls but not of arches. In addition, ridge counts are always performed in the proximal area of the fingerprint, where the *triradii* are located, and since the count line is not always perpendicular to ridge direction, this type of count does not permit ridge breadth estimation.

The first method that enabled ridge breadth assessment, based on ridge density in all types of pattern, and therefore in both finger and palm prints alike, was developed by Cummins et al., (1941). This ridge count was performed along a 1-cm line perpendicular to ridge flow, located at different areas of the finger and palm impressions. This and subsequent studies revealed ridge breadth differences according to the topological area of the finger and palm (with an increasing disto-proximal gradient in ridge breadth), by sex and between populations (Cummins et al., 1941; Ohler and Cummins, 1942; Penrose and Loesch, 1967; Jantz and Parham, 1978). More recently, Acree (1999) proposed performing ridge counts along the diagonal of a square measuring 5 mm x 5 mm and located on the radial side of the impression (without specifying the location in any greater detail). In addition, based on differences in ridge density by sex, the same author calculated the likelihood ratio as a forensic method to infer sex from marks revealed at the scene of a crime.

Subsequently, Gutiérrez-Redomero et al. (2008), proposed increasing the areas counted to three; two in distal areas (radial and ulnar) and one in a proximal area, standardising their location on the fingerprint. This method enabled assessment of topological differences on the fingerprint surface for all main pattern types, including the arch, as well as inference of ridge breadth. The studies conducted to date in different populations using this method have found a common ridge density distribution pattern which indicates the existence of topological, digital and sex differences. Epidermal ridge breadth on the finger surfaces is lower in the distal region and higher in the proximal region. In addition, differences were observed between fingers; thus, in distal areas, ring fingers present the thinner ridges, followed by little and middle fingers, whereas thumbs and index fingers present the thickest ridges. However, this pattern is reversed in the proximal zone. Furthermore, differences by sex were detected in all distal areas and all fingers, whereby female present thinner ridges than male (Argentina:

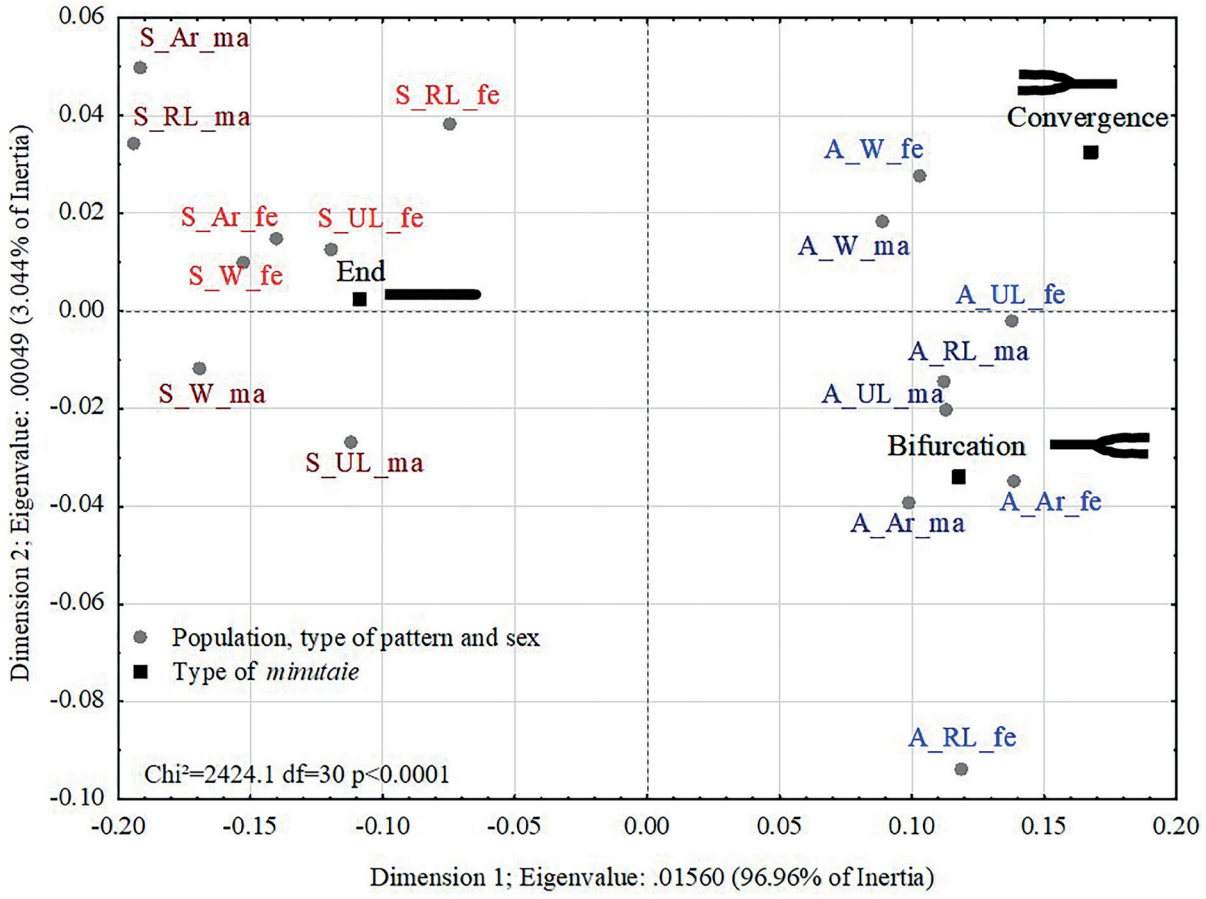

Fig. 4.- Analysis of correspondence between the types of minutiae (end, bifurcation and convergence) and Argentinian (A) and Spanish (S) population samples by sex (fe: females; ma: males) and type of pattern (Ar: arch; RL: radial loop; UL: ulnar loop; W: whorl).

Gutiérrez-Redomero et al., 2011b, 2013a; Rivaldería et al., 2016; North of India: Krishan et al., 2013; sub-Saharan population: Gutiérrez-Redomero et al., 2013b; Thailand: Soanboon et al., 2016; Sudanese population: Ahmed and Osman, 2016). Differences by sex in ridge density and thickness have also been observed in all studies that have solely assessed the radial area, using Acree's method (Acree, 1999; Gungadin, 2007; Nayak et al., 2010a; 2010b; Kaur and Garg, 2011; Nithin et al., 2011; Agnihotri et al., 2012).

The effect of age on topological, finger and sex distribution of ridge density was assessed by Sánchez-Andrés et al., (2018) through a comparison between groups of young adults (18 to 30 years old) and older adults (50 to 66 years old). The results showed that although ridge breadth increases with age in distal areas, it does not do so in proximal areas. Furthermore, the study demonstrated that the differences by sex observed in all previous studies persist with age, with females presenting thinner ridges than male in the same age group.

These differences by sex in ridge density detected in all population samples assessed support the idea of a universal pattern of sex dimorphism for this characteristic, indicating that it could be applied in identification to infer the most probable sex. Ridge density in the different topological areas also presents statistically significant population differences, and therefore could also be applied to infer the origin of the individual (Gutiérrez-Redomero et al., 2013a, 2013b; Rivaldería, 2016).

However, it should be borne in mind that considerable and consistent differences in mean ridge density have been obtained between studies using Acree's method (Acree, 1999), some of which have not indicated the method used to obtain fingerprint impressions (rolled or plain), and those conducted by standardising the area according to the method described by Gutiérrez-Redomero et al. (2008). This discrepancy prompted a study to assess the effect of count area location (core or out) and method of obtaining the fingerprint (rolled or plain) on ridge density counts in different areas (Gutierrez-Redomero et al., 2014) (Figure 5). The results showed significant differences in ridge counts according to count area location (core or out) and method used to obtain the impression (rolled or plain). In both rolled and plain impressions, ridge density is higher in central (core) than in peripheral areas (out), regardless of sex, and the central area also presents the thinnest ridges. In addition, significant differences were observed in ridge density according to the method employed to the fingerprint impression (rolled or plain), due to the different distribution of pressure exerted with one method or the other. Therefore, in order to ensure that the results obtained in different studies are

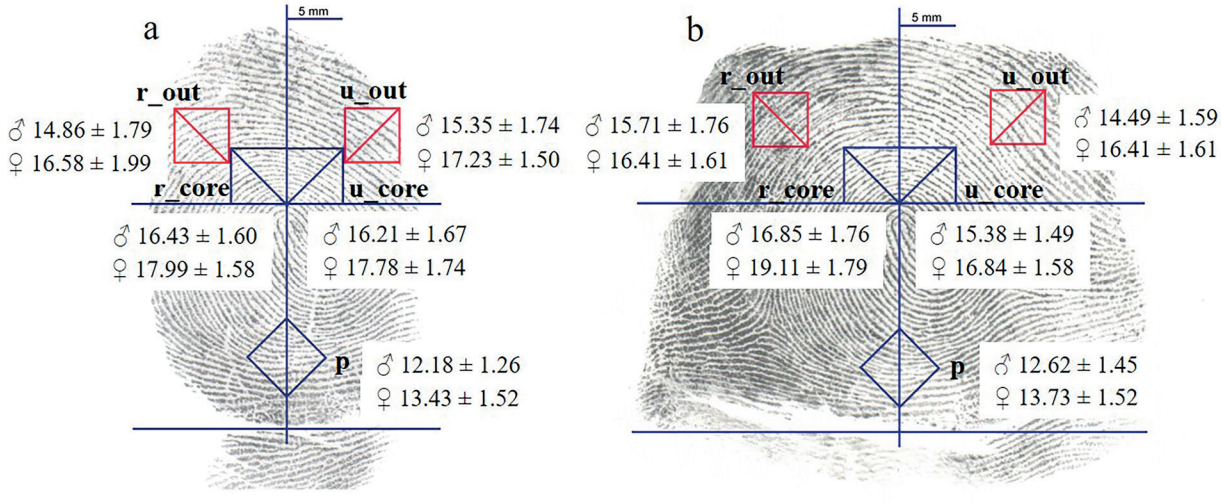

Fig. 5.- Location of the count areas on the right thumb fingerprint of the same subject based on the reproduction Gutiérrez-Redomero et al. (2014). a) plain fingerprint, b) rolled fingerprint; r: radial; u: ulnar; p: proximal; out: external area count; core: core area count.

comparable, it will be necessary to standardise the count location and the method used to obtain the impression.

Use of LR values to infer population of origin and sex from a finger-mark of unknown origin might be useful in some circumstances, but bearing in mind the limitations indicated by research, caution should be exercised since the strength of the indication in terms of LRs is low.

After a century of using fingerprints for human identification, forensic scientists are calling for better and more extensive knowledge of their variability and heritability. Everything suggests that the days will soon be long gone when expert testimony was based on experience as the sole guarantee of expertise, and that, instead, the weight of evidence of fingerprint comparison will be based on a full description of the methods and criteria employed and the application of statistical models. This transition will require solutions to the challenges encountered in developing a valid statistical model suitable for operational implementation, as well as better knowledge of variability in the fingerprint characteristics used for comparison and continuous training of experts to ensure correct application in forensic science.

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Confocal reflection microscopy applicability for human remains: Two cases of forensic anthropology

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SUMMARY

Forensic anthropology has witnessed tremendous advances over the past few decades. With these advances, the need for more precise tools, especially imaging technologies, has become mandatory. This work presents the application of confocal reflection microscopy in two historical forensic cases, Puig Castellar from the fifth-second century BC and Les Roquetes from the 14th century AD. The aim of this paper is to evaluate its virtues/strengths and shortcomings/weaknesses in both cases. On the one hand, regarding Puig Castellar, the technique allowed us to identify the use of a possible skinning tool while comparing skinning marks found on four crania fragments with those made according to an experimental protocol. On the other hand, regarding Les Roquetes, confocal reflection microscopy allowed us to understand the nature, orientation and sequence of a series of cut marks found on human remains after a violent episode.

However, this technology has some limitations, such as the specific size and shape requirements of a piece to study due to its spatial limitations, the need for well-preserved cortical bone for the analysis and a clean bone surface to avoid unwanted reflections from the sample.

Due to these limitations, it is important to develop a broad range of imaging techniques for the characterization of anthropological samples that will work synergistically to extract all of the available information.

Key words: Confocal laser scanning microscopy (CLSM) – Confocal reflection profilometer – Cutmarks – Skinning marks – Human remains – Pogrom

INTRODUCTION

The forensic sciences, as a field of science, gain strength with the incorporation of disciplines and with the application of new methodologies:

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among them, the application of advanced microscopic technologies to investigate human remains (Meijer et al., 2019). Imaging techniques are a major tool used by forensic anthropologists to identify and understand the evidence present in human remains, such as identifying cut marks on bone surfaces and interpreting the causes and how the process was performed (Garvin and Stock, 2016; Lewis, 2008; Gilbert and Richards, 2000; Bello, 2011; Archer and Braun, 2013).

Binocular and simple optical microscopes were the first tools that provided access to the new microscopic dimension in this field (Shipman, 1981; Olsen and Shipman, 1988; Greenfield, 1999, 2006; Smith and Brickley, 2004; Lewis, 2008). Scanning electron microscopy allowed a higher resolution (Shipman, 1981; Olsen and Shipman, 1988; Greenfield, 1999, 2006; Smith and Brickley, 2004; Lewis, 2008) and, in combination with specific detectors, enabled the analysis of the elemental composition in specific regions of the sample, allowing us to identify the raw material, such as stone, metal or wood (Domínguez-Rodrigo et al., 2009).

When applied to human remains, the features of butchery marks can easily be mistaken for those with a diagenetic origin (Fernández-Jalvo et al., 1999; Saladié et al., 2012; Boulestin et al., 2015). Some studies have shown that macroscopic and microscopic descriptions of some characteristics of the marks, such as the location, path or shape, are the best way to assess their origin (Olsen and Shipman, 1988; Bello and Soligo, 2008; Domínguez-Rodrigo et al., 2009; Fernández-Jalvo and Andrews, 2016). Further studies incorporated microtomography of the bone surface to capture the shape of the mark at different focal layers, capturing a three-dimensional (3D) model. This technique is a laborious and time-consuming task that is not suitable for samples where more accuracy is required (Bello et al., 2013, Pante et al., 2017; Otárola-Castillo et al., 2018).

The recent incorporation of 3D analysis techniques and the use of geometric morphometrics have permitted analysis of the angle of the incision and the identification of stone tool cut marks and mammalian carnivore tooth marks (Pante et al., 2017).

Autofluorescence is characteristic of ancient human bone, and the use of confocal laser scanning microscopy (CLSM) for imaging in histomorphometric analyses of bone is now gaining in popularity, and its application to the research of hard tissues is increasingly widespread (Capasso et al., 2001, Maggiano, 2005; Archer and Braun, 2013). Compared with the conventional optical microscopes, CLSM is a non-destructive characterization method, capable of eliminating out-of-focus light in thick specimens (Claxton et al., 2006) and of providing extremely precise 3D imaging with a greater z-axis resolution, macroscale high-contrast scanning, and a simple and fast sample preparation procedure (Biggerstaff et al., 1997; Song et al., 2013).

The confocal reflection profilometer is a recently applied technique in the field of anthropology, used to evaluate incisions and to discriminate their origin. It is presented as a fast and precise non-tactile method that allows for quantitative studies. In this technology, image formation comes from the reflection of the incident laser from the surface of the sample, in contrast to conventional CLSM, where the image is formed with the light that comes from the sample (fluorescence). The confocal reflectance microscopy images are based on the refractive index of the sample, and the structures with the highest refractive index are the brightest. This technology uses a short wavelength excitation (blue range), which, when combined with precise motorized movements of the sweep, the objective lens and the mechanical elimination of light from unfocused planes, allows for the acquisition of high-resolution lateral images (120 nm) and axial (10 nm). With the use of reflection, it is possible to obtain a topography of the material surface similar to what one could obtain using a scanning electron microscope with the great advantage of non-invasive sample preparation.

This technique opens a range of possibilities regarding the study of large samples, such as bones, where processing the sample could affect its morphological or structural aspects. Furthermore, this type of profilometer allows for the acquisition of a 3D model of the incision or mark and different measurements (Casanova

et al., 2020). The type and shape of the marks observed may differ depending on their purpose or origin (e.g., skinning, dismembering, defleshing or wounds) and the material of the tools used to cause them (e.g., stone, wood, metal, etc.), which is closely related to the specific historical period.

This paper is focused on the identification of anthropogenic evidence found on the surface of bones of human remains through the application of confocal profilometry, aiming to establish differences between marks resulting from an episode of violence or a funerary treatment of the cadaver, and the differentiation of alterations due to environmental agents, vascularization marks or suture-related evidence. The remains studied came from two historical cases, Puig Castellar from the fifth-second century BC and Les Roquetes from the 14th century AD.

In summary, the main goal of the paper was to evaluate the advantages and disadvantages of the application of confocal reflection microscopy on these two different sets of marks and the interpretations of the causes and the typology of the marks. In the case of Puig Castellar, we will use these findings to also hypothesize about which were the most likely tools.

MATERIALS AND METHODS

The two samples chosen are the skinning case from Puig Castellar (Santa Coloma de Gramanet, Barcelona) (Subirà, 2014; Casanova, 2015; Subirà and Rovira, 2019) and the violent pogrom case from Les Roquetes (Tàrrega, Lleida) (Clausell, 2017; Clausell et al., in press).

CASES OF STUDY

Puig Castellar

Puig Castellar (Santa Coloma de Gramanet, Barcelona) is an Iberian period village (s. V-II B.C.) known for the presence of two nailed skulls. Subirà and Rovira (2019) identified different cranial fragments from the site corresponding to a minimum of 12 individuals. The samples studied correspond to four of those fragments. All of them are from a part of the anterior skull vault (frontal/frontoparietal) with a region of the temporal fossa where skinning marks can be observed. The cranium fragments studied correspond to a male adult of unknown specific age (fragments s.n., 393 and 394), and a male with an assessed age of twenty years old (individual 391) (Subirà, 2014; Casanova, 2015).

Previous studies had already described and distinguished cutmarks, vascularization evidence, skinning marks and other bone surface modifications (Casanova, 2015; Casanova et al., 2020).

To identify the tools used for skinning marks in these cranial fragments, a comparison with Iberian tools and an experimental study was performed to determine the shape and size of the tools. A set of possible Iberian tools stored in the Museu d'Arqueologia de Catalunya (MAC) were selected and measured *in situ* in the museum and compared to the museum's database and their photographic catalogue. The museum's database and previous studies (Sanmartí et al., 1992) allowed for a morphometric study of the pieces. These pieces are heritage and therefore are not available to be used in experimentation.

The experimental analysis was performed using cuts made in human dry bones (an occipital bone and a clavicle from the Biological Anthropology Unit in *Universitat Autònoma de Barcelona* laboratory collection) that mimicked the activities of skinning and defleshing. Based on the usual tools of the Iberian period, three different tools were chosen as the source of the butchery-related marks and used to perform an experimental study: a flat screwdriver, an awl, and a small knife. Two marks per tool were applied to the surface of the dry bones (an occipital bone and a clavicle), emulating the activity of skinning.

Les Roquetes

Les Roquetes is a Jewish medieval cemetery located in Tàrrega (Lleida), which contains 6 mass graves related to a 1348 pogrom in the Jewish quarters (1280 y 1391 CE cal 2σ; CNA1644). For the study, 10 individuals showing a larger number of violence-related wounds were selected to undergo measurement and trajectory analyses of these wounds.

The study included 5 females and 5 males found in grave #161 (individual 1180), #162 (individuals 1162 and 1166), #163 (individual 1185) and #164 (individuals 1204, 1215, 1216, 1218, 1219 and 1221). Clausell (2017; Clausell et al., in press) previously defined all of the marks due to the pogrom episode first by the naked eye and/or with the help of a stereoscopic magnifying glass.

All of the marks were measured, first with an anthropological caliper; second, the lesions were photographed, and the images were analysed using ImageJ software (Rasband, 1997-2018). The variables analysed using this methodology were length and width and were posteriorly compared using statistical analysis.

Confocal imaging

Marks imaging for the study of both cases was performed by a Confocal Profilometer Leica DCM 3D (Leica Microsystems GmbH, Wetzlar, Germany) with a 5× objective (HCxPL Fluotar), provided by the Servei de Microscòpia from the Universitat Autònoma de Barcelona. This technology allowed a quantitative approach to the study of the bone marks.

The images obtained were then manipulated with the LeicaMap v.6.2 software that obtains different measurements of the mark in every profile: depth, canal breadth, breadth at the bottom, the breadth ratio, the aperture angle (opening angle of the mark), and length (measurement from end to end of the superficial mark).

Regarding the Puig Castellar sample, five profiles were extracted: four simple profiles (most cranial, most caudal, plus two additional points in between) as well as a mean profile of the whole analysed surface. From the profiles obtained, the depth, breadth (canal breadth, breadth at the bottom, and breadth ratio) and overture angle were measured. In addition, the length between the most superficial point and the deepest point was measured.

In addition to these measurements, the software also allows for the calculation of a parameter called complexity, a percentage that explains the amount of the surface that is deeper than the surrounding area. High values of surface complexity indicate a deeper and more complex pattern of the studied mark.

$$\text{Surface Complexity} = \frac{\text{Developed area} - \text{Horizontal area}}{\text{Horizontal area}} \times 100$$

The use of this parameter proves useful while identifying the origin of the mark, since its depth and morphology should be characteristic of its source. Thus, surface complexity was used in the Puig Castellar case, where it allowed for the distinction of the different types of marks and their origin.

In the Roquetes case, a violence-related episode, the parameter "Angle of entry" was also included to evaluate the way in which the tool performed the mark. The goal was to obtain more information related to the possible sequence of the aggressions.

Statistics

To compare the measurements obtained using the caliper and ImageJ, a paired difference test was applied. Then, a Wilcoxon signed-rank test was performed to compare the measurements obtained using the caliper and the photographic study in order to determine the presence or absence of a relationship between them for the Les Roquetes wound marks. These studies were carried out using Past 3.x software37 and the IBM SPSS Statistics package version 23.38.

RESULTS

Puig Castellar

In a previous study, Casanova et al. (2020) differentiated skinning marks in the cranial fragments with respect to the other marks: vascular, suture, and other environmental marks. With the measurements of confocal reflection microscopy, a discriminant function was generated taking into account only depth. This function classified marks as skinning marks with a 65.4% reliability. Then, confocal reflection microscopy made it possible to define the dimensions of the skinning marks. Now we ask ourselves whether, based on experimentation, if confocal reflection microscopy helps us to identify the tools that caused them.

First, we made a selection of possible skinning tools with the dimensions obtained in the collection of Iberian daily material deposited on MAC. This consists of several pieces of bone needles and awls (18 in total), a bone handle and a bronze claw. The measurable tools ranged from 35 to 200 millimeters in length and from 0.5 to 10 millimeters in width.

The material deposited in the museum is part of the cultural heritage of the museum itself. For this reason, it cannot be used in experimentation. In this sense, current tools were sought that had the dimensions of the museum's tools in order to carry out the experimentation. Given these characteristics, a set of three current tools that mimicked them in measures were selected, and an experimental protocol was performed: a flat screwdriver, an awl (Fig. 1) and a knife (as small as a razor). The experimental marks were then analysed following the protocol, which showed different profiles for each tool (Fig. 2):

Flat Screwdriver: a mark of approximately 0.6 mm in width and 220 µm was obtained. It appeared as a continuous, flat mark (\ /) deeper than the surface of the bone.

Awl: The mark appeared as an open U-shape, with a depth of 180 µm and a width of 0.35 mm.

Knife: It appeared as the clearest profile of all with a height and width near 0.5 mm. The profile

Fig. 1.- Bone needle from the Iberian Culture stored in the Museu d'Arqueologia de Catalunya.

showed an abrupt disruption of the flatness of the bone surface. In addition, in this case, a rise surrounding the cutmark was present.

Since access to the tools and weapons was limited, and they were stored in the museum, the exact source of the marks could not be found. However, the experimental marks allowed us to develop a hypothesis of the type of tool used in the studied cases.

Once the profiles were differentiated, it was noticeable that the most similar profile to the study case was the one obtained with the awl. According to these results, it is logical to assume that the most likely tools used in the skinning Iberian practice are comparable to those obtained with an awl of similar dimensions (~1 mm at the distal point).

Les Roquetes

The case of Les Roquetes was focused on the study of 10 individuals. The first step was to determine and describe the number and characteristics of the lesions.

A total of 124 lesions have been observed, but only 116 have been previously measured. In all cases, it has been possible to take measurements with a caliper, but this number has decreased when studying photographs (ImageJ) (Table 1) or confocal images.

Fig. 2.- Profiles of the experimental marks obtained by Leica DCM 3D and processed by Leica Map software.

Table 1. Total of marks studied according to the anatomical region and the method used. Supplementary data. Measures from caliper and from ImageJ software.

	Caliper	ImageJ	Confocal
Skull	66	66	0
Lower Limb	42	19	23
Upper Limb	3	3	0
Other	5	1	4
Total	**116**	**89**	**27**

		Length	
Individual		**Caliper (cm)**	**ImageJ (mm)**
162-1162	Femur D #1	11,3	1,146
	Femur D #2	24,6	2,317
	Femur D #3	15,8	1,938
	Femur D #4	38,2	5,259
	Femur E #1	6,9	0,704
	Femur E #2	6,8	0,847
	Femur E #3	29,5	3,079
	Femur E #4	21,6	2,491
	Femur E #5	23	2,602
	Femur E #6	7,2	1,007
	Tibia D	9,6	1,135
162-1180	Rib E	2,5	0,302
	Ulna E	9,3	1,08
	Femur D-1	11,8	1,235
		8,2*	0,774*
	Femur D-2	10,1	0,852
	Femur D-3	16,3	2,105
		5,4*	0,661*
	Femur E-1	12,9	1,315
		3,2*	0,317*
	Femur E-2	5,2	0,527
	Tibia E	7,4	0,866
		3,4*	0,445*
164-1204	Tibia D	9,4	0,984
		3*	0,305*
	Fibula D	4,5	0,518
164-1215	Clavicle E	8,1	0,792
	Tibia D #1	3,291	0,743
	Tibia D #2	3,295	1,902
	Tibia D #3	1,834	1,66
164-1218	Fibula D	11,7	0,907
	Tibia D	13,3	1,55
164-1219	Clavicle D	8,2	0,932
	Humer D	8,1	0,735
	Tibia D #1	14,7	1,552
	Tibia D #2	5	0,681
	Tibia D #3	5	0,686
	Fibula D #1	12,1	1,309
	Fibula D #2	5,7	0,508

*Width

The decline in measurements from the photographic studies of marks on the long bones is due to the location of the injury and the curvature of the bone, parameters that distorted the image, not allowing analysis.

The use of confocal microscopy presents different limitations. Often the dimensions of the pieces, such as from skulls, make the study impossible. In other cases, the location of the lesion is an added difficulty.

A statistical comparison between measures taken using caliper and ImageJ software (supplementary data) was conducted in order to test this technique and its efficiency. Even though the intraclass correlation, considering one group as the measurements taken using the caliper and the second using photographic analysis, was 98.8% between both groups, the statistical analysis showed significant differences between them (Wilcoxon, $p<0,01$).

In this particular case, the main problem was the marks on the cranium vault. As they are on a curved surface, conventional photography is not suitable to capture the whole shape, so the measurements are altered. This hypothesis was confirmed after performing the same statistical analysis of the cranial marks and postcranial marks separately. Therefore, within the group of cranial marks, the correlation remained high (98,6%), and the difference was statistically significant (Wilcoxon, $p<0,01$), but, in the postcranial marks group, the differences between the measurements were not significant and maintained the correlation (Wilcoxon, $p= 0,077$; 98,8% correlation). This

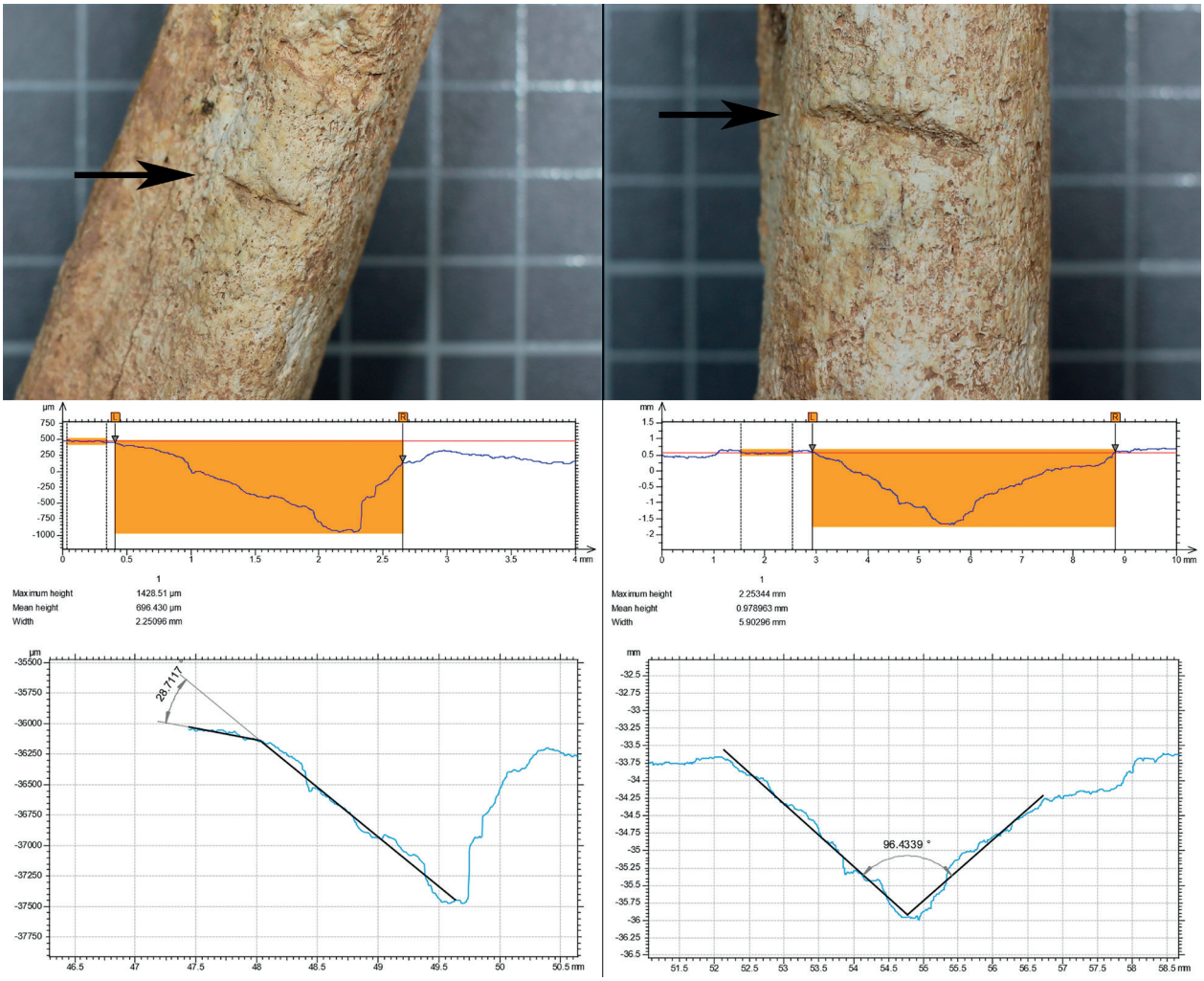

Fig. 3.- FS161-UE1180, right femur, frontal view. Position of the two marks selected for the example: Proximal (left) and distal (right) halves of the diaphysis. From top to bottom: photography of the cutmark on the bone surface, profilometer result showing the depth and width of the incision, and estimation of the angle of entry of the tool. The profilometer profiles show the shape of the incision and the dimensions (coloured); the angle of entry of the tool shows the way the tool entered the surface of the bone, the left one shows an inclining entry while the right one shows a perpendicular entry when using the cortical as reference, enabling us, in this case, to calculate the aperture angle of the blade.

result confirmed the initial hypothesis, and the difference was attributed to the difficulty of capturing larger marks on curved surfaces such as the cranium vault. This result showed that a more precise calibration of the technique or a new approach is needed.

A particular example was chosen to exemplify the profiles obtained by confocal reflection microscopy and their interpretation in a forensic case, the right femur of FS161-UE1180. This piece showed three marks, two on the proximal area and the other one on the distal part of the femur shaft. For this example, two of the three marks, one from each end of the bone, were selected (Fig. 3, top images). Both marks presented a clear profile, and the cortical part of the bone was well preserved.

This femur presents two clear patterns. The proximal mark was L-shaped (Figure 3, central left), consistent with an angled entrance of a tool, 28.7º from the cortical bone and a posterior cut of the cleaved cortical bone (Fig. 3, bottom left). This profile and the angle described are coherent with a wound caused while the victim was standing and subjected to a downward motion of the tool.

The distal mark was V-shaped (Fig. 3, central right), consistent with a perpendicular entrance of the tool considering the cortical bone as a reference. As the image shows, the bone captures the shape of the tool, and the aperture angle of the blade can be measured as 96.4º (Fig. 3, bottom right). This profile is consistent with a wound caused by the victim being in a supine position and the tool entering perpendicular to the victim in a downwards motion.

DISCUSSION

Studies using state of the art 3D reconstructions of bone surface modifications are increasingly used in taphonomic studies. In recent years, there has been a rapid growth of work using quantification of the internal morphology of marks produced by known agents (carnivore modifications, weathering abrasion raw material and the types of stone tools used in butchery activities) to identify diagnostic criteria that will assist in the interpretation of bone assemblage marks (Vietti, 2016; Pante et al., 2017; Courtenay et al.2018; Gümrükçü and Pante, 2018; Yravedra et al., 2018).

In this work, in the Puig Castellar study, images from a confocal profilometer provided an approximation of the possible tool used for skinning the cranial remains. The dimensions obtained allow us to discard tools similar to a knife or flat screwdriver and to clarify that the tools are more similar to and precise as an awl. Consistently, awls and bone needles, both of a similar width, constitute part of the MAC's collection of tools from the Iberian periods.

For the second study case, Les Roquetes, the results were also remarkable. The presence of marks on the forearm bones coincided with blocking moves of protection, and the number of impacts and marks on the skull and inferior extremities matched the initial hypotheses about the bodies coming from a violence-related episode already documented: the 1348 riot at the Jewish quarters of Tàrrega.

There was no pattern for the inflicted marks found, coinciding with the fact that, as recorded in the documents about this period, they were villagers and not a trained militia, and it was, in fact, an unplanned attack. Although it is unknown how the attack occurred, the position and angle of some of the wounds allowed us to describe a plausible hypothesis about the position of the victim at the moment of the attack, and the superposition of some wounds on the same bone allowed us to also describe a sequence of different attacks on the same victim. Then, a reconstruction of the sequence of the execution of blows and cuts was performed on each individual, supporting the interpretation of this episode as a riot.

2D imaging methods have succeeded repeatedly in differentiating structurally similar marks, showing similar rates of correct mark classification as 3D models (Courtenay et al., 2018). However, Otárola- Castillo et al. (2018) argued that 2D models are insufficient because they are affected by the irregular use of landmarks. Our statistical analysis was performed using the data from measures with a caliper, and the ones obtained with the software showed interesting

results. The difference between these measures was hypothesized to be caused by the shape of the bones and the curvature of the marks, which could not be captured using 2D photography. In this particular statistical case, the main problem was the marks on the cranium vault, since they were on a curved surface where conventional photography is not able to capture the whole shape and the measurements are altered. This hypothesis was confirmed after performing the same statistical analysis of the cranial marks and postcranial marks separately. The result of the statistical test separating these groups confirmed the hypothesis, and the difference was attributed to the difficulty of capturing larger marks on curved surfaces such as the cranium vault. This result shows that a more precise calibration of the technique or a new approach is needed.

In our study, we observed that 3D morphometric profiles obtained by confocal microscopy yielded higher definitions than 2D imaging. Confocal reflection microscopy has demonstrated that it is a good technique in this field and is probably better in forensic cases than in historical cases, where the tool used cannot always be elucidated.

The 3D confocal microscopy technique allowed us to capture a profile of the described wounds and the different measurements, such as the angle of entry of the weapon or tool used, and the aperture angle of the edge and the depth using cortical bone as a spatial reference. However, some limitations were found during tests of this technique. One of them is the spatial limitations due to the requirements of the size and shape of the piece. For example, a complete skull does not fit under the microscope and it will not be appropriate for the study. Another important limitation is bone cortical conservation. For the profilometer to show an accurate profile, a well-preserved cortical area is needed to serve as a reference for the measurements. This study identified many cases in which the cortical area was too damaged, showing in some cases spongy bone, which made it unsuitable for analysis. Finally, the reflective surface of the profilometer beam sweeps the bone surface and it is sensitive to particles that can alter the process, such as dust particles, reflective surfaces due to adhesive residue, etc.

CONCLUSIONS

The use of the confocal profilometer proved to be a great technique to analyse the changes in the surfaces of the bone, and in these particular cases, to show the shape of the marks and the angle at which the wounds were inflicted. It also contributed to elucidating the tool that caused the marks on the bone. However, some cases presented some methodological difficulties, and they had to be excluded from this study utilizing this kind of microscopy.

Due to these difficulties, the use of other evaluated techniques, complementary to the use of confocal imaging, allowed for a description of the superficial shape of the wound and proved to be useful to extract all of the information possible to describe the mark.

Complex anthropological studies with very limited samples, such as the ones above, which make it necessary to extract hypotheses from past events, require the use of many synergic techniques working in a complementary way and sophisticated software packages to extract all of the information available. Taphonomists can dispose of several options depending on their resources. In these cases, confocal reflection images have incredible potential, and in the near future the artificial intelligence algorithms associated with those microscopes will provide a great tool to identify these marks and classify them more quickly and accurately, allowing them to obtain reference data for greater precision in the attribution of tools to the marks.

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Natural mummifications in Castile & Leon (Spain)

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SUMMARY

The study of mummified bodies is of great interest because, on many occasions, clothing, utensils and documents are also found that provide additional information about the individual. In recent years, two disciplines have considerably advanced the study of mummified bodies: imaging via multislice CT scans (computed tomography) and genetic techniques, which are becoming increasingly accurate. Multislice CT is a non-invasive technique that provides 3D reconstructions and views of the inside of a body without dissection, conditions that tend to be required by archaeologists and museums. On the other hand, new genetic techniques enable analysis using minimal amounts of tissue, also avoiding any alteration to the body. The bodies studied have all been naturally mummified and still have much of their skin, hair, nails, muscles and viscera. Sometimes there is evidence of attempts to preserve certain uncovered areas, such as the face. In addition to an anthropological study, analyses have also been carried out by historians, physicists, geneticists, restorers, radiologists and pathologists, enhancing the findings and providing a global view of the individual and their context.

Key words: Mummies – Palaeopathology

INTRODUCTION

In anthropology, most of the time bones are the only resource available to identify and get to know the background of individuals, containing both a mineral component and also organic matter such as collagen and DNA. However, when soft tissue is found together with the skeleton, the amount of information we can obtain is multiplied. In modern analyses, skin, hair, muscle, viscera and tendons can reveal aspects not only of physical characteristics but also information about pathologies and genetic profiles regarding an individual's geographical origin and kinship relations. Sometimes the preservation of a body has not been intentional and, in many cases, environmental conditions have played a decisive role in its conservation. This is the case of the mummified corpses in this study, most of them well-known historical figures. In the best of these cases, only the uncovered parts of the bodies were slightly preserved before their burial. This type of study requires coordination of multidisciplinary teams including anthropologists, historians, restorers, physicists, chemists, radiologists and geneticists.

The aim of this paper is to analyse 12 bodies that have been mummified, most of them naturally, found in the region of Castile & León

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in Spain. Complete bodies or mummified parts are those that preserve the soft tissue along with the skeleton, and the body's external morphology has been maintained to a greater or lesser extent. In general, there are two reasons why a body can become mummified: human intervention, to preserve it over time; or as a consequence of environmental factors related to the terrain and climate.

Castile & León is a region in north-west Spain, inhabited since the Pleistocene (Bermudez de Castro et al., 1997) and later by pre-Roman peoples. Its high point was from the 15th to 17th century, when its monarchs were among the most influential in the world. The historical dating of the bodies ranges from the 11th to the 16th century.

All the mummifications we are going to look at here are natural and result from the burial conditions; in stone tombs in cold, well-ventilated locations. In some cases, there is evidence of preservatives having been used for aesthetic purposes and to delay disfigurement.

In almost all the cases studied, a study was carried out post-exhumation using multislice computed tomography (CT) and subsequent 3D reconstruction, providing us with details which could previously only be obtained by opening the coffins or bodies. Nowadays the aim is to preserve the morphology of the bodies as much as possible.

In addition to studying the body, the associated objects, documents and clothing have also provided valuable information about the era, and in some instances have also helped to date the burial more accurately.

In most cases, the studies have been multidisciplinary, involving historians, anthropologists, archaeologists, restorers, physicists, geneticists, radiologists and pathologists. (Ubelaker, 1978; Brothwell, 1987; Botella, 2000; Campillo, 2001; Pastor 2001a; Herrero et al., 2012).

MATERIALS AND METHODS

The mummified bodies studied were as follows:

- 1 THE PRINCE AND PRINCESS OF CASTILE. MUSEUM OF THE CITY OF VALLADOLID (1286-1291).
- 2 THREE NOBLES AND TWO CHILDREN. CHURCH OF SAN ESTEBAN, CUÉLLAR, SEGOVIA (1370-1530).
- 3 UNKNOWN GENTLEMAN. CHURCH OF "LA PEREGRINA", SAHAGÚN. LEÓN.
- 4 ELEANOR OF CASTILE. MONASTERY OF CALERUEGA. BURGOS. (1256-1275).
- 5 DON ARIAS GONZALO. CATHEDRAL OF ZAMORA (1020 to 1030 -?).
- 6 BISHOP PEDRO II. CATHEDRAL OF ZAMORA (?-1302).
- 7 BISHOP BERNARD DE PÉRIGORD. CATHEDRAL OF ZAMORA (? -1149).

In almost all the cases, except for the Castilian prince and princess (13th century), a study was carried out post-exhumation using multislice computed tomography (CT) and subsequent 3D reconstruction, providing details that could previously only be obtained by opening the coffins or bodies. A Toshiba CT device was used, specifically the Aquilion model with 64 detectors. 2mm slices were made every 1.5 mm. The 3D reconstruction was carried out using the 3D Slicer Program ©

Electron microscope scan: a Jeol JSM-T300 SEM and a Quanta FEG-200 ESEM were used. Backscattered electron techniques (BSE) and dispersive energy x-ray microanalysis (EDXA) were used to determine the chemical elements, both qualitatively and quantitatively. SEM was used to visualise skin samples and clothing.

Soft tissues were rehydrated using Sandison's (1955) formula for processing via conventional histology and haematoxylin and eosin staining.

Epiphyseal closure and dental eruption have been taken into account to determine the age. To determine sex, morphology and bone robustness were evaluated, although genetic analysis was also decisive in those cases where this was carried out. Trotter and Gleser's (1952) tables, based on the length of long bones in both males and females, have been used to estimate height.

Genetic analysis was carried out in the cases of the Castilian prince and princess, the nobles from

Cuellar and Princess Eleanor. The genetic profiles were produced by Prof. E. Arroyo-Pardo at the forensic genetics laboratory of the Complutense University of Madrid (Gamba et al., 2010). Mitochondrial and nuclear DNA analyses were carried out. In the case of Princess Eleanor, an external visible characteristics (EVC) analysis was performed to predict her physical appearance in terms of hair, eye and skin colour by means of autosomal SNPs.

A complete genetic analysis was performed in accordance with the authenticity criteria and analysis protocols established by our laboratory (Palomo-Díez, 2015; Gomes et al., 2017), carrying out sample selection, cleaning, grinding, DNA and mitochondrial extraction. DNA amplifications were carried out. EVC and bio-geographical SNP were analysed in accordance with the protocols of the "Luis Concheiro" Institute of Forensic Science (University of Santiago de Compostela, Spain).

RESULTS

THE PRINCE AND PRINCESS OF CASTILE. MUSEUM OF THE CITY OF VALLADOLID (1286-1291)

In 2000, the government de Castile & León (Spain) commissioned us to anthropologically authenticate the remains of Prince Alfonso, third son of Sancho IV of Castile, and Maria de Molina, who died at the age of 5 years old (1286-1291). After opening the sarcophagus, the remains of three partially mummified children were found. The hands were X-rayed and the ages estimated at 3.5 to 4 years, 2 to 2.5 years, and 8 to 10 months. Greulich and Pyle (1959) tables were used to determine the ages. The only body whose DNA could be analysed was the one of intermediate age, confirming that he was male. Most likely, and considering this is a secondary burial, the 3.5 to 4-year-old is Prince Alfonso, the discrepancy between his chronological and biological age resulting from retarded growth. It should also be noted that the comparative tables of Greulich and Pyle are from 1959 whereas the remains date from 7 centuries earlier (Pastor et al., 2001b).

THREE NOBLES AND TWO CHILDREN. CHURCH OF SAN ESTEBAN, CUÉLLAR, SEGOVIA (1370-1530)

In 2008, during an archaeological intervention at the church of San Esteban in Cuéllar (Segovia), several bodies were found in the archosolia of the High Altar. There were 2 skeletons, 3 mummified adults and 2 mummified children (Fig. 1). The three mummified adults were identified historically: Martín López de Córdoba Hinestrosa (ca. 1465-1530), his wife Isabel de Zuazo (ca. 1460-1520) and Alfonso García de León (ca. 1370-1409) (Pastor et al., 2011a, 2011c).

The five mummified bodies were scanned using multislice CT and then 3D reconstructions

Fig. 1.- Mummified bodies from the church of San Esteban in Cuellar, Segovia.

were created. Samples were also taken from all the bodies to determine the genetic profile and establish possible kinship relations.

The two children were male (genetically determined), did not present any alterations and, according to the dental eruption (3D reconstructions), they were one and three months old. They were wrapped in stitched sackcloth that entirely covered the body.

Alfonso García de León: According to dentition and ossification, he had a biological age of between 30 and 40 years and a height of approximately 1.70 m. Genetically confirmed male. No relevant palaeopathologies were found, except for a star-shaped calcium structure that might be a coral-shaped kidney stone.

Isabel de Zuazo: She was dressed in a linen shroud that exposed her ankles and feet, which had become skeletonised. Her forearms were crossed over her chest but at some distance from it, so that a large space could be observed over the chest. According to dentition and ossification, she would have been between 60 and 70 years old and approximately 1.73 m tall. Genetically confirmed female. Several palaeopathologies were detected, the most relevant being the following: the teeth were badly damaged, with loss and alveolar resorption. There was also bilateral thinning of the parietal bones in the skull (Fig. 2) which is considered to be an epigenetic variation (Pastor et. al., 2001a) and was a chance finding from the x-ray carried out on the skull. The spine had moderate scoliosis and there were evident signs of widespread osteoporosis and arthropathy in almost all the vertebral joints and extremities. On the CT images, a mass about 20 cm thick appeared between the thighs (Fig. 2). Macroscopically, the shroud was removed from this area and the mass was discovered to be a conglomerate of printed papers. The archaeologists were informed and they extracted all the material, which was composed of 66 papal bulls. Once restored, they were dated over a long period, between 1484 and 1544. Twelve of them are prior to 1501, so they are considered to be "incunabula". This is the most numerous set found to date. In view of this finding, all the evidence seems to indicate that, initially, Isabel had been holding the bulls over her chest, protected by her hands. In the days following her death, and due to the processes of decomposition, body movements caused the documents to slide into their final location.

Martín López de Córdoba Hinestrosa: He was wrapped in a Franciscan habit of wool, fastened by a rope. He had traces of a beard. According to the dentition and ossification, he had been between 55 and 70 years old and about 1.66 m tall.

Fig 2.- Front section of the body of Isabel de Zuazo. The thin arrows indicate bilateral parietal thinning. The large arrow indicates the series of Papal Bulls. Box: 3D image of the skull.

Genetically confirmed male. The paleopathology is diverse: there were several teeth missing, alveolar resorption, wear and periodontitis. There is a rounded osteolytic lesion in the left parietal region, approximately 1 cm in diameter, affecting the entire wall of the neurocranium. (Fig. 3). This type of injury is typical of some infectious diseases when they become chronic, such as tuberculosis, syphilis and brucellosis. In the SEM analysis (scanning electron microscope), the most significant finding was an alteration in the structure of the hair, consisting of the presence of large cavities, giving the hairs the appearance of hollow tubes. Such lesions are compatible with ringworm. There was a cowhorn cover next to the body.

UNKNOWN GENTLEMAN. CHURCH OF "LA PEREGRINA", SAHAGÚN. LEÓN.

In 2008, a sarcophagus found in the church of "La Peregrina" in Sahagún (León) was taken to our university laboratory in order to produce an anthropological report on the remains. The church dates it from 1257, although the coffin is from a later period, archaeologists dating it to around the 15th century. Another period suggested for the remains is the time of Pope Gregory XI (1329-1378), who held the papacy between 1370 and 1378, since there was a lead seal at the bottom of the coffin for this Pope. Inside the coffin, two adult female skeletons and a mummified body with skeletonised legs and feet were detected. Given the absence of a large number of bones in the feet and hands of both the skeletons and the mummified body (feet), this is a secondary burial for all three individuals. Here we will only deal with the mummified body.

Mummified body: The body was in a supine position with the forearms in front of the abdomen, the right one higher than the left. According to the cranial and pelvic morphology, the body is male and, given the overall ossification, the age would have been between 45 and 55 years. The height was approximately 1.75 m. The palaeopathology consists of almost complete loss of dentition with bone resorption and periodontitis. Extensive breakdown in all the osseous elements can be observed in the left elbow, more than likely causing pseudoarthrosis, leaving the elbow in flexion with hardly any mobility (Fig. 4). The CT scans revealed an image inside the pelvis

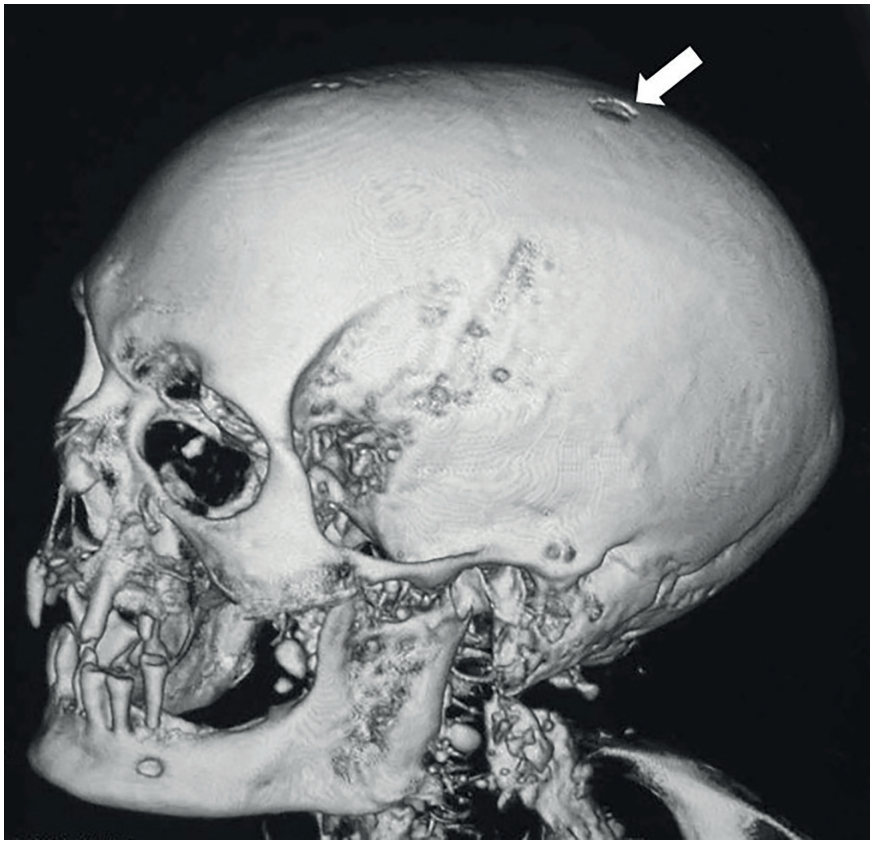

Fig. 3.- Osteolitic lesion in the skull of Martín López de Córdoba Hinestrosa.

consisting of two high-density hemispheres. After direct exploration, two complementary semi-ovoids were removed, calcareous and hollow and measuring approximately 2 x 2 cm. This was a hollow bladder stone that had ruptured (Fig. 5) (Pastor et al., 2010b).

PRINCESS ELEANOR OF CASTILE. MONASTERY OF CALERUEGA. BURGOS. (1256-1275)

Eleanor's remains were studied in 2014 during an archaeological intervention on her sarcophagus. Eleanor of Castile (1256-1275) was the legitimate daughter of King Alfonso X "The Wise" and Violante of Aragon. During a trip to France made by the King with his whole family, Princess Eleanor died suddenly in Perpignan, her father ordering that she should be buried at the Monastery of Caleruega (Burgos). It probably took several days to transfer the body from Perpignan to Burgos.

Before opening the coffin, it was studied via a multislice CT scan, which allowed us to see inside and create a 3D reconstruction of the contents. The study of the remains involved anthropologists, geneticists, radiologists, pathologists, chemists, physicists and tissue restoration specialists. The direct inspection and sampling of the remains was carried out at the Monastery of Caleruega itself. The coffin in which the remains were found was not the original. In 1933 the initial box was exchanged for a smaller one in which the body was now bent over itself, as well as including a few unconnected bones from at least two other individuals. The body was wrapped in a sheet with an embroidered border of golden thread, and dressed in various overlapping items of clothing. According to the ossification in general, and especially that of the iliac crest (Risser sign (1958)), the age was about 17 to 18 years and the height about 1.70 m. The genetic study confirmed the remains were female. The rest of the analyses, such as histopathology and scanning electron microscopy, did not reveal any pathologies. The scan used was an ESEM (environmental scanning electron microscope) to study the skin, carried out with backscattered electrons (BSE), with some areas subsequently being subjected to dispersive energy x-ray microanalysis (EDXA) to determine the chemical composition. The results revealed the presence of common elements due

Fig. 4.- 3D reconstruction of the left elbow of the mummified body from the church of "La Peregrina", showing trauma with pseudoarthrosis.

to biological waste and also two elements in unusually high quantities: sulphur and silver (Fig. 6). Ointments for therapeutic purposes and aromatic products used to cover up the smell of decomposition were already being used in the Middle Ages. Probably in anticipation of the long journey the body had to make, the uncovered parts were smeared with a silver sulphate-based balm. In addition to confirming the sex, the DNA study also revealed that Eleanor was of European origin and would phenotypically have had dark hair and green eyes (Fig. 7) (Pastor et al., 2014).

DON ARIAS GONZALO. CATHEDRAL OF ZAMORA (1020 to 1030 -?)

In 2015, during the restoration of a stone tomb in Zamora Cathedral, the mummified remains appeared of what archaeologists believe could be Don Arias Gonzalo, a renowned man from Zamora who was loyal to Ferdinand I, King Consort of Leon, and who was killed in the 11th century. Both he and his sons confronted King Sancho II of Castile at the siege of Zamora. The remains reveal partial mummification of an incomplete body that is missing part of the abdomen, pelvis and both lower limbs. The right upper limb is not preserved either. The head is bent over the left clavicle and the elbow is bent, with the hand over the upper abdomen. The chronicles explain that the body had been embalmed for burial, but years later it was dug up and the body desecrated, so that only some parts have been preserved. After inspection, a multislice CT scan was carried out, determining that the man had been over 65 years of age and about 1.67 m tall. The most striking palaeopathology was found in the dentition, with a large number of teeth missing and also alveolar resorption, the presence of calculus to differing degrees, periodontitis and apical abscesses. In the posterior part of the right parietal region there was a round osteolytic lesion of 10 mm in diameter affecting the outer table and cranial diploe. These types of lesions may be compatible with brucellosis, tuberculosis or syphilis. Histological studies revealed nothing significant. The entire body was dark in colour and there were linear markings on the forehead, indicating that there must have been a tape around the head. Scanning electron microscopy (SEM) revealed that the facial hair had been cut flush with the skin a short time

Fig. 5.- Horizontal slice from the pelvis via multislice CT scan showing two semi-circular images (fine arrows) and a coprolite (star). Box: Broken bladder stone.

before burial, as the tip of the hairs was bevelled. By means of backscattered electrons (BSE) and energy dispersive x-ray microanalysis (EDX), unexpected elements such as sulphur and silver were found in the facial skin. This had also been found during the study of Princess Eleanor using the same techniques. Possibly silver sulphide was also used in the embalming (De Lera, 1999; Pastor et al., 2015a).

BISHOP PEDRO II. CATHEDRAL OF ZAMORA (?-1302)

In 2015, a simple sarcophagus made of planks in 1621, which contained the remains of Pedro II, Bishop-elect of Zamora, was moved from the Chapel of St. Inés in Zamora Cathedral to the Laboratory of Anthropology of Valladolid University. The bishopric of Pedro II lasted from 1286 to 1302, the date of his death. There is a wealth of documentation on the bishop, including his will, written in the Romance language. An intellectual who was praised by Pope Innocent IV, he possessed an interesting library with books on medicine and law. His body was partially mummified and dressed in episcopal clothing. When the sarcophagus was removed from the stone tomb, the following objects belonging to his funeral trousseau were taken out of the coffin: an episcopal staff, chalice and paten. The body was in a supine position with both limbs stretched out beside the body. The lower limbs were in external rotation. The head was separate from the body. Mummification was incomplete as some parts had become skeletonised. The sarcophagus was subjected to a multislice CT scan, and the body was determined to be male due to the cranial and pelvic morphology. According to the state of ossification, his age would have been over 65 years and his height would have been about 1.52 m. The palaeopathology found consists of arthrosis in the cervical spine, rhizarthrosis in the right hand, as well as arthropathy in the joints of the last two phalanges of all the fingers of the left hand. He also had a deviated left nasal septum. Dental pathology was very abundant: loss of several teeth with bone resorption, periodontitis, calculus formation and intense wear. Alveolar destruction, a consequence of abscesses, was observed in several areas (De Lera, 1999; De Lera, 2002; Pastor et al., 2015b).

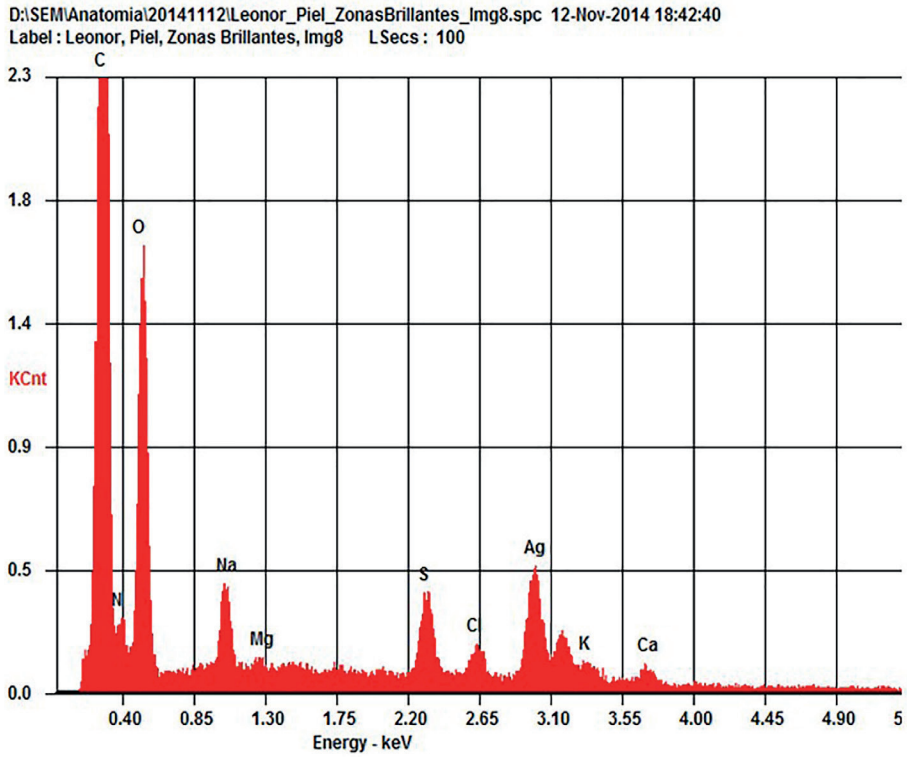

Fig. 6.- Determination of elements using backscattered electrons on the skin of Princess Eleanor's face. A peak of silver (Ag) can be observed.

BISHOP BERNARD DE PÉRIGORD. CATHEDRAL OF ZAMORA (? -1149)

In 2018 a stone tomb was opened in Zamora Cathedral containing the remains of the first bishop of Zamora, Bernard de Périgord (? -1149). After the remains had been removed and the stone tomb and its contents thoroughly inspected, the remains were taken to the Laboratory of Anthropology of the Department of Anatomy and Radiology at Valladolid University. The body was dressed in multiple items of clothing and partially mummified, with the head disconnected from the neck, as well as the forearm from the upper arm on the left. Next to the body were some unconnected bones from another individual and a large amount of soil of different textures and sizes. Due to the general state of ossification, his age could have been between 65 and 80 years. According to the cranial and pelvic morphology, the body is male and his height was about 1.65 m. After carrying out the multislice CT scan, the following was noted:

Head: typical dental pathology of the time. Remains of brain mass in the occipital region indicate that it was a primary burial without any changes in body position.

Chest: in the middle of the eighth right rib, a callus from an old fracture could be seen (Fig. 8). The T7 to T10 vertebrae showed signs of degenerative arthropathy with fusion of the vertebrae.

Lower extremities: in the upper third of the left fibula there was a long, thin area of exostosis that could be due to an old trauma. Two epigenetic variations were found: bregmatic bone and lambdoid ossicles. In the soil between the upper part of the femora there was a rounded mass of about 12 mm in diameter and the density of metal (3071 UH). Its origin is uncertain although it could be a barite conglomerate, given the high density (De Lera, 1999; Pastor et al., 2018).

DISCUSSION

For centuries, the conservation of bodies had been a key concern for shamans and guardians of the dead, as can be seen from the examples found in Ancient Egypt, the Inca Empire and Imperial China. Even St. John, in his Gospel, tells how

Fig. 7.- Princess Eleanor's mummified head.

Nicodemus brought 100 pounds of myrrh and aloe to embalm the body of Jesus Christ (Grilletto, 1989; Mayer, 1990)

Such interest in preserving bodies has also been seen in our own era. In the 17th century, Ruysch (1638-1731) (Bernardo et al., 2014) started to carry out conservation for research and teaching purposes, and was the first person to begin systemising injections with intravascular mercury salts (cinnabar) and alcohol preservatives. Subsequently, there have been many attempts to preserve bodies to ensure that they last over time, until we reach what could be called a huge revolution in this respect: in 1863 Hofmann (1866) synthesised formaldehyde, a product that would later be used as a preservative by the botanist Julius Cohn (Musial et al., 2016).

In the last two centuries, the method of Salafia (1869-1933) (Edward et al., 1993), of Tranchina (1797-1837) (Tranchina, 1835) in Naples and Gannal (1791-1852) (Gannal, 1841) in Paris, at the beginning of the 19th century, used arsenic acid mixed with sulphuric and acetic acids. Pedro Ara (1891-1973) (Ara, 1938) made extensive use of the paraffinisation method previously devised by the Belgian Leo Fréderiq in 1876, (Aranega, 2010) and treated the bodies of Eva Perón and Manuel de Falla during his stay in Argentina. In 1979, the German von Hagens (1945) (von Hagens, 1979) patented a method he called "plastination" which, given its high quality, has not been equalled by any other since.

An uneven state of conservation, even in different parts of the same body, is a result of how and where it has been located. Parts treated with ointments and balsams are better preserved, as are those protected by a large muscle mass. In contrast, legs, feet and hands, in addition to

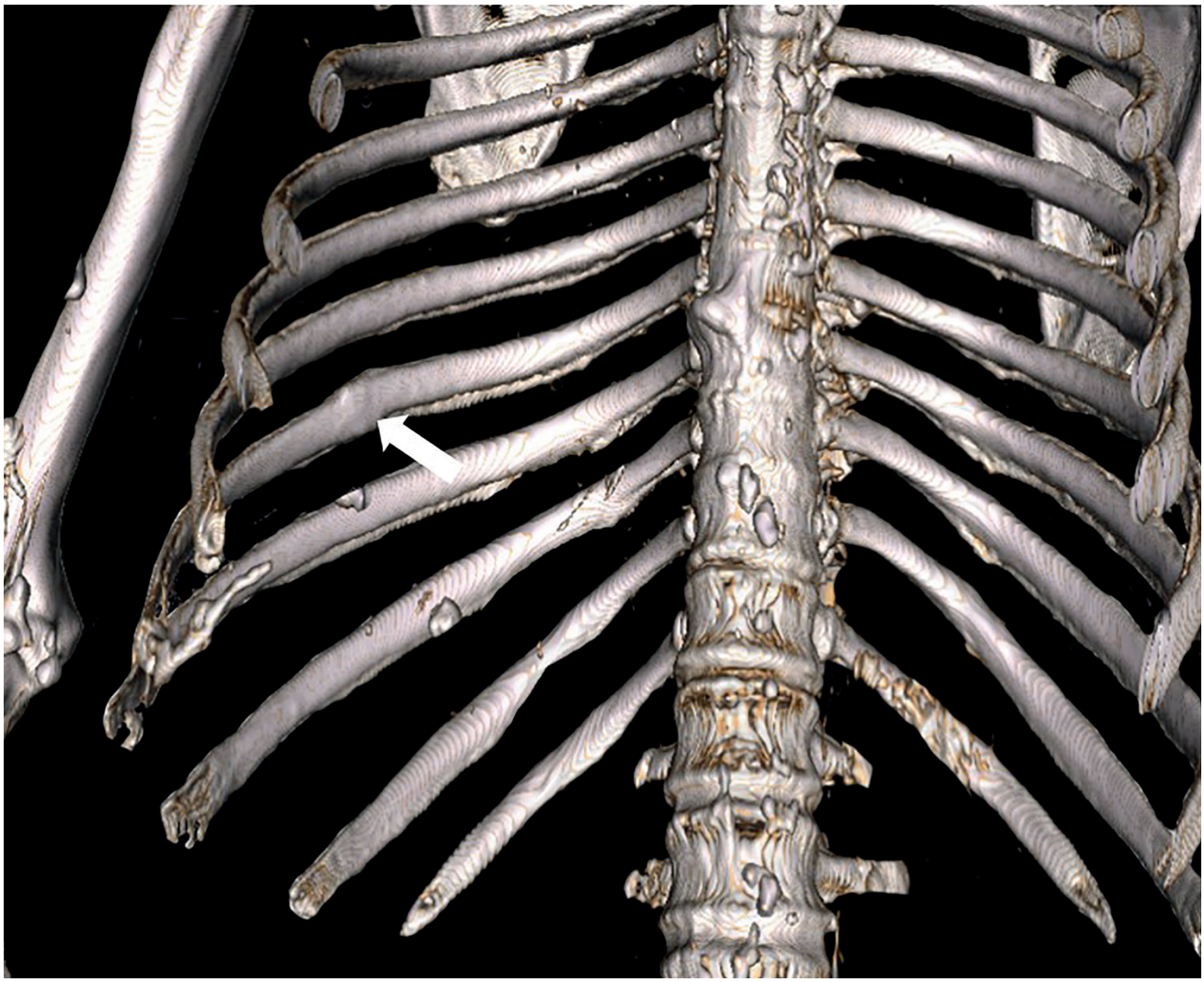

Fig. 8.- 3D reconstruction of the thorax of Bishop Bernard de Périgord showing a bone callus on the eighth right rib (arrow).

having less muscle mass, are generally more exposed to environmental changes, and their state of conservation suffers accordingly. We have not managed to achieve good results when rehydrating skin and muscle using the Sandison method (1955), perhaps because it has been designed for previously embalmed tissue.

The use of multislice CT has been fundamental and has provided a great deal of information, guiding access to the inside of the body when necessary. Good results have been obtained even with bodies inside coffins, including those lined with zinc.

SEM and ESEM have proved to be of great help in determining some pathologies, and the backscattered electron technique (EDX) has revealed the existence of elements that can guide us regarding the procedures used to embalm the bodies or other deliberate treatments.

Genetic studies have been very important for the following: kinship relations and confirmation of sex and, in the case of Princess Eleanor, the findings showed that mummified remains can provide information regarding geographical origin and phenotypical characteristics.

In addition to the study of the body, the associated objects, documents and clothing also provide valuable information about the time of death and the historical context. In all cases, care must be taken to ensure that studies are multidisciplinary and involve historians, anthropologists, archaeologists, restorers, physicists, geneticists, radiologists and pathologists.

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New Trends in Forensic Anthropology

PRESENTATION

New Trends in Forensic Anthropology I
Carme Rissech

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