



Solving cold cases: The importance of new identification techniques in old cases of forensic anthropology

Gabriela Araujo dos Santos^{a,*,1}, Talita Lima de Castro Espicalsky^{b,2},
Leticia Regina Alfaia Custódio^{c,3}, Melina Calmon Silva^{d,4}, Eugenia Cunha^{e,f,5}

^a University of Coimbra, Center for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, Portugal

^b Legal Medical Institute of Porto Velho, Flores da Cunha St., 4359, Porto Velho, Rondônia 78.930-830, Brazil

^c Faculty of Dentistry of Piracicaba, University of Campinas, Limeira Av., 901, Piracicaba, São Paulo 13414-903, Brazil

^d National Center for the Dissemination of Forensic Sciences, Brazilian Federal Police, Distrito Federal, Brazil

^e University of Coimbra, Centre for Functional Ecology, Laboratory of Forensic Anthropology Department of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, Portugal

^f National Institute of Legal Medicine and Forensic Sciences, Lisbon, Portugal

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ABSTRACT

Scientific and technological developments experienced by forensic sciences have increased the possibility of human identification. Forensic anthropology has developed worldwide and has made significant progress in Brazil in the last 10 years. This context has allowed forensic medicine institutes to use new techniques to resolve cold cases of human identification. This work aimed to report the methodology, development, and partial results of the LAFOL Identifies Operation, carried out at the Forensic Anthropology and Forensic Dentistry Laboratory (LAFOL) of the Medico-Legal Institute of Porto Velho. We carried out a multidisciplinary expert approach on human skeletal remains archived over the last 20 years to identify these individuals using current scientific methods. Eighty-one cold cases of skeletonized and/or mummified human skeletal remains were reanalyzed; they were archived in the LAFOL Laboratory collection as unidentified. Anthropological analyses showed that the collection is predominantly composed of adult individuals (86.4%), between 21 and 39 years old (55.6%), and males (62.9%). In the human skeletal remains identified, forensic anthropology played a key role in the case screening process. We expected that the results presented in this work will inspire other Medico-Legal Institutes in Brazil and worldwide to establish task forces for the reanalysis of cold cases in their institutes, promoting new forensic dentistry and anthropological comparisons and collecting biological samples to feed the database of genetic profiles of missing persons.

1. Introduction

About 70–80 thousand individuals disappear in Brazil each year, but only half of these individuals are found alive [1,2]. The discovery of an unidentified body leads to two expectations: identifying it and returning it to its family. However, a recent regional survey estimated that around 30% of the bodies examined in the Amazon region remain unidentified after the conventional thanatoscopic analyses [3], making it impossible

to return them to their families for burial. These unidentified bodies are buried as unknowns or archived in ossuaries at Medico-Legal Institutes or cemeteries and are considered cold cases over time.

The scientific and technological development in forensic sciences worldwide and, more recently, in Brazil has increased the identification of unidentified bodies in forensic medicine institutes. The use of smiling photos of the missing person for forensic confrontation and radiological comparative analysis and the use of anthropological characteristics for

* Corresponding author.

E-mail address: gas.archaeology@gmail.com (G.A. dos Santos).

¹ ORCID: 0000-0003-3594-7555

² ORCID: 0000-0002-4226-4464

³ ORCID: 0000-0002-1108-8808

⁴ ORCID: 0000-0002-7622-4460

⁵ ORCID: 0000-0003-2998-371X

human identification combined with the implementation of laboratories for DNA analysis in Brazilian Medico-Legal Institutes in the last 15 years [4–6] represent an important development to reduce the number of unidentified bodies by the Medico-Legal Institutes in Brazil [7,8]. Increasingly, human identification is a holistic process in which everything must be considered, and the interaction among the various identifiers must be a priority.

This context has allowed Medico-Legal Institutes to use new techniques to reanalyze cold cases of human identification [9–12]. Currently, there is no standard operating procedure for cold case analysis in Brazil despite the availability of several methods and techniques.

Thus, the present work aims to report the methodology, development, and partial results of the LAFOL Identifies Operation, carried out at the Forensic Anthropology and Forensic Dentistry Laboratory (LAFOL) of the Medico-Legal Institute of Porto Velho. This project involved a specialized multidisciplinary approach on human skeletal remains archived over the last 20 years to identify these individuals using current scientific methods.

2. Materials and methods

The LAFOL Identifies Operation was carried out between 2019 and 2023 at the LAFOL Laboratory of the Medico-Legal Institute of Porto Velho in the Brazilian Amazon region. It reanalyzed 81 cold cases of skeletonized and/or mummified human skeletal remains from the LAFOL Laboratory collection. Cases presenting complete documentation (previous reports, police reports, and anthropological data collected at the time) and those without any related data, information, or documents were included. We also included cases with any entry date before 2021, in addition to cases that did not have a record of the entry date.

An official forensic dentist responsible for coordination, a doctoral student in Forensic Anthropology, a master’s degree student in Forensic Dentistry, and a specialist in Forensic Dentistry participated in the operation. The LAFOL Identifies Operation was structured into six phases, five of which have already been completed, and the sixth is in progress (see Table 1).

The first phase corresponded to anthropological collection management. This included the survey of cases to be reanalyzed, the records of cases available, and an extensive reading of each document related to the cases.

In the second phase, boxes were reopened, and the osteological material was subjected to careful cleaning according to the internal cleaning protocol. Since the old boxes and labels had significant damage that could put the chain of custody of the material at risk, the human skeletal remains were placed in new boxes acquired by the project and cataloged in a standardized way with new labels. They received a code containing the acronym RNI (Non-Identified Remains) and a number that started with the oldest case and continued in ascending order until the most recent case, in addition to the Medico-Legal Institute of Porto Velho entry protocol number that accompanied the unidentified bodies in a standard way.

The third phase consisted of the reanalysis of each archived human skeletal remains. This was carried out by a Standard Operating Procedure (SOP) based on current methods and techniques of anthropological analysis, preferably validated or in the process of validation in Brazil, selected after a balance of qualities and limitations of each one. The biological profile (sex, age, population affinity, and height) was estimated for each human skeletal remain. The presence of individualizing signs, pathologies, and traumas were also recorded. Dental characteristics were photographed and noted, in addition to being x-rayed when necessary. All data collected at this stage was entered into the RNI Database, specially created for LAFOL Laboratory during the LAFOL Identifies Operation, to enable consultations and comparisons to identify the alleged victims.

The fourth phase involved the active search to find possible family members. This protocol was divided between cases with and without the

Table 1
Summary of the protocol applied in the LAFOL Identifies Operation.

Phase	OPERATIONAL ROUTINE		
1	✓ Anthropological collection management ✓ Selection of cases to be reanalyzed ✓ Documentary search related to each case		
2	✓ Registration of cases with the name of the alleged victim ✓ Opening the boxes and photos of the contents ✓ Cleaning the material according to specific SOP and conditions of the remaining materials		
		Planned Action	
	Conditions		
	Human skeletal remains with soft tissues	Maceration by cooking at 90 °C followed by manual cleaning with bushings, brushes, dental material, water, and neutral detergent	
	Human skeletal remains without soft tissue, in good condition, however, with impregnated sediments	Manual cleaning with a loofah, brush, dental material, water, and neutral detergent	
	Friable skeletal remains, however, with impregnated sediments	Manual dry cleaning with brushing, brushes, and dental materials	
	Human skeletal remains already cleaned, possibly from previous procedures, or with mummified tissues (totally or partially)	No cleaning procedures were necessary in these cases	
3	✓ Anthropological and dental analysis of the collection ✓ Registration of individualizing characteristics ✓ Registration of antemortem, perimortem, and postmortem trauma		
	Methods used in biological profile estimation		
	Characteristic	Method	Bone
	Sex	Diagnosis Sexuelle Probabiliste (DSP2) – Bruzek et al. (2017) [13,14]	Pelvis
		Analysis of morphological characteristics – Phenice (1969), Walker (2008) [15–17]	Pelvis
		Analysis of morphometric traits – Wasterlain et al. (2000), Curate et al. (2016) [18,19]	Femur and Humerus
		Analysis of morphological characteristics – Phenice (1969), Walker (2008) [16,17, 20]	Skull
	Age (not adult)	Analysis of tooth eruption and mineralization – Nicodemo	Teeth

(continued on next page)

Table 1 (continued)

Phase	OPERATIONAL ROUTINE	
	et al. (1974), Arbenz (1987), Alqahtani (2012), France (2017) [21–24]	All bones available
Age (adult)	Analysis of the development and process of bone fusion – Scheuer et al. (2000), Schaefer et al. (2009) [25,26]	Teeth
	Root transparency – Lamendin et al. (1992) [27]	
	Analysis of degenerative processes through phase systems – Brooks et al. (1990), Calce (2012) [28,29]	Pelvis
	Analysis of degenerative processes using the scoring system – Buckberry et al. (2002) [30]	Pelvis
Stature	Fusion stages of spheno-occipital synchondrosis – Scheuer et al. (2000) [26]	Skull
	Fusion stages of the medial epiphysis of the clavicle – Scheuer et al. (2000) [26]	Clavicle
	Linear regression equation – Freire (2000), Mellega (2004) [31–33]	Humerus, radius, ulna, femur, tibia, and fibula
	Linear regression equation – Albanese (2016) [34]	Humerus, radius, ulna, femur, tibia, and fibula
Population affinity	Non-metric cranial traits – Hefner (2014) [35–37]	Skull
4	✓ Active search for family members, prioritizing cases with the name of an alleged victim ✓ Campaigns in media and on the internet to find possible family members for cases without the name of an alleged victim	
5	✓ Biological sample collection according to the following requirements: - Cutting a window in the femoral shaft or a long bone (in the case of absence of the femur) - If it is impossible to collect long bones, collect teeth (molars), considered as the last option given the importance of the dentition for identification	

Table 1 (continued)

Phase	OPERATIONAL ROUTINE
6	✓ Sorting process for the missing vs. remaining. ✓ Dental data comparison ✓ Confrontation of genetic profiles ✓ Return of the identified individuals to their families

name of an alleged victim. To clarify cases in which the human skeletal remains were unknown without any suspicion of identity, campaigns were carried out in the local press to publicize the LAFOL Identifies Operation and to encourage family members of missing persons to come to the Medico-Legal Institute of Porto Velho to register and collect DNA samples.

In each case, data previously collected within the existing previous investigation were considered. For cases in which the documents showed the name of the alleged victim, the families were searched by name through the available police systems, including the databases of federal and state agencies and the National System for the Location and Identification of Missing Persons (SINALID) linked to the Public Ministry, which registers the family members of missing persons in Brazil. When the search in official systems was unsuccessful, data available online was searched on social networks, internet pages, news, or any online publications that showed contact information for the family members of the alleged victims. When located, they were asked to provide photographs and medical and/or dental documentation of the missing person and a biological sample for DNA testing.

The fifth stage consisted of collecting biological samples from the human skeletal remains for DNA analysis according to local protocol. The closed cases, in which biological samples from the family members of the alleged victim were available, were sent to the laboratory for comparative DNA analysis. In open cases, in which there were no supposed family members available for collection, the biological samples from the human skeletal remains were included in the “National Bank of Genetic Profiles” (BNPG).

The sixth phase consisted of comparing the information collected from the human skeletal remains with the data of the missing persons who appeared as alleged victims in the cases under investigation. This included comparing the genetic profiles obtained through DNA testing.

3. Results

LAFOL Identifies Operation produced individual reports on each of the remaining 81 cold cases, extracting important information. About 83.9% (n = 68) of the human skeletal remains were already skeletonized, 12.4% (n = 10) were calcined, and 3.7% (n = 3) showed signs of mummification in parts of the body tissues. In 33% (n = 26) of cases, the available material consisted of just a skeletonized skull. Most of the material (96.2%) was already cleaned or contained mummified tissue, meaning no cleaning procedure was necessary. In 3.8% (n = 3) of cases, sediments had to be removed with manual dry cleaning since the bones were friable.

The anthropological collection, cleaning, relocating in new boxes, and cataloging of each human skeletal remains took around three months. This allowed the human skeletal remains to be stored more satisfactorily in the collection room (see Fig. 1), facilitating subsequent work.

Among the cases presenting documentation, we can observe that the human skeletal remains analyzed were admitted to the Medico-Legal Institute of Porto Velho over 17 years ago (2004–2021), representing an average of three archived cases per year with a standard deviation of 1.41. Around 18.5% (n = 6) were found in Porto Velho or its surroundings, and around 81.5% (n = 29) were from other cities in the interior of the state of Rondônia, in the Brazilian Amazon region.

Anthropological analyses showed that the collection is predominantly composed of adults (96.3%), average age between 21 and 35

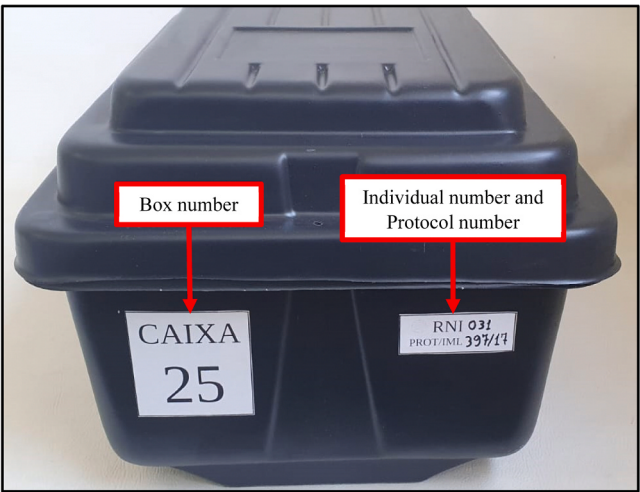


Fig. 1. Box after the cataloging procedure, with new labels containing box number (left) and registration number of each human skeletal remains (right).

years old (24.7%), and males (37.1%). The estimation of population affinity demonstrated a great diversity of biogeographical origins for most individuals (16.1%), as presented in Table 2.

Thirteen individuals (16.05%) had peri mortem trauma, which is relevant to understanding events close to death or shortly after death. The most common types of injuries found were blunt, sharp, and sharp-blunt trauma. In eight individuals, we observed important individualizing characteristics that can significantly contribute to the

Table 2
Biological profile of the LAFOL Laboratory anthropological collection.

Biological Profile Estimation		
Sex estimation	Amount	Percentage
Male	30	37.1%
Female	10	12.3%
Undetermined	15	18.5%
Not Observable (non-adult and human skeletal remains that cannot be analyzed)	26	32.1%
Age estimation* [38]	Amount	Percentage
Fetus (1–9 intrauterine months)	1	1.2%
Newborn (1month old)	0	0%
1st childhood (2 months old to 6 years old)	1	1.2%
2nd childhood (7–12 years old)	0	0%
Adolescence (13–20 years old)	1	1.2%
Young adult (21–35 years old)	19	24.7%
Middle-aged adult (36–50 years old)	11	13.6%
Elderly adult (51 years old or older)	4	4.8%
Undetermined	15	18.5%
Unobservable	29	34.8%
Height estimation (meters)** [39]	Amount	Percentage
Short male (1.62 – 1.67)	3	3.7%
Average male (1.68 – 1.73)	12	14.8%
Tall male (1.74 or above)	5	6.2%
Short female (1.48 – 1.43)	0	0%
Average female (1.54 – 1.61)	1	1.2%
Tall female (1.62 or more)	two	2.5%
Undetermined	27	33.3%
Unobservable	31	38.3%
Population affinity estimation	Amount	Percentage
Africa	9	11.1%
Asia	1	1.2%
America	0	0%
Europe	12	14.8%
Undetermined (results with a lot of mixing)	13	16.1%
Not observable (skull absent or with a lot of fragmentation)	46	56.8%

*Age group classified according to Baccino et al. (2013).

**Height is divided into classes according to the latest census from the Brazilian Institute of Geography and Statistics (IBGE, 2022).

identification process. Fig. 2 shows a radius with a metal rod with the serial number, Fig. 3 corresponds to a skull with signs of craniotomy, Fig. 4 is a pelvic girdle and T-6 to T-8 vertebrae with signs of Diffuse Idiopathic Skeletal Hyperostosis, and Fig. 5 is a skull with changes in the nasal cavity. However, no family member has sought out the Medico-Legal Institute of Porto Velho in search of an individual with any of these individualizing characteristics.

In 70.6% (n = 57) of the human skeletal remains analyzed, the identification by forensic dentistry was limited since there was no dental information available during cadaveric examination. This occurred due to the absence of the skull in the material (35.9%, n = 29), carbonization and/or extreme fragmentation of the material (23.5%, n = 19), or the post-mortem loss of existing teeth (11, 2%, n =9) in which the information could not be obtained or was insignificant. However, in 29.4% (n = 24) of cases, the identification can be made through dental comparisons if the family member of the missing person is located.

After carrying out campaigns to search for family members of the missing persons, 52 missing persons were registered through interviews with family members who went to the Medico-Legal Institute of Porto Velho between 2017 and 2023 in search of someone. The oldest report of disappearance dates from the 1980 s. Most of the cases occurred in Porto Velho or nearby cities. The profile of the missing persons is mostly adult, 21 and 34 years old, male, brown, and approximately 1.70 m tall.

After the interviews, the family members were sent to biological sample collection for DNA testing. Then, they were compared with the samples in closed cases (in which human skeletal remains presented a biological profile compatible with the description of the missing person) or inserted into the National Bank of Genetic Profile (BNPG) in open cases (in which, to date, no human skeletal remains with a profile compatible with the missing person have been found).

Based on records from investigations at the time or documents and belongings that accompanied the unidentified body, we identified 11 human skeletal remains with the name of the alleged victim, which had priority in DNA tests. By August 2023, the LAFOL Laboratory received DNA test reports from two cases in which individuals were identified. Their family members finally received the human skeletal remains and the death documents necessary for burial (see Fig. 6). The remaining cases are still undergoing genetic analysis and awaiting results.

4. Identified persons

4.1. Case 1

One of the success stories of LAFOL Identifies Operation relates to a 32-year-old man who had mental disorders and lived with his father in the countryside. In 2011, after leaving home without warning, the man disappeared, and his father reported the disappearance. The year following his disappearance, incomplete human skeletal remains containing only the skull, tibias, left femur, and left pelvis were received at

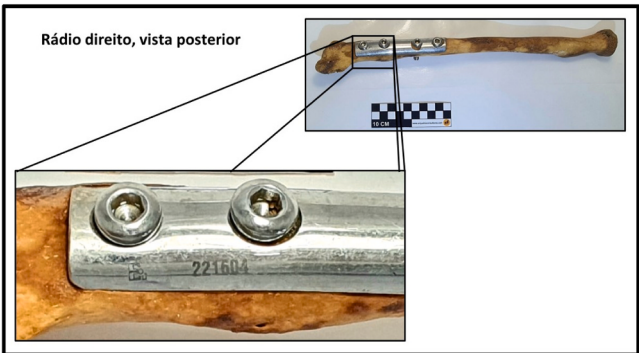


Fig. 2. Metal rod in the distal third of the right radius, in posterior view, and the serial number.

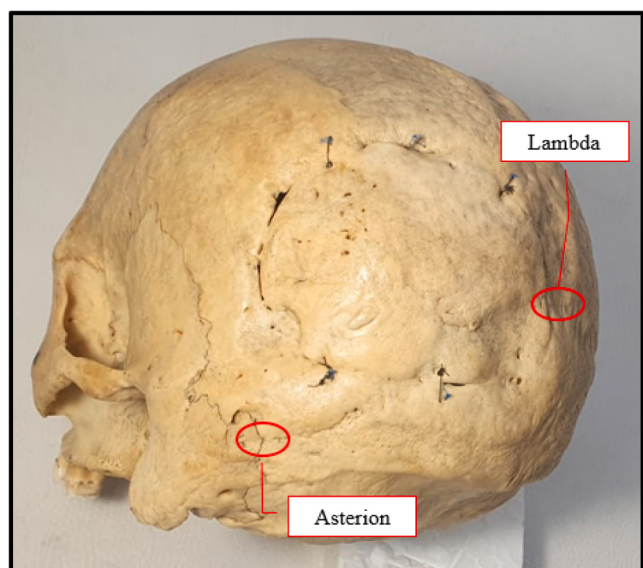


Fig. 3. Left posterior part of the skull, surgery healing region.

the Medico-Legal Institute of Porto Velho, accompanied by personal documents. Despite the team's efforts at the time, they could not identify the body using the methods available in 2012 since there was no record of the man that could be used in dental or anthropological confrontation, and due to the lack of a laboratory to carry out genetic tests in the region. In 2021, LAFOL Identifies Operation determined the reopening of the case, and the genetic comparison report requested confirmed the man's identity. In 2022, when the coordination of the LAFOL Identifies Operation sought out family members related to the case to return the body, the man's father had already passed away, unfortunately, before receiving the news confirming his son's identity.

4.2. Case 2

The second successful case is a 35-year-old Bolivian immigrant man who worked as a caretaker on a farm about 100 km from Porto Velho

and who disappeared in 2015. A year later, an incomplete human skeletal remains containing only a few bones from the chest, upper limbs, and skull was found in a region of the Amazon Rainforest. The human skeletal remains were examined at that time, but it was not possible to identify them, and the investigations were shelved in 2016. The case was reopened in 2021, during LAFOL Identifies Operation, and due to the similarity of anthropological characteristics between the human skeletal remains and the Bolivian man, genetic DNA tests were carried out comparing him to the missing man's sister. The human skeletal remains were confirmed to be the Bolivian man who had disappeared a few years earlier.

5. Discussion

The LAFOL Identifies Operation took a closer look at cold cases of unidentified bodies and a new investigative approach to these human skeletal remains in the state of Rondônia. Anthropological analysis and estimation of the biological profile constitute a solid basis for investigating unidentified bodies. They allow initial screening of cold cases by comparing anthropological data, belongings, and information on the human skeletal remains with files and data on the alleged victims and their disappearance [40].

The human skeletal remains at the LAFOL Laboratory collection consisted of a large representation of young adult males between 21 and 35 years old. This aligns with the information outlined by the 17th Brazilian Yearbook of Public Security, which highlights that approximately 90% of victims of intentional violent deaths are males aged between 17 and 35 years [2].

Unfortunately, many human skeletal remains corresponded only to the skull, which prevented the application of some methods to obtain the biological profile. The arrival of only the skull at the Medico-Legal Institute of Porto Velho is common in forensic medicine institutes in Brazil [41–43]. This can be due to the faster decomposition of head tissues compared to other parts of the body, often being the first to become totally or partially skeletonized [44,45]. Therefore, it can be transported by fauna or fall down slopes to places further away from the rest of the body.

The human skeletal remains with the oldest entry into the collection are from 2004. However, we cannot confirm whether it is the oldest case



Fig. 4. Signs of Diffuse Idiopathic Skeletal Hyperostosis (DISH). A) Pelvic girdle, top view, highlighting the fusion in the sacroiliac joint. B) T-6 to T-8 vertebrae, left lateral view, highlighting the calcification of the anterior vertebral ligament.

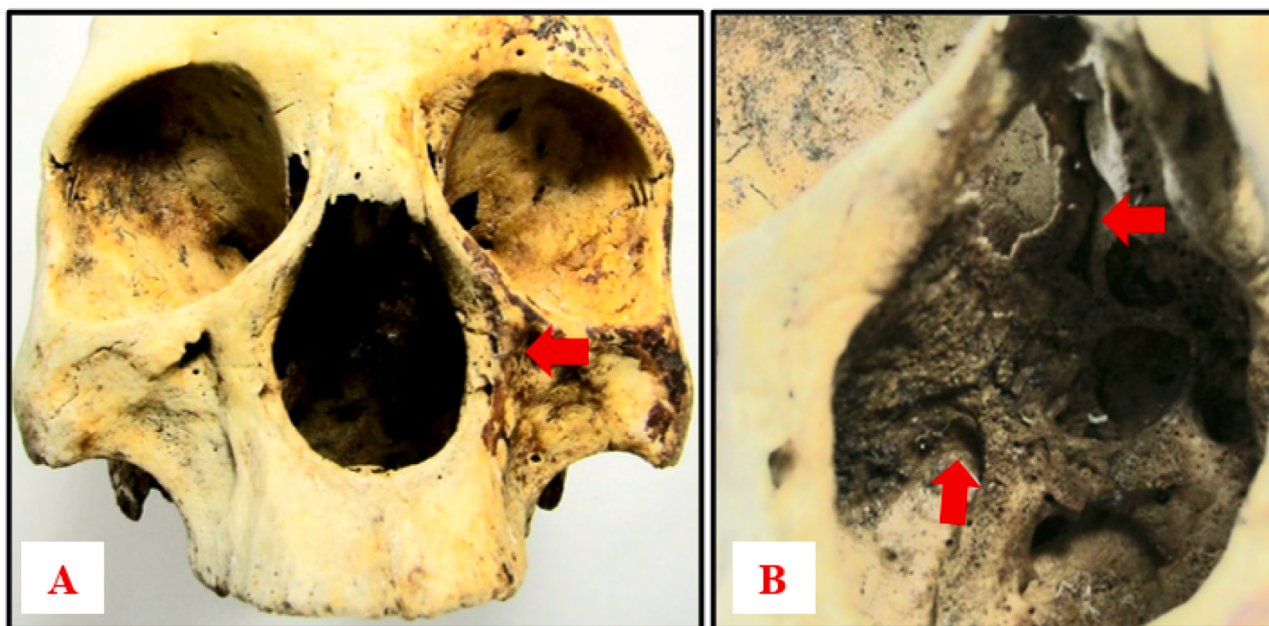


Fig. 5. Pathology in the nasal cavity. A) Skull, anterior view, highlighting the bone remodeling of the edge of the pyriform opening into the nasal cavity. B) Right superolateral part of the nasal cavity with prominent granularity and bone erosion.



Fig. 6. Symbolic act of returning the human skeletal remains to the family member carried out by the LAFOL Identifies Operation team.

since around 56.8% ($n = 46$) of the human skeletal remains in the osuary did not have registration tags or any chain of custody tools that could link the unidentified body to documents or data related to it. This prevented the data collection from these unidentified bodies, such as their date of entry, origin, and the name of any alleged victim that may have existed when it was found.

The operational procedures created for the complete collection of information and data processing carried out during the LAFOL Identifies Operation, maintaining the appropriate chain of custody, were crucial to the results obtained and contributed to establishing the chain of custody guidelines by Law No. 13,964 of December 24, 2019 [46]. The chain of custody in Forensic Anthropology and other criminal sciences is essential and aims at documented and continuous control of the history, location, transfer, custody, and manipulation of traces from the moment they are collected until their use in legal proceedings [47–51]. According to Damascena et al. [50], an ineffective chain of custody can lead to a breakdown in the traceability of evidence and interfere with the adequate analysis of traces, consequently, making it difficult to obtain

concrete answers in each case. The loss of labels and contextual data from some of the human skeletal remains at the LAFOL Laboratory occurred during building relocation and changes in the physical structure of the collection in previous decades. This reflects the lack of chain of custody during this period, which substantially made it difficult to investigate the identity of these individuals.

To avoid these problems in the future, the LAFOL Identifies Operation proposed protocols for cataloging each archived human skeletal remains by implementing labels in each case and creating a database with the information collected from the human skeletal remains of the LAFOL Laboratory collection. This allowed us to manage the material of the anthropological collection and maintain the chain of custody. In addition, this demonstrates caution and care required when investigating cold cases [52], also represented by standard procedures and best practices established by experts.

Considering that every unidentified body is, or could be, a missing person and the LAFOL Identifies Operation initiated before Law No. 13,812/2019 [53], which established the National System for the Location and Identification of Missing Persons (SINALID), we can state that the LAFOL Identifies Operation acted in a visionary way, anticipating the resolution of the problem of missing persons before national legislators. The implementation of standardized administrative and methodological procedures can build an indispensable process around the problem of missing persons and unidentified bodies. Brazil does not yet have an efficient and integrated database for missing persons that contains all the information necessary to carry out systematic searches and comparisons between data on missing persons and unidentified bodies in the Medico-Legal Institutes. This reduces the possibility of identifying these individuals and contributes to remaining missing [1, 54–56]. These databases are essential, and the software that manages this information can standardize data collection, which is important for any investigative work [55].

The Program for the Location and Identification of Missing Persons (PLID), created in 2012 by the Public Ministry of Rio de Janeiro, covers the National System for the Location and Identification of Missing Persons (SINALID), a national database since 2017 to store and cross-reference data on missing persons and people found alive or dead (recent, putrefied, or skeletonized corpses) in Brazil [1,56]. In 2022, the program became active in all states of Brazil and had already helped

resolve 18,837 cases. In 2023, 6951 new disappearances were registered, totaling 96,428 records of missing persons [57]. However, the National System for the Location and Identification of Missing Persons (SINALID) is not yet fully in use due to the lack of power given to the system by the states, even though it is available throughout the country. In October 2022, the Medico-Legal Institute of Porto Velho joined SINALID and fed the system with information collected during the LAFOL Identifies Operation, previously stored in the local database created during the project.

In Brazil, professionals who work with forensic anthropology, including forensic dentists, are law enforcement officers, which guarantees the admissibility of the collected information in court. They are the most suitable professionals to coordinate and/or carry out the family interview and search for ante mortem documents phases, with the assistance of other law enforcement officers in these phases.

The protocols created by the LAFOL Identifies Operation can enrich the forensic scientific community in Brazil, serving as a guideline to encourage and increase identification efforts in cold cases by demonstrating the problems faced and the resources required in an organized and consistent format. This information can direct future operations related to the preparation of the accommodation site and the planning of the cataloging, cleaning, anthropological analysis, packaging, database creation, campaigns, interviews, collection of biological samples, identification, and delivery of the human remains to the family.

The difficulty in obtaining financial resources to purchase minimum laboratory supplies limits the implementation of large action projects like this, even though they only require a minimum chain of custody materials, such as new bone boxes, new quality labels, new shelves, and quality product cleaning [58–61]. Investments are not systematic and may vary from one Brazilian state to another, depending on the political context [40].

The lack of human resources for an operation of this scale also stands out. The Medico-Legal Institute of Porto Velho has only two forensic dentists who had to balance the analysis of cold cases while completing requests for routine cases, which required effort and commitment. The LAFOL Identifies Operation was performed by three volunteer researchers: a master's degree student in Forensic Dentistry from Campinas State University (Unicamp), a specialist in Forensic Dentistry, and a doctoral student in Forensic Anthropology from the University of Coimbra.

Additionally, we highlight the fundamental interrelationship between a forensic case reanalysis operation and its humanitarian importance in guaranteeing human rights. International Humanitarian Law and International Human Rights Law defend the guarantee that family members know the location of their missing relatives [62]. For families, receiving the body and paying tribute to the victims is fundamental to the process of healing and overcoming the loss of someone. The disappearance of someone and the absence of their body are obstacles to this process, leaving family members in a permanent search situation, feeling anguish, anxiety, depression, and somatic difficulties [63]. In Sri Lanka, research showed that the levels of diseases reduced by more than 75% in the affected family members after they received psychosocial support from the Red Cross and held ceremonies to honor their missing relatives [63].

Thus, even when identification is unlikely, cold cases of missing persons deserve a full investigation. These individuals represent the most vulnerable among the population, and their unidentified status embodies the structural inequalities and violence they have likely suffered in life [12].

The results are worth the effort, and the success stories demonstrate the need for Medico-Legal Institutes to dedicate more time, effort, and personnel to analyzing cold cases. Identifying two individuals among the 81 evaluated may seem small, but it allowed two families to bury loved ones and mourn each one. These two identifications were obtained among the cases with an alleged victim's name available. Such cases should always be prioritized in an operation to reanalyze cold cases

since they present a greater chance of positive results.

Although methodological and technical advances in recent years have allowed us to reanalyze archived human skeletal remains, there is an inescapable truth. Time passes, reducing the quantity and quality of DNA in these remains, making it increasingly difficult to compare genetic profiles conclusively.

6. Conclusions

The LAFOL Identifies Operation included collection management, anthropological and dental analysis, and genetic testing of individuals who have been sought for almost two decades, in addition to campaigns and interviews with family members to compare biological profiles and allow identification. The project achieved notable results and included collaboration between a multidisciplinary team with an organized and consistent protocol that accelerated collection, with careful prior planning, and the discussion of crucial information for the investigation of cold cases.

Understanding the strengths and limitations of current technologies and resources available in human identification is important for law enforcement and forensic communities. Reanalysis operations on human skeletal remains by collecting important data can fill in the missing gaps to identify the archived unidentified bodies in the Medico-Legal Institutes in Brazil.

CRedit authorship contribution statement

Gabriela Araujo dos Santos: Writing – original draft, Methodology, Investigation, Conceptualization. **Talita Lima de Castro Espicalsky:** riting – review & editing, Supervision, Project administration, Conceptualization. **Leticia Regina Alfaia Custódio:** Writing – original draft. **Melina Calmon Silva:** Writing – review & editing, Supervision. **Eugenia Cunha:** Writing – review & editing, Supervision.

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Declaration of Competing Interest

We declare that there is no conflict of interest in the preparation of this research and in the submission of the manuscript.

References

- [1] M. Calmon, C.E. Palhares Machado, The problem of the Disappeared in Brazil, in: C.E. Palhares Machado, A.R. Deitos, J. Antonio Velho, E. Cunha (Eds.), *Treatise on Forensic Anthropology: Fundamentals and Methodologies Applied to Expert Practice*, 1st ed., Millennium, Campinas, 2022.
- [2] FBSP, Brazilian Public Security Yearbook, 2023. <https://forumseguranca.org.br/anuario-brasileiro-seguranca-publica/> (accessed August 14, 2023).
- [3] Federal Regional Court of the 4th Region, Electronic Information System - SEI, Ministry of Economy. (2022).
- [4] Palácio do Planalto, Law No. 12.654 of May 28, 2012. Amends Laws No. 12.037 of October 1, 2009, and 7,210, of July 11, 1984 - Criminal Execution Law, to provide for the collection of genetic profiles as a form of criminal identification, and provides other measures., Brazil, 2012.
- [5] C. Marques, E. Abdalla, National Bank of Criminal Genetic Profiles: a bioethical discussion, *Rev. Bras. De Bioética* 8 (2012) 31–46, <https://doi.org/10.26512/rbb.v8i1-4.7775>.
- [6] R.G. Garrido, E.L. Rodrigues, The Brazilian Genetic Profiles Bank Three Years after Law No. 12.654, *Rev Bioet. Derecho*. (2015) 94–107.
- [7] E. Cunha, C. Cattaneo, Historical routes and current practice for personal identification, in: *P5 Medicine and Justice: Innovation, Unitariness and Evidence*, Springer International Publishing, 2018, pp. 398–411, https://doi.org/10.1007/978-3-319-67092-8_25.
- [8] D.H. Ubelaker, H. Khosrowshahi, Estimation of age in forensic anthropology: historical perspective and recent methodological advances, *Forensic Sci. Res* 4 (2019) 1–9, <https://doi.org/10.1080/20961790.2018.1549711>.
- [9] M.H. Mulawka, I.M. Sebetan, P.C. Stein, A uniform protocol to address unidentified human remains and missing persons, *Journa Forensic Identif.* 60 (2010) 748–758.

- [10] E.H. Kimmerle, Practicing Forensic Anthropology: a human rights approach to the global problem of missing and unidentified persons, *Ann. Anthr. Pr.* 38 (2014) 1–6, <https://doi.org/10.1111/napa.12038>.
- [11] M. Hinkes, Forensic anthropology in cold cases, *Cold Case Homicides* (2019), <https://doi.org/10.1201/9781315228259-20>.
- [12] G. Goad, Expanding humanitarian forensic action: an approach to US cold cases, *Forensic Anthropol.* 3 (2020), <https://doi.org/10.5744/fa.2020.1006>.
- [13] J. Bruzek, A method for visual determination of sex, using the human hip bone, *Am. J. Phys. Anthr.* 117 (2002) 157–168, <https://doi.org/10.1002/ajpa.10012>.
- [14] P. Murail, J. Bruzek, F. Houët, E. Cunha, DSP: a tool for probabilistic sex diagnosis using worldwide variability in hip-bone measurements, *Bull. Mem. Soc. Anthr. Paris* 17 (2005) 167–176, <https://doi.org/10.4000/bmsap.1157>.
- [15] A.R. Kales, S.D. Ousley, J.M. Vollner, A revised method of sexing the human innominate using Phenice's nonmetric traits and statistical methods, *Am. J. Phys. Anthr.* 149 (2012) 104–114, <https://doi.org/10.1002/ajpa.22102>.
- [16] T.W. Phenice, A newly developed visual method for sexing the os pubis, *Am. J. Phys. Anthropol.* 30 (1969) 297–302.
- [17] J.E. Buikstra, D.H. Ubelaker, Standards for Data Collection from Human Skeletal Remains: Proceedings of a Seminar at the Field Museum of Natural History, 1st ed., Arkansas Archeological Survey, Fayetteville, 1994.
- [18] S.N. Wasterlain, E. Cunha, Comparative Performance of Femur and Humerus Epiphysis for Sex Diagnosis, *Biométrie Humaine et Anthropologie*. 18 (2000) 9–13.
- [19] F. Curate, J. Coelho, D. Gonçalves, C. Coelho, M.T. Ferreira, D. Navega, E. Cunha, A method for sex estimation using the proximal femur, *Forensic Sci. Int.* 266 (2016), 579. e1–579.e7. <https://doi.org/10.1016/j.forsciint.2016.06.011>.
- [20] P.L. Walker, Sexing skulls using discriminant function analysis of visually assessed traits, *Am. J. Phys. Anthr.* 136 (2008) 39–50, <https://doi.org/10.1002/ajpa.20776>.
- [21] G.V. França, Medicina Legal, 11th ed., Guanabara Koogan, Rio de Janeiro, 2017.
- [22] R.A. Nicodemo, L.C. Moraes, E. Medici, Filho, Chronological table of mineralization of permanent teeth among Brazilians, *Rev. Fac. Odontol. Sao Jose Dos Campos* 33 (1974) 55–56.
- [23] S.J. Alqahtani, The London Atlas: developing and atlas of tooth development and testing its quality and performance measures, Dr. Thesis, Queen Mary, Univ. Lond. (2012).
- [24] G.O. Arbenz, Legal Medicine and Forensic Anthropology, Atheneu, São Paulo, 1987.
- [25] M. Schaefer, S. Black, L. Scheuer, Juvenile osteology: a laboratory and field manual, Elsevier, United States, 2009.
- [26] L. Scheuer, S. Black, Developmental Juvenile Osteology, Elsevier, London, 2000.
- [27] H. Lamendin, E. Baccino, J.F. Humbert, J.C. Tavernier, R.M. Nossintchouk, A. Zerilli, A simple technique for age estimation in adult corpses: the two criteria dental method, *J. Forensic Sci.* 37 (1992) 1373–1379.
- [28] S. Brooks, J.M. Suchey, Skeletal age determination based on the pubis: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods, *Hum. Evol.* 5 (1990) 227–238.
- [29] S.E. Calce, A new method to estimate adult age-at-death using the acetabulum, *Am. J. Phys. Anthr.* 148 (2012) 11–23, <https://doi.org/10.1002/ajpa.22026>.
- [30] J.L. Buckberry, A.T. Chamberlain, Age estimation from the auricular surface of the ilium: a revised method, *Am. J. Phys. Anthr.* 119 (2002) 231–239, <https://doi.org/10.1002/ajpa.10130>.
- [31] R. Mellega, Validation of the Main Existing Height Determination Techniques Applied to Brazilian Cadaver Samples, Master's Dissertation, University of Campinas, 2004.
- [32] J.J.B. Freire, Height as a fundamental data in forensic anthropology: defining a standard for estimating height in Brazil, *Perspect. Leg. Med. Med. Expert.* 1 (2016).
- [33] J.J.B. Freire, Height: Fundamental data in Forensic Anthropology, Master's Dissertation, University of Campinas, 2000.
- [34] J. Albanese, A. Tuck, J. Gomes, H.F.V. Cardoso, An alternative approach for estimating stature from long bones that is not population- or group-specific, *Forensic Sci. Int.* 259 (2016) 59–68, <https://doi.org/10.1016/j.forsciint.2015.12.011>.
- [35] J.T. Hefner, S.D. Ousley, Statistical classification methods for estimating ancestry using morphoscopic traits, *J. Forensic Sci.* 59 (2014), <https://doi.org/10.1111/1556-4029.12421>.
- [36] J.T. Hefner, The Statistical Determination of Ancestry Using Cranial Nonmetric Traits, Doctoral Thesis, University of Florida, 2007.
- [37] J.T. Hefner, Cranial nonmetric variation and estimating ancestry, *J. Forensic Sci.* 54 (2009) 985–995, <https://doi.org/10.1111/j.1556-4029.2009.01118.x>.
- [38] E. Baccino, E. Cunha, C. Cattaneo, Aging the dead and the living, in: *Encyclopedia of Forensic Sciences: Second Edition*, Elsevier Inc., 2013: pp. 42–48. <https://doi.org/10.1016/B978-0-12-382165-2.00009-X>.
- [39] Brazilian Institute of Geography and Statistics - IBGE, IBGE Census, (2022). <https://censo2022.ibge.gov.br/> (accessed August 14, 2023).
- [40] M. Meucci, E. Verna, C. Costedoat, The Skeletal Remains of Soldiers from the Two World Wars: Between Identification, Health Research and Memorial Issues, *Genes (Basel)* 13 (2022) 1852, <https://doi.org/10.3390/genes13101852>.
- [41] L.C.C. Galvão, Sex identification through cranial measurements, Master's Dissertation, State University of Campinas, 1994.
- [42] A.C.K. Furlan, B. de S. Nogueira, A.T.W. Demetrio, L.F. Lolli, Validation of the Carrea Method in the Northwest Region of the State of Paraná, Brazil, *RBOL- Braz. J. Forensic Dent.* (2016), <https://doi.org/10.21117/rbol.v3i1.31>.
- [43] R.C. Cabral, Craniometry in identifying sex in human skeletons - Literature review, Course Completion Work, Universidade Federal Rural de Pernambuco, 2019.
- [44] M.Y. Iscan, M. Steyn, The Human Skeleton in Forensic Medicine, 3rd ed., Charles C. Thomas Publisher Ltd., Illinois, 2013.
- [45] K.R. Burns, Forensic Anthropology Training Manual, 3rd ed., Routledge, 2017. <https://doi.org/10.4324/9781315664163>.
- [46] Palácio do Planalto, Law No. 13.964, of December 24, 2019. Improves criminal and criminal procedural legislation., Brazil, 2019.
- [47] M.M. Evans, P.A. Stagner, Maintaining the Chain of Custody Evidence Handling in Forensic Cases, *AORN J.* 78 (2003) 563–569. [https://doi.org/10.1016/S0001-2092\(06\)60664-9](https://doi.org/10.1016/S0001-2092(06)60664-9).
- [48] J. Prahlow, Introduction to forensic sciences. in: *Forensic Pathology for Police, Death Investigators, Attorneys, and Forensic Scientists*, Humana Press, Totowa, NJ, 2010, pp. 17–33, https://doi.org/10.1007/978-1-59745-404-9_2.
- [49] C.E. Palhares Machado, A.R. Deitos, J.A. Velho, E. Cunha, Treatise on Forensic Anthropology: foundations and methodologies applied to forensic practice., 1st ed., Millennium, Campinas, 2022.
- [50] N.P. Damascena, M. Calmon Silva, A.R. Deitos, R.P. Baldasso, R.C.G. Ferreira, C. Kotinda Júnior, C.E. Palhares Machado, Recommendations for procedures related to the evidence chain of custody in forensic anthropology in Brazil, *Forensic Sci. Res* 7 (2022) 609–622, <https://doi.org/10.1080/20961790.2022.2076984>.
- [51] T. D'Anna, M. Puntarello, G. Cannella, G. Scalzo, R. Buscemi, S. Zerbo, A. Argo, The chain of custody in the era of modern forensics: from the classic procedures for gathering evidence to the new challenges related to digital data, *Healthcare* 11 (2023) 634, <https://doi.org/10.3390/healthcare11050634>.
- [52] R. Hanzlick, Revisiting old cases of unidentified decedents: problems encountered, lessons learned, and suggestions for others, 1008–1008, *J. Forensic Sci.* 53 (2008), <https://doi.org/10.1111/j.1556-4029.2008.00788.x>.
- [53] Palácio do Planalto, Law No. 13.812, of March 16, 2019, Establishes the National Policy for the Search for Missing Persons, creates the National Registry of Missing Persons and amends Law No. 8.069, of July 13, 1990 (Child and Adolescent Statute), Brazil, 2019. https://www.planalto.gov.br/ccivil_03/_ato2019-2022/2019/lei/113812.htm#:~:text=1%C2%BA%20This%20Law%20establishes%20a,District%20Federal%20e%20aos%20Terri%C3%B3rios. (accessed August 14, 2023).
- [54] M. Calmon, Forensic anthropology and missing persons: a Brazilian perspective, *Forensic Sci. Int.* 298 (2019), <https://doi.org/10.1016/j.forsciint.2019.03.032>.
- [55] M. Calmon, Forensic data management and database systems in forensic investigations for cases of missing and unidentified persons in Brazil, *Forensic Sci. Res* 7 (2022), <https://doi.org/10.1080/20961790.2022.2076994>.
- [56] E. Cunha, B. Algee-Hewitt, M. Calmon, The reality of the dead in Brazil: perspectives on identification in forensic anthropology, *Forensic Sci. Res* 7 (2022) 577–579, <https://doi.org/10.1080/20961790.2022.2125414>.
- [57] National Council of the Public Ministry, CNMP and MPMG provide a system for locating and identifying victims of the tragedy that occurred in Brumadinho, Secretariat of Social Communication. (2019).
- [58] D.R. de C. Cerqueira, W.J.A. Lobão, A.X.Y. de Carvalho, The game of seven myths and the misery of public security in Brazil, Rio de Janeiro, 2005.
- [59] LE Soares, Public security: present and future, *Advanced Studies.* 20 (2006) 91–106. <https://doi.org/10.1590/S0103-40142006000100008>.
- [60] R.S. De Lima, J. Sinhorretto, S. Bueno, The management of life and public security in Brazil, *Society and State.* 30 (2015) 123–144. <https://doi.org/10.1590/S0102-69922015000100008>.
- [61] U. Peres, S. Bueno, C.K.D.S. Leite, R.S. De Lima, Public security: reflections on the financing of public policies in the federative context, *Rev. Bras. De Segurança Pública* 8 (2014).
- [62] International Committee of the Red Cross, International Humanitarian Law and International Human Rights Law: analogies and differences, (2004).
- [63] I. Andersen, B. Poudyal, A. Abeypala, C. Uriarte, R. Rossi, Mental health and psychosocial support for families of missing persons in Sri Lanka: a retrospective cohort study, *Confl. Health* 14 (2020), <https://doi.org/10.1186/s13031-020-00266-0>.