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PhD THESIS

**FORENSIC ANTHROPOLOGY - IDENTIFICATION OF
SKELETAL FRAGMENTS OF HUMAN AND NON-HUMAN
ORIGIN**

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A B S T R A C T

Professionals responsible for identifying deceased individuals encounter significant obstacles and bear a considerable burden of responsibility. The lack of certainty regarding the location of a missing family member can directly impact all members of the family and, in certain instances, the entire community as well (1).

Forensic anthropology involves the analysis of human skeletal remains by law enforcement agencies in order to establish the identity of unidentified bones. Forensic anthropologists have a vital role in recognizing undetermined remains, particularly in recent times due to the growing number of cases where investigators neglect to take into account the skeletal evidence at the scene of an event (2).

The main focus of this thesis was on an area of significant interest and importance within this complex and challenging landscape. The motivation for this research arises from the growing demand for precise identification techniques in forensic cases, particularly in instances involving fragmented or incomplete remains. This thesis centres on three primary domains: enhancing DNA extraction protocols, validating sex estimation methods, and utilising histomorphological techniques to distinguish human bones from non-human bones.

The primary objectives of this research are:

- To create an enhanced DNA extraction method from bones and teeth that does not require liquid nitrogen. The goal is to achieve higher DNA yields and improved accuracy in forensic identifications.
- To validate the Diagnose Sexuelle Probabiliste (DSP) method for sex estimation in the Romanian population using CT images.
- To review and demonstrate the effectiveness of histomorphological techniques in distinguishing human bones from those of non-human origin.

The thesis is structured into two parts: the General Part and the Special Part. The General Part introduces forensic anthropology, initial assessments of skeletal remains, and the role of biological profiles in forensic sciences. The Special Part, the largest and most detailed section of the thesis, will be devoted to the presentation and analysis of three separate studies, each approaching the subject from a specific perspective and using varied methods to investigate different techniques for forensic identification.

The research approach involved the development of specific research hypotheses. These hypotheses were carefully formulated to address critical components of the overarching general hypothesis. By breaking down the main hypothesis into more focused, testable propositions, we aimed to systematically investigate various aspects of our research question. This methodological approach allows for a comprehensive examination of the subject matter and facilitates a more nuanced understanding of the research topic.

The initial research hypothesis aimed to evaluate the effectiveness of Automated DNA extraction protocols that do not rely on liquid nitrogen in producing a greater amount and higher quality of DNA from skeletal remains, as compared to traditional methods. This improvement is expected to enhance the efficiency and accuracy of forensic identifications. Additionally, it aimed to assess the variation in DNA yield among different skeletal elements. It was found that teeth and petrous temporal bones consistently yielded higher DNA concentrations compared to other bone types. Consequently, these skeletal elements are considered more reliable sources for forensic DNA analysis. The hypothesis will evaluate integrating morphological assessments of skeletal features with genetic profiling, a more comprehensive and accurate method for identifying human remains. This can be achieved, surpassing the effectiveness of using either approach individually. This integration enhances the precision and reliability of forensic identifications.

The first study presents the development of a DNA extraction protocol from bones and teeth without using liquid nitrogen, aimed at identifying human skeletal remains. The study analyzed ten cases of unidentified skeletal remains received at the Institute of Forensic Medicine in Timisoara, Romania, between 2019 and 2023. Samples included teeth, petrous temporal bones, and femur bones. The DNA extraction was conducted using automated protocols, and the DNA concentration was quantified using the PowerQuant® System. The results indicated that teeth provided the highest DNA concentration, averaging 3.68 ng/μL, followed by petrous temporal bones at 0.936 ng/μL and femur bones at 0.633 ng/μL. The study demonstrated that combining anthropological examination with DNA analysis significantly enhances the accuracy of

human identification. The DNA profiles obtained were successfully used for identifying relationships and confirming identities through comparative analysis with reference samples. Teeth are the most reliable source for DNA extraction in forensic contexts due to their high genetic material concentration. The adoption of automated DNA extraction protocols without liquid nitrogen represents a significant advancement in forensic DNA technology, providing a more efficient, less labor-intensive method for high-quality DNA extraction from damaged bone and tooth samples. This protocol improves the reliability and speed of forensic identifications, emphasizing the importance of selecting appropriate skeletal elements for genetic analyses.

The second study aimed to evaluate the applicability of the Diagnose Sexuelle Probabiliste (DSP) software for sex estimation using computed tomography (CT) images of the os coxae in the contemporary Romanian population. A total of 80 pelvic CT scans were analyzed, with an equal distribution of 40 males and 40 females, ranging in age from 22 to 93 years. The CT images were obtained using standardized protocols, and four measurements were selected from the DSP method for analysis. Repeatability analysis was conducted to ensure the reliability of the measurements. The DSP software correctly estimated sex in 71.25% of the cases, with an overall accuracy rate of 98.24%. For females, the accuracy was 100%, with 34 out of 40 correctly classified and no misclassifications. For males, the accuracy was 95.65%, with 22 out of 40 correctly classified and one misclassification. The study noted that undetermined cases comprised 42.5% of males and 15% of females, highlighting the impact of precision in sex estimation. The DSP software is a reliable tool for sex estimation in the Romanian population, achieving high accuracy rates and demonstrating the importance of using CT

imaging for forensic anthropology. Despite the presence of undetermined cases, the minimal misclassification rate supports the robustness of the DSP method. The results emphasize the significance of accuracy in forensic sex estimation, especially in legal contexts, and suggest that the DSP software can be confidently used as a primary or supplementary technique in forensic investigations.

The third study, represented by a review, critically examines histological techniques for differentiating human bone from animal bone, highlighting the microscopic features and histomorphometric methods employed in various studies. The review synthesizes data from numerous studies that have utilized techniques such as histomorphological analysis, histomorphometric analysis, and discriminant function analysis to identify species-specific characteristics in bone samples. These methods involve analyzing the Haversian systems, osteon structure, and other microscopic features in bone tissue. The review includes a comprehensive comparison of human and non-human bones, emphasizing the differences in the Haversian canal diameter, osteon diameter, and osteon density per mm². The results from various studies indicate that histomorphological analysis is effective in distinguishing human from non-human bones based on the organization and structure of Haversian systems and osteons. Human bones typically exhibit a higher density of well-defined osteons with smaller Haversian canals, while non-human bones vary significantly depending on the species. For example, hen bones have a high density of small osteons, whereas bovine bones have larger osteons with less defined borders. The review also highlights the success of discriminant function analysis in accurately classifying bone samples into human or non-human categories, with notable studies achieving high accuracy rates. Histological techniques,

particularly histomorphological and histomorphometric methods, are valuable tools for differentiating human from non-human bones, especially in forensic and archaeological contexts. These methods are crucial for cases involving fragmented, deteriorated, or burned bones where macroscopic identification is challenging. The review emphasizes the importance of combining multiple histological techniques to increase accuracy and reliability in bone identification. It also suggests the need for further research to refine these methods and address limitations, such as the influence of age and sex on histomorphometric measurements.

Expanding on the discoveries of this PhD thesis, numerous potential avenues for future research can be explored to further progress the field of forensic anthropology and enhance the ability to identify skeletal fragments from both human and non-human sources.

Substantial individual contributions were made to progress the field of forensic anthropology, particularly in the identification of skeletal fragments of both human and non-human nature. An important contribution was the introduction and implementation of a novel DNA extraction procedure that eliminates the need for liquid nitrogen, making the process simpler while still producing DNA of excellent quality. The protocol underwent thorough testing and was found to be effective in analyzing DNA from different skeletal elements, such as teeth and petrous temporal bones. This method can be relied upon for forensic DNA analysis. In addition, thorough comparative analyses were performed to determine the most effective sources of genetic material, which has practical implications for forensic investigations. Another significant contribution was the amalgamation of anthropological investigation with genetic analysis, showcasing that a multidisciplinary approach can greatly augment the precision of human identification. Moreover, the verification

of the Diagnose Sexuelle Probabiliste (DSP) software for determining sex based on CT-derived variables in the Romanian population signifies a significant progress, providing a dependable instrument for forensic experts. Finally, the thorough examination of literature and the implementation of histomorphological techniques to distinguish human bones from non-human bones have equipped forensic anthropologists with reliable methods to accurately identify skeletal remains in situations involving multiple species. These individual contributions collectively enhance the development of forensic anthropology practices and provide valuable methodologies for future research and practical applications in the field.