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USEFULNESS OF RAMAN SPECTROSCOPY AND LIPID ANALYSIS OF DECOMPOSED HUMAN BONES IN FORENSIC GENETICS AND MOLECULAR TAPHONOMY

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ABSTRACT
Bones are among the structures most likely to be recovered after death, making their study extremely relevant in forensics. Raman spectroscopy (RS) and gas chromatography-mass spectrometry (GC/MS), have the potential to be useful as screening tools for DNA analysis. The aim o four study was to assess the usefulness of such molecular investigations. Femur samples from 50 decomposing human bodies were subjected to RS and GC/MS. Assessment of nuclear DNA quantity and STR genotyping were also performed. Fatty acids, hydrocarbons, and fatty acid methyl esters (FAMEs) were detected. The main phosphate peak position in RS was significantly correlated with DNA, while significantly more STR alleles were detected in bones containing methyl hexadecenoate. Detection of FAMEs in the bone matrix suggests a reaction between methanol produced by bacteria and fatty acids. RS and GC/MS can be useful in molecular taphonomy and forensic genetics.

Keywords: Forensic genetics, bones, DNA, Raman spectroscopy, gas chromatography/ mass spectrometry.

Introduction
Molecular taphonomy studies the transformation of biomolecules after death, of which DNA is considered the most informative. For over 30 years, DNA analysis of postmortem bones has allowed the identification of decomposed human bodies, since they are most likely to be recovered. However, DNA analysis of postmortem bone is technically challenging. Therefore, new methods for DNA screening have been extensively studied. RS is a potential screening tool due to its ability to monitor organic and inorganic components, minimally destructive, time- and cost-efficient nature. In the same sense, GC/MS analysis of bone lipids has never been performed in forensics nor archaeoanthropology with that scope.

Objectives
To assess biomarkers in forensic human bones that might be associated with DNA using Raman Spectroscopy and GC/MS.

Methods
Femoral diaphysis samples were collected from 50 decomposing corpses found in Mato Grosso, Brazil, with diverse burial environments, causes of death, and PMIs (6 to 13 years). Methods employed are detailed on Table 1.

Table 1. Description of methods employed.

Analysis	Technique	Target
Human DNA	Real-time PCR	67 bp; 180 bp
STR genotyping	PCR/CE	16 STRs + Amel
Physicochemical	RS	Molecular bonds
Lipid	GC/MS	Total lipids

Results and Discussion
We hypothesize FAMEs are formed during bone putrefaction, where microbial activity is high. Bacterial release of methanol, gases, and other by-products results in pH changes and high internal pressure, which could promote the reaction between free fatty acids and methanol. Statistically significant associations observed are presented on Table 2.

Table 2. Significant associations observed.

Variable	Associated with	p-value
$_{v1}PO_4^{3-}$ position	180 bp human DNA	0.0371
Methyl hexadecenoate	STRs detected	0.0424

Conclusion
RS and GC/MS may be useful in molecular taphonomy and forensic genetics studies.

References
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