

Applying multidisciplinary methods to forensic casework in North Carolina

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Abstract

A multidisciplinary approach in the investigation of unidentified decedents employs the use of a variety of scientific approaches. This approach is applied in casework in North Carolina, USA and has led to successful identifications using anthropological analysis and population affinity estimation using 3D-ID, investigative genetic genealogy (IGG), and isotopic analyses. Results from one scientific approach can help to inform the others, providing more information about the decedent, and thus enhancing the investigation. This case report outlines three cases from North Carolina that combine each of these scientific approaches and serve as examples of collaboration in a multidisciplinary approach.

Key points

- Anthropological analyses including population affinity using 3D-ID can be used in conjunction with isotopic analyses in estimating geographic origin.
- Results from anthropological and isotopic analyses can help to narrow down or direct IGG.
- Multidisciplinary collaboration in the investigation of unidentified decedents enhances the investigation.

Keywords: forensic sciences; isotope analysis; forensic anthropology; investigative genetic genealogy; cold case analysis

Introduction

As of 26 February 2024, there are 158 unidentified persons cases listed in the National Missing and Unidentified Persons System (NamUs) in the state of North Carolina (Figure 1). A multidisciplinary approach has been adopted at the North Carolina State University Human Identification and Forensic Analysis Laboratory (NCSUHIFAL) [1]. Modern methods of skeletal analyses are employed including developing a biological profile (e.g. biological sex, age at death, stature, and population affinity) for unidentified decedents, radiographic comparisons for identification, and trauma analysis, to assist Medical Examiner offices. Additional techniques are employed to develop standard operating procedures for North Carolina casework. An interinstitutional grant awarded in 2019 to North Carolina State University (NCSU) and Western Carolina University (WCU) funded a pilot project to implement a holistic approach to identification involving standard forensic anthropological analysis, investigative genetic genealogy (IGG), and isotopic signatures [1]. Furthermore, NCSUHIFAL partnered with the NC State Bureau of Investigation on a grant funded by a Bureau of Justice Assistance under their Missing and Unidentified Human Remains (MUHR) programme to advance the multidisciplinary approach to reduce the backlog of unidentified cases in North Carolina. A more comprehensive overview of the multidisciplinary endeavour to cold case work within the state of North Carolina has

been outlined by Ross and Passalacqua [1]. Recently, bone and tooth samples from nine decedents were sent to Washington State University (WSU) for isotopic analyses. Here, we present the results of isotopic investigations from three of those cases using geolocation and dietary isotopes, anthropological analyses including population affinity estimation using 3D-ID [2], and IGG. The purpose of this paper is to highlight the multidisciplinary approach and show the added value of using advanced testing in forensic anthropology casework in North Carolina.

Isotope analysis—background

Tissues analyzed

Collagen is the main organic component and apatite is the mineralized component in bone structure and physiology [3]. Different skeletal elements remodel throughout life at varying rates, and therefore, isotopic signatures measured in bone are reflective of isotopic sources from the most recent decades of one's life [4–6]. Tooth enamel is a highly mineralized tissue that forms during childhood and does not remodel once formed [7]. Isotopic values in tooth enamel thus reflect childhood diet and place of residence. Due to the compact mineralized structure, tooth enamel is also resistant to the effects of diagenesis or taphonomic alteration [8].

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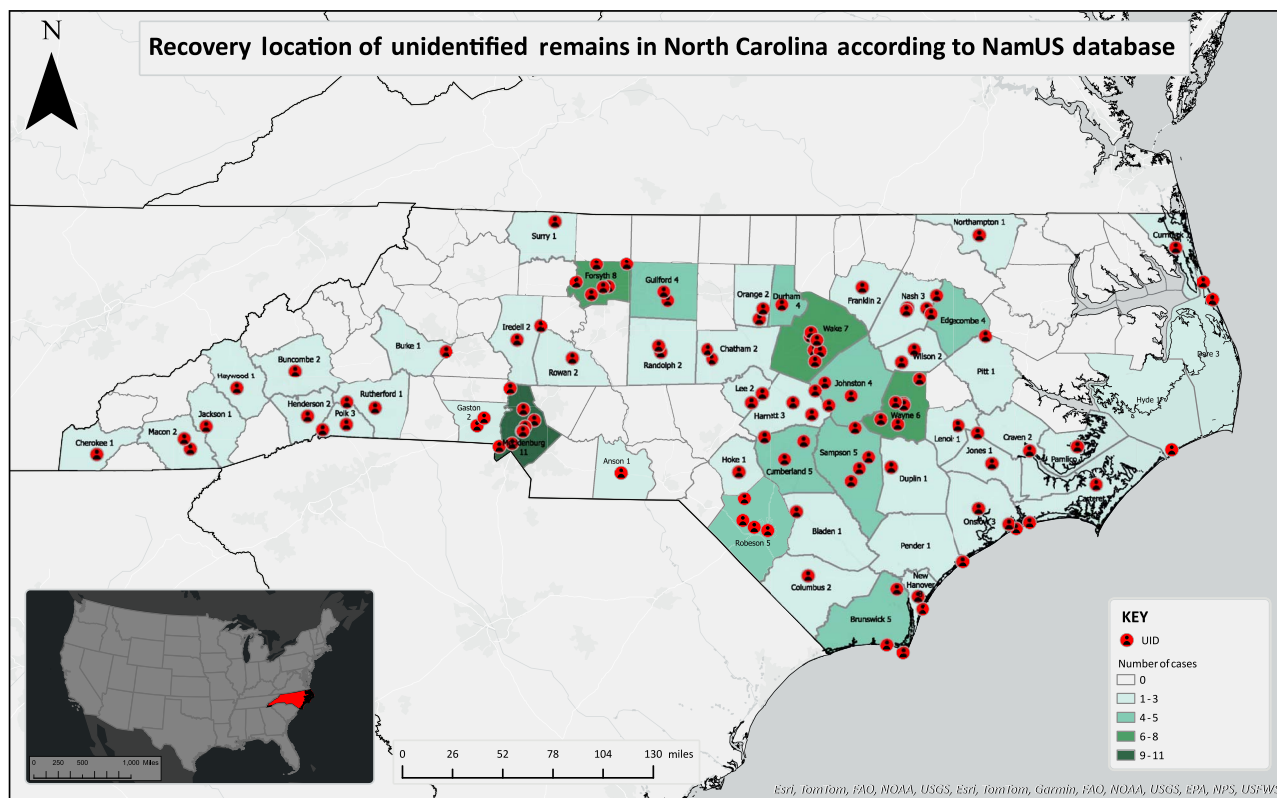


Figure 1 Map displaying recovery location of unidentified skeletalized human remains in North Carolina, USA by county. UID: unidentified.

$^{87/86}\text{Sr}$ analysis—tooth enamel and bone: geolocation

Analysis of $^{87/86}\text{Sr}$ in human skeletal tissues is commonly used to reconstruct human mobility in archaeological and forensic contexts [9, 10]. $^{87/86}\text{Sr}$ varies across the landscape based on the age, chemical composition, and weathering rates of the underlying geology [9]. As humans feed and drink, the $^{87/86}\text{Sr}$ of consumed food and water is incorporated into their skeletal tissues [10]. Strontium isotopes are not significantly fractionated as they are passed up the food chain, so skeletal $^{87/86}\text{Sr}$ reflects the local biologically available $^{87/86}\text{Sr}$ in soil, plants, and water. $^{87/86}\text{Sr}$ measured in tooth enamel corresponds to the location of origin during childhood, while bone represents an average of the past 5–25 years of location due to remodeling [11].

$\delta^{18}\text{O}$ analysis—tooth enamel: geolocation

Stable oxygen isotope ratios ($^{18}\text{O}:^{16}\text{O}$) are similarly used as a proxy for human mobility. Oxygen isotope values are expressed using $\delta^{18}\text{O}$ notation in ‰ difference of the ratio of ^{18}O to ^{16}O compared with an international standard [12–14]. $\delta^{18}\text{O}$ varies due to the fractionation of oxygen isotopes within the water cycle. Factors such as evaporation, condensation, precipitation, temperature, latitude, elevation, and distance from large bodies of water affect $\delta^{18}\text{O}$ [15–17]. Oxygen isotopes are incorporated into phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) groups in bones and teeth, which can be directly compared with measured values from individuals of known origin, or converted to drinking water values, which have been modeled using isoscapes [18, 19]. Direct comparison between measured tooth enamel from known populations is a simplified approach to utilizing $\delta^{18}\text{O}$ isotope as a geolocation

tool and is useful when drinking water models are not yet available [20].

$\delta^{13}\text{C}$ analysis—tooth enamel and bone collagen

Carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) are also expressed in ‰ using δ -notation [21]. Carbon isotopes in plants vary according to their photosynthetic pathway (C3, C4, and crassulacean acid metabolism CAM), which affects how they fractionate atmospheric carbon. C3 plants discriminate more against the heavier ^{13}C resulting in lower $\delta^{13}\text{C}$ in comparison to C4 plants. CAM plants have intermediate $\delta^{13}\text{C}$, which can be influenced by local environmental conditions [22–24]. C3 plants comprise a majority of plants on the planet including fruits, vegetables, nuts, and grains such as wheat and rice. C4 plants consumed by humans include corn, sugarcane, amaranth, millet, and sorghum. CAM plants are not typically major components of the human diet and include succulents and cacti [25, 26]. Marine plants follow the same photosynthetic pathways as terrestrial plants, but plants in ocean water derive carbon from ocean bicarbonate, which is more enriched in ^{13}C than atmospheric carbon [27]. Bone and tooth enamel carbonate $\delta^{13}\text{C}$ reflects total dietary carbon including protein, lipids, and carbohydrates, while isotope values measured in bone or dentine collagen preferentially reflect carbon isotope ratios from dietary protein [28].

$\delta^{15}\text{N}$ analysis—bone collagen

Nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) are similarly expressed using δ -notation ($\delta^{15}\text{N}$) with atmospheric air (0‰) as the standard [29]. In bone collagen, $\delta^{15}\text{N}$ reflects dietary sources of animal protein. Metabolic fractionation of nitrogen corresponds to the trophic level of an organism: the higher

the trophic level, the higher $\delta^{15}\text{N}$ [30]. Additionally, marine resources have higher $\delta^{15}\text{N}$ in comparison to terrestrial food resources since the source of nitrogen in marine systems is dissolved nitrogen, which is $\sim 4\%$ higher *vs.* atmospheric air (0‰) [31]. Anthropogenic sources (e.g. fertilizer, sewage, and animal waste) can increase $\delta^{15}\text{N}$ in consumed foods [32]. A multi-isotope approach can help to minimize the impact of these inputs when interpreting values. Detailed isotopic laboratory methods are discussed in the [Supplementary material](#).

Identified Mecklenburg John Doe 2021

Case background

Human remains in a state of advanced decomposition were discovered in a wooded area in Mecklenburg County, North Carolina in June of 2021. Based on anthropological analysis conducted at NCSU in June of 2022, the decedent was estimated to be a 30- to 50-year-old European-American male, with a mean stature of 5 feet 5 inches (1.651 m) [2, 33–35]. The *hair-on-end* appearance or the perpendicular trabecular proliferation resulting from severe anemias was visible on the postmortem radiographic images (Figure 2) [36]. No perimortem trauma was observed on the remains available for analysis. Bone samples were selected for DNA extraction and sequencing, and isotopic analyses ($^{87/86}\text{Sr}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in 2022.

Bone apatite ($^{87/86}\text{Sr}$) for recent geographic movement

Bone apatite cannot confirm natal origin but reflects recent movements. The strontium ($^{87/86}\text{Sr}$) isotope value of the bone sample (0.708307) is consistent with modern Americans (Table 1) [37–39]. Bioavailable strontium isoscapes indicate that the strontium values are consistent with the Southeast

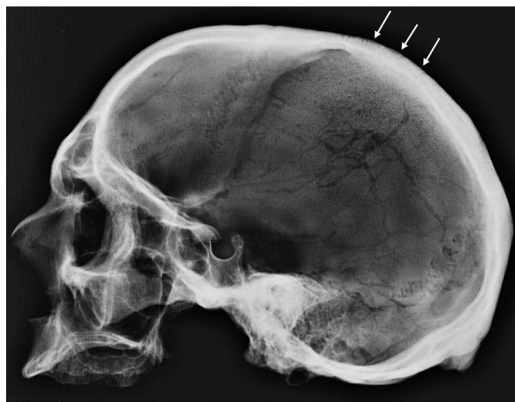


Figure 2 Lateral radiograph of the cranium of Identified Mecklenburg John Doe 2021 showing *hair-on-end* appearance (arrows) indicative of anemia.

region of the USA [40, 41]. Additionally, strontium isotopes have been shown to vary across the continental USA and used alone may not be useful in establishing recent movements [39].

Bone collagen $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ for diet markers

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values reflect locally available dietary sources [28, 29]. The $\delta^{13}\text{C}$ from the bone collagen is consistent with a C₄-based diet observed in US populations (-15.98%) [42].

Investigative Genetic Genealogy

A relative reported that a first cousin was missing in January of 2023, whose profile matched the Mecklenburg County John Doe. Using DNA kits from private genealogy databases, familial matches from the first cousin who submitted the missing person’s report, and subsequently a biological son of the potential decedent, were used to positively identify the Mecklenburg County John Doe.

Summary

The decedent was positively identified *via* IGG in March of 2023. He was originally from South Carolina and was known to live on the streets of Charlotte. Anthropological analyses along with isotopic analyses are consistent with the demographic information provided in the missing person’s report, and these analyses were used as a road map for IGG.

Unidentified Mecklenburg Jane Doe 2011

Case background

An incomplete set of skeletal remains was discovered by the Department of Transportation workers in a wooded area of Mecklenburg County, North Carolina in March 2011. Forensic anthropological analyses were conducted as part of the cold case initiative at NCSU in March of 2024. The decedent was estimated to be a 23- to 49-year-old Mesoamerican female using the software 3D-ID and web-based system rASUDAS [2, 43–45]. Extensive carnivore scavenging was present throughout the postcranial elements. There are dark gray dental amalgam fillings on the left and right mandibular first molars. A mandibular incisor and a rib were selected for isotopic analyses ($^{87/86}\text{Sr}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in 2022.

Enamel and bone apatite dual isotopic ($^{87/86}\text{Sr}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$) signatures for geographic origin

The strontium ($^{87/86}\text{Sr}$) and oxygen ($\delta^{18}\text{O}$) data are consistent with geographic origin outside of the USA (Table 1) [37–39, 46–48]. Using data from Keller et al. [39], Laffoon et al.

Table 1. Isotope ratios from measured osteological samples.

Cases	Material	$^{87/86}\text{Sr}$	$\delta^{18}\text{O}_{\text{vpdb}}$ (‰)	$\delta^{13}\text{C}_{\text{vpdb}}$ (‰)	$\delta^{13}\text{C}_{\text{coll}}$ (‰)	$\delta^{15}\text{N}$ (‰)
Identified Mecklenburg John Doe 2021	Bone	0.708307	-	-	-15.98	10.05
Unidentified Mecklenburg Jane Doe 2011	Tooth enamel	0.706827	-2.01	-7.10	-	-
	Bone	0.708043	-	-	-13.41	10.97
Identified Mecklenburg John Doe 1987	Tooth enamel	0.708905	-4.22	-8.93	-	-

[46], Juarez [47], and Wright [48] of US Cadets, Circum-Caribbean born, modern Mexican-born, and Guatemalan-born individuals, respectively, the measured values from the tooth enamel cluster on a bivariate graph with the Mesoamerican and Caribbean born group for strontium (0.7068), oxygen (-2.01‰), and the Caribbean group for oxygen and

carbon (-7.10‰) (Figures 3 and 4). Measured strontium values between enamel and bone apatite (0.706827) and bone apatite (0.708043) vary, with the observed bone apatite value trending closer to the normal range of modern Americans [37]. Based on remodeling rates of ribs, these results suggest the decedent relocated to the USA within the last 5–10 years of their life [49].

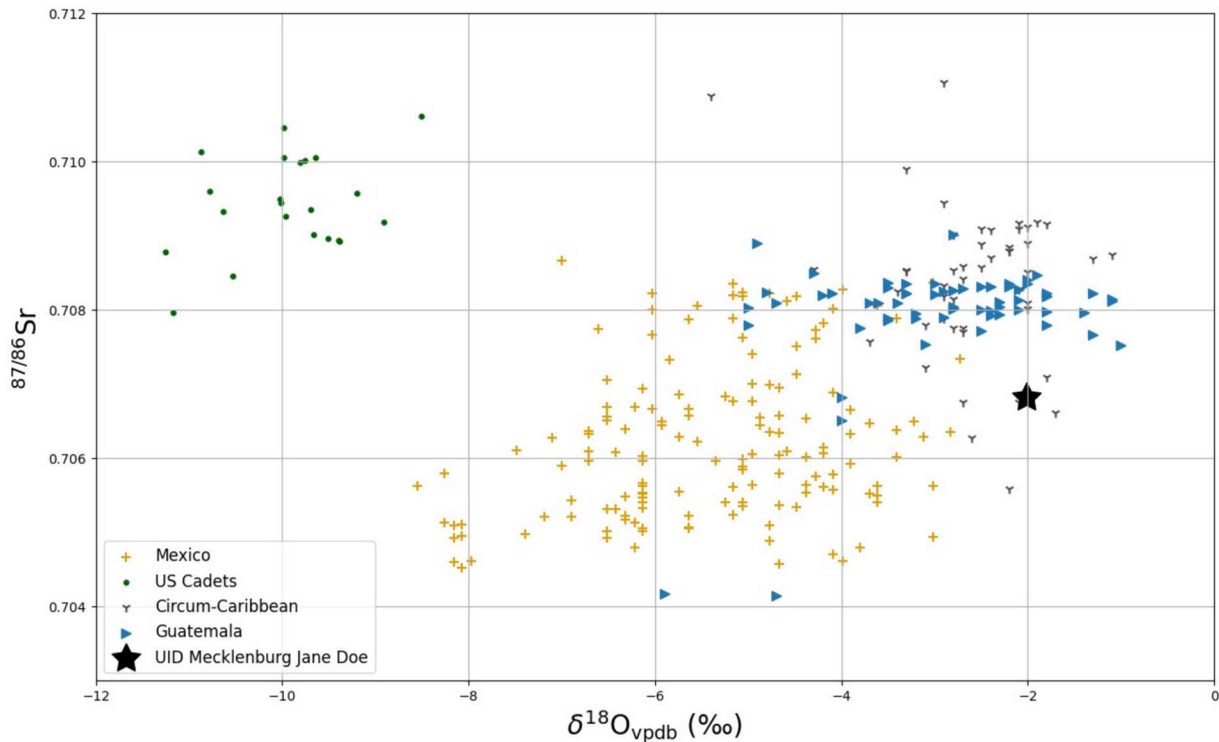


Figure 3 $\delta^{18}\text{O}_{\text{vpdb}}$ values vs. $^{87/86}\text{Sr}$ of Unidentified (UID) Mecklenburg Jane Doe plotted against known samples from regions within the USA and known samples from Mesoamerica and the Circum-Caribbean [39, 46–48].

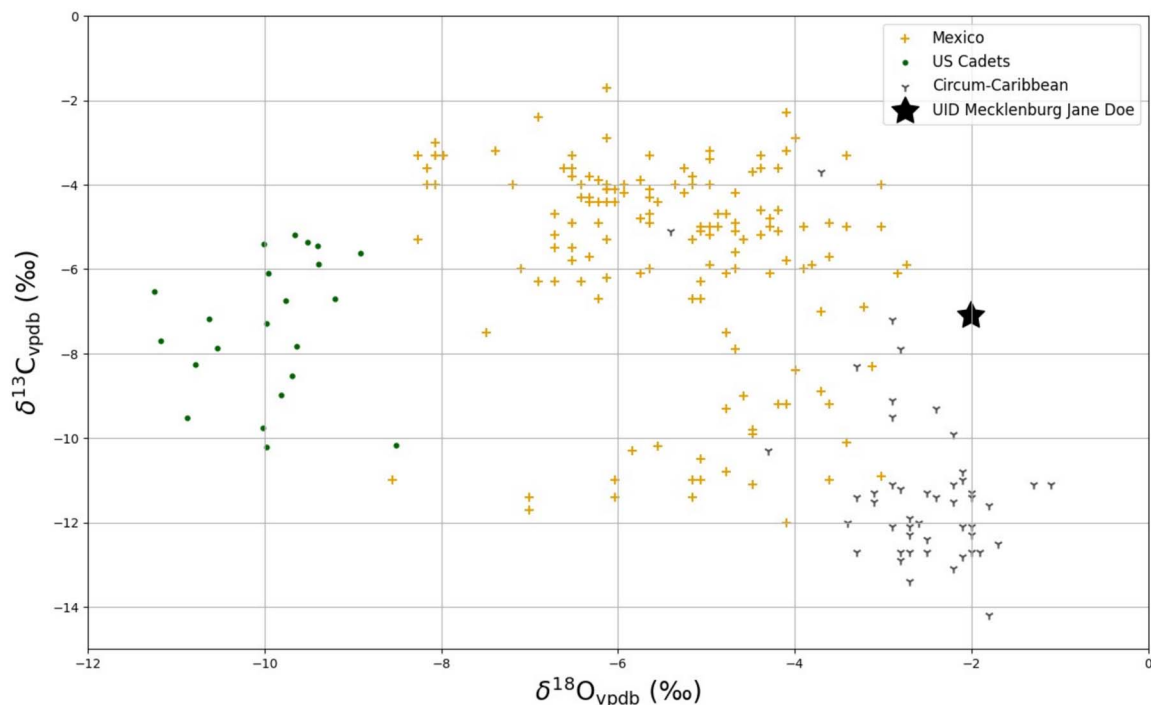


Figure 4 $\delta^{18}\text{O}_{\text{vpdb}}$ values vs. $\delta^{13}\text{C}_{\text{vpdb}}$ of Unidentified (UID) Mecklenburg Jane Doe plotted against known samples from regions within the USA and known samples from Mesoamerica and the Circum-Caribbean [39, 46–48].

Enamel and bone collagen isotopic signatures for diet and identity ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$)

Measured $\delta^{13}\text{C}$ from both bone collagen (-13.41‰) and tooth enamel (-7.10‰) are more enriched than a typical US diet, consistent with a higher proportion of C4 plants (namely corn and corn products) in the diet during childhood and in recent decades [50]. Marine diets and oceanic environments also affect dietary isotopic values of carbon and nitrogen [51]. When plotted on a bivariate graph, the bone sample clusters with Circum-Caribbean samples with oceanic or marine contributions to diet (Figure 5).

Summary

The decedent remains unidentified. The enamel isotopic results suggest the decedent was born in the Circum-Caribbean or Mesoamerican regions and bone apatite signatures suggest that they had recently relocated to North Carolina within the last 5–10 years. The assessment of population affinity using modern techniques (e.g. 3D-ID and rASUDAS) was consistent with isotopic results. This individual is most likely undocumented and will be triaged for IGG.

Identified Mecklenburg John Doe 1987

Case background

A cranium was recovered in Mecklenburg County, North Carolina from a creek bed. Forensic anthropological analysis was conducted at NCSU in January of 2024. Population affinity was estimated to be a male of West African/Nigerian origin using 3D-ID software, with a posterior probability of 0.8364 and a typicality of 0.0422 [2]. The typicality probability is low, meaning that the decedent is not well represented within the reference samples of the software. Bomb pulse dating was applied to confirm the antiquity of the remains and rule out historical origin. The second maxillary premolar was used to estimate the year of birth, which was estimated to be around 1955 and to estimate an age-at-death range

of 30–40 years old [52]. No trauma or pathologies were noted on the cranium. A right first maxillary premolar was selected for isotopic analyses ($^{87/86}\text{Sr}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$) in 2022. A maxillary premolar was selected for bomb pulse dating in 2023.

Enamel dual isotopic ($^{87/86}\text{Sr}$, $\delta^{18}\text{O}$) signatures for geographic origin

The strontium ($^{87/86}\text{Sr}$) and oxygen ($\delta^{18}\text{O}$) data are consistent with geographic origin within North America (Table 1) [37–39]. Using data from Keller et al. [39] of US Cadets, the measured oxygen and strontium values from the enamel cluster on the bivariate graph on the edge of the Southeast region of the USA (Figure 6). These values are consistent with the area where the remains were recovered. When considering population affinity estimation, data from Keller et al. [39] of US cadets, Schroeder et al. [46] for Barbadian-born and African-born individuals, and Laffoon et al. [53] for Circum-Caribbean born individuals, the decedent clusters between the USA and the Caribbean-born group for strontium (0.708905), oxygen (-4.22‰), and carbon (-8.93‰) (Figures 7 and 8). However, other regions of origin within North America cannot be ruled out.

Investigative Genetic Genealogy

This individual was identified in May 2024 *via* IGG. Estimates of year-of-birth and West African origin aided in the direction of the IGG investigation.

Summary

The decedent was positively identified *via* IGG. The family confirmed the age-at-death when he went missing in 1987, the family's West African origin, and that he was born in the Charlotte, NC area. Anthropological analyses and isotope results are consistent with the information provided by the family. However, the isotope results were broadly consistent with this finding and could not be used to

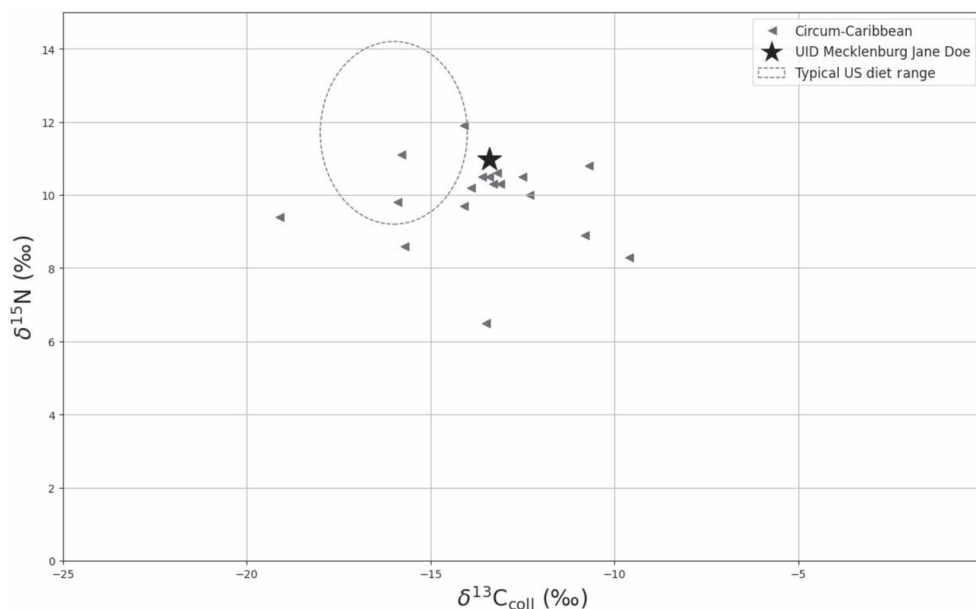


Figure 5 $\delta^{13}\text{C}_{\text{coll}}$ values vs. $\delta^{15}\text{N}$ from bone collagen of Unidentified (UID) Mecklenburg Jane Doe plotted against observed range of the US American diet and known samples within the Mesoamerica and the Circum-Caribbean [51, 52].

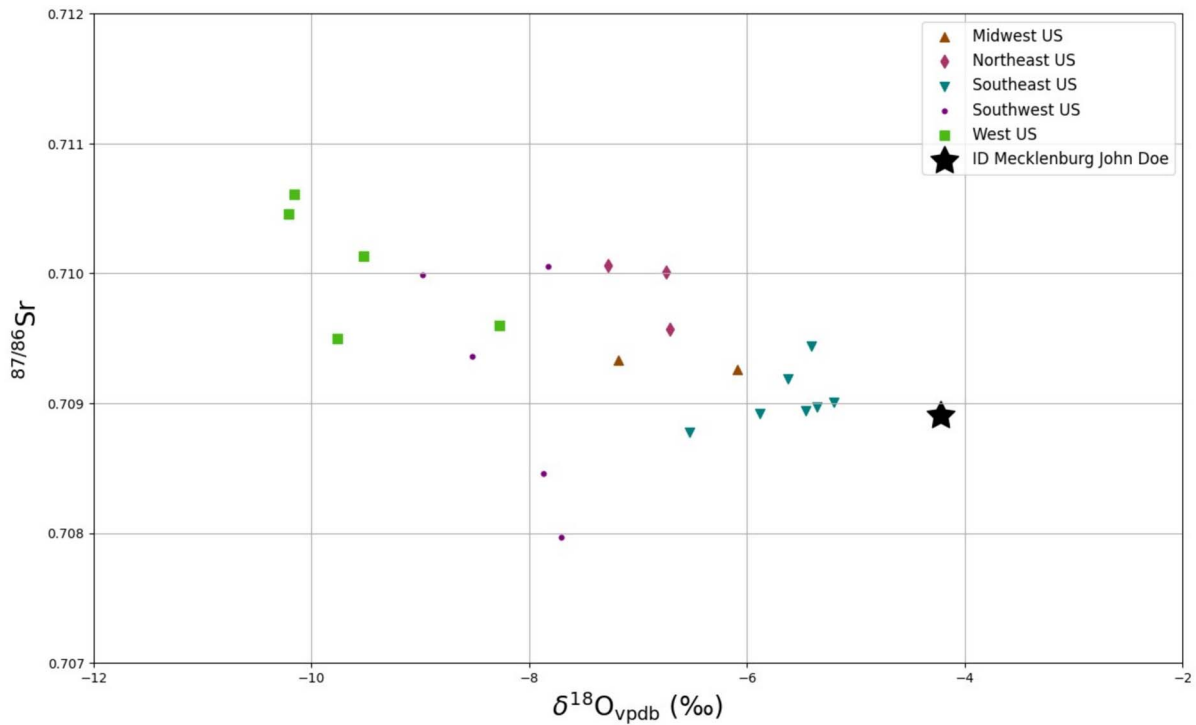


Figure 6 $\delta^{18}\text{O}_{\text{vpdb}}$ values vs. $^{87}/^{86}\text{Sr}$ of Identified (ID) Mecklenburg John Doe plotted against known samples from regions within the USA [39].

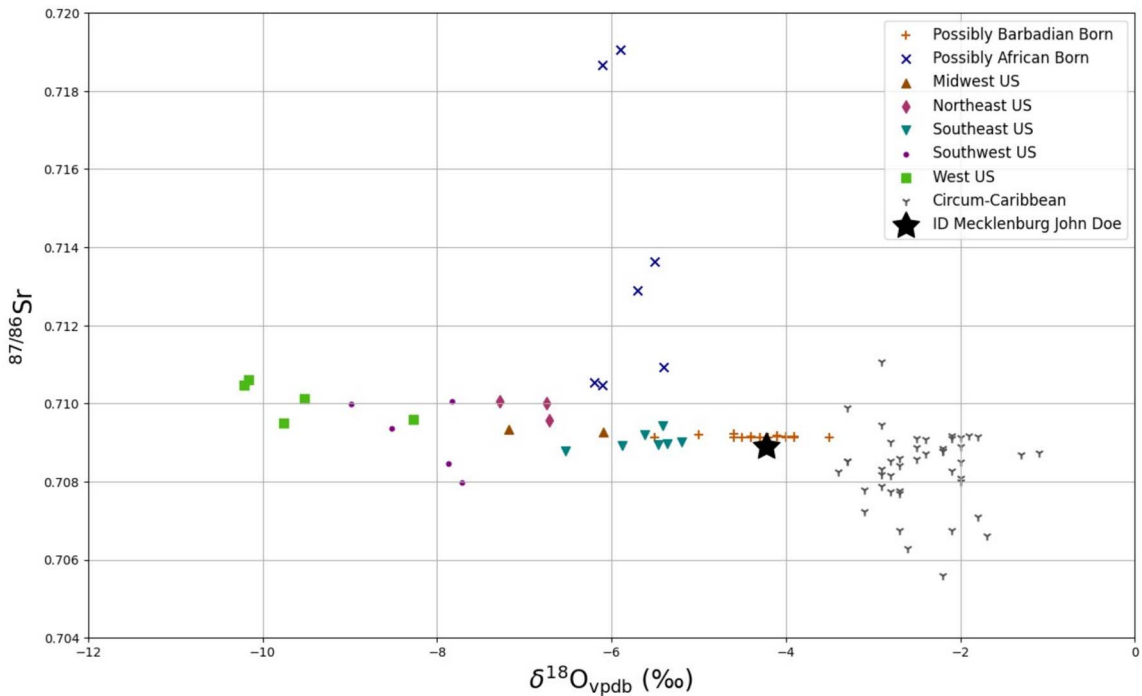


Figure 7 $\delta^{18}\text{O}_{\text{vpdb}}$ values vs. $^{87}/^{86}\text{Sr}$ of Identified (ID) Mecklenburg John Doe plotted against known samples from regions within the USA, known samples from the Circum-Caribbean and, possibly Barbadian-born and African-born individuals [39, 46, 54].

narrow down a region of origin more specific than North America.

Discussion

The human right to personal identity is internationally recognized through various declarations and conventions

(e.g. United Nations Convention on the Rights of the Child, Article 8). According to the FBI's National Crime Information Center statistics, there were 8415 records of unidentified persons in 2021. NamUs states that our missing and unidentified are the nation's silent mass disaster. NamUs lists 158 unidentified persons in the state of North Carolina

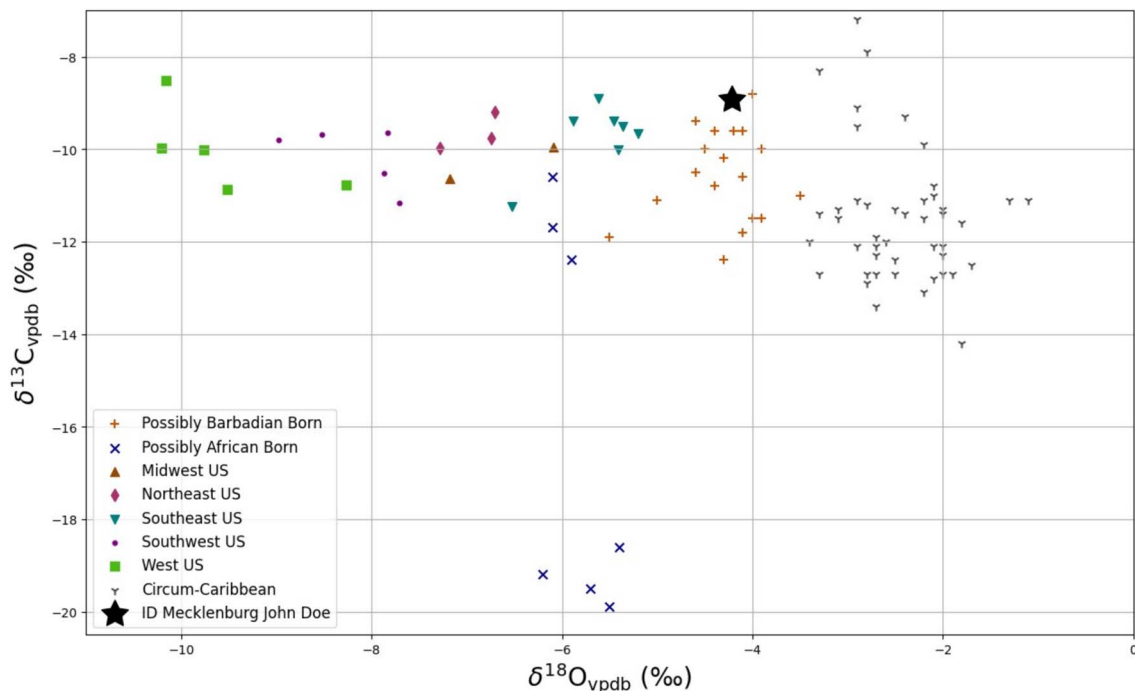


Figure 8 $\delta^{18}\text{O}_{\text{Vpdbb}}$ values vs. $\delta^{13}\text{C}_{\text{Vpdbb}}$ of Identified (ID) Mecklenburg John Doe plotted against known samples from regions within the USA, known samples from the Circum-Caribbean and, possibly Barbadian-born and African-born individuals [39, 46, 54].

with over 100 being skeletonized with national estimates of 4400 unidentified bodies recovered each year [54]. However, this is likely an underestimate as the data in this reference are almost 20 years old [1]. Further complicating identifications, foreign-born and undocumented migrants pose unique challenges as conventional identification tools such as familial DNA reference databases target US citizens [55]. While the ability to solve these cases is multifactorial, information regarding the region of origin using modern tools such as 3D-ID [2] and isotopic analyses [56, 57] is critical to estimating the place of origin. Anthropological analyses draw on modern population affinity techniques such as rASUDAS and 3D-ID. Historical events, gene flow, and migration have shaped similarities and differences observed in modern populations, and population affinity is a statistical approach to examining skeletal variation without the use of racial and typological axioms [58]. When combined with isotopic analyses, such as presented in this multidisciplinary approach, an individual's place of origin can be more confidently estimated. However, both population affinity and isotopic analyses rely on reference populations and reference data with which to compare individual measurements or values. Continued collection and publication of these data is essential to obtain global coverage.

Applying these methods requires a systematic process to determine which method(s) to apply to each case (Figure 9). Initially, skeletonized remains undergo anthropological analyses, utilizing current methods of estimating the biological profile, trauma, and/or pathologies (i.e. [2, 33–35, 43–45]). In cases where taphonomic or postmortem alteration is minimal, there is a higher likelihood that further analyses (i.e. DNA or isotope analyses) will yield viable results. Bone and tooth samples are selected and sent to State and

private laboratories for DNA and isotope analyses. When the circumstances of discovery are not straightforward, radiocarbon bomb-pulse dating can be used to establish antiquity and forensic significance. Ultimately, the application of these methods still may not provide an identification. The last method applied is IGG, which draws on all previous analyses in genealogical research. IGG is very costly, relying on grant and law enforcement funding, and is used as a last resort with cases being prioritized based on the preservation of remains, circumstances of death, and investigative information.

Conclusion

The cases outlined in this report utilize a collaborative approach to casework, leading to the successful identification of several decedents, including the two outlined above. Where investigations are ongoing, input from anthropological and isotopic analyses can now be considered in further evaluations and methods, such as IGG. This multidisciplinary approach to forensic casework in North Carolina continues to inform investigators in cold case reanalysis and is the first step in addressing the cold caseload within the state.

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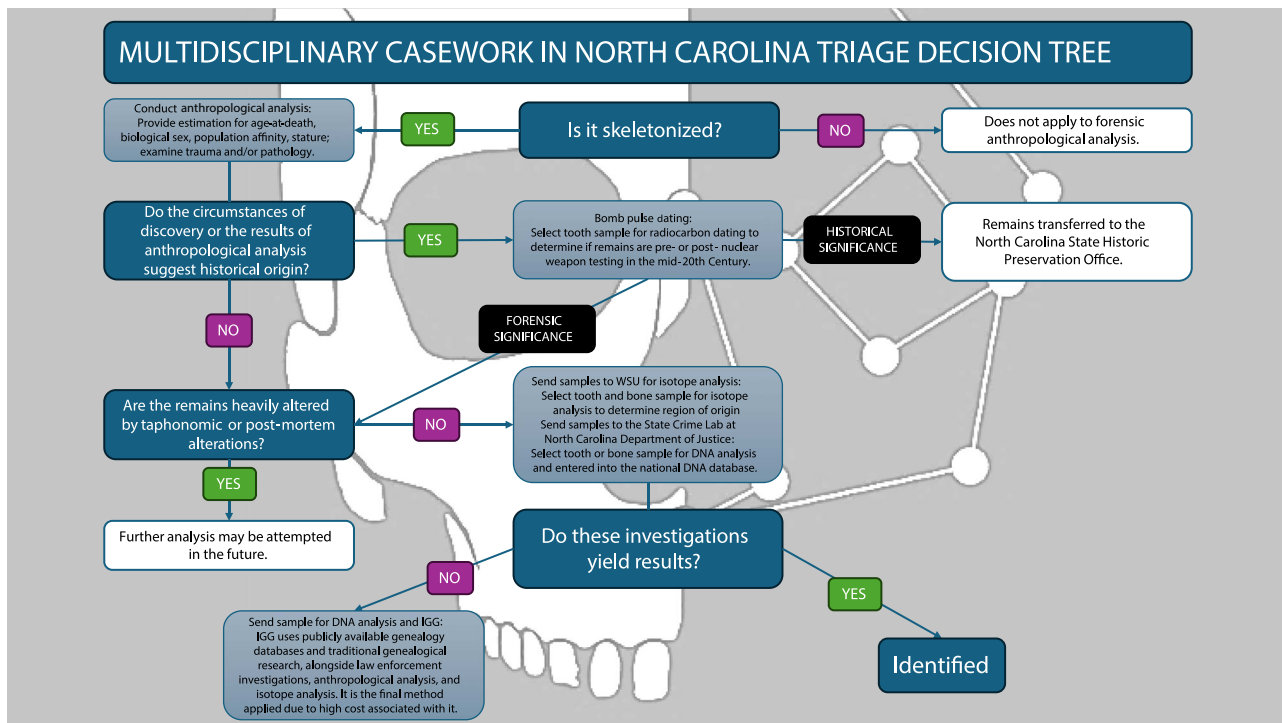


Figure 9 Decision tree demonstrating the process of applying various methods to current cases of unidentified remains and to cold case re-examination. These analyses are completed under grant and law enforcement funding at the North Carolina State University Human Identification and Forensic Analysis Laboratory.

genetic genealogical research. We thank North Carolina Medical Examiner Offices and law enforcement for their assistance and continued collaboration.

Authors' contributions

Nicole Long helped conceive the project, led the design and coordination of the report, assisted with anthropological analyses, and drafted the manuscript; Kimberly Sheets conducted the isotopic lab work and helped draft the isotopic methods section; Erin Kennedy Thornton supervised and assisted with the isotopic analyses and helped to draft the manuscript; Ann H. Ross helped conceive the project, reviewed anthropological casework, participated in the design and coordination of the report, and helped to draft the manuscript. All authors contributed to the final text and approved it.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

Ann H. Ross initial holds the position of Editorial Board Member for the *Forensic Sciences Research* and is blinded from reviewing or making decisions for the manuscript.

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