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RESEARCH ARTICLE

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Strontium isotope ratios related to childhood mobility: Revisiting sampling strategies of the calcined human pars petrosa ossis temporalis

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Rationale: Strontium isotope analysis can be applied to the calcined human otic capsule in the petrous part (pars petrosa ossis temporalis; PP) to gain information on childhood mobility in archaeological and forensic contexts. However, only a thin layer of the otic capsule, the inner cortex, demonstrates virtually no remodelling. This paper proposes an improved sampling method for the accurate sampling of the inner cortex of the otic capsule to ensure that $^{87}Sr/^{86}Sr$ ratios related to early childhood are obtained.

Methods: Calcined rib and diaphyseal fragments and PP from ten cremation deposits are sampled for strontium isotope analysis, whereby our improved sampling strategy is applied to sample the inner cortex of the otic capsule. This allows inter- and intraskeletal $^{87}Sr/^{86}Sr$ comparison within an Iron Age collection from Oss, The Netherlands.

Results: Forty percent (4/10) of the calcined PP that were evaluated for this study show marked differences in $^{87}Sr/^{86}Sr$ (0.00035–0.00065) between the inner cortex and the bone sample surrounding this layer, the external cortex that has higher remodelling rates. Differences in $^{87}Sr/^{86}Sr$ between various skeletal elements also aided in the identification of the minimum number of individuals.

Conclusions: Our study demonstrates the problematic nature of the external cortex and stresses the need for a precise sampling method of the correct areas of the otic capsule. This can only be obtained by cutting the calcined PP midmodiolarly to enable adequate combustion degree assessment, and the correct identification and sampling of the inner cortex of the otic capsule.

1 | INTRODUCTION

Analysis of strontium isotope ratios ($^{87}Sr/^{86}Sr$) in human and faunal skeletal material has proven to be a reliable and only slightly destructive tool in archaeological[1–4] and forensic[5,6] provenance studies. In archaeological contexts, due to the increased susceptibility of bone to diagenetic alterations compared with dental enamel, bone has mostly been excluded from strontium isotope analysis studies.[7–9] Hence, dental enamel has become the preferred material that allows for investigation of childhood palaeomobility patterns. The preference for dental enamel, however, excludes a major component of archaeological skeletal remains: cremations.

In 2015, Snoeck and co-workers demonstrated calcined bone to be a reliable substrate for the determination of biogenic strontium isotope ratios reflecting the later years of an individual's life.[10] Earlier, Harvig et al[11] evaluated the relationship between the $^{87}Sr/^{86}Sr$ ratios of premolar enamel and the otic capsule (also known as the bony or osseous labyrinth) of nine unburnt pars petrosa ossis temporalis

(the petrous parts of the temporal bone; hereafter referred to as PP), one burnt PP, and the enamel of one calcined second molar enamel. As virtually no bone remodelling of the otic capsule occurs after the age of about 2 years,[12,13] it can be concluded that otic capsules from inhumed and cremated human individuals can be used as a proxy for archaeological childhood mobility. Recent research by Sebald et al[14] assessed the potential of heavily burnt dentine for archaeological provenance studies and Taylor et al[15] used burnt dental enamel for forensic provenancing. Although both types of skeletal tissues look promising, dental enamel rarely survives the cremation process (apart from the unerupted crowns from non-adult individuals that are protected by the mandible) and also calcined dentine is rarely available. This would hinder obtaining childhood strontium isotope ratios.

The PP usually survives the cremation process better than dental elements and, in recent years, several researchers have used burnt PP for provenancing.[16–18] If the dentine from the same cremation deposit was available, both types of tissue could be analysed to enable cross-checking of the results. The otic capsule is usually sampled following the methodology of Jørkov et al,[19] whereby the PP is drilled at a right vertical angle into the otic capsule (*ca* 0.5–0.8 cm down) between the internal acoustic meatus and the subarcuate fossa (Figure 1). The powdered outer surface (*ca* 2 mm) of the PP is discarded to avoid the inclusion of powdered bone from other parts of the PP that have different turnover rates. However, PP that appear fully calcined on the outside may have experienced lower temperatures on the inside, being charred instead of calcined, excluding them for Sr isotope analysis.[20] Furthermore, differences in morphology exist due to inter- and intraindividual variation and heat-induced changes may further alter the size and shape of the otic capsule (Figure 2; see also Figure 1B).

Drilling from the outside inwards hinders (macroscopic) assessment of the combustion degree of the deeper segments of the PP. This way, not fully calcined bone (bone that is not fully white) may be included in the sample, which could lead to unreliable results.[10] In addition, variations in morphology may impair the sampling of the correct part of the PP, which was acknowledged by Jørkov and co-workers[19] who recommended serial sectioning of a PP to achieve the correct sampling of the otic capsule. However, the description of their sampling strategy is incomplete, since serial sectioning of the PP makes drilling from the outside towards the otic capsule redundant, and information about the orientation of the anatomical plane through which the sections were made is lacking. A precise understanding and the use of the correct and standardized sampling strategy of the otic capsule are vital for the development of childhood mobility research, especially for populations that practiced cremation.

The aim of our study is to develop an improved and unambiguous sampling method to ensure accurate sampling of the correct area of the PP (i.e. where bone turnover is minimal), which will aid other researchers in detecting early childhood mobility in archaeological populations and forensic cases. A detailed description of the growth, development, and morphology of the PP and the otic capsule is provided, which is then used to describe in great detail an improved and accurate sampling strategy. Strontium isotope analyses are performed on rib, long bone, and PP samples from ten individuals from a Middle Iron Age cremation site in Oss, The Netherlands. The results allow comparison of the intra- and interskeletal $^{87}Sr/^{86}Sr$ and clearly underline the need for an accurate sampling methodology.

2 | GROWTH AND DEVELOPMENT OF THE OTIC CAPSULE

The mammalian inner ear consists of the cochlea that is involved in sound perception and the vestibular organs (vestibulum and three semicircular canals) that are involved in the perception of spatial orientation and balance. As depicted in Figure 2B, the otic capsule is the portion of the PP that surrounds the human inner ear.[21,22] Due to its unique morphology and development, the PP is the most robust cranial bone.[23,24]

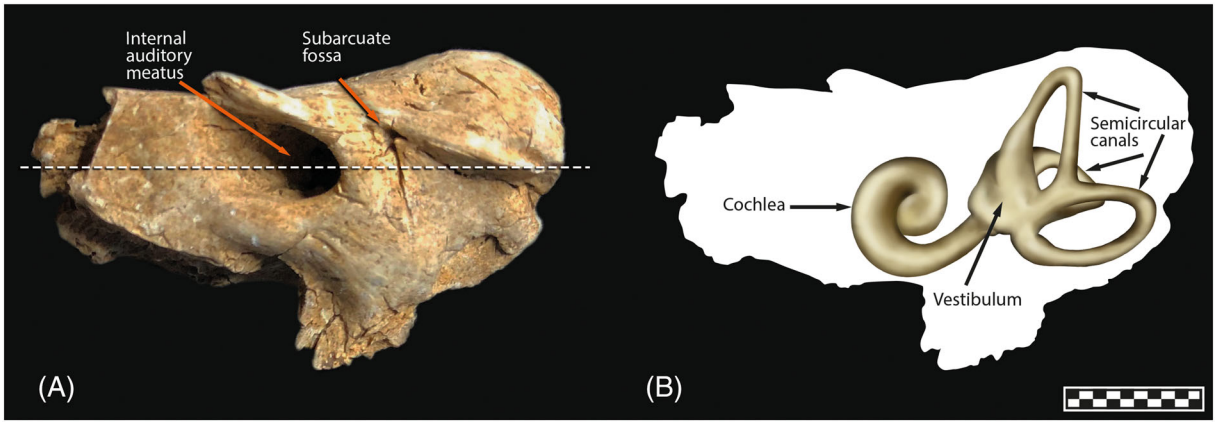

FIGURE 1 (A) Calcined PP (right) showing the medial side frontally with midmodiolar section line. Method of Jørkov et al[19] samples between both arrows. (B) Schematic overview of the otic capsule within the right PP consisting of the cochlea, the vestibulum, and the semicircular canals. Scale is in mm

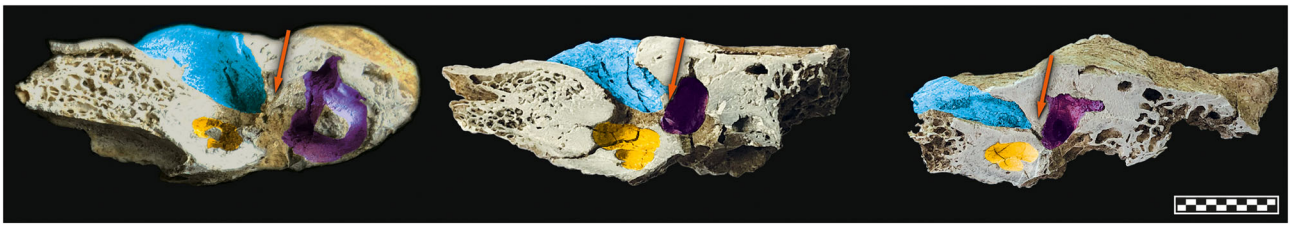

FIGURE 2 Midmodiolar cut from three right calcined PP, showing clear differences in the morphology and location of the internal auditory meatus (blue), cochlea (yellow), and semicircular canals (purple). The arrows point towards the drilling location as described by Jørkov et al[19] Scale is in mm

The foetal otic capsule from the 8th to 16th week *in utero* consists entirely of cartilage and is a product of cranial neural-crest-derived mesenchyme that surrounds the early otic vesicle. Part of this mesenchyme passes through pre-cartilage and cartilage stages and forms a complete cartilage model of the bony otic capsule between the 16th and 17th week and is completely ossified at the 34th week.[21,22] Several key aspects set the otic capsule apart from other bony structures. First, already during foetal development, the various parts of the labyrinth grow to adult proportions before the start of ossification. Second, in the second trimester, fourteen ossification centres appear successively at specific points in the cartilaginous capsule. These ossification centres subsequently fuse without leaving sites for future epiphyseal growth. Third, the bony capsule has a trilaminate structure, consisting of the endosteal layer delineating the lumen of the membranous labyrinth, an intermediate or endochondral layer, and the periosteal layer, which can be divided in an inner and an outer layer.[25] In this trilaminate structure both endochondral and membranous ossification takes place. Fourth, this foetal architecture is maintained and no adult features of bone remodelling such as osteons and Haversian canals will develop later on in the endosteal and enchondral layers.[21,22,26]

In contrast to the other bones in the human skeleton, the otic capsule forms a unique functional unit in which growth, modelling, and remodelling in bone are virtually absent in childhood and adult life,[27–29] which is supported by the observations of persistent microfissures in the bony otic capsule and that larger fractures rarely heal.[28] Only where the outermost (periosteal) layer is involved does osteogenesis seem to occur whereas the innermost (i.e. endosteal and endochondral) layers remains inactive.[28,30] Experimental studies investigating bone turnover rates in the otic capsules of dogs and rabbits demonstrate that bone remodelling by osteoclasts and osteoblasts occurs at an incredibly low rate, with an overall capsular turnover of approximately 2% per year, and with nearly full inhibition in the endosteal and endochondral layers of the otic capsule at a rate of *ca* 0.1% per year, which is 100 times slower than in the surrounding extracapsular cranial bones.[28,29,31]

Nevertheless, certain pathological conditions can affect this slow turnover rate. Higher rates of bone remodelling within the otic capsule may occur in adult individuals suffering from systemic bone disorders, such as Paget's disease, fibrous dysplasia, and osteogenesis imperfecta,[32] but is also seen in patients suffering from chronic renal failure[33] and otosclerosis.[29,34] Yet, Paget's disease, fibrous dysplasia, and osteogenesis imperfecta are relatively rare,[35–37] and about 50% of individuals suffering from chronic renal failure (11% to 13% of modern individuals) develop hearing loss as a result of abnormal otic capsule remodelling.[33,38]

Clinical otosclerosis affects 0.25–1.20% of modern individuals in Europe and North America.[27,29,39,40] Otosclerosis is the process of abnormal bone remodelling in the otic capsule, and about 3.4% of modern individuals show post-mortem histologic evidence of otosclerosis.[39,40] A higher turnover rate in the otic capsule may imply to some extent that childhood $^{87}Sr/^{86}Sr$ are lost due to remodelling, whereby usually the cochlea is affected.[41] However, systemic bone disorders are rare and the prevalence of otoscleroris is relatively low.

The virtually non-existent remodelling of the endosteal and endochondral layer of the otic capsule under 'typical' circumstances most likely results in the retention of childhood Sr isotope compositions. This makes the (burnt) ossified otic capsule an ideal candidate for palaeomobility studies, enabling the analysis of endogenous strontium isotope ratios that can be used to trace mobility patterns in early childhood.[11,19] The calcined PP is often found separated from the cranium (see Figure 2A), but usually recovered almost complete (>75%) due to its robustness. The morphology of the bony otic capsule, which surrounds the cochlea, the vestibulum, and the semicircular canals, and its location within the PP are depicted in Figure 2B.

3 | MATERIALS AND METHODS

For the purpose of this study, ten individuals from the Middle Iron Age cremated human bone collection of Oss-IJsselstraat, The Netherlands, were sampled.[42] Table 1 provides information on age and sex of the selected individuals. For all cremation deposits, the minimum number of individuals was one, apart from V07, V351, and V352 that included the remains of at least two individuals. From each PP, a sample from the endosteal and enchondral layers (hereafter referred to as the internal cortex, IC) of the cochlea, the vestibulum, and semicircular canals (see Figure 3A and the green area in Figure 3B) and one from the surrounding bone (i.e. the periosteal layers; hereafter referred to as the external cortex, EC) were taken (Figure 3A and the red area in Figure 3B).

TABLE 1 Age and sex estimations for each individual[42]

Cremation	Age (years)	Sex
GP	18+	I
V07	7–12 and 0–6	NA and NA
V14	20–40	M
V16	7–12	NA
V219	20–40	I
V225	20–40	M
V226	18+	M
V351	20–40	F and M
V352	20–40	M and M
V356	20–40	F

I, indeterminate; F, female; M, male; NA, not applicable.

To add context to the $^{87}Sr/^{86}Sr$ of the otic capsule, a rib and diaphyseal fragment from each cremation deposit were also sampled. This was also done for the cremation deposits that contain the remains of at least two individuals; consequently the sampled PP does not necessarily belong to the same individual as the sampled rib or the diaphyseal fragment. Still, cremation deposits that contain at least one individual based on the macroscopic osteoarchaeological analysis may in fact contain multiple individuals that were not identifiable macroscopically. By sampling the PP, rib, and diaphysis from each cremation deposit, not only can the $^{87}Sr/^{86}Sr$ of the otic capsule be contextualized, but the strontium isotope compositions of the rib and diaphysis may highlight the challenging nature of using cremation deposits for osteoarchaeological analysis and potentially to aid in the identification of the minimum number of individuals.

Each skeletal element effectively represents a different time period in life due to differences in turnover (remodelling) rates.[43,44] The processes of growth and (re)modelling influence non-adult bone, while in the adult skeleton, growth and modelling have ceased and only remodelling takes place. Growth results in the enlargement of skeletal elements to adult size, while modelling refers to adaptation of the skeleton to growth and changes in functional demands.[45] During remodelling, bone is renewed to maintain bone strength and mineral homeostasis.[43] The average bone turnover rate of adult cortical bone amounts to 2–3% per year,[43,46] while trabecular bone has an annual turnover rate of approximately 28% per year.[47] A large number of factors (e.g. the cortical-to-trabecular bone ratio of each skeletal element, mechanical loading, genetic predisposition) will influence the overall bone turnover rate.[43,44]

To what extent the strontium isotope composition of bone is affected by remodelling is poorly understood. Still, variation in bone remodelling needs to be considered, and the sampling of various types of skeletal elements could enable the detection of $^{87}Sr/^{86}Sr$ related to different periods of an individual's lifetime,[48] allowing reconstruction of palaeomobility patterns. Ribs are considered to have overall higher turnover rates than femoral bone partially due to a higher trabecular-to-cortical bone ratio.[49] Considering the proportion of cortical and trabecular bone in the PP, it is possible to assume that extracapsular bone (i.e. bone surrounding the otic capsule) would have a turnover rate between that of the rib and the femur. Therefore, it is expected that the $^{87}Sr/^{86}Sr$ of the ribs will reflect the origin of the foods and water consumed closer to the individual's death than extracapsular or femoral bone,[50] though more research is needed to confirm this.

The total thickness of the otic capsule in unburnt PP is approximately 2–3 mm, with the endosteal and endochondral layers together (i.e. the IC) measuring about 0.5 mm in thickness.[22,34,51] To ensure the precise sampling of the otic capsule, each PP was cut transversally in half through the internal auditory meatus using clean cut-off wheels on a handheld Dremel (available in various hardware stores) at Université Libre de Bruxelles, Brussels, Belgium (ULB) or a Buehler (Esslingen, Germany) IsoMet 1000 precision saw at Vrije Universiteit Amsterdam, The Netherlands (VU). This creates a midmodiolar section through the cochlea, exposing the IC of the various features (see Figure 2C). Only completely calcined bone was sampled. The visible surface of the cochlea, the vestibulum, and semicircular canals were mechanically cleaned by removing the surface (*ca* 0.05 mm) to minimize contamination by soil (see also Snoeck et al[10]). Sampling of the IC (*ca* 10 mg) was undertaken by drilling the areas indicated in green (see Figure 1D) with an acid-cleaned diamond-tipped burr. Only the IC of the otic capsule is considered to have a nearly inhibited turnover rate. Therefore, just the visible surface of the otic capsule was sampled (*ca* 0.5 mm) to avoid sampling layers of otic capsule with higher turnover rates. Due to the compactness of the calcined PP, it is inevitable that to some

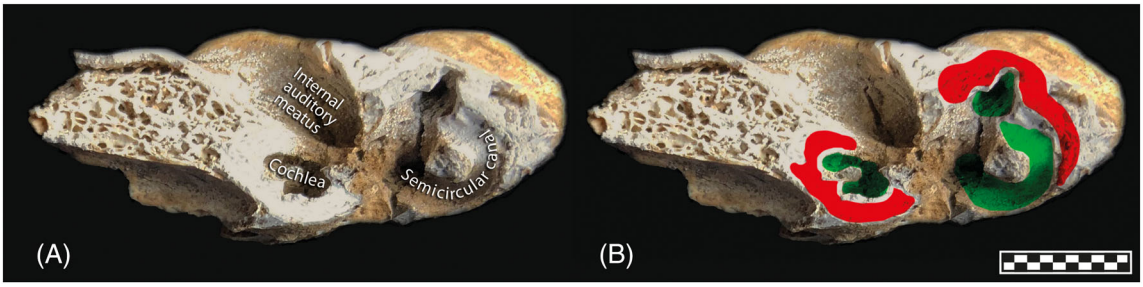

FIGURE 3 (A) Midmodiolar cut of a right calcined PP, exposing the cochlea, and parts of the semicircular canals. Medial side points superiorly. (B) Sampling areas for IC (green) and EC (red). Scale is in mm

extent layers of otic capsule with a slightly higher remodelling rate (i.e. the periosteal layer) were included in the sample. However, the proportion is postulated to be relatively small and the influence on strontium isotope ratios minimal. The EC of the otic capsule was sampled (*ca* 10 mg) from the red areas avoiding direct contact with the green areas (see Figure 2D). This provides a bone sample that is reported to have an overall turnover rate of *ca* 10%, which is 100 times higher than the IC of the otic capsule.[28,31]

All ribs and diaphyses were processed at ULB, as well as PP samples of V07, V219, and V351. The remaining samples were analysed at VU. Approximately 50 mg of calcined rib and diaphysis fragments was collected and rinsed three times ultrasonically for 10 min in MilliQ water (Millipore, Bedford, MA, USA). Next, following Snoeck et al,[10] the bone samples were transferred to clean glass vials and pre-treated with 1 M acetic acid (CH_3COOH) in an ultrasonicator for 3 to 10 min (10 mg of bone: 1 mL of 1 M acetic acid), followed by three MilliQ rinses and 10 min of ultrasonication in MilliQ water.[10,16,52] The powdered PP samples underwent the same treatment, but for samples V07, V219, and V351 0.1 M acetic acid was only used for 1 min. All ULB processed samples were dried overnight in an oven at 50°C. The VU processed samples were dried overnight on a hotplate at 50°C.

The rib and diaphysis samples, and PP samples of V07, V219, andV351 were transferred to a class 1000 clean laboratory facility with class 100 laminar flow hoods at ULB. Strontium was extracted from the samples and purified following the protocol described in Snoeck et al[10] and measured using a Nu Plasma MC-ICP mass spectrometer (Nu Instruments, Wrexham, UK). The purity of the Ar gas used inside the spectrometer prevented any interference on the strontium isotope masses. All the Sr isotopes (84, 86, 87, and 88) were measured, while monitoring masses 83 (Kr) and 84 (Rb), allowing for interference corrections on the masses 84, 86 (Kr), and 87 (Rb) as described by Snoeck et al.[10] During the course of this study, repeated measurements of the NBS987 standard yielded $^{87}Sr/^{86}Sr = 0.710246 \pm 0.000045$ (2σ for $n > 300$), which is, for our purpose, sufficiently consistent with the mean value of 0.710252 ± 0.000013 (2σ for analyses) obtained using thermal ionization mass spectrometry (TIMS) instrumentation.[53] The remaining PP samples were transferred to a class 100 clean laboratory facility at VU. The samples were leached and dissolved following the protocol of Snoeck et al.[10] A detailed description of the Sr column extraction and the sample loading procedures is given in Kootker et al.[54] The strontium isotope ratios were measured with a Thermo Fisher Scientific (Waltham, MA, USA) Triton Plus thermal ionization mass spectrometer. The ratios were determined using a static routine and were corrected for mass fractionation. The intra-run NBS987 gave a mean $^{87}Sr/^{86}Sr$ of 0.710251 ± 0.000008 (2σ, $n = 4$). All MC-ICP-MS and TIMS sample measurements were normalized using a standard bracketing method with the recommended value of $^{87}Sr/^{86}Sr = 0.710248$.[53] Both the ULB and the VU procedural blanks were considered negligible (ULB: total Sr (V) of max 0.02 versus 7–8 V for analyses, i.e. *ca* 0.3%; VU: less than 17 pg of strontium). For each sample, the $^{87}Sr/^{86}Sr$ ratios are reported with 2SE representing the analytical uncertainty on each individual sample calculated from the 60 measurements within each run. A recent isotope study by Sengeløv et al[55] confirmed that there is no significant difference in the strontium isotope ratios obtained at the two facilities.

4 | RESULTS

The strontium isotope ratio for each sample per cremation deposit is presented in Table 2. Figure 4 shows four distinct patterns in the differences in $^{87}Sr/^{86}Sr$ between the IC and the EC, the diaphysis, and the rib per cremation deposit, henceforth $\Delta^{87}Sr/^{86}Sr$. The lines more or less represent bone turnover rate, whereby the ICs have the lowest and the ribs are assumed to have the highest turnover rates. However, bone turnover rates are influenced by many factors, including sampling location. Therefore, the *x*-axis was not defined as representing bone turnover rate.

The $\Delta^{87}Sr/^{86}Sr$ values for the differences between the IC and EC from cremations deposits V07, V16, V225 and V226, V352 and V356 are small (≤ 0.00022) and possibly within the biological variation,[56] while the $\Delta^{87}Sr/^{86}Sr$ values between IC and EC in cremation deposits

TABLE 2 Overview of $^{87}Sr/^{86}Sr$ per sample with 2SE per cremation deposit

Context	IC	2SE	EC	2SE	D	2SE	R	2SE
GP	0.709382	0.000008	0.710032	0.000006	0.710014	0.000013	0.710000	0.000013
V07	0.710230	0.000010	0.710264	0.000011	0.710154	0.000011	0.711111	0.000013
V14	0.709907	0.000009	0.710500	0.000011	0.709960	0.000010	0.709929	0.000011
V16	0.709506	0.000009	0.709289	0.000009	0.709919	0.000014	0.709867	0.000011
V219	0.710451	0.000013	0.710097	0.000011	0.710131	0.000010	0.710025	0.000014
V225	0.709867	0.000009	0.709867	0.000011	0.709954	0.000010	0.709702	0.000010
V226	0.709597	0.000010	0.709585	0.000007	0.709959	0.000011	0.709942	0.000015
V351	0.710051	0.000017	0.710526	0.000014	0.709930	0.000014	0.709919	0.000013
V352	0.708964	0.000007	0.709025	0.000008	0.709852	0.000013	0.709903	0.000016
V356	0.708924	0.000013	0.709102	0.000007	0.709051	0.000013	0.709080	0.000013

IC, inner cortex consisting of endosteal and endochondral layer; EC, external cortex consisting of periosteal layer; D, diaphysis; R, rib.

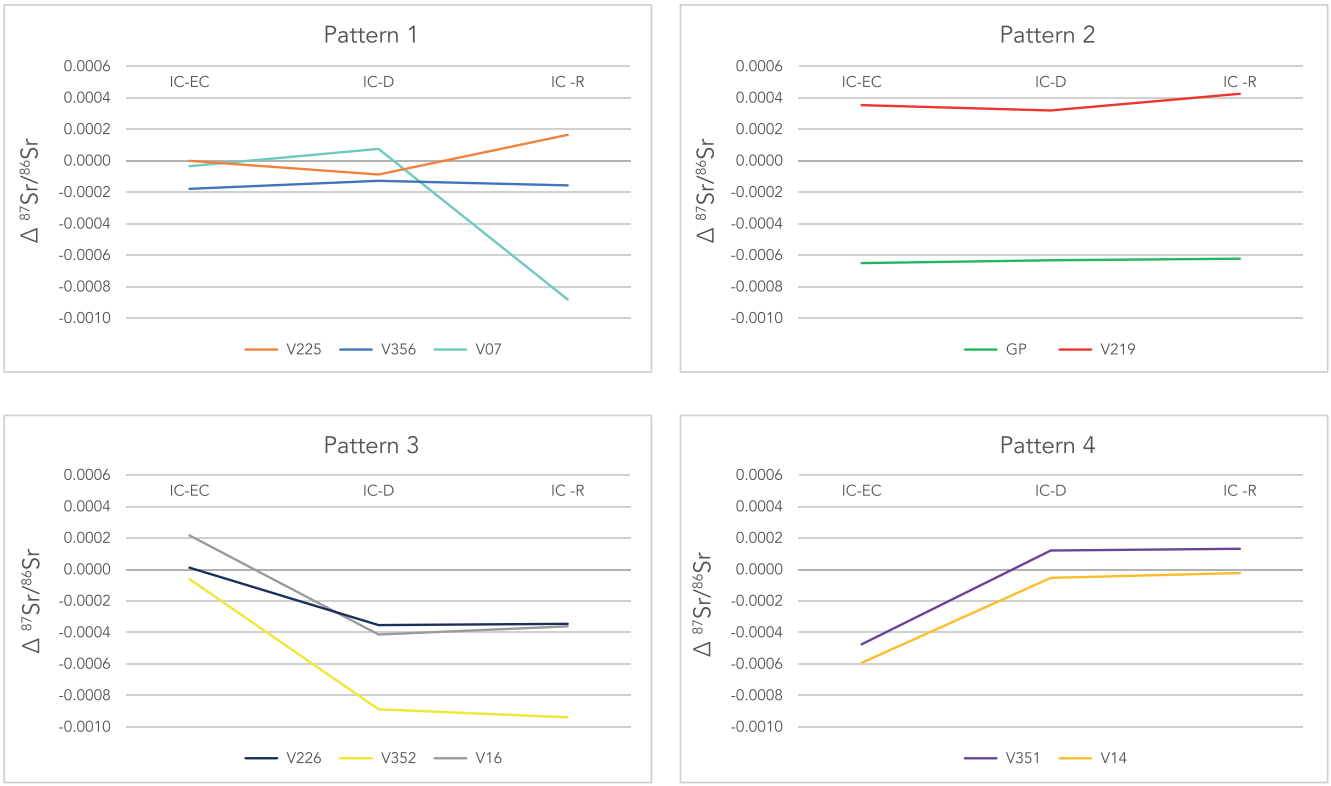

FIGURE 4 Overview of observed patterns in the $\Delta^{87}Sr/^{86}Sr$ per cremation deposit per sample. IC, inner cortex consisting of the endosteal and endochondral layer; EC, external cortex consisting of the periosteal layer; D, diaphysis; R, rib

GP, V14, V219, and V351 are ≥ 0.00035. All the analysed samples from deposits V225 and V356 show little change in strontium ratios (Pattern 1, $\Delta^{87}Sr/^{86}Sr \leq 0.00016$), while GP and V219 only show a marked difference between the $^{87}Sr/^{86}Sr$ of the IC and those of the remainder of the samples (Pattern 2). V16, V226, and V352 show limited difference between IC and EC, but a larger difference between the samples taken from the otic capsule and the rib and diaphyseal samples (Pattern 3). Cremation deposits of V14 and V351 (Pattern 4) present a marked difference between the IC and EC (≥ 0.0004), while the differences between the IC and the diaphysis and rib are very small (≤ 0.00013). V07 presents similar strontium ratios for the IC, EC, and diaphysis, while the rib shows a marked difference (0.0009).

5 | DISCUSSION

Variations in morphological characteristics and heat-induced changes may result in significant differences in the location and shape of the cochlea, the vestibulum, and semicircular canals (Figure 2), thus requiring the cutting of the PP along a midmodiolar plane. This will expose both the IC and the EC of the otic capsule, enabling macroscopic assessment of its burning degree. Furthermore, it allows the identification of the cochlea, vestibulum, and semicircular canals and enables the precise sampling of the IC, which is the layer that has virtually no remodelling and provides information on childhood mobility.

In Pattern 1 (Figure 4), the samples from V225 and V356 show similar $^{87}Sr/^{86}Sr$ ratios over time (≤ 0.0002), regardless of the type of bone or the skeletal element. The absence of isotopic differences between the different skeletal elements suggests a palaeodiet with a consistent Sr isotopic signature throughout life. Cremation deposit V07 shows no large differences in $^{87}Sr/^{86}Sr$ between IC, EC, and diaphysis, but the $^{87}Sr/^{86}Sr$ of the rib is markedly different (0.0009). Although this could suggest that the individual moved or significantly changed diet recently before death, osteoarchaeological analysis has shown that this cremation included the (partial) remains of at least two non-adults, one aged between 0 and 6 years and the other between 7 and 12 years. In non-adults, turnover rates are higher due to the combined processes of growth and (re)modelling,[46] and it is postulated that the differences in $^{87}Sr/^{86}Sr$ are likely to be smaller in the various bone samples. The relatively large difference in strontium isotope ratios between the diaphysis and rib suggests that these samples come from two different individuals, further supporting the presence of more than one individual in this cremation context.

GP and V219, Pattern 2, show marked differences in $^{87}Sr/^{86}Sr$ between IC and the other samples, suggesting a change in the geographical origin of the foods and water consumed during adulthood compared with early in life resulting from mobility, a change in landscape use, and/or change in diet. The fact that all three samples (EC, diaphysis, and rib) show similar $^{87}Sr/^{86}Sr$ ratios implies that these individuals moved or changed their diets relatively early in

life and then did not move or change their diets anymore during the rest of their lives. Crucially, the differing strontium ratios of the IC and EC samples underline the difference in turnover rates of the different layers of the otic capsule and stress the need for correct sampling of the endosteal and endochondral layers of the otic capsule.

V16, V226, and V352 also show relatively large difference in $^{87}Sr/^{86}Sr$ between IC and diaphysis (≥ 0.00035), but the differences between the IC and EC strontium ratios are relatively small (≤ 0.00022; see Pattern 3). The differences in $^{87}Sr/^{86}Sr$ between the IC and diaphyseal samples in these cremation contexts suggest mobility or changes in the diet later in life. Another explanation for this difference in V352 is that osteoarchaeological analysis identified the remains of at least two individuals. It is possible that the PP of one individual and the diaphysis and rib of another individual were sampled, and that the two individuals consumed foods of different geographical origins. The similarity in strontium ratios of the IC and the EC samples may suggest that the turnover rate of the EC is lower than that of the diaphysis, which would contradict expectations. After all, if the turnover rate of the EC was comparable with that of the diaphysis, the strontium ratios of the EC sample are expected to be similar to those of the diaphysis and not the IC. Another possibility is that parts of the IC were sampled as EC, resulting in similar strontium isotope ratios.

Cremation deposits V14 and V351 show a different pattern from the rest (Pattern 4). The strontium isotope ratios of the IC, the diaphysis, and the rib are similar, whereas the difference between IC and EC is relatively large (> 0.00045). This may suggest that the turnover rate of the EC sample is higher than that of the ribs, which would contradict assumptions. Another possibility is that the PP belonged to one individual (who moved and/or changed diet) and the diaphysis and rib samples to another whose food originated from a similar region to where the first individual lived the first few years of life. Although osteoarchaeological analysis identified one individual in V14 and two in V351, it is possible that V14 also contained more than one individual. Hence, the combination of strontium isotope with osteoarchaeological analysis may aid in determining a more accurate number of the individuals within a cremation deposit. However, more research on the application of strontium isotope analysis for this purpose is needed. In any case, the large difference in $^{87}Sr/^{86}Sr$ between the IC and EC of deposits V14 and V351 further highlights the problematic nature of EC.

The various patterns, (1) no difference in $^{87}Sr/^{86}Sr$ between skeletal elements, (2) large difference in $^{87}Sr/^{86}Sr$ between IC and the other skeletal elements, and (3) small difference in $^{87}Sr/^{86}Sr$ between IC and EC, but large difference between IC and the rest of the skeletal elements, show the complexity of conducting multi-skeletal element analysis of a single cremation deposit and how differences in turnover rate can influence strontium isotope ratios. These results underline the need for future research on bone turnover rates of various skeletal elements to improve our understanding of this process and how it may affect the strontium isotope composition of different skeletal elements. Most importantly, the results of this study demonstrate the problems when the bone surrounding the IC is sampled. In 40% (4/10) of the cases, the $^{87}Sr/^{86}Sr$ of the IC samples differed from those of the EC samples (between 0.00035 and 0.00065), while the rest showed virtually no differences (≤ 0.00022). The lack of differences between $^{87}Sr/^{86}Sr$ in the IC and EC samples could be due to sampling strategy, whereby part of the IC was sampled as EC. The absence of an isotopic difference, however, may also simply point towards the possibility that the individuals did not move or significantly change their diets during life. Since only the endosteal and endochondral layers of the otic capsule display virtually no remodelling, it is vital to sample just those layers to enable the detection of childhood strontium isotope ratios.

6 | CONCLUSIONS

Our study has clearly shown that careful sampling of the IC of the otic capsule is needed to obtain childhood $^{87}Sr/^{86}Sr$ by comparing the $^{87}Sr/^{86}Sr$ ratios of the IC with those of the EC and other skeletal elements. This can only be obtained by sectioning the PP midmodiolarly, assessing the combustion degree of the bone, identifying the various features of the otic capsule, and sampling only the IC (cleaned) layer of bone, thus minimizing contamination of the sample by soil and/or parts of the otic capsule with a higher bone turnover rate (EC).

As demonstrated by the results, the comparisons of $^{87}Sr/^{86}Sr$ of various skeletal elements show different patterns that may suggest mobility or changes in diet and/or landscape use. However, not all observed patterns are fully understood in terms of bone turnover rates. This stresses the need for further research on differences in bone turnover rate in various skeletal elements and its influence on strontium isotope composition in bone, to improve our understanding of changes in $^{87}Sr/^{86}Sr$ over the course of an individual's life.

Using differences in strontium isotope ratios in addition to the results of macroscopic osteoarchaeological analysis of cremated remains improved the evaluation of the number of individuals within a cremation deposit in this study. The wider application of strontium isotope analysis as a way of determining the minimum number of individuals in combination with macroscopic osteoarchaeological analysis needs to be evaluated further.

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PEER REVIEW

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