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Sexing the bony labyrinth: A morphometric investigation in a subadult and adult Belgian identified sample

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Abstract

In forensic anthropology, sex estimation is a fundamental step in assessing individual biological profiles when analyzing human skeletons. Yet, current methods are not reliable enough to allow an accurate sex identification of highly fragmented, burnt, or subadult remains. This paper aims to investigate sexual dimorphism of the bony labyrinth on both identified subadult and adult individuals. The bony labyrinth is of particular interest for sex estimation since it is alleged to complete size and maturation pre-pubertally and is located inside the petrous part of the temporal bone which protects it from taphonomic processes. The study was performed on 93 CT scans of identified individuals from two Belgian osteological collections (19–20th century) and from current pediatric images (Erasme hospital, Brussels). Linear and angular measurements were taken on 2D slices of right bony labyrinths. Intra- and interobservers error measurements were calculated. Statistical tests were used to unravel any morphological variations between subadult and adult bony labyrinths and to highlight differences between females and males, separately in subadults and adults. Linear discriminant functions were established by cross-validation and tested on an independent sample from Belgium. Some measurements were significantly different between subadults and adults, and between females and males within both subadult and adult samples. Univariate functions achieved 72.7% in subadults and 68.4% in adults whereas multivariate equations increased accuracy respectively up to 84.9% and 78.4%. This study entails promising results to design a sex estimation method suitable for fragmented and/or subadult remains. Further metric approaches are needed to explore bony labyrinth sexual dimorphism.

KEYWORDS
bony labyrinth, computed tomography, forensic anthropology, fragmented bones, sexual dimorphism, subadult remains

1 | INTRODUCTION

In both osteoarchaeology and forensic anthropology [1,2], establishing the sex of an individual is an essential step in the study of human remains. Sex is a crucial component in determining individual identity, and morphologic and paleodemographic patterns of past populations [1]. It is also extremely valuable for victim identification and forensic investigation resolution [2]. Sex assessment is necessary prior to estimating age and stature as most of these methods are sex-specific [3,4].

The accuracy of sex estimation varies depending on the degree of sexual dimorphism expressed by skeletal elements and on their completeness and their state of preservation when exhumed [5,6]. It is generally asserted that *os coxa* is the most reliable anatomical element for assessing sex since its structure responds to parturition requirements [7,8]. Qualitative and quantitative methods based on this bone are nowadays routinely used in anthropology, allowing a classification accuracy of a minimum of 90% [7–9]. However, the efficiency and reliability of sex estimations can be jeopardized in situations in which the *os coxae* are absent or when the remains are incomplete or burnt [5,6,10].

To design a method suitable for fragmentary remains, it appears necessary to develop methods of sex estimation based on single bone elements that are displaying both a high degree of sexual dimorphism and a resistant architecture prone to be less affected by taphonomic or anthropic processes [11,12]. Located inside the petrous part of the temporal bone, one of the sturdiest parts of the human skeleton, the inner ear seems particularly interesting in this respect as it is likely to be well preserved from both inhumation and cremation [13,14]. Additionally, this original anatomical structure presents another advantage since it is alleged to attain its adult size and shape *in utero* between the 17th and 25th fetal weeks [15,16]. Investigating sexual dimorphism of an element that completes maturation before birth and that is relatively unaffected during postnatal growth will help overcome current issues in subadult sex estimation. Currently, methods are either based on skeletal elements expressing a high degree of sexual dimorphism after puberty (*e.g.*, *os coxae*, skull) [17–23], or on the size of deciduous teeth [24]. The accuracy of these methods therefore varies depending on the age range of the individual studied [20,24]. Even though new developments in the study of long bones are promising [25], to date the most reliable alternative to sex a subadult skeleton remains DNA analysis [26,27]. Yet, DNA analysis can be destructive, time-consuming, and expensive to conduct [26–28]. Additionally, sex assessment using this method is more likely to be affected by issues of contamination and degradation [26–28].

The inner ear is an intraosseous structure located into successive cavities of the petrous part of the temporal bone. It is composed of the internal acoustic meatus and both bony and membranous labyrinths. The bony labyrinth is surrounding and protecting the membranous part that contains the organs associated with hearing and balance [15]. It is composed of five distinct elements (Figure 1): the cochlea, a spiral-shaped structure that contains the organs necessary for sound reception and conversion [29,30], the vestibule that hosts the otolith organs responsible for capturing information linked to linear head acceleration [30] and the three semicircular canals (SCC, the anterior, posterior and lateral) that contain semicircular ducts and ampullas that help maintain balance by coordinating both angular head rotation and body movements during locomotion [15,29,30]. This close relationship between bony and membranous labyrinths offers the opportunity to evaluate the morphometry of this bony structure and its implications on auditive and balance functions in various species [31,32]. Previously analyzed through destructive techniques, the architecture and ontogeny of the bony labyrinth are now examined using non-invasive methods [16,29,32] that facilitate access to this hidden anatomical part.

Highlights

- Human right bony labyrinths were examined with traditional morphometric approach.
- Six out of 39 linear and angular measurements were different between subadult and adult labyrinths.
- Significant sexual dimorphism was expressed in vestibular apparatus of both subadults and adults.
- Multivariate discriminant functions reached accuracy up to 84.9% in subadults and 78.4% in adults.
- Bony labyrinth seems promising for sexing fragmented or subadult remains.

Numerous studies have explored the bony labyrinth morphology in the fossil record to gain insight into paleoanthropological salient questions, such as differentiating locomotor patterns between species [33] and seeking to understand the shift to human bipedalism [34,35]. Additionally, it has also been used for phylogenetic purposes to chart human migrations [36].

However, few researchers have investigated the intraspecific variations of this skeletal element, and even fewer have analyzed its sexual dimorphism. Studies assessing the normal range of variation of the bony labyrinth have provided contradictory results about sex differences [29,37–40]. Until recently, the only study that investigated sexual dimorphism of the bony labyrinth from an anthropological perspective was the study conducted by Osipov et al. in 2013 [41]. By analyzing a series of linear measurements, initially developed by Spoor and Zonneveld (1995) [42], based on the right and left bony labyrinths of a sample of adult Cretans of known age and sex (n = 94), Osipov et al. found significant sex differences in the three semicircular canals. The univariate and multivariate discriminant functions established achieved respective rates of classification accuracy of 75% and 82.4%. Uhl et al. recently tested Osipov's multivariate function 1 separately on three different populations (North-American, German and Zulu) and found lower accuracy levels (from 66.6% to 73.3%) [43]. Yet, in both studies some of the linear and angular measurements defined by Spoor and Zonneveld were not considered and, more importantly, subadult sexual dimorphism of the bony labyrinth was not examined. In 2019, a new 3D approach was developed on the bony labyrinth to particularly explore the variations of torsion degree of the cochlear shape between males and females. Based on a non-parametric representation of the 3D cochlear curve from clinical images, researchers obtained highly reliable results with an accuracy rate of 93% in adults and 91% in subadults [44].

The present paper proposes to examine sexual dimorphism of the bony labyrinth using a metric approach based on 2D linear and angular measurements, as defined by Spoor and Zonneveld [42]. The innovative aspect of the study relies on the fact that it applies such method to both subadults and adults for the first time. After assessing the repeatability and reproducibility of the data acquisition

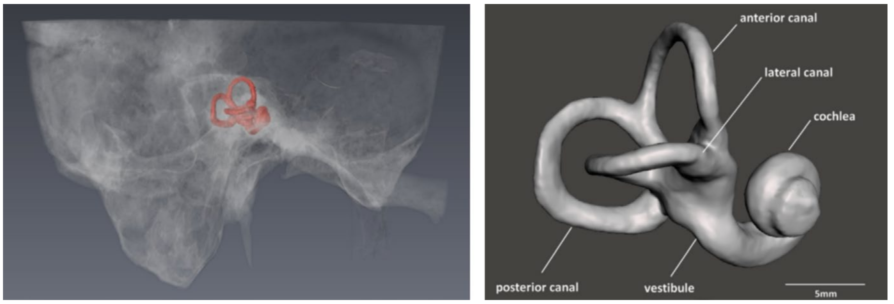

FIGURE 1 Position of the bony labyrinth (in red) inside the petrous part of the temporal bone (lateral view) and identification of its distinct elements on a 3D reconstruction executed with ©Avizo 7.0 from CT scans slices of a male individual (Châtelet 8, 76 years old, Royal Belgian Institute of Natural Sciences, Brussels) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Age and sex distribution in the training sample (Châtelet, Schoten, and Erasme hospital)

Age class	Females	Males	Total
0–1 year	0	1	1
5–9 years	4	3	7
10–14 years	6	5	11
15–19 years	7	7	14
20–29 years	0	1	1
30–39 years	1	3	4
40–49 years	5	8	13
50–59 years	2	4	6
60–69 years	2	4	6
70–79 years	5	8	13
80–89 years	9	3	12
>90 years	3	0	3
adults	2	0	2
Total	46	47	93

protocol, the first objective was to examine differences between subadult and adult morphology to discuss the possibilities of developing an age-independent sex assessment method. Secondly, sexual dimorphism present in both subadult and adult bony labyrinths was quantified through a discriminant analysis to determine whether a more accurate sexing method for fragmented remains and/or subadult remains could be developed.

2 | MATERIAL

The sample for this study includes the CT scans of 93 right bony labyrinths. Sixty-three of them were acquired from dry crania of two Belgian 19–20th century documented collections, curated at the Royal Belgian Institute of Natural Sciences (Châtelet and Schoten collections). Among them, 60 belong to adults aged from 29 years to 94 years (mean age = 63.6 years, SD = 18.6) and three subadults aged from 15 to 19 years old (mean age = 17.6 years, SD = 2.3). The Schoten collection (n = 50) was gathered in 1946 by Pr. Fr. Twiesselmann after the dismantlement of the Schoten cemetery (Antwerp province, Belgium). The individuals were born between 1837 and 1916 and died between 1930 and 1931 [45]. Information regarding the individuals' age and sex is known from death certificates and cemetery records, except for the exact age of two adult females (complete dental development and bone maturation indicated however that adulthood was reached). The second documented collection is composed of 13 individuals exhumed in 2006–2009 from the cemeteries of Châtelet (Hainaut province, Belgium) by the authors (C. P. and Ph. L.). The individuals were born between 1796 and 1877 and died between 1870 and 1963. Information regarding the individuals' age and sex is known from cemetery and population registers. Although the collections date back to the 19th century, the fact that they include skeletonized individuals that were subjected to long-term burial periods, whose effects are comparable to those of taphonomic processes experienced even currently in both clandestine or mass graves, is particularly interesting for forensic purposes [2].

The second part of the corpus is composed of CT scans from pediatric patients with hearing issues acquired at the Erasme hospital in the years 2010. Individuals suffering from pathologies that could impact the morphology of their inner ear, that is, congenital malformation, tympanic perforation, and vestibular dysmorphias, were excluded from the study sample. Thirty individuals, 15 girls and 15 boys, aged between 7 months and 19 years, were selected (mean age = 11.9 years, SD = 4.8). The access to these subadult images was authorized by the Ethical Commission of Erasme hospital (ref: P069/296). The data were anonymized, and only the patients' age and sex were communicated. To reinforce confidentiality, the CT images were specifically restricted to the petrous part of the temporal.

Overall, the sample used for the present study was composed of 93 individuals and included 60 adults and 33 subadults (Table 1). To facilitate the analysis, subadults from the Schoten documented collection (n = 3) were analyzed together with individuals from the pediatric group (n = 33; mean age = 12.5 years, SD = 4.9). Altogether, the subadult group was composed of 17 females and 16 males and the adult group included 29 females and 31 males. The two groups were analyzed separately.

TABLE 2 Age and sex distribution in the test sample (KULeuven)

Age class	Females	Males	Total
0–4 years	4	0	4
5–9 years	2	2	4
10–14 years	0	2	2
15–19 years	1	3	4
20–29 years	2	1	3
30–39 years	1	1	2
40–49 years	1	2	3
50–59 years	1	1	2
60–69 years	2	1	3
70–79 years	0	1	1
Total	14	14	28

Spoor demonstrated in 1993 that there are less intraindividual differences than interindividual differences in labyrinth measurements [29]. Therefore, in the context of this study, only the right bony labyrinth has been selected for analysis, assuming that it will reflect the same amount of sexual dimorphism than the left one.

To test our discriminant functions, we used a validation sample composed of 28 CT scan images from forensic cases autopsied at the Forensic Medicine Department of the Katholieke Universiteit Leuven (KULeuven). This sample was composed of 14 subadults aged from 3 to 17 years old (mean age = 9.3 years, SD = 5.4) and 14 adults aged from 23 to 79 years old (mean age = 47.3 years, SD = 16.5; Table 2). The access has been authorized by the Ethical Commission of Leuven Hospital (ref: NH019 2020-02-01). To ensure anonymity, these whole-body CT scans were restricted to the petrous part of the temporal bone and only the individuals' age and sex were communicated.

3 | METHODS

3.1 | CT scan procedure

Sixty-three skulls from the Schoten and Châtelet collections were CT-scanned in 2017 within the Radiology Department of Erasme Hospital using a Siemens Force (384 RAM, spatial resolution of 0.24 mm). The CT scans were acquired using a tube current of 250 mAs, a tube voltage of 12 kV, collimation of 64 RAM × 0.6 mm, a rotation time of 1 s, and a pitch of 0.5. Scans were made with a slice thickness of 0.4 mm every 0.1 mm. Voxels size is therefore 0.24 × 0.24 × 0.1 mm^3. Those parameters are in line with a specific windowing method routinely used for inner ear zoom reconstructions on living patients. The thirty remaining scans were collected from the Radiology Department of the Erasme Hospital. Scans were performed with the same machine and using the same parameters. Adjustments were done when analyzing these children patients, that is, intensity varying from 126 to 270 mAs and voltage from 100 to 130 kV. CT scans were done in an orbitomeatal plane. For the dry crania, the systematic position was maintained using polystyrene pieces. Regarding the test sample, CT scans were performed at the Forensic Medicine Department of KULeuven prior to autopsies with a Siemens Definition Flash and based on the following settings: a voltage of 120 kV, an intensity of 400 mAs, a collimation of 128 × 0. 6 mm, a rotation time of 0.5 s, a slice thickness of 1 mm, and a slice increment of 0.9 mm.

3.2 | Segmentation protocol

3D reconstructions of the right bony labyrinth were carried out by the main author through manual segmentation using the software ©Avizo 7.0. To extract bone structures and separate them from the air, and soft tissues in patients, a threshold of 550–650 Hounsfield units was selected.

As recommended by Spoor and Zonneveld to capture the entire morphology of the bony labyrinth, those 3D reconstructions were then used to reformat the images in the transverse and sagittal planes, to facilitate measurements. The transverse plane is parallel to the planar orientation of the lateral semicircular canal and perpendicular to the one of the anterior and posterior canals. The sagittal plane is perpendicular to the transverse plane [42].

3.3 | Measurements

In this study, metric variables were taken on the 2D slices using ©ImageJ v.1.8. Forty-three measurements (28 linear and 15 angular) were conducted to get a complete picture of each element composing the bony labyrinth: the cochlea, the three semicircular canals (SCC), and the vestibule [29,34,40]. Most of those measurements had previously been defined by Spoor [31,42], and some of the linear measurements had already been examined for sexual dimorphism in the study of Osipov and collaborators [41].

The measurements were defined by landmarks that were positioned either in the center of a lumen (*e.g.*, for the semicircular canals and cochlea), or on the boundary of a structure, especially at the edge of the vestibule (Figure 2). Metric variables are listed in Table S1, and we recommend looking at Spoor and Zonneveld's paper for a precise definition [42].

Linear measurements were composed of variables of height, width, and radius of curvature. The relative radius of curvature for each canal was also calculated in relation to the sum of the radius of curvature for all three canals (%R) [31]. Two new measurements were added to record the size of the vestibule: the vestibule length and width [40]. Additionally, ten ratios were calculated to characterize the shape of each element (height/width * 100) and to record the percentage of the posterior canal that lies below the plane of the lateral canal (SLI = SLIi / (SLIs +SLIi) * 100) and the relative size and orientation of the arcs of the lateral and posterior semicircular canals in the transverse plane (TLI = TLIp / (TLIa +TLIp) * 100) (Table S1). Finally, torsion angles of each canal and angles between labyrinth elements were calculated through the positioning of specific vectors on the 2D

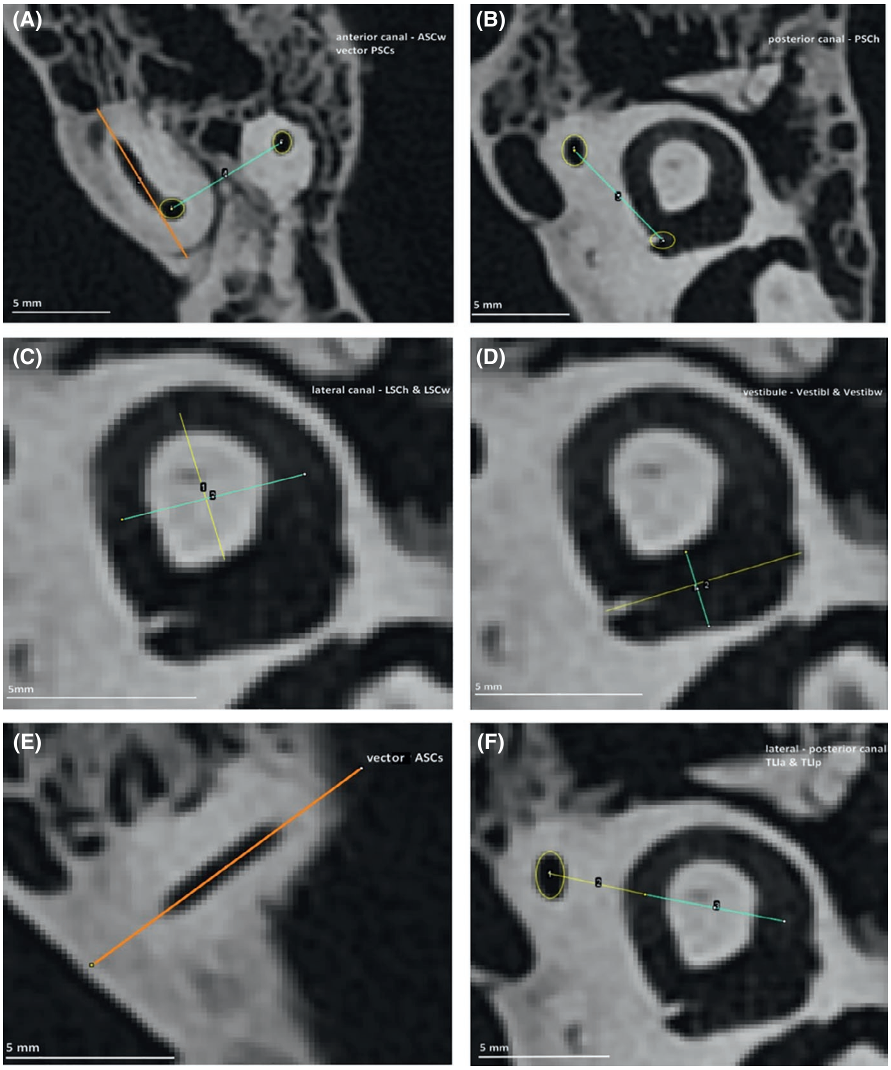

FIGURE 2 Example of some variables collected with ©ImageJ (transverse plane), A: ASCw - anterior canal width, PSCs - vector taken on the superior limb of the posterior canal for angle ASCs<PSCs; B: PSCh - posterior canal height; C: LSCh and LSCw - lateral canal height and width; D: Vestibl and Vestibw - vestibule length and width; E: ASCs - vector taken on superior limb of the anterior canal for angle ASCs<PSCs; F: TLIa and TLIp (see definitions in Table S1; Châtelet 8, 76 years old, Royal Belgian Institute of Natural Sciences, Brussels), vectors are in orange and measurements in blue or yellow [Color figure can be viewed at wileyonlinelibrary.com]

slices (Table S1, Figure 2). Those measurements' use is novel as they were not examined in the previous study of Osipov [41].

Measurements were taken in millimeters and rounded up to the nearest hundredth whereas angles were rounded up to the tenth degree.

3.4 | Statistical analyses

Measurements were taken by the main author a second time within a two-month interval on a sub-sample of 40 individuals, composed of both subadult and adult individuals. A second observer redefined, from the 3D reconstructions, the two planes of observation and recorded measurements on a sub-sample of 30 individuals. To evaluate intra- and interobserver errors of each metric variable, we calculated an intraclass correlation coefficient (ICC). We considered that a measurement was repeatable and reproducible when ICC was above 0.70 [46].

Before conducting any statistical analysis, normality and homoscedasticity of the distribution were checked through the computation of normality probability plots, Shapiro's tests and Bartlett's tests. As some variables did not respect the normality and homoscedasticity conditions (21%), we used non-parametric tests for further means comparisons.

Mann–Whitney U-tests were thus performed between subadult and adult sets to determine the existence of differences in their bony labyrinth morphology. As differences between subadult and adult groups were found, it was decided to investigate sexual dimorphism separately between the two groups. To compare female and male bony labyrinth measurements, within subadult and adult sets, Mann–Whitney *U*-tests were used once again.

Linear direct discriminant function analyses were performed separately within the subadult and adult groups based on the set of significantly dimorphic variables. The data were mean centered. We used a threshold of 0.5 to separate and classify males and females; consequently, accuracy represents the ratio between the number of individuals that were correctly estimated and the total number of individuals. The sectioning point for all discriminant functions was calibrated to zero. Posterior probabilities were calculated for the most accurate univariate and multivariate functions. A leave-one-out cross-validation was carried out on each optimal combination of variables. Additionally, a Wilks' lambda was obtained to evaluate the strength of the discrimination. The Lambda value usually varies between 0 (perfect discrimination) and 1 (no discrimination). The functions were then tested on an independent sample (KULeuven) to validate their reliability. All of the statistical analyses were performed in R©, v3.4, with a *P*-value significance threshold of 0.05.

4 | RESULTS

4.1 | Intra- and interobserver errors

Intraclass correlation coefficients calculated for each variable are presented in Table S2. It appears that one measurement is not repeatable when registered by the same observer, that is the anterior canal index (height/width). Three other ratios are not reproducible when collected by an independent trained observer, the posterior canal index and anterior and posterior relative radius of curvature. Those four measurements were deleted from the protocol and were not included in further statistical analyses.

4.2 | Differences between adult and subadult bony labyrinth morphology

Six metric variables showed a significant *P*-value below 0.05: half of them are linear measurements, and half are angular ones (Table S3). The dimension of the posterior canal located below the lateral one (abbreviated SLIs), the sagittal labyrinthine index and the vestibular index were significantly different between subadults and adults. Moreover, the three angles associated with the lateral canal were significant: angle between lateral canal and the common crus (LSC<CCR), and the ampullar line (LSCm<APA), and the vestibulo-cochlear line (LSCm<VC). For SLIs measurement, subadults displayed a lower value than adults. For all other significant variables, subadults showed a higher value than adults.

4.3 | Differences between female and male bony labyrinth morphology

4.3.1 | Within the subadult sample

Results of Mann–Whitney U-tests carried out on the subadult sample showed that nine metric variables were significantly different between males and females (Table S4). One measurement is characterizing the vestibule (length), one the anterior semicircular canal (width), two concern the posterior canal (height and radius of curvature), and three were dealing with the lateral canal (height, width, and radius of curvature). Same findings were highlighted for the distance between the center of the ampulla and the center of the posterior limb of the lateral canal (TLIa). In addition, one angular measurement showed significant differences between males and females, the angle between the bisector of the opening angle between anterior and posterior canal and the symmetry axis of lateral canal (VSC<LSCt). For all these measurements, females exhibited significantly lower values.

4.3.2 | Within the adult sample

Five measurements displayed a significant *P*-value below 0.05 (Table S5). Two of them were linear measurements characterizing the height of the posterior semicircular canal (PSCh) and distance between the center of the posterior limb of the lateral canal and the center of the posterior canal (TLIp). Two other variables were indices that correspond to vestibule and lateral canal index (VestibI/w, LSCh/w). The last angular measurement to be significantly different by sex was the angle between superior limb of anterior canal and the superior limb of posterior canal (ASCs<PSCs). For each of these metric variables, females displayed significantly lower measurements or angles than males.

4.4 | Discriminant function analysis

All variables, except vestibule length within the subadult set, followed a normal distribution and were homoscedastic. To avoid collinearity in discriminant analysis, both radii of curvature of lateral and posterior canals that were found dimorphic in the subadult sample were removed (LSC.R and PSC.R).

4.4.1 | Within the subadult sample

Demarking points and rates of accuracy of the three best univariate functions are given in Table 3. The variables giving the highest cross-validated accuracy were anterior canal width and TLIa (distance between the center of the ampulla and the center of the posterior limb of the lateral canal) with 72.7% of accuracy for both. Yet, sex ratio was relatively unbalanced for both variables. Looking at results in

TABLE 3 Demarking points and accuracy rates of the three best univariate discriminant functions obtained with leave-one-out cross-validation (n = 33) and in test sample (n = 14) within the subadult group

Variable	Demarking points	Cross-validated %accuracies			Test sample %accuracies		
		Males	Females	Total	Males	Females	Total
TLIa	F < 4.99 < M	81.3	64.7	72.7	42.9	16.7	28.6
Vestibl	F < 6.42 < M	50.0	88.2	69.7	57.1	71.4	64.3
ASCw	F < 6.83 < M	62.5	82.5	72.7	71.4	71.4	71.4

TABLE 4 Accuracy rates of multivariate discriminant functions obtained with leave-one-out cross-validation (*n* = 33) and in the test sample (*n* = 14), within the subadult group

Variables	Function 1	Function 2	Function 3	Function 4	Function 5	Function 6	Function 7
Standardized function coefficient							
TLIa	4.829	2.094	3.566	3.572	3.507	3.829	
Vestibl			1.461	1.461	1.514	1.466	2.557
ASCw	1.106	0.807	1.242	1.243	1.546	1.275	1.976
PSCh			0.222	0.224		0.237	
LSCh			0.012		0.082		
LSCw	−4.460		−3.883	−3.880	−3.917	−3.770	
VSC<LSCt	0.058		0.056	0.055	0.057		
Constant	−16.432	−15.972	−24.548	−24.550	−27.474	−20.346	−29.914
Cross-validated %accuracies							
Males	87.5	87.5	75	75	75	68.8	68.8
Females	82.4	76.4	82.4	82.4	82.4	82.4	82.4
Total	84.9	81.8	78.8	78.8	78.8	75.8	75.8
Wilks' lambda	*0.59*	*0.70*	*0.55*	*0.55*	*0.55*	*0.56*	*0.65*
Test sample %accuracies							
Males	71.4	71.4	85.7	85.7	85.7	71.4	71.4
Females	28.6	42.9	57.1	57.1	57.1	57.1	57.1
Total	50	57.1	71.4	71.4	71.4	64.3	64.3

TABLE 5 Demarking points and accuracy rates of the three best univariate discriminant functions obtained with leave-one-out cross-validation (*n* = 60) and in test sample (*n* = 14) within the adult group

Variable	Demarking points	Cross-validated %accuracies			Test sample %accuracies		
		Males	Females	Total	Males	Females	Total
VestibL/w	F < 228.56 < M	65.6	60.7	63.3	85.7	14.3	50
LSCh/w	F < 91.12 < M	65.6	60.7	63.3	71.4	42.9	57.1
ASCs<PSCs	F < 78.14 < M	71.9	64.3	68.4	85.7	28.6	57.1

the test sample, the highest accuracy was achieved by anterior canal width with 71.4% (Table 3).

The seven best multivariate discriminant functions are presented in Table 4. The highest cross-validated accuracy was given by the combination of four variables, 84.9% (F1). However, rates of accuracy in the test sample were relatively low. Equations that gave balanced sex ratio and good accuracies by cross-validation and in the test sample were F3, F4, and F5, composed of seven and six variables. They reached 78.8% of accuracy (Table 4). The lowest Wilks' lambda (*i.e.*, best discrimination) was also obtained from these three equations. In the test sample, they perform almost equally if we looked at results within each four distinct age group, that is up to 75%. The only exception is in the 10-14 age group in which all the individuals are correctly identified but that means only 2 boys.

4.4.2 | Within the adult sample

The best univariate functions found in the adult group are presented in Table 5. The angle ASCs<PSCs had the highest cross-validated accuracy rate with 68.4%. Accuracy in the test sample was however unsatisfactory.

Results of multivariate equations in adult sample are given in Table 6. Combination of all five variables resulted in 70% of cross-validated accuracy and the lowest Wilks' lambda (F6). The best multivariate equation was F1 with 78.4% of accuracy in cross-validation and a sex ratio in favor of males. F2 and F3, composed of three and two variables, resulted in a more balanced sex ratio. For each function, accuracy in the test sample was not satisfactory (*i.e.*, just above 50%).

5 | DISCUSSION

5.1 | Data acquisition

In this study, we used the protocol defined by Spoor and Zonneveld to characterize morphometry of the bony labyrinth [29,42]. Back then, first generations of CT scan machines enabled the visualization of the structure and the measurements of elements with

TABLE 6 Accuracy rates of multivariate discriminant functions obtained with leave-one-out cross-validation (n = 60) and in test sample (n = 14) within the adult group

Variables	Function 1	Function 2	Function 3	Function 4	Function 5	Function 6
Standardized function coefficient						
TLIp	0.988	1.367			1.226	1.034
VestibL/w	0.029	0.028	0.029	0.027	0.024	0.025
PSCh	0.444			0.667		0.245
LSCh/w				0.048	0.058	0.051
ASCs<PSCs	0.103	0.120	0.161	0.099	0.103	0.096
Constant	−21.045	−20.409	−19.187	−22.647	−22.939	−23.014
Cross-validated %accuracies						
Males	81.3	75	71.9	71.9	68.7	68.7
Females	75	75	71.4	67.8	71.4	71.4
Total	78.4	75	71.7	70	70	70
Wilks' lambda	*0.69*	*0.71*	*0.78*	*0.70*	*0.68*	*0.67*
Test sample %accuracies						
Males	85.7	100	85.7	100	85.7	100
Females	14.3	14.3	14.3	14.3	14.3	14.3
Total	50	57.1	50	57.1	50	57.1

maximal errors of 0.1 mm for linear measurement and 1° for angles [29]. In the present study, images of the petrous part were acquired with high-resolution CT scans (for most individual voxel size was: 0.24 × 0.24 × 0.1 mm^3) that allows to obtain framed high-quality images of bony labyrinths. As seen in Figure 2, no alterations of the structure were noticed on Châtelet and Schoten individuals, despite a prolonged period of burial in their postmortem intervals.

Prior to developing any identification method, it is fundamental to check the repeatability and reproducibility of the data acquisition procedure [47]. Yet, this has not always been done in previous studies researching bony labyrinth [37–40]. In the present study, significant error measurements concern four variables, exclusively ratios, that were subsequently removed from the protocol.

5.2 | Subadult versus adult bony labyrinth morphology

Previous research projects examining the ontogeny of the bony labyrinth morphology state that the size of the bony labyrinth is not impacted by postnatal changes after the ossification of the otic capsule happening around the 24th and 31st fetal week [15,16]. On the other hand, some studies demonstrate modifications in the shape of the bony labyrinth and suggest that morphological changes can still happen after ossification [15,29].

In the present study, significant differences between subadult and adult bony labyrinths were noticed. Most of the variables concerned shape and orientation of the labyrinth, two ratios (SLI, Vestibl/w) and three angles linked to lateral canal (LSCm<CCR, LSCm<APA, LSCm<VC). These results are consistent with previous observations [15,29,48]. According to some researchers, these differences can be interpreted as a consequence of the growth of the external acoustic meatus and mastoid [48]. Petrous bone is known to reach half of its adult size within the first two years of an individual's life. Differences observed in the present study between subadult and adult labyrinths could be explained by the continuous growth of the petrous bone between childhood and puberty that induce some modifications in the shape and orientation of the bony labyrinth [12]. It is however difficult to fully understand the reasons for these differences, especially considering the chronological gap between the subadult sample (pediatric images) and the adult one (19-20th century dry skulls) . However, changes in orientations could have possibly occurred in the bony labyrinth during postnatal life, with specific remodeling around the lateral canal. Hence, if a sexing method were to be developed, it could not strictly be age-independent [41].

5.3 | Sexual dimorphism of the bony labyrinth

Nine linear measurements were found to be significantly dimorphic between girls and boys in the subadult sample. These variables concerned the vestibular apparatus including the vestibule, the three SCC and one angle between anterior, posterior, and lateral canals (VSC<LSCt). In the adult set, five measurements of the vestibule and SCC, among them one angle between anterior and posterior canal (ASCs<PSCs), were significantly different by sex which confirms previous observations [39–41].

Only the height of posterior canal and measurements linked to the vestibule (length of the vestibule for subadults, vestibular index for adults) were dimorphic in both subadult and adult groups. To some extent, sexual dimorphism appears to persist in adult bony

TABLE 7 Results of previous and current researches that investigate sexual dimorphism of bony labyrinth morphology with distinct methodologies (DFA: discriminant functions analysis, CV: cross-validation, SCC: semicircular canals)

Reference	Population	Age of individuals	Training sample	Test sample	Elements investigated	Methodology	Dimorphic measurements	Results
[41]	Cretan identified individuals (19th cemetery collection)	Adults (from 19 to 97 years)	$n = 94$	*NA*	Cochlea SCC	Linear measurements and indexes taken on 3D reconstructions of right and left labyrinths and averaged	$n = 9$ (SCC)	DFA with CV: from 77.5% to 82.4% overall accuracy
[44]	French and South African identified individuals (CT scans of current patients)	Subadults (from 2 months to 10 years) Adults (>18 years)	Adults $n = 40$	Subadults $n = 22$ Adults $n = 54$	Cochlear 3D curve	Non parametric representation of 3D cochlear shape of left labyrinths	Differences in torsion	Regression model and Fréchet means: overall subadult accuracy: 91% overall adult accuracy: 93%
Present paper	Belgian identified individuals (19–20th cemetery collections and CT scans of current patients + autopsied individuals)	Subadults (from 7 months to 19 years) Adults (from 29 to 94 years)	Subadults $n = 33$ Adults $n = 60$	Subadults $n = 14$ Adults $n = 14$	Cochlea SCC Vestibule	Linear, angular measurements and indexes taken on 2D slices of right labyrinths	Subadults: $n = 9$ (SCC and vestibule) Adults: $n = 5$ (SCC and vestibule)	DFA with CV: Subadults: from 75.8% to 84.9% overall accuracy Test sample (subadults): 50-71.4% Adults: from 70% to 78.4% overall accuracy Test sample (adults): 50-57.1%

labyrinth in both posterior and lateral canals and on the vestibule but is less apparent on the anterior one. In both sets, females displayed systematically lower values than males. No linear or angular measurements were found to be sex-specific for the cochlea, which validates some studies [37,41] and contradicts others [38,40,44].

Regarding discriminant functions, the results concerning adult individuals are similar to the ones found by Osipov et al. [41], when looking at the overall accuracy of both univariate and multivariate equations after cross-validation (respectively here in univariate 63.3%–68.4% vs. 73.9%–75% in Osipov's study and here in multivariate 70%–78.4% vs. 75%–82.4%). Slight discrepancies may be due to variations in the degree of sexual dimorphism found in those two European populations (continental vs. insular) [41,43]. Yet, comparisons are somewhat restricted because of differences in measurement sets and acquisition protocols (Table 7).

On the other hand, discriminant functions established here for the subadult sample are unprecedented. Highly significant variables were underlined and led to a higher classification rate after cross-validation, 84.9%. This finding is surprising as studies frequently found a lower level of sexual dimorphism in subadults compared to adults, even for anatomical elements subjected to an early development [49]. Those differences could possibly be linked to the chronological gap existing between both training samples (19-20th century collections vs. 21st current patients) and more probably to the distinct nature of the CT scan images (dry crania vs. *in vivo* images). Both of these aspects could explain why a higher degree of sexual dimorphism was observed in subadults.

Discriminant functions were then tested on an independent sample (KULeuven, $n = 28$). Accuracy rate dropped to *ca*. 50% for adults, which is too low to effectively estimate sex [47]. However, results in the test sample were quite promising for the subadult group (>70% of correct classification). Again, the equations performed better in subadults than in adults. Inconsistencies between accuracies obtained in the training and test samples for adults could be linked to chronological differences existing between both samples (19-20th century vs. current population) or more likely to distinct data collection processes (dry crania vs. fully fleshed bodies). The posterior probabilities calculated per discriminant function for each individual of the KULeuven test sample are available in Tables S6 and S7. Further validation of these functions on larger samples and other populations will be needed.

5.4 | Understanding sexual dimorphism of bony labyrinth in size and shape

Our results suggest that sexual dimorphism is expressed in the bony labyrinth and that it can be captured by some linear and angular measurements, exclusively on the vestibular apparatus (vestibule and SCC). Yet, this traditional osteometric method makes it difficult to isolate size and to differentiate shape variables which depend directly on size (allometry) from those that are function of other factors such as sex. The sexual dimorphism highlighted in the present study possibly reflects the differences of size and body mass that exist between males and females [29,34,44], which vary among human populations [41,43], more than pure shape differences by sex. To further explore modalities of sexual dimorphism of the bony labyrinth using a geometric morphometric approach could be valuable. It would indeed allow for a more detailed examination by quantifying shape changes and by isolating size. A specific protocol for bony labyrinth has been designed but has not yet been used to examine the sexual dimorphism of this structure in humans [50]. More recently, a study proposed to outdo common limitations of geometric morphometrics by introducing a new approach which favors analyzing pure shape of the cochlea through a non-parametric representation of its curve. The authors obtained high sex classification rates, with 93% of accuracy for adults and 91% for subadults. Yet, this method needs to be further validated on other populations before being applicable to forensic cases [44].

5.5 | Implications for sex estimation methods

The bony labyrinth presents a significant advantage compared to other skeletal elements. It is likely to be found complete and unaltered in various forensic contexts thanks to its hidden location inside the petrous part of the temporal bone [11–14]. As demonstrated in our training sample, it remains accessible and intact in skeletons that experience longtime burials (Figure 2). Hence, developing a sex estimation method based on the bony labyrinth would be extremely valuable when analyzing fragmented human remains for which sex cannot be assessed using the *os coxae* or skulls or long bones. The present study solely analyzed the right bony labyrinth and assumed that sexual dimorphism occurred in a similar way on the left bony labyrinth. Previous studies showed indeed that measurements on bony labyrinth of both sides were comparable [29,51]. This represents a great advantage for the analysis of fragmented remains as it would make it possible to estimate the sex of an individual through the study of only one petrous bone side.

Another advantage of using the bony labyrinth for sex estimation relies on the fact that this body part is usually considered to attain its full size and shape while *in utero* [15,16] which facilitates the use and development of age-independent sex methods [41]. Yet, our results showed that some bony labyrinth measurements were different between subadults and adults. Consequently, predictive models were developed separately for the two groups. When the maturation degree of the individual is unknown, as it is often the case with fragmented remains, these distinctive models can represent a limitation of applicability, even though other bones can be used to complement the age assessment. In cases where it is clearly impossible to assess the individual maturation, discriminant functions based on a pooled sample of subadults and adults are likely to be necessary (Tables S8–S10).

In this study, we demonstrated that bony labyrinth sexual dimorphism was expressed early in postnatal life. In the adult sample, the discriminant functions after cross-validation allowed a classification rate above 75%, which can be considered as moderate. However, in the validation sample, accuracies dropped to low levels. Results for subadults are more promising as classification rates reached over 80% of accuracy in the training sample and above 70% in the test sample. These accuracies are close to the ones obtained when examining complete pelvis or skull in subadult remains [20]. These results should therefore not be discarded as they appear acceptable for sexing damaged remains. A synthesis of the studies that have examined bony labyrinth sexual dimorphism to date with distinct methodologies is available in Table 7.

When applying any discriminant function to an individual of unknown sex, posterior probabilities must always be clearly appraised by the forensic scientist and discussed in the decision-making process. In discriminant analysis, a threshold of 0.5 is commonly used to separate sex so that every individual received a classification as either female or male. However, an assessment based on an individual posterior probability between 0.5 and 0.7 should not be considered reliable for sex diagnosis because of the strong possibility of overlapping results between sexes [47]. Sex assessment based on bony labyrinth morphology, examined either on fragmented or subadults remains, must take this point into consideration.

It has previously been stated that osteometric methods based on the petrous part of the temporal bone could be highly valuable for the initial assessment of sex in cremated remains [11–13]. It could also be the case of sex estimation based on the bony labyrinth as the dense and resistant structure of the petrous part offers it adequate protection. This also represents an alternative solution to DNA analyses that currently have relatively low success rates in cremated cases [53]. Nevertheless, evaluating the impact of shrinkage on the bony labyrinth structure in diverse heating processes, the way it has been done for other skeletal elements [54], will be needed before implementing this method on cremated bones.

Finally, it is crucial to remember that sex estimation based on bony labyrinth morphology will undoubtedly require access to a CT scan apparatus (micro CT scan is not necessary), which is not always easy to obtain in forensic situations such as for domestic cases or international humanitarian investigations. Additionally, analyzing bony labyrinth slices require a peculiar knowledge of its structure and a specialized software to read and correctly segment medical images. With an experienced observer, the sex estimation of an individual from acquisition to measurements can be done in less than three hours.

6 | CONCLUSION

This study further explored the question of bony labyrinth sexual dimorphism by evaluating metric variations of that structure by sex, on the right side, in both subadult and adult individuals. It is the first of its kind to demonstrate that sexual dimorphism exists in subadult bony labyrinths, based on a large sample. The protocol of acquisition of linear and angular measurements on the 2D slices revealed a high rate of repeatability and reproducibility, with minimal errors of measurements. Significant differences were found between subadult and adult bony labyrinth morphology. Despite of the size and nature of the sample (dry crania images from 19 to 20th century collections and *in vivo* images of current patients), it could not be excluded that shape changes occur in bony labyrinth in postnatal life. Consequently, a sexing method based on bony labyrinth morphology could not be strictly age-independent. While examining groups separately, significant sexual dimorphism was detected in vestibule and semicircular canal size and shape in both sets, with dimorphic measurements more frequently found in the subadult set. After cross-validation, univariate equations achieved 72.7% of accuracy in subadult group and 68.4% in adult group while multivariate functions yielded respectively 84.9% and 78.4% of accuracy. In the validation sample, results for subadult equations were promising (>70%) but unsatisfactory for adults (*ca.* 50%). Further validations of these equations will be needed on larger and distinct independent samples. Bony labyrinth appears to be an encouraging skeletal element for developing sexual diagnosis methods adapted to fragmented or subadult remains. Heat-induced changes of the structure however need to be explored before applying this method to cremated bones. Employing 3D approaches such as geometric morphometrics and curve shape analysis to investigate bony labyrinth could entail a full comprehensive understanding of its sexual dimorphism.

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SUPPORTING INFORMATION

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