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Can bony labyrinth dimensions predict biological sex in archaeological samples?

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ABSTRACT

Osipov and colleagues [American Journal of Physical Anthropology, 2(151) 2013] previously posited that the dimensions of the bony labyrinth exhibit sexual dimorphism. Using a recent sample of known sex, they produced an age-independent, multivariate equation to predict biological sex using several of these dimensions. We aim to test the applicability of this equation to archaeological samples, where the method could prove useful with poorly preserved remains. Using µCT data, we collected a standard set of linear dimensions from the bony labyrinth in two groups from the ancient Nile Valley that show body size differences associated with diet and disease. We determined the biological sex of these individuals using established skeletal features and with Osipov and colleagues' (2013) equation. Results of each were compared with McNemar tests, and raw measurements were examined within each group for dimorphism with a Discriminant Function Analysis. Biological sex predicted by labyrinthine dimensions overidentified individuals from both groups as female. Adjustments to the equation constant balanced predicted sex numbers and increased consistency both on an individual level (31.3% to 67.4%) and model level (χ^2 < critical value). Discriminant function analysis consistently classified 60% of el-Badari and 50% of Kerma individuals. Absolute bony labyrinth dimensions and the results of Osipov and colleagues' (2013) equation did not produce sex estimations consistent with those made from assessment of pelvic and cranial features. As these assessments also have error, further exploration of sexual dimorphism in the labyrinth should be undertaken in collections with known sex.

1. Introduction

In the past several decades, X-ray computed tomography (CT) has allowed non-destructive analyses of the bony labyrinth (Zonneveld et al., 1989). The bony labyrinth is a space within the petrous portion of the temporal, filled with the membranous labyrinth and fluid (Gray, 1918). The labyrinth consists of three regions: the cochlea, vestibule, and three semicircular canals, which relate to neurons positioned to detect sensory input respectively related to sound, translation, and orientation (Milton, 1808). With the ability to visualize the labyrinth's morphology in detail, biological anthropologists are able to explore the potential of the bony labyrinth as a tool to understand variation in skeletal morphology related to modern human variation and human evolution (among others, see: Spoor and Zonneveld, 1995; Trinkaus, 2018).

The bony labyrinth is unique for several reasons. The structure completes development early in gestation, between 17 and 19 weeks, and so is thought to be protected from some elements of developmental plasticity (Jeffery and Spoor, 2004; Richard et al., 2010). The bone around the labyrinthine margin has been found to remodel at 3% of the rate of the rest of the skeleton on average, and therefore may be resistant to shape change during life (Frisch et al., 2000; Sørensen, 1994; Sørensen et al., 1992). The highly mineralized nature of the petrous portion means that it preserves well in archaeological and paleoan-thropological contexts, even in less-than-ideal taphonomic circumstances (Ponce De León and Zollikofer, 1999). Finally, the morphology

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of the bony labyrinth is hypothesized to be closely related to the morphology of the organs within, and hence closely associated with their sensory function (Jáuregui et al., 2016).

Many morphological aspects of the bony labyrinth have been studied, particularly for its association with locomotor adaptations in extinct and extant primates. For example, a relatively larger semicircular canal diameter has been associated with higher sensitivity to head rotation and therefore greater agility (Hullar, 2006; Ryan et al., 2012; Silcox et al., 2009; Spoor et al., 2007; Walker et al., 2008; Yang and Hullar, 2007). Even though the results of these studies have not always been consistent (Cox and Jeffery, 2010; Malinzak et al., 2012), functional signals continue to be investigated, particularly for the purpose of inferring vestibular function in extinct taxa (David et al., 2010). More recent work indicates that intraspecific analyses have the potential to contribute to our understanding of functionally significant morphological variation (Perier et al., 2016).

Indeed, some assessment of labyrinthine shape has already extended to intraspecific analyses in humans and non-human primates. Largely these studies have focused on comparing humans and close relatives, such as Neanderthals. Although some morphological differences have been identified between these groups, significant morphological overlap is present (Bouchneb and Crevecoeur, 2009; Ponce De León and Zollikofer, 1999; Spoor et al., 2003; Spoor et al., 2002; Uhl et al., 2016; Wu et al., 2014). More recent work explores how labyrinthine morphological variation follows expected patterns of neutral population divergence within modern humans (Ponce de León et al., 2018).

At a within-population level, bony labyrinth morphology has also been studied for sexual dimorphism. Older studies, collecting physical measurements from the bony labyrinth, found that males had greater height, width, and length of the anterior and posterior semicircular canals than females (Schönemann, 1906; Turkewitsch, 1930). These results seem to support evidence that other features of the ear, such as cochlear length (Miller, 2007), internal acoustic meatus angle (Akansel et al., 2008; Goncalves et al., 2011; Graw et al., 2005; Lynnerup et al., 2006; Norén et al., 2005), and overall metric analysis of the petrous portion (Wahl and Graw, 2001) exhibit sexual dimorphism. Early analysis of CT data by Sato and colleagues (Sato et al., 1991, 1992) found sexual dimorphism in the cochlea and semicircular canals, but this study was based on a small sample of only 9 individuals. In contrast, Spoor (1993) found no significant differences between male and female labyrinthine shape or size in a larger sample of modern humans of geographically diverse origins assessed through linear measurements on CT data.

Osipov and colleagues published the most detailed analysis of human labyrinthine sexual dimorphism in 2013. In this study, a knownsex collection of individuals born between 1876 and 1956 in Crete was found to have dimensions of the bony labyrinth that correlated significantly with biological sex (Osipov et al., 2013). For some dimensions, the correlation was strong enough to use a DFA to calculate an age-independent equation for determining sex from the bony labyrinth alone, with up to 82.4% accuracy (Osipov et al., 2013). The equation has been applied in one other study (Uhl et al., 2016), where it was used to produce a male determination for biological sex of the Cioclovina Early Modern European calvaria. To date, the equation has not been applied or re-tested in intraspecific analyses of geographically diverse modern *Homo sapiens* groups.

If measures of the bony labyrinth accurately and consistently correlate with sexual dimorphism, their use would eliminate two of the major drawbacks to the macroscopic methods widely used to determine sex from the skeleton: age dependence and preservation. These methods rely on dimorphism in pelvic morphology related to the size and shape of the birth canal, which is fragile and often poorly preserved, (Rogers and Saunders, 1994) and cranial morphology related to overall robusticity (Buikstra and Ubelaker, 1994). Both the pelvis and cranium become sexually dimorphic during puberty (White et al., 2011). Traits such as nuchal crest development, mastoid process volume, supraorbital margin sharpness, glabella prominence, mental eminence size, sciatic notch breadth, and ventral arc width are generally scored from 1 to 5 against illustrated morphological standards and scores are combined to create a composite for each individual (Buikstra and Ubelaker, 1994; Phenice, 1969). Pelvic traits are preferable to cranial traits when making assessments that extend between populations, because the pattern and extent of dimorphism in cranial morphology also varies across populations (White et al., 2011). In addition, younger adult crania are more likely to be scored as female and older crania are more likely to be scored as male due to hormone levels (Meindl et al., 1985). Accuracy of sex estimation from pelvic traits is known to range from 84% to 95%, and so higher than cranial traits, which range in accuracy between 74% and 80% depending on the traits used (Bruzek and Murail, 2006; Buikstra and Ubelaker, 1994; Garvin et al., 2014; Rogers, 2009; Rogers and Saunders, 1994; Stevenson et al., 2009; Steyn and İşcan, 2008; Walker, 2008; White et al., 2011; Williams and Rogers, 2006).

As mentioned previously, the bony labyrinth completes growth well before development of secondary sexual characteristics associated with cranial and pelvic indicators of biological sex, thus supporting the potential age-independence of a method based on this region. To explain the relationship between biological sex and labyrinthine dimensions, *Osipov and colleagues (2013)* suggest that sexual dimorphism in the bony labyrinth may be related to body size dimorphism. Spoor and Zonneveld identified a positive correlation between labyrinthine dimensions and body size in interspecific analyses of primates (Spoor and Zonneveld, 1995), but a similar correlation has not been found within humans (Ward, 2016). Body size does vary in modern humans, but for many reasons in addition to sexual dimorphism (Katzmarzyk and Leonard, 1998; Tanner, 1987; Will et al., 2017), making it an imperfect indicator of biological sex. Therefore, bony labyrinth size and shape might vary for reasons related to sex other than body size.

The bony labyrinth has the potential to be an important tool for indicating biological sex, especially in non-adults (where sex cannot be reliably determined from macroscopic evidence) or in cases of poor preservation. However, the degree and patterning of sexual dimorphism in the bony labyrinth and its variability across populations has not been fully explored. In the current study, we 1) assess the bony labyrinthbased, age independent method for estimating biological sex (Osipov et al., 2013) and 2) explore labyrinthine dimorphism based on linear measurements in two groups. These groups are previously published archaeological collections, which include a range of body sizes as well as individuals assigned male, female, and unknown sex identification. We aim to test the following hypotheses: (1) Estimations of biological sex produced from measurements of the bony labyrinth are consistent with those made by assessing preserved macroscopic features of the pelvis and skull; and (2) Absolute labyrinthine measurements consistently discriminate between individuals identified as male and female using macroscopic methods.

2. Materials and methods

We test our hypotheses using two archaeological groups: the el-Badari and the Kerma. These skeletal collections span the transition to agriculture in the Nile region of north-east Africa (Fig. 1) and are currently curated in the Duckworth Collection at the University of Cambridge, UK.

The el-Badari culture, with associated contracted burials, dates to between 4000 and 5500 BCE (Brunton, 1927; Brunton and Caton-Thompson, 1928; Zakrzewski, 2002, 2007) (Fig. 1). Although the el-Badari were early adopters of domesticated plants and animals, they were semi-nomadic and heavily reliant on hunting, fishing, and gathering for food (Arkell and Ucko, 1965; Brewer and Friedman, 1989; Brewer et al., 1994; Brunton, 1927, 1948; Hassan, 1988; Hendrickx and Vermeersch, 2000; Holmes and Friedman, 1994; Midant-Reynes, 2000; Zakrzewski, 2007). The el-Badari are considered early pastoralists and



Fig. 1. Map of the Nile Valley, showing the locations of the el-Badari and Kerma cultures.

represent a population subsisting at the beginning of the transition to agriculture in the Nile Valley (Hendrickx and Vermeersch, 2000; Midant-Reynes, 2000).

The Kerma culture is more recent than the el-Badari, dating to between 2100 and 1500 BCE (Kemp, 1989; Reisner, 1923; Trigger, 1976; Zakrzewski, 2002, 2007) (Fig. 1). Between the el-Badari and Kerma cultures, increases in sedentism and the creation of proto-city states are seen by 3500 BCE, but widespread irrigation did not facilitate largescale agriculture until about 2000 BCE (Brewer et al., 1994; Kemp, 1989; Starling and Stock, 2007; Stock et al., 2011). Sedentism and reliance on agriculture lead to complex stratified social systems and government (Kemp, 1989; Zakrzewski, 2002, 2003).

The el-Badari and Kerma are from a similar climatic and geographic region, but are chronologically and culturally disparate. These two groups have been studied (among others) in the Nile Valley and represent extremes through the transition from hunting and gathering to agriculture (Starling and Stock, 2007; Stock et al., 2011; Zakrzewski, 2002). Differences are especially evident through examination of body size differences between the groups. The el-Badari exhibit smaller estimated body mass and stature (Table 1) than the Kerma, but the Kerma

Table 1

Body mass and stature estimated for the el-Badari and Kerma groups, reproduced from Stock and colleagues (2011). Stature estimates from Raxter et al. (2008) and body mass estimations calculated using the mean of three equations from Ruff et al. (1997).

El-Badari Mean Stature (cm)		Mean Body Mass (kg)		
Male	164.9	Male	57.4	
Female	152.5	Female	53.2	
All	157.1	All	54.8	
Kerma				
Mean Stature (cm)		Mean Body Mass (kg)		
Male	170.8	Male	64.7	
Female	157.0	Female	55.0	
All	163.6	All	59.7	

are more sexually dimorphic, particularly in body mass. Relatively smaller body size in the el-Badari is accompanied by a higher frequency of linear enamel hypoplasia (LEH) and cribra orbitalia than in the Kerma (Starling & Stock, 2007; Stock et al., 2011; Zakrzewski, 2003). Stock and colleagues (2011) attribute body size and LEH differences to poor health during the initial transition to agriculture, followed by a recovery. Zakrzewski (2003) interprets the overall positive trend in body size through time as evidence of stronger social hierarchy, and interprets increasingly sexually dimorphic body size as preferential provisioning of males.

Osipov and colleagues' (2013) equation was proposed for application to individuals from any particular group, even in forensic contexts. The current study tests that assumption by examining the utility of the equation in populations that vary in respect to external environmental variables, introduced by different subsistence patterns, infrastructure, and state formation. In doing so, the study will examine if the equation is measuring intrinsic sexual dimorphism, independent of these factors.

For analyses here, biological sex determination was previously made by JTS (Stock et al., 2011), using standard skull and pelvic traits (Buikstra & Ubelaker, 1994; Stevenson et al., 2009; Ubelaker, 1989; Walker, 2008; White et al., 2011). Morphological features of the pelvis were scored using the Phenice (1969) traits of the subpubic region (ventral arc, subpubic concavity, and the ischiopubic ramus ridge), and the shape of the greater sciatic notch (Buikstra & Ubelaker, 1994). When pelvic traits were unobservable or ambiguous, cranio-mandibular traits including the morphology of the mastoid process, supra-orbital margin, glabella, mental trigone, and gonial angle were observed. Crania from both groups were seriated on the basis of dimorphic traits to determine the range of expression within the samples, and scores reflected population specific diversity. In the case of the Kerma sample there were three individuals without associated ossa coxae; two of these had strong expression of traits and were classified as female and male, while one was more ambiguous in expression but with male characteristics and was scored as 'probable male' and included among the male subsample. Sex determinations were independently verified by DLW using the same methods (Table 2).

All bony labyrinth data collection was performed on μ CT data. Each cranium was μ CT scanned with a Nikon XT H 225 ST (Nikon Metrology, Inc., Brighton, MI, USA) in the Cambridge Biotomography Centre by

Table 2

Abbreviations and descriptions of bony labyrinth measurements based on Spoor (1993).

Abbreviation	Definition
PSCh	Height of the Posterior Semicircular Canal
PSCw	Width of the Posterior Semicircular Canal
LSCh	Height of the Lateral Semicircular Canal
LSCw	Width of the Lateral Semicircular Canal



Fig. 2. Left bony labyrinth volume (left: superior view, right: postero-lateral view), segmented from within the cranium, illustrating measurements collected in this study. PSCw: posterior semicircular width, PSCh: posterior semicircular canal height, LSCw: lateral semicircular canal width, LSCh: lateral semicircular canal height. Measurements adapted from Spoor (1993).

DLW. Crania were placed on a rotating platform between the X-ray source and detector, and scanned using optimized energy settings (120Kv and 147microAmps, 1000 ms exposure, and 1080 views with one frame per projection). After scanning, CTPro3D (Nikon Metrology, Inc., Brighton, MI, USA) was used to reconstruct a 3D model of the cranium from the radiographs. The resolution of each scan varied between 125 and 95 μ m depending on the portion of each cranium that was preserved. Reconstructed TIFF stacks from CTPro3D were imported into Avizo 8.1 (Visualization Sciences Group, Burlington, MA, USA) which was used to take all measurements.

The volume of both right and left bony labyrinths were segmented from the cranium for each individual using the half-maximum-height (HMH) principle, which has been shown to be reliable for visually distinguishing bone density material from air density material in µCT data (Gunz et al., 2012; Spoor, 1993). Measurements listed in Table 2 were collected from these visualizations, as shown in Fig. 2, and are comparable to those collected in relevant studies of labyrinthine shape (Osipov et al., 2013; Uhl et al., 2016). These are a subset of standard measurements originally described by Spoor (1993), corresponding to variables required for the equation published by Osipov and colleagues (2013). All labyrinthine measurements were taken in duplicate for three crania of each population, el-Badari and Kerma, to test observer error. Each set of measurements was taken on different days by DLW, with no reference to the previous measurements. Percent error ranged from 2.05% for the height of the posterior semicircular canal to 3.75% for the height of the lateral semicircular canal (Table 3). This is within the range of error considered acceptable in comparable studies (Uhl et al., 2016). All statistical testing and comparisons, including those for observer error, were completed using RStudio 3.2.2 "Fire Safety" (RStudio Team, 2015) and PAST (Hammer et al., 2001).

3. Theory and calculations

The average of the left and right measurements was used, preservation permitting, to determine sex using Osipov et al.'s (2013)

 Table 3

 Error testing on measurements collected from the bony labyrinths.

Variable	Mean (mm)	Standard Deviation	Percent Error
PSCh	5.38	0.08	2.05%
PSCw	4.52	0.10	3.19%
LSCh	3.63	0.10	3.75%
LSCw	3.21	0.07	2.98%

equation. The equation is:

 $(PSChM \times 0.728) + (PSCwM \times 1.407) + (LSCh/wM \times 6.217) - 18.749$

where PSChM is posterior semicircular canal height, PSCw is the width of the posterior semicircular canal, and LSCh/wM is the height of the lateral semicircular canals divided by the width (Osipov et al., 2013). A value greater than 0 indicates male sex, while values less than 0 (i.e., negative values) indicate female sex.

First, we compared the results of the multivariate equation to the original morphological sex assessments by examining proportions of male and female individuals, and the percent of each sex consistently identified in each population and in both populations combined (Table 4). To test whether the proportions differed significantly, McNemar tests (McNemar, 1947) were conducted for the el-Badari and Kerma groups separately (Table 5A). The McNemar test is a test for nominal non-independent (paired) data, in this case applied to assess whether the method employed for the original morphological assessments produced statistically different results from the determinations obtained from the equation. A binomial correction was employed following Edwards (Edwards, 1948) given the small sample sizes.

The results of the multivariate equation were then plotted to explore the distribution of sex determinations. The mean result fell below zero (-1.91), suggesting an over-identification of female individuals, since macroscopic methods indicated an overall sample composition of 39%

Table 4

Sample composition by population and by sex determined from pelvic and cranial traits, determined by bony labyrinth dimensions, using the multivariate equation from Osipov and colleagues (2013), and determined by the same equation with the mean of all results adjusted to zero, by adding 1.91 to every result.

Pelvic and Cranial Sex Estimation					
	Male	Female	Sum		
el-Badari	6	14	25		
Kerma	12	14	26		
Sum	18	28	51		
Bony Labyrinth Sex Estimation					
	Male	Female	Sum		
el-Badari	1	19	20		
Kerma	2	24	26		
Sum	3	43	46		
Adjusted Bony Labyrinth Estimation					
	Male	Female	Sum		
el-Badari	10	10	20		
Kerma	13	13	26		
Sum	23	23	46		

Table 5A

Bony Labyrinth Sex Estimation Frequency Table.

el-Badari	Male (Equation)	Female (Equation)	Kerma	Male (Equation)	Female (Equation)
Male	0	6	Male	1	11
Female	1	13	Female	1	13

Table 5B

Adjusted Bony Labyrinth Sex Estimation Frequency Table.

el-Badari	Male (Equation)	Female (Equation)	Kerma	Male (Equation)	Female (Equation)
Male	5	1	Male	8	4
Female	5	9	Female	6	8

males and 61% females. To attempt to correct for the over-prediction of female individuals, we shifted the constant associated with the multivariate equation by taking the absolute value of the mean, 1.91, and adding it to each result. This adjustment assumes an even sex ratio, and while females are more numerous than males in our sample, a Chi-square test indicates this is not a statistically significant departure from a balanced sex ratio ($\chi^2_{df} = _{20} = 1.1$, p = 0.3). This shifted the mean result to zero. The resulting proportions of male and female individuals were first assessed again, as well as the percentage of each sex consistently identified (Table 4). Then, frequency tables were re-tested against the macroscopic morphological sex estimations via McNemar tests (Table 5B).

Because the performance of the Osipov et al. (2013) multivariate equation was poor in our study sample, we used Discriminant Function Analysis (DFA) to assess the ability of our multivariate bony labyrinth measurements to distinguish between the sexes in the el-Badari and Kerma. To ensure assumptions for DFA were met, we verified multivariate normality and equivalence of covariance matrices for the four bony labyrinth measures collected. We then applied DFA to each group.

4. Results

Sex estimations produced by Osipov and colleagues' (2013) multivariate equation are generally inconsistent with those produced by macroscopic morphological assessment. The distribution of equation results (Fig. 3) is skewed negatively, indicating that more individuals were determined to be female than male. The mean of all multivariate equation results is -1.91.

More precisely, sex estimation from bony labyrinth measurements resulted in 93.5% of individuals being assessed as female and 6.5% of individuals being assessed as male, of the 46 pooled individuals with preserved labyrinthine morphology and assessed biological sex. This result is in marked contrast to the results from the assessment of pelvic and cranial features, for which the sexes were roughly equally represented (Stock et al., 2011). In sum, the equation predicted biological sex with 31.3% consistency when compared to morphological sex estimations. When each group was considered separately, the consistency was 35% for el-Badari individuals and 26.1% for Kerma.

Next, biological sex determinations from the equation and from macroscopic methods were compared. The data for these comparisons are listed in frequency tables, separated according to group (Tables 5A & B). For the el-Badari, the McNemar test using an exact binomial distribution produced a non-rejection χ^2 (1, N = 20) = 3.57, p = 0.13, indicating that the difference between the proportion of individuals determined to be female and those determined to be male do not differ significantly between the two methods. However, the odds ratio (OR = 6, 95% CI [0.73, 275.99]), a measure of effect size, indicates that the equation is 6 times more likely to identify an individual as female than male. For the Kerma, the McNemar test using an exact

binomial distribution produced a rejection of the hypothesis of equal proportions χ^2 (1, N = 26) = 8.33, p = 0.01, indicating that there is a statistically significant difference between the two biological sex estimation methods. This is consistent with the odds ratio (OR = 11, 95% CI [1.60, 473.48]) which indicates that the equation is 11 times more likely to identify an individual as female than male, meaning consistent identification of male individuals is very low. Although the McNemar test did not identify difference in sex estimation between the two methods with the el-Badari and did with the Kerma, this does not indicate consistency on an individual level, as the test is examining proportions of each sex.

Adjusting the Osipov et al. equation to give a mean result of 0 in our sample (Fig. 3) shifted the frequency of each sex to be 50%, but did not alter the shape of the distribution in any way. Each McNemar test was repeated with the adjusted frequencies (Table 5B). Both analyses performed with these adjusted frequencies, one for each archaeological group, produced non-rejections (el-Badari: χ^2 (1, N = 20) = 2.67, p = 0.22; Kerma: $\chi^2 (1, N = 26) = 0.4$, p = 0.75), indicating that the results of the adjusted labyrinth-based equation produced proportions of males and females that are not significantly different from the proportions based on sex assessments made using macroscopic methods, as expected given adjustments made to the equation. By shifting the mean to zero, we adjusted the equation to produce equal proportions of male and female individuals, which does not validate the equation. The odds ratios calculated for these adjusted data now indicate that for both archaeological groups it is more likely that the equation identifies an individual as male than female, although the values are not statistically significant (el-Badari: OR = 0.2, 95% CI [0.004, 1.79]; Kerma: OR = 0.67, 95% CI [0.14, 2.81]).

The adjusted model also identified male and female individuals more consistently with morphological determinations across both groups. Seventy percent of el-Badari individuals and 65% of Kerma individuals were consistently identified, leading to 67.4% consistency across both groups pooled or around half of female individuals inconsistently assigned.

Prior to the DFA, data from each group were confirmed to be multivariate normal (p > 0.05), and a Box's M test produced a nonrejection for the homogeneity of variance-covariance matrices (el-Badari: M = 9.012, p > 0.001; Kerma: 10.126, p > 0.001). Results of the DFA are roughly consistent with those from multivariate estimate consistency assessment. Raw classification accuracy of el-Badari individuals as either male or female was 80%, and the jackknifed accuracy was 60%. Posterior semicircular canal measurements had more impact on classification (loadings height: 0.332, width: 0.393) than lateral semicircular canals (loadings height: 0.075, width: -0.021) (Fig. 4A). Raw classification accuracy of Kerma individuals as either male of female was 65.38%, and the jackknifed classification accuracy was 50%. Again, posterior semicircular canal measurements had more impact on classification (loadings height: 0.197, width: 0.344) than lateral semicircular canals (loadings height: -0.006, width: -0.00) (Fig. 4B). The height and width of the lateral semicircular canal had relatively less impact in sex determination of Kerma individuals than in el-Badari individuals, and the width of the posterior semicircular canal had slightly more impact than posterior semicircular canal height in both groups.

5. Discussion

Sex estimation across the el-Badari and Kerma groups based on measurements of the bony labyrinth over-predicted the frequency of female individuals. As Osipov and colleagues suggest, dimorphism in the labyrinth may be related to body size dimorphism in *Homo sapiens* as opposed to intrinsic labyrinthine morphological dimorphism (Osipov et al., 2013). As the body sizes of Osipov and colleagues' reference population are not published, we cannot compare body mass and stature estimates with the el-Badari and Kerma. As stated previously, body



Fig. 3. Histogram of results from multivariate bony labyrinth measurement equation proposed by Osipov and colleagues (2013) applied to skeletal samples from el-Badari and Kerma. According to Osipov et al., values above zero indicate "male" and values below zero indicate "female". Results of equation application are on the x axis, and the mean result for the combined el-Badari and Kerma samples, -1.91, is indicated with a star. The frequency of individuals with any given result is on the y axis.



Fig. 4A. Results of Discriminant Function Analysis of labyrinthine measurements for the el-Badari. The x axis is the first canonical variate axis, and the y axis is frequency. Loadings on this axis are: lateral semicircular canal height: 0.075, lateral semicircular canal width: -0.021, posterior semicircular canal height: 0.332, and posterior semicircular canal width: 0.393. Purple is female individuals, pink is male, and magenta is the overlap between male and female individuals.



Fig. 4B. Results of Discriminant Function Analysis of labyrinthine measurements for the Kerma. The \times axis is the first canonical variate axis, and the y axis is frequency. Loadings on this axis are: lateral semicircular canal height: -0.006, lateral semicircular canal width: 0.000, posterior semicircular canal height: 0.197, and posterior semicircular canal width: 0.344. Purple is female individuals, pink is male, and magenta is the overlap between male and female individuals.

size variation has many causes which may or may not be related to labyrinth morphology. Based on larger body size and stronger body size dimorphism in the Kerma (Stock et al., 2011), we might expect more consistency in labyrinthine sex estimation in the Kerma, but that result is not found. Between population differences may be a better explanation for the lack of labyrinthine sexual dimorphism identified with Osipov and colleagues' (2013) equation. Recent work has identified neutral population divergence in labyrinthine morphology, which may account for inconsistencies in sex determination (Ponce de León et al., 2018). Body size comparisons alongside study of labyrinthine morphology may have the potential to elucidate intraspecific variation in *Homo sapiens* in the future.

To increase the consistency of macroscopic and equation-based sex determinations, we adjusted the distribution of sex determinations by shifting the constant. Although this adjustment did make the model fit much better with the determinations based on skeletal morphology, this cannot be taken as an indicator that the equation results have been made more accurate. Indeed, the proportion of male and female individuals in each group is much more consistent with the original estimations, but the consistency in sex estimation on an individual level is still poor. Shifting the constant increased the correspondence with the original determinations from 31.3% to 67.4%, whereas accuracy using pelvic traits ranges from 84 to 98% and accuracy using cranial traits ranges from 74 to 80% (Bruzek, 2002; Bruzek and Murail, 2006; Buikstra and Ubelaker, 1994; Garvin et al., 2014; Rogers, 2009; Rogers and Saunders, 1994; Stevenson et al., 2009; Stevn and İşcan, 2008; Walker, 2008; White et al., 2011; Williams and Rogers, 2006). Results of the McNemar test indicate difference in labyrinthine prediction consistency with macroscopic skeletal methods only in the Kerma, a result which is not present when the equation constant is adjusted. In both groups, McNemar testing indicated that the equation is between 6 and 11 times more likely to predict an individual to be female than male. Results of McNemar testing do not suggest that the equation is predicting sex with better consistency, as both males and female individuals are misidentified.

Results of discriminant analysis show that analyses of raw linear labyrinthine measurements alone are not able to show sexual dimorphism in either group. Jackknifed accuracy was 60% in the el-Badari and 50% in the Kerma. This is lower than multivariate equation results and not as high as macroscopic pelvic or cranial based methods. Results of the DFA show that the multivariate equation may not have been consistent with pelvic and cranial sex assessments because of an inherent lack of sexual dimorphism in these labyrinthine measurements. However, as the multivariate equation increased in consistency by shifting the constant, body size still may be a factor affecting inaccuracy in the results of the multivariate equation proposed by Osipov and colleagues (2013). If body size were a factor in labyrinthine dimorphism, it would be more likely to be seen in the Kerma, based on previously identified body size dimorphism and suggested preferential resource provisioning to male individuals (Zakrzewski, 2003). Our analysis shows this is not the case. As neither the corrected nor DFA results were as accurate as macroscopic pelvic and cranial methods reported by other studies, our results indicate that sexual dimorphism cannot be confidently identified in the bony labyrinth, at least with the measurements used here.

It should be noted, however, that we compare the bony labyrinth's ability to indicate biological sex to assessments based on pelvic and cranial morphology, and cannot therefore claim to be testing true accuracy. Osipov and colleagues mitigate this drawback using a partially documented collection. Archaeological collections are less suited to testing such methods, as no direct sex information is available. Accuracy of sex estimation from pelvic and cranial traits is not perfect and therefore introduces inherent error into this study. Ideally, by combining as many pelvic and cranial features as possible for sex estimation, potential error is minimized, but cannot be ruled out. Nothing about the associated archaeological record suggests that the burial behavior in either sample studied here was sex-biased (Brunton, 1927; Brunton and Caton-Thompson, 1928; Kemp, 1989; Reisner, 1923; Trigger, 1976; Zakrzewski, 2002, 2007), or that a preservation differential exists between male and female individuals, which would have

produced a largely female group.

6. Conclusion

We conclude by rejecting our first hypothesis, that both methods of sex estimation tested here would produce the same results, and fail to reject our second hypothesis, that a discriminant function analysis would be able to predict biological sex consistently with macroscopic methods. We suggest that further investigation into labyrinthine dimorphism and intraspecific variation is warranted. The contribution of body size variation as well as other variables known to influence bone shape and size, such as climate and diet, needs to be better understood. This research should be conducted on diverse samples with good preservation, known sex, and genetic data, to support the most accurate test of correlation between labyrinthine size and biological sex. Particularly, with recent research focusing on the labyrinth's potential as an indicator of population history (Ponce de León et al., 2018; Trinkaus, 2018), the causes and types of variability in the modern human bony labyrinth must be explored more in the future.

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Appendix A. Supplementary data

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