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PAPER

ANTHROPOLOGY

Sarah C. Kindschuh,[1] *M.A.; Tosha L. Dupras,*[2] *Ph.D.; and Libby W. Cowgill,*[3] *Ph.D.*

Exploring Ancestral Variation of the Hyoid

ABSTRACT: This study utilizes metric analysis to examine size and shape variation between hyoids of Africans and Europeans in the Robert J. Terry Anatomical Collection. A total of 200 fused and unfused hyoids were measured and three statistical methods were employed to explore variation between ancestries. First, independent sample *t*-tests showed that some significant size differences do occur between ancestries. Second, to examine shape variation, skeletal measurements were regressed on the geometric mean using least squares linear regression with the residuals used to evaluate size-corrected shape differences. Finally, discriminant function analysis was used to develop two functions for ancestry prediction with overall accuracies of 73% and 77%. Results of the analyses suggest hyoid size and shape differences do occur between ancestries, notably that European hyoids are broader than African hyoids, while the African hyoid is longer than Europeans.

KEYWORDS: forensic science, forensic anthropology, hyoid, ancestry, discriminate function analysis, Terry collection

As one of the four components of the biological profile, skeletal variation occurring among individuals of different ancestries has long been studied by physical anthropologists. While these skeletal differences provide no direct evidence for traits generally thought of as racial, such as skin color, they do allow for an estimation of ancestral geographic origin of the individual in question (1). As population migration and admixture continue to occur at very high rates, accurate methodology for the identification of individual ancestral variation has become increasingly important for the physical anthropologist in general, and the forensic anthropologist in particular. This study explores the occurrence of hyoid size and shape variation in individuals identified as either Black (of African ancestry) or White (of European ancestry) in the Robert J. Terry Anatomical Collection. In addition, we also test the ability of the hyoid to be used in ancestry assessment.

Unfortunately, the body of research focusing on ancestral differences in the hyoid is extremely limited. Kim et al. state that based on their research involving sexual dimorphism within a sample of Korean hyoids, “the hyoid…will prove helpful in distinguishing them [Koreans] from other populations” (2, p. 984). This statement was based on a comparison between their measurements and those of a previous study by Miller et al., which focused on hyoid morphology in “Whites and Hispanics” (3, p. 1138). For many of the measurements shared between the two studies, differences were significant, which suggests that size differences between populations could be used to distinguish between the groups, although Kim et al. (2) do not propose a method for doing so.

Although considered by Kim et al. (2), ancestral shape variation in the hyoid, whether examined metrically or morphologically, has not yet been approached by other researchers. In addition, one significant difference between this study and that performed by Kim et al. (2) is the inclusion of unfused hyoids as part of the sample. The majority of hyoid research performed up to this point, including Kim et al.’s research (2), has used hyoids that were removed from the individual during autopsy. While this is a convenient method that ensures all components of the hyoid are kept for study, if the greater cornua are not actually ossified to the body, they remain attached by a small amount of tissue so as to simulate a completely fused hyoid. Therefore, this method does not permit the analysis of ancestral variation of the unfused hyoid. Given the high probability that a hyoid will be found incomplete and/or unfused in forensic and archaeological settings, it is important to have a complete understanding of size and shape differences that occur in both fused and unfused hyoids.

Given previous research, this study attempts to address several gaps in our current knowledge about hyoid morphology and variation. First, our analysis uses independent *t*-tests and regression analysis to achieve a more comprehensive understanding of ancestral size and shape variation in the hyoid. Second, we use discriminate function analysis to generate predictive formulae for ancestry determination that can be used on both fused and unfused hyoids. This research expands our current body of knowledge on ancestral variation in the human skeleton and provides simple, easy-to-use formulae for the prediction of ancestry from the hyoid, which will aid forensic anthropologists in the compilation of an individual’s biological profile.

Materials and Methods

All hyoids utilized in this study are part of the Robert J. Terry Anatomical Collection, housed at the Smithsonian Institute’s National Museum of Natural History (NMNH) in Washington, DC. For further information regarding the history and composition of the Terry Collection, refer to Hunt and Albanese (4). A total of 100 African and 100 European hyoids were chosen as a subset of a larger sample for this analysis based on the completeness of the bone and the ability of each measurement to be taken. Because the

[1]Department of Anthropology, Binghamton University, State University of New York, P.O. Box 6000, Binghamton, NY 13902.
[2]Department of Anthropology, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816.
[3]Department of Anthropology, University of Missouri, 107 Swallow Hall, Columbia, MO 65201.

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larger sample was biased against European individuals, we decided to assemble a smaller sample population with an equal number of hyoids from each ancestry. Within each respective ancestral group, 50 hyoids were fused, with both greater cornua ossified to the body, and 50 were unfused, with neither of the greater cornua fused to the body. Further attempts were made to provide a uniform sample by including as equal numbers of males and females as possible within the subgroups of African and European hyoids. However, the numbers of male and female European hyoids are slightly uneven because of underrepresentation of one or the other. While the number of fused and unfused hyoids, as well as the overall total, remains the same within each ancestral group, the numbers of each sex vary within the European sample. Sample distribution by ancestry and sex are shown in Table 1.

Two sets of measurements were developed and used based on whether the hyoid was fused or unfused. Owing to the lack of research dealing with ancestry determination of the hyoid, measurements were chosen to demonstrate size differences, and because they had been used in previous research dealing with sexual dimorphism (2,3,5–8). These measurements, outlined in Table 2 and demonstrated in Figs 1 and 2, were taken to the nearest hundredth of a millimeter using Mitutoyo Absolute Digimatic calipers (model CD-6″CS; Mitutoyo America Corporation, Aurora, IL). Although these calipers were used based upon the amount of accuracy they

TABLE 1—*Breakdown of the sample population.*

Ancestry	Sex	Condition	Number	Total
African	Male	Unfused	25	100
		Fused	25	
	Female	Unfused	25	
		Fused	25	
European	Male	Unfused	29	100
		Fused	23	
	Female	Unfused	21	
		Fused	27	

TABLE 2—*Fused and unfused hyoid measurements (also see Figs 1 and 2). All measurements were, and should be, taken as though the hyoid was in anatomical position.*

Dimension	Description
BL	Maximum length of body taken from the most laterally projecting points
BH	Maximum height of body taken from the most superiorly and inferiorly projecting points
CWI	Width of greater cornu at fusion point with body (inferior end)
CHI	Height of greater cornu at fusion point with body (inferior end); taken perpendicular to CWI
CL	Maximum length of greater cornu from the inferior to superior end. When the hyoid is fused this measurement should be taken from the fusion line between the body and greater cornu to the superior end of the cornu
CWS	Greatest width of superior end of greater cornu
CHS	Greatest height of superior end of greater cornu; taken perpendicular to CWS
THL	Total hyoid length—maximum length of hyoid, from anterior surface of the body to the superior ends of greater cornua (fused hyoids only)
THW	Total hyoid width—maximum distance between the widest points of the greater cornua (not pictured; fused hyoids only); in some cases this measurement may be equivalent to WCS
WCS	Total width between the superior ends of the right and left greater cornua; taken from lateral edges (fused hyoids only)

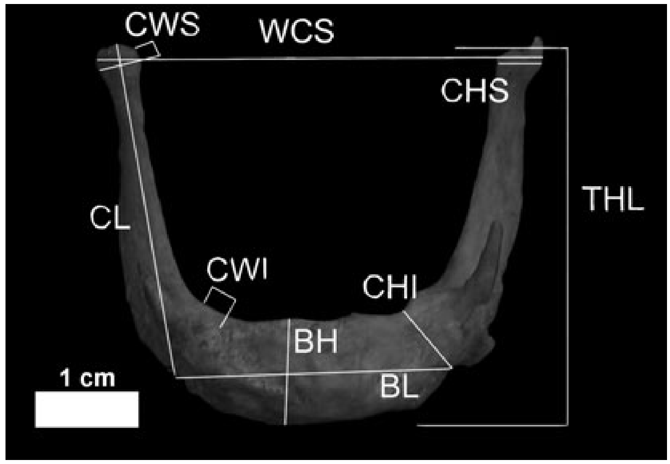

FIG. 1—*Fused hyoid measurements (NMNH Terry Collection #561).*

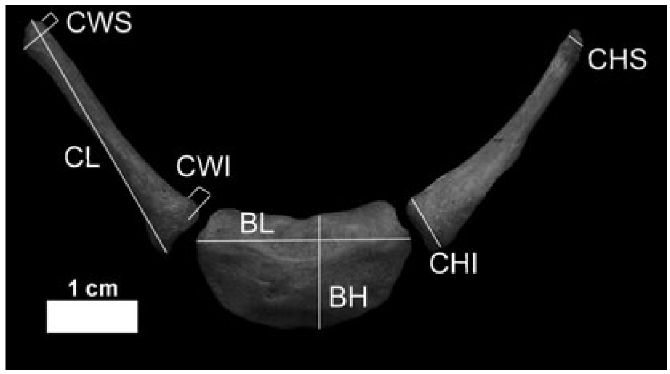

FIG. 2—*Unfused hyoid measurements (NMNH Terry Collection #908).*

provided, it is believed that those providing measurements to the nearest tenth or hundredth of a millimeter will also suffice. In some cases, the hyoids displayed small bony growths or spicules, particularly in the vicinity of the joint between the greater cornua and body, which may have interfered with or added to the measurements. When possible these growths were not included in the measurements. Previous research (9) has shown that there is very little asymmetry between sides in the hyoid, and therefore, the average of both sizes was used in all statistical analyses. Given this low level of asymmetry, future researchers using the formulae presented here should be able to use measurements from whatever side of the hyoid is available and/or preserved.

Because the opportunity to measure inter-observer error was not available during this study, a random subsample of hyoids was selected and remeasured to quantify intra-observer measurement error. Percent measurement error for 20 fused and 20 unfused hyoids (equal sex representation in each group) is shown in Table 3. The average amount of error for each measurement was below 5%. In addition, the amount of percent error for each measurement used in the discriminant functions was both added to and subtracted from the original measurement value of each hyoid in the overall sample. The discriminant functions were then calculated using these new values to test whether the error had a significant effect on the ability of the functions to classify the hyoids. It was found that for fused hyoids, when the error was both added and subtracted the function accurately classified the hyoids 78% of the time. For unfused hyoids, when the error was added the function correctly classified 73% of cases and when error was subtracted it correctly

TABLE 3—*Average percentage of error of fused and unfused hyoids.*

	Average Error (%)	
Measurement	Fused	Unfused
BL	4.72	0.91
BH	2.08	2.44
CWI	4.52	3.58
CHI	3.01	3.43
CL	2.19	0.47
CWS	3.04	4.70
CHS	4.44	4.67
THL	1.35	
THW	0.85	
WCS	0.40	

For abbreviations see Table 2.

classified 72% of cases. These values are comparable with the original cross-validated accuracies of the functions and suggest that the accuracies of the functions are not significantly compromised by the amount of error identified in this study.

Statistical Methods

A one-sample Kolmogorov–Smirnov test was performed on the entire hyoid sample ($N = 200$), including both fused and unfused hyoids to determine whether the data are distributed normally. The test confirmed that the data are distributed normally, and therefore, parametric statistical methods, including discriminant function analysis and independent samples *t*-tests, are appropriate for this analysis. In addition, Box's *M* tests were used to test for an equality of covariance among the samples used to develop the discriminant functions ($p = 0.05$). The results of the Box's *M* test were nonsignificant ($p = 0.220$ for fused hyoids and $p = 0.399$ for unfused hyoids), suggesting that the covariances are homogeneous and that the assumptions of discriminant function analysis have not been violated.

Two statistical methods were used to test the null hypothesis, which state that the hyoids of both Africans and Europeans are equal in size and shape. First, independent samples *t*-tests were used to determine whether differences in size between Africans and Europeans existed. Second, the geometric mean for all variables of each individual was calculated and the skeletal measurements of each variable were regressed on the geometric mean using least squares linear regression. Residuals from these regressions, therefore, reflect "size-corrected" variation in shape between African and European populations. For example, individuals displaying positive residuals are relatively larger in a given measurement once size, as quantified by the geometric mean, has been corrected for. Independent samples *t*-tests were then performed using the regression residuals for each measurement for Africans and Europeans to test whether the differences in size-corrected variables were statistically significant.

Once variation in size and shape had been identified and explored, discriminant function analysis was performed in an attempt to develop equations that would utilize a combination of measurements to classify a hyoid as either African or European. In developing the discriminant functions, the significance of each independent variable in the analysis was determined using stepwise statistics to find the variables that set the Wilks' lambda at a minimum. For each analysis that was performed, each step was statistically significant at $p < 0.001$ and the Wilks' lambda was significant at <0.001 for each function, suggesting the function is producing significantly different discriminant scores for each group. Once the stepwise analysis identified which variables were most valuable in determining ancestry, a second analysis was performed, in which these variables were added independently. To maximize the number of hyoids used in the analysis, the option to include the mean of each missing variable was chosen. This resulted in 83 fused hyoids and all 100 unfused hyoids being used in the analyses. Leave-one-out classification was performed to test the ability of the function to classify a hyoid. The discrimination ability of the function was evaluated based on the canonical correlation and the closeness of the eigenvalue to 1.0. High canonical correlations and eigenvalues close to 1.0 indicate that the distinction ability of the function is high. All statistical procedures were performed using SPSS 17.0 (IBM Corporation, Armonk, NY).

Results

Independent Sample t*-Test Results*

Independent samples *t*-tests performed using the fused hyoid measurements show statistically significant differences in size between Africans and Europeans for five of the 10 measurements (Table 4). In general, the hyoid of European individuals is larger than those from African individuals. Most significant differences are in those measurements of the overall width of the hyoid, such as the length of the body, total hyoid width, and the distance between the greater cornua. In addition, although only five measurements display statistically significant differences in size, the majority of the average measurements are larger in the European sample than in the African sample, even if by only a slim margin. Tests performed on unfused hyoids display similar results (Table 5), although only two of the seven measurements reached statistical significance between the two ancestral groups. As with

TABLE 4—*Average measurements (in mm) and* p*-values of fused hyoids (mean ± standard deviation.*

Measurement	African	European	*p*-Value
BL	21.39 ± 2.99	23.26 ± 2.74	0.002*
BH	11.39 ± 1.45	11.31 ± 1.35	0.766
CWI	4.25 ± 0.63	4.67 ± 0.94	0.011*
CHI	7.28 ± 0.96	7.42 ± 1.36	0.556
CL	28.89 ± 3.36	29.61 ± 3.75	0.338
CWS	3.17 ± 0.64	3.32 ± 0.95	0.384
CHS	3.98 ± 0.78	4.41 ± 0.84	0.014*
THL	35.79 ± 4.07	35.0 ± 4.52	0.376
THW	38.90 ± 4.18	42.54 ± 5.12	< 0.001*
WCS	39.28 ± 5.63	43.48 ± 6.02	0.003*

For abbreviations see Table 2.
*Statistically significant.

TABLE 5—*Average measurements (in mm) and* p*-values of unfused hyoids (mean ± standard deviation).*

Measurement	African	European	*p*-Value
BL	21.69 ± 2.69	23.20 ± 2.86	0.008*
BH	11.89 ± 1.51	11.44 ± 1.57	0.149
CWI	3.70 ± 0.53	4.10 ± 0.74	0.003*
CHI	6.28 ± 0.98	6.43 ± 1.04	0.454
CL	29.01 ± 3.08	29.49 ± 3.74	0.501
CWS	3.07 ± 0.51	3.07 ± 0.73	0.953
CHS	4.28 ± 0.76	4.26 ± 0.74	0.876

For abbreviations see Table 2.
*Statistically significant.

the fused hyoids, these measurements were larger for the European hyoids than the African.

Linear Regression Analysis Results

Of the 10 measurements taken from fused hyoids, three measurements exhibited statistically significant differences in shape between ancestral groups, once size-corrected through regression on the geometric mean (Table 6). When the regression analysis was applied to the unfused hyoid measurements, three measurements displayed statistically significant differences in shape between ancestral groups (Table 7).

Discriminant Function Analysis Results

Table 8 shows the functions, the condition of the hyoid in which they are used, their cut-off points, and overall accuracies. Function 1, developed using fused hyoids, utilizes the hyoid's total length, total hyoid width, maximum length of the body, height of the greater cornu at its superior end, and maximum cornu length. When the discriminant function analysis was performed on the unfused hyoid sample, only the two measurements taken on the body were utilized in the functions. Therefore, Function 2 requires only the maximum length and height of the body and can be used regardless of whether the hyoid is unfused and complete or only the body is recovered. Overall accuracies were *c.* 78% and 73% for Functions 1 and 2, respectively. Cross-validation provided accuracies of 76% and 70% for Functions 1 and 2, respectively.

TABLE 6—*Regression residuals and* p-*values used to demonstrate differences in shape of fused hyoids.*

Measurement	African	European	*p*-Value
BL	−0.253	0.261	0.033*
BH	0.072	−0.074	0.552
CWI	−0.115	0.118	0.341
CHI	0.089	−0.092	0.458
CL	0.179	−0.184	0.135
CWS	0.108	−0.111	0.372
CHS	−0.174	0.179	0.147
THL	0.529	−0.545	<0.001*
THW	−0.284	0.293	0.016*
WCS	−0.154	0.159	0.200

For abbreviations see Table 2.
*Statistically significant.

TABLE 7—*Regression residuals and* p-*values used to demonstrate differences in shape of unfused hyoids.*

Measurement	African	European	*p*-Value
BL	−0.218	0.341	<0.001*
BH	0.176	−0.275	0.001*
CWI	−0.116	0.181	0.035*
CHI	0.054	−0.084	0.331
CL	−0.067	0.105	0.224
CWS	0.058	−0.091	0.290
CHS	0.064	−0.100	0.248

For abbreviations see Table 2.
*Statistically significant.

Discussion

Results of the statistical analyses suggest several differences in hyoid size and shape occur between those of African and European ancestries. Although the majority of the size differences indicated by the independent samples *t*-tests are not statistically significant ($p = 0.05$; $n = 100$ for both fused and unfused hyoids), those that attained significance provide insight into possible overall differences. When looking exclusively at fused hyoids, there are three dimensions which display significant differences that could be indicators of overall differences in size. In all three dimensions (total hyoid width, maximum length of the body, and width between the distal ends of the greater cornua), the European hyoid is significantly larger. The significance of these three measurements reflects the general pattern of broader hyoids among individuals of European ancestries. In addition, the length of the body remains one of the only three measurements that is dimorphic on unfused hyoids, so the difference in size is likely not a product of ossification of the greater cornua to the body. This suggests that the length of the hyoid body is one of the most dimorphic indicators of ancestry in the hyoid and supports its use in both of the discriminant functions produced from this study.

The analysis of the size-corrected residuals partially confirms these results and provides additional information about hyoid shape. Three major size-corrected variables differ between African and European groups: total length, total width, and maximum length of the body. In general, even when overall size is removed from the analysis, European hyoids are relatively short and broad; African hyoids are relatively long and narrow. The relatively smaller maximum body length of African hyoids likely contributes to the African hyoids being narrower than Europeans.

Although there are no studies of ancestry determination using the hyoid with which to compare this research, achieving cross-validated accuracies of 75% and 70% suggests a significant amount of variation is present between ancestries, making discrimination between the two possible. Function 1 likely performs better than Function 2 because of the use of five, as opposed to two, variables, providing more opportunity for the overall size and shape of the bone to be known. In addition, four of the five variables used in Function 1 are indicative of overall length or width of the bone, something that cannot be determined from an unfused hyoid, suggesting they are great contributors to the difference in accuracies. However, Function 2 may still prove useful, as the greater cornua

TABLE 8—*Discriminant functions for determining ancestry from unfused and fused hyoids. See Table 2 and Figs 1 and 2 for measurements. Values above cut-off points are European, values below are African.*

Function Number	Hyoid Condition	Discriminant Functions 1 and 2	Cut-Off Point	Total Function Accuracy (%)	Cross-Validation Accuracy (%)
Function 1	Fused	D = (−0.346) (THL) + (0.1) (THW) + (0.265) (BL) + (0.218) (CL) + (0.717) (CHS) − 7.078	0.076	78	76
Function 2	Unfused/Body only	D = (0.542) (BL) + (−0.901) (BH) − 1.657	0.0	73.0	70.0

For abbreviations see Table 2.

can be easily damaged, leaving the body as the only available component of the bone for determining ancestry.

While the discriminant function analysis of the hyoid has not been previously conducted for the determination of ancestry, discriminant function analysis of this element has been used in four previous cases to determine sex. Miller et al. (3) used five measurements to develop an equation with an overall accuracy of *c*. 72%, Kim et al. (2) used three measurements to develop an equation of *c*. 88%, and Reesink et al. (6) used three measurements of the hyoid body to develop an equation with an overall accuracy of 76%. Most recently, Kindschuh et al. (9) developed three discriminant functions to be used on fused or unfused hyoids or the hyoid body, ranging in accuracy from *c*. 83 to 85%. These results suggest that the hyoid displays significant sexual dimorphism in addition to the ancestral size and shape variation. However, the accuracies of the ancestry functions presented here are comparable to the accuracies of the sexual dimorphism function reported in previous literature.

Because of the hyoid's relative independence from the remainder of an individual's skeletal structure, it is difficult to surmise why size and shape variation would occur between ancestries. The primary muscle and ligament attachments found on the hyoid are those involved in speaking and chewing, suggesting a possible link between the mandible and mid- and lower facial region. One of the most prominent and well recognized differences between skulls of African and European ancestry is the relative degree of prognathism. Therefore, it may be possible that the size and shape differences seen between ancestries, particularly in the length of the bone, are a product of increased prognathism in African individuals.

Previous research has shown that the relationship between the mandible and the position of the hyoid generally remains constant (10), so that changes in mandibular positioning results in changes in the position of the hyoid (11). In addition, while hyoid positioning is related to the mandible, the fact that it is not actually articulated with the mandible means it is likely that hyoid positioning relies mostly on the musculature that surrounds it (12). It is therefore possible that the forward projection of the mid-facial region and mandible in African individuals has influenced the musculature of the face and neck which uses the hyoid as an attachment site. This in turn may have influenced the shape of African hyoids, resulting in straighter greater cornua and overall longer shape than Europeans. Unfortunately, little to no research addressing the effects of ancestral prognathism on the hyoid has been undertaken.

Limitations of Study

This study has several potential limitations. First, hyoids used to develop the discriminant functions here may not be comparable in size and shape to those in modern or pre-Terry Collection populations. This issue of representativeness is one that is common in metric studies based on the Terry Collection (and other collections) and as such the user of the discriminant functions should be aware of possible discrepancies between the size and shape of the individual in question and those individuals in the Terry Collection and use the functions in conjunction with other methods of ancestry estimation (13). Although the functions presented here have not been tested on an outside population so that their ability to classify hyoids outside the Terry Collection is still unknown, it is believed that the functions can be useful in both modern and archaeological contexts.

The second limitation of the Terry Collection is the lack of representation of ancestries other than African or European. Unfortunately, of the 1728 documented individuals comprising the collection, only five were listed as being of Asian ancestry (4), none of which were used in the study. No other ancestral or ethnic groups are represented (e.g., Native American, Australian Aborigine, Hispanic, etc.). Obviously, this creates a dilemma for the physical anthropologist and the discriminant functions presented here should be used with caution. However, the study performed by Kim et al. (2) in which measurements of their Asian population were compared with Miller et al.'s (3) "White and Hispanic" population, gives some indication that ancestral differences can be identified if an Asian sample can be included in the analysis. We hope this study will serve as a basis for future hyoid studies that can include data from a variety of ancestries, thereby increasing the accuracy of methods of ancestry determination based on the hyoid.

Third, there are likely other sources of morphological variation in the hyoid other than ancestry. As stated earlier, several previous studies have explored levels of sexual dimorphism in the hyoid and found significant size differences between the sexes (2,3,5,6,9). In addition, recent work has indicated that there may be significant interactions between ancestry and sex—individuals of African descent may be less sexually dimorphic in some hyoid measurements than Europeans (9). However, most of the variables used in the current analysis showed no statistically significant interactions between sex and ancestry, and it seems unlikely that the minimal interactions between sex and ancestry will significantly affect the use of these formulae in the determination of ancestry.

Conclusion

Hyoids have been known to vary in size and shape by sex, but until this point, ancestral variation has remained unexplored. The results presented here have identified both size and shape variation, particularly in fully fused hyoids, between African and European ancestries. Because of this variation, it is possible to classify a hyoid with reasonable accuracy as African or European. Unfortunately, because of the lack of representation of ancestries other than African or European in the Terry Collection, it is still unknown how the hyoids of other ancestries will vary in size and shape. This study provides a starting point for future studies regarding the utility of the hyoid and its potential to aid in ancestry determination.

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Additional information and reprints requests:
Sarah C. Kindschuh, M.A.
Department of Anthropology
Binghamton University
P.O. Box 6000
Binghamton, NY 13902-6000
E-mail: skindsc1@binghamton.edu