



Evaluating postcranial macromorphoscopic traits to estimate population variation among modern South Africans

N.P. Bothma^{*,1}, E.N. L'abbé², L. Liebenberg³

University of Pretoria, Department of Anatomy, Pretoria, South Africa, Private Bag x323, Gezina 0031, South Africa

ARTICLE INFO

Keywords:

Forensic anthropology
Population affinity
Observer agreement
Random Forest Modelling
Classification accuracy
Variable importance

ABSTRACT

Population overlap and the variation within and among populations have been globally observed but is often difficult to quantify. To achieve this, numerous different methods need to be explored and validated to assist with the creation of an accurate biological profile. The current lack of databases for postcranial macromorphoscopic traits indicates the need to further investigate if the method can be employed repeatedly in a forensic context. The current study aimed to assess the prevalence of eleven postcranial macromorphoscopic traits in a South African sample. A total of 271 postcrania of adult black, coloured, and white South Africans were assessed. The intra- and inter-observer agreement ranged from fair to almost perfect except for the accessory transverse foramen of C1, which had poor agreement between observers. Only seven traits differed significantly between at least two of the groups. Univariate and multivariate random forest models were created to test the positive predictive performance of the traits to classify population affinity. The classification accuracies for the univariate models ranged from 33.3% to 53.0% and ranged from 54.6% to 62.1% for the multivariate models. Based on the variable importance, the traits assessing spinous process bifurcation were the most discriminatory variables. The results indicate that the postcranial MMS approach does not outperform current methods employed to estimate population affinity. Further research needs to be done for the method to have practical applicability for medicolegal casework in South Africa.

1. INTRODUCTION

The estimation of population affinity contributes valuable information to the biological profile and is particularly important in countries with diverse populations, such as South Africa. South Africa has a heterogeneous population that consists of socially identified black, white, coloured, and Indian or Asian individuals [1]. Each group has unique population origins and histories. The black South African population initially descended from Bantu-speaking groups that migrated from West Africa to sub-Saharan Africa and settled south of the Limpopo River (by AD 300) [2,3]. The English and Afrikaans-speaking white South African population are descendants of European settlers who migrated to the country in the 1600s [2,4,5]. The Cape coloured group, also known as the South African coloured population, is a heterogeneous population with the widest variety of global genetic contributions that are both intra- and inter-continental [5]. "Coloured" is a social term that

is still used within South Africa [6]. Heterogeneous populations display skeletal variation among the populations; however, there is also substantial group overlap [7–9]. Because of its diversity, it is imperative to have access to numerous methods of population affinity estimation for the South African population.

Anthropological practitioners have developed various methods to assess population affinity, which include both metric and non-metric approaches using several different skeletal elements [7,8,10,11]. Standard metric methods quantify the size of skeletal elements through the use of measuring equipment, like calipers. In comparison, morphological, or non-metric methods entail the visual assessment of non-pathological skeletal variants that may vary in size, shape or expression among populations [10,12]. In the past, non-metric traits have popularly been used to assess and distinguish differences among global populations through biodistance studies. However, this approach has notably been criticized for use in medicolegal casework due to

* Correspondence to: Department of Anatomy, University of Pretoria, Private Bag x323, Arcadia 0007, South Africa.

E-mail addresses: u11049571@tuks.co.za, bothmapearl@gmail.com (N.P. Bothma).

¹ ORCID: 0000-0001-5116-8186

² ORCID: 0000-0002-3289-1122

³ ORCID: 0000-0003-4310-5489

inherent subjectivity and a lack of standardization [11,13,14].

The issues associated with the non-metric approach have been addressed in methods commonly employed for sex estimation [15–17]. Hefner [11] introduced similar standardization of non-metric trait analysis to avoid subjectivity when using the cranium to assess population affinity. The standardization included the introduction of line drawings, more descriptive definitions, and robust statistics [11]. As a result, the scoring of the traits, commonly referred to as macro-morphoscopic (MMS) traits, became a more valid avenue to assess non-metric variation. Currently, the cranial MMS traits have been thoroughly explored in many populations across the globe and remain the most developed non-metric method for population affinity estimation in forensic analyses [18,19]. More recently, Spiros [20] and Spiros and Hefner [21] presented similar work assessing postcranial MMS traits. Spiros [20] created trait definitions and illustrations for eleven postcranial traits and assessed the reliability of the scoring technique and the frequency distribution of the postcranial MMS traits in a sample of black and white North Americans.

Many studies have noted differences between modern South Africans and North Americans (e.g. 5,15), which have prompted ongoing work to modify existing standards before adopting them for skeletal analyses in South Africa. Currently, several South African-specific databases exist which contain metric standards for skeletal analyses [7,8,10]. However, non-metric methods to estimate population affinity have not received as much attention in the country. The addition of reliable postcranial MMS methods to estimate population affinity can assist in learning more about human variation among the populations and possibly develop reference samples that can be used for future research and forensic case analysis. However, the postcranial MMS method needs to be validated for analyses involving South Africans because research on non-metric traits for population affinity estimation has been limited. This study aims to explore postcranial variation and the prevalence of eleven postcranial MMS traits as a tool to estimate population affinity among black, coloured, and white South Africans. The current study is also the first to investigate the accuracy with which the postcranial MMS method can predict population affinity in a modern South African sample.

2. Materials and methods

The sample consists of the postcrania of 271 black, coloured, and white South Africans (Table 1). More specifically, the cervical vertebrae, sternum, scapula, humerus, femur, patella, and calcaneus were used (Table 2). All individuals in the sample were adults, older than 18 years of age. Any individuals with excessive post-mortem damage or pathological lesions that prevented the accurate scoring of the traits were excluded. Additionally, individuals that were missing more than four traits, were excluded from the sample.

The sample was obtained from two South African collections: the Pretoria Bone Collection (PBC) and the Kirsten Skeletal Collection (KSC) located at the University of Pretoria and the Stellenbosch University, respectively. The PBC is composed of donated bodies of known sex, age, stature, population affinity, and cause of death [23,24]. The KSC is also a collection of willed and unclaimed whole-body donations of persons who died from natural causes [25]. Willed and unclaimed whole-body donations to South African medical schools are governed by the National Health Act of 2003, which states that anyone can donate their body for tissue transplants, research, and medical training [26]. Ethical

Table 1
Sample distribution.

Population	Males	Females	Total
Black	46	41	87
White	49	41	90
Coloured	47	47	94
Total	142	129	271

Table 2

Summary of the trait names and abbreviations and their associated scores taken from Spiros [20].

Trait	Abbreviation	Location	Score	Description
Accessory Transverse Foramen	ATF	Cervical vertebra (C1, C3, C4, C5, C6, C7)	0	Absent
			1	Unilateral
			2	Bilateral
Posterior Bridging	PB	Cervical vertebra	0	Absent
			1	Unilateral
			2	Bilateral
Double Superior Articular Facets	DSAF	Cervical vertebra	0	Absent
			1	Unilateral
			2	Bilateral
Spinous Process Bifurcation	SPB	Cervical vertebra (C3, C4, C5, C6)	0	Non-bifid
			1	Partially bifid
			2	Completely bifid
Suprascapular Foramen	SF	Scapula	0	Absent
			1	Present
			0	Absent
Sternal Aperture	STA	Sternum	0	Absent
			1	Present
			0	Absent
Supra-condyloid Process	SCP (L & R)	Humerus	0	Absent
			1	Present
			0	Absent
Septal Aperture	SA (L & R)	Humerus	0	Absent
			1	Translucent
			2	Small perforation
Third Trochanter	TT (L & R)	Femur	0	Absent
			1	Present
			0	Absent
Vastus Notch	VN (L & R)	Patella	0	Absent
			1	Present
			0	No anterior facet
Anterior and Middle Calcaneal Facets	AMCF (L & R)	Calcaneus	0	No anterior facet
			1	Single elongated facet
			2	Small anterior facet
			3	Large anterior facet

approval was obtained from the Human Research Ethics Committee (HREC) of the Faculty of Health Sciences (Ref 610/2021).

A total of eleven postcranial MMS traits were visually assessed and scored by applying the methodology described by Spiros [20], and Spiros and Hefner [21]. Table 2 presents the traits and their descriptions. In the case of bilateral traits, both the left and right sides were assessed.

All statistical analyses were conducted using R (version 4.1.0) [27], and included tests to measure the observer agreement, exploratory analyses to test for significant group differences, and classification models to test the accuracy with which population affinity can be assigned using the traits. The inter- and intra-observer agreement was tested with Cohen’s kappa, using the Landis and Koch scale [28] to describe the degree of repeatability. Nine individuals were randomly selected to test inter- and intra-observer agreement. Frequency distributions were created for the traits, and Kruskal-Wallis tests were applied to determine if there are significant differences between the sexes and among the groups for each trait. A *post-hoc* Dunn’s test was also applied (with a Holm’s adjustment) to further investigate group differences and overlap. Random forest modelling (RFM) was employed to create classification models and to see if the traits are useful for population affinity estimation. As a classification method, RFM refers to a combination of decision trees that are generated using a non-parametric algorithm integrating random sampling with replacement and majority voting [29]. Through a series of nodes or rules, RFM predicts a categorical variable (such as population affinity) from a set of measurements or observations on one or more predictor variables (such as postcranial scores) [30–32].

Training data sets are used to create classification models, after which a hold-out (or “out-of-bag”) testing set is used to simultaneously evaluate the models using an independent sample. Essentially, a training data set is the known or collected data that is used to fit a classification model, and a testing data set is unseen data that evaluates if the training model works adequately for classification. In the current study, the sample was divided so that 75% constituted the training set (to create the models), and the remaining 25% was kept as the out-of-bag (OOB) testing set (to validate the models). In the case of missing data, the mode was calculated for each sex and population group separately within each trait and the result was used as the score of that missing trait. The mode was used as an imputation value specifically because it appears the most in a set of values which in this case, in a population or sex group, most individuals are likely to depict that value. Both univariate and multivariate models were employed to evaluate the performance of the traits when tested both individually and in a group. The univariate models assist to determine the performance of each trait and the multivariate models determine the performance of the traits when tested in combination. Three different multivariate models were tested: the first model included all the traits; for the second model all traits with variable importance below one (as calculated from the first model) were removed; and finally, the third model included only the variables that were found to be significantly different with the Kruskal-Wallis tests. A total of 2500 classification trees were used for each model with four variables at each split. The classification accuracy (for both the training and testing samples), Kappa values, and variable importance were recorded for each model. Both the classification accuracy and Kappa values are measures of model accuracy. The classification accuracy presents the percentage of correctly classified individuals out of all of the individuals, whereas the Kappa value presents the percentage of correctly classified individuals while taking random chance into account. The Kappa value is a particularly useful metric in the case of unbalanced classes (e.g., where traits can be scored as zero or one, but a score of 1 is a fairly rare occurrence). With variable importance, the higher the value, the more a variable contributes to the classification.

3. Results

3.1. Inter- and intra-observer agreement

The intra-observer agreement ranged between moderate and almost perfect ($\kappa = 0.58$ to 1.00) (Table 3). Overall, six of the eleven traits

Table 3
Kappa values for the inter- and intra-observer agreement with the associated description following Landis and Koch [17].

Trait	Intra-observer	Description	Inter-observer	Description
ATF_C1	1.00	Almost perfect	-0.11	Poor
ATF_C3	N/A*	-	N/A*	-
ATF_C4	N/A*	-	N/A*	-
ATF_C5	0.84	Almost perfect	0.85	Almost perfect
ATF_C6	1.00	Almost perfect	0.67	Substantial
ATF_C7	0.87	Almost perfect	0.14	Slight
PB	1.00	Almost perfect	N/A*	-
DSAF	1.00	Almost perfect	1.00	Almost perfect
SPB_C3	0.86	Almost perfect	0.40	Fair
SPB_C4	0.58	Moderate	0.51	Moderate
SPB_C5	0.72	Substantial	0.63	Substantial
SPB_C6	N/A*	-	0.64	Substantial
SSF	N/A*	-	N/A*	-
STA	1.00	Almost perfect	1.00	Almost perfect
SCP	N/A*	-	N/A*	-
SA	0.88	Almost perfect	0.68	Substantial
TT	1.00	Almost perfect	N/A*	-
VN	0.75	Substantial	0.62	Substantial
AMCF	1.00	Almost perfect	1.00	Almost perfect

* N/A: “not applicable” Kappa value outcome due to lack of variation in sample

demonstrated almost perfect agreement ($\kappa = 1.00$). The trait with the lowest agreement was the spinous process bifurcation of C4 ($\kappa = 0.58$). The inter-observer agreement was substantially lower than the intra-observer agreement, ranging between poor and almost perfect ($\kappa = -0.11$ to 1.00). When comparing the scores between two different observers, only four of the eleven traits demonstrated almost perfect agreement. Additionally, the accessory transverse foramen of C1 presented with agreement poorer than randomly allocating a score ($\kappa = -0.11$). Some traits had a “non-applicable” outcome such as the accessory transverse foramen for C3 and C4, suprascapular foramen, posterior bridging, supracondylar process and the third trochanter. The “non-applicable” Kappa outcome is due to the lack of variation observed among the three population groups, as none of the randomly selected specimens had the traits and thus all received the same score (i.e. a score of 0 to indicate absence) [28]. Despite the “non-applicable” Kappa outcome, the traits were included for further analysis.

3.2. Frequency distribution

Table 4 presents the frequency distributions for the traits among the three populations and between the sexes, as well as the results for the Kruskal-Wallis tests. For population affinity, the results of the Kruskal-Wallis test revealed that seven of the traits were significantly different; this includes the accessory transverse foramen (only for C4, C5 and C6), double superior articular facet, spinous process bifurcation (C3 to C6), suprascapular foramen, septal aperture, vastus notch and the anterior and medial calcaneal facets traits. Notably, for two of the bilateral traits (septal aperture and vastus notch), only the right side was observed to differ significantly among the groups ($p < 0.05$). Not a single instance of a supra-condyloid process was recorded in the sample, indicating that it will not be a useful trait to distinguish among the groups in South Africa.

A *post-hoc* Dunn’s test was then conducted to further explore the variation of the traits among the groups (see Table 5 for the breakdown of population group overlap). None of the traits demonstrated significant differences among all three groups; in other words, at least two of the groups showed overlap for the traits that were noted to differ significantly. More specifically, the black and coloured South Africans demonstrated the most similarities and subsequent group overlap, while the white South Africans typically demonstrated greater differences for at least four traits (see Table 4 for trait frequencies). Overall, the double superior articular facets and bifid spinous processes of the cervical vertebrae were noted more frequently in white South Africans, while both black and coloured South Africans had single facets and non-bifid spinous processes. Coloured South Africans were more likely to possess a vastus notch on the patella, and a translucent septal aperture on the humerus than the other groups.

The frequencies of the traits were also compared between males and females, with the population groups pooled together (Table 4). Only four traits were noted to be statistically significantly different, namely spinous process bifurcation (C3 to C6), suprascapular foramen, septal aperture and vastus notch. All the above-mentioned traits were also significantly different when compared among the three populations. It should be acknowledged that while only the right septal aperture and vastus notch were significant for population affinity, both the left and right sides for both traits were significant when assessing sex. This indicates substantial differences between the left and right sides. Overall, females were more likely to present with a spinous process bifurcation and a vastus notch on the patella compared to males.

3.2.1. Univariate models

Table 6 presents the classification results for the univariate models. Three measures of performance were recorded for each trait: [1] the training accuracy, which is based on the training sample which includes 75% of the total sample; [2] the testing accuracy, which is based on the hold-out sample (the remaining 25% of the total sample) that was not

Table 4

Trait frequencies among population groups (black, coloured, and white) and between the sexes (males and females). Refer to Table 2 for trait names and abbreviations. $p < 0.05$ indicates significant differences.

Score	Population affinity						Sex			
	Black		Coloured		White		Females		Males	
	n	%	n	%	n	%	n	%	n	%
ATF (C1)										
0	56	74.67	71	78.02	67	84.8	92	76.67	102	81.60
1	14	18.67	14	15.39	8	10.13	21	17.50	15	12.50
2	5	6.67	6	6.59	4	5.06	7	5.83	8	6.67
	p = 0.31						p = 0.39			
ATF (C3)										
0	85	100.00	90	100.00	80	100.00	123	99.19	132	100.00
1	0	0	0	0	0	0	1	0.81	0	0
2	0	0	0	0	0	0	0	0	0	0
	p = 0.34						p = 0.30			
ATF (C4)										
0	80	97.56	87	95.60	77	87.50	116	92.80	128	98.46
1	2	2.44	4	4.40	9	10.23	8	6.40	1	0.77
2	0	0	0	0	2	2.27	1	0.80	1	0.77
	p = 0.02						p = 0.67			
ATF (C5)										
0	69	83.13	67	72.04	52	60.47	85	67.46	103	75.74
1	12	14.46	21	22.58	25	29.07	32	25.40	26	19.12
2	2	2.41	5	5.38	9	10.47	9	7.14	7	5.15
	p < 0.01						p = 0.14			
ATF (C6)										
0	53	63.86	52	59.77	37	44.05	61	51.26	81	60.00
1	18	21.69	25	28.74	24	28.57	35	29.41	32	23.70
2	12	14.46	10	11.49	23	27.38	23	19.33	22	16.30
	p < 0.01						p = 0.19			
ATF (C7)										
0	72	90.00	71	92.21	69	81.18	103	89.57	110	85.94
1	7	8.75	6	7.79	12	14.12	10	8.70	15	11.72
2	1	1.25	0	0	4	4.71	2	1.74	3	2.34
	p = 0.07						p = 0.41			
PB										
0	62	82.67	74	80.43	68	86.08	103	85.12	101	80.80
1	8	10.67	13	14.13	6	7.60	13	10.74	14	11.20
2	5	6.67	5	5.44	5	6.33	5	4.13	10	8.00
	p = 0.67						p = 0.33			
DSAF										
0	65	86.67	78	84.78	48	60.76	100	82.64	91	72.80
1	7	9.33	8	8.70	19	24.05	10	8.26	24	19.20
2	3	4.00	6	6.52	12	15.19	11	9.09	10	8.00
	p < 0.01						p = 0.10			
SPB (C3)										
0	65	80.25	62	73.81	23	29.87	82	67.77	68	56.20
1	11	13.58	9	10.71	18	23.38	18	14.88	20	16.53
2	5	6.17	13	15.48	36	46.75	21	17.36	33	27.28
	p < 0.01						p < 0.01			
SPB (C4)										
0	72	61.54	60	72.29	16	20.78	82	67.77	50	41.67
1	15	12.82	7	8.43	7	9.09	18	14.88	20	16.67
2	30	25.64	16	19.28	54	70.13	21	17.36	50	41.67
	p < 0.01						p < 0.01			
SPB (C5)										
0	33	41.25	50	56.82	13	15.66	57	47.11	39	30.00
1	16	20.00	10	11.36	9	10.84	12	9.92	23	17.69
2	31	38.75	28	31.82	61	73.49	52	42.98	68	52.31
	p < 0.01						p = 0.03			
SPB (C6)										
0	49	61.25	65	71.43	32	38.10	78	69.64	68	50.37
1	8	10.00	12	13.19	8	9.52	13	11.61	15	11.11
2	23	28.75	14	15.39	44	52.38	21	18.75	52	38.52
	p < 0.01						p = 0.01			
SSF										
0	85	100.00	86	92.47	79	87.78	115	89.84	135	96.43
1	0	0.00	7	7.53	11	12.22	13	10.16	5	3.57
	p < 0.01						p = 0.03			
STA										
0	65	90.28	72	90.00	63	96.92	95	94.06	105	90.52
1	7	9.72	8	10.00	2	3.08	6	5.94	11	9.48
	p = 0.23						p = 0.33			
SCP (L)										
0	87	100.00	93	100.0	88	100.00	128	100.00	140	100.00
1	0	0	0	0	0	0	0	0	0	0

(continued on next page)

Table 4 (continued)

Score	Population affinity						Sex			
	Black		Coloured		White		Females		Males	
	n	%	n	%	n	%	n	%	n	%
SCP (R)	p = NA						p = NA			
0	87	100.00	93	100.00	90	100.00	128	100.0	142	100.00
1	0	0	0	0	0	0	0	0	0	0
SA (L)	p = NA						p = NA			
0	27	31.03	8	8.60	26	30.59	18	14.06	43	31.39
1	30	34.48	64	68.82	40	47.06	63	49.22	71	51.83
2	11	12.64	12	12.90	6	7.06	19	14.84	10	7.30
3	19	21.84	9	9.68	13	15.29	28	21.88	13	9.49
SA (R)	p = 0.18						p < 0.01			
0	27	31.77	11	11.83	30	34.48	22	17.46	46	33.09
1	29	34.12	62	66.67	45	51.72	63	50.00	73	52.52
2	10	11.77	8	8.60	6	6.90	14	11.11	10	7.19
3	19	22.35	12	12.90	6	6.90	27	21.43	10	7.19
TT (L)	p < 0.01						p < 0.01			
0	83	95.40	81	89.01	74	87.06	115	90.56	123	90.44
1	4	4.60	10	10.99	11	12.94	12	9.45	13	9.56
TT (R)	p = 0.15						p = 0.96			
0	83	96.51	79	88.76	82	93.18	119	94.44	125	91.24
1	3	3.49	10	11.24	6	6.82	7	5.56	12	8.76
VN (L)	p = 0.14						p = 0.32			
0	55	66.27	56	60.87	65	77.38	92	74.19	84	62.22
1	28	33.73	36	39.13	19	22.62	32	25.81	51	37.78
VN (R)	p = 0.06						p = 0.04			
0	59	71.08	46	51.69	61	75.31	89	72.36	77	59.23
1	24	28.92	43	48.32	20	24.69	34	27.64	53	40.77
AMCF (L)	p < 0.01						p = 0.03			
0	1	1.28	2	2.44	2	2.70	3	2.75	2	1.60
1	57	73.08	60	73.17	38	51.35	75	68.81	80	64.00
2	20	25.64	20	24.39	27	36.49	27	24.77	40	32.00
3	0	0.00	0	0.00	7	9.46	4	3.67	3	2.400
AMCF (R)	p < 0.01						p = 0.34			
0	0	0.00	2	2.44	3	3.90	3	2.70	2	1.61
1	59	77.63	64	78.05	42	54.54	83	74.78	82	66.13
2	17	22.37	16	19.51	26	33.77	21	18.92	38	30.64
3	0	0.00	0	0.00	6	7.79	4	3.60	2	1.61
	p < 0.01						p = 0.11			

Table 5

Breakdown of group overlap based on the Kruskal-Wallis and Dunn's test results comparing the population groups. Refer to Table 2 for trait names and abbreviations.

No groups overlap	All groups overlap	Black and coloured overlap	Black and white overlap	White and coloured overlap
-	ATF (C1) ATF (C3) ATF (C7) PB STA SA (L) TT (L) TT (R) VN (L)	ATF (C4) ATF (C5) ATF (C6) DSAF SPB (C3) SPB (C4) SPB (C5) SPB (C6) SSF SA (R) AMCF (R) AMCF (R)	SA (R) VN (R)	ATF (C4) ATF (C5) SSF

used to train the model; and [3] the Kappa value, which is based on the entire sample while taking random chance into account. For the training sample, the classification accuracy ranged from 3.9% to 60.0%. When

the univariate models were validated with the testing sample, the classification accuracy ranged from 33.3% to 53.0%. Overall, the spinous process bifurcation for C4 had the highest classification accuracy for both training and testing samples. The accessory transverse foramen for C1 had the lowest classification accuracy for the testing sample. The Kappa values for the testing sample ranged from 0.0% to 29.0%.

3.2.2. Multivariate models

Three multivariate models were tested for classification. The first model included all the traits; the second model included only the traits that demonstrated higher variable importance in the first model (Var-Imp values > 1), and the third model included only the traits that showed significant differences among the population groups. The classification accuracy for the first model was 63.5% for the training sample and 54.6% for the testing sample with a Kappa value of 32.0% (see Table 7 for the comparison of all three multivariate models). Black South Africans presented with a classification error rate of 42.4%, with 30.3% misclassifying as coloured South Africans. White South Africans had the lowest classification error. Black and coloured South Africans mostly misclassified as one another. White South Africans misclassified equally as both black and coloured with no specific trend. Fig. 1 presents the variable importance for the first multivariate model. The trait with the

Table 6
Positive predictive performance of each trait using univariate RFM to estimate population affinity. Refer to Table 2 for trait abbreviations.

Variable	Training sample accuracy (%)	Testing sample accuracy (%)	Kappa value (%)
ATF_C1	35.8	33.3	0.0
ATF_C3	33.3	34.9	0.0
ATF_C4	36.3	36.4	2.0
ATF_C5	28.4	42.4	15.0
* ATF_C6	36.3	34.9	1.0
* ATF_C7	35.9	42.4	12.0
PB	34.1	34.9	2.0
DSAF*	42.5	34.9	4.0
SPB_C3*	26.5	43.9	17.0
SPB_C4*	60.0	53.0	29.0
SPB_C5*	53.8	50.0	25.0
SPB_C6*	46.3	50.0	24.0
SSF*	3.9	36.4	7.0
STA	13.7	36.4	5.0
SA	35.6	42.4	13.0
TT	6.6	36.4	3.0
VN	39.1	39.4	9.0
AMCF*	39.1	36.4	3.0

* Traits that showed significant differences among the population affinity groups.

Table 7
Confusion matrix showing patterns of overlap and misclassification among the groups for the training model for the first model (all the traits).

Classifies into:		Black	Coloured	White	Classification error
Group:	Black	38	20	8	42.4%
	Coloured	23	39	9	45.1%
	White	8	7	53	22.1%

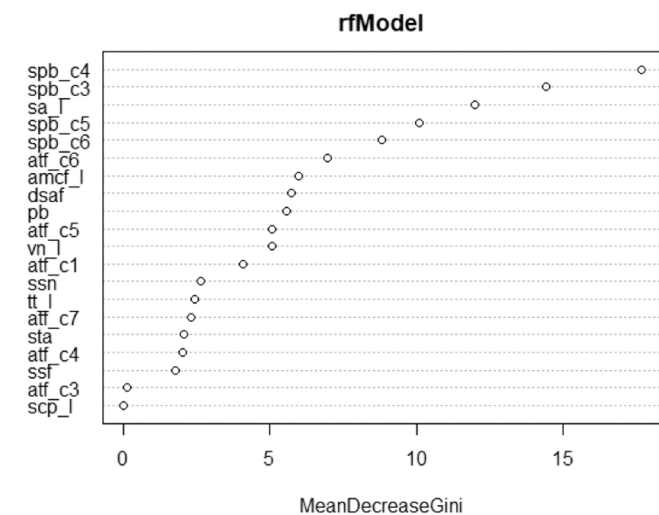


Fig. 1. Comparison of variable importance for the multivariate model employing all of the traits.

highest variable importance was the spinous process bifurcation of C4. Two traits – supracondylar process and accessory transverse foramen of C3 – had variable importance values of 0.0 (i.e., do not contribute any information to the model).

For the second multivariate model, traits with variable importance below one were removed, these include the accessory transverse foramen of C3 and the supracondylar process. The removal of the variables

led to a 0.5% decrease for the training accuracy, while both the testing accuracy and Kappa value increased by 2.0%. When assessing the confusion matrix (see Table 8), white South Africans had the lowest error rate and coloured South Africans had the highest, which was a similar result to the model that used all variables. Similar patterns of misclassifications were observed for both the first and second models. The spinous process bifurcation of C4 was still considered the trait with the highest variable importance and the suprascapular foramen had the least variable importance.

For the third multivariate model, only variables that were noted to be significantly different with the Kruskal-Wallis tests were selected. This includes the accessory transverse foramen of C3 to C6, spinous process bifurcation of C4 to C6, double superior articular facet, suprascapular foramen, and the anterior and medial articular facets. The training accuracy for the third model was 2.5% lower than the first model and 2.0% lower than the second model (see Table 9). However, there was a marked increase in the testing accuracy and the Kappa value. Table 10 presents a comparison of all of the models. Once again, similar patterns of misclassification were observed. Similar to the second model, the spinous process bifurcation of C4 had the highest variable importance and the suprascapular foramen had the lowest.

4. Discussion and conclusion

This is the first study to assess the use of postcranial MMS traits on modern South African populations with the methodology proposed by Spiros [20]. The study aimed to test the repeatability with which the traits can be scored and to explore the variation of the traits among socially defined South African groups.

Overall, the repeatability (particularly intra-observer repeatability) of the postcranial MMS traits was better than reported for the cranial MMS approach when applied to the same population [22]. This is largely assumed to be the result of the scoring system itself. More specifically, the recordation scale of the postcranial traits is dichotomous (either present or absent) or related to the bilaterality of the trait. Cranial MMS traits are mostly ordinal and quantify quasi-continuous variation where traits can be classified as either small, intermediate, or large, or with minor shape variations [11]. Ordinal traits may be more difficult to score because it potentially introduces more subjectivity to the scoring process; one observer may view a trait as small, whereas another observer may view the same trait expression as intermediate [33]. The amount of overlap between the trait expressions and among the population groups may also further complicate scoring in the case of ordinal variables. Therefore, the postcranial MMS traits seem easier to score reliably than cranial MMS traits. Despite the intra-observer agreement of the traits being quite high, some of the traits were noted to be much less repeatable when the scores were compared between multiple observers (e.g., accessory transverse foramen for C1 and C7 and the spinous process bifurcation for C3). The reason for the decreased agreement may be due to a lack of experience with the postcranial MMS method or less experience with osteological variations, particularly in the case of traits that are as rare as some of the ones included in this study. Similar research assessing morphoscopic variation has also emphasized the role that experience can play in the scoring process [19,34,35]. It is recommended that students, researchers and practicing forensic

Table 8
Confusion matrix showing patterns of overlap and misclassification among the groups for the training model for the traits that demonstrated high variable importance.

Classifies into:		Black	Coloured	White	Classification error
Group:	Black	38	20	8	42.4%
	Coloured	23	38	10	46.5%
	White	7	8	53	22.1%

Table 9

Confusion matrix showing patterns of overlap and misclassification among the groups for the training model for the traits that demonstrated significant differences.

Classifies into:		Black	Coloured	White	Classification error
Group:	Black	24	25	8	48.5%
	Coloured	22	39	10	46.0%
	White	9	7	52	23.5%

Table 10

A comparison of the training, testing and Kappa values for the three multivariate models.

	Training accuracy (%)	Testing accuracy (%)	Kappa value (%)
All Traits model	63.5	54.6	32.0
Variable importance model	63.0	56.1	34.0
Significant difference model	61.0	62.1	43.4

anthropologists develop the necessary experience with the postcranial MMS methodology before employing it in studies or skeletal analyses.

Spiros [20] demonstrated almost perfect inter-observer agreement for nine out of the eleven traits, with the remaining traits demonstrating substantial agreement. In comparison, the current study yielded inter-observer agreement levels ranging from poor to almost perfect. The difference in inter-observer agreement levels between the two studies can most likely be explained by the fact that Spiros [20] was involved in the development of the method and possess a better understanding of the trait definitions and more subtle expressions, especially in the case of trait variations. For example, the accessory transverse foramen is one of the traits that demonstrated the most variation in terms of inter-observer repeatability, resulting in a poor agreement score. The poor agreement for the accessory transverse foramen is assumed to be the result of observed variations, such as an incomplete bridge (see Fig. 2). The trait definitions do not indicate how to approach such variations, so researchers may resolve to score the variant morphologies in different ways [34]. Incomplete bridges were also observed with the posterior bridging trait.

Trait variation needs to be considered as many variations were also observed with other traits, specifically with the suprascapular foramen (see Fig. 3). For example, a few individuals had both a scapular notch and a suprascapular foramen inferior to the coracoid process. Spiros [20] mentioned that a suprascapular foramen inferior to the coracoid process should be marked as absent, as the trait is rare [36]. However, for an inexperienced observer, the trait can be confused with the

traditional suprascapular foramen. Furthermore, different degrees of expression for the scapular notch as discussed by Hrdlička [37] were observed with some displaying an incomplete bridge over the suprascapular foramen. The variation observed on the scapula should be considered for future research to investigate whether the variation of the trait can affect the frequency distribution of the trait.

Spiros [20] also cautioned about enthesophytes being confused as the third trochanter and distinguishing between the two was a challenge with some specimens in the current study (see Fig. 4 for images comparing an enthesophyte and a third trochanter). Other indications of pathology, such as myositis ossificans traumatica, were also features to be cognizant of when scoring, specifically with the supracondylar process of the humerus [38,39]. The supracondylar process was not observed in any of the specimens, but a bony spur consistent with myositis ossificans traumatica was present on one of the specimens and mimicked the trait (see Fig. 5 for the feature consistent with myositis ossificans). One of the deterrents of scoring the anomaly as a present supracondylar process trait was that the feature was located on the lateral side of the humerus and was therefore ruled out. Again, familiarity with the trait definitions and locations is essential to score traits accurately. Furthermore, osteological knowledge and an understanding of normal morphology will contribute to greater accuracy in recognizing the traits and any variations of the traits.

The trait frequencies observed in the current study demonstrate similar patterns of group overlap among the South African populations as previously noted with cranial macromorphoscopic, craniometric and postcraniometric data [6,7,42]. More specifically, black and coloured South Africans displayed the most overlap, while white South Africans were more dissimilar. This is similar to the patterns of variation observed with osteometric studies in South Africa and has largely been attributed to socio-political circumstances and positive assortative mating. [7,10,40]. Historically, mixed marriages between coloured and black South Africans occurred more frequently in comparison to coloured-white or black-white mixed marriages [41,42]. Legislation against mixed marriages such as the Prohibition Act of Mixed Marriages Act No. 55 of 1949, was not enforced by the apartheid government as strictly on mixed marriages between black and coloured South Africans as it was between white South Africans and other populations.

The postcranial MMS method has only been evaluated in a few studies [13,14]. The current study reported seven traits that showed significant differences, while Spiros [20] found four traits (spinous process bifurcation for C3 and C4, third trochanter, and the anterior and medial calcaneal facet) with significant differences when assessing black and white North Americans. The spinous process bifurcation was the only trait between the North American and South African samples that demonstrated a common significant difference outcome. Overall, the South African sample yielded more traits with significant differences. Interestingly, Spiros [10] did not find significant differences between

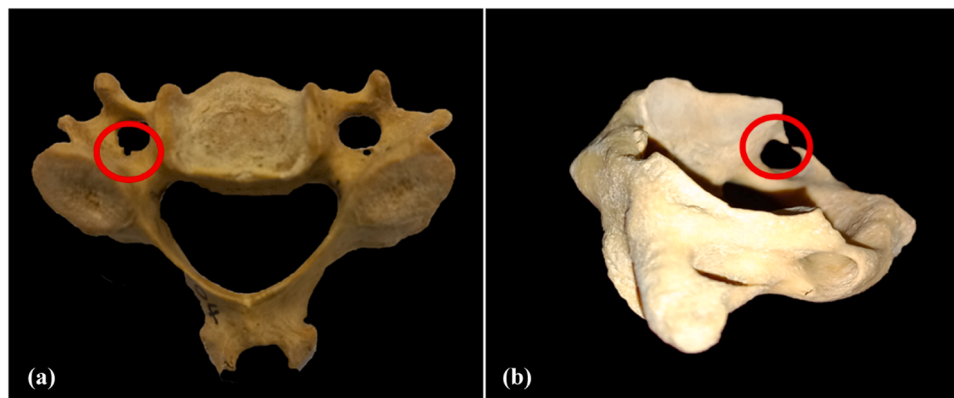


Fig. 2. Incomplete bridge (encircled) on the (a) accessory transverse foramen (superior view) and (b) posterior bridging (lateral view) traits on the vertebrae.

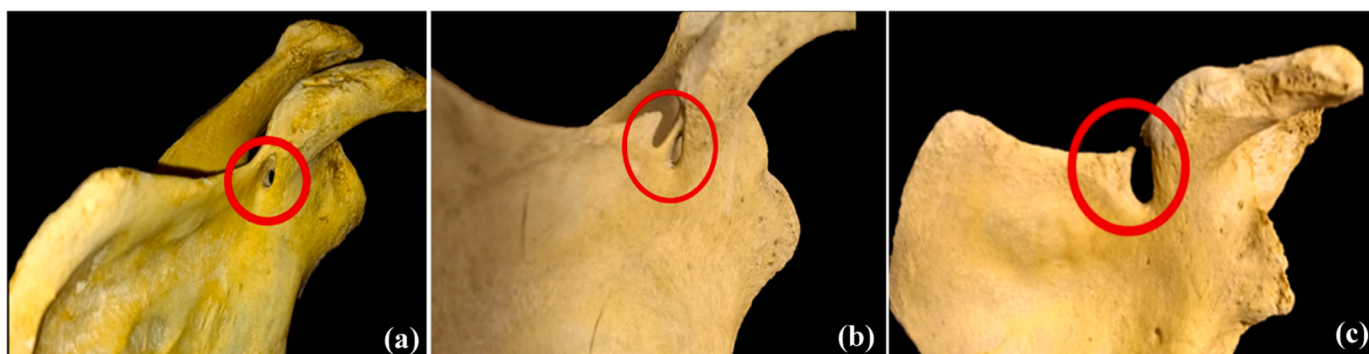


Fig. 3. Variation observed on the scapula or scapular foramen. (a) Scapular foramen below the coracoid process; (b) suprascapular notch with scapular foramen; (c) incomplete bridge over suprascapular foramen.

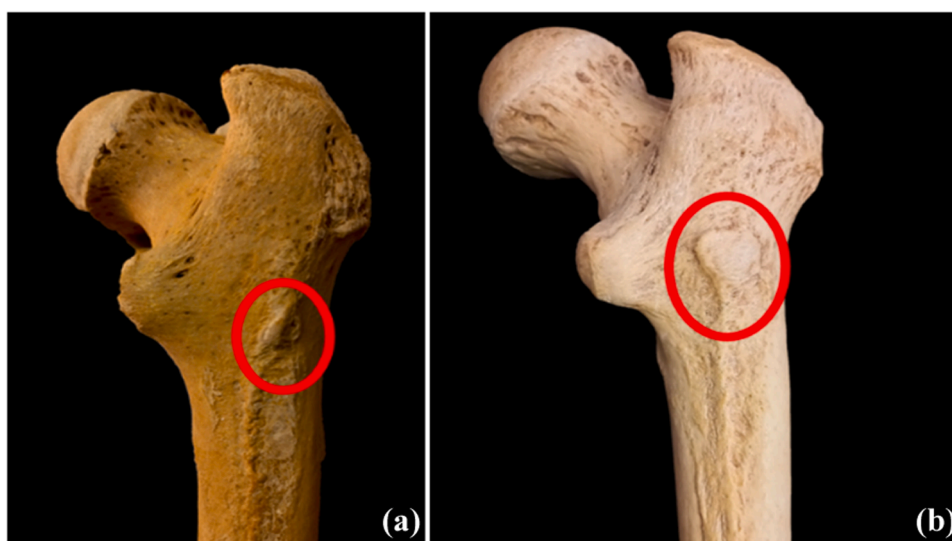


Fig. 4. Comparison of (a) enthesophyte and (b) third trochanter (posterior view).

the sexes; however, the current study noted significant differences for four of the traits. Thus, the effects of sex on the traits should be further explored. The difference in frequency distribution and statistical difference between the South African and the North American samples supports the notion that the traits are believed to have a genetic component that can be modified by epigenetic factors resulting from the environment and internal physiology [43,44]. The two samples are from different geographical environments with different population histories, which can possibly explain the variation of the trait expressions. Furthermore, with the traits showing similar patterns of variation among South Africans compared to osteometric data, which has been shown to reflect genetic relationships and heritability, postcranial MMS traits may be useful in attempting to classify population affinity [45].

Spiros and Hefner [21] produced promising results in their assessment of population affinity using combined cranial and postcranial macromorphoscopic models (with accuracies between 89.5% to 92.1%). Further research should be conducted to explore the application of the combined traits in classification models to estimate population affinity among modern South Africans. The implications of sex and asymmetry on the expression of the traits should also be evaluated.

The current study was the first to evaluate the postcranial MMS method to assess population affinity on a modern South African sample. The current study differed from the Spiros [10] study on which it was modelled by the fact that a sample with three groups was used, whereas, with the original study, only two groups were compared. Additionally, two cervical vertebrae (C5 and C6) were added for the analysis of the

accessory transverse foramen. The Spiros [10] study only assessed the frequency distribution of the traits between black and white North Americans and did not assess the predictive performance of the traits. This is likely because limited significant differences were observed in their sample. In a follow-up study, Spiros and Hefner [21] combined cranial and postcranial MMS traits and attempted to estimate population affinity using a variety of statistical methods. When employing RFM, the authors reported an 88.0% correct classification for the testing sample. While it should be acknowledged that their results are not directly comparable to the current study because of the combination of crania and postcrania, these are the only published error rates that included the postcranial MMS traits. The current study used both univariate and multivariate models, where the highest accuracy obtained was for the multivariate model that employed only variables that have been shown to differ significantly among black, white and coloured South Africans. However, the classification accuracy was fairly low with both the testing and training accuracies in the lower 60%. It has been recommended that classification methods yield accuracies at least 50% better than chance to be considered for skeletal analyses [12]. This was not the case with the postcranial MMS traits. This is likely why Spiros and Hefner [12] only present combined crania and postcrania results rather than presenting postcrania results on their own. Compared to methods currently employed in South African medicolegal casework, the postcranial MMS traits did not perform well. For example, the current craniometric standards yield accuracies of 73.0% and postcraniometric standards yield accuracies of 85.0% when using discriminant analysis on the same

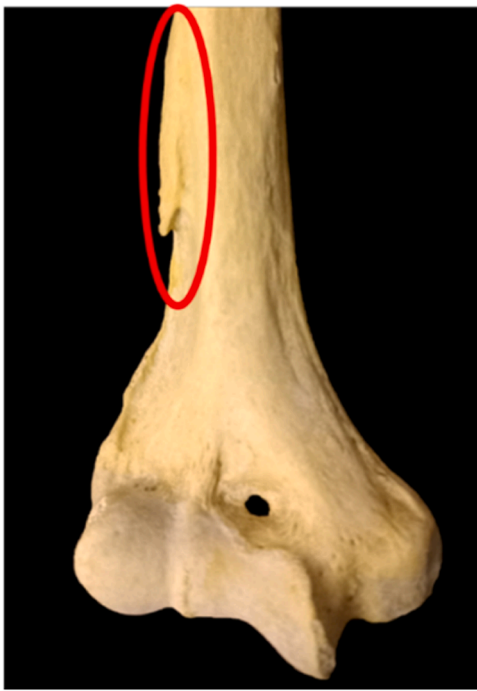


Fig. 5. A feature consistent with myositis ossificans traumatica on the lateral aspect of the humerus.

population [7,10]. Thus, the postcranial MMS traits do not outperform existing methods and should not be added to standard operating procedures simply because it is a novel method. The combination of cranial and postcranial traits does yield higher accuracies [21], thereby suggesting that the postcranial MMS method may have some potential for population affinity estimation, although further research is required.

Similar to the Spiros and Hefner [21] study, the current study observed the spinous process bifurcation to be the most discriminatory variable. However, the Kappa values for the spinous process bifurcation (C3 to C6) in the current study were much lower than the classification accuracies, which may make the trait inadequate for population affinity estimation specifically on its own. Overall, the multivariate models performed better than the univariate models. This is not unexpected and has previously been shown in other studies [10]. However, univariate analyses are also necessary to determine how each variable works on its own and if that particular variable can be used in instances where there is limited skeletal material to assess such as missing skeletal elements and fragmented remains. For the current method, the multivariate approach is recommended as the univariate models did not perform well because the classification accuracies were low. A multivariate approach is best in forensic case analysis because there is reduced bias and it encompasses more variation as multiple variables are assessed because skeletal traits are not unique to just one group [12,46–49]. The analysis of just one variable or trait in a univariate approach may exclude the variation within a population as the traits cannot be limited to one population group. In addition, traits are also dependent on their frequency distribution within a population. Assessing multiple traits makes up for a trait that may not be present on skeletal remains being assessed in forensic case analysis. There is an overlap and different groups share similarities and certain traits as previously observed in postcranial metric analyses [7]. Therefore, a combination of traits that have different patterns of overlap and dissimilarities is necessary to be able to distinguish between population groups. As such, even when limited skeletal material is available for analysis, it is recommended that as many traits from as many different methods as possible be used for classification; the use of univariate postcranial MMS traits should be a last resort.

The postcranial MMS approach satisfies the *Daubert* criteria in terms of reporting error rates and classification accuracies [50]. However, the accuracies are too low for the method to be used on its own and as it stands may only be used to confirm results obtained with another method. Future research should explore a combination of both cranial and postcranial MMS traits to estimate population affinity in South Africa.

CRediT authorship contribution statement

NP BOTHMA is the primary investigator. E.N. L'ABBÉ is the co-supervisor. L. LIEBENBERG is the main supervisor.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the EU Erasmus+ grant [597924-EPP-1-2018].

Bakeng se Afrika for providing financial support for this project.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.forsciint.2024.111954](https://doi.org/10.1016/j.forsciint.2024.111954).

References

- [1] G.C. Krüger, L. Liebenberg, J. Myburgh, A. Meyer, A.C. Oettlé, D. Botha, et al., Chapter 27 - Forensic Anthropology and the Biological Profile in South Africa, in: K.E. Latham, E.J. Bartelink, M. Finnegan (Eds.), *New perspectives in forensic human skeletal identification*, Academic Press, 2018, pp. 313–321.
- [2] L.M. Thompson. *A History of South Africa*, Third edition., Yale University Press., 2001, p. 416.
- [3] I. Ribot, Differentiation of modern sub-Saharan African populations: craniometric interpretations in relation to geography and history, *Bull. Mém Société D'Anthropologie Paris* [Internet 16 (3-4) (2004) 16. Available from: <http://journals.openedition.org/bmsap/3873>.
- [4] T. Davenport, C. Saunders, *South Afr.: A Mod. Hist.* Springer (2000) 831.
- [5] N. Patterson, D.C. Petersen, R.E. van der Ross, H. Sudoyo, R.H. Glashoff, S. Marzuki, et al., Genetic structure of a unique admixed population: implications for medical research (Available from:), *Hum. Mol. Genet* [Internet] 19 (3) (2010) 411–419, <https://doi.org/10.1093/hmg/ddp505>.
- [6] Mngadi Z., Tibane E., editors. *Official Guide to South Africa 2018/19*. 16th ed. Pretoria: Government Communications (GCIS); 2019.
- [7] L. Liebenberg, E.N. L'Abbé, K.E. Stull, Population differences in the postcrania of modern South Africans and the implications for ancestry estimation, *Forensic Sci. Int.* 257 (2015) 522–529.
- [8] L. Liebenberg, G.C. Krüger, E.N. L'Abbé, K.E. Stull, Postcraniometric sex and ancestry estimation in South Africa: a validation study, *Int. J. Leg. Med.* 133 (1) (2019) 289–296.
- [9] K.E. Stull, M.W. Kenyhercz, E.N. L'Abbé, Ancestry estimation in South Africa using craniometrics and geometric morphometrics, *Forensic Sci. Int.* (2020). Mar 26]; 245:206.e1-206.e7. Available from: <http://www.sciencedirect.com/science/article/pii/S0379073814004277>.
- [10] E.N. L'Abbé, M. Kenyhercz, K.E. Stull, N. Keough, S. Nawrocki, Application of Fordisc 3.0 to Explore Differences Among Crania of North American and South African Blacks and Whites, *J. Forensic Sci.* 58 (6) (2013) 1579–1583.
- [11] J.T. Hefner, Cranial nonmetric variation and estimating ancestry, *J. Forensic Sci.* 54 (5) (2009) 985–995, cited 2020 Apr 4].
- [12] Ousley S.D., Jantz R.L. *Fordisc 3 and Statistical Methods for Estimating Sex and Ancestry*. In: Dirkmaat DC, editor. *A Companion to Forensic Anthropology*. West Sussex: Wiley-Blackwell; 2012. p. 311–27.
- [13] E.A. Hooton, *Methods of Racial Analysis*, *Science* 63 (1621) (1926) 75–81. Available from: <https://www.jstor.org/stable/1649373>.
- [14] S. Rhine, *Non-metric skull racing*. In: *Skeletal attribution of race: Methods for forensic anthropology*, Maxwell Museum of Anthropology, 1990, pp. 9–20.
- [15] P.L. Walker, Greater sciatic notch morphology: sex, age, and population differences, *Am. J. Phys. Anthr.* 127 (4) (2005) 385–391. Available from: (<http://onlinelibrary.wiley.com/doi/abs/10.1002/ajpa.10422>).

- [16] P.L. Walker, Sexing skulls using discriminant function analysis of visually assessed traits, *Am. J. Phys. Anthr.* [Internet] 136 (1) (2008) 39–50. Available from: (<http://onlinelibrary.wiley.com/doi/abs/10.1002/ajpa.20776>).
- [17] A.R. Klales, S.D. Ousley, J.M. Vollner, A revised method of sexing the human innominate using Phenice's nonmetric traits and statistical methods, *Am. J. Phys. Anthr.* 149 (1) (2012) 104–114.
- [18] J.T. Hefner, S.D. Ousley, Statistical classification methods for estimating ancestry using morphoscopic traits, *J. Forensic Sci.* [Internet] 59 (4) (2014) 883–890. Available from: (<http://onlinelibrary.wiley.com/doi/abs/10.1111/1556-4029.12421>).
- [19] A.R. Klales, M.W. Kenyhercz, Morphological assessment of ancestry using cranial macromorphoscopies, *J. Forensic Sci.* [Internet] 60 (1) (2015) 13–20. Available from: (<https://onlinelibrary.wiley.com/doi/abs/10.1111/1556-4029.12563>).
- [20] M.C. Spiros, Standardization of postcranial nonmetric traits and their utility in ancestry analysis, *Forensic Anthr.* [Internet] 2 (1) (2019) 29–44, 29–44. Available from: (<http://journals.upress.ufl.edu/fa/article/view/747>).
- [21] M.C. Spiros, J.T. Hefner, Ancestry estimation using cranial and postcranial macromorphoscopic traits, *J. Forensic Sci.* 65 (3) (2020) 921–929.
- [22] E.N. L'Abbé, C. Van Rooyen, S.P. Nawrocki, P.J. Becker, An evaluation of non-metric cranial traits used to estimate ancestry in a South African sample, *Forensic Sci. Int.* 209 (2011) 195.e1–195.e7.
- [23] E.N. L'Abbé, M. Loots, J.H. Meiring, The Pretoria bone collection: a modern south african skeletal sample, *Homo Int Z. Vgl Forsch. Am. Mensch* 56 (2) (2005) 197–205.
- [24] E.N. L'Abbé, G.C. Krüger, C.E.G. Theye, A.C. Hagg, O. Sapó, The Pretoria bone collection: a 21st century skeletal collection in South Africa, *Forensic Sci.* [Internet] 1 (3) (2021) 220–227. Available from: (<https://www.mdpi.com/2673-6756/1/3/20>).
- [25] A. Alblas, L.M. Greyling, E.M. Geldenhuys, Composition of the Kirsten Skeletal Collection at Stellenbosch University, *South Afr. J. Sci.* [Internet] 114 (1–2) (2018) 1–6. Available from: http://www.scielo.org.za/scielo.php?script=sci_abstract&pid=S0038-23532018000100012&lng=en&nrm=iso&tng=en.
- [26] National Health Act. National Health Act 61 of 2003 | South African Government Gazette [Internet]. Sect. 62 2003 p. 66. Available from: (<https://www.gov.za/documents/national-health-act>).
- [27] J. Verzani, *Getting Started with RStudio*, O'Reilly Media, Inc., 2011, p. 97.
- [28] J.R. Landis, G.G. Koch, The measurement of observer agreement for categorical data, *Biom.* [Internet] 33 (1) (1977) 159–174. Available from: <https://www.jstor.org/stable/2529310>.
- [29] L. Breiman, Random Forests (Available from:), *Mach. Learn* [Internet] 45 (1) (2001) 5–32, <https://doi.org/10.1023/A:1010933404324>.
- [30] G. Biau, *Anal. a Random For. Model* 33 (2012).
- [31] J.T. Hefner, M.K. Spradley, B. Anderson, Ancestry assessment using random forest modeling, *J. Forensic Sci.* [Internet] 59 (3) (2014) 583–589. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/1556-4029.12402>.
- [32] D. Navega, C. Coelho, R. Vicente, M.T. Ferreira, S. Wasterlain, E. Cunha, Ancestrees: ancestry estimation with randomized decision trees, *Int J. Leg. Med* 129 (5) (2015) 1145–1153.
- [33] C. Maier, Evaluating mixed-methods models for the estimation of ancestry from skeletal remains, *Forensic Anthr.* [Internet] 2 (1) (2019) 45–56. Available from: (<https://www.proquest.com/docview/2232215078/abstract/8B935163ECA7488DPQ/1>).
- [34] Wilczak C.A., Mariotti V., Pany-Kucera D., Villotte S., Henderson C.Y. Training and interobserver reliability in qualitative scoring of skeletal samples. *J Archaeol Sci Rep* [Internet]. 2017 Feb 1 [cited 2022 Oct 3];11:69–79. Available from: (<http://www.sciencedirect.com/science/article/pii/S2352409X16307507>).
- [35] A. Klales, Current state of sex estimation in forensic anthropology, *Forensic Anthr.* 4 (2) (2022) 118–127 (Available from:), (<https://journals.upress.ufl.edu/fa/article/view/1415>).
- [36] J.L. Voisin, M. Ropars, H. Thomazeau, Anatomical evidence for a uniquely positioned suprascapular foramen (Available from:), *Surg. Radio. Anat.* [Internet] 38 (4) (2016) 489–492, <https://doi.org/10.1007/s00276-015-1558-9>.
- [37] A. Hrdlička, The scapula: visual observations, *Am. J. Phys. Anthr.* [Internet] 29 (1) (1942) 73–94. Available from: (<https://onlinelibrary.wiley.com/doi/abs/10.1002/ajpa.1330290107>).
- [38] A.J. Thorndike, Myositis ossificans traumatica, *JBJS* [Internet] 22 (2) (2022) 315–323. Available from: https://journals.lww.com/jbjsjournal/abstract/1940/22020/myositis_ossificans_traumatica.5.aspx.
- [39] Beiner J.M., Jokl P. Muscle Contusion Injury and Myositis Ossificans Traumatica. *Clin Orthop Relat Res* 1976–2007 [Internet]. 2002 Oct [cited 2022 Aug 2];403: S110. Available from: https://journals.lww.com/corr/Abstract/2002/10001/Muscle_Contusion_Injury_and_Myositis_Ossificans.13.aspx.
- [40] K.E. Stull, M.W. Kenyhercz, M.L. Tise, E.N. L'Abbé, P. Tuamsuk, Chapter 13 - The Craniometric Implications of a Complex Population History in South Africa. In: Pilloud MA, Hefner JT, editors. *Biological Distance Analysis*, San Diego: Academic Press, 2016, pp. 245–263.
- [41] Sofer C. Some Aspects of Inter-Racial Marriages in South Africa, 1925–461. *Africa* [Internet]. 1949 Jul [cited 2022 Aug 3];19(3):187–203. Available from: <https://www.cambridge.org/core/journals/africa/article/abs/some-aspects-of-interracial-marriages-in-south-africa-1925461>.
- [42] Hyslop J. White Working-class Women and the Invention of Apartheid: 'Purified' Afrikaner Nationalist Agitation for Legislation Against 'Mixed' Marriages, 1934–9. *J Afr Hist* [Internet]. 1995 Mar [cited 2022 Aug 3];36(1):57–81. Available from: <https://www.cambridge.org/core/journals/journal-of-african-history/article/abs/white-working-class-women-and-the-invention-of-apartheid-purified-afrikaner-nationalist-agitation-for-legislation-against-mixed-marriages-19349/0B10E8B54AC60671ADC86E940FB98A23>.
- [43] S.R. Saunders, Nonmetric skeletal variation, *Reconstr. Life Skelet.* [Internet] (1989) 95–108 (Available from:), (<https://ci.nii.ac.jp/naid/10003963475/>).
- [44] Mann R.W., Hunt D.R. Non-metric traits and anatomical variants that can mimic trauma in the human skeleton. *Forensic Sci Int* [Internet]. 2019 Aug 1 [cited 2020 Sep 24];301:202–24. Available from: <http://www.sciencedirect.com/science/article/pii/S0379073819302233>.
- [45] S. Ousley, R. Jantz, D. Freid, Understanding race and human variation: why forensic anthropologists are good at identifying race, *Am. J. Phys. Anthr.* [Internet] 139 (1) (2009) 68–76. Available from: (<http://onlinelibrary.wiley.com/doi/abs/10.1002/ajpa.21006>).
- [46] H.R. Maier, G.C. Dandy, Determining inputs for neural network models of multivariate time series, *Comput.-Aided Civ. Infrastruct. Eng.* [Internet] 12 (5) (1997) 353–368. Available from: (<https://onlinelibrary.wiley.com/doi/abs/10.1111/0885-9507.00069>).
- [47] M.A. Frelat, P. Mittereöcker, Postnatal ontogeny of tibia and femur form in two human populations: a multivariate morphometric analysis, *Am. J. Hum. Biol. J. Hum. Biol. Counc.* 23 (6) (2011) 796–804.
- [48] B.G. Tabachnick, L.S. Fidell, J.B. Ullman. *Using multivariate statistics*, Seventh edition., Pearson, NY, NY, 2019, p. 832.
- [49] E. Alladio, B. Poggiali, G. Cosenza, E. Pilli, Multivariate statistical approach and machine learning for the evaluation of biogeographical ancestry inference in the forensic field, *Sci. Rep.* [Internet] 12 (1) (2022) 8974. Available from: (<https://www.nature.com/articles/s41598-022-12903-0>).
- [50] Grivas C., Komar D. Kumho, Daubert, and the Nature of Scientific Inquiry: Implications for Forensic Anthropology. *J Forensic Sci.* 2008 Aug 1;53:771–776.