

The Casado (2017) method for sex estimation of contemporary brazilian skulls: a validation study

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Editor: Dr. Altair A. Del Bel Cury

Received: September 09, 2023

Accepted: March 14, 2024

Casado (2017) described a low-cost and practical method for sex estimation of adult skulls using precision calipers to evaluate anatomical landmarks. **Aim:** This study aimed to validate this method in Brazilian skulls. **Methods:** Measurements of 202 skulls (118 male and 84 female) were collected, according to Casado (2017). **Results:** The results were very promising. Except for the external occipital protuberance, the anatomical landmarks were related to sex ($p < 0.0001$). The anatomical landmarks from both sexes were included, and 72.16% of the skulls were correctly classified. The individual percentage per sex was similar, with 71.40% accuracy in male skulls and 72.92% in female skulls. **Conclusion:** The method efficiently identified sex differences in anatomical landmarks of Brazilian skulls regardless of age and ancestry.

Keywords: Forensic sciences. Craniometry. Forensic anthropology. Skull. Sex differences.



Introduction

The worldwide population increased from one to six billion over the past 200 years, especially in urban areas. The number of deaths caused by diseases, natural disasters, and violent crimes also increased, highlighting the importance of person identification using their bones¹.

Currently Brazil has over 210 million inhabitants and high miscegenation, which provides heterogeneous characteristics in the population, hindering forensic anthropology^{2,3}. Miscegenation provided heterogeneous characteristics in the Brazilian population, making forensic anthropology challenging in this population³. Moreover, studies indicate secular skull changes in other populations⁴⁻⁸ however, whether these changes affected the Brazilian population is unclear.

The efficacy of sex estimation using only anatomical landmarks is not a consensus⁹. However, the skull is considered one of the best tools for sex estimation in the lack of pelvic or postcranial elements. The skull is resistant to adverse conditions and can be easily submitted to quantitative evaluations^{3,10-12}. In addition, the best way to achieve efficient sex estimation is by using many variables¹³⁻¹⁵.

The usual method to identify sex from adult skulls comprises a quantitative or visual analysis of skeletal traits using an ordinal scale; its accuracy relies on a trained evaluator. Imaging tests are also widely used to study sexual dimorphism with high accuracy¹⁶⁻¹⁸. However, the high-cost equipment hinders access in many forensic analysis institutes^{19,20}.

Simple, low-cost, and accessible techniques (e.g., precision calipers) for sex estimation that requires little training have demonstrated high reliability²¹. Casado¹¹ (2017) proposed a low-cost and easy-to-replicate method using precision calipers compared with computed tomography. In Brazil, low-cost methods for sex estimation are important due to the miscegenation and varied socioeconomic characteristics between regions. Therefore, this study aimed to test and validate the method proposed by Casado¹¹ to identify sexual dimorphism in Brazilian skulls.

Materials and Methods

This cross-sectional study was conducted at the Skull Museum of the Federal University of São Paulo, Brazil. Ethical approval was obtained from the University's Ethics and Research Committee (no.: 3.035.353/2018).

Sample

Dry adult skulls with complete biological profiles (sex, age, and ancestry) were included. Those with fragmentation or trauma that hindered or could alter the measurements were excluded. From the original sample (n = 403), only 202 skulls (118 male and 84 women) were selected for analysis (Table 1).

Table 1. Biological characteristics of the sample.

Sex	Frequency	%
Male	118	58.42
Female	84	41.58
Total	202	100.00
Age	Mean	SD
Male	42.95	12.75
Female	40.75	16.33
Total	42.05	14.32
Ancestry	Frequency	%
White	90	44.55
Black	112	55.45
Total	202	100.00

SD = Standard deviation.

The skulls were from deaths documented between 1933 and 1970, representing changes in a modern population^{4-6,8,22}.

A precision compass (Mitutoyo®, São Paulo, Brazil) was used to measure the linear distances between the anatomical landmarks to determine the external projection of each trace. A digital depth caliper (Digimess®, São Paulo, Brazil) was used to measure the depth of bony protuberances and capture the three-dimensional nature of an anatomical landmark¹¹.

Procedures

Twelve measurements were performed on six anatomical landmarks: three sites of muscle attachment (external occipital protuberance, nuchal protuberance [right {R}, left {L}, and center], and mastoid process [R and L]) and three anatomical landmarks evaluated due to hormonal differences (glabella, supraorbital ridge [R and L], and frontal boss [R, L, and center])¹¹. The skulls were fixed on specific support, and one evaluator performed all measurements. For each measurement, the evaluator moved the depth compass (i.e., the central arm) to find the most protruding point of each anatomical landmark to determine the external projection. The measurements were systematized and followed the order described in Table 2.

Table 2. Anatomical landmarks.

Anterior View
From point A to point B (Casado, 2017)
1R - Right supraorbital ridge
1L - Left supraorbital ridge
2 - Glabella

Continue

Continuation
From bregma to frontomolare temporale
3R - Right frontal boss
3L - Left frontal boss
From bregma to nasion
3C - Central frontal boss
Posterior View
From lambda to opisthion
4 - External occipital protuberance
From lambda to mastoid process groove
5R - Right nuchal protuberance
5L - Left nuchal protuberance
From inion to opisthion
5C - Central nuchal protuberance
Lateral View
From external auditory meatus to mastoid process groove
6R - Right mastoid process
6L - Left mastoid process

Statistical analysis

The Shapiro-Wilk test analyzed the data distribution. The Mann-Whitney verified the relationship between sex and skull anatomical landmarks measurements. The multivariate analysis using discriminant functions was applied to find which group the skulls belonged to (0 = male; 1 = female). The Chi-square test analyzed the association between the results and the expected distribution. The analyses were performed in GraphPad Prism®, and the significance was set at $p < 0.05$.

To set the cut-off point for sex estimation accuracy, we considered the mean, standard deviation, and value range of each sex. The cut-off point was used to estimate the sex for each measurement. Then, the estimative was divided by the correct number of skulls from each sex. The same calculus was made for the overall classification.

Results

The sample was not normally distributed. Except for the external occipital protuberance, the anatomical landmarks were related to sex (Table 3, Figure 1).

Table 3. Descriptive statistics by anatomical landmarks.

	Male (n = 118)				Female (n = 84)			
	Mean	SE	W	R	Mean	SE	W	R
Right supraorbital ridge	21.11 ± 3.26	0.2981	0.898	< 0.0001	25.46 ± 3.77	0.406	0.823	< 0.0001
Left supraorbital ridge	21.56 ± 3.4	0.3108	0.900	< 0.0001	26.37 ± 3.8	0.410	0.807	< 0.0001

Continue

Continuation								
Glabella	20.86 ± 3.53	0.3225	0.899	< 0.0001	23.07 ± 3.52	0.379	0.845	< 0.0001
Right frontal boss	22.19 ± 3.36	0.307	0.897	< 0.0001	22.74 ± 3.59	0.387	0.854	< 0.0001
Left frontal boss	23.12 ± 3.57	0.3258	0.864	< 0.0001	23.62 ± 3.63	0.392	0.762	< 0.0001
Central frontal boss	13.91 ± 3.06	0.2796	0.976	0.0311	14.42 ± 3.06	0.330	0.971	0.0498
External occipital protuberance	12.52 ± 3.73	0.3405	0.986	0.2618	14.93 ± 3.82	0.412	0.987	0.5127
Right nuchal protuberance	20.70 ± 3.45	0.3154	0.934	< 0.0001	22.08 ± 3.8	0.410	0.889	< 0.0001
Left nuchal protuberance	20.58 ± 3.63	0.3319	0.955	0.0006	21.46 ± 3.59	0.388	0.901	< 0.0001
Central nuchal protuberance	36.60 ± 4.11	0.3754	0.604	< 0.0001	36.53 ± 4.22	0.455	0.459	< 0.0001
Right mastoid process	29.98 ± 3.3	0.3009	0.624	< 0.0001	30.94 ± 3.59	0.387	0.477	< 0.0001
Left mastoid process	30.16 ± 3.29	0.3004	0.620	< 0.0001	31.43 ± 3.68	0.397	0.498	< 0.0001

Mean ± standard deviation in millimeters; SE = standard error; W = Shapiro-Wilk (alpha = 0.05); R = Mann-Whitney (p < 0.05).

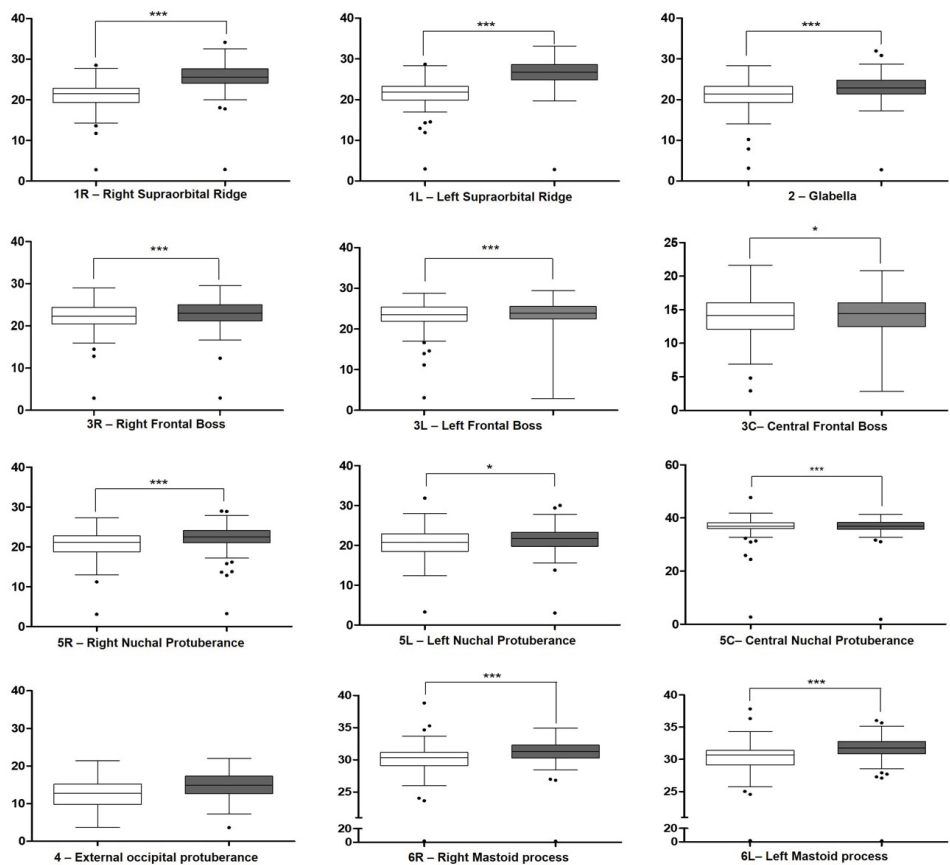


Figure 1. Boxplot of Shapiro-Wilk distribution. White boxes represent male skulls and gray boxes represent female skulls. ***p < 0.001; *p < 0.05.

The measures differed between the sexes ($p=0.0009$), showing that the method effectively identified a sexual dimorphism in the skulls (Table 4).

Table 4. Chi-square test comparing male and female skulls.

Function test	Chi-square	df	P
1	10.96	1	0.0009

In the general classification, we found an overall accuracy of 72.16%, including all anatomical landmarks. Moreover, the accuracy in each group was similar (71.40% accuracy in male and 72.92% in female skulls) (Table 5).

Table 5. Overall classification of the skulls. The numbers in bold indicate the percentage of correct identification of each sex.

	Planned participation in the group		
	Male	Female	Total
Original count			
Male	61	57	118
Female	23	61	84
%			
Male	71.40	28.6	100
Female	27.08	72.92	100

72.16% of the original cases grouped were correctly classified.

The accuracy values were separated by anatomical landmark and sex (Table 6); values varied between 64.30% and 82.20%. The anatomical landmarks that least identified the sexual dimorphism were the external occipital protuberance on male skulls (66.10%) and the left supraorbital ridge on female skulls (64.30%). Additionally, the ones that most identified it were the central nuchal protuberance (82.20%) on male skulls and the left frontal boss (82.10%) on female skulls.

Table 6. Percentage of correct identification by variable.

	Male (n = 118)	Female (n = 84)
1R - Right supraorbital ridge	68.6	75.0
1L - Left supraorbital ridge	76.3	64.3*
2 - Glabella	72.0	71.4
3R - Right frontal boss	69.5	70.2
3L - Left frontal boss	71.2	82.1**

Continue

Continuation		
3C - Central frontal boss	72.0	66.7
4 - External occipital protuberance	66.1*	70.2
5R - Right nuchal protuberance	67.8	75.0
5L - Left nuchal protuberance	72.0	75.0
5C - Central nuchal protuberance	82.2**	75.0
6R - Right mastoid process	70.3	72.6
6L - Left mastoid process	68.6	77.4

* Lower accuracy; ** Higher accuracy.

Discussion

Although studies indicated secular changes in the skulls^{4,8}, whether they affected the sexual dimorphism in Brazilian skulls is unclear. The evaluated adult skulls were similar to those analyzed by Casado¹¹ (2017). However, our sample included skulls from mixed-race people due to the miscegenation of the Brazilian population from immigration or migratory movements^{2,3}. Thus, validating a method in Brazilian skulls must consider every ethnic group.

Although Brazilians have varied ethnic groups, their skulls exhibit similar sexual dimorphism. Studies stated that most differences exist within geographic populations, not between them²³⁻²⁵. However, Brazilian forensic anthropologists suggested that bones must be analyzed in each population to identify sexual differences, considering the notorious level of variation and miscegenation of contemporary human beings^{1,3,12,26}.

The methods used to determine the external projection of anatomical landmarks can be subjective or objective. Subjective methods, such as visual inspection, relies on an experienced evaluator, taking time and being costly. Objective methods use measurements to quantify the anatomical landmarks and compare intra- and inter-populations. In the latter, the geometric analysis of photographs and imaging tests depend on specific software, computers, and specialized evaluators. Imaging tests are high-priced and far from reality in most forensic institutes.

In this study, the depth caliper measured and captured the three-dimensional nature of the anatomical landmarks of the skulls. Our results showed that using calipers is simple and effective and achieves values similar to traditional methods (visual inspection or imaging) in adult skulls, they are affordable, easy, and do not require specific training or long-time experience, making their use accessible to identify sexual dimorphism^{3,12,21,26-28}. Although some studies separated the skulls into ethnic groups, this study considered all skulls as a single group.

In this study, the sex estimation accuracy was similar to the values found by Casado¹¹ (2017) (71.4% *versus* 69.6% for men; 72.9% *versus* 74.7% for women, respectively). The overall classification (72.20%) was also the same as presented by Casado¹¹ (2017), suggesting high accuracy and reliability of the method. Walker²⁵ (2008) obtained sim-

ilar results using visual inspection (78% accuracy for Native Americans and between 84% and 88% for Non-natives). In addition, Garvin et al.²⁹ (2014) reported 82% accuracy for the overall sample and between 63% and 79% for each group, Krüger et al.³⁰ (2015) found between 68% and 84% in the overall sample (without considering ancestry), and Godde et al.³¹ (2018) 80% accuracy in the overall sample.

Another method for sex estimation is the analysis of the geometry of anatomical landmarks in photographs. Nikita and Michopoulou¹³ (2018) reported accuracy of 85.10% and 94.60% in identifying male and female skulls, respectively. Also, Nikita³² (2019) reported 87% accuracy for the overall sample. Both studies used Greek skulls, a population with little miscegenation, possibly justifying the high accuracy. González-Colmenares et al.³³ (2019) used the same technique in radiographs of Colombian skulls and found 88.60% accuracy. Other authors performed the same technique using computed tomography: Kimmerle et al.¹⁶ (2008) reported 77.80% accuracy; Bulut et al.³⁴ (2016), 77.50%; and Čechová et al.³⁵ (2021), 72.80%. All values were similar to ours (72.20%), suggesting the reliability of our results and allowing method comparison.

Petaros et al.¹⁵ (2021), examined the volume of mastoid processes in Medieval Croatian skulls using visual inspection, precision calipers, and geometric analysis and obtained 67.1%, 69.4%, and 81.0% accuracy, respectively. This study was relevant to understand the contribution of each method. The more qualitative and quantitative information we can extract from the skulls, the greater the identification accuracy, greater population specificity susceptible to secular changes, and lesser challenge in the judicial sphere once judicial precedents require accurate and quantifiable methods to be presented in forensic testimony¹². Therefore, we believe that the method proposed by Casado¹¹ (2017) has high admissibility in court.

In conclusion, although the method proposed by Casado was developed for American skulls, we found similar measure values in Brazilian skulls. Moreover, the method efficiently classified the sexual dimorphism of Brazilian skulls, regardless of age and ancestry.

Ethical Approval

The research protocol was submitted for evaluation and approved by the Ethical Committee of the Federal University of São Paulo São Paulo, Brazil (nº 3.035.353/2018). The stages of the study were carried out according to the approved guidelines.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article and no external financial support.

Data availability

Datasets related to this article will be available to the corresponding author upon request.

Author Contribution

Silvio Antonio Garbelotti Junior: project conception and design, drafting the article, analysis and interpretation of data, final approval of the version to be published. **Rodrigo Barbosa de Souza:** analysis and interpretation of data, statistical analysis, final approval of the version to be published. **Marco Antonio de Angelis:** critical review of content, final approval of the version to be published. **Sérgio Ricardo Marques:** critical review of content, drafting the article, final approval of the version to be published. **Ricardo Luiz Smith:** project conception and design, analysis and interpretation of data, critical review of content, final approval of the version to be published. All authors actively revised and approved the final version of the manuscript.

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