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Sex estimation based on glabella morphology in contemporary Brazilian human skulls

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Abstract

Background Estimating sex from a dry skull depends on studying validated and specific traits. One such reference area exhibiting sexual dimorphism is the glabella, located on the frontal bone. This anatomical landmark is easily classified and shows promising results in sexual identification due to its magnitude variations between sexes. The present study is methodologically based on Walker's approach, which visually analyzes cranial traits and scores them according to their morphology. This study aimed to investigate the sex estimation of dry skulls from Brazilian human skeletons for human identification through macroscopic analysis of the glabella. This analytical, quantitative, cross-sectional study utilized a sample of 471 skulls from both sexes, aged 25 years or older, belonging to the contemporary collection of the Center for Studies in Forensic Anthropology at the Faculty of Dentistry, University of Pernambuco (CEAF/FOP/UPE), Brazil. Data were recorded in an Excel spreadsheet and subsequently subjected to statistical analysis using IBM© SPSS (version 22.0).

Results Initial observation of the skeletons excluded 52 (11.0%) due to age below 25 years, absence of a skull, or compromised glabella region, resulting in a final sample of 419 skulls. The mean age was 66.6 years, with a median of 67, a minimum of 25, and a maximum of 109 years. Regarding glabella types, score 2 showed the highest frequency (35.6%), while score 5 had the lowest percentage (7.9%). A statistically significant association was found between female sex and glabella scores 1 and 2. Also, individuals aged over 60 presented more glabella scores 1 and 2. The sex estimation accuracy for the sample was 76.1%, with females having a higher percentage of correct observations than males.

Conclusions The morphology of the glabella provides useful information for estimating sex in the biological profile. However, further studies on this morphological trait in other Brazilian osteological collections are encouraged to establish a national profile and contribute to human identification procedures in the country.

Keywords Human identification, Forensic Anthropology, Sexual dimorphism, Glabella

Background

Forensic dentistry has gained prominence in justice and forensic anthropology (Lopes 2020; Trevisol et al. 2021). This specialized field plays a crucial role in criminal investigations, forensic thanatology, and human identification. Its purpose extends to the examination of physical, chemical, biological, and psychological signs

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related to individuals in various states —whether living, deceased, or skeletal remains (Coutinho et al. 2013).

In cases of human identification, the condition of the body (such as charred, dismembered, or significantly decomposed) significantly influences the methods employed. Consequently, the forensic odontologist assumes a pivotal role in analyzing skeletal skulls, as this study contributes to species identification and provides estimates of sex, stature, age, and population affinity for the individual (Silveira 2008). Given the versatility of cranial characteristics, this field has witnessed global growth (Nunes, Gonçalves, 2014).

The study of cranial morphology reveals distinct variations between sexes, facilitating the identification and comparison of features unique to male and female skulls (Trevisol et al. 2021). Commonly, male skulls exhibit more robust and pronounced characteristics, while female skulls display delicate and less prominent structures (Mestekova et al. 2015). This morphological difference underscores the advantage of utilizing cranial techniques in identification processes (Trevisol et al. 2021).

Forensic anthropology has adeptly adapted to the changes brought about by globalization (Cunha 2017). Despite technological advancements and the development of metric techniques, visually assessing sexually dimorphic skull traits remains recognized and reliable. Visual analysis offers advantages such as ease of data collection, cost-effectiveness, and practicality in handling fragmented forensic findings (Walker 2008).

Estimating sex from a dry skull depends on studying validated and specific traits. One such reference area exhibiting sexual dimorphism is the glabella, located on the frontal bone (Nikita & Michopoulou 2018; Walker, 2023). This anatomical landmark is easily classified and shows promising results in sexual identification due to its magnitude variations between sexes (Perlaza 2014; Rosa 2020).

The present study is methodologically based on Walker's approach (2008), which visually analyzes cranial traits and scores them according to their morphology. Among the structures examined by Walker (2008), the glabellar area demonstrated significant sexual dimorphism, with a high percentage of correct sex attributions. For instance, a study conducted by Carvalho et al. (2017) analyzed the characteristics of 66 skulls using Walker's method (2008), where the glabella emerged as one of the most visually dimorphic regions. The results provided strong sex indicators through this method and underscored the importance of further analyses due to anthropological variations across different populations.

Brazil boasts a highly heterogeneous population with Indigenous, African, European, Asian, and Arab ancestries, among others. However, studies are scarce in the country regarding sexual estimation methods based on macromorphoscopic examination of skulls (Rosa 2020). Therefore, research aimed at sex estimation and cataloging cranioscopic traits within the Brazilian population is relevant and contributes to forensic science in Brazil (Nunes & Gonçalves 2014).

Because studies investigating cranial traits in Brazil are still scarce when compared to other countries, the present study aims to evaluate glabellar morphology in identified skulls from Northeast Brazil, building upon Walker's studies (2008), to establish the glabellar profile and study sexual dimorphism in this sample.

Methods

The study was conducted at the Center for Forensic Anthropology (CEAF), located at the Faculty of Dentistry of Pernambuco (FOP/UPE) in Recife, Pernambuco, Brazil. The CEAF/FOP/UPE collection comprises 471 identified Brazilian skeletons of both sexes (227 female and 244 male) aged 0 to 109 years.

For the present study, the glabellae of all adult individuals aged 25 years or older ($n=419$), regardless of sex, from the osteological collection at CEAF/FOP/UPE were macroscopically analyzed and classified according to the scoring system established by Walker (2008). The scores range from 1 to 5, with scores 1 and 2 corresponding to feminine characteristics (non-prominent or very discreet glabellae), scores 4 and 5 representing male morphological features (pronounced or very pronounced glabellae), and score 3 indicating an intermediate zone between the mentioned scores (Fig. 1). Skulls exhibiting traumatic injuries, bone substance loss due to taphonomy, anomalies, or pathologies that could compromise their study were excluded from this research.

A preliminary training phase was conducted to standardize the observations of the glabellae. During this phase, the examiner's observations were initially compared with the results of an evaluation performed by an examiner experienced in forensic anthropological expertise, which was considered the gold standard. An excellent level of agreement was observed between both examiners, with an inter-examiner Kappa value of 0.949. After 15 days of data collection, the researcher re-examined 40 skulls, repeating their analysis in the subsequent month, obtaining an intra-examiner Kappa value of 0.972. Landis & Koch (1977) developed a six-level scale to interpret Kappa values, where values of zero are considered as no agreement; 0.00 to 0.20, as slight agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; and values above 0.81, as almost perfect agreement. The maximum acceptable intra-observer error for the present study was 10%,

Fig. 1 Illustration of the Walker's (2008) scores adopted for this study. Scores 1 to 5, from left to right. Source: CEAF/FOP/UPE Collection

even considering that this was the researcher's first experience in the anthropological analysis of human skulls. Data collection was conducted blindly by the researcher, based on the registration number, without prior knowledge of the skeleton's sex. The data collection sheet for the study only included the skeleton's registration number. Only after data collection did the researcher request information from the collection's coordination regarding the sex of the examined skulls.

The collected data were entered into an IBM© SPSS spreadsheet (version 22.0), where both descriptive and inferential statistics were performed. Pearson's chi-squared tests were applied to assess associations between variables, along with correlation and logistic regression analysis. Diagnostic performance was evaluated, and percentages of accuracy, specificity, sensitivity, precision, and area under the curve were reported. The significance level (p-value) for statistical tests was set at 5%.

Results

A total of 471 skeletons were examined. Among these, 52 (11.0%) were excluded due to their age being below 25 years, absence of a skull, or presence of glabellar bone trauma or substance loss that would compromise analysis. The final sample ($n=419$) exhibited a balanced composition, with a slight predominance of female individuals ($n=213$; 50.8%) compared to males ($n=206$; 49.2%).

Age information was available for 379 skeletons in the studied sample, revealing an average age of 66.6 years (dp = 16.6), a median of 67, a mode of 62, a minimum age of 25, and a maximum age of 109 years. Regarding glabellar types in the total sample, those classified as type 2 showed the highest frequency (35.6%), followed by type 1, as detailed in Table 1.

The Chi-square test was used to check whether there was an association between sex and the type of glabella. Cramer's V was also used to measure the strength of the association between the variables analyzed, bearing in mind that the stronger the association, the closer the test result is to 1. It was found that glabella type 1 was more strongly associated with females, with a statistically significant difference between these two variables, as shown in Table 2.

Table 1 Distribution of the sample, in percentage and absolute values, according to glabella classification

Classification	Frequency	Percentage
1	132	31.5
2	149	35.6
3	51	12.2
4	54	12.9
5	33	7.9
Total	419	100.0

Table 2 Investigation of the association between the variables sex and glabella classification

Classification	Sex		Total *N*(%)
	Male *N*(%)	Female *N*(%)	
1	14 (3.3)	118 (28.2)[a]	166 (31,5)
2	69 (16.5)	80 (19.1)	149 (35.6)
3	41 (9.8)	10 (2.4)	51 (12.2)
4	52 (12.4)	2 (0.5)	54 (12.9)
5	30 (7.2)	3 (0.7)	33 (7.9)
Total	206 (49.2)	213 (50.8)	419 (100.0)

[a] Pearson's Chi-square: 170.0; $p<0.001$. Cramer's V: 0.637

In this study, the Spearman test, a non-parametric measure of the strength of the relationship between two variables, was applied to assess the correlation between glabellar type and sex. The correlation coefficient can range from -1 to + 1. In Spearman correlation, an absolute value of 1 indicates perfectly linear data, meaning that a higher value of one variable is associated with a higher value of another variable. Conversely, an absolute value of -1 in Spearman correlation indicates that

Table 3 Investigation of the correlation between the variables sex and glabella

			Sex	Glabella classification
Spearman's rho (ρ)	**Sex**	Correlation Coefficient	1.000	0.631[a]
		Sig. (2 extremities)	-	0.000
		N	419	419
	Glabella classification	Correlation Coefficient	0.631	1.000
		Sig. (2 extremities)	0.000	-
		N	419	419

[a] 95% CI: Minimum: 0.569; Maximum: 0.685

Table 4 Distribution of the sample according to the categorization of the glabella classification

Categorization	Frequency	Percentage
Glabelas 1 and 2	281	67.1
Glabelas 4 and 5	87	20.7
Undetermined	51	12.2
Total	419	100.0

Table 5 Distribution of the sample according to the categorization of the glabella classification, by sex

Categorization	Sex		Total *N*(%)
	Male *N*(%)	Female *N*(%)	
Glabellas 1 and 2	83 (22.5)	198 (53.8)[a]	281 (76.4)
Glabellas 4 and 5	82 (22.3)	5 (1.4)	87 (23.6)
Total	165 (44.8)	203 (55.2)	368 (100.0)

[a] Pearson's Chi-square: 112.5; $p < 0.001$. Cramer's V: 0.553

Table 6 Investigation of the correlation between the sex and categorized glabella

			Sex	Categorized glabella
Spearman's rho (ρ)	**Sex**	Correlation Coefficient	1.000	0.553[a]
		Sig. (2 extremities)	-	<0.001
		N	368	368
	Categorized glabella	Correlation Coefficient	0.553	1.000
		Sig. (2 extremities)	<0.001	-
		N	368	368

[a] 95% CI: Minimum: 0.478; Maximum: 0.620

Table 7 Distribution of the sample according to the categorization of the glabella classification, by age categories

Age	Categorization		Total *N*(%)
	Glabellas 1 and 2 *N*(%)	Glabellas 4 and 5 *N*(%)	
25 to 40 years	13 (5.1)	10 (12.8)	23 (6.9)
41 to 60 years	53 (20.9)	32 (41.0)	85 (25.7)
61 to 80 years	117 (46.2)	29 (37.2)	146 (44.1)*
Over 80 years	70 (27.7)	7 (9.0)	77 (23.3)
Total	253 (100.0)	78 (100.0)	331 (100.0)

the highest value for one variable is associated with the lowest value for the other variable. These variables can be positively or negatively correlated, and this correlation can be strong or weak meaning that the variables "move" together either positively or negatively. In the present study, a positive and moderate correlation was observed between glabellar type and sex (Table 3).

Considering Walker's classification (2008), where scores 1 and 2 correspond to the estimated female sex, scores 4 and 5 correspond to the estimated male sex, and score 3 represents an intermediate "gray zone," we opted to distribute the sample into three categories: "Glabellas 1 and 2," "Glabellas 4 and 5," and "Indeterminate." Notably, the first category ("Glabellas 1 and 2") exhibited the highest percentage in the studied sample (67.1%), as demonstrated in Table 4.

To further evaluate the behavior of glabellar-type categories concerning sex, we chose to perform analyses considering only the categories "Glabellas 1 and 2" and "Glabellas 4 and 5," resulting in a sample of 368 individuals. A statistically significant moderate association between the variables was observed, as shown in Table 5. In addition to being statistically associated, the variables sex and glabella categories were positively correlated, albeit moderately, as shown in Table 6.

Analyzing the classification of the glabella, considering categories "1 and 2" and "4 and 5" according to age, there was a statistically significant association between individuals aged over 60 and the highest number of "1 and 2" classifications, as shown in Table 7.

The results of the logistic regression analysis show that both glabella morphology and age had significant effects on the estimation of sex (Table 8). The analysis shows that with an increase in one age category, the chance of a given outcome increases by 62.3%, with a statistically significant effect ($p = 0.003$). This suggests that age is an important factor in shaping the outcome. Concerning the glabella classification, the negative coefficient indicates that an increase in the Glabella category is associated with a substantial reduction in the chance of the outcome (around 97.4%). The low

Table 8 Binominal logistic regression to verify sex prediction

		B	S.E	Wald	df	Sig	Odds ratio	95% IC to Odds ratio	
								Inferior	Superior
Step 1	Age	0.484	0.163	8.808	1	0.003	1.623	1.179	2.235
	Glabella_Cat	-3.661	0.535	46.882	1	< 0.001	0.026	0.009	0.073
	Constant	3.161	0.761	17.267	1	< 0,001	23.586		

Table 9 Results of the diagnostic performance of the glabella evaluation for estimating sex

	Predicted		
Observed	**Male**	**Female**	**% correct**
Male	82	83	49.7
Female	5	198	97.5
% Accuracy			76.1
Precision			94.3
AUC			73.6[a]

[a] *P*-value: < 0.001; CI: 0.682 – 0.790

probability is highly significant ($p < 0.001$), showing that Glabella is a strong predictor in the model.

Regarding the diagnostic performance of glabellar morphology assessment for sex estimation, an overall accuracy of 76.1% was achieved, with females exhibiting a higher percentage of correct observations than males (see Table 9). While accuracy reflects how closely the observations align with reality, as it relates to the percentage of correct predictions, precision indicates how closely these observations cluster together, signifying method consistency and reproducibility. The area under the curve (AUC) represents the estimate of the probability of correctly classifying an individual by chance. According to Metz (1978), AUC can be classified as follows: 0.5–0.6 (poor), 0.6–0.7 (fair), 0.7–0.8 (regular), 0.8–0.9 (good), and > 0.9 (excellent). In the present study, an AUC value of 0.736 was obtained, indicating a poor probability of correct classification.

Discussion

In the construction of a biological profile, it is essential to establish an effective method for sex estimation that considers both the condition of skeletal remains and the analyzed population. In the present study, using Walker's scoring method (2008) based on non-metric cranial features, advantages such as practicality, ease of data collection, and the elimination of specialized equipment were observed during the macroscopic analysis of skulls. In various forensic contexts, this approach serves as a robust alternative due to its easy adaptability, particularly in specific cases involving mass disasters or small, degraded samples where other methods may be impractical (Garcovich et al. 2022).

Concerning the analysis of the sexually dimorphic potential of the glabella, the present study observed a positive correlation between glabellar types and sex, corroborating the findings of Walker (2008) that the glabella is one of the most sexually dimorphic features evaluated in the skull. In a similar analysis, Stasievski et al. (2021) examined cranial characteristics in a Brazilian sample of 192 skulls, ranging in age from 22 to 97 years, with 108 male and 84 female individuals. Among their results, the glabella exhibited a high degree of sexual dimorphism. These findings may be related to changes in the morphological expression of the glabellar region and the presence of muscle insertions in this area, where muscle traction can elicit bone responses, in addition to the influence of testosterone levels on cranial shape (Godde; Thompson; Hens, 2018; Whitehouse et al. 2015). Furthermore, it is pertinent to mention the study by Del Bove et al. (2020), which analyzed anatomical traits of the frontal bone in 161 European and North American individuals, highlighting the glabella as a structure strongly associated with sex. Similarly, Garcovich et al. (2022), through radiographic image analyses of skulls, emphasized the morphological differences between male and female glabellas, underscoring this cranial region as valuable for sex estimation.

One of the main objectives of the present research was to describe the frequency of glabellar morphological types based on Walker's scoring system (2008). It was observed that the glabellar type with a classification of 2 exhibited the highest frequency, followed by types 1, 4, 3, and finally, score 5. Based on these results, it can be suggested that the analyzed sample tended to display less robust traits. Examining the frequency distribution of scores, Carvalho et al. (2017), in a sex estimation study using a sample of 66 skulls from the southeastern Brazilian population, found that the most common glabellar classification was 3, followed by 2, 4, 1, and finally 5. These differences in results, despite applying the same analytical method, may be attributed to factors such as significant size disparities between the two samples or potential variations in anatomical traits across different

populations, influenced by genetic, nutritional, and cultural factors, which impact craniofacial growth (Carvalho et al. 2017).

Regarding the diagnostic performance of glabellar assessment for sex estimation, an overall accuracy of 76.1% was achieved, which does not represent a favorable classification probability. One possible reason for this percentage is the visual classification of cranial features being more sensitive for females than for males in the sample. This finding aligns with previous research (Krueger et al., 2015; Langley; Dudzik; Cloutier, 2018), where the female sex tends to benefit due to slower development of robustness and rugosity in male skulls (Langley; Dudzik; Cloutier, 2018). Another potential factor is secular changes in sexual dimorphism patterns (Godde; Thompson; Hens, 2018), along with morphological variation in cranial traits across different populations, particularly in a Brazilian sample characterized by significant admixture (Carvalho et al. 2020).

The higher percentage of correct classifications for females compared to males suggests a potential confusion between males exhibiting more graceful and less robust traits. Different results from the present study indicate variations when the method was tested across different populations. Dereli et al. (2018), using three-dimensional modeling of volumetric computed tomography images based on cranial morphological scoring, achieved a higher percentage of accurate sex estimation for males compared to females. Similarly, in the study by Cappella et al. (2022), Walker's method was applied to a contemporary Italian population of 177 individuals, revealing higher precision in the male sample, even outperforming Walker's original male population. Among the explanations for these divergent results, differences in sample origins play a significant role, as also noted by Capella et al. (2022).

Regarding secular changes in cranial anatomical traits, the study by Godde et al. (2018) tested the hypothesis of sexual dimorphism variation across populations by evaluating cranial morphological features in three different samples from various time periods. From this study, the authors supported the idea that populations exhibit distinct patterns of sexual dimorphism, and therefore, sex estimation methods do not yield consistent results across all populations. In their sample analysis, the authors revealed that in males, supraorbital margins and the glabella exhibited a decrease, which could lead to incorrect sex classifications due to variations in the expression of these features across different populations.

Therefore, understanding the profile of cranial anatomical characteristics within each population holds immeasurable value. From the observations obtained, sex estimation methods can be validated, adapted, or even new methods can be developed based on information collected from the specific population. The goal is to comprehend the patterns unique to each population, allowing for adjustments in method application to yield reliable results and evaluate sexual dimorphism trends across diverse populations (Cappella et al. 2022).

In the morphological assessment of cranial traits, genetic, environmental, social, and cultural issues must be taken into account as population factors that influence the manifestation of different morphological characteristics, including when studying sexual dimorphism (Campanacho et al 2021; Rogers et al. 2024). Populations of Asian origin, for example, have been reported in the literature as capable of presenting individuals with more graceful characteristics, including males. The consequence of this, as explained by Rogers et al. (2024), is that when methods are applied to evaluate morphological characteristics that are classically present in the literature, there may be a mistaken estimate of sex, i.e. a male individual may be estimated to be female. These data reinforce the issue regarding the potential influence that the studied population may exert on sexual dimorphism. Although the present study did not aim to specifically evaluate population affinity and its association or lack thereof with glabella classification for sex estimation purposes, the authors believe that conducting studies in this regard should be encouraged, especially in the Brazilian population, given its extensive and continuous miscegenation.

Studies also indicate that bone density and the definition of glabella characteristics may decrease with age, which may attenuate the differences between the sexes. In the present study, individuals aged over 60 had more glabella classifications 1 and 2, i.e. more feminine glabella morphological characteristics. Albert et al. (2007) pointed out that ageing can result in bone resorption and loss of bone mass, which can soften the sexually dimorphic characteristics of the glabella. Furthermore, Tallman (2019) observed that, in Asian populations, sexual dimorphism in the glabella is less pronounced in older individuals, suggesting that age may be a confounding factor in forensic analyses using this region. These changes in glabella morphology with age have significant implications for forensic anthropology. In older populations, sex estimation based on the glabella may become less accurate, requiring the use of multiple cranial indicators or the application of complementary methods for a more robust analysis.

These changes in glabella morphology with age have significant implications for forensic anthropology. In older populations, sex estimation based on the glabella may become less accurate, requiring the use of multiple cranial indicators or the application of complementary

methods for a more robust analysis. Based on the potential causes for the mentioned variations, research indicates that sex estimation performance is superior when multiple anatomical traits are combined. Langley et al. (2018) reported that associating anatomical features with greater discriminatory capabilities enhances the precision of sex estimation. Their most accurate method included the glabella, zygomatic extension, and mastoid process. Garcovich et al. (2022) assert that, in forensic contexts, sex estimates based on a single anatomical trait should be avoided, and imprecision in results is significantly reduced through multimethod approaches. Similarly, the findings of Nikita and Michopoulou (2018) pointed to greater sex estimation efficacy when combining three structures: glabella, mastoid process, and external occipital protuberance. However, in many forensic cases, findings are fragmented, unfortunately limiting simultaneous analysis of different anatomical regions.

Finally, it is essential to highlight the significance of osteological collections, particularly those consisting of contemporary identified skeletons. Recent Brazilian collections, such as the one utilized in the present study from CEAF/FOP/UPE, can provide findings that closely reflect the population in a temporally proximate manner. These collections further facilitate studies examining the expression of both morphological and metric characteristics over time.

Conclusions

Based on the findings presented, it was observed that the most common morphological type of the glabella was score 2 for the total group, with a statistically significant association between sex and glabellar type. The female sex exhibited a stronger correlation with glabellar classification and a higher percentage of correct observations. Individuals aged over 60 presented more glabella classifications "1 and 2", i.e. more feminine glabella morphological characteristics. Additionally, sex estimation based on glabellar morphological types achieved an accuracy of 76.1%, suggesting that this analytical method should be used in conjunction with others for sex diagnosis.

Given the potential influence of different populations on cranial traits, particularly in a country like Brazil, characterized by diverse features resulting from significant admixture, studies examining the expression of sexually dimorphic cranial traits, such as the glabella, should be encouraged, especially within Brazilian osteological collections. By doing so, a national profile of this morphological trait can be established, contributing to human identification procedures in the country.

Abbreviations

CEAF/FOP/UPE	Center for Studies in Forensic Anthropology at the Faculty of Dentistry, University of Pernambuco
FOP/UPE	Faculty of Dentistry of Pernambuco
AUC	Area under the curve

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Authors' contributions

EPS conceived the study, participated in its design and coordination, and approved the version to be published. MVDC participated in the design and coordination of the study. JNMFG, AMNFC, and GMAS performed the examinations at the CEAF/FOP/UPE and drafted the article.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The procedures for conducting this research adhered to guidelines and regulations governing studies involving human subjects, as approved by Resolution No. 466 of December 12, 2012, issued by the National Health Council. This study is a component of a larger research project currently underway at CEAF/UPE (Center for Forensic Anthropology Studies, University of Pernambuco, Brazil), which has received proper approval from the Research Ethics Committee (Approval No. 4,972,915; CAAE: 50354121.2.0000.5207).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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