




## RESEARCH ARTICLE

# A geometric morphometric approach to the study of sexual dimorphism in the modern human frontal bone

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## Abstract

**Objectives:** We analyzed the main anatomical traits found in the human frontal bone by using a geometric morphometric approach. The objectives of this study are to explore how the frontal bone morphology varies between the sexes and to detect which part of the frontal bone are sexually dimorphic.

**Materials and methods:** The sample is composed of 161 skulls of European and North American individuals of known sex. For each cranium, we collected 3D landmarks and semilandmarks on the frontal bone, to examine the entire morphology and separate modules (frontal squama, supraorbital ridges, glabellar region, temporal lines, and mid-sagittal profile). We used Procrustes ANOVAs and LDAs (linear discriminant analyses) to evaluate the relation between frontal bone morphology and sexual dimorphism and to calculate precision and accuracy in the classification of sex.

**Results:** All the frontal bone traits are influenced by sexual dimorphism, though each in a different manner. Variation in shape and size differs between the sexes, and this study confirmed that the supraorbital ridges and glabella are the most important regions for sex determination, although there is no covariation between them. The variable size does not contribute significantly to the discrimination between sexes. Thanks to a geometric morphometric analysis, it was found that the size variable is not an important element for the determination of sex in the frontal bone.

**Conclusion:** The usage of geometric morphometrics in analyzing the frontal bone has led to new knowledge on the morphological variations due to sexual dimorphism. The proposed protocol permits to quantify morphological covariation between modules, to calculate the shape variations related to sexual dimorphism including or omitting the variable size.

## KEYWORDS

sexual dimorphism, frontal bone, geometric morphometrics, LDA

## 1 | INTRODUCTION

Determination of sex is a crucial in all branches of physical anthropology. Many standards for building biological profiles, such as those for age, stature, and body mass estimation, are sex-specific (e.g., Brooks & Suchey, 1990; Formicola & Franceschi, 1996; Garvin & Ruff, 2012;

Scheuer & Black, 2000; Trotter & Gleser, 1951). In forensic anthropology, correctly sexing an individual can drastically reduce possible matches of missing persons (DiGangi & Moore, 2013). In bio-archaeology, sexing individuals can provide unexpected insights into the culture of ancient populations, their funerary rituals, and their gender concepts (Hedenstierna-Jonson et al., 2017; Pearson, 1996). In

palaeoanthropology, size variation among specimens of early *Homo* has been alternatively interpreted as related to taxonomic differences or to sexual dimorphism (Plavcan, 2012; Spoor et al., 2007; Wood, Li, & Willoughby, 1991).

The difference between the sexes in terms of body size and shape, development, and behavior is called sexual dimorphism (DiGangi & Moore, 2013). In *Homo sapiens*, sexual dimorphism in the skeleton arises during growth and is determined and influenced by many genetic and environmental factors, such as hormones, development, and nutrition (Buikstra & Ubelaker, 1994; Frayer & Wolpoff, 1985; Lorenzo, Carretero, Arsuaga, Gracia, & Martínez, 1998; Steyn & Yaşar İçsan, 1998; Walrath, Turner, & Bruzek, 2004).

Physical anthropologists use sexually dimorphic traits in the skeleton to distinguish males and females through visual assessment or metric estimation (DiGangi & Moore, 2013). Sex assessment by visual inspection is widely used because, when highly dimorphic traits such as the pelvis are observable, it is fast and, if applied by trained scholars, its accuracy can reach more than 90% (Phenice, 1969; Ubelaker & Volk, 2002). In other circumstances, for example, when skeletal remains are incomplete or poorly preserved, measurement-based methods estimate sex are preferable (Spradley & Jantz, 2011). Many approaches to sex estimation have been developed, both on cranial and postcranial elements, based on single measurements and multivariate techniques (i.e., Konigsberg, Algee-Hewitt, & Steadman, 2009; Saini et al., 2011; Spradley & Jantz, 2011), or using a geometric morphometric (GM) approach (Bigoni, Velemínská, & Brůžek, 2010; Bytheway & Ross, 2010; Franklin, Oxnard, O'Higgins, & Dadour, 2007; Gonzalez, Bernal, & Perez, 2011; Kimmerle, Ross, & Slice, 2008; Perlaza, 2014; Pretorius, Steyn, & Scholtz, 2006; Rmoutilová, Dupej, Velemínská, & Brůžek, 2017).

## 1.1 | GMs and sex estimation

Since the first studies applying discriminant functions to linear measurements on skulls (Giles & Elliot, 1963; Uytterschaut, 1986), many authors have felt the need to find new quantitative methods to distinguish between sexes (i.e. Luo, Wang, Zhang, & Congbo, 2018; Small, Schepartz, Hemingway, & Brits, 2018; Suazo Galdames, Perez Russo, Zavando Matamala, & Luiz Smith, 2009). Among them, a number of scholars opted for a GM approach, and applied it to mandibles (Franklin et al., 2007), pelvis (Bytheway & Ross, 2010; Rmoutilová et al., 2017), and skulls (Bigoni et al., 2010; Gonzalez et al., 2011; Kimmerle et al., 2008; Pretorius et al., 2006). In the last decades, GM proved itself to be a powerful tool for studying shape variation and its relationship to other variables like sex and population (Adams, Rohlf, & Slice, 2004; Baylac & Friess, 2005; Gunz et al., 2004; Rohlf & Leslie, 1993; Weber, 2015).

The GM method allows for the study of specific anatomical regions/traits by defining a set of geometric points (i.e., landmarks and semilandmarks). Through the study of the geometry of anatomical traits paired with multivariate statistical analyses, we can analyze shape components in detail, independent of dimensions, and

relate changes therein to sex differences and other variables (Bookstein, 1989).

## 1.2 | Sexual dimorphism in the frontal bone

One of the elements most used for sexing individuals is the skull, considered by some anthropologists to be the second-most dimorphic area after the pelvis (but see discussion in Spradley & Jantz, 2011). In the human skull, morphological differences due to sexual dimorphism are recognizable in different anatomical regions, with males generally having a more robust appearance (Buikstra & Ubelaker, 1994; Frayer & Wolpoff, 1985; Walker, 2008).

The cranium is a complex structure organized in modules. From a morphological point of view, a module is defined as a within integrated unit and modules could be integrated and/or modular each other due to the interactions between them (Esteve-Altava, Diogo, Smith, Boughner, & Rasskin-Gutman, 2015; Klingenberg, 2014).

In accordance with “the Palimpsest Model of Covariation Structure” proposed by Hallgrímsson et al. (2009) the phenotype is influenced by the covariation between modules. Subsequently, the study of the pattern of integration between the parts composing the structure under investigation is determinant to interpret the morphological variations and differences in the light of functional and adaptive processes. The analysis of the pattern of covariation between sub-modules belonging to the same bone has been studied far less than the relation between bones.

The frontal bone, which is the focus of this article, is one of the most sexually dimorphic anatomical areas of the human cranium (Acsádi & Nemeskéri, 1970; Buikstra & Ubelaker, 1994). It is one of the largest cranial bones and represents part of the interface between the neurocranium and the upper face, articulating with the parietals, nasals, maxillae, sphenoid, ethmoid, lacrimal, and zygomatic bones (Bruner, Athreya, De La Cuétara, & Marks, 2013; White & Folkens, 2005).

In addition to frontal bone morphology, physical anthropologists analyze the size and shape of specific anatomical regions such as the supraorbital ridges, the frontal squama, the glabellar region, and the temporal lines during sex determination (Acsádi & Nemeskéri, 1970; Walker, 2008). Studies have found consistent differences in frontal bone morphology between females and males (Bulut, Petaros, Hizliol, Wärmländer, & Hekimoglu, 2016; Garvin & Ruff, 2012; Perlaza, 2014; Petaros, Garvin, Sholts, Schlager, & Wärmländer, 2017; Shearer, Sholts, Garvin, & Wärmländer, 2012). The frontal bone in males is more inclined, whereas in females it appears more vertical and rounded (Bulut et al., 2016). In general, the brow ridge and supraorbital region are the most sexually dimorphic areas of the frontal bone; specifically, supraorbital ridges in males tend to be extended inferiorly as compared to the relatively flat appearance of females (Garvin & Ruff, 2012; Perlaza, 2014; Shearer et al., 2012). The glabellar region has also been suggested in the literature as being a good proxy for sex assessment, as it is much larger in males (Perlaza, 2014; Petaros et al., 2017).

In this article, we propose a workflow developed in R (R Core Team, 2020) for the study of sexual dimorphism using a GM approach. The main purpose of this study is to analyze differences in shape and size in the frontal bone due to sexual dimorphism and to highlight how the frontal bone varies between the sexes in *Homo sapiens*. In detail, we tested the following hypotheses: (i) the human frontal bone shows a uniform degree of sexual dimorphism; (ii) in the frontal bone, the size of anatomical modules (i.e., supraorbital ridges, frontal squama, glabella, frontal profile, and temporal lines) has a relationship with the sexual dimorphism signal; and (iii) in the frontal bone the degree of sexual dimorphism detected is related to the pattern of integration and modularity between modules.

We measured how sexual dimorphism affects frontal bone morphology analyzing five different a priori anatomical modules (i.e., supraorbital ridges, frontal squama, glabella, frontal midsagittal profile, and temporal lines) by placing on them a set of curves or surface semilandmarks and we calculated the pattern of integration and modularity between the five modules defined on the frontal bone. These modules are those commonly investigated by physical anthropologists in assigning sex-to-sex-unknown collection. We also propose a standardized protocol for quantifying sexual dimorphism in the frontal bone to produce shape variations related only to sexual dimorphism, that can act as a standard for the detection of sex in osteological collection. For maximum certainty regarding the relationship between form and sex, we used only specimens of known sex; however, this markedly reduced our ability to use a global sample and prevented us from looking at interpopulation variability.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample

The sample size consisted of 161 crania (74 females and 87 males) belonging to four digital osteological repositories: (i) the Lynn Copes digital Collection (Black Americans) from the Anthropology department at the National Museum of Natural History, Washington, DC (Copes, 2012); (ii) the Museum of Anthropology "G. Sergi" from Sapienza University of Rome (Rubini & Scarani, 1989); (iii) the Oloriz Collection in Spain; and iv) the Anthropological Museum of Florence (University of Florence) (Moggi Cecchi & Stanyon, 2014) (Table 1). The collections consisted of 3D models of crania of adult specimens of known sex. The 3D models were obtained via photogrammetry and CT scan.

### 2.2 | Data acquisition

In the case of samples obtained via photogrammetry, we used Agisoft PhotoScan software (version 1.4) to build 3D models.

On each specimen, we collected 13 fixed landmarks (Table 2) placed on the entire frontal bone using Avizo software (Version 7). The anatomical traits belonging to the frontal bone were defined by

**TABLE 1** List of collection used in this study

Population	Repository	Total	Females	Males
Fiorentini	A	58	27	31
Sardi	A	29	12	17
Siracusani	A	16	5	11
Bolognesi	B	26	16	10
Oloriz	C	11	3	8
Terry collection	D	21	11	10

Abbreviations: A, Museum of Anthropology and Ethnology, University of Florence; B, Museum of Anthropology "G. Sergi" from Sapienza University of Rome; C, Nespos repository and D, Terry Collection of the National Museum of Natural History, Washington, DC.

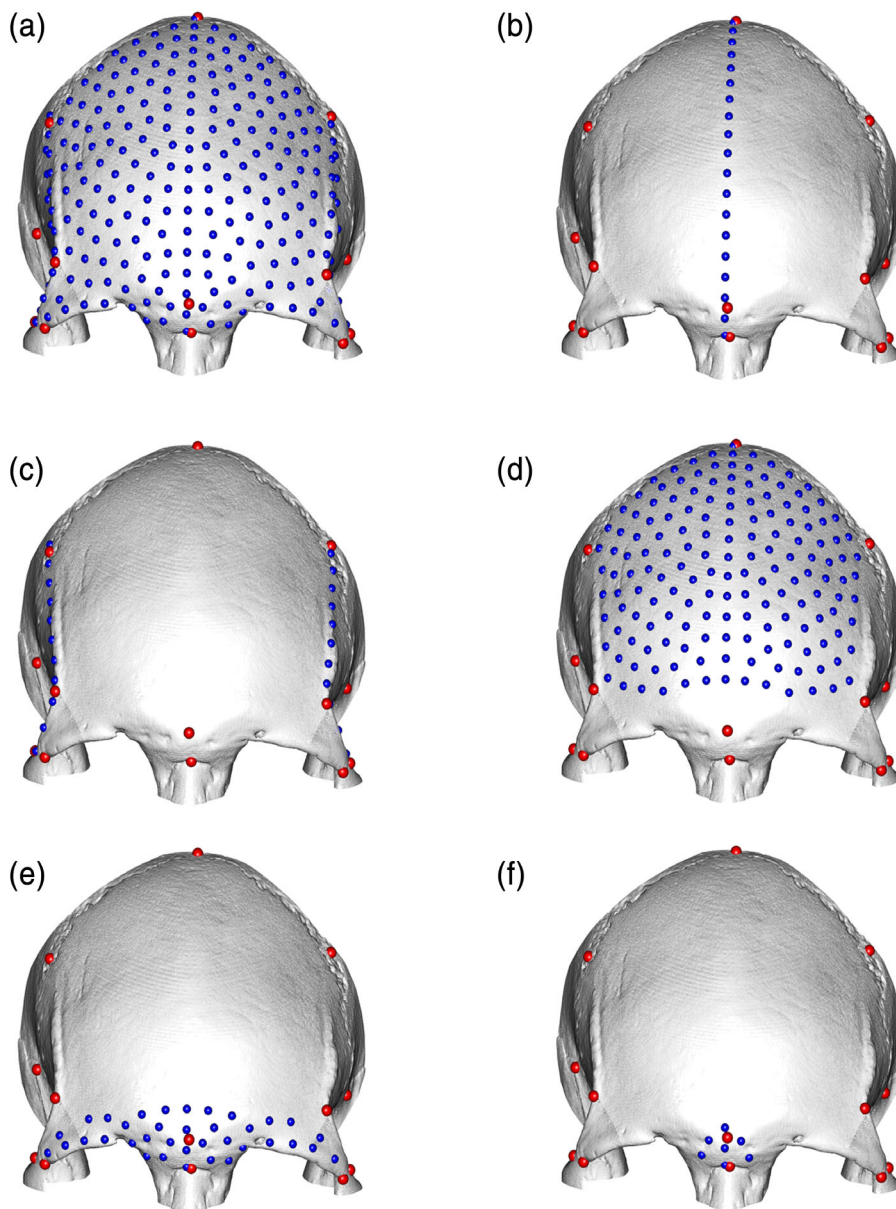
**TABLE 2** List of cranial landmarks with definitions (White & Folkens, 2005).

Numbers	Landmarks	Definition
1	Nasion	The midline point where the two nasal bones and the frontal intersect
2	Glabella	The most anterior midline point on the frontal bone
3	Bregma	The ectocranial point where the coronal and sagittal sutures intersect
4-5	Ectoconchion	The most lateral point on the orbital margin
6-7	Frontomolare temporale	The point where the frontozygomatic suture crosses the temporal line
8-9	Frontotemporale	The point where the temporal line reaches its most anteromedial position on the frontal
10-11	Stephanion	The point where the coronal suture crosses the temporal line
12-13	Pterion	The point, where the frontal, temporal, parietal, and sphenoid meet on the side of the vault

placing semilandmarks sets. The average distance calculated on the mean shape between each semi-landmark and its neighborhood (the closest 10 semilandmarks) is  $1.06 \pm 0.11$  mm.

We opted to record the frontal midsagittal profile (19 points) and the temporal lines (22 points) by acquiring three curves. The frontal midsagittal profile starts from the frontonasal suture (i.e., *nasion*) to the coronal suture (i.e., *bregma*). The two temporal lines start from zygomaticofrontal suture (i.e., *frontomolare temporale*) to the coronal suture (i.e., *stephanion*; Figure 1). Before shape analysis, the R package Morpho was used to slide the curves to minimize bending energy (Schlager, 2017).

We defined frontal squama (161 semilandmarks), supraorbital ridges (36 semilandmarks), and glabellar regions (7 semilandmarks) by building a unique symmetric reference template. Subsequently, we



**FIGURE 1** Landmark and semilandmark configurations used in this study. In red anatomical landmarks, in blue surface semilandmarks and curves. The modules tested are as follows: entire frontal shape (a), mid-sagittal profile (b), temporal lines (c), frontal squama (d), supraorbital ridges (e), glabellar region (f)

split the entire slid semilandmark patch into three sub-modules: one for each anatomical trait (Figure 1). The sliding procedure was performed by sliding the entire patch (Morpho R package).

### 2.3 | Linear discriminant analysis, integration, modularity, and shape variations

The morphology of the entire frontal bone was analyzed by performing a PCA on the semilandmarks sets after generalized procrustes analysis (GPA). We performed the analysis in the shape and form space (the log of the centroid size [CS] is added as variable to the coordinates after the GPA) in order to investigate the relation between sex and frontal bone morphology including and excluding the size variable.

We evaluated the influence of sex on shape and size by using the Procrustes ANOVA embedded in the geomorph R package (Adams, Collyer, & Kaliontzopoulou, 2019).

In addition, we performed an linear discriminant analysis (LDA) using PC scores (defined as 95% of the shape information) as dependent variable; the log of the Centroid Size (logCS) and sex are considered as independent variables. We used the logCS as a proxy for the actual size of the anatomical regions under investigation. Subsequently, we calculated the accuracy of the model in classifying individuals based on sex by designing a bootstrap procedure (1,000 iterations). At each iteration, we randomly sampled 100 individuals, at the end of the iterative procedure we calculated the mean accuracy and the standard deviation of the model in discriminating sex.

We calculated the pattern of integration and modularity among all the combinations (10 pairs of comparisons) of the five

morphological modules defined on the frontal bone in the female and male sample. We evaluated integration within the frontal bone by applying a partial least square (PLS) between two modules at a time (Rohlf & Corti, 2000). We considered as signal of integration the correlation coefficient between PLS scores of the first PLS axis. The modularity between modules (Adams, 2016; Klingenberg, 2009, 2013) has been assessed by calculating the covariance ratio (CR) between pairs of modules.

For each morphological module defined in the frontal bone, we built the shape variations associated to female and male morphology. We consider as shape the first PC scores accounting the 95% of the total explained variance. On each PC, we test differences in means between females and males applying the T-Student test. In the definition of the shape variation of each module, we considered only the PC scores statistically different between male and female groups summing their contribution. Though canonical variate analysis (CVA) produces a high degree of accuracy, we used PCA because CVA standardizes total variance by the within-group variance, thus altering the pattern of differentiation among groups (Renaud, Dufour, Hardouin, Ledevin, & Auffray, 2015).

### 3 | RESULTS

#### 3.1 | Procrustes ANOVA

Results from the Procrustes ANOVA show that variables "sex" and "size" are significantly related to the morphology of the entire frontal bone and its sub-modules (frontal squama, supraorbital ridges, glabellar region, temporal line, and frontal mid-sagittal profile) except for the relation between size and temporal lines in the shape space (Table 3). The inclusion of "size" in the shape variable increases the  $R^2$  in all the modules tested.

The interaction between "sex" and "size" is not significant in all the investigated modules. This means that the significances observed

for the independent variables "sex" and "size" is not due to their interaction.

#### 3.2 | Linear discriminant analysis

The results of the LDA differ depending on whether CS is included in the analysis. Accuracy and precision in sex estimation are usually higher when CS is excluded (Table 4). The best success in discriminating sexes was achieved by LDAs on the "supraorbital ridges" when size is excluded with  $86 \pm 1\%$  success rate. The "temporal lines" in both shape space and form spaces show the worst rate of success with 63 and 69%, respectively. In sum, when size is excluded from the analysis only the "supraorbital ridges" (86%) show higher or equal success rate in discriminating sex than the entire frontal bone morphology (82%). In the form space only the "supraorbital ridges" (85%) shows a level of accuracy higher than the entire frontal bone (80%; Figure 2).

#### 3.3 | Integration and modularity in the frontal bone

In the PLS analysis, the "glabella" shows in males and females a low degree of covariation with the "squama" and the "temporal lines" (PLS-corr = .29,  $p = .30$  in females and PLS-corr = .34,  $p = .03$ ; Table 5). Instead, we observed in females a moderate degree of covariation between "glabella" and "supraorbital ridges" (PLS-corr = .89,  $p < .01$ ) and a low degree of covariation between these two modules in males (PLS-corr = .74,  $p < .01$ ). The test of modularity between all the combinations possible with the five modules show a general pattern of integration (the CR is close to 1) except in the comparison between "glabella and squama" (CR = 0.77,  $p < .01$  in females, and CR = 0.82,  $p < .01$  in males), "glabella and temporal lines" (CR = 0.79,  $p < .01$  in

**TABLE 3** Summary of the results of procrustes ANOVA performed on the semilandmark configurations after GPA in the shape and form space

Shape space	$R^2$ (sex)	$p$ -value	$R^2$ (size)	$p$ -value	$R^2$ (sex*size)	$p$ -value
Entire	.030	.001	.034	.001	.004	.627
Glabella	.074	.001	.106	.001	.008	.198
Squama	.023	.011	.062	.001	.003	.698
Midsagittal profile	.039	.002	.058	.001	.002	.770
Supraorbital ridges	.025	.002	.021	.010	.004	.728
Temporal lines	.024	.025	.017	.065	.001	.914
Form space	$R^2$ (sex)	$p$ -value	$R^2$ (size)	$p$ -value	$R^2$ (sex*size)	$p$ -value
Entire	.078	.001	.400	.001	.002	.627
Glabella	.076	.001	.450	.001	.005	.198
Squama	.056	.001	.501	.001	.001	.698
Midsagittal profile	.098	.001	.642	.001	.001	.770
Supraorbital ridges	.090	.001	.595	.001	.001	.728
Temporal lines	.086	.001	.457	.001	.001	.914

Note: Statistically significant results in bold.

**TABLE 4** Precision and accuracy (expressed as percentage) calculated on the modules defined in the frontal bone in classifying sex in the shape space and form space

Shape Space	Accuracy upper	Accuracy mean	Accuracy lower	Precision female upper	Precision female mean	Precision female lower	Precision male upper	Precision male mean	Precision male lower
Entire	81.996	81.784	81.572	81.993	81.743	81.493	82.120	81.897	81.674
Glabella	78.755	78.556	78.357	76.308	76.079	75.850	80.876	80.659	80.442
Squama	73.469	73.273	73.077	71.777	71.543	71.309	74.841	74.639	74.437
Midsagittal profile	79.129	78.912	78.695	77.154	76.891	76.628	80.932	80.697	80.462
Supraorbital ridges	85.721	85.545	85.369	85.075	84.837	84.599	86.344	86.146	85.948
Temporal lines	62.827	62.633	62.439	59.929	59.490	59.051	64.330	64.124	63.918
Form Space	Accuracy upper	Accuracy mean	Accuracy lower	Precision Female upper	Precision Female mean	Precision Female lower	Precision Male upper	Precision Male mean	Precision Male lower
Entire	80.083	79.879	79.675	78.293	78.053	77.813	81.454	81.247	81.040
Glabella	73.657	73.467	73.277	71.220	71.002	70.784	75.675	75.456	75.237
Squama	73.075	72.872	72.669	70.625	70.367	70.109	75.136	74.934	74.732
Midsagittal profile	76.570	76.371	76.172	76.413	76.198	75.983	76.710	76.488	76.266
Supraorbital ridges	85.104	84.913	84.722	83.306	83.064	82.822	86.700	86.506	86.312
Temporal lines	69.712	69.494	69.276	67.533	67.265	66.997	71.385	71.165	70.945

females, and CR = 0.78,  $p < .01$  in males). Despite the CR being close to 1 when the modularity test is performed between “glabella” and “supraorbital ridges”, the  $p$ -value is not statistically significant (Figure 3).

### 3.4 | Shape analysis

When looking at the entire set of semilandmarks, males are characterized by an antero-posteriorly elongated frontal bone; the temporal lines are closer to the midsagittal plane than in females, and the frontal squama are oriented horizontally. The frontal squama in females are shorter than those observed in males and their orientation, as noted above, is vertical on females and horizontal in males. Compared to males, the central superior margin of the supraorbital ridges of females are slightly expanded as compared to males. The glabellar region in females is related to horizontal expansion of the frontal bone and appears more vertically elongated, while males have a protruded glabellar region. Temporal lines in females are shorter vertically and restricted laterally, because the intersection between the coronal suture and the stephanion is positioned lower and anteriorly.

The frontal midsagittal profile in males is horizontal and a bulging is detectable at the level of the glabellar region. The female profile is vertical and short. In females, bulging is visible in the frontal eminence and the glabellar portion appears deflated. Additionally, midsagittal lines emphasize the male-horizontal and female-vertical feature of frontal bone. The male frontal bone morphology is characterized by an expansion of the squama in the anterior portion and by the presence of longer temporal lines. The supraorbital ridge in males is

expanded laterally and reduced medially in correspondence of the glabella.

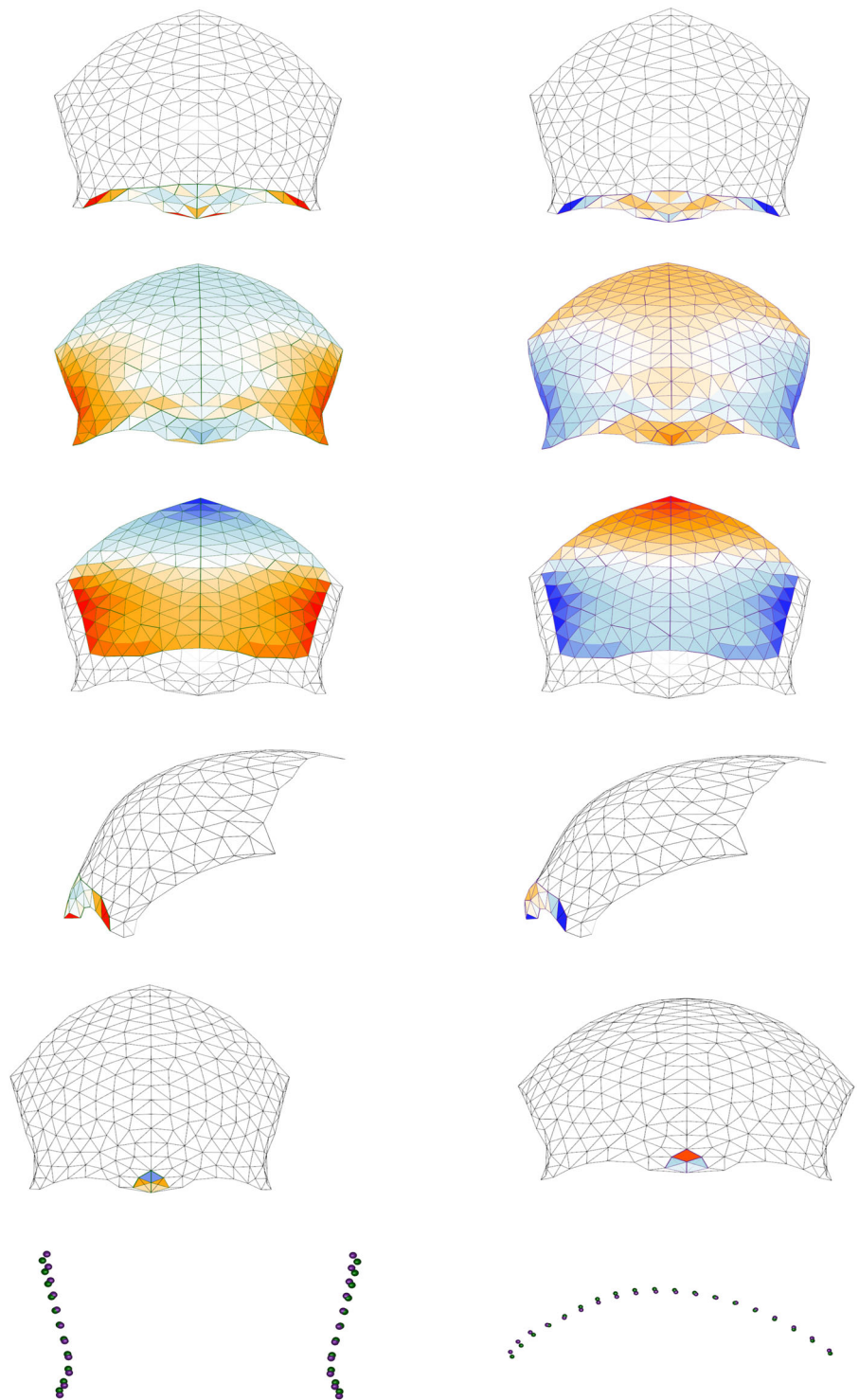
## 4 | DISCUSSION

The approach used in this study was specifically designed to encompass all the steps made by physical anthropologists during sex determination. In fact, the frontal bone shape was acquired in its entirety. In the analysis, we considered both local (module) and whole morphologies. A great advantage to using GM is the decomposition of the variance along single and independent shape variables. For each defined module, we took into consideration only the PC scores which were statistically significant for sex discrimination. The use of a sample of individuals of known sex eliminated researcher subjectivity in sex determination, and so the accuracy values are reliable. In addition, this approach may be used by other researchers on other anatomical regions.

We built six pairs (female and male) of shape variations (entire morphology + five modules) adding in each pair only the PC scores separating female from male individuals. The results of these shape variations are specifically related to morphological differences due to sexual dimorphism and could supply clear guidelines for sex determination of specimens of unknown sex.

This study, through a GM approach, demonstrates that sexual dimorphism significantly influences the morphology of the frontal bone in all the modules analyzed. Frontal bone morphology is influenced by both sex and size. Analyzing sexual dimorphism in each trait can help us understand how specific differences are due to sexual dimorphism. From the shape variations, most of the differences

**FIGURE 2** Shape variation associated to the female (left) and male (right) morphology for each module considered in this study. Warm colors are associated to regions characterized by local contraction (of area), cold colors indicate local expansion of area. In the first four rows, the shape variations related to the female morphology are reported on the left, those related to the male morphology on the right. The shape variations of the temporal lines and mid-sagittal profile related to the female and male morphology are shown respectively in dark green and violet



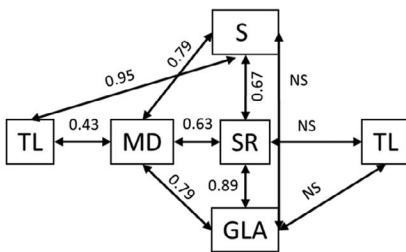
between sexes in the squamous regions are found in the frontal eminence, in agreement with a prior study on the inclination of frontal bone (Bulut et al., 2016). The morphology of the supraorbital ridges is different in female and male specimens and is particularly important for detecting sex differences between males and females (Table 4). In females the supraorbital ridges is flattened medially while in males it is well-developed medially. The degree of shape variation of the frontal bone demonstrates that the supraorbital ridges is particularly

important for detecting sex differences between males and females. In the literature, the glabella is often held to be the most informative trait for determining sex in osteological collections. Using this variable, our models were 79% (excluding CS) and 73% (including CS) accurate at predicting sex, indicating that this anatomical trait is a good indicator of sex. Many studies (Acsádi & Nemeskéri, 1970; Buikstra & Ubelaker, 1994; White & Folkens, 2005) have indicated that the glabellar region is expanded in males and contracted in females. A recent

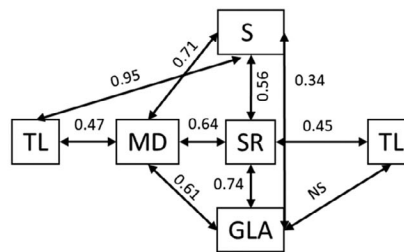
Female	PLS corr	PLS <i>p</i> -value	CR corr	CR <i>p</i> -value
Glabella versus squama	.29	.30	.77	<.01
Glabella versus midsagittal profile	.79	.02	1.01	.06
Glabella versus supraorbital ridges	.89	<.01	.97	.07
Glabella versus temporal lines	.32	.16	.79	<.01
Squama versus midsagittal profile	.79	<.01	.89	<.01
Squama versus supraorbital ridges	.67	<.01	.86	<.01
Squama versus temporal lines	.95	<.01	.94	<.01
Midsagittal profile versus supraorbital ridges	.63	<.01	.77	<.01
Midsagittal profile versus temporal lines	.43	<.01	.79	<.01
Supraorbital versus temporal lines	.61	.16	.88	<.01
Males	PLS-corr	PLS- <i>p</i> -value	CR-corr	CR- <i>p</i> -value
Glabella versus squama	.34	.03	.82	<.01
Glabella versus midsagittal profile	.61	<.01	.98	.03
Glabella versus supraorbital ridges	.74	<.01	1.01	.20
Glabella versus temporal lines	.24	.45	.78	<.01
Squama versus midsagittal profile	.71	<.01	.92	<.01
Squama versus supraorbital ridges	.56	<.01	.89	<.01
Squama versus temporal lines	.95	<.01	.94	<.01
Midsagittal profile versus supraorbital ridges	.64	<.01	.88	<.01
Midsagittal profile versus temporal lines	.47	<.01	.82	<.01
Supraorbital versus temporal lines	.45	.01	.89	<.01

Note: On the first column are shown the pairs of tested modules in males and females. Statistically significant results are in bold.

### Female frontal bone



### Male frontal bone



**TABLE 5** Results of the PLS (integration) and CR (modularity) analyses

**FIGURE 3** Network of integration between the modules defined in the frontal bone. The numbers indicate the correlation coefficients between the PLS scores of the first PLS axis (NS, result not statistically significant). On the left and right the networks for respectively female and male subsamples. GLA, glabella; MD, midsagittal profile; S, squama; SR, supraorbital ridges; TL, temporal lines

article about the inclination of the glabella (Petaros et al., 2017) suggests that the morphology of the glabellar region is related to the population variable. The five modules investigated in this work show a low level of integration (Table 5), except for a clear signal of morphological integration between the frontal squama and the temporal lines, suggesting that the sexual dimorphic signal we observed in the glabellar region and in the supraorbital ridges is not due to the covariation between these two modules.

Unexpectedly, the visual perception that the anatomical traits of the frontal bone in males appear bigger than those in females is not confirmed by both Procrustes ANOVA and LDA. From Procrustes ANOVA (Table 3) the interaction between the variables sex and size is not statistically significant in both shape and size space indicating the level of the sex variable does not depend on the size variable. In

addition, in the LDA analysis the inclusion of size led to the reduction of the accuracy except for the “temporal lines.” These findings are also confirmed when comparing the predicted sexes from the LDA analysis by using separately the variables size and shape. The percentage of the specimens correctly classified by sex in both analyses ranged between 28.57 and 54.66% (entire = 46.58%, glabellar region = 47.82%, squama = 28.57%, midsagittal profile = 43.48%, supraorbital ridge = 54.66%, temporal lines = 34.16%). This confirms that the variable size should be treated with caution in sexing archaeological collections basing on frontal bone morphology.

In sum, these results reject the hypothesis that human frontal bone shows a uniform signal of sexual dimorphism. The results reject the hypothesis that the variable size increases the accuracy in detecting sex. Last, the hypothesis that “in the frontal bone the degree



**TABLE 6** Summary of previous studies on the sexual dimorphism signal found in the frontal bone

Anatomical region	Methodology	Number of individuals	Accuracy	Reference
Supraorbital ridges	T-test on 3D volume	128	NA	Shearer et al., 2012
Frontal bone inclination	Trait scoring	413	76.1% 81.1% 74.8% 66.3%	Petaros et al., 2017
2D sagittal profile on the glabellar region and supraorbital regions	LDA	60	84.31%	Perlaza, 2014
Entire frontal bone	LDA	160	77.5 %	Bulut et al., 2016
Supraorbital ridges	Cross-validation LOOCV	119	91.6%, 79.8%	Garvin & Ruff, 2012
Entire frontal bone	Cross-validation	103	83.49% 86.41%	Čechová et al., 2019
Glabellar region	Trait scoring	90	84.0 % 86.0 %	Çelbiş, İşcan, Soysal, & Çağdır, 2001

of sexual dimorphism detected is related to the pattern of integration and modularity between modules” is falsified because the two most dimorphic modules “glabella” and “supraorbital ridges” are not strongly integrated (Table 5).

Prior research has demonstrated that ancestry interacts with sex in shaping cranial morphology. Not all traits are subject to the influence of ancestry, nor are they affected equally: for example, the glabellar region, the nuchal crest, and the temporal lines show significant correlations between sex and ancestry (Bakken, Dale, & Schork, 2011; Garvin, Sholts, & Mosca, 2014; Jurda & Urbanová, 2016). Our study used only samples from individuals of known sex, which reduced our ability to investigate ancestry in a worldwide sample; moreover, the primary focus of our research was on the use of GM methodologies to study sexual dimorphism.

The shape variation of temporal lines shows how the morphology of this region is different in female and male individuals. Temporal lines in males are longer and narrower than in females. The different lengths of the temporal lines in females and males is linked to differences in the position of the stephanion. The accuracy of the model based on the temporal lines is equal to 63% (without CS), but when CS is taken into account the accuracy increases to 69% (Table 4), suggesting that size is an important factor in determining sexual differences.

Analyses of the mid-sagittal profile are relatively simple because its morphology is easily observable in lateral view. The sex shape variation model for this module highlights as the projection of the frontal mid-sagittal along the frontal squama is vertical in females and horizontal in males, confirming previous studies (Bigoni et al., 2010; Rosas & Bastir, 2002).

One goal of this study was to create a scale of importance of single traits in determining sex; based on the accuracy of our models when size is excluded, the supraorbital ridges (86%) is the most dimorphic trait, followed by the glabella (79%), the midsagittal profile (79%),

the squama (73%), and lastly the temporal lines (63%). If the size is included in the analysis the supraorbital ridges (85%) is the most dimorphic trait followed by the midsagittal profile (76%), the glabella (73%), the squama (73%), and the temporal lines (69%).

Importantly, this study has allowed us to reconsider which traits of the frontal bone are most critical for determining sex. Through GM, it is possible to bypass the variables and consider the importance of traits individually. Thanks to the methodological approach we designed it is possible to investigate in detail the contribution of both shape and size in discriminating sex in osteological collections. We confirmed the previous literature (Table 6) about the importance of analyzing the supraorbital ridges and the glabellar region in classifying sex-unknown osteological individuals. On the contrary, we found that the size of the morphological traits of the frontal bone is not statistically related with sex in the sample we investigated; the only exception is found in the supraorbital ridges with a slight increment in detecting males (86% excluding size and 87% including size).

The same methodological approach on the entire cranium subdivided in modules should be considered by physical anthropologists to update the trait scores used in the classification of specimens as females or males. In addition, another advantage of a GM approach is the opportunity to build 3D models specifically accounting for sexual dimorphism as we demonstrated in this case study.

## 5 | CONCLUSION

We want to emphasize that one of the most important outcomes of this study has been an updated hierarchy of importance of sexually dimorphic areas of the frontal bone (supraorbital ridges, glabellar region, midsagittal profile, frontal squama, and temporal lines). Specifically, the supraorbital ridge region impacts more than

entire frontal bone. The results of this study suggest that size differences in the frontal bone modules between males and females should be reconsidered as an important factor in sexing individuals.

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## AUTHOR CONTRIBUTIONS

**Antonietta Del Bove:** Conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. **Antonio Profico:** Conceptualization; formal analysis; methodology; project administration; software; supervision; writing-original draft; writing-review and editing. **Alessandro Riga:** Investigation; writing-original draft; writing-review and editing. **Ana Bucchi:** Methodology; validation; writing-review and editing. **Carlos Lorenzo:** Conceptualization; methodology; project administration; supervision; writing-original draft; writing-review and editing.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Code and full data are available on Zenodo (10.5281/zenodo.3940597).

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