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BRIEF COMMUNICATION

Sexual dimorphism analysis in permanent human mandibular canines by geometric morphometrics

Análisis del dimorfismo sexual en caninos mandibulares permanentes humanos mediante morfometría geométrica

Gonzalo Garizoain[1,2] | Virginia A. Cobos[2,3*]

1) Laboratorio de Investigaciones en Ciencias Forenses (LICiF), Facultad de Ciencias Médicas (FCM), Universidad Nacional de La Plata (UNLP), Argentina. **2)** Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. **3)** División Antropología del Museo de La Plata, Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata (UNLP), Argentina.

Abstract

Sex estimation of unknown human remains is a common form of assessment in bioarchaeological and forensic practice. When remains are poorly preserved, teeth become important. Research regarding the use of teeth for sex estimation mainly uses odontometrics. Nevertheless, to date, few studies have aimed to systematically evaluate sexual dimorphism in tooth shape. The purpose of this study is to analyze and describe sexual dimorphism in the mandibular canine. Landmarks and semilandmarks were placed on the occlusal, mesiodistal and buccolingual views of mandibular canines of 56 individuals (37 males and 19 females) from the "Prof. Dr. Rómulo Lambre" collection. Moreover, the impact of dental wear in morphometric analyses on sexual dimorphism was explored. The shape of male and female canines differed significantly in the three views analyzed. Female canines showed a larger crown in relation to its root, while male canines showed greater roots in relation to their crown. While female canines showed a large development of the cingulum, male's teeth exhibited less development of this trait. For these reasons, this study constitutes a first approach to show the usefulness of shape for estimating the sex of canine teeth. These differences might be the result of dissimilarities in the total amount of dentin and enamel due to the effect of sex-linked genes in the growth of these tissues. Rev Arg Antrop Biol 26(1), 075, 2024. https://doi.org/10.24215/18536387e075

Keywords: forensic odontology; human remains; morphometrics; sex estimation

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*Corresponding author: Virginia A. Cobos. Laboratorios Anexo Museo, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Av. 60 y 122, 1900, La Plata, Argentina. Email: virginiacobos@fcnym.unlp.edu.ar

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Resumen
La estimación del sexo de restos humanos no identificados es una tarea habitual de la práctica bioarqueológica y forense. Cuando los restos están mal preservados, los dientes cobran especial relevancia. Las investigaciones sobre el uso de la dentición para la estimación sexual utilizan principalmente la odontometría. Sin embargo, hasta la fecha se han realizado pocos estudios destinados a evaluar sistemáticamente el dimorfismo sexual en la forma de los dientes. El objetivo de este estudio es analizar y describir el dimorfismo sexual en el canino mandibular. Se colocaron landmarks y semilandmarks en vistas oclusal, mesiodistal y bucolingual de caninos mandibulares de 56 individuos (37 masculinos y 19 femeninos) de la colección "Prof. Dr. Rómulo Lambre". Asimismo, se exploró el impacto del desgaste dental en los análisis morfométricos de dimorfismo sexual. La forma de los caninos masculinos y femeninos difirió significativamente en las tres vistas analizadas. Los caninos femeninos mostraron una corona más grande en relación con la raíz, mientras que los caninos masculinos mostraron mayores raíces en relación con la corona. Los caninos femeninos exhibieron un gran desarrollo del cíngulo y los dientes masculinos presentaron un menor desarrollo de este rasgo. El presente estudio constituye una primera aproximación para mostrar la utilidad de la forma para la estimación del sexo de los dientes caninos. Las discrepancias observadas en estas estructuras podrían ser el resultado de diferencias en la cantidad total de dentina y esmalte debido al efecto de genes ubicados en los cromosomas sexuales involucrados en el desarrollo de estos tejidos. Rev Arg Antrop Biol 26(1), 075, 2024. https://doi.org/10.24215/18536387e075

Palabras Clave: odontología forense; restos humanos; morfometría; estimación del sexo

Sex estimation is one of the main assessments that involves the usual bioarchaeological or forensic practice (Prabhu & Acharya, 2009). This is possible due to the existence of morphological sexual differences within species (Anuthama *et al*., 2011; Christensen *et al*., 2014). Investigations regarding such differences in human skeleton remains have been well studied in many populations around the world. In the last decades, studies of sexual dimorphism in teeth have been carried out extensively (Anuthama *et al*., 2011; Isçan & Kedici, 2003; Kazzazi & Kranioti, 2017; Khamis *et al*., 2014; Prabhu & Acharya, 2009; Viciano, 2013). The relevance of dental studies is related to the higher resistance to postmortem changes and the value of teeth in the forensic identification of human remains (Acharya & Mainali, 2007; Anuthama *et al*., 2011; Bañuls *et al*., 2014; Prabhu & Acharya, 2009). Hence, the use of these elements in forensic and bioarchaeological investigations is important when bones are fragmented, skeletal secondary sexual characteristics are not fully developed or DNA analysis is not feasible. Accordingly, in the last decades numerous studies have analyzed the shape and size of teeth in order to explore the dynamics of prehistoric populations or even sexual differences (Cucina *et al*., 2015; Polychronis *et al*., 2013; Popovici *et al*., 2022; Yamada *et al*., 2021).

Several studies have revealed that sexual variation in the size of dental elements exists (Acharya & Mainali, 2007; Garn *et al*., 1966; Viciano, 2013; Vodanovic *et al*., 2007). Canine tooth sexual dimorphism is common among anthropoids, e.g. in some cercopithecoid species males exceed females in height by 400% (Plavcan, 2001). Despite a decrease in sexual dimorphism throughout the human evolutionary process, differences between male and female dentition are still present in modern humans (Banarjee *et al*., 2016; Ghandi *et al*., 2017; Plavcan, 2001; Vodanovic *et al*., 2007). These differences have been found mainly in canines, both in mesiodistal and buccolingual dimensions (Kazzazi & Kranioti, 2017; Viciano *et al*., 2013). Isçan & Kedici (2003) have reported that canines allow to correctly classify sex with a 77% accuracy in Turkish individuals, while the rest of the dentition exhibits lower percentages. Furthermore, Pettenati-Soubayroux *et al.* (2002) evaluated sexual dimorphism in canines, central and lateral incisors of both dental arches. The authors concluded that the canine was the most dimorphic tooth, followed by both upper incisors (Pettenati-Soubayroux *et al*., 2002). Another odontometric study led by Viciano (2012) in a Mediterranean osteological sample showed that the canines and first mandibular molar are the teeth that best display sexual dimorphism.

Most of the investigations regarding the sexual variation in dental features were made by using odontometric analysis (Acharya & Mainali, 2007; Isçan & Kedici, 2003; Kazzazi & Kranioti, 2017; Viciano, 2013; Viciano *et al*., 2013; Viciano *et al*., 2015). However, the shape differences between sexes in human dentition have been less explored and the few studies that addressed this issue have mainly analyzed population differences or singularities within populations (Bernal, 2007; Kieser *et al*., 2007; Perez *et al*., 2006). Two investigations of dental variation in modern human molar crowns have revealed no differences in shape between sexes employing a geometrical analysis (Ferrario *et al*., 1999; Polychronis *et al*., 2013). Despite finding no differences in shape, Polychronis *et al.* (2013) have described male molars as being more squared and having higher cusp heights than female molars. More recently, López-Lázaro *et al.* (2020) have explored the sexual dimorphism in the occlusal view of the maxillary postcanine dentition employing geometric morphometric techniques. Since the results of the cross-validation analysis were not satisfactory, the postcanine maxillary dentition is not useful for sex estimation.

Considering that morphology is constituted by two components (size and shape), the lack of studies on the second dimension in dental anthropology is a matter to reverse. Particularly, despite the large number of investigations on dental size, one of the aspects that has been less studied is shape variation related to sex. Therefore, this work aims to analyze and describe dental sexual variation in shape and size focusing on the analysis of mandibular canines by means of geometric morphometrics.

MATERIALS AND METHODS

Sample

The sample included in this study belongs to the "Prof. Dr. Rómulo Lambre" collection (*Facultad de Ciencias Médicas, Universidad Nacional de La Plata*), which is constituted by individuals from the late 20th century with associated documentary information. A total of 56 right mandibular canines from 37 male and 19 female individuals with known age-at-death and sex information were selected, being the mean age of 55.25 years (Supplementary Table S1). The decision to consider only the right canine was based on the fact that it is the most represented side of both mandibular canines (Garizoain *et al*., 2018). In order to obtain more consistent results, no samples with dental anomalies or pathologies were included. This investigation was conducted by taking into account the national ethics codes (Aranda *et al*., 2014). Furthermore, it was approved by the Bioethics Committee of the *Facultad de Ciencias Médicas, Universidad Nacional de La Plata*.

It is worth mentioning that in the mesiodistal view, three teeth (from one female and two males) showed dental decay. This fact hindered the possibility of suitably positioning the landmarks to perform the analysis. For this reason, those three cases were excluded from the analysis in that view.

Morphometric analysis

Odontometric analysis was conducted using 2-D geometric morphometrics techniques based on anatomical points (landmarks) and curves (semilandmarks) registered on three views of the canine tooth, occlusal (OV), mesiodistal (MV) and buccolingual (BV) views (Fig. 1). Images of the teeth were obtained (G. Garizoain) employing a Canon EOS Rebel T3i digital camera. The teeth were positioned on dental modeling wax sheets next to a scale. For the OV images, the camera lens was located at a distance of 470 mm from the occlusal surface of the teeth. For the MV and BV, photographs were taken at a distance of 480 mm from the canine's surfaces. Coordinates of nine semilandmarks and six landmarks were surveyed for the OV (Fig. 1A), 10 semilandmarks and five landmarks for the MV (Fig. 1B), and 10 semilandmarks and seven landmarks for the BV (Fig. 1C).

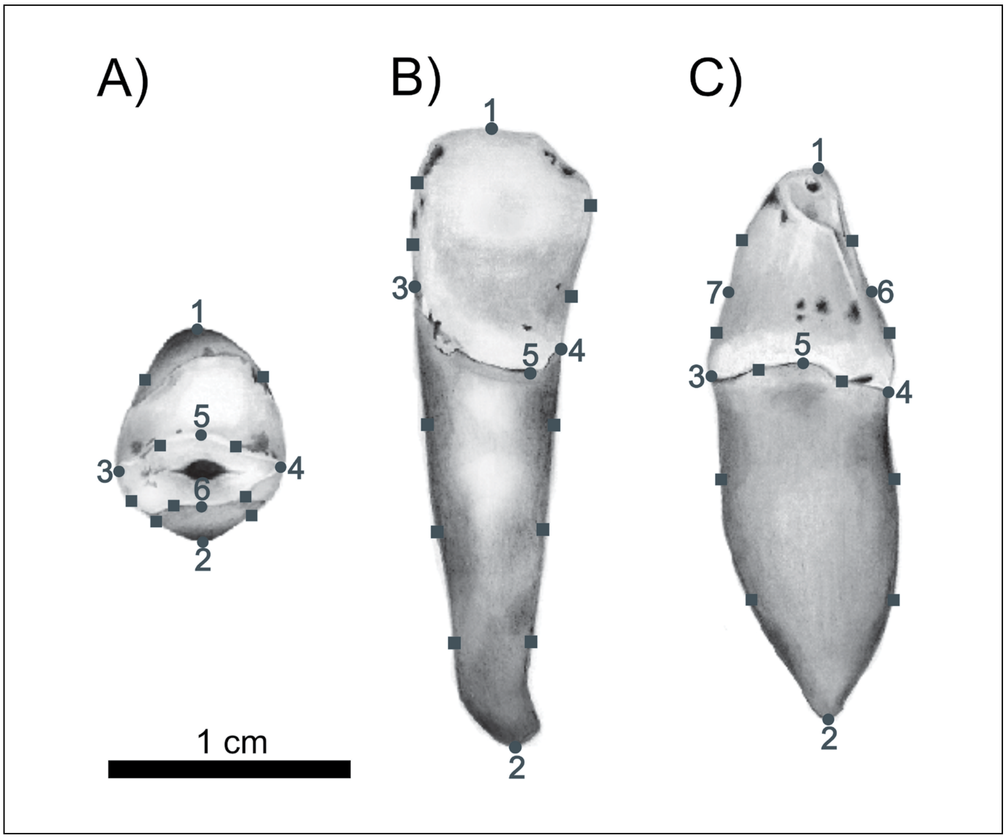

FIGURE 1. Landmarks (circles) and semilandmarks (squares). **A)** 1. Outermost point of the labial margin of the tooth; 2. Outermost point of the lingual margin of the tooth; 3. Outermost point of the distal margin of the crown; 4. Outermost point of the mesial margin of the crown; 5. Outermost point of the labial margin of the cusp; 6. Outermost point of the lingual margin of the cusp. **B)** 1. Highest point of the crown; 2. Lowest point of the root; 3. Point at the enamel-cementum junction on the distal margin; 4. Point at the enamel-cementum junction on the mesial margin; 5. Lowest point of the enamel-cementum junction on the labial surface. **C)** 1. Highest point of the crown; 2. Lowest point of the root; 3. Point at the enamel-cementum junction on the labial margin; 4. Point at the enamel-cementum junction on the lingual margin; 5. Highest point of the enamel-cementum junction on the mesial surface; 6. Highest point on the lingual margin where the inflection of the cingulum begins; 7. Point on the labial margin, opposite to landmark (Illustrations by Adumbratio Ilustración Científica, Laura Blanco and Vanesa Gaido).

The coordinates were recorded using the tpsDig2 Version 2.31 software (Rohlf, 2017) and analyzed separately. The recording of the variables was made by a single observer (V. A. Cobos). The estimation of the nature and magnitude of the error in the registration of the coordinates of landmarks and the curves described by the semilandmarks, which can appreciably affect the results of morphometrics analyses, was carried out through repeated measures ANOVA and correlation intraclass (Bernal *et al*., 2004). These estimations were calculated employing the R software Version 4.0.3 (R Development Core Team, 2020). The results displayed low levels of intraobserver error for both data sets.

Superimposed coordinates, or shape variables, were obtained from the original coordinates of landmarks and semilandmarks using the Generalized Procrustes Analysis (Rohlf & Slice, 1990). This method translates, scales, and rotates coordinates of semilandmarks and landmarks. The semilandmarks were slid along tangents to the outline of the curve minimizing bending energy (Bookstein, 1997; Bookstein *et al*., 2002), using the tpsRelw Version 1.70 (Rohlf, 2019). From superimposed coordinates, a covariance matrix was calculated and Canonical Variate Analysis (CVA) was performed to explore the pattern of intersex variation in the occlusal, mesiodistal and buccolingual shape. The shape changes in relation to the consensus form associated with the major axes were visualized as deformation grids based on each CVA axis. In order to explore the accuracy of the canine shape for sex-group allocation, a Discriminant Function Analysis with cross-validation was performed. The centroid size (CS) of the specimens (the square root of the summed squared distances from all landmarks and semilandmarks to the configuration centroid) was measured for each canine view (Bookstein, 1991). Subsequently, a regression of the CS versus the Procrustes coordinates was performed to examine how much of the shape was dependent on the size. All these analyses were performed using the MorphoJ software Version 1.06d (Klingenberg, 2011).

To show the sexual dimorphism explained by the CS, a box plot representation was executed. Finally, an analysis of the variance (ANOVA) was carried out to observe the significance of the sexual dimorphism differences with the CS and the percentage of sexual dimorphism was calculated according to the formulae proposed by Garn *et al.* (1967). These analyses were performed with the R program Version 4.0.3 (R Development Core Team, 2020).

Finally, to explore the impact of moderate dental wear on the results obtained, all the analyses were repeated including only those teeth with a degree of wear equal to or less than 4 on Smith's scoring system (Smith, 1984; Supplementary Table S1). Higher wear degrees imply the loss of anatomical traits such as the cingulum of the crown, which also implies that dental decay affects more than half of the tooth. Thus, this subsample excluded a total of three female and ten male individuals.

RESULTS

In the CVA, the relative size of the crown cusp varies from negative to positive values for the OV. The cusps of the teeth on the positive side of the CVA axis are smaller than the consensus value, while the canines on the negative side are bigger. In the canines to the right of the axis, the cingulum exhibits more development than the labial side. The opposite pattern can be appreciated in canines to the left of the axis, where the cingulum is less developed than the opposite side (Fig. 2A). Likewise, the analysis in the MV and BV show that the canines on the right have relatively larger crowns than the ones on the left: The root is larger concerning the crown in the male individuals. However, those patterns are more marked in the MV of the canines (Fig. 2B and 2C). The morphology described for individuals with positive values is associated with female individuals while the one described for the canines with negative values is associated with male individuals for the views analyzed. The same pattern is observed for the three canines' views: The canines from female individuals (right) are clearly segregated from those from the male ones (left) throughout the VC1 (Fig. 2). When considering tooth wear, the CVA results obtained showed the same trend as when the entire sample was analyzed (Supplementary Information, Fig. S1).

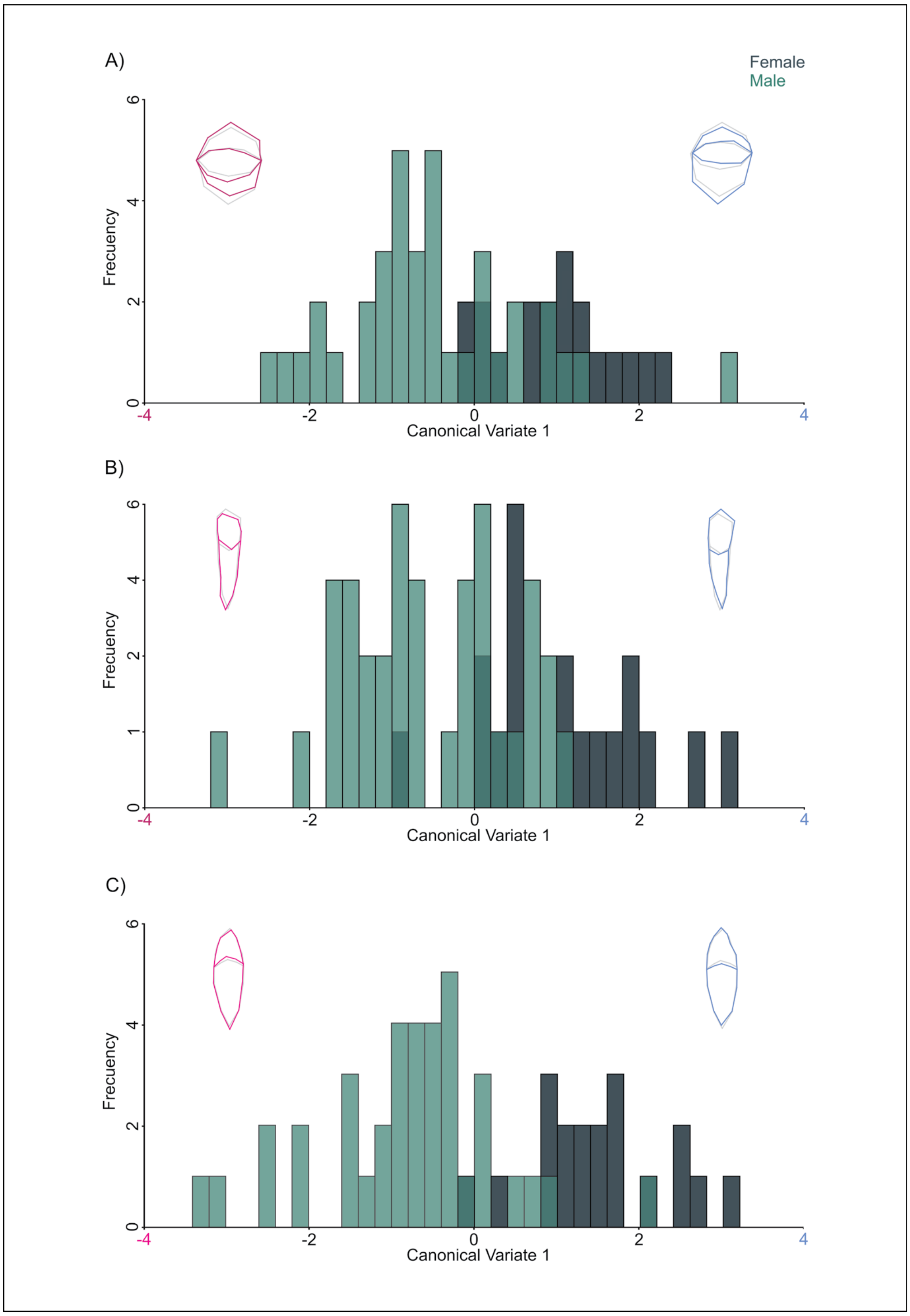

FIGURE 2. Canonical variate analysis (CVA) and wireframes of the canines (consensus in grey, extreme shapes of the x-axis in color). **A)** Occlusal view. **B)** Mesiodistal view. **C)** Buccolingual view.

Revista Argentina de Antropología Biológica

The results of the discriminant analysis show that the percentage of individuals whose sex was correctly assigned is over 75% in all the tooth views examined (Table 1). Furthermore, there is no difference in these percentages between the sexes, such that overclassification of individuals of one sex may be discarded. Nevertheless, when performing a cross-validation to test the results of the discriminant function, the percentages of correctly assigned individuals decrease considerably, also revealing an overclassification of samples as male (Table 1). Including only those teeth with a score of dental wear equal to or less than 4 according to Smith's classification in the discriminant analysis, we achieved percentages of correctly assigned cases higher than 75% in all views. However, when the cross-validation analysis was performed, the percentages of correctly classified individuals were around 50% (Supplementary Table S2).

TABLE 1. Number and percentages of correct sex estimations for the three dental views.

		Discriminant Analysis			Cross-Validation		
		Correctly assigned	Incorrectly assigned	% correctly assigned	Correctly assigned	Incorrectly assigned	% correctly assigned
OV	F	15	4	78.94	8	11	42.10
	M	29	8	78.37	20	17	54.05
	Total	44	12	78.57	28	28	50
BV	F	14	4	77.77	6	12	33.33
	M	28	7	80	19	16	54.28
	Total	42	11	79.24	25	28	47.16
MV	F	17	2	89.47	7	12	36.84
	M	33	4	89.18	19	18	51.35
	Total	50	6	89.28	26	30	46.42

OV: occlusal, **MV:** mesiodistal, **BV:** buccolingual; **F:** female; **M:** male

Figure 3 shows that the CS means of the female canines are smaller than those of the male for all the views analyzed. For the MV and BV males, it shows a larger dispersion in the CS values than females (Fig. 3B and 3C). However, for the OV females, more dispersion is observed, with sizes almost as large as those for males (Fig. 3A). The average values obtained for the centroid size are higher for male individuals (OV = 1.24; MV = 2.12; BV = 3.25) than females (OV = 1.18; MV = 2.88; BV = 3.02) in OV and BV views. Likewise, slightly higher values are obtained in both views when only including those individuals with dental wear equal to or less than 4. However, in this last analysis, higher values for males are also obtained in the MV view (MV male subsample = 3.15; MV female subsample = 2.86).

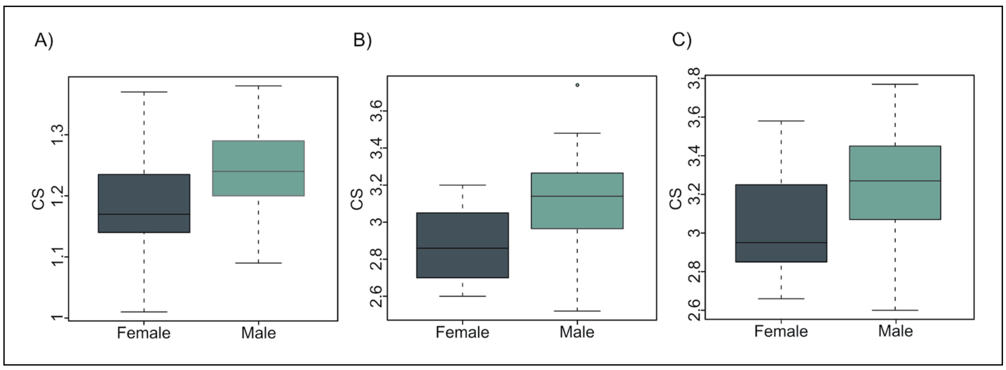

FIGURE 3. Boxplot representation of the centroid size (CS) values. **A)** Occlusal view. **B)** Mesiodistal view. **C)** Buccolingual view.

The regression of the CS versus the Procrustes coordinates is significant for the MV (p-value < .001; Fig. 4A) and BV (p-value = .001; Fig. 4B), while no significant correlation was observed in the OV (p-value = .211). Nevertheless, the change in size explains a small shape variation (8.05% for the MV and 7.75% for the BV). In both analyses, large sizes are related to relatively large roots. The female canines show smaller sizes than the male canines (Fig. 4). These results match those obtained by the ANOVA performed with the CS values (Table 2). For this analysis, significant p-values were obtained for all views (Table 2). By performing the analyses including only canines with a score less than 5, the same pattern is obtained (MV p-value <.001; BV p-value <.001; OV p-value = .447). However, the change in size explained by shape variation is greater than when considering the whole sample (10.33% for the MV and 11.03% for the BV). Also, the ANOVA performed with the CS values provided significant p-values for all views (Supplementary Table S3).

Finally, the percentage of sexual dimorphism points out that the MV is the most dimorphic dimension (8.24%), followed by the BV (7.55%) and the OV (4.36%). On the other hand, the sexual dimorphism calculated with the subsample that only considers those individuals with dental wear equal to or less than 4 in Smith's (1984) classification is similar except for the MV. Thus, for the OV and the BV, the estimated percentages were 4.87% and 7.97% respectively, while for the MV was 10.13%.

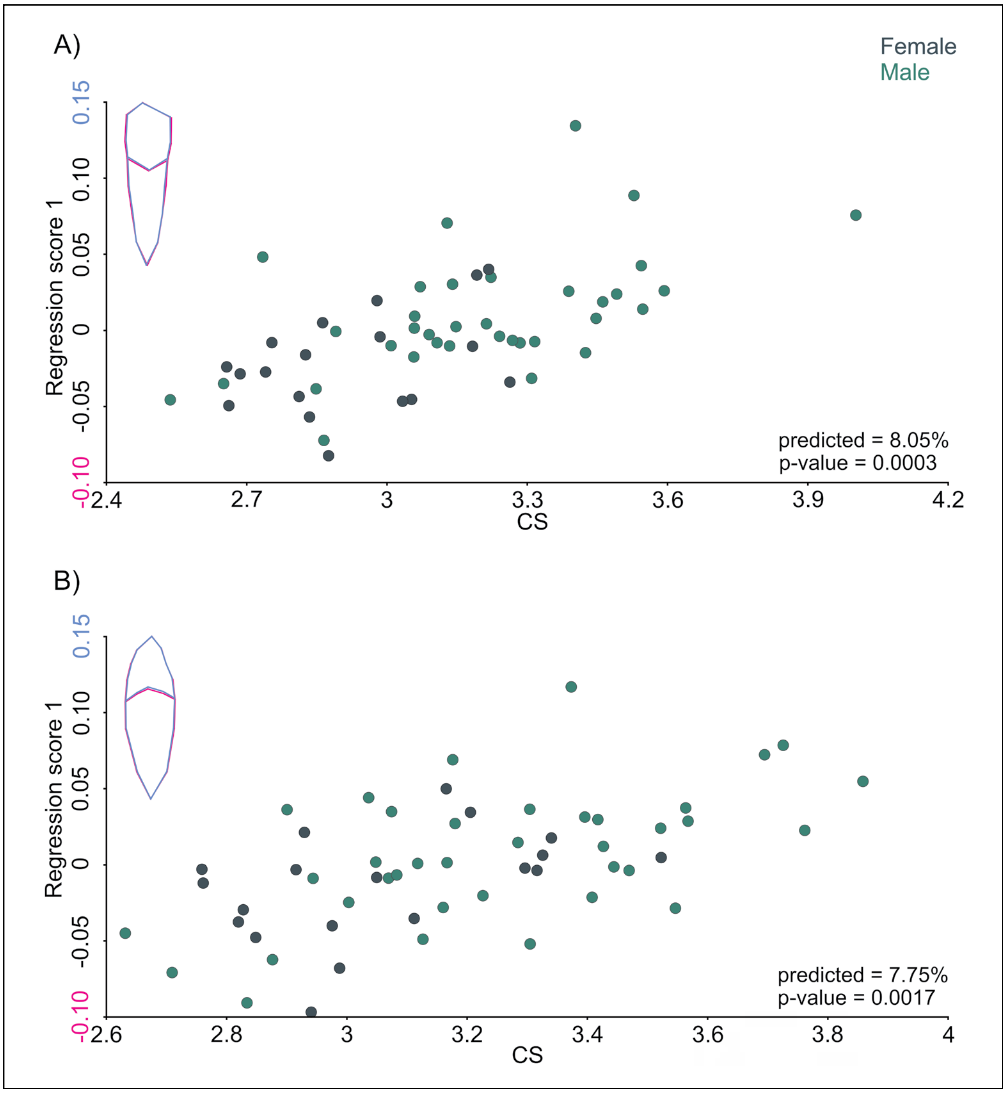

FIGURE 4. Regression of the centroid size (CS) versus the Procrustes coordinates and wireframes of extreme values (y-axis) of the Procrustes coordinates. **A)** Mesiodistal view. **B)** Buccolingual view.

TABLE 2. Results of the analysis of variance (ANOVA), performed for the three views of the canine using the centroid size values.

View	F value	Pr (>F)
Occlusal	5.06	0.0285*
Mesiodistal	11.71	0.0012**
Buccolingual	8.12	0.0061**

* p < 0.05; ** p < 0.01

DISCUSSION

Different studies focused on sexual dimorphism in teeth size have found that differences in enamel and dentin proportion were related to the sexual chromosomes (Alvesalo, 1971, 1997, 2009; Alvesalo *et al*., 1977). Evidence provided by analysis of aneuploidy cases has suggested that the X chromosome contributes to the enamel development and the Y chromosome is involved in both enamel and dentine development (Alvesalo, 2009; Alvesalo & Tammisalo, 1981). These results imply that differences in overall size between sexes lie in a greater proportion of dentine in males which generates size differences, being male dentition slightly larger than female. However, the sexual dimorphism in shape dentition has been less explored.

In the present study, it was found that male and female canine shapes differ, although there is an overlap in their morphologies, as expected in all biological traits within a species. On the occlusal surface, it was observed a transition from a female shape, with a great development of the cingulum, to a male one, where this trait is less developed. Likewise, when analyzing the mesiodistal and buccolingual views, the female crown occupies a greater proportion of the whole teeth in relation to the root, while the male canine is characterized by having a greater root in relation to the crown. These sexual differences in the proportions that crown and root occupy in canines may be due to disparities in the total amount of enamel and dentine between individuals of different sexes. In this work, we have shown that a moderate degree of dental wear -i.e., equal to or less than 4 in Smith scale- does not modify these results. Other studies have concluded that male dental pieces are characterized by presenting larger amounts of dentine in comparison to females (Alvesalo *et al*., 1991; Graziano *et al*., 1984; Stroud *et al*., 1994). These differences also have consequences in root size, which agree with the results of other authors (Alvesalo, 2009; Lähdesmäki, 2006; Lähdesmäki & Alvesalo, 2004, 2005).

On the other hand, it is held by other studies that female enamel caps tend to be thicker than male's (Saunders *et al*., 2007; Schwarz & Dean, 2005). Considering these results, it might be argued that the greater crown proportion in relation to the root present in female canines, is due to the minor dentinal component that such teeth have. Likewise, male canines, which present significantly more dentine, show larger roots that occupy a greater proportion of the whole teeth. These differences, as shown by other studies, stem from the influence of the Y chromosome on root growth, which significantly differs from the effect of the X chromosome (Alvesalo, 2009; Alvesalo *et al*., 1991; Graziano *et al*., 1984; Lähdesmäki, 2006; Lähdesmäki & Alvesalo, 2004, 2005; Schwarz & Dean, 2005).

Previous studies have reported significant statistical differences between sexes in root length (Alvesalo, 2009; Garizoain *et al*., 2018; Lähdesmäki, 2006; Lähdesmäki & Alvesalo, 2004, 2005). The results of this work are consistent with what is known about sexual size differences. In the mesiodistal and buccolingual view, sexual shape differences are explained mostly by size differences in the roots and crowns of the canine. Moreover, we show that these results cannot be attributed to modifications resulting from dental wear. On the other hand, higher values of centroid size were obtained for males in all views when dental wear was considered.

The results of the discriminant analysis indicate that the use of shape differences between the sexes in the lower canines does not currently have sufficient classificatory power to be used for sex estimation. Although the initial results were acceptable, the correct classifications were low in the cross-validation used to test the models. As it was previously pointed out, a considerable amount of overlap between male and female forms was observed in all three views, initially suggesting that the discriminatory ability of this geometric morphological analysis is limited. However, the fact that canines have been identified in several studies (Acharya & Mainali, 2007; Anuthama *et al*., 2011; Garizoain, 2019; Luna, 2019; Viciano, 2013) as the teeth that best predict biological sex (based on odontometric analysis) suggests that further studies of this type are needed. When considering tooth wear, the analysis performed showed no differences in the discriminant analysis with the whole sample. This may indicate that it could be possible to apply this kind of analysis to teeth with moderate wear without affecting sex differences in canine shape.

Finally, when calculating the percentage of sexual dimorphism in the different views it was found that the mesiodistal view of the canines was the one with greater differences exhibited, followed by the buccolingual view. Moreover, the percentage of sexual dimorphism shown by the occlusal view was considerably lower than the other views, which might be due to external factors such as dental wear. Nevertheless, a low percentage of sexual dimorphism is observed in centroid size for all views. Since the regression analysis indicated a significant positive co-relationship between size and shape, despite only a small percentage of the differences in shape being explained by size (for the BV and the MV), it is concluded that the main distinctions between the sexes stem from variation in the form of the canine. When we consider dental wear, only for the mesiodistal view there was a considerable difference in the percentage of sexual dimorphism in relation to the values obtained with the whole sample. Nonetheless, our results also suggested that when considering the impact of dental wear, a greater amount of change in size may be explained by shape variation. Therefore, this result leads us to conclude the importance of taking into account the impact of dental wear when assessing sexual dimorphism, particularly in archaeological samples with high degrees of dental decay.

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CONCLUSIONS

In the present study, shape differences between sexes were found. Our results emphasize an aspect of morphology that has not been deeply studied in the context of forensic anthropological investigations. Concerning forensic anthropology studies, most of the sex studies conducted to date in dentition utilize size differences in the development of discriminant functions, with varying degrees of precision. It is generally assumed that sexual differences in tooth size result from differences in total tissue amount. For this reason, geometric morphometric analysis on sex differences in teeth should be promoted to fully understand sex variation in shape in order to develop methods for sex estimation. From the results obtained, it is possible to conclude that the geometric morphometric analysis of the canines would not be useful for the estimation of sex. However, to fully understand the utility of this type of methodological approach to sex estimation, further analysis will be needed. A discriminant analysis with a larger sample will allow us to evaluate reliably the classificatory capacity that canine shape presents for sex estimation. Besides, further studies on the impact of dental decay on sexual dimorphism are needed.

AUTHORS' CONTRIBUTIONS

Gonzalo Garizoain (Conceptualization, Writing, Research, Photo compilation, Interpretation of the results), Virginia A. Cobos (Conceptualization, Writing, Research, Formal analysis, Interpretation of the results, Figure Edition).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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