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Are deciduous upper molars and lower canines useful for sex estimation?

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ABSTRACT

Objective: This paper investigates whether deciduous upper molars and lower canines have sexual dimorphic features, exploring these teeth' dimensions and the presence of Zuckerkandl's tubercle and Carabelli's cusp on the first and second upper molars.

Design: We analyzed 64 pairs of dental plaster casts from 34 females and 30 males aged between 3 and 12 years. We measured the first and second deciduous upper molars and the lower deciduous canines (maximum mesio-distal and buccolingual length), and we registered the presence of the Zuckerkandl's tubercle and the Carabelli's cusp on the first and second upper molars, respectively.

Results: Regarding the differentiation between sexes using Carabelli's cusp and Zuckerkandl's tubercle, the classification was not independent of Carabelli's cusp presence only for tooth 65 ($p = 0.035$). In all other teeth, whether for Carabelli's cusp or Zuckerkandl's tubercle, their presence was similar for both sexes. There were statistically significant differences between sexes ($p < 0.05$) for the buccolingual measurements of both upper second molars, the first right upper molar, and the right canine. The developed model allowed for a 64.1% accuracy in sex estimation.

Conclusions: The study suggests that while Carabelli's cusp and Zuckerkandl's tubercle in upper deciduous molars don't consistently differ between sexes, tooth size, particularly the buccolingual measurements of certain teeth, including upper deciduous molars and lower canines, may provide a more reliable criterion for sex estimation. The developed model depicted moderate accuracy, underscoring the need for a multifactorial approach when estimating sex from skeletal remains. It suggests that while dental features can contribute to sex estimation, they should be used in conjunction with other skeletal or molecular markers to improve accuracy.

1. Introduction

Sex estimation in children's skeletal remains has been a long-time challenge for forensic medicine (Black, 1978). In adults, sex can be estimated with osteological methods, mainly using the pelvis and skull analysis (Krishan et al., 2016; Schutkowski, 1993); however, in immature human remains, this diagnosis is more complex because sexually differentiated skeletal features are not yet discernible (Schutkowski, 1993).

Numerous studies have been carried out to develop methods for estimating sex in prepubertal age (Fan et al., 2019; Noble, Cardini, Flavel, & Franklin, 2019; Schutkowski, 1993; Viciano, Tanga, D'Anastasio, Belcastro, & Capasso, 2021). However, as stated, these are not as accurate as those used to estimate sex in adults (Lopez-Lazaro, Aleman, Viciano, Irurita, & Botella, 2018). These studies analyzed sex differences in several anatomical regions, including the pelvis (Blake, 2011; Estévez Campo, López-Lázaro, López-Morago Rodríguez, Alemán Aguilera, & Botella López, 2018), the long bones (Stull & Godde, 2013), the mandible (Schutkowski, 1993), the skull (Noble et al., 2019), the tarsal bones (Harris & Case, 2012) and teeth (Adler & Donlon, 2010; Black, 1978; Cardoso, 2010; Harris & Couch Jr., 2006; Harris & Lease, 2005; Holman & Jones, 2003; Introna, Cantatore, Dragone, & Colonna, 1993;

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Macaluso, 2011; Tanguay, Demirjian, & Thibault, 1984). Some claim sexual diagnosis in subadults is more accurate using odontometric rather than skeletal methods (Paknahad, Vossoughi, & Ahmadi Zeydabadi, 2016). The fact that most teeth complete their development before skeletal maturation may account for that evidence (Shireen & Ara, 2016). The existence of sexual dimorphism in specific dimensions in the permanent dentition (Azevedo, Pereira, Gouveia, Tavares, & Caldas, 2019; Ditch & Rose, 1972; Garn, Lewis, & Kerewsky, 1964; Jensen, Kai-Jen Yen, Moorrees, & Thomsen, 1957; Silva et al., 2016), suggested that this may also be the case for the primary dentition (De Vito & Saunders, 1990).

Meanwhile, several studies on sex estimation using deciduous teeth have been performed, based on metric features (Anderson, 2005; Axelsson & Kirveskari, 1984; Brown, Margetts, & Townsend, 1980; De Vito & Saunders, 1990; Harris & Lease, 2005; Introna et al., 1993; Kondo et al., 1998; Liu, Dung, & Yang, 2000; Lu et al., 2022; Margetts & Brown, 1978; Shankar et al., 2013; Vaughan & Harris, 1992; Viciano, Lopez-Lazaro, & Aleman, 2013; Viciano et al., 2021) concerning a non-contemporary population (Viciano, Aleman, D'Anastasio, Capasso, & Botella, 2011; Viciano, D'Anastasio, & Capasso, 2015), using the occlusal surface features of the first upper molar (Lopez-Lazaro et al., 2018), exploiting metric traits (crown size, trigonid size, and talonid size) and morphological features (Carabelli's cusp and molar cusp number) (Adler & Donlon, 2010), performed on x-rays (Harris & Couch Jr., 2006; Paknahad et al., 2016), considering teeth emergence (Holman & Jones, 2003; Tanguay et al., 1984) and only one was performed on a small (n = 46) Portuguese sample (Cardoso, 2010). Yet, metric traits in deciduous dentition among different populations have shown that tooth sizes and patterns have diverse dissimilarities in other ethnicities (Anderson, 2005; Axelsson & Kirveskari, 1984; Margetts & Brown, 1978; Vaughan & Harris, 1992).

This work explored whether sexual dimorphism exists in deciduous dentition, specifically in the upper molars and lower canines, using metric and non-metric traits in a Portuguese sample.

2. Materials and methods

A sample of 64 pairs of plaster dental casts was selected. Casts belonged to children attending a private practice in Madeira Archipelago, Portugal. All children were born in Portugal, not necessarily in Madeira, and the parents' nationality was Portuguese. Portuguese people are one of the most ethnically homogenous countries in the world, with about 95% of the population being ethnic Portuguese (Santos, Romão, & Cerqueira, 2023). Most casts belonged to females (n = 34, 53.1%) with mixed dentition, aged between 3 years and six months and 11 years and seven months. The models were coded to preserve anonymity and avoid classification bias.

Inclusion criteria were a) complete eruption of deciduous teeth, b) absence of caries or restorations, c) absence of previous orthodontic treatment, d) presence of deciduous canines and molars, and e) absence of defects in the plaster casts.

Exclusion criteria were the presence of caries, hypoplastic defects, traumatic lesions, anomalies in number, size, and shape, accentuated tooth wear, and other nationalities that were not Portuguese.

The upper first and second deciduous molars and the lower deciduous canines were selected for metric data collection. The upper first molars were evaluated for the presence of Zuckerkandl's tubercle, and the upper second molars were studied for Carabelli's cusp expression. Teeth are described using the FDI notation and a non-clinical nomenclature, both portrayed in Table 1.

Metric data were obtained using a 150 mm high-precision digital caliper (Mitutoyo ®) with a 0.01 mm resolution. The mesiodistal and buccolingual diameters of the upper molars and lower canines (54,55,64,65,73 and 83) were measured. The mesiodistal diameter was defined as the maximum distance between the mesial and distal contact points, with the caliper parallel to the occlusal and buccal surface. The buccolingual diameter was defined as the maximum distance between the buccal and lingual surfaces, with the caliper placed perpendicular to the mesiodistal distance. For rotated or poorly positioned teeth, measurements were taken from the proximal faces of the crowns, where a point of contact with adjacent teeth would be expected (Adler & Donlon, 2010).

Table 1
Nomenclature used for dental identification.

Tooth	FDI notation	Non-clinical nomenclature
Upper right first deciduous molar	54	urdm1
Upper left first deciduous molar	55	uldm1
Upper right second deciduous molar	64	urdm2
Upper left second deciduous molar	65	uldm2
Lower right deciduous canine	83	lrdc
Lower left deciduous canine	73	lldc

Two plates, inspired by the Arizona State University Dental Anthropology System (ASUDAS) plates (Scott & Irish, 2017), were created: one for the Zuckerkandl's tubercle (Fig. 1) and another for the Carabelli's cusp (Fig. 2). The plates were built from casts observed by two authors, both experienced dentists with a background in dental anthropology, and used to classify the two morphological features.

To assess interobserver and intra-observer agreement and reliability, the Intraclass Correlation Coefficient (ICC) was used for continuous variables (mesiodistal and buccolingual diameters) and Cohen's Kappa test for categorical variables (Zuckerkandl's tubercle and Carabelli's cusp). Interobserver agreement was tested by randomly selecting a subsample of 12 upper and 12 lower casts, and two observers performed the measurements using the same caliper. The procedure was repeated for non-metric data, using the plates for classification purposes. Intra-observer agreement was tested by randomly selecting a subsample of 12 upper and 12 lower models, and the same observer performed the measurements and used the plates twice at an interval of two weeks.

The intraclass correlation coefficient for intra- and inter-observer error analysis were assessed for reliability based on a random sample of 6 teeth and 12 casts. In the case of intraclass correlation, the estimates and the corresponding 95% confidence intervals were based on two observations by the same investigator (2-input mixed effects model, absolute agreement). In contrast, the interclass correlation was based on the readings of two observers (2-input mixed effects model, consistency). According to (Fleiss, 1986), ICC values below 0.4 indicate poor reliability, values between 0.4 and 0.75 indicate moderate to good reliability, and values above 0.75 indicate excellent reliability.

The procedure was repeated for non-metric data, with intervals of two weeks, using the same classification boards.

As for the agreement and reliability of the categorical variables, the classifications obtained were evaluated using Cohen's Kappa test, as suggested by Landis and Kock (Landis & Koch, 1977).

Descriptive statistics were calculated, describing the mean and standard deviation for continuous variables and frequency and percentage values for categorical variables for each variable. Independent Student's t-test was applied after confirming, through the Kolmogorov-

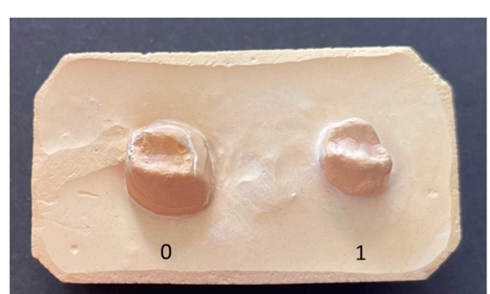

Fig. 1. Plate for classifying Zuckerkandl's tubercle (0 – absent; 1- present).

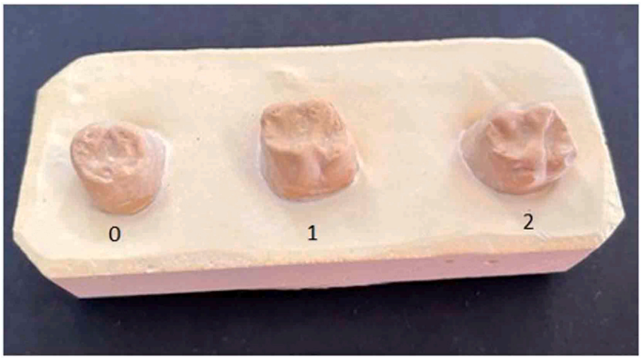

Fig. 2. Plate used for classifying Carabelli's cusp (No expression-0; Medium expression-1; Maximum expression-2).

Smirnov normality test, that the variables under analysis have an approximately normal distribution. Moreover, homoscedasticity was evaluated using a scatterplot of residuals versus predicted values, and no clear pattern in the distribution was found.

A discriminant analysis was carried out to verify if there was a linear relationship between the mesiodistal and buccolingual diameters of the lower canines and the upper first and second molars (independent variables) and sex (dependent variable). The Chi-Square test was used to analyze the categorical variables to verify if there is a relationship between the presence or expression of Zuckerkandl's tubercle and Carabelli's cusp, respectively, and sex.

Statistical analysis was performed using SPSS software (Statistical Package for Social Sciences), version 28.0. Significance was set to $p < 0.05$.

The present study obtained a favorable recommendation from the Ethics Committee of the Faculty of Dental Medicine of the University of Porto (Recommendation number 28/2021).

3. Results

The mean ICC for inter-observer error analysis was 0.965 for buccolingual and 0.933 for mesiodistal, with an overall mean value of 0.949. Regarding the intra-observer error analysis, the mean ICC values were 0.912 for buccolingual and 0.853 for mesiodistal, with an overall mean value of 0.883. Altogether, the values of the global and measured coefficients indicate excellent reliability and reproducibility.

The interobserver agreement showed substantial and significant agreement for the two measures, with Kappa values of 0.679 ($p < 0.001$) and 0.625 ($p < 0.001$) for the classifications of Carabelli's cusp and Zuckerkandl's tubercle, respectively. In the analysis of the intra-observer agreement, the values obtained indicated a very high agreement, with $k = 0.888$ and $k = 0.882$ ($p < 0.001$, in both).

Table 2
Carabelli's cusp distribution, by sex, in the upper second deciduous molars, n (%).

Expression	Sex				TOTAL	
	Female, n (%)		Male, n (%)		n (%)	
	urdm2	uldm2	urdm2	uldm2	urdm2	uldm2
1 (Absent)	11 (55.0)	3(25.0)	9 (45.0)	9(75.0)	20 (100)	12 (100)
2 (Mild)	16 (53.3)	26 (65.0)	14 (46.7)	14 (35.0)	30 (100)	40 (100)
3 (Full)	7 (50.0)	5(41.7)	7 (50.0)	7(58.3)	14 (100)	12 (100)
TOTAL	34 (100)	34(100)	30 (100)	30 (100)	64 (100)	64 (100)

abbreviations: 1st letter - u=upper; l=lower; 2nd letter - r = right; l=left; d=deciduous; m=molar; e.g. uldm2 =upper, left deciduous molar second.

Table 2 depicts the Carabelli's cusp distribution by sex. In upper right second molar, the distribution between sexes was similar, and no statistically significant differences were found (χ^2 (2) $= 0.084$, $p = 0.959$); as for the left tooth, most cases classified with absence corresponded to males (75.0%), being the differences between sexes statistically significant (χ^2 (2)$= 6.710$, $p = 0.035$); when classified with a mild expression, 65% of the cases were female, and, with full expression, 58.3% were male.

As for Zuckerkandl's tubercle (Table 3), it was equally distributed in both sexes, and its presence was not associated with sex (χ^2 (1) $= 0.084$, $p = 0.772$, and χ^2 (1)$= 0.759$, $p = 0.384$, for tooth 54 and 64, respectively).

In metric features (Table 4), there were statistically significant differences between sexes ($p < 0.05$) for the buccolingual measurements of both upper second molars (55 and 65), the first right upper molar (54), and the right canine (83). On average, males had a significantly higher value in the buccolingual diameter than females for all teeth except the upper left first molar and the lower left canine (73). Considering mesiodistal measurements, statistically significant differences between sexes were only found in tooth 65 (the upper left second deciduous molar). On average, males had higher values in all measurements, except for the lower left canine mesiodistal diameter and the upper right first molar mesiodistal width, where females had a higher mean.

The discriminant analysis assumed a normal multivariate distribution. In the absence of a formal test for multivariate normality, it was found that the different variables observed the Kolmogorov-Smirnov normality test, except for the buccolingual dimension of the upper right first molar.

Comparing the means with the t-test, the mesiodistal dimension of the upper second molars and the buccolingual dimensions of the lower right canine, upper first, and second molars presented statistically significant differences for sex at the level of 10%.

Initially, the model with all variables was studied. Later, the stepwise variable selection method was used to generate a tighter model, and we only included the variable buccolingual dimension of the upper second left molar. Likewise, the model with only significant variables resulted in a discriminant function with a single variable (buccolingual dimension of the upper second left molar). As these models are susceptible to multicollinearity problems, the correlation between these variables was investigated, and high correlations were observed, as shown in Table 5.

The generated function was as follows: $Z = -18.497 + 1.911\ BL65$ (with BL65 representing the buccolingual dimension of the upper second left molar).

to which correspond the centroids of -0.299 for females and 0.399 for males. The cut-off value for classifying future observations, weighted by the number of observations in each group, was 0. Based on this cut-off value, which guarantees the minimization of classification errors, Table 6 presents the classification of observations, verifying that 59.4% of the original observations were correctly classified.

This result corresponds to taking only the variable BL65 (the buccolingual dimension of the upper second left molar) as a discriminant. However, forcing only the significant variables at the 5% level to be

Table 3
Zuckerkandl's tubercle by sex in upper first deciduous molars, n(%).

Expression	Sex				TOTAL	
	Female, n (%)		Male, n (%)		n (%)	
	urdm1	uldm1	urdm1	Uldm1	urdm1	uldm1
0 (Absent)	26 (54.2)	28 (56.0)	22 (45.8)	22 (44.0)	48 (100)	50 (100)
1 (Present)	8 (50.0)	6(42.9)	8(50.0)	8(57.1)	16 (100)	14 (100)
TOTAL	34 (100)	34(100)	30 (100)	30 (100)	64 (100)	64 (100)

abbreviations: 1st letter - u=upper; l=lower; 2nd letter - r = right; l=left; d=deciduous; m=molar; e.g. uldm1 =upper, left deciduous molar first.

Table 4
Difference in the mean measurements between females and males (Student's t-student's test independent samples) (significant values in bold).

Measurements	Tooth	Sex	Mean value (mm)	Standard deviation	P value
Mesiodistal	lldc	Female	6.080	0.357	0.737
		Male	6.048	0.390	
	lrdc	Female	5.927	0.346	0.363
		Male	6.007	0.059	
	urdm1	Female	7.135	0.523	0.887
		Male	7.117	0.524	
	urdm2	Female	8.844	0.482	0.077
		Male	9.107	0.680	
	uldm1	Female	6.923	0.459	0.779
		Male	6.958	0.534	
	uldm2	Female	8.806	0.461	**0.023**
		Male	9.118	0.606	
Bucco-lingual	lldc	Female	5.514	0.478	0.204
		Male	5.662	0.443	
	lrdc	Female	5.408	0.407	**0.043**
		Male	5.639	0.486	
	urdm1	Female	8.084	0.546	**0.034**
		Male	8.387	0.568	
	urdm2	Female	9.428	0.495	**0.018**
		Male	9.755	0.583	
	uldm1	Female	8.110	0.546	0.075
		Male	8.357	0.541	
	uldm2	Female	9.522	0.490	**0.013**
		Male	9.856	0.559	

abbreviations: 1st letter - u=upper; l=lower; 2nd letter - r = right; l=left; d=deciduous; m=molar; e.g. uldm2 =upper, left deciduous molar second.

Table 5
Difference in the mean measurements between females and males (Student's t-student's test independent samples) (significant values in bold).

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	urdm1	Female	8.084	0.546	**0.034**
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incorporated in the model (the mesiodistal dimension of the upper second molar, the buccolingual dimension of the lower right canine, upper right first molar, and upper second molars), the discriminant function generated was as follows:

$$Z = -19{\cdot}446 + 0{\cdot}521\ MD65 + 0{\cdot}435\ BL83 + 0{\cdot}395\ BL54 + 0{\cdot}251\ BL55 + 0{\cdot}732\ BL65$$

(MD65, BL83, BL54, BL55, and BL65 correspond to the mesiodistal dimension of the upper second molar, the buccolingual dimension of the lower right canine, upper right first molar, and upper second molars), to which correspond the centroids of − 0.333 for females and 0.377 for males. The corresponding leaderboard, based on a weighted cut-off value of 0, is visible in Table 7. This model led to 64.1% of the original observations being correctly classified.

Table 6
Classification of the observations resulting from classifying the sample using the developed model.

Predicted Original	Female	Male	Total
Female	22	12	34
Male	14	16	30
% Female	64.7	35.3	100.0
% Male	46.7	53.3	100.0

4. Discussion

The significance of dental metrics in anthropology, forensic science, and clinical applications is well-documented in (da Silva et al., 2019), underscoring the abundance of tooth size studies in existing literature. However, a gap remains concerning the primary dentition, signaling a potential area for further exploration (Eswara et al., 2014). The established occurrence of sexual dimorphism in permanent teeth (Black, 1978; Garn et al., 1964; Ingaleshwar et al., 2018) suggests that sexual dimorphism also occurs in the primary dentition as well (Ingaleshwar et al., 2018; Jensen et al., 1957; Margetts & Brown, 1978; Singh, Bhatia, Sood, & Sharma, 2017; Sujitha, Bhavyaa, Muthu, Nirmal, & Patil, 2022; Viciano et al., 2013), though the degree of dimorphism is often more pronounced in the former (Garn, Lewis, Swindler, & Kerewsky, 1967). Notably, some studies argue that certain deciduous teeth exhibit more marked sexual dimorphism than their permanent counterparts (Kondo et al., 1998). In this context, our research focuses on lower canines and upper molars, selected based on their noted sexual dimorphism in previous studies (Adler & Donlon, 2010; Axelsson & Kirveskari, 1984; Viciano et al., 2011; Viciano et al., 2015; Viciano et al., 2021). These data are far from consensual, with other authors reporting other results. Some have claimed that the upper central and the lower lateral incisors are less informative (Singh et al., 2017). The first maxillary molar has been referred to as the deciduous tooth presenting most sexual dimorphim, using mesiodistal and buccolingual measurements (Kuswandari & Nishino, 2004; Rodriguez-Florez, Mangeaud, Colantonio, & Fonseca, 2008; Singh et al., 2017). Harris & Lease (2005) referred to the usefulness of the first upper molars and reported that the lower canine was equally valuable. Others stated that the only tooth presenting sexual dimorphism was the lower canine (Moyers, van der Linden, Riolo, & McNamara, 1976).

This choice is further justified by the practical challenges in forensic human identification, where the preservation of certain teeth, like lower canines and molars, is more likely due to their robustness. In fact, in this context, extensive fragmentation and decay usually exist when dental techniques are required. Therefore, it is common for the mandible and single root teeth to be absent, except in lower canines, which may persist (if the lower jaw is not lost) due to their long root (Carneiro, Santos, Magalhaes, Afonso, & Caldas, 2015).

As for the traits chosen, we've decided to study molar characteristics

Table 7
Classification of the observations resulting from classifying the sample using a model with the significant variables at the 5% level.

Predicted Original	Female	Male	Total
Female	22	12	34
Male	11	19	30
% Female	64.7	35.3	100.0
% Male	36.7	63.3	100.0

that occur in the buccal and lingual surfaces rather than the cusp number. This is because, in Portugal, sealant placement in children is free, and almost all children have it. Sealant placement, caries, and caries treatment can alter the occlusal surface and cusp number and can obscure critical morphological traits. The selected features are characteristics that are easy to identify and observe and are conserved despite dental wear and most dental treatments. This approach aligns with the necessity to adapt research methodologies to contemporary dental practices.

The Carabelli's cusp occurs on the molars' mesiolingual or lingual surface, on cusp 1 (protocone), and is the most studied variant on teeth. As for the Zuckerkandl's tubercle, regardless of being much more common than the Carabelli's cusp, it is less known, and some call it even obscure (Eramo, Mascolo, Lisi, Calabrese, & Barraco, 2023). It presents as a slight enamel relief near the mesial cervical margin of the vestibular face of the crown of the first deciduous molars (Mahoney, 2011).

Dental casts for data collection may be troublesome, as tooth measurements done by the direct (intraoral) or indirect (dental casts) methods may differ. Yet, Anderson (2005) compared direct and indirect measurement techniques and established no statistically significant difference between the mean values obtained for tooth dimensions. Moreover, it has been found that measuring teeth directly is more complex than measuring on dental casts and that the mesiodistal measurements, when taken directly from the mouth, produced reduced values compared to the indirect method (Hunter & Priest, 1960). Problems can arise from inadequate cast preparation methodologies and long-term and improper storage techniques, leading to dimensional inconsistencies. To avoid those, dental casts were made by the same person immediately after the dental print collection and analyzed 24 h after making the cast, following the cast manufacturer's instructions.

The study's methodological rigor extends to metric and non-metric data analyses, particularly on buccolingual measurements, due to their higher reliability and reproducibility than mesiodistal measurements. Most studies have focused solely on mesiodistal measurements, and studies evaluating buccolingual dimensions are scarce (Sujitha et al., 2022). Some state that both measures are equally sexually dimorphic (Lukacs, 2022). In contrast, others refer both should be done, but if choosing only one, the mesiodistal dimension should be preferred, as the buccolingual maximum width is often located subgingival. Therefore, it cannot be adequately measured on plaster casts without introducing a bias in the results (Daniele, Matilde, Maria, Rafael, & Milagros, 2020). Non-metric classifications also presented high reliability and reproducibility scores, which agrees with data from other investigations (Diaz et al., 2014; Kieser, 1984).

Our results regarding mesiodistal measurements highlight the upper left second molar had significantly sexual dimorphism, offering a nuanced contribution to the discourse on dental sexual dimorphism (Cardoso, 2010; Liu et al., 2000). On average, males had higher values in all measurements except for the lower left canine and the upper right first molar mesiodistal width, where females had a higher mean. Our findings contradict other studies that stated males have larger teeth than females (Axelsson & Kirveskari, 1984; Margetts & Brown, 1978; Reade, 1965). The reported studies are antique, and an evolutionary change may have happened (Hinton, Smith, & Smith, 1980). However, this technique can also be useless in sex estimation. In this regard, the diameters of maxillary and mandibular canines show that some variability exists (Liu et al., 2000), and Tsai (2001) stated that "Variability is an important component in the make-up of the human dental structure."

For mesiodistal measurements, only the upper left second molar depicted statistically significant differences between sexes, with males having a significantly higher mean value; the upper right second molar presented a close to significance value (with males also having a higher mean value), and in the remaining measurements, no statistically significant differences were found. These results indicate a more significant sexual dimorphism of the second deciduous molar, which agrees with other authors, (Cardoso, 2010; Liu et al., 2000). Yet, other authors found other tooth measurements to depict sexual dimorphism. For example, Kuswandari & Nishino (2004) and Lukacs (2022) referred to the mesiodistal dimensions of the first upper molar as the ones depicting more sexual dimorphism, and a population effect may exist.

Our model presented a low accuracy in sex estimation, with the best performance leading to 64.1% of the original observations being correctly classified. These results are not different from those of Cardoso (2010), who tested several discriminant functions in a Portuguese population, obtaining poor overall accuracy (33.3–75%) and poor cross-validation (46.2–60.0%) in sexual diagnosis, suggesting that this approach alone, may be insufficient for sex estimation.

As for non-metric features, we have found only a few studies regarding Carabelli's cusp in primary dentition and one study concerning Zuckerkandl's tubercle. Some report the Carabelli's cusp as a sex dimorphic trait (Goose & Lee, 1971; Kaul & Prakash, 1981; Kieser, 1984; Kieser & Preston, 1981), whereas others said there is no significant sex dimorphism (Diaz et al., 2014; Kieser, 1984). As for Zuckerkandl's tubercle, Lopez-Lazaro et al. (2018) obtained statistically significant values regarding sexual dimorphism. Our study found that Zuckerkandl's tubercle presence did not show sexual dimorphism. The Carabelli's cusp presence depicted sexual dimorphism only in tooth 65 (the upper left second deciduous molar), suggesting a population bias may exist. The Carabelli's cusp may be used as a non-metric trait in sexual diagnosis using the primary dentition in the present sample.

Compared to other authors, our results point to significant variability in the expression of sexual dimorphism in deciduous teeth. According to Lukacs (2022), the primary dentition sexual dimorphism shifts across samples from incisors to canines to molars. The explanation behind this remains unknown, and the developmental, hormonal, and sex-chromosomal forces are considered the main influences (Guatelli-Steinberg et al., 2008). Lukacs (2022) stated that factors responsible for sexual dimorphism in permanent teeth, namely sex chromosomes and hormones, may have less direct influence on sexual dimorphism in deciduous teeth.

5. Conclusions

Based on the provided results, we can draw several conclusions regarding the differentiation between sexes using dental morphological features in the upper deciduous molars, namely the Carabelli's cusp and Zuckerkandl's tubercle, along with teeth measurements from upper deciduous molars and lower canines.

The presence of Carabelli's cusp and Zuckerkandl's tubercle does not significantly differ between sexes except for the upper left second molar, suggesting that these features, in isolation, are unreliable indicators for sex differentiation. On the other hand, tooth size, precisely the buccolingual dimension of certain teeth, can be a more effective criterion for sex estimation.

The study's model had a significant predictive capability above random chance. Yet, there is room for improvement, namely by using a more substantial sample or incorporating additional dental or skeletal markers.

Statement

All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the Ethics Committee of the Faculty of Dental Medicine of the University of Porto (Recommendation number 28/2021). The privacy rights of human subjects were consistently observed.

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Authors' contribution

All authors participated in the study's conceptualization and design.

Valente C collected, analyzed, and interpreted the data, and Teixeira A, Oliveira P, and Caldas IM revised and supervised these stages. Caldas IM wrote the draft of the article, and Valente C, Oliveira P, and Teixeira A revised it critically for content. All authors approved the final version of the paper submitted.

CRediT authorship contribution statement

Inês Morais Caldas: Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pedro Oliveira:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Alexandra Teixeira:** Writing – review & editing, Validation, Supervision, Conceptualization. **Cármen Valente:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no existing conflict of interests.

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