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Dental age estimation in individuals from a Brazilian archaeological collection through the analysis of the pulp/tooth area ratio in canines*

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Abstract

To estimate the age at death of archaeological individuals through a radiographic analysis of the pulp/tooth area ratio in canines, according to Cameriere et al.'s method (2007) and its corresponding version adapted and validated for the contemporary Brazilian population. Sixty canines recovered from 30 individuals in five pre-colonial archaeological sites (Buracão, Piaçaguera, Tenório, Moraes, and Capelinha) in the state of São Paulo, Brazil, were examined. The specimens are under the custody of the Museum of Archeology and Ethnology at the University of São Paulo (MAE-USP). Pulp and tooth radiographic areas were measured by two examiners in the free software ImageJ®. The data were applied to formulas to obtain age estimates. The intraclass correlation coefficient (ICC, $p \leq 0.05$) was calculated to analyze intra- and inter-examiner agreement. The estimates were compared against those previously cataloged by the MAE-USP using other anthropological methods. There was a significant intra-examiner (ICC = 0.888 to 0.99) and inter-examiner (ICC = 0.842 to 0.908) reproducibility regarding the estimated ages. Our data indicated that the accuracy of age estimates obtained through the analysis of pulp/tooth area ratio were similar to, or greater than, those previously determined by techniques commonly used in bioarchaeology. The method described herein can be accurately applied to individuals from a Brazilian archaeological collection, with the possibility of estimating their age based on the examination of a single tooth. However, this method should be ideally combined with other techniques and further studies should be performed using sample populations from different archaeological sites

Keywords: dental age determination; canine teeth; archaeology; bioanthropology; Brazil

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Introduction

Estimating the age at death and elucidating the sex of archaeological bones has been proven a valuable tool to reconstruct the demographics, health-disease processes, and social and symbolic aspects of past civilizations. Yet, while sex estimation methods for well-preserved adult skeletons have shown accurate results (1), post-maturation age estimates may be inconclusive and unreliable (2). For instance, some methods described in the literature often underestimate the age of older adult individuals (3).

To date, several approaches are available to estimate an individual's age through the analysis of bone alterations during their development and aging. However, bone alterations can vary considerably from one individual to another and postmortem modifications of the bones due to taphonomic processes (e.g., weather, humidity, soil, etc.) may substantially reduce the accuracy of the estimates (2,4,5).

The mineral composition of the dental enamel renders teeth more resistant than bones to taphonomic and weather-related deterioration. Hence, the analysis of dental elements is considered an accurate approach to estimating the age at death of archaeological individuals. The possibility of estimating the age of archaeological bones using only a single tooth or a few teeth is promising. Previous studies have successfully estimated the dental age based on a radiographic examination of the pulp/tooth area ratio in canines (6,4,7,8,9). Such radiographic analysis considers a slow-paced secondary dentin deposition by odontoblasts onto the side walls of the pulp chamber since tooth eruption, while the tooth remains alive. As the individual ages, dentin deposition gradually narrows down the diameter of the pulp canal in a sound tooth (10). Secondary dentin production is regular and continuous in adult individuals and can only be interrupted by caries or severe dental wear (11).

In 2004, Cameriere et al. (12) carried out a study to estimate the age of living adults based on the analysis of secondary dentin deposition in the maxillary right canine. The authors used panoramic radiographs to perform the measurements and observed a significant correlation between the individuals' age and their pulp/tooth area ratio. Further studies estimated the dental age based on secondary dentin deposition in canines using periapical radiographs, including samples from historic archaeological and bone collections with known and unknown age information (4,13,14,15). Ever since, this method has been successfully applied in representative living and bone samples in Italy, Mexico, Brazil, Portugal, India, and Spain, among other countries (16,17,7,18,19,20,21). According to De Luca et al. (21), the same regression equations used for age estimation based on secondary dentin deposition in canines can be used in diverse populations, with no significant differences.

De Luca et al. (7) suggested the use of the pulp/tooth area ratio for paleodemographic studies, which is based on the construction of population profiles. However, this area of study has received a lot of criticism regarding the standardization of techniques and the definition of acceptable error rates in unidentified archaeological populations (1,2,7). The interpretation and the analysis of age distribution in these population groups is essential to understand their demographic profile. It is important to ponder that most findings are based on estimates and not official records.

While there is a plethora of methods for dental age estimation, some of them may be complex and/or destructive. The analysis of tooth wear is commonly used by anthropologists and archaeologists, and factors such as diet, habits, and culture are known to influence the severity and pattern of dental wear (4). Moreover, this analysis may bias other dental estimation methods since tooth wear can be influenced by secondary dentin deposition and/or the method can be unfeasible to apply.

Documented evidence has shown that sex does not play a significant role in the equations for age estimation (12,13,19,20,22,23). This can be considered another advantage of the Cameriere et al.'s method (4) since archaeological remains may not always provide enough elements to determine an individual's sex. Accordingly, the method may more accurately estimate the age, particularly of older individuals, regardless of sexual dimorphism (17).

In this study, we estimated the age at death of archaeological individuals from pre-colonial archaeological sites (Buracão, Piaçaguera, Tenório, Moraes, and Capelinha 1) in the state of São Paulo, Brazil. A radiographic analysis of the pulp/tooth area ratio in canines was carried out according to Cameriere et al.'s (4) method and its corresponding version that was adapted and validated (19) for the contemporary Brazilian population.

Material and Methods

This study was previously approved by the Research Ethics Committee at the University of

São Paulo School of Dentistry, under protocol number 2.943.917/18.

A total of 136 canines from 52 individuals recovered from five archeological sites in the state of São Paulo were radiographed and measured as described by Cameriere et al. (4).

1. Piaçaguera (n=23), coastal shellmound located in the city of Cubatão, Baixada Santista, dating approximately to 7169-4684 years before the present (B.P.) (24,25,26);
2. Buracão (n=4), a coastal shellmound close to Santo Amaro island, Baixada Santista, dating approximately to 1728-1402 years B.P. (24,26,27);
3. Tenório (n=15), a shallow site located in the city of Ubatuba, North Coast of the State of São Paulo, dating to 1986-1557 years B.P. (25);
4. Moraes (n=9), a fluvial shellmound located in the city of Miracatu, Vale do Ribeira do Iguape, dating to 6792-4971 years B.P. (28);
5. Capelinha (n=1), a fluvial shellmound located in the city of Cajati, Vale do Ribeira, dating to 10175-9625 years B. P. (28,29,30).

The radiocarbon dating of the archaeological sites selected for this study was recalibrated, and the results can be consulted in Supplementary Information 1 (SI1). The dating was recalibrated in the OxCal v4.4.3 program (31). Land samples and individuals exhumed from fluvial shellmounds were calibrated by the SHCal 20 curve (32) and the marine samples were calibrated by the Marine20 curve (33). Human remains exhumed from coastal sites were calibrated using a mixed curve (50/50, SHCal20, and Marine20). Reservoir effects for the samples calibrated through the marine curve were calculated based on the study by Alves et al. (34). The individuals examined are part of the collection of human remains under the custody of the Museum of Archeology and Ethnology at the University of São Paulo (MAE-USP). The selected sample consisted of 22 females, 19 males, and 11 unidentified individuals (convenience-selected based on the collection, specifically those who had one or more canines). Maxillary (n=31) and mandibular (n=29) canines with a closed apex and wear score up to grade 5 of Molnar's wear table (35) were included in the analysis. Canines with a severe degree of wear and/or atypical wear, with caries, without apical closure, fractured, with hypercementosis, and root shortening were excluded from the analysis. All canines were radiographed at the Division of Radiology, School of Dentistry, using a portable direct-current X-ray device (Nomad, Aribex, Utah, USA) and an indirect digital sensor with photostimulating plates from the Digora Optime digital system (Soredex, Tuusula, Finland). The sensor was exposed to X-rays and laser-scanned in the Digora Optime equipment. Radiographic images were obtained in the Digital Imaging and Communication in Medicine (DICOM) format and stored in the Digora program for Windows (Soredex, Tuusula, Finland).

In the original method, described by Cameriere et al. (12), the measurements of the pulp/tooth area were obtained from high-resolution digital radiographs in JPEG format in AutoCAD2000® and followed by several studies (4,7,18,19,36). In our study, the images were analyzed in DICOM format in the free ImageJ® program. When needed, brightness and contrast adjustments and/or colour inversion of the radiograph were performed.

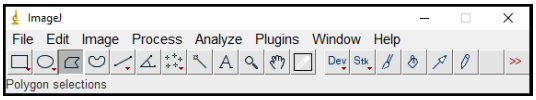

Figure 1. Toolbar of the ImageJ® program showing the selection of the polygonal lasso tool.

Figure 2. The contour of the tooth area (A); the contour of the pulp area (B).

The contours of the tooth and pulp areas were selected using the polygonal lasso tool (Figure 1). In the original method (12), the contours of the pulp and total tooth area were made with 10 and 20 points, respectively. Here, based on previous publications, we opted for using at least 10 points for the pulp area and at least 20 points for the total tooth area (Figures 2: A and B) to increase the accuracy of the measurements (7,18,19,11,21,36,37). To determine the number

of pixels in the tooth and pulp areas, the selected structures were measured in the program using the Analyze > Measure command or by activating the “Ctrl+M” keys.

Before the analysis, two expert dentists in anthropology and forensic dentistry (DRM & MICB) were trained to improve their technical skills. Firstly, the intraclass correlation coefficient (ICC, p≤0.05) was calculated to analyze intra- and inter-examiner agreement in 20% of the sample, with an interval of one month between the first and second analysis. Secondly, the analysis of inter-examiner agreement in the entire sample was performed. Agreement tests were performed in the Stata 13.0® program.

The dental age of the sample was estimated based on the number of selected pixels of the pulp and total tooth areas applied to the equations described by Cameriere et al. (4) (original formula - OF) and Azevedo et al. (19) (formula validated for the Brazilian population - VF). As shown in Table 1, each author described three formulas for the maxillary canines, mandibular canines, and both (Table 1).

The formulas were applied according to the availability of maxillary and/or mandibular canines in each individual and, whenever possible, the formula for both canines was also applied. Subsequently, the estimates were compared against those previously published in the literature or available from the database of the Laboratory for the Study of Zooarchaeology and Bioarchaeology (LEZB-MAE), whose data were kindly provided by a collaborator (VW).

The individuals from Moraes and Piaçaguera bone collections had their age estimated by Fischer (38) (Master’s Thesis) through the application of the anthropological methods described by Buikstra and Ubelaker (39), with an association of multiple markers, when applicable. The age range of two individuals from Buracão shellmound (SB_1 and SB_21) was previously estimated by Silva (27) in their Ph.D. dissertation based on the stages of tooth eruption, the fusion of ossification nuclei, and the closure of cranial sutures. The age of the third individual (SB_19A) was estimated by one of the coauthors of our study following the anthropological methods described by Buikstra and Ubelaker (39) while participating in the project for the re-curatorship of MAE-USP’s human bone collections.

The individual from Capelinha 1 included in our study had craniosynostosis (early fusion of the cranial sutures) and, for this reason, his chronological age was estimated based on the postcranial skeleton, such as the appearance of the auricular surface and fusion of the iliac crest (40).

More recently, the individuals from Tenório archaeological site had their ages estimated by Fidalgo, Wesolowski, & Hubbe (41,42) through the anthropological methods described by Buikstra and Ubelaker (39).

The information about identification (initials) and sex, index teeth, the severity of tooth wear, measurements of the pulp and total tooth area, and age estimates (OF and VF) were entered into 2020 Microsoft Office Excel® spreadsheets. Subsequently, the previously estimated age was added to the table and the data were compared. Individuals were named according to their burial site and number (in some sites by Arabic numerals and others by Roman numerals, respecting the registration code assigned by the MAE-USP). For example, “Burial 21 – Buracão Archaeological Site”. Here, the nomenclature was abbreviated to contain the initials of the archaeological site followed by the burial number (e.g., SB_21). We note that the original identification codes (initials) were maintained in Portuguese for consistency.

Results

A significant intraexaminer agreement was observed between the age estimates obtained by the OF (ICC = 0.99; ICC = 0.905) and the VF (ICC = 0.986; ICC = 0.888). Interexaminer agreement rates (ICC) ranged from 0.842 to 0.908 (Table 2).

Buracão archaeological site

Nine canines from three individuals were examined, as shown in Table 3. In one individual (SB_1), the OF yielded estimates that were within or close to the age range previously cataloged by the MAE-USP. In contrast, the VF overestimated this individual’s age by at least 4 years for all teeth. Likewise, the application of both the OF and the VF for the maxillary and mandibular canines also overestimated the individual’s age as compared to the MAE’s records.

In individual SB_19A, the age estimates obtained by the OF for both the maxillary and mandibular canines were within the cataloged range and overestimated for one of the canines when using the VF. Lastly, the age of individual SB_21 was estimated at 32.2-40.4 years as compared to 20+ years according to the MAE-USP.

Capelinha 1 archaeological site

As shown in Table 4, the age of individual SC_II estimated by the OF was within the range reported by Neves et al. (40) or was

overestimated by 2.1 years as compared to the MAE-USP's records. The age estimated by the VF based on the analysis of the maxillary left canine (tooth #23) showed the greatest disagreement compared to the records. When combining the left-side canines, the OF estimates were found to be within the age range whereas the VF estimates were overestimated by 1.9 years.

Moraes archaeological site

Four individuals from the Moraes archaeological site were included in the study, and their age estimates can be found in Table 5. In individual SM_VIA, the OF age estimates using the mandibular canines were within the age range previously determined by the MAE-USP, whereas when using the maxillary canines, the age was overestimated by 1.5 years. The VF and the combined formula for both maxillary and mandibular canines overestimated this individual's age compared to the records.

In individual SM_VII, the maxillary right canine (tooth #13) was examined and his age was overestimated by 5.5 (OF) and 6 years (VF). In individual SM_XIIA, the OF underestimated her age by 4.1 years whereas the VF indicated an age estimate within the range previously determined by the MAE-USP. Individual XVA had their age underestimated by 0.3 (VF) and 4 years (OF), except for the VF in the mandibular left canine (tooth #33), which was found to be within the previously determined age range.

Piaçaguera archaeological site

Thirteen individuals from the Piaçaguera archaeological site were included for the analysis of age estimation based on secondary dentin deposition, as shown in Table 6. Individual SP_I had one tooth examined and her age was underestimated by 1.4 years according to the OF or was within the age range when the VF was applied. Individual SP_V had three teeth examined. The analysis of maxillary canines accurately estimated this individual's age, while the measurements of tooth #43 underestimated his age by one year compared to the minimum age range. All estimates obtained by the VF were within the age range. When combining the analysis of both maxillary and mandibular right-side canines, both formulas estimated this individual's age within the reference range.

The OF estimated the age of individual SP_VIII within the age range when considering tooth #13 and overestimated it by 4 years when the measurements of tooth #23 were applied. The VF estimated this individual's age within the range previously determined by the MAE-USP, regardless of the index tooth.

In individual SP_XIV, the OF estimated the age within the cataloged range for both canines while the VF overestimated it by 1.2 years. When combining both canines (teeth #13 and #43), both formulas estimated this individual's age within the reference range.

Three individuals from this site (SP_XV, SP_XXXII, and SP_LIII) had only one tooth evaluated. For both formulas, the ages were estimated at least 10 years above the previously determined ages. In SP_XXXII, the estimates were more than twice those of the records.

Teeth #13, #23, and #33 of individual SP_XXXVI were included for the analysis and, when evaluated individually, all of them overestimated the age by 1.7 years (OF) and 11.1 years (VF). When combining the left-sided canines in the formulas for both canines, there was also overestimation compared to the records.

Individual SP_XXXVIII had his age estimated at over 40 years and, with the application of the formulas, his ages were estimated at under 40 years, except for the VF in tooth #13, which estimated his age at 40.7 years. When combining the maxillary right canine and mandibular left canine, the results were also below the age previously estimated, for both formulas.

In individual SP_XLIV, the age estimates obtained with the VF for each canine individually were within the reference range, while the OF underestimated the age in two canines and accurately estimated it in another canine. When combining the maxillary and mandibular left canines, the VF estimated this individual's age within the reference range in contrast to underestimates by the OF.

Individual SP_52 had two teeth analyzed and her age was estimated, in both formulas, within the previously cataloged range, except for the OF in tooth #33, which underestimated this individual's age by only 0.5 years. When combining the two canines on the left side, the estimated ages coincided with the age range for both the OF and the VF.

Individual SP_19 had only one tooth analyzed and their age was underestimated by the OF by 1.2 years and was within the range when the VF was applied. Both formulas underestimated the age of individual SP_23 by 8 years (OF) and 3.3 years (VF).

Table 1. Age estimation formulas – the original formula (OF)*, and the formula validated for the Brazilian population (VF)*.

OF	Age = 99.937 – 532.775 x (Pixels of the pulp / pixels of the maxillary canine)
VF	Age = 94.706 – 465.358 x (Pixels of the pulp / pixels of the maxillary canine)
Mandibular Canines	
OF	Age = 89.456 – 461.873 x (Pixels of the pulp / pixels of the mandibular canine)
VF	Age = 99.409 – 503.726 x (Pixels of the pulp / pixels of the mandibular canine)
Maxillary and Mandibular Canines	
OF	Age = 114.624 – 431.183 (Pixels of the pulp / pixels of the maxillary canine) - 456.692 x (Pixels of the pulp / pixels of the mandibular canine) + 1798.377 x (Pixels of the pulp/ pixels of the maxillary canine) x (Pixels of the pulp/ pixels of the mandibular canine)
VF	Age = 122.026 – 471.568 (Pixels of the pulp / pixels of the maxillary canine) - 486.891 x (Pixels of the pulp/ pixels of the mandibular canine) + 2122.427 x (Pixels of the pulp/ pixels of the maxillary canine) x (Pixels of the pulp/ pixels of the mandibular canine)

* Cameriere et al. (2007a); **Azevedo et al. (2015).

Table 2. Intraexaminer and interexaminer agreement rates.

Examiner 1		**Confidence interval (95%)**	
ICC OF	0.990	Minimum	0.969
		Maximum	0.997
ICC VF	0.986	Mimimum	0.956
		Maximum	0.995
Examiner 2			
ICC OF	0.905	Minimum	0.703
		Maximum	0.969
ICC VF	0.888	Minimum	0.651
		Maximum	0.964
Inter-examiners*			
ICC OF	0.858	Minimum	0.559
		Maximum	0.955
ICC VF	0.842	Minimum	0.507
		Maximum	0.949
Inter-examiners**			
ICC OF	0.893	Minimum	0.821
		Maximum	0.936
ICC VF	0.885	Minimum	0.808
		Maximum	0.931
Inter-examiners (both canines)			
ICC OF	0.887	Minimum	0.675
		Maximum	0.960
ICC VF	0.908	Minimum	0.736
		Maximum	0.968

*Calibration phase; **Whole sample; ICC: Intraclass correlation coefficient; OF: Original formula; VF: Validated formula.

Table 3. Dental age estimation among individuals from Buracão archaeological site.

Identification	Sex	Tooth	Wear	EA OF	EA VF	EA MAE
SB_1	Undetermined	13	2	17.5	22.7	12-18
		23	2	19.4	24.4	
		33	2	21	24.8	
		23 + 33		22.1	26.1	
SB_19A	Female	13	2	29.4	33.1	20-34
		23	2	34.4	37.5	
SB_21	Female	13	3	37.4	40.1	+20
		23	3	34.8	37.8	
		33	4	32.2	37	
		43	4	38.5	43.8	
		13 + 43		36.9	40.4	
		23 + 33		32.5	36.2	

SB: Buracão archaeological site; EA OF: Age estimated by the original formula; EA VF: Age estimated by the validated formula; EA MAE: Age estimated previously by the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP).

Table 4. Dental age estimation among individuals from Capelinha 1 archaeological site.

Identification	Sex	Tooth	Wear	EA OF	EA VF	EA MAE
SC_II	Male	13	4	30.3	33.9	25-30
		23	3	32.1	35.4	
		33	3	25.3	29.4	
		23+33		28.1	31.9	

SC: Capelinha archaeological site; EA OF: Age estimated by the original formula; EA VF: Age estimated by the validated formula; EA MAE: Age estimated previously by the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP).

Table 5. Dental age estimation among individuals from Moraes archaeological site.

Identification	Sex	Tooth	Wear	EA OF	EA VF	EA MAE
SM_VIA	Undetermined	13	1	15.5	20.9	13-14
		33	1	14.1	17.3	
		43	1	13.5	16.5	
		13 + 43		18	22.5	
SM_VII	Male	13	4	54.5	55	45-49
SM_XIIA	Female	33	3	35.9	40.9	40-44
SM_XVA	Undetermined	33	3	24.4	28.4	25-34
		43	3	21	24.7	

SM: Moraes archaeological site; EA OF: Age estimated by the original formula; EA VF: Age estimated by the validated formula; EA MAE: Age estimated previously by the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP).

Table 6. Dental age estimation among individuals from Piaçaguera archaeological site.

Identification	Sex	Tooth	Wear	EA OF	EA VF	EA MAE
SP_I	Female	33	3	23.6	27.6	25-30
SP_V	Male	13	2	28.9	32.7	25-34
		23	2	26.3	30.7	
		43	3	24	28	
		13 + 43		26.4	30.2	
SP_VIII	Male	13	4	33	36.2	25-34
		23	4	38.3	40.9	
SP_XIV	Undetermined	13	3	31.3	34.5	25-34
		43	3	30.5	35.2	
		13 + 43		30.4	34.1	
SP_XV	Female	13	4	46.1	47.7	25-34
SP_XIX	Undetermined	13	2	23.8	28.2	25-34
SP_XXIII	Female	43	3	32	36.7	40-45
SP_XXXII	Female	23	2	33.5	36.7	15 ± 36m
SP_XXXVI	Female	13	3	32.9	36.1	18-25
		23	3	29	32.8	
		33	3	26.7	31	
		23 + 33		27.7	31.5	
SP_XXXVIII	Male	13	3	38.2	40.7	+40
		33	3	33.5	38.4	
		13 + 33		34.6	38.2	
SP_XLIV	Male	13	2	35.4	38.3	35-40
		23	2	33.7	36.9	
		33	3	31.6	36.4	
		23 + 33		31.8	35.5	
SP_LII	Female	23	2	28.7	32.5	25-34
		33	3	24.5	28.6	
		23 + 33		26.5	30.4	
SP_LIII	Female	23	5	48.8	50	25-34

SP: Piaçaguera archaeological site; EA OF: Age estimated by the original formula; EA VF: Age estimated by the validated formula; EA MAE: Age estimated previously by the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP).

Table 7. Dental age estimation among individuals from Tenório archaeological site.

Identification	Sex	Tooth	Wear	EA OF	EA VF	EA MAE
ST_I	Female	33	3	25.2	29.4	21 to 30
		43	3	25.7	29.9	
ST_II	Undetermined	23	2	27	31	21+
		33	2	26.1	30.3	
		43	2	25.1	29.2	
		23 + 33		26.7	30.5	
ST_III	Male	33	4	36.4	41.5	21+
ST_IV	Male	43	4	33.6	40.7	30+
ST_SN*	N	13	2	24.1	28.5	**
ST_XIII	Female	43	3	30	34.4	±21
ST_XIV	Undetermined	43	2	22.3	26.2	18-25
ST_XVI	Female	13	2	15.6	21	15-16
		23	2	17.7	22.9	
		33	2	15.2	18.4	
		43	2	15.8	19.1	
		13 + 43		18.9	23.3	
		23 + 33		19.3	23.6	
ST_XX	Male	13	2	20.8	26.5	25-29
		23	2	25.2	29.4	
		33	2	22.7	26.6	
		43	2	20.5	24.2	
		13 + 43		22.3	26.4	
		23 + 33		26.6	28.5	

ST: Tenório archaeological site; EA OF: Age estimated by the original formula; EA VF: Age estimated by the validated formula; EA MAE: Age estimated previously by the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP); *Unidentified burial (no background); **no age information available.

Tenório archaeological site

Nine individuals from the Tenório archaeological site were included in the analysis, as shown in Table 7. Both mandibular canines were measured in Individual ST_I and her age estimates by the OF and the VF were consistent with those reported by Fidalgo, Wesolowski, & Hubbe (42).

Individual ST_II had their age previously estimated by the MAE-USP at 21+ years. In our analysis, the OF and the VF applied to three canines individually estimated their age between 25.1 and 27 years and 29.2 and 31 years, respectively. When the maxillary and mandibular left canines were combined, the estimated ages were 26.7 (OF) and 30.5 (VF). Two other individuals (ST_III and ST_XIII) had their ages estimated at 21+ years by Fidalgo, Wesolowski, & Hubbe (42) as compared to 30+ years in our analysis.

Individual ST_IV had their age previously estimated at over 30 years by the MAE-USP. In our study, this individual's age was estimated by the original and validated formulas in tooth #43 at 33.6 and 40.7 years, respectively. Individual ST_SN has no record of age estimation by the MAE. In our study, the OF and VF in tooth #13 estimated this individual's age at 24.1 and 28.5 years, respectively.

The age of individual ST_XIV was within the range previously determined by Fidalgo, Wesolowski, & Hubbe (42) when the OF was applied and was overestimated by 1.2 years when the validated formula was applied. Of the four canines examined in individual ST_XVI, three accurately estimated her age when the OF was applied, whereas the measurements of tooth #23 overestimated her age by 1.7 years. When the VF was applied, all teeth overestimated this individual's age as compared to the records. When combining left-side or right-side maxillary and mandibular canines, this individual's age was overestimated by both the OF and the VF.

Four canines were examined in individual ST_XX. Of these, the measurements of three canines applied to the OF underestimated this individual's age by 2.3 to 4.5 years, whereas another canine accurately estimated his age within the range reported by Fidalgo, Wesolowski, & Hubbe (42). When the VF was applied, three canines provided estimates within the reference range whereas the other canine underestimated this individual's age by 0.8 year. When the measurements of the right-side and left-side canines were applied to both formulas, age estimates were within the reference range, except for the OF in teeth #13 and #43.

Discussion

In this study, we estimated the age of Brazilian archaeological individuals based on the analysis of secondary dentin deposition in canines developed by Cameriere et al. (4). Subsequently, the estimates were compared against those previously determined by common anthropological methods in archaeology.

The measurement of secondary dentin deposition has some limitations to consider in the field of archaeology, that is, it requires an imaging examination (radiograph), a design program, and a calibrated examiner. In addition, several archaeology research groups may experience difficult in terms of affordability, time restriction, equipment, and knowledge to apply these types of techniques (43). The method described in our study can be used to estimate the age at death of archaeological individuals. This is a conservative approach with good reproducibility after examiner's calibration, as seen in our findings.

Some forensic methods may provide age estimates closer to the actual age of the individual, but they require additional steps, such as tooth sectioning for microscopic analysis. Previously, Cameriere et al.'s method (4) was applied to microscopic images of sectioned canines from archaeological individuals. The authors observed an increase in the accuracy of the age estimates in older individuals (17). Yet, in the archaeology context, an irreversible damage to a tooth can be a great limitation to the practicality of this method (44).

In our study, a convenience-sample was selected from the collection of human remains under the custody of the MAE-USP, resulting in a sample from different archaeological sites, spanning various time periods. Despite the populations having lived in different periods, ranging from 1728 years BP to 10175 years BP, no significant differences were observed in the accuracy of age estimation using the Cameriere et al.'s method (4).

All shellmounds are located in the state of São Paulo, from the Mesoregion of the South Coast (Capelinha, Moraes), through the Baixada Santista (Piaçaguera and Buracão), to the North Coast (Tenório). The greatest distance between the current municipalities where they are located is approximately 460 kilometers (from Cajati to Ubatuba). Studies indicate that the cranial morphology of individuals from the shellmounds of Baixada Santista shows a strong relationship

among them, with indications of Asian ancestry. However, it was also observed that the cranial morphology of individuals from the Tenório site (North Coast) does not differ much from those of Baixada Santista. Individuals from the Moraes sites have biological affinities, cranial measurements and non-metric dental characteristics indicate similarities with those from the coast, while non-metric cranial traits indicate a greater affinity with people from the plateau. However, the individual from the Capelinha site presented Paleoamerican cranial morphology (26,40,45,46).

Despite the differences and similarities among the populations, what seems to influence the results of the method applied in this study is more the degree of wear of the analyzed tooth and the presence of pathological or traumatic processes that may have caused pulp sclerosis. These factors may be related to the location where the populations settled, which dictates their habits and oral health.

The differences in lifestyle between fluvial shellmounds (Capelinha and Moraes) and coastal shellmounds (Tenório, Piaçaguera, and Buracão) are evident. In the fluvial sites, the diet was more focused on terrestrial resources, which also included starches, contributing to the presence of caries and calculus lesions, and a lower overall wear. In contrast, in the coastal shellmounds, the diet was predominantly marine, and individuals generally had a lower prevalence of caries and greater dental wear. At the Buracão site, hypercementosis and a high antemortem loss of anterior teeth were also observed. Individuals from the Piaçaguera site presented severe dental wear, mainly in the anterior dentition, suggesting the use of teeth as "tools." In the Tenório site, the paramasticatory use of teeth was also observed in some individuals (45). In our analysis, only individuals with canines with a wear level up to 5 on the Molnar's wear table (35) were included. Despite differences in population lifestyles impacting the teeth of individuals, no differences were observed in the results between sites. Differences in results were observed individually, depending on the condition of the analyzed canine, which will be discussed next.

Estimating the age at death of older adult individuals remains a challenge in the field of bioarcheology. In our study, we observed that the OF accurately estimated the age of most subadult individuals (SB_1, SM_VIA, ST_VI) compared to that determined by anthropological methods. Considering that younger individuals commonly have little or insignificant tooth wear, we were able to estimate their ages within a narrower range than that previously determined by the MAE-USP. However, we note that the VF overestimated the age of this population as compared to the reference range. This can be explained by the fact that the VF was developed with specific adjustments for application in the Brazilian population (19). Historically, this population has undergone a very large process of miscegenation since the colonization of Brazil. However, the archaeological sample studied herein had not yet undergone this miscegenation process. In addition, individuals under 20 years of age were not included in the validation survey for Brazilians.

Despite the little tooth wear shown by younger individuals, in one individual (SB_1) in which wear was classified as 2 of Molnar's classification (35), the estimated age was closer to the oldest age in the age range estimated by the MAE (18 years). In contrast, in another study (47), which estimated the age of the same individual based on the analysis of third molar mineralization stages, the estimation of age approached the younger extreme of the MAE's age range (12 years). This difference can be explained by the fact that dental development is less influenced by external factors, such as lifestyle, nutrition, and wear, among others.

Another individual in the sample (SP_32) had an estimated age between 12 and 18 years, while the secondary dentin deposition method indicated an age of 33.5 years (OF) and 36.7 years (VF). The index tooth analyzed in this individual may have had tertiary dentin deposition as a result of a pathological process (10). We noted a narrowing of the pulp canal that is unusual for a young tooth, but it is not possible to differentiate secondary from tertiary dentin in radiographic examinations. This same individual had its age estimated by the third molar mineralization stage, as conducted by Maciel et al. (47), and the age was estimated to be 15.8 years, which reinforces the hypothesis that the canine analyzed in this study may have undergone some pathological process.

According to Hatice et al. (48), According to Hatice et al. (48), an error of up to 10 years is acceptable in predicting the age of adults, allowing for both overestimation and underestimation. In the study that validated the method for the Brazilian population (19), higher mean errors were found in the younger group (20 to 29 years) and in the older adult groups (60 to 69 years and 70 to 79 years). The most accurate

estimates were observed among individuals aged 30 to 59 years. In our study, we observed that the formula validated for Brazilians tends to overestimate the age compared to the OF, which gradually decreases with aging. This suggests that age estimates obtained by the VF or the OF are more accurate in older adults. The oldest individual (SM_VII) analyzed in this study had an estimated age between 45 and 49 years as compared to 54.5 (OF) and 55 years (VF) when applying the analysis of secondary dentin deposition. In other words, both formulas overestimated the age of this individual within an acceptable error, with only a small difference between them.

Individuals with an estimated age range between 25 and 34 years (SM_XVA, SP_I, SP_V, SP_VII, SP_XIV, SP_XIX, SP_LII) had their ages estimated beyond the reference range or with a difference of a few years more or less than the age group limits, both for the formulas that consider the canines individually and for the formulas of both canines. However, two individuals from the Piaçaguera site (SP_XV, SPL_III) had an estimated age of more than 10 years above that previously estimated by the MAE, with grade 4 and grade 5 tooth wear. However, other individuals from the same site classified in the same age group, including some teeth with grade 4 wear, showed accurate estimates with both the VF and the OF. This age range corresponds to the “adult phase” and is used when an individual does not have bone elements to estimate a more adjusted age by the usual methods, such as the analysis of the auricular surface, sacrum, or femoral head. The same happens with those individuals whose age range is estimated at 18 to 25 years, as there are elements to classify them as “young adults” but not enough to provide a narrow age range. Hence, the analysis of the pulp/tooth area ratio in canines may increase the accuracy of estimates in cases that do not present ideal conditions for a more detailed bone analysis.

The applicability of the method described herein for age estimation in archaeology may be substantially affected by the state of conservation and presence of tooth wear, and the available sample size. In our study, only 60 canines from 30 individuals were included for the analysis, as many were excluded due to post-mortem fractures or excessive wear; in addition, some individuals had only one tooth analyzed, while others had four.

In this study, as previously mentioned, canines that did not show wear greater than grade 5 of Molnar’s wear table (35) were included for analysis. This decision was based on the recommendations by Fabbri et al. (11), who suggested that wear up to grade 5 does not affect the pulp chamber and thus is acceptable when applying the Cameriere’s method to archaeological canines.

According to Fabbri et al. (11), the analysis of secondary dentin deposition may slightly underestimate age in teeth with wear. In our study, the age of individuals with tooth wear was overestimated in most cases. This may be due to the deposition of tertiary dentin around the pulp due to tooth wear, causing a narrowing of the pulp canal.

Another difficulty experienced in our study was the available age ranges for confrontation. Some ranges were wider or narrower whereas the analysis of secondary dentin deposition provides an “exact” age. Comparing these data can be a little complicated, as a certain difference in age estimation is acceptable, but when the numbers go beyond the limits of that age group, the comparison may be subject to the researcher's subjectivity. In this study, when the maximum age of the range was overestimated or the minimum age was underestimated, the difference in years was calculated according to the age range limit.

When the estimate used for comparison is given only as greater or less than a certain age, the comparison becomes even more complex. For example, in this study, in individual SB_21, who was estimated to be over 20 years old, all four canines evaluated estimated her age at over 30 years, that is, greater than 20, but probably beyond the age previously determined by the archaeologists.

The remains of individuals from the Buracão archaeological site were incomplete and fragmented, which can compromise the determination of their age by traditional methods. Thus, the method applied in this study can help in the estimation of the age of individuals from the archaeological population when there are no other available elements. Here, most of the canines analyzed individually provided estimates that crossed or were very close to reference age range limits. Combining this analysis with other methods is preferable to provide more specific age estimates in some cases as well as a more comprehensive data interpretation.

Conclusion

Our data confirm that the pulp/tooth area ratio can be accurately applied to individuals from an archaeological collection with closed-apex

canines and little tooth wear, with estimates close to or within the age ranges determined by other methods. Further studies with different archaeological populations and combined methodological strategies are encouraged to narrow down the estimation of the age range for unidentified individuals. Taken altogether, this contributes to a more realistic reconstruction of the demographic profile of archaeological populations.

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Declaration of Interest
The authors declare no conflict of interest.

Author Contributions
The authors contributed to this work, as follows: conceived and designed the experiments (DRM, MICB, VW, DF, MGHB), performed the experiments (CC, DRM, MICB), analyzed the data (MGHB, EMC), contributed materials/analysis tools (CC, MGHB, EMC), wrote the paper (DRM, MGHB), edited the final version of the submitted paper (DRM, MICB, DF, VW, MGHB, EMC), read and approved the final revised version (DRM, MICB, DF, CC, EMC, VW, MGHB).

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