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Morphological characterization of mandibular molars in a Portuguese population and its potential application in ancestry estimation: An exploratory study

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Abstract: Forensic Dentistry is the branch of dental medicine that, in a legal context, examines dental evidence by comparing teeth to antemortem dental records, helping the human identification process. In forensic contexts, the study of mandibular teeth may be particularly relevant given that the mandibula, being the only moving part of the skull, can be found in isolation. Human dentition can present several variations in its morphological structures. It can be translated at the level of the cusps and roots, particularly in the pattern of grooves and shape of the cusps. Several studies have been carried out in different world populations to describe the main morphological characteristics, namely, the most specific ones that aid the identification process. To initiate this characterization in a contemporary Portuguese population, data were collected on the morphology of mandibular molars from intraoral analysis and photographic records of 83 patients to evaluate and record the number of cusps and the morphological pattern that characterizes the first and second mandibular molars of the contemporary Portuguese population and to determine its possible interest to the estimation of population affinity. Our results suggest that there are potentially relevant differences between the frequency of the number of cusps and the anatomy of the grooves in the mandibular molars of the studied population, compared with other described populations, namely the absence of +5, +6, and X patterns in the mandibular first molars and a higher percentage of teeth with 6 cusps in the mandibular second molars. However, given the small sample used, more studies are needed to validate these observations.

Keywords: mandibular molars; crown; morphology; ancestry; groove pattern; cusps

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Introduction

Human remains are not always found in conditions conducive to their identification, for instance, when fragmented, burned, or with a high degree of decomposition. In these circumstances, teeth (considered the toughest tissue in the body), are often the only material available to aid in the identification process [1]. This is the domain of forensic dentistry, specializing in the examination of dental findings to be used in a legal context [2]. Thus, the dental pieces can be examined and later compared with dental antemortem records, to obtain the possible identification of the individual [2,3]. In a forensic context, the study of

mandibular teeth may also be relevant for determining biological profile parameters [4], particularly because the mandible, being the only mobile part of the skull, can be found separately. Human dentition can present several variations in its morphological structures. These variations, studied by forensic dentistry, can manifest themselves at the level of the cusps and roots, namely in the pattern of grooves, ridges, number of roots, and shape of the cusps [5–8]. The variability of anatomical structures of mandibular molars, combined with their statistical study, allows for data collection capable of characterizing a population. In this way, it is also possible to understand the degree of variation between different populations [9]. The purpose of these studies is to understand how different populations are related, giving special attention to ancestry, one of the parameters of the biological profile, with relevance in human identification [9]. Ancestry estimation is also relevant since it may affect other parameters of the biological profile, namely sex and stature estimation [10] and may also provide clues and help identify the region of origin of unidentified missing individuals [11]. The assessment of ancestry is done by examining certain morphological particularities of teeth, including the crown and root [6] usually classified by the Arizona State University Dental Anthropology System (ASUDAS) [12], which helps characterize dental traits in different populations [8,13]. Teeth, especially molars, have structures whose characteristics are considered partially hereditary and which, once formed, do not change, such as the groove pattern [1,9,14]. According to Hasund and Bang [15], a study published by Jorgensen [16] showed that the occlusal pattern of mandibular molars can be used to differentiate populations. However, for these traits to be valid in ancestry estimation, they must occur with frequencies and specificities that are sufficiently distinct from all other populations. Thus, the study of dental variation can be a useful tool to evaluate and validate specific characteristics of each contemporary population. The detailed study of the structure and morphology of the teeth, whether retained in the mandible or not, as well as their variations and anomalies, is therefore relevant in the process of identifying skeletonized remains [17].

The objective of this preliminary study was to evaluate the variation in the number of cusps and groove patterns present in mandibular molars within a contemporary Portuguese population, aiming to assess their potential application in estimating population affinity.

Materials and Methods

For this study, intraoral photos were collected from a non-random sample of patients at the Filinto Baptista University Clinic of the Polytechnic and University Higher Education Cooperative (CESPU), having obtained a favorable opinion from its Clinical Director and the Ethics Committee of the University Institute of Health Sciences (IUCS-CESPU), with registration number 13/CE-IUCS/2020. All participants signed an informed consent specifying the purpose of the study, and the confidentiality of the information collected for statistical purposes was guaranteed. After this procedure, the data required to determine the biological profile was collected through an identification form, as well as an intraoral photograph of the mandibular molars, with the photos later irreversibly pseudo-anonymized.

The study sample consisted of 83 individuals (54 women and 29 men, between 16 and 69 years of age). The participants were selected using the following inclusion criteria: a) Portuguese nationality and ancestry (verified up to the 2nd generation); b) To have at least one intact mandibular molar (excluding the third molars, not presenting occlusal caries, abrasion, erosion, or attrition lesions that altered the occlusal surface, nor previous treatments on the occlusal and interproximal surface); c) Absence of systemic and/or dental conditions and pathologies that affect normal dental development. The number of cusps and the pattern of the grooves of the mandibular first and second molars were evaluated through clinical examination to select the participants, followed by detailed photographic analysis using a 14-megapixel camera, and the ASUDAS Guide (Fig. 1) [12].

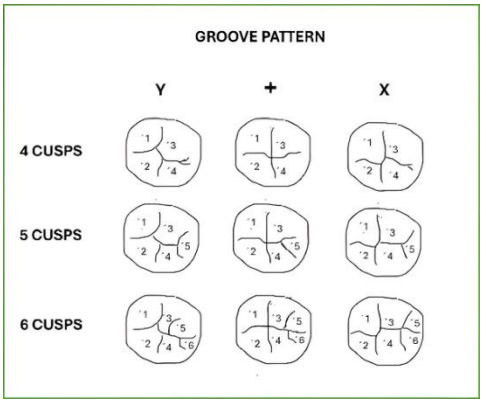

Figure 1. Schematic representation of the occlusal morphologies (number of cusps and groove patterns) of mandibular molars according to the ASUDAS guide [12].

The process of counting cusps and groove pattern analysis was subjected to calibration by two independent observers (BP and DP-M), using 25 teeth, to determine the intra-observer agreement. The analysis of the whole sample was repeated twice and confirmed by the same observer, on different days, to ensure the reliability of the data. All dental pieces whose groove pattern and number of cusps raised doubts were excluded from the study. The data obtained were entered into Microsoft Excel Office 365. Cohen's Kappa value was determined to measure the level of intra-observer agreement. A descriptive statistical analysis was also performed using the Statistical Package for Social Sciences (SPSS) software, version 25.0 (IBM).

Results

A total of 85 mandibular first molars and 111 mandibular second molars belonging to 54 women and 29 men aged between 16 and 69 years were studied, following a calibration step, using 25 teeth (Kappa = 0.949 for the number of cusps and Kappa = 0.724 for the groove patterns). After clinical and photographic examination of the mandibular molars of all participants, we observed that the maximum number of cusps present in the first mandibular molar, which was also the most common, was 5. Regarding the mandibular second molar, the maximum number of cusps observed was 6, but most of them had 4 cusps (Tables 1 and 2). Regarding the groove pattern, the predominance of the "Y" pattern in the first molars, and "+" in the second molars, stood out (Tables 3 and 4). The occurrence, on a smaller scale, of first molars with a "+" pattern and second molars with a "Y" pattern was also verified. In contrast, no teeth with an "X" pattern were observed.

Table 1. Data collected regarding the number of mandibular first molar cusps.

Teeth		No. of cusps	Frequency	Percentage	Valid Percentage	Cumulative Percentage
36	Valid Teeth	4	6	7.2%	13%	13%
		5	40	48.2%	87.0%	100%
		6	0	0%	0%	
		Total	46	55.4%	100%	
	Invalid Teeth	Excluded	37	44.6%		
	Total		**83**	**100%**		
46	Valid Teeth	4	9	10.8%	23.1%	23.1%
		5	30	36.1%	76.9%	100%
		6	0	0%	0%	
		Total	39	47.0%	100%	
	Invalid Teeth	Excluded	44	53%		
	Total		**83**	**100%**		

Table 2. Data collected regarding the number of mandibular second molar cusps.

Teeth		No. of cusps	Frequency	Percentage	Valid Percentage	Cumulative Percentage
37	Valid Teeth	4	57	68.7%	98.3%	98.3%
		5	0	0%	0%	98.3%
		6	1	1.2%	1.7%	100%
		Total	58	69.9%	100%	
	Invalid Teeth	Excluded	25	30.1%		
	Total		**83**	**100%**		
47	Valid Teeth	4	50	60.2%	94.3%	94.3%
		5	1	1.2%	1.9%	96.2%
		6	2	2.4%	3.8%	100%
		Total	53	63.9%	100%	
	Invalid Teeth	Excluded	30	36.1%		
	Total		**83**	**100%**		

Table 3. Data collected regarding the groove patterns of first mandibular molars.

Teeth		Groove Patterns	Frequency	Percentage	Valid Percentage	Cumulative Percentage
36	Valid Teeth	Y	40	48.2%	87.0%	87%
		+	6	7.2%	13.0%	100%
		X	0	0.0%	0.0%	
		Total	46	55.4%	100%	
	Invalid Teeth	Excluded	37	44.6%		
	Total		**83**	**100%**		
46	Valid Teeth	Y	30	36.1%	76.9%	76.9%
		+	9	10.8%	23.1%	100%
		X	0	0.0%	0.0%	
		Total	39	47.0%	100%	
	Invalid Teeth	Excluded	44	53.0%		
	Total		**83**	**100%**		

Table 4. Data collected regarding the groove patterns of second mandibular molars.

Teeth		Groove Patterns	Frequency	Percentage	Valid Percentage	Cumulative Percentage
37	Valid Teeth	Y	1	1.2%	1.7%	1.7%
		+	57	68.7%	98.3%	100%
		X	0	0%	0%	
		Total	58	69.9%	100%	
	Invalid Teeth	Excluded	25	30.1%		
	Total		**83**	**100%**		
47	Valid Teeth	Y	2	2.4%	3.8%	3.8%
		+	51	61.4%	96.2%	100%
		X	0	0%	0%	
		Total	53	63.9%	100%	
	Invalid Teeth	Excluded	30	36.1%		
	Total		**83**	**100%**		

Discussion

Teeth can maintain their integrity when submitted to adverse conditions and, therefore, constitute excellent study material for living and non-living populations regarding genetic, anthropological, forensic, and dental investigations [18,19]. Mandibular molars are the largest and most robust teeth in the mandible and their study is relevant in the process of human identification in a forensic context [1,3] including in the determination of parameters of the biological profile, such as ancestry and sex estimation [1,4]. However, it should be noted that, for characteristics associated with ancestry to be useful, they must be observed in a specific population, and their frequencies must be sufficiently different from those of other populations. Furthermore, the presence of rare anatomical features can be important in the individualization process and contribute to human identification [20]. These anatomical variations are therefore relevant in the identification process and are the basis for carrying out this exploratory study, given the lack of data in a contemporary Portuguese population. To this purpose, a clinical examination was carried out, with a subsequent photographic recording of the mandibular molars, in a sample of Portuguese patients, aged between 16 and 69 years, clearly showing the cusps and the groove pattern, to study their morphological variations. A review of published studies referring to contemporary populations worldwide, to assess the frequency of the number of cusps present in first and second molars, as well as the groove patterns in these teeth, are presented in Tables 5, 6, 7, and 8, respectively. Analyzing the first molars, we observed the presence of 5 cusps in 82.4% and 4 cusps in 17.7% of the population studied (Table 5). The data obtained in the present study does not present significant differences in comparison to the percentage of 4 and 5 cusps reported in other populations; nevertheless, it should be noted that teeth with 6 or 7 cusps could not be found, unlike other populations studied [16,19,21–27] (Table 5).

Table 5. Percentage of the number of first molar cusps reported in contemporary populations.

1st Molar				
Contemporary Sample	**No. of cusps (%)**			
	4	**5**	**6**	**7**
Portugal (present study)	17.65	82.35	-	-
Portugal (late 19th/early 20th century) [21]	ND	73.8	0.5	4.3
Northern Portugal (early 20th century) [21]	ND	72.2	-	3.8
Spain [22]	ND	ND	12.5	3.6
Spain/Basque Country [22]	ND	ND	17.8	8.8
Basque Country [22]	ND	ND	18.2	7.9
The Netherlands [16]	10.6	89.4	ND	ND
Oriental Africa [23]	4.9	88.5	6.6	ND
Jordan [24]	ND	ND	21.67	15.83
India (Madhya Pradesh) [18]	-	-	6.95	11.75
India (Gujarati) [25]	18	71.5	10.5	-
Saudi Arabia [26]	11	85	ND	ND
Japan [19]	8.51	80.85	10.64	ND
India (Mangalore) [27]	4	82	12	ND

ND: not determined

Analyzing the second molars, we observed that 96.4% of the population in this study had 4 cusps, while teeth with 5 and 6 cusps were present in a much smaller percentage (0.9% and 2.7%, respectively), and teeth with 7 cusps were not found (Table 6). Interestingly, the percentage of teeth presenting 5 cusps (0.9%) is small even in comparison to the results observed in the Portuguese population of the late 19th/early 20th century (13.1%) [21], as well as other contemporary populations (between 6.5% and 26.42%) [16,19,22,23,25–29] (Table 6).

Table 6. Percentage of the number of second molar cusps reported in contemporary populations.

2nd Molar				
Contemporary Sample	**No. of cusps (%)**			
	4	**5**	**6**	**7**
Portugal (present study)	96.4	0.9	2.7	-
Portugal (late 19th/early 20th century) [21]	ND	13.1	0.4	0.2
Northern Portugal (early 20th century) [21]	ND	12.6	0.5	-
Spain [22]	85	ND	ND	ND
Spain/Basque Country [22]	93.3	ND	ND	ND
Basque Country [22]	88.6	ND	ND	ND
The Netherlands [16]	88.4	11.6	ND	ND
Oriental Africa [23]	81.4	18.3	0.3	ND
Singapore (Chinese Population) [28]	ND	43.1	ND	ND
Iran [29]	86	13	0.94	ND
India (Gujarati) [25]	93.5	6.5	-	-
Saudi Arabia [26]	82	16	ND	ND
Japan [19]	71.23	26.42	2.36	ND
India (Mangalore) [27]	86	12	ND	ND

ND: not determined

Concerning the groove patterns, 82.4% of the first molar teeth studied presented a Y5 pattern and, in contrast, teeth with an X pattern were not found. The principal differences observed when comparing our data to the remaining populations are the high frequency of the +4 pattern (17.6%) and the absence of +5, +6, and X patterns [15,16,18,19,23,25–27] (Table 7). Nevertheless, further studies should be carried out to assess the relevance of this characteristic for forensic identification, since these results may be due to the reduced sample size. It would be equally interesting to evaluate this characteristic in other populations, namely European ones, to assess its differentiating potential.

Table 7. Percentage of the number of first molar groove patterns reported in contemporary populations.

1st Molar															
Contemporary Sample	**Groove patterns (%)**														
	Y	**Y4**	**Y5**	**Y6**	**Y7**	**+**	**+4**	**+5**	**+6**	**+7**	**X**	**X4**	**X5**	**X6**	**X7**
Portugal (present study)	82.4	-	82.4	-	-	17.6	17.6	-	-	-	-	-	-	-	-
The Netherlands [16]	72.4	ND	ND	ND	ND	16.3	ND	ND	ND	ND	11.3	ND	ND	ND	ND
Oriental Africa [23]	ND	4.1	77.9	4.9	ND	ND	0.8	10.6	1.7	ND	ND	ND	ND	ND	ND
India (Madhya Pradesh)* [18]	50.36	-	-	-	-	44.61	-	-	-	-	1.25	-	-	-	-
Alaska (Eskimo population) [15]	ND	-	77.8	ND	ND	ND	1.4	20.8	ND	ND	ND	ND	ND	ND	ND
India (Gujarati) [25]	60.5	ND	47	ND	ND	39.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Saudi Arabia [26]	80	ND	ND	ND	ND	16	ND	ND	ND	ND	ND	ND	ND	ND	ND
Japan [19]	71.28	2.13	63.83	5.32	-	22.87	4.78	15.47	2.26	ND	5.85	1.60	1.60	2.66	ND
India (Mangalore) [27]	65	ND	ND	ND	ND	28	ND	ND	ND	ND	ND	ND	ND	ND	ND

*In this study, a fourth type of sulcus, Ɣ, was considered, with a 3.78% prevalence in the population. ND: not determined

In the analysis of the second molars, a relatively low frequency of Y-groove patterns was verified (2.7%) when compared with other populations [15,19,22,23,25–27,29] and they were only observed in 6-cusp teeth (Y6). It should be noted that the most common pattern observed was +4 (96.4%), with 2.7% showing a Y6 pattern, and 0.9% a +5 pattern, while X pattern teeth could not be found (Table 8). The low percentage of the +5 pattern observed in our population is the most striking difference observed in comparison to other populations studied (Table 8); however, further studies, with a larger sample, are essential to confirm these results and their potential relevance for ancestry estimation. Dahlberg, in his 1951 study, cited by Hasund and Bang [15] described that the most common pattern is Y5 for the 1st molar and +4 for the 2nd molar, the latter being considered the pattern that presents the greatest evolution. The results of the present study are in line with these findings, although not all teeth have the standard morphology. Thus, it is important to highlight all patterns that deviate from the norm, as they may be relevant in the identification process.

Table 8. Percentage of the number of second molar groove patterns reported in contemporary populations.

2nd Molar															
Contemporary Sample	**Groove patterns (%)**														
	Y	**Y4**	**Y5**	**Y6**	**Y7**	**+**	**+4**	**+5**	**+6**	**+7**	**X**	**X4**	**X5**	**X6**	**X7**
Portugal (present study)	2.7	-	-	2.7	-	97.3	96.4	0.9	-	-	-	-	-	-	-
Spain [22]	22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spain/Basque Country [22]	23.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Basque Country [22]	19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oriental Africa [23]	ND	14.5	5.4	0.3	ND	ND	66.9	12.9	-	ND	ND	ND	ND	ND	ND
Alaska (Eskimo population) [15]	ND	-	13.3	ND	ND	ND	33.3	53.3	ND	ND	ND	ND	ND	ND	ND
Iran [29]	12.4	9.2	3.1	0.06	ND	87.6	76.9	9.8	0.9	ND	ND	ND	ND	ND	ND
India (Gujarati) [25]	6.5	ND	ND	ND	ND	93.5	88.5	ND	ND	ND	ND	ND	ND	ND	ND
Saudi Arabia [26]	11	ND	ND	ND	ND	88	ND	ND	ND	ND	ND	ND	ND	ND	ND
Japan [19]	4.25	0.94	1.89	1.41	-	41.04	32.08	8.96	-	ND	54.71	38.2	15.57	0.94	-
India (Mangalore) [27]	11	ND	ND	ND	ND	85	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: not determined

A large part of the dental morphological characterization studies carried out in the European population refer to ancestral populations [30]. Thus, the data published may not fully correspond to the reality of more contemporary populations, and more studies should be conducted to address this question. Regarding the Portuguese population, there is one study that was carried out on a cranial collection, from the University of Coimbra, of individuals deceased between 1896 and 1938 [21]. In the present study, the main difference observed in the first molars, compared with the population of the late 19th/early 20th century, was the absence of 6 and 7 cusps, although this difference may be explained by the size of the sample used (Tables 7 and 8). Regarding the second molars, the main differences observed were a significant reduction in the number of teeth with 5 cusps (only 0.9% of the population in the present study *vs.* 13.1% in the population of the late 19th/early 20th century) and an increase in individuals with 6 cusps (2.7% in our study *vs.* 0.4% in the population of the late 19th/early 20th century). These differences, if confirmed, may indicate a secular trend concerning the number of mandibular second molar cusps, which justifies the characterization of these parameters in the contemporary population, envisaging a possible forensic application. There are several limitations to this study, namely the small sample size, as molar teeth are frequently subjected to composite restorations, which prevent groove analysis [31] and the need to obtain good-quality intraoral photos, to avoid bias. Therefore, it is of the utmost importance to emphasize that future studies should be carried out with representative samples from the various regions of the country, to confirm the patterns described in the current population and characterize these parameters in the various national regions, highlighting features specific to each. However, it should be considered that the miscegenation of people, resulting from the greater mobility of populations or, for example, from recent migratory flows in Europe, may be factors that, in the future, minimize the presence of anatomical characteristics specific to a given population.

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Author Contributions

BP was responsible for the experimental execution of the work, clinical examination, photograph classification, and writing the initial version of the manuscript. AA contributed to the statistical study, critical reading, and review of the final version of the manuscript. DP-M participated in photograph classification for sample calibration, critical reading, and review of the final version of the manuscript. AT planned the experimental work, wrote the final version, and submitted the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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