

# Dimension variability of the M2 human molar teeth: comparisons between prehistoric and medieval samples

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## SUMMARY

Teeth are a valuable source of information for studies regarding past human populations in archaeological and forensic contexts. In dental anthropology, the linear measurements of tooth crowns are used for assessing morphological variability and sexual dimorphism in both modern and past human populations. The aim of this research is to evaluate the M2 molar crown variability in archaeological human populations from Prehistory (Chalcolithic and Bronze Age, ~ 5000-1150 BCE) and Middle Ages (13<sup>th</sup>-17<sup>th</sup> centuries) discovered in sites from North-Eastern Romania. The objectives of this study emphasize on the diachronic comparison of the M2 molar crown variables between prehistoric and medieval samples (1), and the assessment of sexual dimorphism expression (2). The two crown measurements, mesio-distal (MD) and bucco-lingual (BL) diameters, were performed using ImageJ software on occlusal digital images acquired stereo-microscopically. The crown index (CI), crown area (CA) and the sexual dimorphism index (SDI), along with the two linear measurements, were subjected to univariate and multivariate statistical analysis.

Our results show that the variation coefficient (CV) differs for the MD variable in the female upper M2 molars, being higher in the medieval sample than the prehistoric one; also, a higher variability is remarked for the mandibular molar in the medieval sample than in prehistoric one. In females, the MD and CA variables for mandibular M2 molars and the BL and CA for maxillary molars showed significant statistical differences between the medieval and prehistoric mandibular teeth, with higher values for the exemplar from Middle Ages. Similar result was obtained in males, for the CA variable in the upper M2 molars. In our study, the sexual dimorphism manifested at the M2 crown molar was highlighted in the prehistoric sample, though less in the medieval one.

**Key words:** M2 molar teeth – Crown measurements – Sexual dimorphism – Archaeological human populations – Nord-Eastern Romania

## INTRODUCTION

Dental anthropology is an interdisciplinary research area that involves several directions of study such as physical anthropology, dentistry,

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biology, paleontology, and paleopathology (Moreno-Gómez, 2013). The unique preservation of teeth is based on their hard tissues (i.e., enamel, dentine, and cementum), teeth being a valuable source of information for studies regarding the past human populations in archaeological and forensic contexts (Eboh, 2017; Jeon et al., 2020; Higgins and Austin, 2013; Hillson, 2005; Watson and Haas, 2017). In physical anthropology, teeth have been studied by various methodologies concerning age at death estimation (Bertrand et al., 2019; Brothwell, 1981), dental wear and dental calculus as bioindicators of diet (Green and Croft, 2018; Cummings et al., 2018; Fiorenza et al., 2018), cultural modifications (Wasterlain et al., 2016; Smith-Guzmán et al., 2020), dental diseases (Hillson, 2001; Wasterlain et al., 2020; Garot et al., 2019; Marchewka et al., 2014), inter- and intra-population variations of shape and size (Gómez-Robles et al., 2015; Black, 2014; Takahashi et al., 2007; Kondo et al., 2005).

A number of studies showed that genetic, environmental, and epigenetic factors have a great influence on dental morphology (Kondo and Manabe, 2016; Townsend et al., 2012; Farzin et al., 2020).

Several studies of dental anthropology approached the linear measurements of tooth crowns for assessing the sexual dimorphism in both modern human populations (Zorba et al., 2011; Zúñiga et al., 2021) and past ones (Petraru et al., 2020; Yoo et al., 2016; Górká et al., 2015), as well the dental variability between ethnic groups (Brook et al., 2009; Hasegawa et al., 2007). Most studies of the tooth crown size variability are based on the measurement of the mesio-distal and bucco-lingual diameters (Petraru et al., 2020; Zorba et al., 2011; Kondo et al., 2005; Yoo et al., 2016), although other alternative measurements have been reported (Hillson et al., 2005; Zorba et al., 2013). Due to their sexual dimorphism and resistance to postmortem alteration process, teeth, by crown dimensions, are essential in assessing sex when other skeletal parameters are not reliable (Zorba et al., 2013; Petraru et al., 2020). Several studies showed that the canine (particularly the mandibular canine) is the most sexually dimorphic tooth (Scherer,

2018; Magalhães et al., 2021). The adjacent teeth around the canines (i.e., lateral incisors and first premolars), along with the molar teeth (especially the mandibular teeth) also show dimensional variations due to sexual dimorphism (Zorba et al., 2011; Kondo et al., 2005).

Although odontometric research is well-known in both archaeological human populations and forensic contexts, there are few studies regarding dental variability and sexual dimorphism on molar crown in samples from North-Eastern Romania (Petraru et al., 2020; Petraru et al., 2017). Considering the consequences of genetic influence, environmental factors, like variation of food resources exploitation, and the interference of cultural factors, we suppose that dental variability is increasing in time being correlated with a decreasing sexual dimorphism in teeth.

The aim of this study is to assess M2 molar crown variability in samples of archaeological human populations discovered in North-Eastern Romania. Knowing the importance of teeth as indicators of the intra- and inter-population variability, the objectives of this study emphasize the diachronic comparison of the M2 crown variables (mesio-distal and bucco-lingual diameters, crown area, and crown index) between medieval and prehistoric samples (1), and the expression of sexual dimorphism at the M2 molar crown (2).

## MATERIALS AND METHODS

### Material

The dental material is represented by the M2 molars (maxillary and mandibular molars) (Fig. 1) (n = 284) belonging to human skeletons discovered in archaeological sites from North-Eastern Romania (Fig. 2; Table 1). The M2 molar was chosen, while for the first molar - M1, was avoided due to its variable morphology and usually high degree of wear. Furthermore, only the molars without pathologies, an integrated tooth crown and an occlusal wear that does not exceed a 'moderately advanced wear' (Smith, 1984, Tomczyk et al., 2020) were considered. All molars, related to different age categories (Table 1), were considered for the size analysis, as, once

formed, teeth do not change their size (Górka et al., 2015). The material under study dates, according to the archaeological inventory, from Prehistory (Chalcolithic and Bronze Age ~ 5000-1150 BCE), and the Middle Ages (13<sup>th</sup>-17<sup>th</sup> centuries) (Table 2). Some published data regarding the dimensional variability of M2 molar dating from the Bronze Age (n = 54) (i.e., Truşeşti and Căndeşti samples published previously by Petraru et al. (2020)) were included in this research due to the comparative approach. The molars from the “Curtea Domnească” necropolis of the 17<sup>th</sup> century, which have been subjected to a previously odontometric study based on caliper measurements (Petraru et al., 2017), were reanalyzed with Image J (n = 133) and no statistically significant differences were obtained between the two dimensioning (BL: t = 0.321, p = 0.749; MD: z = 0.289 p = 0.772); thus, all dental material was studied by the same

method in order to avoid error interpretation. The human skeletons discovered in the mentioned archaeological sites were previously studied in terms of age at death and sex estimations, demography, pathologies and odontometrics (Petraru et al., 2017; Petraru et al., 2020) (Table 2). The dental material used in this study is currently preserved in the osteological collection of the “Olga Necrasov” Center of Anthropological Research, Romanian Academy - Iaşi Branch.

## Methods

The molars selected to be studied were prepared for measurements by removing the exogenous grit/ impurities using ethanol, hydrogen peroxide, and cotton wool (Petraru et al., 2020). The teeth were placed under the stereomicroscope being situated in the dental alveoli, or positioned in the specific anatomical orientation if the teeth

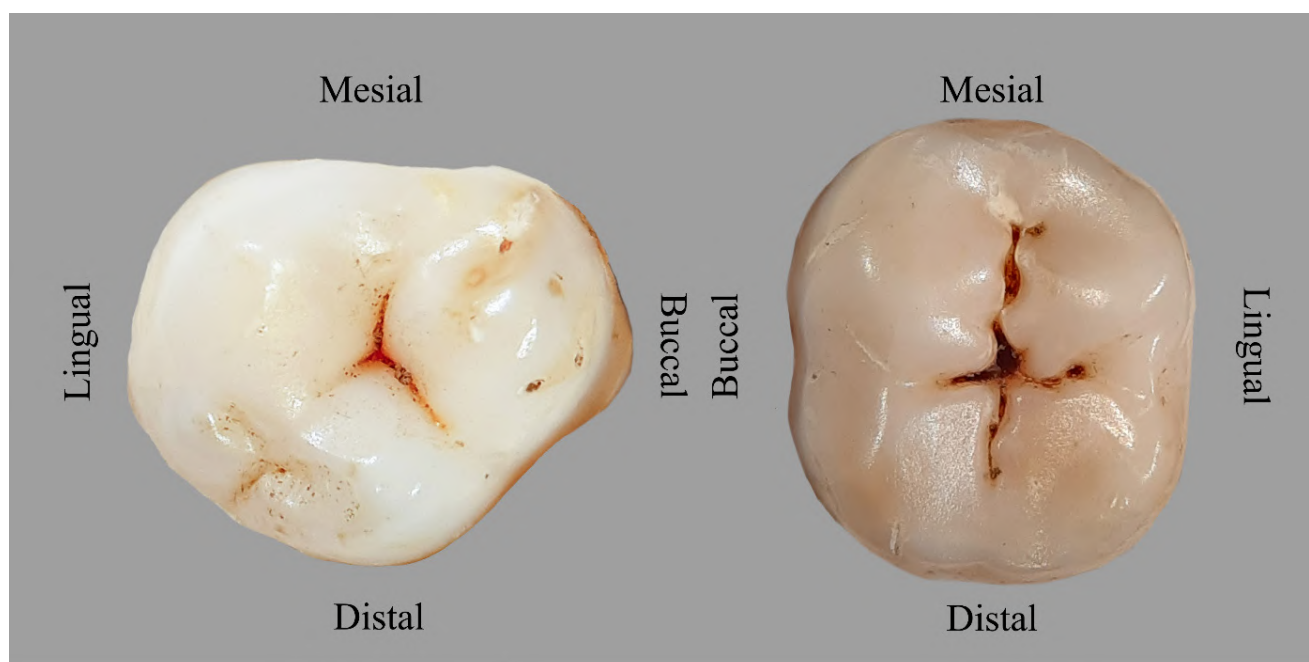
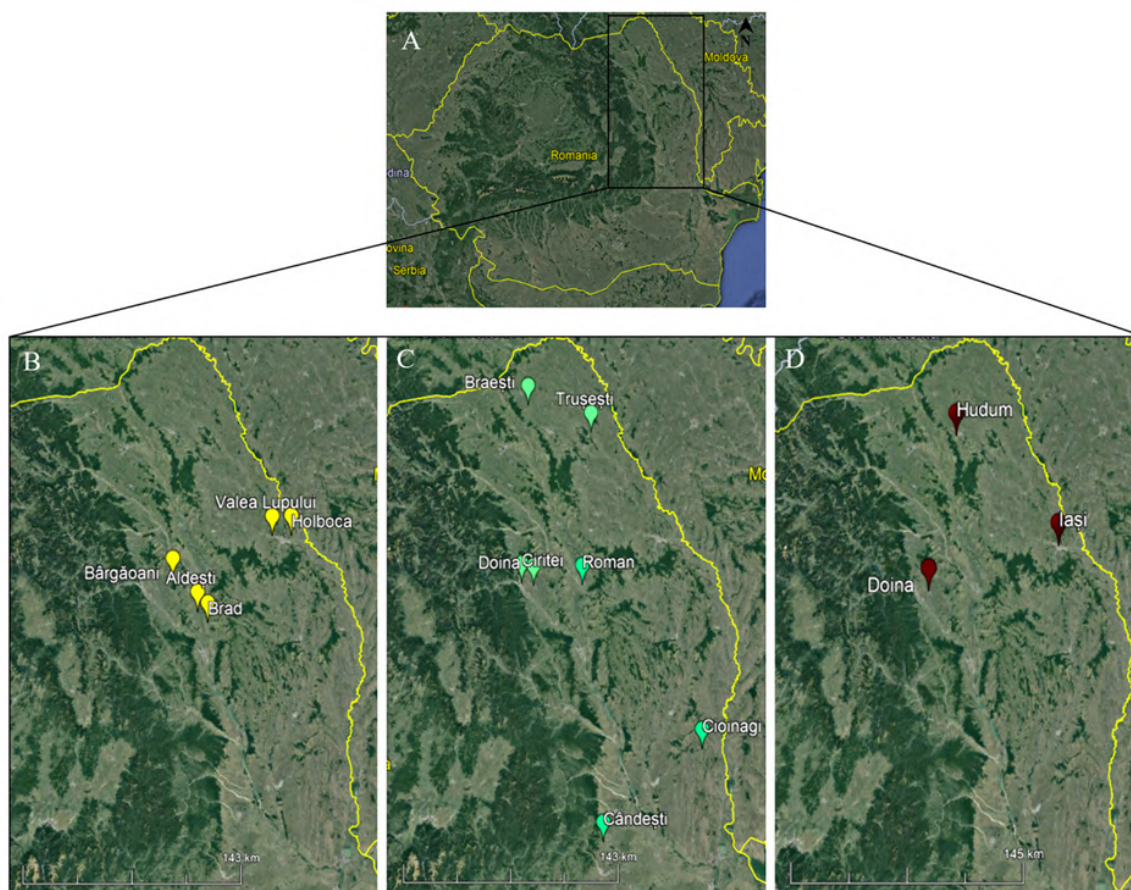


Fig. 1.- Occlusal surface of left maxillary M<sup>2</sup> molar (left) and left mandibular M<sub>2</sub> molar (right).

Table 1. M2 molar teeth selected for the study.

<i>Distribution of M2 molars according to the position in the skull</i>			
Upper right M2 molar (M <sup>2<sub>r</sub></sup> )	Upper left M2 molar (M <sup>2<sub>l</sub></sup> )	Lower right M2 molar (M <sub>2<sub>r</sub></sub> )	Lower left M2 molar (M <sub>2<sub>l</sub></sub> )
n = 71	n = 70	n = 79	n = 64
<i>Distribution of M2 molars according to age at death category proposed by Buikstra and Ubelaker (1994)</i>			
Adolescents (12-20 years)	Young adults (20-35 years)	Middle adults (35-50)	Old adults (> 50 years)
n = 28	n = 118	n = 115	n = 23



**Fig. 2.-** Location of archaeological sites: **A** – Nord-Eastern Romania area; **B** – archaeological sites from Chalcolithic (5000–3800/3700 BCE), **C** – archaeological sites from Bronze Age (3500–1200/1150 BCE); **D** – archaeological sites from Middle Ages (13<sup>th</sup> – 17<sup>th</sup> centuries).

**Table 2.** List of archaeological sites considered in the study.

Period		Archaeological site / County	Dental samples	Skeleton sample Reference	Odontometric ImageJ data Reference
Prehistory	Chalcolithic	Brad / Neamț	n = 3	Necrasov and Onofrei (1972)	Inedit
		Aldești / Neamț	n = 3		Inedit
		Valea Lupului / Iași	n = 3	Antoniou and Roșca-Gramatopol (1966)	Inedit
		Holboca / Iași	n = 19	Necrasov and Cristescu (1957)	Inedit
		Bârgăoani / Neamț	n = 3	Necrasov et al. (1972)	Inedit
	Bronze Age	Cioinagi / Galați	n = 7	Cristescu et al. (1965)	Inedit
		Căndești / Vrancea	n = 20	Miu (1999)	(Petraru et al., 2020)
		Roman / Neamț	n = 3	Diaconu et al. (2016)	Inedit
		Trusești / Botoșani	n = 34	Cristescu and Miu (1999)	(Petraru et al., 2020)
		Ciritei / Neamț	n = 3	Cristescu (1961)	Inedit
Middle Ages	13 <sup>th</sup> -14 <sup>th</sup> centuries	Braești / Botoșani	n = 4	Miu (1992)	Inedit
		Doina / Neamț	n = 2	Archive data*	Inedit
	17 <sup>th</sup> century	Hudum / Botoșani	n = 34	Miu et al. (2003); Archive data*	Inedit
		Doina / Neamț	n = 13	Necrasov and Botezatu (1964); Archive data*	Inedit
		“Curtea Domnească” / Iași	n = 133	Groza (2015)	Inedit

\* Data from the Archive of “O. Necrasov” Centre of Anthropological Research

were detached from the skull (Petraru and Bejenaru, 2019). The teeth were placed under stereomicroscope so that the cement-enamel junction plane was perpendicularly situated to the optical axis of the camera lens (Galbany et al., 2016; Górká, 2016; De Castro and Nicolas, 1995; Al-Khatib et al., 2011; de Castro et al., 2001; Martín-Albaladejo et al., 2017).

### Dental micrometry

Digital images of occlusal surfaces of the M2 molars were recorded using a Carl Zeiss Stemi 2000-C stereomicroscope with a Canon Power Shot G9 attached. The images were processed and calibrated (in mm) using ImageJ software (Abràmoff et al., 2004). Two maximum crown diameters were taken by ImageJ software: bucco-lingual (BL) diameter and mesio-distal (MD) diameter. The MD diameter was taken as the maximum width of the tooth crown in the mesio-distal plane (Kazzazi and Kranioti, 2018), while BL diameter was taken as the widest point of the tooth crown in the bucco-lingual plane, perpendicular to the MD (Nava et al., 2021; Takahashi et al., 2007; Pilloud and Scott, 2020). In the dental samples in which inter-proximal facets were observed, the MD length was estimated according to the method proposed by Wood and Abbott (1983). The crown index (CI = BL diameter x 100/MD diameter), and the crown area (CA = BL diameter x MD diameter) were used to characterize the overall crown size, but not the shape (Kondo et al., 2005; Kondo and Manabe, 2016). The sexual dimorphism index (SDI = M-F/ F x 100; M - male mean value; F - females mean value) was also used (Harris and Foster, 2014; Zorba et al., 2011).

### Statistical analysis

Intra-observer error of dental measurements was achieved by blind tests in which 20 molars

were randomly examined. The BL, and MD variables were measured twice with one month between measurements, by the same researcher. The Shapiro-Wilk test was computed for the data distribution in order to perform the asymmetry analysis, bivariate analysis of the M2 molar variables (BL, MD, CA, CI). Student's T-test and Mann-Whitney tests were used to compare the BL and MD values as independent groups of the left and right mandibular/maxillary molars.

Descriptive statistics and relationships between measurements were investigated through univariate and multivariate statistical analysis using XLSTAT and PAST softwares (Hammer et al., 2001). Pearson's and Spearman's tests were used to analyze the correlations between the BL and MD measurements (Ratner, 2009; Razali and Wah, 2011).

To evaluate the accuracy of the BL, MD, CA and IC variables in assessing sexual dimorphism, the Discriminant Analysis (DA) was used. The analysis was achieved for every category of data: upper and lower M2 molars in every period (Prehistory and Middle Ages). Hotelling's test indicates how convenient is a given molar variable in the discriminant analysis; the F Statistic determines how much variation exists between the sexes and the significance level of the variance.

## RESULTS

The result of the *t* test was not statistically significant (BL: N = 20; *t* = 0.27; *p* > 0.78; MD: N = 20, *t* = 0.37, *p* = 0.71).

The diameters' asymmetry between the left and right mandibular/maxillary molars was analysed and no statistically significant result was revealed (Table 3); therefore the left and right mandibular/maxillary molars were analyzed simultaneously.

**Table 3.** Test results for asymmetry between the left and right mandibular/maxillary BL and MD.

Test type	$M_{2\text{ right}} \text{ vs. } M_{2\text{ left}}$		$M^{2\text{ right}} \text{ vs. } M^{2\text{ left}}$	
	BL diameter	MD diameter	BL diameter	MD diameter
Shapiro-Wilk	W = 0.98, <i>p</i> = 0.66 W = 0.96, <i>p</i> = 0.1	W = 0.95, <i>p</i> = 0.005 W = 0.95, <i>p</i> = 0.019	W = 0.97, <i>p</i> = 0.28 W = 27, <i>p</i> = 0.26	W = 0.98, <i>p</i> = 0.50 W = 0.98, <i>p</i> = 0.60
<i>T</i> test/ Mann-Whitney	<i>t</i> = 1.02, <b><i>p</i> = 0.307</b>	Z = 0.62, <b><i>p</i> = 0.53</b>	<i>t</i> = 1.47, <b><i>p</i> = 0.16</b>	<i>t</i> = 0.19, <b><i>p</i> = 0.84</b>

The summary statistics of the bidimensional diameters are comparative shown by sex, position of the molars (mandibular and maxillary), and period, in Table 4. The bidimensional diameters for the mandibular M<sub>2</sub> molars are characterized by a higher mean in males comparative to females, probably due to sexual dimorphisms (BL = 9.42 mm in females and 9.72 mm in males; MD = 10.10 mm in females and 10.43 mm in males). The differences between the BL and MD variables in males vs. females are statistically significant (BL:  $z = 3.05$ ,  $p = 0.002$ ; MD:  $z = 2.60$ ,  $p = 0.009$ ). A difference was also observed for the superior molars M<sup>2</sup> but not supported by statistical significance (BL t test:  $t = 0.518$ ,  $p = 0.605$ ; MD t test:  $t = 0.906$ ,  $p = 0.3665$ ). When the dimensions were divided by period (*i.e.*, Prehistory, Middle Ages), the BL and MD values were similar for the inferior M<sub>2</sub> molars. In this study, the variability degree of the M2 dimensions is indicated by the coefficient of variation (CV%). Thus, constant CV% values were observed for the mandibular molars dating from Prehistory (CV% = 6.71 for BL diameter, and 6.87 for MD diameter), while a greater variability was observed for the medieval teeth from both 13<sup>th</sup>-14<sup>th</sup> centuries (CV% = 7.99 for BL diameter, and 8.22 for MD diameter) and 17<sup>th</sup> century (CV% = 5.93 for BL diameter, and 6.83 for MD diameter). In contrast with the dimension variability of the mandibular molars, for the superior M<sup>2</sup> molars a higher variability coefficient is observed for both diameters in the 13<sup>th</sup>-14<sup>th</sup> centuries (CV% = 10.73 for BL diameter, and 10.79 for MD diameter). Similar high CV% was noted for the MD diameter of the M<sup>2</sup> from prehistoric sample (CV% = 10.53), while BL diameter was characterized by lower variability (CV% = 7.23).

To compare the molar dimensions in terms of diachronic and sex criteria, the medieval teeth of 13<sup>th</sup>-14<sup>th</sup> centuries and those of the 17<sup>th</sup> century were considered together. In order to merge the samples, parametric and non-parametric tests, depending on the data distribution, were performed to test whether there are differences between the odontometric data of the 13<sup>th</sup> -14<sup>th</sup> centuries and those of the 17<sup>th</sup> century. The results showed no statistical differences between the variables of the mandibular M2 molars (BL:

$t = 0.73$ ,  $p = 0.46$ ; MD:  $z = 0.03$ ,  $p = 0.96$ ) and the maxillary ones (BL:  $t = 0.08$ ,  $p = 0.93$ ; MD:  $t = 1.44$ ,  $p = 0.15$ ). The upper M<sup>2</sup> molars from the female dataset are characterized by a similar variability of the mesio-distal diameter for both prehistoric and medieval samples (CV% = 8.76, and 8.20 respectively), in contrast with the variability of the bucco-lingual diameter which varies less in the prehistoric molars (CV% = 4.43) than in medieval ones (CV% = 7.05). In contrast with the dimensions of the superior molar in females, the dimensions of the mandibular molar are characterized by lower variability in the sample from Prehistory (CV% = 3.45 in BL diameter, and 4.75 in MD diameter), while in the medieval sample the variability is higher (CV% = 7.22 in BL diameter, and 6.41 in MD diameter). In the male samples, comparable CV% values were observed for the inferior molars, and for the BL diameter in the maxillary M<sup>2</sup> molars (CV% = 8.14 in prehistoric molars, and 7.69 in medieval ones), while different CV% values were obtained for the MD diameter in the prehistoric molars (CV% = 11.24), and medieval ones (CV% = 8.82).

The bidimensional measurements analyzed on the criterium of tooth position in the skull (mandibular and maxillary) have shown a non-normal distribution in the mandibular M<sub>2</sub> molars for the MD diameter ( $W = 0.95$ ,  $p = 0.0002$ ), and a normal distribution for the BL diameter ( $W = 0.98$ ,  $p = 0.08$ ). In the maxillary M<sup>2</sup> molars, the diameter values showed a normal distribution (BL diameter:  $W = 0.98$ ,  $p = 0.08$ ; MD diameter:  $W = 0.99$ ,  $p = 0.48$ ). When sex criteria were applied, the data for the mandibular male molars showed a non-normal distribution for the MD diameter ( $W = 0.96$ ,  $p = 0.01$ ), and a normal distribution data for the BL diameter ( $W = 0.98$ ,  $p = 0.45$ ). In the male maxillary molars, both diameter values showed a normal distribution (BL diameter:  $W = 0.98$ ,  $p = 0.40$ ; MD diameter:  $W = 0.97$ ,  $p = 0.11$ ). Similar distribution of data diameters was observed in the female molars: non-normal distribution data in the mandibular molars (BL diameter:  $W = 0.86$ ,  $p = 0.0003$ ; MD diameter:  $W = 0.91$ ,  $p = 0.009$ ), and normal data distribution in the maxillary ones (BL and MD diameters:  $W = 0.95$ ,  $p = 0.15$ ).

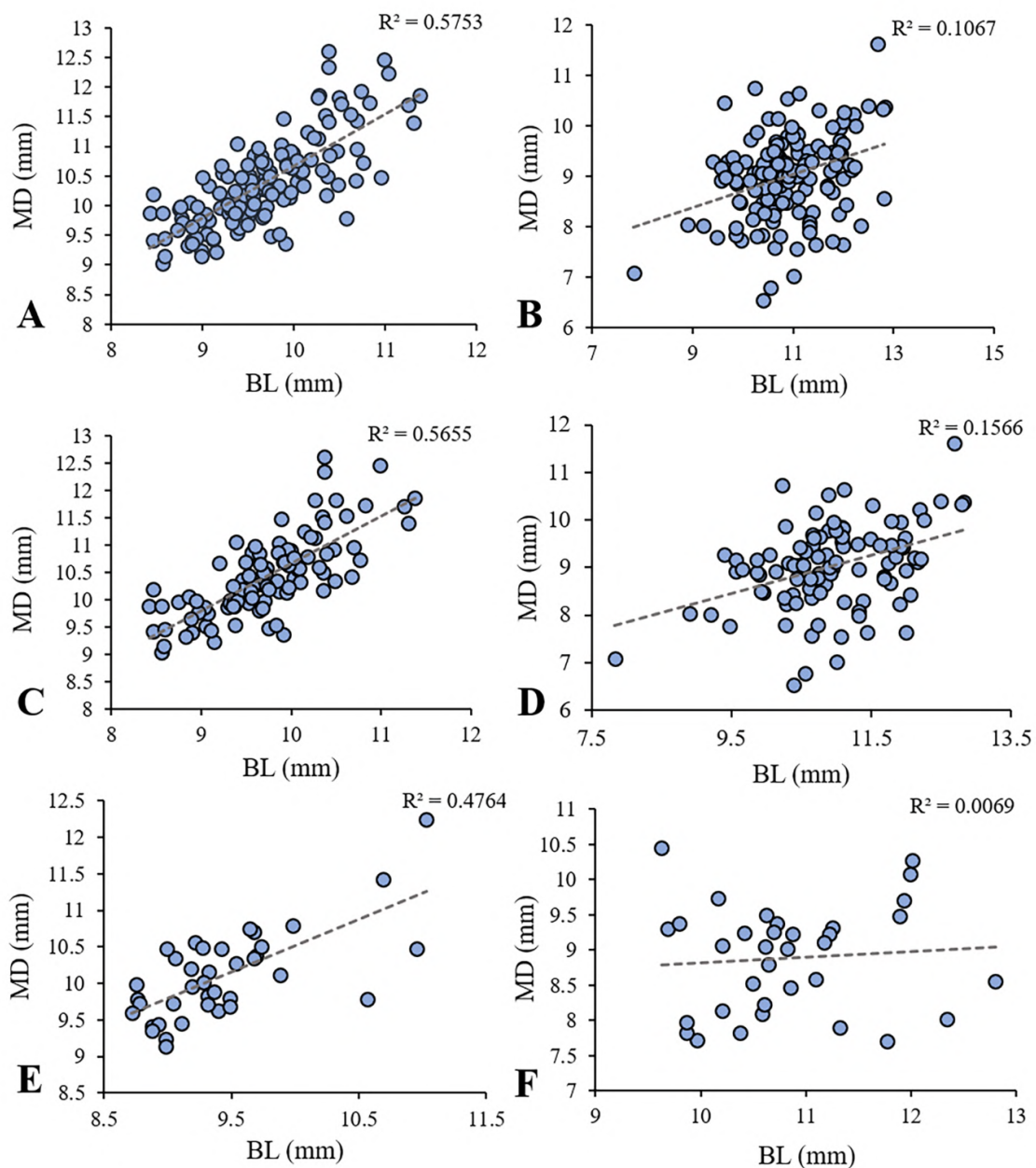
**Table 4.** Summary statistics of bucco-lingual (BL) and mesio-distal (ML) diameters in the M2 molars by position in skull, sex, and periods (N – number, Min – minimum, Max – maximum, Std. error – standard error, Var. – variance, Std. dev. – standard deviation, Coeff. Var. – coefficient of variance).

Tooth	N	Sex	Variable	Min	Max	Mean	Std. error	Var.	Std. dev.	Median	Coeff. Var.
M <sub>2</sub>	143	-	BL	8.41	11.37	9.65	0.05	0.41	0.64	9.64	6.64
			MD	9.02	12.61	10.37	0.06	0.55	0.74	10.27	7.14
M <sup>2</sup>	141	-	BL	7.82	12.82	10.9	0.07	0.70	0.83	10.84	7.67
			MD	6.53	11.61	9.00	0.07	0.71	0.84	9.05	9.36
<i>Dimension variability of M2 molars by sex</i>											
M <sub>2</sub>	101	Male	BL	8.41	11.37	9.72	0.06	0.41	0.64	9.7	6.59
			MD	9.02	12.61	10.43	0.07	0.54	0.73	10.33	7.08
M <sub>2</sub>	38	Female	BL	8.71	11.03	9.42	0.09	0.33	0.58	9.31	6.17
			MD	9.14	12.24	10.10	0.09	0.37	0.61	9.99	6.05
M <sup>2</sup>	102	Male	BL	7.82	12.82	10.92	0.08	0.74	0.86	10.88	7.92
			MD	6.53	11.61	9.03	0.08	0.77	0.88	9.07	9.74
M <sup>2</sup>	34	Female	BL	9.62	12.8	10.83	0.13	0.64	0.80	10.66	7.42
			MD	7.7	10.45	8.88	0.13	0.57	0.76	9.05	8.55
<i>Dimension variability of M2 molars by period</i>											
<i>Prehistory (~ 5000-1150 BCE)</i>											
M <sub>2</sub>	53	-	BL	8.42	11.3	9.59	0.08	0.41	0.64	9.51	6.71
			MD	9.14	12.46	10.24	0.09	0.49	0.70	10.08	6.87
M <sup>2</sup>	51	-	BL	8.9	12.68	10.6	0.10	0.58	0.76	10.61	7.23
			MD	6.53	11.62	8.79	0.12	0.82	0.91	8.86	10.53
<i>Middle Ages (13<sup>th</sup>-14<sup>th</sup> centuries)</i>											
M <sub>2</sub>	27	-	BL	8.56	11.38	9.77	0.15	0.61	0.78	9.88	7.99
			MD	9.03	11.94	10.45	0.16	0.73	0.85	10.33	8.22
M <sup>2</sup>	20	-	BL	7.83	12.82	11.05	0.26	1.40	1.18	11.34	10.73
			MD	7.08	10.37	8.90	0.21	0.92	0.96	9.01	10.79
<i>Middle Ages (17<sup>th</sup> century)</i>											
M <sub>2</sub>	63	-	BL	8.46	11.03	9.66	0.07	0.32	0.57	9.6	5.93
			MD	9.22	12.61	10.44	0.08	0.50	0.71	10.42	6.83
M <sup>2</sup>	70	-	BL	9.56	12.8	11.07	0.08	0.50	0.71	10.97	6.43
			MD	7.02	10.74	9.18	0.08	0.51	0.72	9.22	7.83
<i>Dimension variability of M2 molars by period and sex*</i>											
<i>Prehistory (~ 5000-1150 BCE)</i>											
M <sub>2</sub>	34	Male	BL	8.42	11.3	9.76	0.11	0.48	0.69	9.72	7.15
			MD	9.36	12.46	10.37	0.12	0.50	0.70	10.21	6.84
M <sup>2</sup>	33	Male	BL	8.9	12.68	10.70	0.15	0.76	0.87	10.64	8.14
			MD	6.53	11.62	8.80	0.17	0.97	0.98	8.89	11.24
M <sub>2</sub>	17	Female	BL	8.72	9.98	9.22	0.07	0.10	0.31	9.19	3.45
			MD	9.14	10.79	9.87	0.11	0.22	0.46	9.8	4.75
M <sup>2</sup>	15	Female	BL	9.63	11.1	10.32	0.11	0.20	0.45	10.37	4.43
			MD	7.72	10.46	8.68	0.19	0.58	0.76	8.59	8.76
<i>Middle Ages (13<sup>th</sup> – 17<sup>th</sup> centuries)</i>											
M <sub>2</sub>	67	Male	BL	8.46	11.38	9.70	0.07	0.37	0.61	9.69	6.33
			MD	9.03	12.61	10.46	0.09	0.57	0.75	10.43	7.22
M <sup>2</sup>	69	Male	BL	7.83	12.82	11.02	0.10	0.72	0.84	10.96	7.69
			MD	7.02	10.74	9.15	0.09	0.65	0.80	9.19	8.82
M <sub>2</sub>	21	Female	BL	8.76	11.03	9.59	0.15	0.48	0.69	9.42	7.22
			MD	9.36	12.24	10.28	0.14	0.43	0.65	10.27	6.41
M <sup>2</sup>	19	Female	BL	9.86	12.8	11.24	0.18	0.62	0.79	11.22	7.05
			MD	7.7	10.27	9.04	0.17	0.55	0.74	9.22	8.20

\* 9 molars were removed from the descriptive statistics due to the indeterminate sex of the individuals

Our results show a strong and positive correlation between the BL and MD diameters for the mandibular M<sub>2</sub> molars (Spearman coefficient = 0.75,  $p < 0.001$ ), and moderate one in the maxillary M<sup>2</sup> molars (Pearson coefficient = 0.32,  $p < 0.001$ ) (Fig. 3A, B). When a sex criterium was applied, the dental measurements in the mandibular M<sub>2</sub> molars (i.e., BL, and MD) were strong correlated in males (Spearman coefficient = 0.76,  $p < 0.001$ ), and moderate positive correlated in females (Spearman coefficient = 0.64,  $p < 0.001$ ) (Fig. 3C, E). In contrast with these results, for the maxillary

M<sup>2</sup> molars, dental measurements showed a moderate positive correlation in males (Pearson coefficient = 0.39,  $p < 0.001$ ), and no correlation in females (Pearson coefficient = 0.083,  $p < 0.64$ ) (Fig. 3D, F). The MD and BL diameters were less correlated in the maxillary M<sup>2</sup> molar than in the mandibular M<sub>2</sub> (10%, and 57% respectively). This aspect is highlighted especially in the female molars (47% in the mandibular M<sub>2</sub>, and 0.69% in the maxillary M<sup>2</sup>) in contrast with the male molars (56% in mandibular M<sub>2</sub> and 15% in the maxillary M<sup>2</sup>).



**Fig. 3.-** Dimension variability of the M2 human molars belonging to the archaeological populations from North-Eastern Romania: **A** – dimensions of the M<sub>2</sub> molars; **B** – dimensions of the M<sup>2</sup> molars; **C** – dimensions of the M<sub>2</sub> molars in males; **D** – dimensions of the M<sup>2</sup> molars in males; **E** – dimensions of the M<sub>2</sub> molars in females; **F** – dimensions of the M<sup>2</sup> molars in females.



The crown index (CI) can be used to obtain data regarding the crown proportions, especially in a comparative approach (Kondo et al., 2005). The CI frequency values are shown by sex and tooth position in the skull in Fig. 4. The distribution of the CI values was assessed using the Shapiro-Wilk test. The results showed that the CI values have non-normal distribution in the maxillary M<sup>2</sup> molars in males ( $W = 0.94$ ,  $p = 0.002$ ); therefore, the Mann-Whitney pairwise test was used. According to the Mann-Whitney test, a significant statistical difference was revealed between the mandibular M<sub>2</sub> molars in female vs. male ( $p < 0.001$ ), probably due to the sexual dimorphism that influenced the crown proportion.

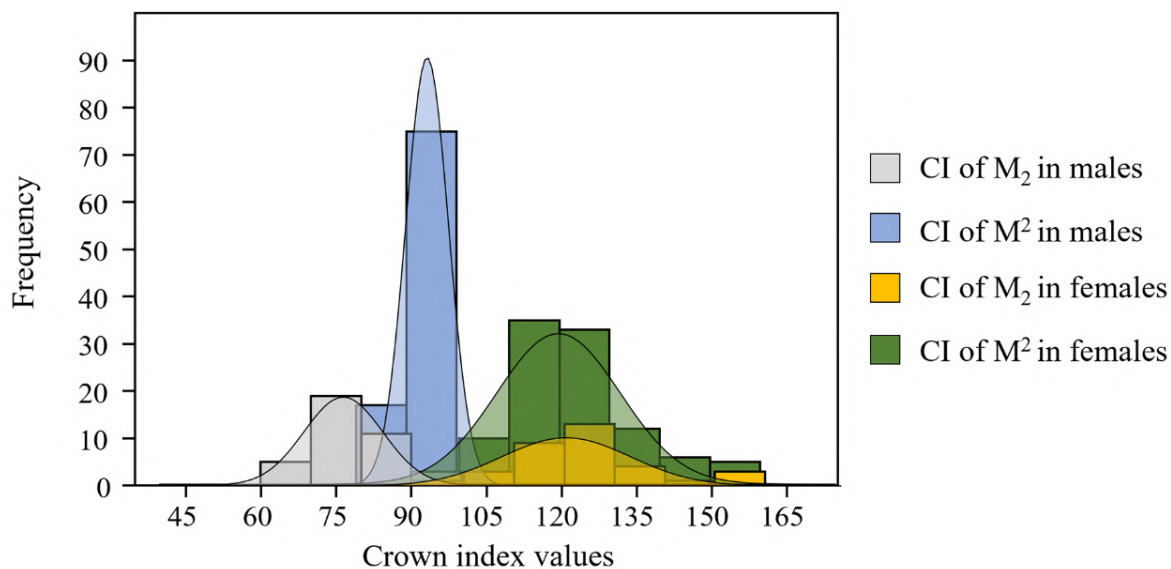


Fig. 4.- Crown index of the M2 molars by sex.

To analyze the dynamic of the tooth crown proportion in the upper and lower M2 molars, the medio-distal (MD) and bucco-lingual (BL) measurements, along with the crown area (CA), and crown index (CI) were diachronically compared. The results of data normality test are shown in Table 5.

Three variables register statistically significant differences between the prehistoric and the medieval periods in the female teeth (Fig. 5A). For the lower M2 molar, the mesio-distal (MD) diameter recorded a mean value greater in the medieval sample (MD = 10.28 mm) comparative with that of prehistoric one (MD = 9.87 mm) (U

test = 109;  $p = 0.042$ ). Also, the crown area (CA) of the mandibular molar M<sub>2</sub> recorded higher values in the medieval sample (98.97 mm<sup>2</sup>) compared to the prehistoric one (91.08 mm<sup>2</sup>) (U test = 102;  $p = 0.02$ ).

In the upper M<sup>2</sup> molars, the bucco-lingual diameter (BL) and the crown area (CA) registered significant higher values for the medieval sample (U test = 46;  $p = 0.00$  and U test = 51;  $p = 0.001$ ), namely 11.24 mm in BL and 101.66 mm<sup>2</sup> in CA, comparative with 10.32 mm and 89.56 mm<sup>2</sup> for prehistoric sample.

In the male dataset, the Mann-Whitney test results highlight significant differences between

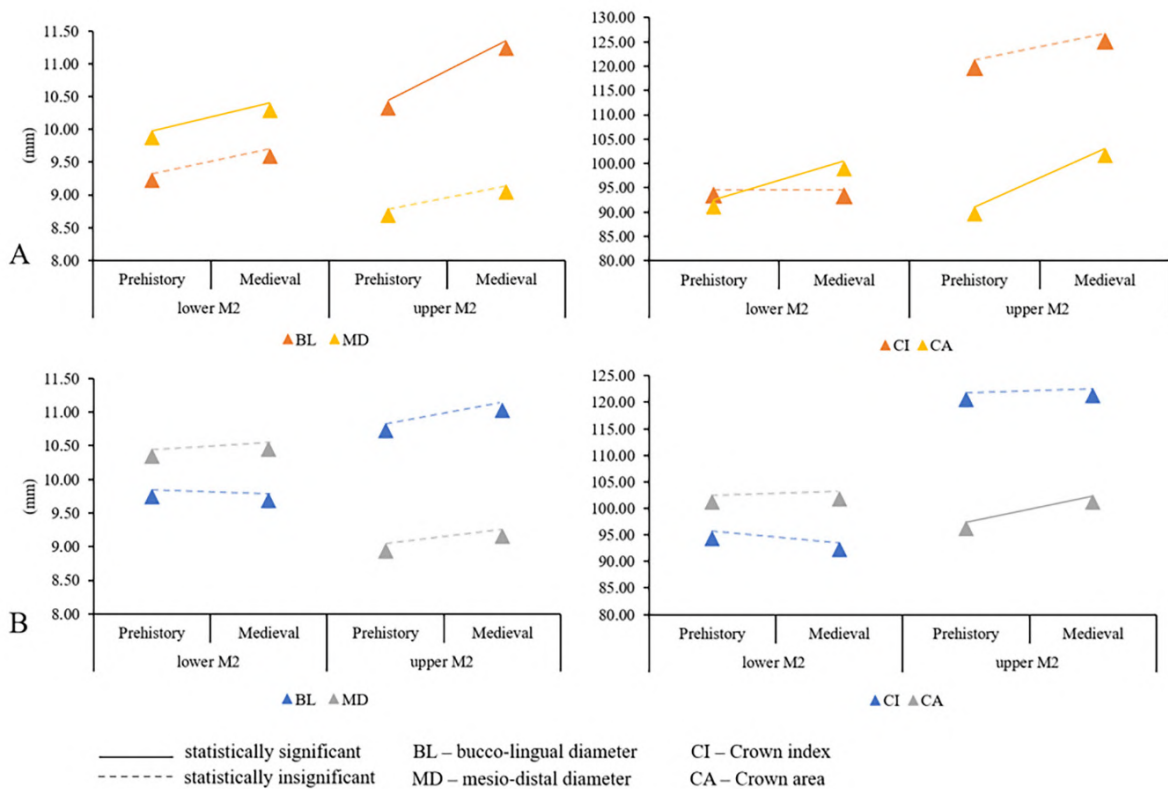
the mean values only for the crown area (CA) of upper M<sup>2</sup> molars (U test = 743;  $p = 0.008$ ) (Fig. 5B). The CA registered higher values for this molar in the medieval sample (101 mm<sup>2</sup>) than in the prehistoric one (96.22 mm<sup>2</sup>).

### Sexual dimorphism

In our study, the sexual dimorphism was calculated for each period separately: Prehistory and Middle Ages. In the prehistoric mandibular M<sub>2</sub>, of the four variables investigated (MD, BL, CI, CA), the sexual dimorphism was manifested in three of them: BL (U test = 112;  $p = 0.001$ ), MD (U test = 138;  $p = 0.01$ ), and CA (U test = 114;  $p = 0.001$ ).

**Table 5.** Normality test result for BL, MD, CA, CI variables.

Prehistory									
Lower M <sub>2</sub> molar – male					Lower M <sub>2</sub> molar – female				
Normality test	Variables				Normality test	Variables			
	BL	MD	CI	CA		BL	MD	CI	CA
W	0.979	0.868	0.984	0.916	W	0.958	0.963	0.946	0.925
p - value	0.790	0.001	0.921	0.021	p - value	0.592	0.694	0.394	0.176
Upper M <sup>2</sup> molar – male					Upper M <sup>2</sup> molar – female				
Normality test	Variables				Normality test	Variables			
	BL	MD	CI	CA		BL	MD	CI	CA
W	0.986	0.942	0.976	0.874	W	0.946	0.935	0.877	0.950
p - value	0.950	0.088	0.673	0.001	p - value	0.459	0.319	0.043	0.525
Middle Ages									
Lower M <sub>2</sub> molar – male					Lower M <sub>2</sub> molar – female				
Normality test	Variables				Normality test	Variables			
	BL	MD	CI	CA		BL	MD	CI	CA
W	0.985	0.978	0.980	0.975	W	0.893	0.900	0.848	0.886
p - value	0.622	0.303	0.389	0.200	p - value	0.029	0.035	0.004	0.019
Upper M <sup>2</sup> molar – male					Upper M <sup>2</sup> molar – female				
Normality test	Variables				Normality test	Variables			
	BL	MD	CI	CA		BL	MD	CI	CA
W	0.958	0.973	0.941	0.978	W	0.976	0.938	0.836	0.966
p - value	0.020	0.141	0.003	0.274	p - value	0.883	0.246	0.004	0.692



**Fig. 5.-** Comparative representation of the M2 molar variables (BL, MD, CA, CI) between medieval and prehistoric samples: A. Females, B. Males.

**Table 6.** Discriminant analysis statistics.

<b>Stepwise discriminant analysis of dental variables</b>					
<b>Period</b>	<b>Molar</b>	<b>Hotelling's value</b>	<b>F test</b>	<b>P value</b>	
Prehistory	Lower M <sub>2</sub> molar	12.17	2.84	0.03*	
	Upper M <sup>2</sup> molar	3.4	0.8	0.52	
Middle Ages	Lower M <sub>2</sub> molar	1.04	0.25	0.9	
	Upper M <sup>2</sup> molar	2.4	0.58	0.67	
<b>The correlations between the factors (F1) and original variables</b>					
<b>Variables</b>	<b>Lower M<sub>2</sub> molar Prehistory</b>	<b>Upper M<sup>2</sup> molar/ Pre-history</b>	<b>Lower M<sub>2</sub> molar Middle Ages</b>	<b>Upper M<sup>2</sup> molar Middle Ages</b>	
BL	0.926	-0.701	0.62	0.646	
MD	0.771	-0.583	0.893	-0.347	
CI	0.213	0.021	-0.421	0.783	
CA	0.896	-0.796	0.821	0.105	
<b>Canonical discriminant function coefficients and Functions at Group Centroid</b>					
<b>Molar</b>	<b>Variable</b>	<b>F1</b>	<b>Function of Group centroid</b>		
Lower M <sub>2</sub> molar Prehistory	Constant	-39.596	Female: -0.675 Male: 0.383		
	BL	14.077			
	MD	2.583			
	CI	-0.549			
	CA	-0.714			
Upper M <sup>2</sup> molar/ Prehistory	Constant	-74.156	Female: 0.395 Male: -0.185		
	BL	-6.111			
	MD	9.012			
	CI	0.602			
	CA	-0.142			
Lower M <sub>2</sub> molar Middle Ages	Constant	-102.491	Female: -0.194 Male: 0.063		
	BL	-13.542			
	MD	9.865			
	CI	1.198			
	CA	0.191			
Upper M <sup>2</sup> molar Middle Ages	Constant	-38.829	Female: 0.315 Male: -0.087		
	BL	10.339			
	MD	3.255			
	CI	-0.275			
	CA	-0.709			
<b>Confusion matrix for the cross-validation results:</b>					
<b>M2</b>	<b>from \ to</b>	<b>F</b>	<b>M</b>	<b>Total</b>	<b>% Correct</b>
Lower M <sub>2</sub> molar Prehistory	F	14	3	17	82.35%
	M	16	14	30	46.67%
	Total	30	17	47	59.57%
Upper M <sup>2</sup> molar/ Prehistory	F	9	6	15	60.00%
	M	12	20	32	62.50%
	Total	21	26	47	61.70%
Lower M <sub>2</sub> molar Middle Ages	F	1	20	21	4.76%
	M	7	58	65	89.23%
	Total	8	78	86	68.60%
Upper M <sup>2</sup> molar Middle Ages	F	0	19	19	0.00%
	M	5	64	69	92.75%
	Total	5	83	88	72.73%
Discriminant function for M2 mandibular molar – Prehistory: F1= (-39.596) + 14.077*BL + 2.583*MD + (-0.549*CI) + (-0.714*CA).					

\*Statistically significant p-value

The SDI values are close for the two variables: 6.09% for BL, and 5.06% for MD. So, for that we consider that indeed both variables play a more important role in the sexual differentiation. This idea is strengthened by the area of the dental crown (CA), calculated precisely on these two variables (BL x MD). The SDI value of CA shows a much more dimorphic role than for the two variables analyzed separately (SDI = 11.74%).

The role of variables in the sexual discrimination was also tested with Discriminant Analysis. Discriminant Analysis was achieved for every category of data: upper and lower M2 molars, and every period (Prehistory and Middle Ages). The analysis only reinforces the findings of the previous test.

The Table 6 shows the results of Discriminant Analysis. It includes the Hotelling's test, which highlights the role of the dental variables in the stepwise analysis, while F test determines how much variation exists between sexes and the significance level of variance. The DA results show that BL variable is the one that makes the biggest contribution to the functions obtained, and a stronger correlation between the variables is noticed in the test of the mandibular molar from the Prehistoric period. Also, in Table 6 the canonical discriminant coefficient and function at group centroid are presented. A group centroid is the mean discriminant score for each of the sexes. The formula to identify the sex by M2 is derived from the discrimination function coefficients (unstandardized coefficients), clearly categorizing the subjects as male or female. After calculating F value from the formula, it will be compared with the section point which is calculated as 0.

In the present study, classification accuracy in the stepwise discriminant analysis ranged between 59.57-72.73% (Table 6). Maximum accuracy of sex diagnosis was observed in the maxillary second molar (72.73% in Middle Ages, and 61.70% in Prehistory), followed by the mandibular second molar (59.57% in Prehistory, and 68.60% in Middle Ages). If we referred at the function statistically significant ( $p$  value < 0.05), namely the one of the prehistoric samples, we can notice a higher accuracy for females (82.35%) than for males (46.67%).

## DISCUSSION

This study explores the dental variability of permanent molar crowns in past human populations from Prehistory (~ 5000-1150 BCE) and the Middle Ages (13<sup>th</sup>-17<sup>th</sup> centuries), Northeastern Romania. In this research, the M2 molar teeth have been subjected to linear measurements of tooth crown (i.e., medio-distal diameter MD, and bucco-lingual diameter BL), along with two indexes (i.e., crown area CA, and crown index CI). Several studies on tooth characterization used these variables in both past human populations (Brook et al., 2009; Nava et al., 2021; Lukacs, 2019; Viciano et al., 2012) and actual ones (Zúñiga et al., 2021; Abaid et al., 2021). The results of the present study show that mandibular M2 molars in females are characterized by a mean BL value of 9.42 mm, and a mean MD value of 10.10 mm (Table 4). A higher mean value of the BL variable was obtained by Pajević and Glišić (2017) in samples from Mesolithic-Neolithic to Middle Ages from Serbia. In our study, comparable mean values to those attained by Pajević and Glišić (2017) were also obtained for the male mandibular molars. The same study conducted by Pajević and Glišić (2017) showed higher values in the male maxillary M2 molars in comparison with the data obtained for the samples belonging to archaeological sites from North East Romania.

When the period criterium was considered, differences regarding the tooth diameters were observed between the molars from Prehistory and Middle Age.

Several comparative studies on tooth dimension reveal that tooth size decreases from the prehistoric populations to the medieval ones (Christensen, 1998; Brace et al., 1987; Pinhasi et al., 2008). Pajević and Glišić (2017) reported that the BL diameter of the maxillary M2 molar decreases from the Mesolithic-Neolithic samples to the medieval ones. In contrast, our results show that the BL diameter of the maxillary molar increases from Prehistory to the Middle Ages, while the MD diameter values for mandibular molars are higher in the medieval period in comparison with Prehistory. Same increasing tendency of dental diameters was noted for the male molars, but not statistically supported. A study of Mockers et al.

(2004) shows that the MD diameter of mandibular teeth belonging to the prehistoric population from Roaix, Southern France, is smaller when compared to its size in modern Caucasian populations. Moreover, the mean value for the MD diameter of prehistoric mandibular M2 molars from Nord Eastern Romania (10.24 mm) is higher than the value obtained by Mockers et al. (2004) for the prehistoric molars from a population in Roix, France (9.75 mm). Regarding the BL and MD diameters of the medieval mandibular molars, comparable mean values were obtained by Vodanović et al. (2007) in a study approaching dental metrics in medieval samples from Eastern Croatia; the female maxillary molars from North East Romania showed slightly lower values than ones from Croatia. The crown area (CA), also known as the robustness index, is often used to characterize the overall crown size (Hillson et al., 2005; Petraru et al., 2020; Nowaczewska et al., 2013; Schmidt et al., 2011). The M2 molars from North East Romania show an increasing CA from the prehistoric period to the medieval one in both mandibular and maxillary molars in females. The same tendency is maintained for the upper M<sup>2</sup> molar in males. Similar CA values of medieval maxillary M2 molars in females from Croatia were obtained by Vodanović et al. (2007), while higher values were found in both mandibular and maxillary male molars in comparison with the data obtained for the dental samples from Romania.

Sexual dimorphism refers to systematic morphological differences between males and females of the same species (Abaid et al., 2021; Magalhães et al., 2021). Sexual dimorphism can be reflected in the human dentition especially in permanent teeth (Shankar et al., 2013; Zúñiga et al., 2021). The molars are considered among the most dimorphic teeth (El Sheikhi and Bugaighis, 2016) and even if the overall trend is known in any population, the degree of sexual dimorphism of each variable involved remains a population-specific characteristic (Garn et al., 1964; Acharya and Mainali, 2007; Zorba et al., 2011; Shankar et al., 2013). Previous studies have found that there is a statistically significant difference in tooth size between males and females, and this is due to the differences in the body size of the two sexes

that occur in any human population (Schwartz and Dean, 2005; Ateş et al., 2006; Saunders et al., 2007).

While the prehistoric dental sample has been found highly dimorphic by sex, not a similar result was found in the medieval material, according to the Mann-Whitney test. As in another study conducted by İşcan and Kedici (2003), the lower M<sub>2</sub> is more dimorphic than the upper M<sup>2</sup>.

The tooth dimensions typically are larger in males than in females, but the difference is not constant across populations (Barrett et al., 1964; Barrett et al., 1963; Harris and Foster, 2014). There are studies conducted on more recent material suggesting that mandibular BL is more dimorphic than mandibular MD (Yamada and Sakai, 1992; Garn et al., 1966; Zorba et al., 2011; Prabhu and Acharya, 2009), in contrast to others which mention that MD dimension is better suited than BL for sex discrimination; however, they found that better results are obtained when both MD and BL dimensions are used together (Acharya and Mainali, 2007; Barrett et al., 1964; Barrett et al., 1963). Our view is closer to the latter one if we consider the obtained results.

The stepwise discriminant analysis was done to find a reliable result on sex discrimination.

Lakhanpal et al. (2013) considered that, although BL dimension is more easily measured, its ability to correct classifications of individuals is moderate when used independently. Furthermore, if one has the opinion of choosing between the two types of linear measurements, MD should be preferred, even if it is more difficult to measure. We consider that better accuracy can be obtained when several variables are correlated. In our material, CI has a small contribution to sex discrimination, compared to the other three variables (BL, MD and CA). CI is the relationship between BL and MD and while the linear dimensions in males are usually larger than in females, this may not be true when they are taken as a relative measure (Kondo and Townsend, 2004). There are opinions according to which the crown index does not quantify the tooth size, as the crown area and linear measurements do. Harris and Rathburn (1991), and Garn et al. (1967) consider the crown

index to be a description of the tooth shape rather than tooth size, being a more relevant indicator of population variations and not of sex differences.

A comparison between the maxillary and mandibular molars showed that the upper ones were more dimorphic than the mandibular molars, but statistically insignificant ( $p$  value  $> 0.05$ ). Between the two sexes, the classification accuracy was higher in females than in males for cross-validated data. This could mean that males have the greatest variability of dental size and they can more often be misclassified than females. This result is in concordance with those of İşcan and Kedici (2003), Ateş et al. (2006), Peckmann et al. (2015) on crown mesiodistal and buccolingual measurements, which reported higher classification accuracy in females. A study conducted by Zorba et al. (2013) also provided similar results but for the diagonal measurements of molar teeth in a modern Greek population.

## CONCLUSIONS

This paper approached dental measurements in archaeological human populations in order to assess M2 molar crown variability. The results of our study show a greater variability in the medieval female M2 teeth compared to prehistoric sample, in both maxillary (MD variable) and mandibular molars (MD and BL variables). Also, the two diameters were less correlated in the maxillary M<sup>2</sup> molar than in the mandibular M<sub>2</sub> molar, especially in females. In males, a higher variability of the mesio-distal (MD) variable was observed in the prehistory maxillary molars compared with the medieval ones. In the mandibular female teeth, significant statistical differences were obtained between the prehistoric and medieval samples (MD variable and CA variable). Also, in the maxillary molars the bucco-lingual (BL) and crown area (CA) variables showed higher values in the medieval samples.

In the male maxillary molars, the crown area (CA) variable showed significant higher values in the medieval sample than in the prehistoric one.

The discriminant analysis confirmed the sexual dimorphism on the mandibular M<sub>2</sub> crown in the prehistoric sample.

Further multidisciplinary studies are required to provide information concerning the influence of genetic, epigenetic and environmental factors on the dental variations.

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