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Dimensional variability of the human second molar (M2) in Bronze Age populations from North-East Romania

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Abstract. The second molar tooth (M2) is particularly valuable for paleoanthropological research. The crowns of mandibular and maxillary M2 molars from Bronze Age paleoanthropological samples from North-Eastern Romania were investigated. Measurements of tooth crown included mesio-distal (MD) and bucco-lingual (BL) diameters performed on digital images obtained stereo-microscopically using ImageJ software. The crown robustness index was also used: BL diameter x 100/MD diameter. Our results show a high dental crown variability for the M^2 molar in males and a lower variability for the M_2 molar in both males and females. The analyzed molars will be the subject of further studies concerning dental geometric morphometrics and dental wear.
Keywords: human second molar (M2), Bronze Age, dental crown diameters, variability.

Variabilitatea dimensională a celui de al doilea molar (M2) uman în populații de epoca bronzului din nord-estul României. Dintele molar M2 este deosebit de valoros pentru cercetarea paleoantropologică. Coroanele molarilor M2 superiori și inferiori din eșantioane paleoantropologice de epoca bronzului din nord-estul României au fost supuse investigării. Măsurătorile coroanei dentare au cuprins diametrele mezio-distale (MD) și buco-linguale (BL) prelevate pe baza imaginilor obținute la stereomicroscop, folosind ImageJ. De asemenea, a fost utilizat și indicele de robustețe al coroanei: diametrul BL x 100 / diametrul MD. Rezultatele arată o variabilitate mare a coroanei dentare pentru molarul M^2 superior la bărbați și o variabilitate mai mică pentru molarul M_2 inferior, atât la bărbați, cât și la femei. Molarii M2 analizați vor fi obiectul unor studii ulterioare privind geometria morfometrică și uzura dentară.
Cuvinte-cheie: al doilea molar (M2) uman, epoca bronzului, diametrele coroanei dentare, variabilitate.

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Introduction

Teeth are an excellent material for multidisciplinary research and represent a valuable source of information for the paleoanthropological research. Owing to their exceptional preservation based on their hard tissues (*i.e.* enamel, dentine, and cementum), teeth are stable markers and indicators for certain studies regarding the past human populations and also for other mammals assemblages. Teeth have been the subject of various researches such as tooth wear (Petraru, Bejenaru 2019; Hernando et alii 2020), geometric morphometrics (Gómez-Robles et alii 2007; Gómez-Robles et alii 2012; 2015), histological and paleopathological research regarding the health status (Liebe-Harkort 2012; Lorentz et alii 2019; Nava *et alii* 2019).

Several findings regarding tooth size were established on traditional linear measurements of tooth crowns (*i.e.* mesio-distal and bucco-lingual crown diameters) (Kondo, Yamada 2003; Kondo et alii 2005; Peiris et alii 2005; Galdames et alii 2008). Some studies suggest that teeth that develop later ontogenetically tend to be more variable in size, expressing greater sexual dimorphism owing to variations in sex hormone production between males and females (Takahashi et alii 2007). The variation in odontometry is especially important to establish the effect of sexual dimorphism on the size of the teeth, therefore the study of crown dimensions becomes particularly essential when anatomical parameters are not reliable for identifying a particular subject (Galdames et alii 2008). Moreover, several studies have shown that other factors such as age, ethnicity, genetics, environmental condition, can influence the tooth morphology in different populations (Farzin et alii 2020).

This paper represents a preliminary comparative study regarding the dental crown size of the M2 molars in Bronze Age paleoanthropological samples from North-Eastern Romania, depending on sex and position in the dental arch. The human remains come from the two necropolises of Cândești (Vrancea County, 45.539172°N, 27.073576°E, Monteoru culture, 1550-1300 CAL BC) and Trușești (Botoșani County, 47.73605°N 27.01693°E, Noua culture, 1600-1150 CAL BC) (Florescu 1964; Florescu, Florescu 1983). Paleodemographic and morphometrical research of the discovered human remains has been performed by Miu (1999) and Cristescu, Miu (1999). Nowadays, part of the discovered paleoanthropological material is included in the osteological collection of the Romanian Academy - Iași Branch, "Olga Necrasov" Center of Anthropological Research. To increase the sample size, in this study, all M2 teeth were considered as a single group representative for the Bronze Age in north-eastern Romania.

Material and methods

The M2 molars were selected for this analysis; the first (M1) and third molars (M3) were avoided – M1 has a variable morphology and usually a high degree of wear, and M3 is not always present – congenitally absent (Bernal 2007; Gilmore, Grote 2012).

The crowns of M2 molars (n = 54) belonging to males, females and undetermined individuals (**Tab. 1**) were prepared for study according to the following steps: contaminants were removed from the dental surface using ethanol, hydrogen peroxide, and cotton wool; the teeth were placed under the stereomicroscope with the specific anatomical orientation (recommended where teeth were detached from the skull) (Petraru, Bejenaru 2019).

Sex	M2 molar	N
Males	inferior	13
	superior	15
Females	inferior	13
	superior	8
Undetermined	inferior	2
	superior	3

Table 1. Teeth selected for bidimensional analysis
Tab. 1. Dinții selectați pentru analiza bidimensională

Data acquisition. Digital images of occlusal surfaces were recorded using a Carl Zeiss Stemi 2000-C stereomicroscope with a Canon Power Shot G9 attached. The images (1223 x 922 pixels) were processed and calibrated (in mm) using ImageJ software (Abràmoff et alii 2004). The maximum crown diameters were taken: mesio-distal (MD, the largest mesial-to-distal dimension taken parallel to the occlusal surface), and bucco-lingual (BL, the greatest distance between the buccal and lingual surfaces, perpendicular to the mesio-distal diameter) (**Fig. 1**) (Zorba et alii 2011). The shape of the occlusal surface can be characterized by a crown shape index CI = BL diameter x 100/MD diameter (Kondo et alii 2005).

Statistical analysis. Descriptive statistics and relationships between measurements were investigated through univariate and multivariate statistical analysis using XLSTAT Student version and PAST software (Hammer et alii 2001). The Grubs's test was used to correct for measurement errors, and detect outliers in a univariate dataset (Grubbs 1969). Data were assessed for normality using the Shapiro-Wilk test (Shapiro, Wilk 1965). Multivariate analysis of variance (MANOVA) and Canonical Variates Analysis (CVA) were employed to assess the role of variables for discrimination among subsamples, also aiming to categorize the molar with uncertain sex.

Results and Discussion

Normality of data cannot be rejected for any variable according to the Shapiro-Wilk test ($p > 0.05$). **Tab. 2** provides the summary statistics for measurements of the M2 molars. The bucco-lingual mean diameter is almost comparable for both males and females for the inferior M_2 molars (BL diameter = 9.78 mm in males and 9.27 in females), the values being slightly larger in males, probably due to sexual dimorphism. The same trend was observed for the MD diameter in inferior M_2 molars (10.23 mm in males and 9.97 mm in females). When the sex criteria were pooled, and the descriptive analysis involve molars from undetermined skeletons, the MD diameter mean value for the mandibular molar was 10.18 mm and 8.58 in the superior M^2 molars. In a study regarding crown dimensions from the Bronze Age Harappans, the obtained dental measurements for the M2 molars were also slightly larger in males than females (Dutta 1983).

Tooth	Sex	Variable	N	Min (mm)	Max (mm)	Mean (mm)	Std. error	Var.	Stand. dev	Coeff. var
Inferior second molar (M_2)	-	BL	28	8.59	10.76	9.55	0.10	0.27	0.52	5.42
		MD	28	9.23	11.86	10.18	0.11	0.36	0.60	5.88
Superior second molar (M^2)	-	BL	26	9.47	11.75	10.46	0.11	0.32	0.57	5.43
		MD	26	6.53	10.46	8.58	0.18	0.85	0.92	10.71
Inferior second molar (M_2)	Males	BL	13	8.59	10.76	9.78	0.15	0.30	0.55	5.62
		MD	13	9.36	10.95	10.23	0.15	0.29	0.54	5.27
	Females	BL	13	8.72	9.98	9.27	0.09	0.11	0.34	3.64
		MD	13	9.23	10.79	9.97	0.13	0.22	0.47	4.69
Superior second molar (M^2)	Males	BL	15	9.47	11.75	10.49	0.16	0.38	0.61	5.85
		MD	15	6.53	9.82	8.42	0.24	0.86	0.93	11.04
	Females	BL	8	9.63	10.86	10.24	0.16	0.20	0.45	4.40
		MD	8	7.72	10.46	8.63	0.33	0.86	0.93	4.40

Table 2. Summary statistics for measurements of M2 molars.
Abbreviations: BL – bucco-lingual maximum crown diameter; MD – mesio-distal maximum crown diameter; N – number of examined molars; Min. – Minimum; Max. – Maximum; Std. error – standard error; Var. – variance, Stand. dev – standard deviation, CV – coefficient of variation

Tab. 2. Statistica descriptivă pentru măsurătorile molarilor M2.
Abrevieri: BL – diametrul buco-lingual maxim al coroanei; MD – Diametrul mezio-distal maxim al coroanei; N – numarul de molar examinați; Min. – Minimum; Max. – Maximum; Std. error – eroarea standard; Var. – varianța, Stand. dev – deviația standard, CV – coeficientul de variație

In our study, the variability degree of the measurements is shown by the coefficient of variation (CV). A high variability is highlighted for MD diameter in males for the superior M^2 molar (CV = 11.04). In contrast, for the female dataset, a low and constant variability was observed for the superior molars in both MD and BL diameters (CV = 4.40). The bidimensional measurements related to the inferior M_2 molars show a low variability in both males (CV = 5.27 for MD diameters, CV = 5.26 for BL diameters) and females (CV = 4.69 for MD diameters, CV = 3.64 for BL measurements).

The bivariate analysis of the M2 molars is shown in **Fig. 2**. In males, for the inferior M_2 molar a positive correlation was recorded (r = 0.7), while for the superior M^2 molar a very weak correlation was noted (r = 0.2) (**Fig. 2/a, b**). In the female dataset, the Pearson coefficient recorded a value of 0.5 (moderated correlation) for the M_2 inferior molars, and -0.5 (negative correlation) for the superior M^2 molars (**Fig. 2/c, d**). When the sex criteria were pooled, the bidimensional variability for all studied inferior molars recorded a moderate positive correlation (r = 0.6), and a very weak negative correlation (r = -0.1) for the superior molars (**Fig. 2/e, f**).

Dental indexes are revealed to have evolutionary and clinical significance (Acharya, Mainali 2008). The crown area or robustness index is characterized by BL and MD dimensions and can be used to measure the gross dentition size for comparative purposes (Hemphill 2015). Regarding the robustness index, our results show higher values for the male sample comparative to the female sample but without a statistical significance (p = 0.20 for the inferior molars and p = 0.37 for the superior molars) (**Fig. 3**).

The linear combinations between the variables involved in multivariate analysis (*i.e.* MD, BL, and CI), categorize the five molars with uncertain sex as following: three molars belong to females, while two belong to males. This result is also highlighted by the distribution of colour symbols on the biplot of CVA. The graph of CVA is presented in **Fig. 4** and 79% of the among group variation was accounted for the first two canonical axes. Multivariate analysis of variance (MANOVA) highlights significant results (F test 7. 4; $p < 0.05$).

Conclusions & Future perspectives

This work confirms the role of molar dimensions in sexual dimorphism and highlight some peculiarities of the analyzed sample. Our results show a high crown variability for the superior M^2 molar in males and a lower variability for the inferior M_2 molar in both males and females.

New odontometric aspects are emphasized by the bivariate analysis: a positive correlation between MD and BL for the inferior molar, and a weak and insignificant correlation for the superior molar. The multivariate analysis of the three variables

(*i.e.* MD, BL, and CI) could be used as an alternative method of sex estimation in samples where classical morphometric criteria cannot be applied.

As future perspectives, we look towards approaches concerning dental geometric morphometrics and dental wear to characterize the size and shape variation and to obtain teeth abrasiveness data regarding paleodiet.

Acknowledgements

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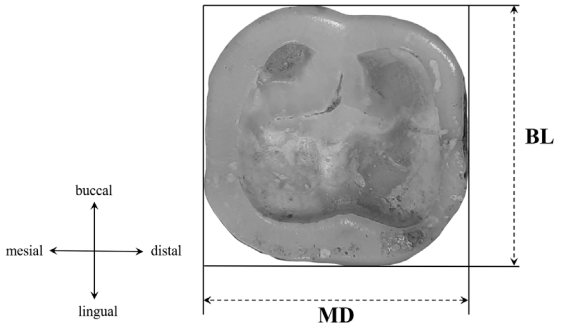

Fig. 1. Crown diameters of M_2 molar; BL – bucco-lingual diameter, MD – mesio-distal diameter
Fig. 1. Diametrele coroanei molarului M_2; diametrul buco-lingual BL, diametrul mezio-distal MD

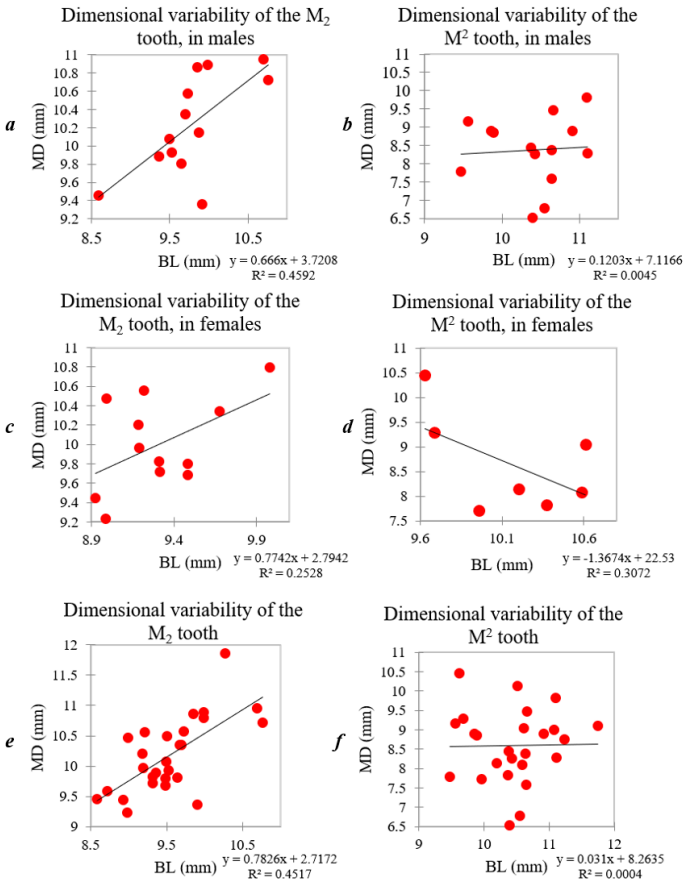

Fig. 2. Dimensional crown variability of the M2 molars in Bronze Age samples
Fig. 2. Variabilitatea dimensiunilor coroanei pentru molarii M2 din probe de epoca bronzului

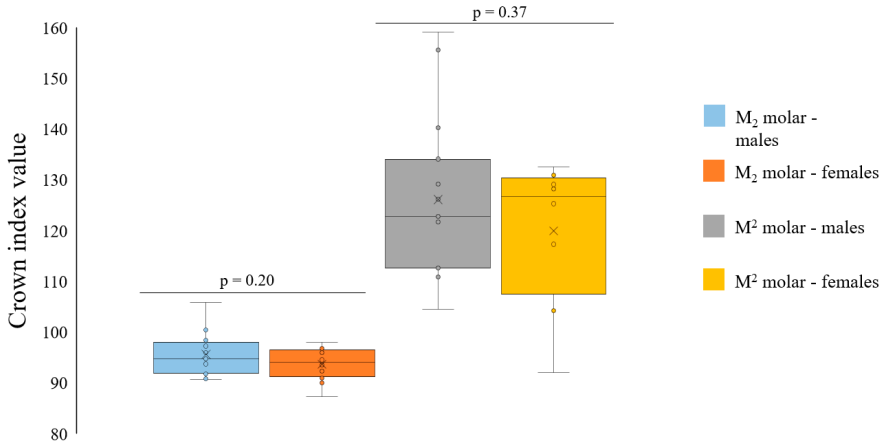

Fig. 3. Crown index shape (mean and median are indicated with 'x' and horizontal bar respectively)
Fig. 3. Indexul de robustețe al coroanei (media și mediana sunt indicate prin „x" și, respectiv, linie orizontală)

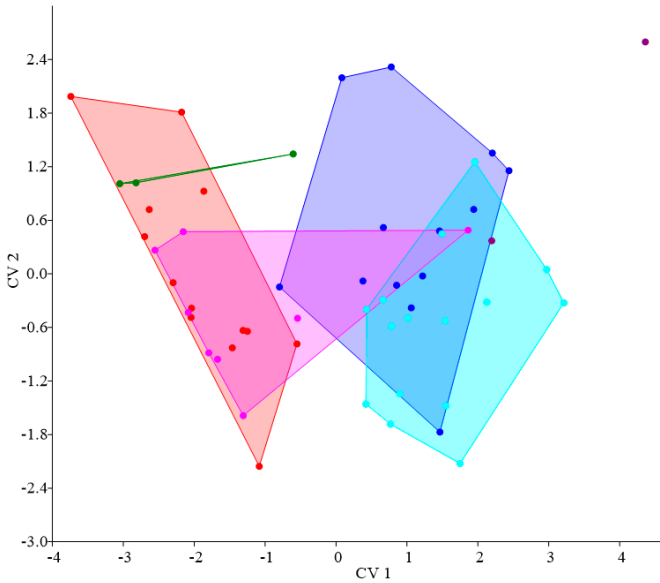

Fig. 4. CVA plot of the molars sample (blue – inferior M_2, males; red – inferior M_2, females; light blue – superior M^2, males; pink – superior M^2, females; mauve – inferior M_2, undetermined sex; green – superior M^2, undetermined sex)
Fig. 4. Graficul CVA pentru molarii studiați (albastru – molari M_2 inferiori, bărbați; roșu – molari M_2 inferiori, femei; albastru deschis – molari M^2 superiori, bărbați; roz – molari M^2 superiori, femei; mov – molari M_2 inferiori, indeterminabili sexual; verde – molari M^2 superiori, indeterminabili sexual)