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Carabelli's trait in Croatian populations over 1800 years

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ABSTRACT

Examination and comparison of the morphological features of tooth crown in archaeological and recent samples can be difficult due to the different levels of tooth wear seen both within and between populations. These differences make the comparison of frequency data for Carabelli trait problematic. The aim of the present study is to detect the frequency and degree of expression of Carabelli's trait in Croatian populations from late antiquity to recent times and to use these data as supplementary evidence of complex population migration.

A total of 1287 individuals from the late antiquity, medieval, early modern and modern periods were examined. Correlation between the presence of Carabelli's trait and tooth crown size was tested.

The results of our analyses show that the frequency of Carabelli's trait is significantly greater in the early modern period (51.3%) and in the 21st century (43.1%) than in the late antiquity (20.4%) and medieval periods (23.4%).

These results are consistent with historical evidence of migration and population change in the territory of present-day Croatia throughout the almost 1800 years covered by this study. The results also provide additional evidence for the complex nature of population change in the transition from the late antiquity to the early medieval period.

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Introduction

Teeth are one of the most enduring types of physical evidence of the existence of an individual after death and as such provide excellent material for various kinds of analyses in bioarchaeology, paleodontology and other related fields. Since the size, form and morphology of teeth are primarily determined by genes (Scott and Turner, 1997), they are a reliable tool for tracking some genetic changes throughout the history of humankind. Morphological variants and anomalies of teeth are highly significant for the study of human evolution, the skeletal biology of ancient populations (especially for inter- and intra-group investigations), and in paleopathology and forensic odontology (Alt et al., 1998). Early French and German anthropologists and odontologists demonstrated that some morphological variants, particularly the cusp number of the upper and lower molars, distinguished the major geographic populations of humankind (Scott and Turner, 1997).

In 1842 the Hungarian court dentist of the Austrian Emperor Franz Joseph, Georg Carabelli (Carabelli György), described a small additional cusp at the mesiopalatal line angle of maxillary first molars which was quite common in European dentitions. In his honor this cusp was named the cusp of Carabelli, or Carabelli's tubercle, or the *tuberculum anomale* of Georg Carabelli. If present, this accessory cusp is usually found on the permanent maxillary first molars or deciduous maxillary second molars. This cusp can also be found on the permanent second and third molars, although considerably less frequently than on the first molars. Carabelli's cusp can be absent in some individuals. Because of numerous variations in cusp size and shape including ridges, pits, single furrows, double furrows, partial cusp outlines and tubercles, it is customary to use the term Carabelli's trait. The term Carabelli's cusp should be used only if there is a real cusp on the mesiopalatal tooth surface. Carabelli's trait is considered a heritable feature and development of this trait is affected by multiple genes (Hunter et al., 2010; Kraus, 1951; Townsend et al., 2009). Lauc (2003) evaluated the influence of increased homozygosity due to inbreeding on the phenotypic distribution of the Carabelli trait and concluded that Carabelli's trait is strongly genetically determined. Additionally, his findings imply it may be controlled by recessive alleles (Lauc, 2003). Generally the trait is bilateral, but some unilateral cases have also been described (Khraisat et al., 2011). The Carabelli's trait is most common among Western Eurasian people and Pacific population groups (Scott and Turner, 1997). Consequently, Carabelli's trait molars, together with shovel trait incisors, are dental features that are commonly used to differentiate between Chinese and Caucasoid populations. Chinese populations differ from Caucasoids by having a high frequency of shovel trait and a low frequency of Carabelli's trait (Hsu et al., 1999). Kashibadze et al. (2011) employed gene geography, anthropophenetics, and the phenogeographical method for anthropological research to reconstruct human history by mapping dental markers in living Eurasian populations. They developed computer maps providing both analysis and visualization of the enormous amount of data accumulated in dental morphology and anthropology in living and ancient human populations with particular emphasis on the Carabelli's trait. Their investigation showed that dental markers provide the best possibility to examine directly time events in populations. They also concluded that to provide clear dating, additional studies in integrating with established genetic, archaeological and linguistic evidence should be launched.

Carabelli's trait is one of the most examined dental variables, and numerous attempts have been made to classify it. Different standards in the recording of this trait render the results of many studies incomparable. This makes tracking the frequency and expression of this feature in different geographical and temporal settings both difficult and unreliable. At present, the Arizona State University Dental Anthropology System (ASU-DAS) with the eight-grade scale developed by Turner and Dahlberg is the most commonly used system to score the Carabelli's trait (Scott and Turner, 1997).

The Carabelli's trait has been used as a critical ethnic indicator for several decades, most likely because it can be simply observed in both living individuals and skeletal material, and can therefore be used to show major ethnic differences in dentition (Hsu et al., 1999). With this in mind, and taking into account the available historical evidence for population migration throughout time in what is modern day Croatia, we propose to test the accumulated historical and archaeological data that suggest considerable population movement and displacement throughout time in Croatia by using dental morphological features (Klaić, 1988). Historical and archaeological data from the antique period onwards in Croatia describe numerous periods of profound cultural, social, economic, and political changes in

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the region of south-eastern Europe (Šlaus, 2008; Steindorff, 2006). Transition from the late antiquity to the early medieval period has, previously been considered as uniformly catastrophic and characterized by the destruction of major urban centres, depopulation, famine and the spread of epidemic diseases. This view is supported by historical and archaeological evidence (Šlaus, 2008; Steindorff, 2006), such as destruction of large urban centres in the Roman provinces of Pannonia (which included modern continental Croatia) and Dalmatia (which included Adriatic Croatia) during the second half of the 6th century and the beginning of the 7th century. The second line of evidence concerns the disappearance of most Roman names of towns in both provinces, while the third relates to historical reports regarding the endeavors of the Church to retrieve captive Christians and stolen relics from the invading Slavs and Avars, who were holding them to ransom. The general picture emerging from this information was that the late antiquity population of Croatia was annihilated, with a small number of surviving refugees fleeing to small defendable centres in the Dinarid mountain range or to the Adriatic islands where they could be protected by the Byzantine fleet. Recent bioarchaeological analyses (Šlaus, 2006) of this transition recorded the frequencies of four markers of health: *cribra orbitalia*, linear enamel hypoplasia, non-specific periostitis and trauma in 981 skeletons from the late antiquity and early medieval series in Croatia. This revealed a much more complicated picture of the transitional period. Comparisons between the late antiquity and early medieval series showed similar frequencies of the health indicators in continental Croatia – suggesting no significant discontinuity of living conditions and a significant increase of *cribra orbitalia*, periostitis, and trauma frequencies during the early medieval period in Adriatic Croatia. Furthermore, this deterioration of living conditions was not evenly distributed, but primarily affected subadults and males. These data suggest that the transition from the late antiquity to the early medieval period in Croatia was not a uniform process, and differentially affected the population, presumably due to local cultural, socio-economical or political factors. There is every reason to believe that the profound uncertainty and instability of everyday life caused by other historically documented upheavals and intrusions: for instance the continuous fighting for control of the rich Dalmatian towns with the Venetians, defeat and later political union with the Kingdom of Hungary, military intrusions by the Mongols and Ottoman Turks and so on also affected the population structure of the past inhabitants of Croatia in complex ways. Analyses of different dental morphological features – particularly the Carabelli's trait, are therefore a good way to gauge the effect that these changes had on the population structure of Croatia.

The aim of the present study was to detect the frequency and degree of expression of the Carabelli's trait in populations from late antiquity to recent times in Croatia. The hypotheses we wanted to test were:

- The frequency of Carabelli's trait in people who inhabited the territory of present-day Croatia changed throughout history.
- Differences in the frequencies of Carabelli's trait can be correlated with historical evidence of population migration.
- There is no significant difference in the frequency of Carabelli's trait between males and females throughout time in Croatia.
- Differences in the frequency of the Carabelli's trait are accompanied by differences in tooth crown dimensions.
- Teeth with Carabelli's trait have, on average, larger crowns than those without the Carabelli's trait.
- Tooth dimensions are, on average, larger in males than in females.

Materials and methods

A total of 1287 individuals (1222 skulls and 65 plaster models) from four periods: late antiquity (3rd–6th centuries), medieval (7–11th centuries), early modern (18th century) and modern (21st century) were examined to determine the expression of the Carabelli's trait (Table 1). Skulls dated from the late antiquity to the early modern period belong to the osteological collection of the Croatian Academy of Sciences and Arts. The sample from the late antiquity consists of 457 skulls from five urban sites located in the eastern part of continental Croatia and along the eastern Adriatic coast.

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Table 1
Dahlberg's scale for the determination of degree and expression of Carabelli's cusps (Scott and Turner, 1997).

0 – No vertical ridges, pits, or other manifestations on the mesiopalatal cusp
1 – Small vertical ridge and groove
2 – Small pit with minor grooves diverging from a depression
3 – Double vertical ridges or slight and incomplete cusp outline
4 – Y form: moderate grooves curving in opposite directions
5 – Small tubercle
6 – Broad cusp outline or moderate tubercle
7 – Large tubercle with free apex in contact with lingual groove (height often approximates that of major cusps)

Preliminary analysis showed no statistically significant differences in the expression of the Carabelli's trait between the urban sites and the sites were grouped in the composite late antiquity sample. The medieval sample consists of 661 skulls from six sites also located in the eastern part of continental Croatia, and along the eastern Adriatic coast. Preliminary analysis showed no statistically significant differences in the expression of the Carabelli's trait between these sites and the sites were grouped in the composite medieval sample. The early modern sample consists of 104 skulls from the crypt of the Požega cathedral. Plaster models of adult dentition from the crypt belong to the collection of the Department of Dental Anthropology, School of Dental Medicine, University of Zagreb. The collection was established primarily for scientific research in the field of dental anthropology and can be considered as representative.

Only skulls and plaster models with at least one permanent maxillary first molar were examined. From each individual only one tooth was included in the analysis (right side teeth were selected for the study; if a tooth from the right side was missing it was replaced by the tooth from the left side). Only permanent maxillary first molars without caries, and tooth wear on the occlusal tooth surface were included in the analysis of the Carabelli's trait.

The sex and age of each individual was determined using standard anthropological criteria. Sex determination was based on cranial and pelvic morphology (Šlaus, 2006). If these elements were missing or poorly preserved, discriminant functions for the femur and tibia developed for antique and medieval Croatian populations were used (Šlaus and Tomičić, 2005). Age was estimated using pubic symphysis morphology, auricular surface morphology, ectocranial suture closure and sternal rib end changes (Šlaus, 2006). Individuals younger than 15 years were considered subadults.

Dahlberg's eight-grade scale (including absence and seven degrees of trait presence) was used to examine the Carabelli's trait (Table 2). Teeth were examined with a magnifying glass (Zeiss hand-held magnifier D8AR, 2× magnification) under direct light.

To check the relationship between the expression of the Carabelli's trait and tooth dimensions, mesiodistal and buccolingual crown diameters were measured for the permanent maxillary first molar examined for the expression of the Carabelli's trait. Permanent maxillary first molars included in the odontometric analysis were intact and unworn (without caries and tooth wear). Measurements were taken with a digital sliding caliper. The mesiodistal diameter of the tooth crown was taken as the greatest mesiodistal dimension, parallel to the occlusal surface. The buccolingual crown diameter was taken as the greatest distance between the facial and lingual surfaces of the crown, taken at right angles to the plane in which the mesiodistal diameter was taken (Vodanović et al., 2007).

Differences between populations regarding the Carabelli's trait were tested with nonparametric Kruskal–Wallis and Mann–Whitney *U* tests. The Spearman correlation was used to test the correlation between presence of the Carabelli's trait and tooth crown dimensions. Analysis of variance (ANOVA) and the Scheffe post hoc test were used to test the differences in tooth dimensions between samples. All statistical analyses were performed using the SPSS statistical package version 15.0 (SPSS Inc); $p < 0.05$ was considered statistically significant. Intra-rater and inter-rater agreement was tested using 10% of randomly selected sample and Cohen Kappa score was calculated.

Results

A total of 1287 individuals were examined. Since only skulls and plaster models with at least one intact, unworn permanent maxillary first molar could be included in the analysis, sample size was

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Table 2
Distribution of the sample according to the historical periods, archaeological sites and sex.

Periods and sites	Century	Males					Females					Subadults					Total				
		N individuals	*N* teeth	*N* UM1-R	*N* UM1-L	UM1-R + UM1-L	*N* individuals	*N* teeth	*N* UM1-R	*N* UM1-L	UM1-R + UM1-L	*N* individuals	*N* teeth	N UM1-R	N UM1-L	UM1-R + UM1-L	*N* individuals	*N* teeth	N UM1-R	N UM1-L	UM1-R + UM1-L
Late antiquity																					
Zadar	1–5	39	1749	53	51	104	30	652	22	22	44	23	282	8	10	18	92	2683	83	83	166
Osijek	3–4	34	435	13	14	27	28	372	9	13	22	10	76	2	3	5	72	883	24	30	54
Štrbinci	4	43	587	15	19	34	38	436	16	13	29	24	122	5	6	11	105	1145	36	38	74
Vinkovci	4	8	151	6	4	10	9	72	3	2	5	8	102	3	3	6	25	325	12	9	21
Zmajevac	4	58	963	29	32	61	61	913	32	36	68	44	461	16	17	33	163	2337	77	85	162
Late antiquity – total	1–5	182	3885	116	120	236	166	2445	82	86	168	109	1043	34	39	73	457	7373	232	245	477
Medieval																					
Stari Jankovci	7–8	23	280	12	13	25	24	133	5	3	8	9	66	3	3	6	56	479	20	19	39
Velim	7–9	55	851	25	31	56	50	673	21	21	42	32	339	13	12	25	137	1863	59	64	123
Privlaka	8–9	76	1119	36	37	73	74	987	29	31	60	60	814	26	26	52	210	2920	91	94	185
Glavice	8–11	24	428	13	13	26	12	177	8	6	14	20	277	11	11	22	56	882	32	30	62
Radašinovci	9	38	494	16	17	33	33	431	20	19	39	42	319	15	16	31	113	1244	51	52	103
Šibenik	9	23	302	11	13	24	33	448	15	13	28	33	279	10	9	19	89	1029	36	35	71
Medieval – total	7–11	239	3474	113	124	237	226	2849	98	93	191	196	2094	78	77	155	661	8417	289	294	583
Early modern																					
Požega	18	65	1168	55	49	104	39	442	32	34	66	0	0	0	0	0	104	1610	87	83	170
21st century																					
Zagreb[a]	21	34	1008	30	29	59	31	899	26	28	54	0	0	0	0	0	65	1907	56	57	113
Total – all sites	1–21	520	9535	314	322	636	462	6635	238	241	479	305	3137	112	116	228	1287	19,307	664	679	1343

N – number; UM1 – upper first molar; R – right; L – left.

[a] Plaster models from the Department of Dental Anthropology, School of Dental Medicine at the University of Zagreb.

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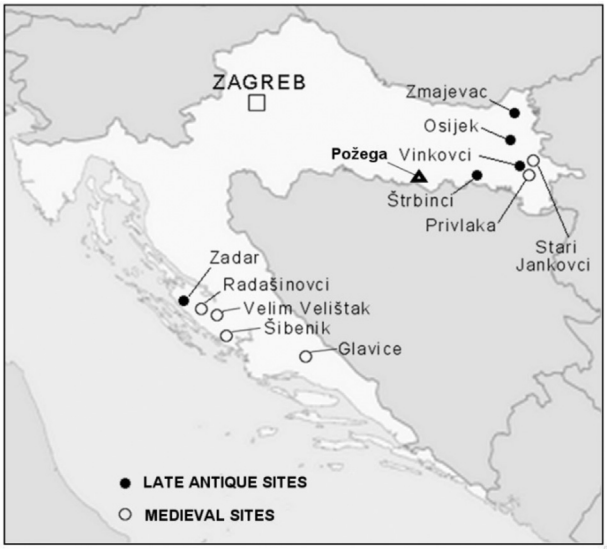

Fig. 1. Geographic distribution of the sites.

significantly reduced (Table 1). Of the 19,307 available teeth, 1343 (7.0%) were permanent maxillary first molars. Of these only 350 (26.1% of the total number of available permanent maxillary first molars) were intact and unworn, and thus suitable for examination of the frequency and expression of the Carabelli's trait. The number of teeth available for examination increased from 11.8% in the late antiquity period (54/457), to 29.0% in the medieval period (192/661), 37.5% (39/104) in the early modern period, and 100.0% in the 21st century (65/65). The final sample for analysis in this study consisted of 350 permanent maxillary first molars from 350 individuals (Table 3). Fig. 1 shows the geographic distribution of the sites.

The results of the analysis of the frequency and expression of the Carabelli's trait are presented in Table 3. There are statistically significant differences between the analyzed populations in the frequency of Carabelli's trait ($\chi^2 = 23.335$, $p = 0.001$). The Carabelli's trait (grade 1–7) was recorded in 20.4% of the late antiquity sample. Cusp forms (grade 5–7) were recorded in 9.3% of the late antiquity sample. In the medieval sample the Carabelli's trait (grade 1–7) was recorded in 23.4% and cusp forms (grade 5–7) were recorded in 6.8% of this sample. The Carabelli's trait (grade 1–7) was recorded in 51.3% of the 18th century sample and 20.5% of teeth had cusp forms grade 5–7. In the 21st century sample the Carabelli's trait (grade 1–7) was recorded in 43.1% and cusp forms (grade 5–7) were recorded in 18.5% of the sample. There was no statistically significant difference in the trait frequency between the late antiquity and medieval samples (Mann–Whitney $U = 5020.000$, $p = 0.631$). A statistically significant difference in the trait frequency was, however, noted between: the late antiquity and early modern samples (Mann–Whitney $U = 721.500$, $p = 0.002$), the late antiquity and 21st century samples (Mann–Whitney $U = 1333.500$, $p = 0.007$), between the medieval and early modern samples (Mann–Whitney $U = 2617$, $p = 0.000$) and between the medieval and 21st century samples (Mann–Whitney $U = 4885$, $p = 0.001$). No significant difference (Mann–Whitney $U = 11{,}675$, $p = 0.462$) in the Carabelli's trait frequency between the early modern and 21st century samples was noted.

A Kruskal–Wallis test results showed statistically significant differences between males from different populations in the frequency of the Carabelli's trait ($\chi^2 = 26.984$, $p = 0.000$). No statistically significant difference in the trait frequency was noted between the late antiquity (0.0%) and medieval (10.3%) male samples (Mann–Whitney $U = 351$, $p = 0.203$). However, significant differences in the trait frequencies were recorded between the late antiquity (0.0%) and early modern (48.3%) male samples (Mann–Whitney $U = 97.500$, $p = 0.012$), the late antiquity (0.0%) and 21st century (50.0%) male samples (Mann–Whitney $U = 110.500$, $p = 0.002$), the medieval (10.3%) and early modern (48.3%) male

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Table 3
Results of the analysis of the Carabelli's trait.

Periods	Grade																		$N1+N2+N3+N4+N5+N6+N7$		Cusp form ($N5+N6+N7$)		Cusp form ($N6+N7$)	
	$N0$	%	$N1$	%	$N2$	%	$N3$	%	$N4$	%	$N5$	%	$N6$	%	$N7$	%	N total	%	N	%	N	%	N	%
Late antiquity																								
Males	13	100.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13	100.0	–	–	–	–	–	–
Females	18	90.0	2	10.0	–	–	–	–	–	–	–	–	–	–	–	–	20	100.0	2	10.0	–	–	–	–
Subadults	12	57.1	3	14.3	1	4.8	–	–	–	–	2	9.5	1	4.8	2	9.5	21	100.0	9	42.9	5	23.8	3	14.3
Total	43	79.6	5	9.3	1	1.9	–	–	–	–	2	3.7	1	1.9	2	3.7	54	100.0	11	20.4	5	9.3	3	5.6
Medieval																								
Males	52	89.7	3	5.2	–	–	–	–	–	–	–	–	1	1.7	2	3.4	58	100.0	6	10.3	3	5.2	3	5.2
Females	53	86.9	3	4.9	2	3.3	2	3.3	–	–	1	1.6	–	–	–	–	61	100.0	8	13.1	1	1.6	–	–
Subadults	42	57.5	15	20.5	5	6.8	2	2.7	–	–	3	4.1	2	2.7	4	5.5	73	100.0	31	42.5	9	12.3	6	8.2
Total	147	76.6	21	10.9	7	3.6	4	2.1	–	–	4	2.1	3	1.6	6	3.1	192	100.0	45	23.4	13	6.8	9	4.7
Early modern																								
Males	15	51.7	4	13.8	–	–	2	6.9	3	10.3	3	10.3	–	–	2	6.9	29	100.0	14	48.3	5	17.2	2	6.9
Females	4	40.0	2	20.0	–	–	–	–	1	10.0	2	20.0	–	–	1	10.0	10	100.0	6	60.0	3	30.0	1	10.0
Subadults	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	19	48.7	6	15.4	–	–	2	5.1	4	10.3	5	12.8	–	–	3	7.7	39	100.0	20	51.3	8	20.5	3	7.7
21st century																								
Males	17	50.0	2	5.9	4	11.8	1	2.9	–	–	2	5.9	1	2.9	7	20.6	34	100.0	17	50.0	10	29.4	8	23.5
Females	20	64.5	4	12.9	5	16.1	–	–	–	–	2	6.5	–	–	–	–	31	100.0	11	35.5	2	6.5	–	–
Subadults	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	37	56.9	6	9.2	9	13.8	1	1.5	0	0.0	4	6.2	1	1.5	7	10.8	65	100.0	28	43.1	12	18.5	8	12.3
Total – all periods	246	70.3	38	10.9	17	4.9	7	2.0	4	1.1	15	4.3	5	1.4	18	5.1	350	100.0	104	29.7	38	10.9	23	6.6

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Table 4
Results of the odontometric analysis.

Periods	Mesiodistal diameter			Bucolingual diameter		
	N	Mean (mm)	SD (mm)	*N*	Mean (mm)	SD (mm)
Late antiquity						
Males	N/A	N/A	N/A	N/A	N/A	N/A
Females	4	9.4	0.3	4	10.6	0.7
Subadults	29	9.7	0.8	29	10.9	0.5
Total	33	9.6	0.8	33	10.9	0.5
Medieval						
Males	25	10.2	0.7	25	11.1	0.6
Females	28	10.3	0.8	28	11.0	0.8
Subadults	75	9.9	0.6	75	10.9	0.9
Total	128	10.1	0.7	128	10.9	0.8
Early modern						
Males	35	10.1	0.6	35	11.2	0.5
Females	14	9.6	0.5	14	10.3	1.4
Subadults	N/A	N/A	N/A	N/A	N/A	N/A
Total	49	10.0	0.6	49	11.0	1.0
21st century						
Males	34	9.7	0.5	34	10.9	0.5
Females	31	9.3	0.6	31	10.7	0.8
Subadults	N/A	N/A	N/A	N/A	N/A	N/A
Total	65	9.5	0.6	65	10.8	0.6
Total – all periods	184	9.9	0.7	184	10.9	0.8

N – number of teeth examined; SD – standard deviation; N/A – not available.
ANOVA results: mesiodistal parameter: $F = 6.361$, $p = 0.000$ and bucolingual parameter: $F = 0.462$, $p = 0.709$.

samples (Mann–Whitney $U = 564.500$, $p = 0.000$), and the medieval (10.3%) and 21st century (50.0%) male samples (Mann–Whitney $U = 564.500$, $p = 0.000$). There were no significant differences in the Carabelli's trait frequency between the early modern (48.3%) and 21st century (50.0%) male samples (Mann–Whitney $U = 454.500$, $p = 0.568$).

The Kruskal–Wallis test also showed significant differences between females from different populations ($\chi^2 = 17.467$, $p = 0.001$). No significant differences in the Carabelli's trait frequencies were noted between the late antiquity (10.0%) and medieval (13.1%) female samples (Mann–Whitney $U = 764.00$, $p = 0.608$). Significant differences were, however, noted between the late antiquity (10.0%) and early modern (60.0%; Mann–Whitney $U = 46.000$, $p = 0.017$), the late antiquity (10.0%) and 21st century (35.5%; Mann–Whitney $U = 224.000$, $p = 0.030$), the medieval (13.1%) and early modern (60.0%; Mann–Whitney $U = 144.000$, $p = 0.000$), and the medieval (13.1%) and 21st century (35.5%; Mann–Whitney $U = 699.000$, $p = 0.016$) female samples. There were no significant differences between the early modern (60.0%) and 21st century (35.5%) female sample (Mann–Whitney $U = 106.000$, $p = 0.143$).

Chi-squared tests were employed to test the differences in frequencies of the Carabelli's trait between males and females from the same populations. No differences were, however, noted in any of the analyzed temporal groups (late antiquity: males 0.0%, females 10.0%, $\chi^2 = 1.342$, $p = 0.247$; medieval: males 10.3%, females 13.1%, $\chi^2 = 0.0825$, $p = 0.775$; early modern: males 48.3%, females 60.0%, $\chi^2 = 0.495$, $p = 0.482$; 21st century: males 50.0, females 35.5%, $\chi^2 = 3.596$, $p = 0.058$).

There are significant differences in mesiodistal tooth crown diameters between different populations ($F = 6.361$, $p = 0.000$; Table 4). A Scheffe post hoc test was employed to determine which populations differed (Table 5). Significant differences in mesiodistal crown diameters exist between medieval and 21st century populations ($p = 0.008$). The mesiodistal crown diameter was larger in the medieval sample (10.1, SD = 0.7 mm), than in the 21st century sample (9.5, SD = 0.6 mm). Significant differences in mesiodistal crown diameters between the early modern and 21st century populations ($p = 0.004$) were also noted. The mesiodistal crown diameter was larger in the early modern sample;

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Table 5
Differences in mesiodistal tooth crown diameter (mm).

Period A	Period B	Mean difference (A – B)	Standard error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Late antiquity	Medieval	−.25156	.13055	.297	−.6193	.1162
	18th century	−.32811	.14183	.151	−.7277	.0714
	21st century	.11577	.13463	.864	−.2635	.4950
Medieval	Late antiquity	.25156	.13055	.297	−.1162	.6193
	18th century	−.07656	.11453	.930	−.3992	.2461
	21st century	.36732[a]	.10547	.008	.0702	.6644
Early modern	Late antiquity	.32811	.14183	.151	−.0714	.7277
	Medieval	.07656	.11453	.930	−.2461	.3992
	21st century	.44388[a]	.11916	.004	.1082	.7796
21st century	Late antiquity	−.11577	.13463	.864	−.4950	.2635
	Medieval	−.36732[a]	.10547	.008	−.6644	−.0702
	18th century	−.44388[a]	.11916	.004	−.7796	−.1082

Sig. – significance.
[a] Statistically significant (Scheffe post hoc tests).

10.0, SD = 0.6 mm compared to 9.5, SD = 0.6 mm in the 21st century sample. Differences between other samples in mesiodistal tooth crown diameters were not found. Scheffe post hoc tests confirmed the statistically significant differences between early modern and 21st century males ($p = 0.03$). The average mesiodistal tooth crown diameter in early modern males was 10.1, SD = 0.6 mm and in 21st century males 9.7, SD = 0.5 mm. There were no statistically significant differences in mesiodistal tooth crown diameters between females in any of the analyzed temporal groups. Significant differences between males and females within the same population in mesiodistal tooth crown diameters were noted only in the early modern sample and in the 21st century sample (there were no male samples in the late antiquity population). The mesiodistal tooth crown diameter of the 18th century males was 10.1, SD = 0.6 mm and females was 9.6, SD = 0.5 mm ($p = 0.015$). The mesiodistal tooth crown diameter of the 21st century males was 9.7, SD = 0.5 mm and females was 9.3, SD = 0.6 mm ($p = 0.004$).

Results of the buccolingual tooth crown diameter analysis are presented in Table 4. No significant differences between the analyzed populations in buccolingual tooth crown diameters were noted ($F = 0.462$, $p = 0.709$). A Scheffe post hoc test did, however, show a significant difference between early modern and 21st century males ($p = 0.048$). The average buccolingual tooth crown diameter in the early modern males was 11.2, SD = 0.5 mm and in the 21st century males 10.9, SD = 0.5 mm. There were no significant differences in buccolingual tooth crown diameters between females from different populations. A significant difference between males and females in buccolingual tooth crown diameters was recorded in the early modern sample (there were no male samples in the late antiquity population). The buccolingual tooth crown diameter of the early modern males was 11.2, SD = 0.5 mm and of females 10.3, SD = 1.4 mm ($p = 0.001$).

A Spearman correlation was used to test the correlation between the presence of Carabelli's trait and tooth crown dimensions. At the level of the total sample there was a weak positive correlation between tooth dimensions and the presence of Carabelli's trait indicating that the frequency of Carabelli's trait increases with an increase of mesiodistal and buccolingual tooth crown diameters (0.214 for the mesiodistal diameter, and 0.276 for the buccolingual diameter, $p = 0.01$). The correlation between tooth dimensions and the presence of the Carabelli's trait was most pronounced in the late antiquity sample (0.516 for the mesiodistal diameter and 0.662 for the buccolingual diameter, $p = 0.05$). In the medieval sample, the correlation was 0.243 for the mesiodistal diameter and 0.139 for the buccolingual diameter, $p = 0.05$. No correlation was noted between tooth dimensions and the presence of the Carabelli's trait in the early modern sample. In the 21st century sample, the correlation was 0.289 for the mesiodistal diameter and 0.433 for the buccolingual diameter, $p = 0.01$.

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On a repeated random sample (10% of the total sample), performed three weeks after the first analysis, similar findings were observed, confirming intra- and inter-examiner reliability. Cohen Kappa score was 1.00.

Discussion

This analysis was performed on a total of 1287 individuals whose lives covered a long period of time, a period of approximately 1800 years. Analyses like this, focusing on a relatively small geographic area and covering such a long time span are extremely rare (Alt et al., 1998; Hasegawa et al., 2010; Scott and Turner, 1997). An analysis of samples from the same geographic area, but from different historical periods, can provide a unique opportunity to analyze and interpret inter- and intra population changes using the frequency and expression of the Carabelli's trait (Kallay, 1974). The use of standardized methods and calibrated, uniform assessments of the examined trait in this analysis guarantees that the obtained results can be compared with other series. This is an important consideration when planning future research. Unfortunately, although our initial sample was fairly large, the final sample available for examination was significantly reduced. The reduction of the sample size is positively correlated with the age of the series. The older the series was, the greater the reduction of the sample size was with reductions ranging from 62.5% in the early modern series to 88.2% in the late antiquity sample. This reduction can be explained by both antemortem and postmortem factors. Permanent maxillary first molars generally appear in the mouth between the ages of six to seven years. Together with the permanent mandibular first molars, they represent the first permanent molars of the human dentition. As such, during the remainder of an individual's life they are used to chew and grind food. Consequently, when compared with other teeth, these teeth are most likely to be affected with both tooth wear (occlusal and approximal), and dental caries. In the archaeological series tooth wear and dental caries were the most common causes of antemortem tooth loss (Vodanović et al., 2005). Even when these teeth were not lost during life, accumulated tooth wear and potential dental caries compromise the examination of the Carabelli's trait and measurement of tooth dimensions. Additionally, antemortem changes and injury to hard dental tissues caused by tooth wear and caries are frequently linked with dental pulp infections that result in periodontitis, abscesses and subsequent weakening of the surrounding alveolar bone. Thus, even if such a tooth was not lost during an individual's lifetime, it had a very good chance of being lost postmortem due to the thin and easily breakable bone. Of course, the length of time that a skull has spent in the soil after inhumation is also an important factor related to the possibility of postmortem damage (Šlaus, 2006).

The results of this study confirmed our initial hypothesis that the frequencies of Carabelli's trait will differ among people who inhabited the territory of modern Croatia during the examined historical periods. The frequency of the Carabelli's trait increased from the late antiquity period to the 21th century, but statistically significant differences were noted only between older (late antiquity and medieval samples) and younger (early modern and 21st century samples) groups. There were no differences in the frequencies of the Carabelli's trait either between the late antiquity and medieval samples, or between the early modern and the 21st century samples. Employing the available historical data this can be interpreted in the following manner. The newly arrived German, Slavic (and from the 8th century on, Croat populations) assimilated within themselves the remains of the late antique population that had previously inhabited Croatia. Consequently, these populations share similar frequencies of the Carabelli's trait. These data are consistent with bioarchaeological analyses that highlight the complex nature of the late antiquity/early medieval transition in Croatia (Goldstein, 1995; Klaić, 1988; Steindorff, 2006) and tentatively suggest that the late antique population that inhabited Croatia was at least partially assimilated into the newly arrived populations.

In order to identify the characteristic diachronic and evolutionary processes and to explain the origin and evolution of anthropological Dinaric type, Mikić (1982) examined 40 skeletal series containing about 5000 individuals. It has been found that the process of brachycephalisation is a diachronic process which can be traced from the earliest Neolithic period to the recent man. The representatives of the anthropological Slavic type did not exist as independent groups or populations for a long time. Mikić (1982) concluded that the Dinaric type genetically derived from the autochthonous neo-eneolithic Mediterranean anthropological substratum. Gradually, following a series of profound changes that

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included both political and military upheavals – defeat by, and later political union with the Kingdom of Hungary in the 12th century, military intrusions by Venetians (between the 10th and 15th centuries), Mongols (the 13th century), and Ottoman Turks (between the 15th and 18th centuries), and catastrophic epidemiological outbreaks of various infectious diseases (the plague in the 14th, 15th and 17th centuries, syphilis from the 16th century) on these populations, they gradually disappeared, and were supplanted by other populations (for instance the early modern population from Požega). Therefore, the modern inhabitants of Croatia – who are their direct descendants, are similar to these latter populations in terms of Carabelli's trait frequencies (Goldstein, 1995; Klaić, 1988; Steindorff, 2006). This may explain the similarities between the late antiquity and medieval samples, and the early modern and 21st century samples, as well as the significant differences between the two older (late antiquity and medieval), and two younger (early modern and 21st century) samples. Of course, full confirmation of this assumption would require further investigations of larger samples from the late antiquity and early modern periods.

The results of analyses dealing with the frequency of the Carabelli's trait made by other authors are broadly similar to our results (20.4% in the late antiquity, 23.4% in the medieval sample, 51.3% in the early modern sample and 43.1% in the 21st century sample), and more importantly, highlight the importance of using standardized methods when analyzing dental traits. Gauta et al. (2010) have investigated 252 human molars from both archaeological and modern Croatian contexts. The molars from archaeological contexts were from the medieval Bijelo Brdo site (10–13th century). The authors also used the Dahlberg's eight-grade scale. The frequency of the Carabelli's trait was 23.3% in the archeological sample, and 7.1% in the modern sample. The observed grades of expression were generally the first 3 grades from the eight-grade scale. The frequency of the Carabelli's trait in our medieval series (23.4%) is almost identical to their result. Similarly, using the Dahlberg's eight-grade scale, Njemirovskij et al. (1999) examined the frequency of the Carabelli's trait in modern Croatians. The total frequency of the cusp form of the Carabelli's trait in their study was 21% in males and 24% in females. Values (for the cusp form only) recorded in our study are 29.4% for males and 6.5% for females. Although the examined population was the same (modern Croatians) Njemirovskij et al. (1999) examined 506 females and 404 males, and in our study only 31 females and 34 males were examined. It is possible that the sample size difference can explain the differences in the frequency of the cusp form of the Carabelli's trait in these two samples.

In order to determine the characteristics of the teeth of medieval Serbians, Djurić-Srejić et al. (1997) investigated the number, size and shape of teeth in skeletal remains from two medieval necropoleis: Čačak and Žiča. Carabelli's trait occurred in 53.8% of maxillary first molars in Čačak and in 34.8% in Žiča. The frequency of Carabelli's trait in Croatian medieval sample was 23.4% which is significantly lower than in the Serbian medieval contemporaries. Unfortunately the authors did not specify which system of classification for Carabelli's trait they employed. Differences in the methods used for recording Carabelli's trait could be one of the reasons for the difference in the frequency of this trait.

From a similar related geographical area Štamfelj et al. (2006) determined the total frequency, expression and asymmetry of the Carabelli's trait in the permanent dentitions of modern Slovenes, and a medieval skeletal sample from the northeast of Slovenia. A total of 254 dental casts from modern Slovene children was examined in this analysis while the medieval series (which dates to the period between the 10th and 15th centuries), was represented by 94 skeletons. A modification of the method of Alvesalo and associates was used to classify the Carabelli's trait with a five-grade scale (Štamfelj et al., 2006). The trait was expressed on the upper first molars of 79.7% of modern Slovenians and 75.8% of the medieval sample. Like in the previous case, differences in the methods used for recording the Carabelli's trait may be one of the reasons for the differences in frequencies of this trait.

Mavrodisz with his colleagues examined the frequency and degree of expression of a Carabelli tubercle in a modern Hungarian population and compared it with the dentition of skulls dating from the 11th century, the so-called Árpád-era (Mavrodisz et al., 2007). The frequency of Carabelli's cusps was 65% in the modern and 34% in the 11th century populations. This difference was statistically significant ($p < 0.01$). Based on these data the authors concluded that modern Hungarians are a mixture of Europeans and Mongoloids. Their data are in agreement with linguistic evidence that shows that distant Hungarian ancestors belonged to the Finno-Ugrian family of people, whose habitats extended from the Baltic to the middle Urals. Springs Pacelli and Márquez-Grant, 2010 examined dental variants

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(including the Crabelli's trait) in 167 skeletons from the 13th century site Molí de Can Fonoll, located in the southwest of Ibiza, Spain. The Carabelli's trait was observed on 434 permanent upper first molars, and was found on 21 teeth, with a frequency of 4.61%.

The crowns of the maxillary molar teeth tend to have four cusps, referred to as the paracone (mesiobuccal), protocone (mesiolingual), metacone (distobuccal), and hypocone (distolingual). The paracone is the first cusp to appear, whereas the hypocone develops last both in terms of ontogeny and phylogeny. Teeth that develop later in ontogeny tend to be more variable in size, and it has been proposed that they display greater sexual dimorphism due to increasing differences in sex hormone production between males and females (Takahashi et al., 2007). Although the question of sexual dimorphism in the Carabelli's trait expression is still controversial (Hsu et al., 1999; Scott and Turner, 1997), in this study no statistically significant differences in the frequency of the Carabelli's trait between males and females from the same population were found. Dental researchers usually find low levels of sexual dimorphism in human crown dimensions. Although male teeth are only 2–6% larger than female teeth, discriminant function analyses of tooth size can correctly classify the sexes in up to 86% of cases (Scott and Turner, 1997). In our study statistically significant differences between males and females within the same population in the mesiodistal tooth crown diameter were found only in the early modern and 21st century samples. Males from these series exhibited an approximately 4% larger mesiodistal crown diameter than females. Statistically significant differences between males and females in the buccolingual tooth crown diameter were found only in the early modern sample, where males, on average, had an approximately 8% larger buccolingual diameter than females. Although it was expected that an increase in the frequency of the Carabelli's trait would be accompanied by an increase of tooth crown dimensions – and that teeth with Carabelli's trait will, on average, have larger crowns than those without the trait, there was only a weak correlation between tooth crown size and the appearance of the Carabelli's trait.

In the research of Hsu and his colleagues however, the buccolingual diameter of teeth with a Carabelli's trait was larger than that of teeth without the trait. In 2006 Kondo and Townsend published a paper about associations between the Carabelli's trait and cusp areas in human permanent maxillary first molars (Kondo and Townsend, 2006). They tested several hypotheses about overall crown size, individual cusp areas, and expression of Carabelli's cusps in human permanent first molars by obtaining data from dental casts and standardized occlusal photographs of 308 Australians of European descent. Their conclusion was that molars with larger crowns will be more likely to display Carabelli's cusps in genetically predisposed individuals, and that smaller molars will tend to be associated with less developed forms of the Carabelli's trait. Khraisat et al. (2011) found that in modern Jordanians the presence of the Carabelli's trait on the first molar was strongly associated with the increase of the buccolingual, but not the mesiodistal diameter. In our study the correlation between mesiodistal and buccolingual diameters and the appearance of the Carabelli's trait was weak. This could be a population specific relationship between tooth size and the expression of the Carabelli's trait.

Conclusion

The investigation of the Carabelli's trait and tooth crown dimensions in archaeological samples is difficult and complicated, primarily because extensive tooth wear significantly reduces the number of teeth available for examination. The results of our analyses show that the frequency of the Carabelli's trait is significantly greater in the early modern period and in the 21st century than in the late antiquity and medieval periods. These data are consistent with historical evidence of migration and population change in the territory of present-day Croatia throughout the almost 1800 years that this study covers. These results also provide additional evidence for the complex nature of transitional population change. The late antiquity/early medieval transition in Croatia may not have been characterized by a complete replacement of the late antiquity population by the newly arriving Slav and Croat peoples. The similar frequencies of the Carabelli's trait that these populations share suggest that at least some of the previous inhabitants of the Roman provinces of Dalmatia and Pannonia were assimilated into the new influx of people. We found no difference in the frequency of the Carabelli's trait between males and females in our series. A significant correlation between the frequency of the Carabelli's trait

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and tooth crown dimensions was also observed in our series. Tooth dimensions are, on average, larger in males than in females.

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