Dental ancestry estimation in a 1500 years old human skeleton from Slovenia using a new web-based application rASUDAS

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KEYWORDS

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ABSTRACT

The present study was performed on a skeleton excavated from the Late Roman Period necropolis in Ptuj, Eastern Slovenia. Previous anthropological analysis has revealed that the skeleton belongs to a male, who died in his early twenties; however, determination of ancestry was problematic. The skull displays artificial cranial deformation of circular fronto-occipital type and shows some Asiatic anthroposcopic features. However, the inter-orbital projection method of Gill and Hughes has placed him into the Western Eurasian group. The aim of the present investigation was to estimate whether this individual belonged to Western Eurasian or Eastern Asian ancestry group by analyzing his dental morphology. Twenty-one key dental traits for ancestry assessment were scored following the Arizona State University Dental Anthropology System (ASUDAS). Morphology of the roots was scored from cone-beam computed tomography (CBCT) images of the dentition. These scores were input into a web application rASUDAS (beta version) which uses a naive Bayes classifier algorithm to assign an individual to a preselected number (from two to seven) of ancestry groups. The analysis revealed a complex tooth crown morphology (moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, multiple molar enamel extensions) and a simplified external root morphology (27 single-rooted teeth out of 32). Both mandibular second molars and the right mandibular third molar possess a C-shaped root canal. In addition, the mandible bilaterally exhibits accessory mental foramina. In a two-group analysis, the application calculated that the probability of assigning the individual from Ptuj to Eastern Asian ancestry group was close to 1.0. The result is in agreement with archaeological evidence which has indicated that this individual was a Hun warrior from the Migration Period of Europe. This study demonstrates a modern approach to estimating ancestry from dental morphology in bioarcheological and forensic contexts.

INTRODUCTION

In 2000, during the excavation of the Late Roman Period necropolis in Ptuj archaeologists discovered an almost complete skeleton with artificially deformed skull (Ptuj-ŠC 2000, grave no. 50). The skeleton was buried in an abandoned Roman lime-kiln together with the following grave-goods: gilded bronze earring, parts of belt set, coin, arrowhead, and iron sword. Archaeological evidence has indicated that the skeleton belongs to a Hun warrior from the 5th century AD.[1] Macroscopic and anthropometric anthropological analysis was done by one of the present authors.[2,3] The results have indicated that the skeleton undoubtedly belongs to a male who died in his early twenties. No clues have been found about the cause or manner of death. Artificial cranial deformation is of a typical circular fronto-occipital type caused by circular head-binding during early childhood (Fig. 1). Findings of artificially deformed skulls are quite rare. Moreover, this grave is considered to be the first material trace of the Huns on the territory of Slovenia. The skeleton is currently housed in the National Museum of Slovenia.

Osteological ancestry assessment has taken into account the artificial cranial deformation and other physical characteristics. The former is, however, not a reliable indicator of ancestry because it has not been observed only in skulls from Hun cemeteries but also in those from Gothic-Alan (Western Eurasian tribes) cemeteries.[4] It is also possible, that in some regions this practice was picked up by the indigenous Romanised population. In Slovenia, deformed crania have been observed in skulls of the Ostrogoths from three Migration Period archaeological sites: Dravlje near Ljubljana,[5] Lajh in Kranj,[6,7] and Miren.[8]

Analysis of physical characteristics has favored Asiatic origin of this individual. Although the inter-orbital projection method of Gill and Hughes has placed him into the Western Eurasian group, the skull also shows some Asiatic anthroposcopic features: unmarked masculine sexual traits, rounded orbits, broad zygomatic bones with inferior zygomatic projection, wide ramus of mandible, elliptic palate with straight suture, and 6-cusped mandibular first molars.[9] Moreover, the individual had a stature of 163 cm, calculated by a variety of different methods (Manouvrier, Dupertius and Hagen, Shitai, Stevenson, and Trotter and Gleser), and a relatively gracile body composition. The aim of the present investigation was to estimate whether this individual belonged to Western Eurasian or Eastern Asian ancestry group by analyzing his dental morphology.

MATERIALS AND METHODS

The dentition is composed of maxillae with 14 permanent teeth (right premolars lost *post mortem*) and a mandible with 16 permanent teeth. The tooth crowns and roots were examined macroscopically (using naked eye and a magnifying glass) and by using cone-beam computed tomography (CBCT).

The CBCT images of the dentition were taken using a Veraviewepocs 3D R100 machine (Morita, Kyoto, Japan) operating at 90.0 kV and 3.0 mA, with exposure time 9.4 s. The isometric voxel size was 0.160 mm and the slice thickness was 0.960 mm. The scans were produced according to the manufacturer's recommended protocol. A field of view of 88.160 mm × 88.160 mm × 80.640 mm was used. The CBCT images were analysed with dedicated software (i-Dixel One Volume Viewer 2.0.0) in a personal computer, with a 19-inch Hewlett Packard LCD screen with a resolution of 1280 × 1024 pixels in a darkroom. The contrast and brightness of the images were adjusted using the image processing tool in the software to ensure optimal visualization of the roots.

Key dental traits for ancestry assessment (14 crown traits, six root traits, and pegged-reduced-missing maxillary third molars) were scored following the Arizona State University Dental Anthropology System (ASUDAS).[10] Two authors (I. Š. and T. H.) scored the traits independently; in the case of variant scoring, a third, joint evaluation was conducted. Scores were input into the beta version of the rASUDAS application (freely available on the link http://apps.osteomics.com/rASUDAS/) and both historically relevant groups (Western Eurasians and Eastern Asians) were selected for analysis.

RESULTS

Excellent preservation of teeth, absence of dental caries and heavy wear, and the use of CBCT for assessment of root morphology have made it possible to score all dental traits included in the rASUDAS except deflecting wrinkle (Table 1). The dentition is characterized by moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, molar enamel extensions (Fig. 2) and simplified external root morphology (Fig. 3). In a two-group analysis, the application assigned the individual from Ptuj to Eastern Asian and Western Eurasian ancestry groups with posterior probabilities of 99.97% and 0.03%, respectively. Additionally, the CBCT images of the dentition revealed two distinctive anatomical features that are not included in the ASUDAS system. First, both mandibular second molars and the right mandibular third molar possess a C-shaped root canal (Fig. 4A). Second, the mandible bilaterally exhibits accessory mental foramina (Fig. 4B).

Table 1. Dental morphological traits which were used for ancestry estimation, scored according to the ASUDAS system

Dental trait	Tooth	Range	Grade
Winging	UI1	0, 1	0
Shoveling	UI1	0-7	2
Interruption groove	UI2	0, 1	0
Hypocone	UM2	0-5	3
Carabelli's trait	UM1	0-7	0
Metaconule	UM1	0-5	2
Enamel extension	UM1	0-3	2
Multiple lingual cusps	LP2	0-3	2
Groove pattern	LM2	x, +, y	x
Cusp number	LM2	4, 5	5
Entoconulid (Cusp 6)	LM1	0-5	2
Metaconulid (Cusp 7)	LM1	0-4	0
Protostylid	LM1	0-7	1
Deflecting wrinkle	LM1	0-3	-
Tomes's root	LP1	0-7	3
Root number	UP1	1, 2	1
Root number	UM2	1-3	1
Root number	LC	1, 2	1
Root number	LM1	1-3	2
Root number	LM2	1-3	1
Pegged-reduced-missing	UM3	0, 1	0

Figure 1. The skull of the individual from Ptuj displaying artificial cranial deformation.

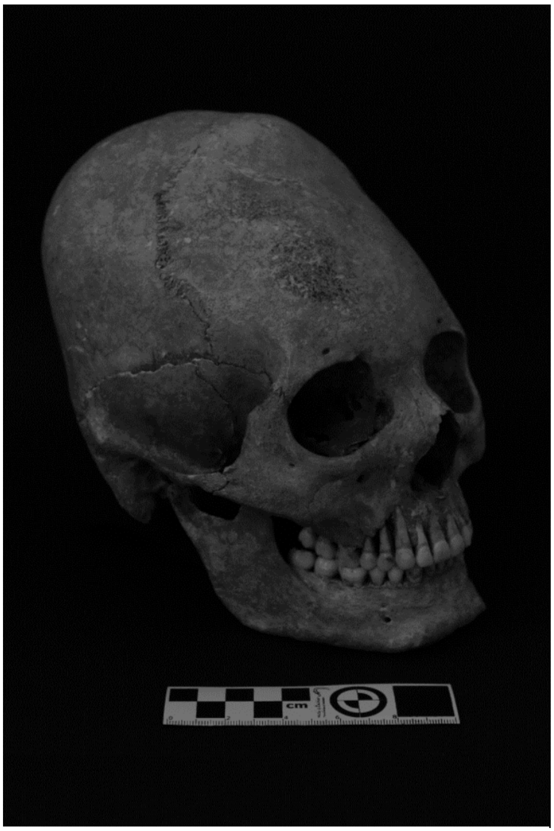


Figure 2. Crown traits of the individual from Ptuj. In maxillary arch (**A**) arrows indicate cusp 5 (metaconule) on occlusal surfaces of both maxillary first molars and the right maxillary second molar. Arrowheads indicate exposed dentine cores of marginal ridges on both maxillary central incisors. In mandibular arch (**B**) arrows indicate cusp 6 (entoconulid) on occlusal surfaces of both mandibular first molars and cusp 5 (hypoconulid) on both mandibular second molars. Lateral view of the jaws in occlusion (**C**). Note enamel extensions (arrows) on buccal side of the right maxillary and mandibular molars.

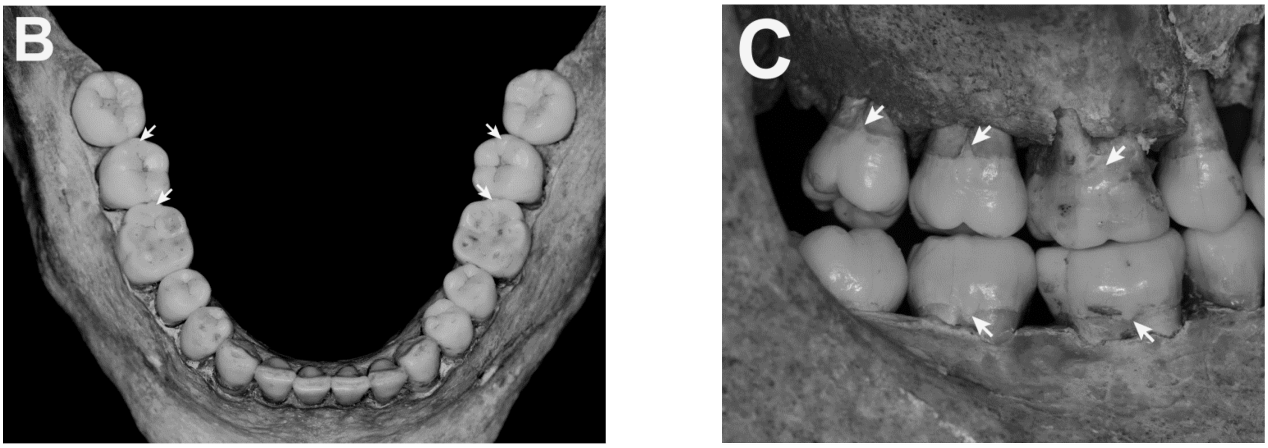


Figure 3. Panoramic CBCT reconstructions showing maxillary (**A**) and mandibular (**B**) teeth. There are five teeth with more than one root: RM1 – right maxillary and mandibular first molars, LM1 – left maxillary and mandibular first molars, LM3 – left mandibular third molar. Arrowheads indicate endosseous canals in the symphysis region of the mandible.

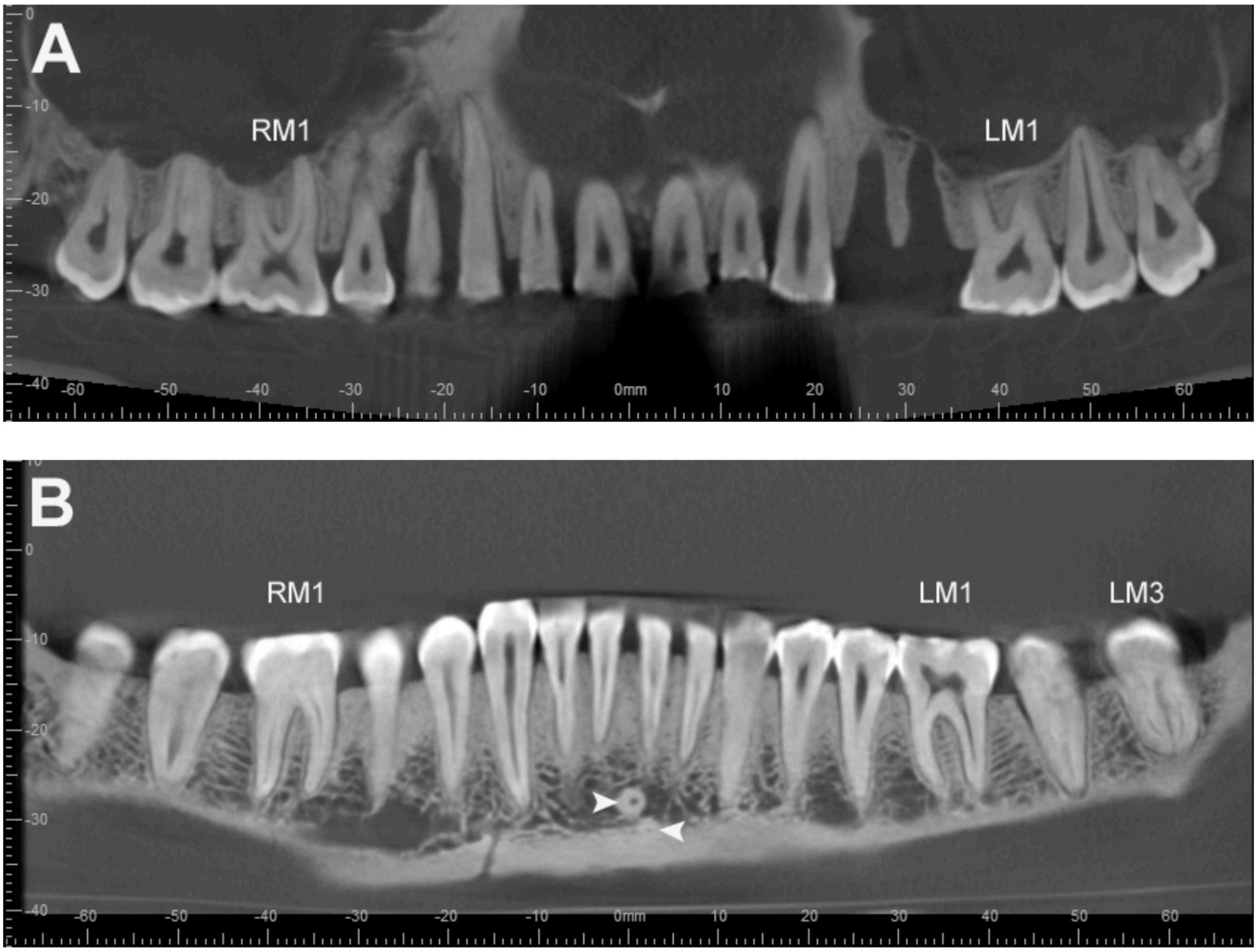
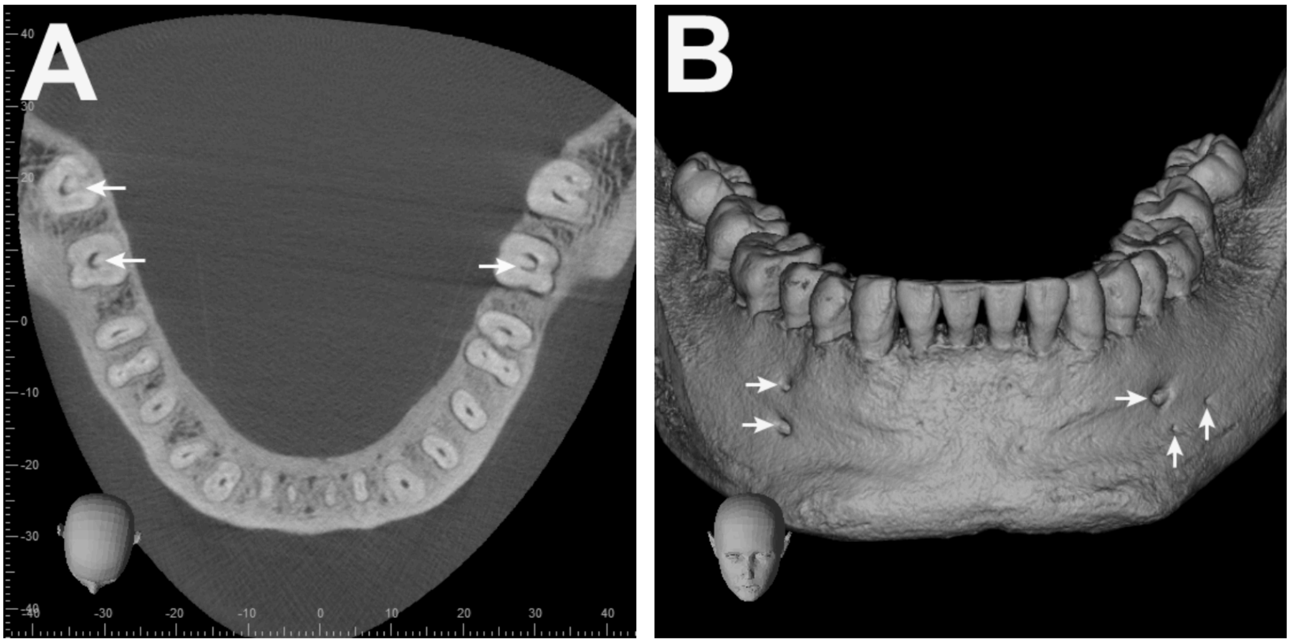


Figure 4. CBCT axial section of the mandible (A) shows a C-shaped canal system (arrows) in pyramidal roots of both mandibular second molars and the right mandibular third molar. 3D reconstructed CBCT image of the mandible (B) displaying multiple mental foramina bilaterally (arrows).



DISCUSSION

The primary role of dental morphology in forensic anthropology is in discerning the ethnic identity or ancestry of an individual when only skeletal-dental remains are available.[11] It has long been acknowledged that morphological dental traits show significant differences in frequency and grade of expression among populations around the world. When viewed as trait constellations, rather than isolated traits, they can provide clues about the individual's ancestry. Nevertheless, this research area has not received much attention until recently. Scott and Turner[12] listed a suite of 12 crown and root traits that could help establish the least and the most likely ethnic affiliation of an individual. Irish[13] developed a method that assigns an individual to one of five groups (East Asian, American Indian, White, Polynesian, and Black) and involves scoring a suite of ten crown traits. The main disadvantage of both methods is that they do not produce associated probabilities. From forensic perspective, such a result could be of little value in court procedures. Edgar[14] presented a method which uses ten crown traits in conjunction with logistic regression to assign individuals to one of four groups (African American, European American, Hispanic from New Mexico, and Hispanic from South Florida). This method produces probabilities but the reference sample is limited for use in the United States.

In 2015, David Navega and João Coelho developed a web application rASUDAS which was based on naive Bayes classification algorithm and generated posterior probability of an individual being assigned to one or more ancestry groups.[15] The application's label refers to the ASUDAS system and R programming language. This alpha-version involved crown and root trait frequencies for 21 ancestry groups taken directly from the appendix of the textbook *The Anthropology of Modern Human Teeth* by Scott and Turner.[12] These trait frequencies are predominantly based on archaeological samples. With further development, the number of ancestry groups was reduced, some traits were added and some removed, some traits were divided into more categories than presence or absence, and the reference sample was expanded with new and improved trait frequencies. This resulted in a beta-version of rASUDAS which involves 21 traits (14 crown traits, six root traits, and pegged-reduced-missing maxillary third molars) and seven ancestry groups (American Arctic and Northeast Asia, Australo-Melanesia and Micronesia, East Asia, American Indian, Southeast Asia and Polynesia, Sub-Saharan Africa, Western Eurasia). The authors tested the application on 150 individuals (with data for a minimum of 12 traits) from the reference sample. The overall accuracy of the application in predicting group assignment was 51.8%; however, it improved with a decrease in the number of ancestry groups to four (66.7%) or three (Western Eurasia, Sub-Saharan Africa, and East Asia; 72.7%).[15] Classification accuracy attained its highest level in pairwise comparisons: East Asians and East Asian–derived populations (Northeast Asian, Arctic, and Native American) could be distinguished from Western Eurasians and Sub-Saharan Africans roughly 90% of the time.[15]

The present study demonstrates a modern approach to estimating ancestry from dental morphology in bioarcheological and forensic contexts. A CBCT was used to evaluate root morphology without damaging the skeletal material. In the presented case, the skeleton under consideration was excavated from the archaeological site in Ptuj, Slovenia and according to the results of archaeological and osteological analyses belongs to a young Hun warrior from the Migration Period of Europe.[1] Literature data indicate that the Huns were a heterogeneous mixture of nomadic tribes who had their origins in China and Inner Mongolia.[16] Scott et al.[17] pointed out that North and East Asians and Native Americans, in contrast to Western Eurasians, exhibit rich crown morphology coupled with the relatively common occurrence of unseparated roots. The dentition of the individual from Ptuj matches to this pattern: the tooth crown morphology is complex (moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, molar enamel extensions); however, the external root morphology is simplified (27 single-rooted teeth out of 32). As pointed out by Scott and Turner,[12] moderate incisor shoveling, molar enamel extensions, 1-rooted maxillary first premolars, and cusp 6 on mandibular first molars would form a set of dental traits rarely found in a Western Eurasian. Posterior probabilities calculated with rASUDAS do not allow any doubt about his Eastern Asian descent (Eastern Asia 99.97%, Western Eurasia 0.03%). Moreover, both mandibular second molars of this individual possess a C-shaped root canal configuration, which is according to a global survey of recent populations significantly more prevalent in China than in any other geographic region.[18] In addition, his mandible bilaterally exhibits accessory mental foramina, which are according to another global survey of recent populations more prevalent in East and Northeast Asians (15%-25%) than in Europeans (5%-15%).[19]The estimation of ancestry is an important part of individual's biological profile in bioarcheological and forensic investigations. There are many methods to estimate ancestry but the most common are based on craniometrics and cranial morphoscopics. Recently, several methods employing dental morphology proved effective at differentiating ancestral groups.[13-15] When a cranium is damaged (e.g. trauma, burning, weathering, soil acidity) or artificially deformed, as in the presented case, the analysis of dental morphology can be the primary method for ancestry estimation. In the present study, we decided to use the rASUDAS application for several reasons. First, the beta version of rASUDAS has a reference sample that is primarily archaeological (approximately 30,000 individuals from seven biogeographic regions with a time span of several thousand years) which makes it particularly useful in bioarcheological contexts. Secondly, the application employs crown and root traits and therefore has the ability to incorporate more information from the dentition compared to methods which employ only crown traits. Nevertheless, this potential can be fully exploited only when the application is used in conjunction with 3D imaging technologies like CBCT which enable evaluation of root morphology without damaging the skeletal material. Thirdly, the application calculates probabilities and has been validated on archaeological material. Moreover, the application is user-friendly an accessible online. Despite these advantages, it should be emphasized that the current version of rASUDAS should not be used as the sole method for ancestry estimation in modern forensic casework; it is still necessary to validate it using forensic cases and expand its reference sample with data on living populations.[15]

To conclude, the dentition of the skeleton from Ptuj is characterized by a suite of specific traits which correspond to the Sinodont dental complex found in East Asia and East Asian–derived populations. This is in agreement with archaeological evidence which indicates that this individual was a Hun warrior from the Migration Period of Europe.

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