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Uncovering the Past: DNA Analysis of Skeletal Remains from the Medieval Bosnian City of Bobovac

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Abstract

Numerous archaeological sites in Bosnia and Herzegovina represent a historical heritage and testify to the rich cultural, social, and political life of medieval Bosnia. Bobovac, the capital of the Bosnian Kingdom after King Tvrtko I's coronation in 1377, featured a royal complex with a palace, church, and fortification. Recent molecular-genetic research on skeletal remains from Bobovac aims to uncover medieval ancestors' customs and genetic origins. Fifteen well-preserved teeth samples from Bobovac were processed. STR amplification employed PowerPlex® Fusion and Investigator® 24plex QS Kits, with Y-STR profiles generated using the PowerPlex® Y23 System. Fourteen partial autosomal STR profiles were obtained, enabling sex determination and kinship analysis. STR amplification success varied due to ancient DNA degradation, with larger loci showing lower amplification rates. Kinship analysis confirmed appropriate marker selection, demonstrating high reliability for determining close relationships. Integrating aDNA analysis with archaeological research enhances our understanding of historical populations, connecting archaeology and forensic genetics to contribute to the broader narrative of human history.

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Introduction

The medieval era in Bosnia was rich in political, cultural, and social events. During this time, Bosnia became the center of significant historical changes, including the rise of the Bosnian Church and the development of a unique cultural identity. Numerous archaeological findings from this period, such as fortifications, churches, and burial tombstones, testify to the complex social life and dynamics of the era. Bobovac, a medieval Bosnian town, is often regarded as the capital of the Bosnian Kingdom. The court is initially referenced in historical records from 1356, and following King Tvrtko I's coronation in 1377, it became the capital city safeguarding the crown and royal treasury. The construction of Bobovac likely commenced under Ban Stjepan II Kotromanić during the early 14th century. Strategically located and seamlessly blended with its natural environment, the royal complex featured two distinct palaces. The site also housed a larger unfinished church, a Franciscan monastery, the royal burial chapel, and various supporting and fortification structures. Archaeological investigations conducted between 1959 and 1969 unearthed numerous important details about this extensive architectural complex and explored the tombs of Bosnian rulers. These findings provide valuable insights into the lifestyle of the highest nobility and the formidable strength of the medieval Bosnian kingdom, which was well-integrated into the broader medieval European court culture (Anđelić 1984; Bujak 2021).

Skeletal remains discovered within the tombs have been the subject of intensive molecular-genetic research since 2015. This research aims to gain a more comprehensive understanding of our medieval ancestors, their customs, and their

genetic origins. The success in extracting usable ancient DNA (aDNA) profiles from archaeological skeletal remains is influenced by various interconnected factors, including environmental conditions, bone sample type, sample age, and laboratory methods (Hagelberg & Clegg 1991; Foran 2006; Hofreiter et al. 2001; Brown & Brown 2011). Research suggests that although the age of bones does not significantly affect bone tissue preservation, external factors such as bone density, structure, and degenerative processes are crucial (Hagelberg et al. 1991; Foran 2006). These external factors also influence the preservation of aDNA. The type of bone used is significant for aDNA isolation quality. Dense and large bones such as the femur are most effective due to their higher density and quantity of bone material (Mulligan 2005). Additionally, studies indicate that teeth are particularly effective for DNA isolation. The hard enamel structure of teeth slows microbial decomposition, making teeth a suitable and often more successful source for DNA isolation (Turner-Walker 2008; Adler et al. 2011). The quantity of isolated aDNA also depends on the particle size of the bone powder and the method used to process the bone. Research comparing different bone material preparation methods has shown that mill-ground samples producing a fine powder yield higher DNA concentrations (Zupanič Pajnič 2020). The investigation of aDNA from archaeological samples faces several challenges due to the degradation of genetic material over centuries. Factors such as the degree of bone preservation, the extent of DNA degradation, and the presence of polymerase chain reaction (PCR) inhibitors complicate the extraction and analysis of viable aDNA. Consequently, choosing appropriate molecular markers is critical to overcoming these limitations. There are two primary methodologies

employed in aDNA analysis: sequencing and profiling. Sequencing aDNA provides comprehensive genetic information, allowing for in-depth reconstruction of genetic lineages and population structures. This approach, although highly informative, is often cost-prohibitive and technically demanding due to the complexity of the procedures involved.

Alternatively, aDNA profiling focuses on specific genetic variations and is particularly adept at detecting endogenous DNA, inferring kinship relationships, and determining the sex of individuals. This method, less resource-intensive than sequencing, is favored in many bioarchaeological studies for its simplicity and effectiveness in addressing common research questions related to gender and kinship. Short Tandem Repeat (STR) markers are frequently utilized in aDNA profiling due to their high polymorphism and reproducibility even in degraded samples. This approach has been essential in identifying historical figures and verifying kinship in archaeological contexts. Notable applications of STR markers include the identification of the Romanov family (Gill et al. 1994), Saint Lukas the Evangelist (Vernesi et al. 2001), and Saint Brigitta of Sweden (Nilsson et al. 2010). Furthermore, STR analysis has been employed to ascertain kinship among skeletal remains from various archaeological sites (Baca et al. 2012; Lacan et al. 2011; Larmuseau & Bodner 2018; Džehverović et al. 2021; Alterauge et al. 2021). In addition to kinship studies, aDNA profiling has been extensively used for sex determination of archaeological remains, which is crucial for constructing demographic profiles of past populations. Significant studies in this area include those by Daskalaki et al. (2011) and Skoglund et al. (2013). The integration of aDNA

analysis with archaeological research not only enriches our understanding of historical populations but also bridges the fields of archaeology and forensic genetics. By utilizing refined techniques developed for degraded and low-copy DNA analysis, researchers can extract meaningful genetic information from ancient remains, thereby contributing to the broader narrative of human history and evolution.

Materials and methods

From the Bobovac site, a total of 15 archaeological skeletal remains were collected and analyzed. To remove surface contaminants, 15 well-preserved teeth samples were treated as follows: bathed in 5% Na-hypochlorite (Sigma Aldrich, USA) for 10 minutes, rinsed three times with distilled water, soaked in absolute ethanol (Sigma Aldrich, USA) for five minutes, and then dried on a clean paper towel for five to six days. Samples were exposed to UV light for three minutes before grinding to fine dental powder using a sterilized IKA Tube mill (IKA®-Werke GmbH & Co. KG, Germany).

DNA extraction

To avoid cross-contamination and contamination with modern DNA, the DNA extraction procedure was conducted in a pre-PCR room irradiated with ultraviolet light for 72 hours (Willerslev & Cooper, 2005). Approximately 0.5 g of dental powder per sample was placed in sterile 50 mL polypropylene tubes and preceded by decalcification with 0.5 M EDTA (Sigma Aldrich, USA) for seven days. Each extraction process was preceded by checking for laboratory-based contamination by leaving an open 1.5 mL tube for two to three hours, which served as a negative

control. Two researchers at the Institute for Genetic Engineering and Biotechnology performed DNA extraction for each sample in temporally separated duplicates, following protocols established by MacHugh et al. (2000).

DNA extraction followed an optimized phenol-chloroform-isoamyl alcohol protocol, which included prolonged incubation for 48 hours. We purified the extracted DNA by treating it with absolute ethanol and washing it with 70% ethanol. The final purification step involved using Amicon Ultra 0.5 mL filter columns (Merck Millipore, Carrigtwohill, Co. Cork, IRL). The DNA was then eluted in 50 µL of TE buffer (10 mM Tris, 1 mM EDTA). The concentration of DNA, presence of inhibitors, and level of DNA degradation were assessed using the Quantifiler Human DNA Quantification Kit (Applied Biosystems, Foster City, California, USA). Real-time PCR amplification was conducted with the Applied Biosystems 7300 Real-Time PCR System.

STR amplification

For STR amplification, two kits were used: PowerPlex® Fusion System (Promega Corp., USA) and Investigator® 24plex QS Kit (Qiagen, Germany). Y-STR profiles were generated using the PowerPlex® Y23 System (Promega Corp., USA). Amplifications were carried out in the GeneAmp™ PCR System 9700 (Applied Biosystems, USA). Amplification products were separated by capillary electrophoresis using the 3500 Genetic Analyzer (Applied Biosystems, USA). Loading mixes, including HiDi™ Formamide and internal lane standards, varied by kit. Electrophoresis used a GS STR POP4 (1 mL) A module. Data were analyzed with 3500 Data Collection Software and GeneMapper™ ID-X

Software v.1.6, using a 50 RFU threshold for allele calling. An elimination database was created to identify potential contamination by recording the genetic profiles of all personnel handling the samples.

Statistical Analysis and Y-Haplogroup Prediction

To evaluate kinship between individuals, the Likelihood Ratio (LR), Overall Likelihood Ratio (OLR), and Kinship Probability (KP) were calculated using FamLink v.2.3 software (<https://www.famlink.se/>). Allele frequencies from the contemporary B&H population were used for calculating LR values.

Y-haplogroup predictions were made using Whit Athey's Haplogroup Predictor (available at: <http://www.hprg.com/hapest5/>) and NEVGEN (available at: <https://www.nevgen.org/>).

Results and Discussion

Although 15 samples were collected, the amplification of STR loci was successful for 14 of them. Consequently, 14 partial autosomal STR profiles were successfully obtained. This enabled further analyses, including sex determination and the establishment of various degrees of kinship such as parent/child, grandparent/grandchild, siblings, half-siblings, and first cousins. Based on the analysis, 10 individuals were identified as male, 3 as female, and for one individual, the sex could not be determined due to the failure of amelogenin amplification. The number of amplified STR loci per sample ranged from eight to 22 successfully amplified loci. The success of amplifying STR loci in the analyzed samples varied, which is expected for ancient specimens. Degraded biological samples undergo DNA

fragmentation due to various factors (Turner-Walker, 2008). Fragmented DNA molecules amplify with varying success during PCR reactions, resulting in differential amplification rates for some STR loci. Specifically, larger loci (greater than 300 bp) are more prone to fragmentation, directly impacting amplification success (Ham et al., 2016). In our study, the Penta E locus exhibited the lowest amplification success, consistent with its size range of 379 to 484 bp (Butler et al., 2022). Conversely, various studies have demonstrated that the Penta E locus is a highly polymorphic marker with a low mutation rate (Ocampos et al., 2009; Lai et al., 2015), underscoring its importance for assessing heterogeneity. The TH01 locus, with a size range of 179 to 203 bp (Butler et al., 2022), was amplified in the highest number of samples. Despite having the fewest detected allelic variants, TH01 is crucial for individualization and kinship determination. The lower number of detected allelic variants at these loci is likely due to weaker amplification resulting from small and degraded DNA quantities. Although SNP tests and systems are more sensitive than most STR kits due to the size of the PCR products, they cannot reliably analyze extremely small DNA quantities (e.g., less than 250 pg) when assessing a large number of loci. The kinship results and the high percentage of calculations confirm the appropriate selection of molecular markers. Although single nucleotide polymorphism (SNP) and small biallelic insertions/deletions (InDel) are commonly used in similar studies worldwide (Dixon et al., 2005; Westen et al., 2009; Pereira et al., 2009; Fondevila et al., 2008), STR markers are frequently used for analyzing kinship relationships of historically significant individuals (Gill et al., 1994, 2020; Katzenberg et al., 2005; Coble et al., 2009;

Džehverović et al., 2021). These markers are highly precise in determining close kinship relationships, and the analysis provides unequivocal results with high statistical reliability (Butler, 2005).

At this archaeological site, kinship was determined for two out of 14 individuals. Despite the samples originating from two different positions within the same site—distinct due to their specific historical contexts (with members of the royal family interred in the burial chapels) — a comparative analysis of samples from both positions was conducted. Comparative analysis of samples A11-03-17 and A13-03-17 showed matching allelic variants at 10 out of the 21 loci analyzed. Statistical analysis suggested that these samples likely originate from individuals who are either brothers or siblings (brother and sister) with a probability of 99.98101% (LR 5267) as depicted in Table 1. Due to the failed amplification of amelogenin in sample A11-03-17, it remains uncertain whether the relationship is between two sisters, a brother and sister, or two brothers. In the comparison of samples A5-03-17 and A4-07-19, common allelic variants were identified at nine out of the 21 loci analyzed. Statistical analysis indicated that a brother/sister relationship could not be excluded, as the LR detected a borderline value of 10 (Table 2).

The recalculated probability of kinship was 91.478%. Kinship analysis is one of many pieces that form a complete picture of the life and customs of people who inhabited these areas in the distant past. Therefore, DNA technologies are of exceptional importance in archaeogenetics, primarily because they alone can provide accurate and unequivocal results regarding kinship relationships. For instance, at the Kopošići site, DNA analysis revealed a father/son relationship

Table 1. Results of statistical kinship analysis between A11-03-17 and A13-03-17 (siblings)

STR loci	A11-03-17	A13-03-17	Common allele	LR		
D3S1358	15 18	15 15	15	1.159		
D1S1656	11 12	12 12	12	1.880		
D2S441	11 14	11 11	11	1.117		
D10S1248	13 14	13 15	13	0.727		
D13S317	11 11	12 12	-	0.25		
Penta E	-	-	-	-		
D16S539	8 10	8 10	8 10	144.522		
D18S51	15 19	15 19	15 19	29.772		
D2S1338	16 16	-	-	-		
CSF1PO	12 12	10 10	-	0.25		
Penta D	10 12	-	-	-		
TH01	6 9.3	6 9.3	6 9.3	2.915		
vWA	16 18	-	-	-		
D21S11	28 28	-	-	-		
D7S820	9 11	10 10	-	0.25		
D5S818	9 10	-	-	-		
TPOX	11	8 11	11	1.844		
D8S1179	10 13	-	-	-		
D12S391	20 22	19.3 -	-	-		
D19S433	13 14	14 14	14	0.947		
FGA	24 25	-	-	-		
D22S1045	15	15 -	15	3.567	OLR	KP(%)
AMEL	-	X -	-	////	5267	99.98101

between two skeletal remains, subsequently confirming the identity of the medieval Bosnian nobleman, Prince Mirko Radojević (Džehverović et al., 2021).

Similarly, at the Divičani site, DNA analysis revealed first and second-degree kinship among three skeletal remains, a highly exclusive finding given that no material or written archaeological source indicated such kinship. Analysis of autosomal STR markers was the method of choice for determining kinship among potential relatives

of Hungarian King Bela III (Olasz et al., 2019), providing unequivocal data on the kinship relationships of the analyzed skeletal remains. For the 10 male individuals, Y-STR locus analysis was successful in five samples, sufficient for Y haplogroup prediction. The detected haplogroups were I2a in three samples and R1a in two samples. The I2a haplogroup is prevalent in Europe, especially in the Western Balkans, Sardinia, and parts of Eastern Europe. It dates back to the Upper Paleolithic era (20 000-25 000 years ago) and is

Table 2. Results of statistical kinship analysis between A5-03-17 and A4-07-19 (siblings)

STR LOCI	A5-03-17	A4-07-19	Common alleles	LR		
D3S1358	15 16	18 18	-	0.25		
D1S1656	14 16	12 17.3	-	0.25		
D2S441	11 15	11 11	11	1.117		
D10S1248	14 15	14 15	14 15	3.308		
D13S317	9 9	9 12	9	3.043		
Penta E	-	-	-	-		
D16S539	9 13	9 11	9	1.545		
D18S51	15 18	15 18	15 18	18.108		
D2S1338	18 20	-	-	-		
CSF1PO	10 10	10 10	10	5.027		
Penta D	9 9	-	-	-		
TH01	9 -	6 6	-	0.25		
vWa	17	17 19	17	1.213		
D21S11	30 32.2	30 32.2	30 32.2	7.660		
D7S820	8 10	12	-	-		
D5S818	12 12	12 13	12	0.907		
TPOX	8 8	11 11	-	0.25		
D8S1179	11 12	14	-	-		
D12S391	19.3 22	20 24	-	0.25		
D19S433	13 14	16 17	-	0.25		
FGA	26	21 22	-	0.25		
D22S1045	-	15 16	-	-	OLR	KP(%)
AMEL	X Y	X X	X	/////	10	91.4788

linked to Mesolithic hunter-gatherer populations. Significant subclades include I2a1 and I2a2, with distinct regional distributions. Recent genetic studies highlight its role in the genetic landscape of prehistoric Europe. The R1a haplogroup is widely distributed across Eurasia, notably in Eastern Europe, Central Asia, and South Asia.

Conclusion

The molecular-genetic analysis of skeletal remains from the medieval Bosnian site of Bobovac has

provided valuable insights into the genetic makeup, kinship structures, and historical significance of the population. This study demonstrates the efficacy of STR markers in aDNA analysis, particularly for kinship determination and historical investigations. Despite challenges related to DNA degradation, the methods employed ensured reliable and reproducible results, contributing significantly to our understanding of medieval Bosnian society. Integrating genetic data with archaeological findings has enhanced the historical narrative,

showcasing the potential of aDNA research in uncovering the past.

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Authors' contributions

Mirela Džehverović:

Investigation, Methodology, Writing - original draft, Writing - editing.

Amela Pilav:

Investigation, Methodology, Project administration.

Belma Jusić:

Investigation, Writing - review & editing.

Edin Bujak:

Resources.

Naris Pojskić:

Resources, Software.

Jasmina Čakar:

Conceptualization, Supervision, Validation.

Conflict of interest

Authors declare no conflict of interest.

References

- Adler CJ, Haak W, Donlon D, Cooper A (2011) Survival and recovery of DNA from ancient teeth and bones. *J Archaeol Sci* 38: 956-964.
- Alterauge A, Lösch S, Sulzer A, Gysi M, Haas C (2021) Beyond simple kinship and identification: aDNA analyses from a 17th-19th century crypt in Germany. *Forensic Sci Int Genet* 53: 102498.
- Anđelić P (1984) Doba srednjovjekovne Bosanske države. Sarajevo: Kulturna istorija Bosne i Hercegovine: 435-436.
- Athey TW (2005): Haplogroup prediction from Y-STR values using an allele-frequency approach. *Journal of Genetic Genealogy* 1: 1-7.
- Baca M, Doan K, Sobczyk M, Stankovic A, Weglenski P (2012) Ancient DNA reveals kinship burial patterns of a pre-Columbian Andean community. *BMC Genet* 13: 30.
- Brown TA, Brown KA (2011): *Biomolecular Archaeology: An Introduction*. Wiley Blackwell, Oxford, UK.
- Bujak E (2021) *Kasnosrednjovjekovna groblja srednje Bosne (doktorska disertacija)*, Filozofski fakultet-Odsjek za arheologiju, Sveučilište u Zagrebu, Zagreb.
- Butler JM (2005) *Forensic DNA Typing (2nd Edition)*. Academic Press, Massachusetts, USA.
- Butler JM, Vallone PM, Gettings KB, Borsuk LA, Ruitberg CM, Reeder DJ (2022) NIST Short Tandem Repeat DNA Internet Database, National Institute of Standards and Technology.
- Coble MD, Loreille OM, Wadhams, MJ, Edson SM, Maynard K, Meyer CE, Niederstatter H, Berger C, Berger B, Falsetti AB, Gill P, Parson W, Finelli LN (2009) Mystery Solved: The Identification of the Two Missing Romanov Children Using DNA Analysis. *PLoS ONE* 4: 4838.
- Daskalaki E, Anderung C, Humphrey L, Götherström A (2011) Further developments in molecular sex assignment: a blind test of 18th and 19th century human skeletons. *J Archaeol Sci* 38: 1326-1330.
- Dixon LA, Murray CM, Archer E, Dobbins A E, Koumi P, Gill P (2005) Validation of a 21 locus autosomal SNP multiplex for forensic identification purposes. *Forensic Sci Int* 154: 62-77.
- Džehverović M, Čakar J, Bujak E, Pilav A, Ramić J, Kalajdžić A, Pojskić N (2021) DNA analysis of skeletal remains of an important historical figure from the period of medieval Bosnia. *Int J Osteoarchaeol* 31: 1-9.
- Fondevila M, Phillips C, Naveran N, Fernandez L, Cerezo M, Salas A, Carracedo A, Lareu M V (2008) Case report: Identification of skeletal remains using short amplicon

- marker analysis of severely degraded DNA extracted from a decomposed and charred femur. *Forensic Sci Int Genet* 2: 212-218.
- Foran D (2006) Increasing the Predictability and Success Rate of Skeletal Evidence Typing: Using Physical Characteristics of Bone as a Metric for DNA Quality and Quantity. National Institute of Justice, Rockville, USA.
- Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K (1996) Identification of the remains of the Romanov family by DNA analysis. *Nat Genet* 6: 130-135.
- Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K (1994) Identification of the remains of the Romanov family by DNA analysis. *Nat Genet* 6: 130-135.
- Hagelberg E, Clegg JB (1991) Isolation and Characterization of DNA from Archaeological Bone. *Proc Royal Soc B* 244: 45-50.
- Ham SK, Kim SY, Seo BY, Woo KM, Lee SH, Choi CY (2016) Differential pre-amplification of STR loci for fragmented forensic DNA profiling. *Electrophoresis* 37: 3002-3009.
- Hofreiter M, Serre D, Poinar HN, Kuch M, Pääbo S (2001) Ancient DNA. *Nat Rev Genet* 2: 353-359.
- Katzenberg MA, Oetelaar G, Oetelaar I, Fitzgerald C, Yang D, Saunders SR (2005) Identification of historical human skeletal remains: A case study using skeletal and dental age, history and DNA. *Int J Osteoarchaeol* 15: 61-72.
- Lacan M, Keyser C, Ricaut F, Brucato N, Duranthon F, Guilaine J, Ludes B (2011) Ancient DNA reveals male diffusion through the Neolithic Mediterranean route. *Proc Natl Acad Sci USA* 108: 9788-9791.
- Lai L, Shen X, Han L, Chen D, Hu J (2015) Genetic study of the Penta E locus and identification of rare alleles. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 32: 657-660.
- Lamuseau MHD, Bodner M (2018) The biological relevance of a medieval king's DNA. *Biochem Soc Trans* 46: 1013-1020.
- Life Technologies Corporation (2014) Quantifiler® Duo DNA Quantification Kit (User Guide, Rev. 2014). Carlsbad, California, USA. (Cat. No. 4387746).
- Mulligan CJ (2005) Isolation and Analysis of DNA from Archaeological, Clinical and Natural History Specimens. *Meth Enzymol* 395: 87-103.
- Nielsen-Marsh CM, Smith CI, Jans MM. E, Nord A, Kars H, Collins MJ (2007) Bone diagenesis in the European Holocene II: taphonomic and environmental considerations. *J Archaeol Sci* 34: 1523-1531.
- Ocampos M, Fernandes RC, Latorre AF, da Silva CM, Komdorfer FP, Giamarusti Ade C, Menezes ME (2009) 15 STR loci frequencies in the population from Santa Catarina, Southern Brazil. *Forensic Sci Int Genet* 3: 129-131.
- Olasz J, Seidenberg V, Hummel S, György S Z, Melegh SB, Kasler M (2019) DNA profiling of Hungarian king Béla III and other skeletal remains originating from the Royal Basilica of Székesfehérvár. *Archaeol Anthropol Sci* 11: 1345-1357.
- Pereira R, Phillips C, Alves C, Amorim A, Carracedo A, Gusmao L (2009) Insertion/deletion polymorphisms: A multiplex assay and forensic applications. *Forensic Sci Int Genet Suppl Ser* 2: 513-515.
- Promega Corp (2020) PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers (Technical Manual, Rev. 7/20). Madison, WI, USA. (Cat. No. TMD0039).
- Promega Corp (2021) PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers (Technical Manual, Rev. 4/21). Madison, WI, USA. (Cat. No. TMD035).
- Qiagen (2021) Investigator® 24plex QS Handbook (Rev. 02/2021). Hilden, Germany. (Cat. No. HB-1860-009).
- Skoglund P, Storå J, Götherström A, Jakobsson M (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J Archaeol Sci* 40: 4477-4482.

- Turner-Walker G (2008) The Chemical and Microbial Degradation of Bones and Teeth. In: Pinhasi R, Mays S (Eds.). *Advances in Human Palaeopathology*. John Wiley & Sons, West Sussex, UK.
- Vemesi C, Di Benedetto G, Caramelli D, Secchieri E, Simoni L, Katti E, Malaspina P, Novelletto A, Marin VT, Barbujani G (2001) Genetic characterization of the body attributed to the evangelist Luke. *Proc Natl Acad Sci USA* 98: 13460-13463.
- Westen AA, Matai AS, Laros J F J, Meiland H C, Jasper M, de Leeuw WJ F, de Knijff P, Sijen T (2009) Tri-allelic SNP markers enable analysis of mixed and degraded DNA samples. *Forensic Sci Int Genet* 3: 233-241.
- Zupanič Pajnič I (2020) Molecular genetic aspects of ancient DNA analyses. *Slovenian Medical Journal* 89: 171-189.