



## **PROJECT "GENDER, SEX AND STATUS IN IRON AGE LATVIA (7TH – 12TH CENTURIES AD)"**

**Project No.:** lzp-2018/1-0395

**Period:** 31 August 2018 – 31 August 2021

**Project costs:** 300 000 EUR

**Principal investigator:** Dr. hist. Guntis Gerhards (University of Latvia)

**Collaboration:** Dr. biol. Renāte Ranka (Latvian Biomedical Research and Study Centre)

The aim of the project is to study burial customs in Iron Age populations from Latvia (7th – 12th centuries AD) in the light of gender, sex and status, employing archaeological materials and bioarchaeological methods, as well as dietary (carbon and nitrogen) isotope, and ancient DNA analyses. To achieve the aim, archaeological cemetery populations with reasonably good preservation of skeletal remains from different regions of Latvia will be analysed. An innovative approach will be employed during the project by using biomolecular (ancient DNA) and biochemical (isotope) methods for the study of burial customs. The ancient DNA data will be used to determine biological sex and kinship of the individuals, while isotope analysis will reveal information about differences in diet and thus, access to resources between different population, sex and age groups. The project will generate considerable amount of unique data, as well as reconsider the existing preconceptions about gender, sex and status in Middle and Late Iron Age populations in Latvia. It is the first interdisciplinary project of this scale in the archaeology of Latvia. The results of the research will be presented in international conferences and published in peer-reviewed media, including in Open Access. Using internationally approved research methods and state of the art technologies will further the development of archaeological research in Latvia. The results of the project will be of importance not only to archaeologists, but also researchers in other fields both in Latvia and abroad, promoting the understanding of similarities and differences in the history of Baltic and European countries.

The Project is in line with one of the approved Latvia's research priorities for the years 2018-2021, "Latvia's statehood, language and values, culture and art", which encourages the use of new methods and technologies in studying archaeological heritage of Latvia. The European Convention on the Protection of the Archaeological Heritage (1992), which is also ratified by the Republic of Latvia (2003), states that archaeological heritage is the source of common European memory, as well as a valuable part of cultural heritage, the study of which helps to understand the history of humanity.

## **Project activities by period:**

### ***August 31, 2018 – December 31, 2018***

1. Data review was started on the middle and late Latvian Iron Age burial grounds (7th – 12th centuries AD), including archaeological reports, published data, etc., at the Repository of Archaeological Material, Institute of Latvian History, University of Latvia (LULVI) and the repository of archaeological material, Museum of National History. Inclusion criteria for the burial grounds to be studied in this project, were also set.
2. Protocols were set for determination of age and sex and other parameters, as well as palaeopathological analysis, for anthropological material from archaeological excavations. Review of skeletal material available at the Repository of Bioarchaeological Material, LULVI, was also started.
3. The collaboration partner, Latvian Biomedical Research and Study Centre (BMC) identified the strategy for the extraction and analysis of ancient DNA (aDNA) for the project, and prepared mitochondrial DNA (mtDNA) libraries of people involved in handling project samples, to be used for checking contamination from most likely sources.
4. During the public event, European Researchers' Night 2018, members of the public interested in the work of the BMC were given an insight into the opportunities of aDNA analysis for archaeological material.
5. The project's management committee was formed from the LULVI and BMC representatives, and the leaders of project activities.

*Date information prepared: 30.12.2018*

### ***January 1, 2019 – April 30, 2019***

1. Data review was continued on the middle and late Latvian Iron Age burial grounds (7th – 12th centuries AD), including archaeological reports, published data, etc., at the Repository of Archaeological Material, Institute of Latvian History, University of Latvia (LULVI) and the repository of archaeological material, Museum of National History.
2. Bioarchaeological analysis was started on the available anthropological material, as well as collection of samples for the various research activities planned in the project.
3. DNA was extracted from the first project samples; modern mtDNA libraries were sequenced; for the project samples, libraries were prepared, and quality control was carried out. Modification and testing of DNA extraction protocol for ancient samples, and modification and testing of aDNA library preparation protocol to reduce loss of sample DNA, was performed.
4. Two abstracts were submitted for participation at the 25th Annual Meeting of the EAA, Going Beyond Paradigms, session "Systemic Approaches to Juvenile Funerary Rituals. Atypical, Deviant or Normative?".
5. In cooperation with the National Heritage Board of Latvia, expert reports (A. Vilcāne) were provided about preservation of the Iron Age archaeological material.
6. For publicity purposes, a public event, The Shadow Day was organised at the BMC, where interested members of the public were introduced to the possibilities and results of aDNA analysis.

7. A review of scientific literature relevant for the project activities, was carried out.

*Date information prepared: 30.04.2019*

***May 1, 2019 – August 31, 2019***

1. Research and analysis of archaeological information gathered during the data review about the Lejasbitēni and Čunkāni – Dreņģeri burial grounds (burial traditions, grave goods, etc.), was continued.
2. Palaeopathological analysis, as well as age determination of non-adult individuals was started on available Iron Age anthropological material. Collection of samples for the various analyses planned in the project was continued.
3. At the aDNA laboratory, BMC, work was continued on aDNA extraction, preparation of libraries, sex determination using PCR methods, and modification and testing of aDNA library preparation protocol to reduce loss of sample DNA.
4. Collection of the first environmental samples (soil) from Iron Age burial grounds was started, in order to determine microbiome profiles with the Ion Torrent Next Generation Sequencing technologies.
5. An abstract was submitted for the participation at the international conference, „Investigating bones: Diet, health, environment in the Baltic region” in Vilnius, Lithuania.
6. The Viļaka Municipality Council was consulted with regard to the preservation of archaeological heritage from the local area, as well as rescue excavations at the Logini ancient burial ground. During the excavations, Iron Age samples were collected for the ancient microbiome analysis.
7. Review of scientific literature relevant to the project activities was continued.

*Date information prepared: 31.08.2019*

***September 1, 2019 – December 31, 2019***

1. Collection and analysis of archaeological data was continued. Female gender and non-adult burials containing weapons were studied in more detail.
2. In cooperation with Poznan Radiocarbon Laboratory, Poland, the first six dates from two Iron Age burial grounds were obtained.
3. The collection and processing of samples was continued, and stable isotope analysis was started.
4. The work on aDNA was continued, with activities including whole genome enrichment and library sequencing, sex determination using PCR methods and sequencing the data, and testing of a new aDNA extraction method from human teeth.
5. Samples from Iron Age burial environments (soil, dental calculus) were analysed. Extraction of aDNA and shotgun sequencing was performed on several human tooth samples.
6. The results obtained during the project were presented in the following international conferences: 25th Annual Meeting of the EAA, Going Beyond Paradigms, in Bern, Switzerland, September 4-7 (two presentations); Investigating bones: Diet, health, environment in the Baltic region, October 4-5, 2019, Vilnius, Lithuania; International

Scientific Conference, History and archaeology of the Daugava region, November 19 – 22, in Minsk, Belarus.

7. The following abstracts were published:

- 1) “Axe in the Iron Age Child Burials (7th – 12th century AD) in the territory of Latvia: Gender, Sex or Status” / Aija Ērkšķe, Antonija Vilcāne, Elīna Pētersone-Gordina, Alisa Kazarina, Jānis Ķimšis, Renāte Ranka, Guntis Gerhards // 25th EAA Annual Meeting, 25 years Beyond Paradigms, September 4-7 2019, Bern: Abstract Book, Bern: European Association of Archaeologists, 2019, p. 64-65.
- 2) “Sex determination of Iron Age Human Remains from Latvia” / Jānis Ķimšis, Elīna Pētersone-Gordina, Alisa Kazarina, Antonija Vilcāne, Aija Ērkšķe, Egija Zole, Guntis Gerhards, Renāte Ranka // Investigating bones: Diet, health, environment in the Baltic region: 10th International Conference of Prof. Jonas Puzinas, October 4-5, 2019, Vilnius: Conference Proceedings, Vilnius: Vilnius University, 2019, p. 23.
- 3) “Two deviant subadult burials at the Čunkāni-Dreņģeri cemetery (8th-11th c.) in the territory of Latvia” / Aija Ērkšķe // 25th EAA Annual Meeting, 25 years Beyond Paradigms, September 4-7, 2019, Bern: Abstract Book, Bern: European Association of Archaeologists, 2019, p. 64.

8. Popular Science publications about the project:

- 1) Ērkšķe, A. “Ieskats Eiropas arheologu asociācijas ikgadējā konferencē Bernē, Šveicē” [An insight into the Annual Meeting of the EAA in Bern, Switzerland] // Zinātnes vēstnesis 15 (578), ISSN 1407-6748, September 30, 2019.
- 2) Vilcāne, A. “Starptautiska zinātniska konference par piedaugavas vēsturi un arheoloģiju Baltkrievijā” [An international scientific conference about the history and archaeology of areas near the river Daugava in Belarus] // Zinātnes vēstnesis 21 (584), ISSN 1407-6748, December 23, 2019.
- 3) Keynote speech (J. Ķimšis), public lecture at the conference organised by the students of the University of Latvia, Homo Et, titled „Feedback: human and innovation”.
- 4) Work was continued on evaluation of data obtained during the project, as well analysis of relevant scientific literature, and preparation of manuscripts for publication.

*Date information prepared: 30.12.2019*

**January 1, 2020 –February 29, 2020**

1. Project activities with regard to analysis of archaeological material, bioarchaeological analysis, and stable isotope and aDNA analyses, were continued.
2. The project results were presented at the International Scientific Conference, XXX Scientific Readings, at Daugavpils University, January 26 – 27, Daugavpils, Latvia (two presentations).
3. The following manuscripts were submitted for publication:
  - 1) Ērkšķe, A. “Children are missing! Some thoughts about the underrepresentation of non-adult burials in Latvian Iron Age cemeteries” (Journal of Estonian archaeology; Web of Science).
  - 2) Gerhards, G. “Gender and status in Iron Age Latgalian society” (in Latvian; the peer-reviewed collection of articles Vēsture: avoti un cilvēki [History: Sources and People].

3) Vilcāne, A. “Funerary traditions near the north-eastern border of Latvia in late Iron Age and early medieval period” (in Latvian; submitted to the peer-reviewed collection of articles, *Vēsture: avoti un cilvēki* [History: Sources and People]).

4) Ērkšķe, A., Vilcāne, A., E. Pētersone – Gordina, Gerhards, G. “Trephination only for the privileged? Case studies from the Iron Age Latvia (7th-10th c AD)” (in Latvian; submitted to peer-reviewed journal *Arheoloģija un Etnogrāfija* [Archaeology and Ethnography]).

4. In cooperation with the National Heritage Board of Latvia expert reports (A. Vilcāne) were provided about dating of the Iron Age archaeological material.
5. Collaboration was commenced with European laboratories (Germany, Estonia) with regard to aDNA analysis of control samples of the project material.
6. An abstract was submitted for the participation at the 26th Annual Meeting of the EAA, session "More than Just Bones - Understanding Past Human Behaviour through the Study of Human Remains", in Budapest, Hungary.

*Date information prepared: 29.02.2020*

### ***March 1, 2020 – May 31, 2020***

1. Due to COVID-19 state of emergency was announced in Latvia and elsewhere in Europe. The epidemiological safety measures significantly affected data collection in museums and repositories, as well as the functioning of laboratories. Planned conferences were either cancelled or postponed.
2. Archaeological material from the Lejasbitēni cemetery (453 burials) was systematically grouped in order to enable statistical analysis of burial traditions.
3. Palaeopathological analyses, as well as age estimation were continued on available Iron Age anthropological material.
4. The work for aDNA extraction, purification and sequencing was performed as planned. In most cases the archaeological samples from Lejasbitēni burials were chosen for the aDNA analysis.
5. Some adjustments for the bioinformatics pipelines have been made in accordance with the most recent aDNA protocols available in scientific literature. The analysis of aDNA data was performed, and obtained results were compared for different protocols/sample groups.
6. Review of scientific literature relevant to the project activities was continued.

*Date information prepared: 31.05.2020*

### ***June 1, 2020 – August 31, 2020***

1. In response to the continuation of COVID-19 pandemic as a *force majeure* situation, the project management committee amended the schedule of project activities, as well as the budget, whereby the funds which had been allocated for travel (conferences, and capacity building for the scientific team in laboratories abroad) were reallocated for additional radiocarbon analysis, as well as amelogenin peptide analysis for sex determination in archaeological human tooth samples.
2. Archaeological material from the Čunkāni – Dreņģeri cemetery (743 burials) was systematically grouped, in order to enable statistical analysis of burial traditions.
3. Analysis of bioarchaeological data from Iron Age (7th – 11th AD) cemeteries was continued.

4. The work for aDNA extraction, purification and sequencing was performed as planned. In most cases the archaeological samples from Čunkāni – Dreņģeri burials were chosen for the aDNA analysis.
5. The bioinformatics analysis pipelines were amended for microbiome/ancient pathogen studies based on aDNA data, and microbiome analysis of archaeological samples was performed.
6. The results obtained during the project were presented in the following international conference: 26th EAA Virtual Annual Meeting, August 24-30.
7. The following abstract was published: Ērkšķe, A., Vilcāne, A., Pētersone-Gordina, E. Gerhards, G. Trephination only for the privileged? Case studies from the Iron Age Latvia (7th – 10th CC AD) // 26th EAA Virtual Annual Meeting, August 24-30, 2020: Abstract Book Prague: European Association of Archaeologists, 2020. P.565-565; ISBN 9788090727076.
8. A paper about non-adult individuals in Iron Age cemeteries from Latvia was published: Ērkšķe, A. 2020. The children are missing! Some thoughts on the underrepresentation of non-adult burials in Latvian Iron Age cemeteries. *Estonian Journal of Archaeology*. Vol 24, N 2, pp. 161-189. doi.org/10.3176/arch.2020.2.03.

*Date information prepared: 31.08.2020*

#### ***September 1, 2020 – December 31, 2020***

1. Work was continued in the repository of archaeological material at the National History Museum of Latvia to obtain comparative data about Iron Age Latgalian and Semigallian cemeteries.
2. Tooth samples were collected for amelogenin peptide analysis for sex determination in archaeological human remains.
3. In cooperation with the Stable Isotope Biogeochemistry Laboratory (SIBL), Department of Archaeology, Durham University, Great Britain, the first stable isotope results from Latvian Iron Age cemeteries were obtained.
4. Standard aDNA analysis workflow was slightly amended for aDNA extraction purposes from poorly preserved archaeological samples.
5. Additional archaeological samples from previously studied individuals were obtained for those cases when the initial aDNA samples were of low quality/showed excessive degradation pattern.
6. The analysis of aDNA sequencing data was performed; both human and microbial aDNA data were assessed.
7. The general public was informed about the implementation of this research project, and the importance of aDNA research field was highlighted during the interview for a local radio channel. <https://lr2.lsm.lv/lv/raksts/nakotnes-pietura/renates-rankas-interesu-loka-seno-infekcijas-slimibu-izpete.a137422/> [from 14:10]

*Date information prepared: 31.12.2020*

#### ***January 1, 2021 – April 2021***

1. Analysis of human remains from the Lejasbitēni and Čunkāni – Dreņģeri cemeteries, as well as the study of burial traditions with regard to gender, sex, and social status, was continued. The typological dating of grave goods, and the estimation of individual social status, was carried out.
2. Overall, aDNA isolation, sequencing and data analysis was continuing.

3. For separate archaeological sample sets biological sex of the individual was assessed based on aDNA data.
4. Metagenomics datasets were thoroughly analysed for the archaeological tooth samples, traces of ancient oral microbiome were studied, and authentication of oral microbial aDNA was performed.
5. The goals, methodology, challenges and benefits of aDNA research field were described and explained at the public event during the 2021 European Researchers' Night (<https://www.facebook.com/LatvianBiomedicalCentre/videos/eiropas-zin%C4%81tnieku-nakts-2021-lbmc/368266991185218/> [from 43:00] <https://www.youtube.com/watch?v=HsdYBlqu8Ps>) The advancement of aDNA research in Latvia and the contribution of this project was highlighted.
6. The project results were presented at International Scientific Conferences, XXXI Scientific Readings, at Daugavpils University, January 28 – 29, Daugavpils, Latvia (two presentations); 79th International Scientific Conference of the University of Latvia on Medicine, April 24, Riga, Latvia.
7. The following abstract was published: Ķīmsis, J., Kazarina, A., Gerhards, G., Ranka, R. 2021. Metagenome analysis of archaeological teeth samples from Latvian Iron Age, 8th -11th centuries AD (Poster Presentations. Basic Medical Science) 79th International Scientific Conference of the University of Latvia on Medicine April 23, p. 49.
8. The following manuscripts were submitted for publication: Ķīmsis, J. Ranka, R., Vilcāne, A. Gehards, G. Molecular sex determination in an ambiguous burial from Latvian Iron Age (in Latvian, the peer-reviewed collection of articles *Vēsture: avoti un cilvēki* [History: Sources and People]. Ērkške, A. Bracelets made from other jewellery as a source about subadults' social status in the Iron Age society: case study of the Čunkāni-Dreņģeri cemetery (8th –11th cc AD) (in Latvian, the peer-reviewed collection of articles *Vēsture: avoti un cilvēki* [History: Sources and People].

*Date information prepared: 31.04.2021*

### ***May 1, 2021 – August 31, 2021***

1. In cooperation with Poznan Radiocarbon Laboratory, Poland, new dates from Lejasbitēni and Čunkāni – Dreņģeri cemeteries were obtained.
2. In cooperation with the Stable Isotope Biogeochemistry Laboratory (SIBL), Department of Archaeology, Durham University, Great Britain, new stable isotope results, and the first results of the amelogenin peptide analysis from Latvian Iron Age cemeteries were obtained.
3. Work was started on evaluation of all material from the Lejasbitēni and Čunkāni – Dreņģeri cemeteries in the light of data obtained from dietary stable isotope and aDNA analyses.
4. Isolation and purification of aDNA from the final archaeological sample sets was performed, the quality of aDNA samples was assessed.
5. Sequencing libraries were prepared, additional size selected, and library enrichment was performed, if necessary.
6. Sequencing of aDNA libraries using Illumina methodology was performed, and raw sequencing data were processed using various bioinformatics tools/pipelines.
7. Work was continued on evaluation of data obtained during the project, as well analysis of relevant scientific literature, and preparation of manuscripts for publication.
8. The project results were presented at the scientific readings of the National History Museum on 14th May: Vilcāne A., *Orientācijas nozīme apbedīšanas tradīcijās. Ieskats*

historiogrāfijā [The significance of burial orientation in funerary ritual in historiography].

*Date information prepared: 31.08.2021*

***September 1, 2021 – December 31, 2021***

1. Work was continued on studying material from the Lejasbitēni and Čunkāni – Dreņģeri cemeteries in the light of data obtained from dietary stable isotope, and bioarchaeological analyses, in order to assess the quality of life of these populations. All results were processed and analysed, and relevant manuscripts were prepared.
2. The obtained aDNA sequencing data sets were thoroughly assessed by using several bioinformatics tools, pipelines, and analysis methods.
3. Human aDNA data sets were rigorously analysed. The authentication of human aDNA was performed, human aDNA amount/degradation in various samples was assessed. For individuals, biological sex identification and mitochondrial DNA analysis was performed.
4. Microbial metagenome datasets were analysed, bacterial diversity and characteristic features in various archaeological samples from two burial sites were evaluated. The possible presence of ancient pathogens was assessed.
5. The obtained results were presented at the conference: 27th EAA Annual Meeting, Biosocial Archaeology: When Ancient DNA Opens the Discussion to Social Structures. Assembling archaeological theory and the archaeological sciences (Widening Horizons, Virtual Meeting), September 6-11, 2021, Kiel. An abstract was published: Ranka, R., Ķimsis, J., Pokšāne, A., Pētersone-Gordina, E., Vilcāne, A., Kazarina, Gerhards, G. 2021 Survival of aDNA in Tooth and Bone Samples from Iron Age (7th -12th Centuries AD) Burials in Latvia. //27th EAA Annual Meeting (Abstract Book Prague: European Association of Archaeologists, P.716.
6. Scientific manuscripts (4) were prepared and submitted to journals which are included in Scopus/ Web of Sciences databases.

*Date information prepared: 30.12.2021*