4th Bone Workshop and Conference 2019

Prague, September 9-10, 2019

Final program

Venue:

Police Museum, Ke Karlovu 1, Prague 2

Organized by:

FDNAS
FORENSIC DNA SERVICE

Biologicals

Institute of the Legal Medicine, 2nd Medical Faculty, Charles University in Prague, Bulovka Hospital, Prague

Nemocnice Na Bulovce

Police Museum Prague

4th Bone Workshop and Conference 2019

Monday September 9th, 2019

12:00-13:00	Registration
13:30-13:40	Conference opening
13:40-14:40	*Michael HOFREITER* HOW TO GET ANCIENT DNA AND WHAT TO DO WITH IT
14:40-16:10	*Bruce BUDOWLE* MOLECULAR ADVANCES IN HUMAN IDENTIFICATION – INCREASING CAPABILITIES TO ANALYZE HUMAN REMAINS
16:10-16:30	Coffee break
16:30-17:30	*Daniel VANEK* EVALUATION OF THE 2nd COLLABORATIVE EXERCISE ON DNA IDENTIFICATION OF BONE SAMPLES AND GUIDELINES FOR THE 3rd COLLABORATIVE EXERCISE
Practical demonstrations	
17:30-18:00	*Adrian LINACRE* VISUALISING LATENT CELLULAR MATERIAL USING DNA BINDING DYES
18:00-18:20	*Paul LYNCH + Daniel Vanek* BONE GRINDING USING CRYOGENIC MILL VS. WARRING MILL
18:20-18:40	*Jiri SINDELAR* FACE RECONTRUCTION OF QUEEN JUDITH FROM DURYN USING SKULL 3D PHOTOGRAMMETRY
18:40-19:00	Guided tour to the church of the Assumption of the Virgin Mary and St. Charles the Great (next to the conference venue)
19:00-??	Dinner and live music (next to the conference venue)

Tuesday September 10th, 2019

8:30-9:40	*Magdalena M. BUS* WHOLE MITOCHONDRIAL GENOME ANALYSES WITH SHORT AMPLICON MULTIPLEXES
9:40-10:00	*Edvard EHLER* AmtDB: DATABASE OF ANCIENT FULL mtDNAs AND SAMPLE METADATA
10:00-10:20	*Martin POSPISEK* DENTAL CALCULUS MICROBIOME FROM MEDIEVAL POPULATIONS IN PILSEN
10:20-10:40	*Jan FROLIK* RESCUE ARCHAEOLOGICAL EXCAVATION AT THE MEDIEVAL OSSUARY IN KUTNÁ HORA – SEDLEC (CENTRAL BOHEMIA)
10:40-11:00	Coffee break
11:00-11:20	*Jan FROLÍK* MEDIAEVAL POPULATION IN THE CENTRE AND COUNTRY. ARCHAEOLOGY, BIOARCHEOLOGY AND GENETICS OF CEMETERIES OF PRAGUE CASTLE, CENTRAL AND EASTERN BOHEMIA
11:20-11:55	*Daniel VANEK* CEMETERIES OF PRAGUE CASTLE, CENTRAL AND EASTERN BOHEMIA: THE RESULTS OF DNA ANALYSIS
11:55-12:10	*Stephen CLIFFORD* DNA EXTRACTION FROM BONE IN FORENSIC CASE WORK
12:10-12:40	*Alain STEVANOVITCH* WORLD WARS SOLDIERS IDENTIFICATION : BRING BACK THE HEROES HOME
12:40-13:00	Conference closing
13:00-14:00	Lunch

ABSTRACTS

MOLECULAR ADVANCES IN HUMAN IDENTIFICATION – INCREASING CAPABILITIES TO ANALYZE HUMAN REMAINS

BRUCE BUDOWLE

Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107, USA

Human remains can be one of the most challenging samples to analyze. They are exposed to the environment and thus may contain chemicals that may inhibit downstream assays, can be altered (such as degradation and oxidation) due to environmental insults, and be of limited quantity. However, the field of forensic genetics/genomics has made great strides in the analysis of biological evidence related to identification of human remains. Massively parallel sequencing (MPS) is the latest innovation that will improve analysis of biological evidence and in turn increase the number of associations developed. MPS has a substantially higher throughput compared with capillary electrophoresis (CE) platforms, which enables more markers and more samples to be analyzed in a single run. Moreover, it provides flexibility and diversity to accommodate alternate methodologies that were not readily adapted to CE-based analyses. MPS enables scanning the entire mitochondrial genome, detecting underlying sequence variation within the amplicons of STRs, simplifying SNP analyses, and typing of short amplicons, which have expanded the field substantially beyond current practices. In addition, large genome scans (by MPS or SNP arrays) enable more accurate kinship analyses beyond reference samples from first and second degree relatives. This presentation will discuss the enhanced capabilities of MPS and how it impacts method development and human identification practices. Topics will include: 1) basics of use of databases and kinship analyses, 2) the technologies that are used, 3) genetic genealogy, 4) increased genetic variation residing within currently used STRs and SNPs, 5) novel STRs with increased variation over current STRs, 6) validation of whole mitochondrial DNA genome methodology, 7) ancestry informative markers, and 8) novel approaches for analysis of highly degraded DNA. Case applications will be presented to appreciate the impact advanced technologies can have on human identification.

WHOLE MITOCHONDRIAL GENOME ANALYSES WITH SHORT AMPLICON MULTIPLEXES

Magdalena M. BUS

Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107, USA

Massively Parallel Sequencing (MPS) technology has provided new opportunities in human identification testing. However, even with MPS highly degraded, contaminated or low copy DNA samples pose challenges to typing. Compromised samples may yield low-quality data manifested as an increased number of sequence errors or artifacts, low read depth and/or lack of results (i.e., drop-out) for a few to the entire battery of DNA markers in a panel. Post-mortem processes severely reduce the total copy number of nuclear DNA in samples such as bones or teeth. Several MPS approaches have been developed to increase the yield of DNA information and to establish highly accurate, reproducible, and robust results. One such approach is the sequencing of mtDNA from human remains. The circular structure of the genome and the higher copy number of mtDNA per cell compared with that of nuclear DNA make the marker an advantageous tool in human identification when aged samples are a source of DNA. Additionally, DNA fragmentation as a natural consequence of degradation has a significant effect on the length of nuclear and mtDNA fragments that can be recovered from a sample. Novel MPS multiplexes that have been designed to target the entire mtDNA genome can amplify short (approximately ≤160bp long) overlapping fragments. With this short target system, data may be obtained beyond the control region, i.e. the coding region, which can increase the genetic information about an individual even from degraded samples.

DNA EXTRACTION FROM BONE IN FORENSIC CASE WORK

Stephen CLIFFORD

Forensic Science Ireland, Dublin, Ireland

In missing person and body identifications, bone may be the only suitable material available for STR genotyping. Specialized techniques are required for the extraction of DNA from bone tissue, and the DNA can be present in low concentrations particularly when the bones are very old or have been exposed to unfavourable environmental conditions, such as bones recovered from the sea. Extracting DNA from skeletal remains at Forensic Science Ireland is typically performer using a modified version of an organic DNA extraction followed by purification using QIAamp® DNA Investigator Kit. A DNA extraction method using Promega Bone DNA extraction chemistry is currently being trialled. The results obtained in forensic case work at Forensic Science Ireland for a range of bone samples of varying age and condition will be discussed.

AmtDB: DATABASE OF ANCIENT FULL mtDNAs AND SAMPLE METADATA

Edvard EHLER[1,2,*]

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In recent years number of ancient DNA studies, amount, and quality of ancient DNA samples have been constantly rising. Thanks to advances in ancient DNA isolation and sequencing techniques, today we are able to access the population variability of many ancient populations. In most of the published studies the samples from Eurasia are being studied, with the focus on end of Pleistocene and beginning of Holocene. Today, autosomal SNPs and uniparental markers, Y chromosome or mitochondrial DNA, provide valuable insight into ancient population genetics. So far, the genotypes of few thousands of prehistoric samples have been published. For researchers, this provides a challenge, as the sequences and additional information about each sample are spread across many publications, in many different formats and places. That's why we have created a hand-curated database of ancient human full mitochondrial genomes, where researchers can find the mtDNA sequences and associated sample descriptors. In the talk I will introduce the database, its basic functionality and results of several studies of the genetic variability of ancient central European population we have performed with the help of this tool. AmtDB database can be found at https://amtdb.org.

RESCUE ARCHAEOLOGICAL EXCAVATION AT THE MEDIEVAL OSSUARY IN KUTNÁ HORA – SEDLEC (CENTRAL BOHEMIA)

JAN FROLÍK

Institute of Archaeology of the Czech Academy of Science, Prague

A rescue archaeological research was carried out at the cemetery chapel of All Saints with the ossuary between 2016 and 2018. Research revealed a total of 1817 skeletons in a two-meter-wide strip of terrain around the ossuary, some of which (about two-thirds) are buried in mass graves. A total of 23 of these have been explored in part or in full. Based on archaeological findings and stratigraphy and relationship to the foundations of the ossuary and written reports, four graves can be identified as famine victims in 1318 and seven graves as victims of plague from 1348 to 1350. It is the largest collection of medieval mass graves in the Czech Republic and probably also in Europe, which provides the opportunity to study precisely dated populations and to observe their changes over time. The first results of anthropological research show that this is a mining-related population (male dominance). A project is currently under preparation to assign all mass graves to one of the aforementioned disasters. Furthermore, the origin of this people and diet customs and interrelationships of the buried should be ascertained.

MEDIAEVAL POPULATION IN THE CENTRE AND COUNTRY. ARCHAEOLOGY, BIOARCHEOLOGY AND GENETICS OF CEMETERIES OF PRAGUE CASTLE, CENTRAL AND EASTERN BOHEMIA. RESULTS OF THE PROJECT.

JAN FROLÍK

Institute of Archaeology of the Czech Academy of Science, Prague

Between 2014 and 2018, the project was under way and made available the results of archaeological excavations in the center of the early medieval Czech state – at Prague Castle. Selected cemeteries in central and eastern Bohemia were evaluated as a comparison. In addition to archaeological evaluation (especially chronology and social status of buried), their origin (isotopes of strontium) and diet (diet isotopes) were studied. DNA research focused on the oldest five generations (c. 880 to 1030 AD) of the ruling prince and later royal dynasty of the Přemyslid family and selected case studies at other cemeteries.

Detailed archaeological evaluation has shown the survival of some still pre-Christian burial customs deep into the Middle Ages. The unusual composition of ceramic ensembles points to the practices associated with worshiping buried. When analyzing anthropological material, attention should also be paid to bones that have been relocated to grave pits or cemetery layers. The most common finding is altered (increased) representation of buried children. The study of strontium isotopes confirmed the supposed considerable mobility at Prague Castle during the 9th to 11th centuries, but surprisingly also for burial grounds in the background of Prague Castle and some remote rural burial grounds (a woman of Mediterranean origin at Žabonosy burial ground). Study of dietary isotopes has shown greater consumption of meat (including fish) in Prague Castle and relatively high millet consumption. The combination of all methods contributed to the knowledge of the oldest Czech rulers and their wives, but also to the identification of people living on the margins of the society at that time (Český Brod).

HOW TO GET ANCIENT DNA AND WHAT TO DO WITH IT

Michael HOFREITER

University of Potsdam, Germany

The analysis of paleogenomes has resulted in remarkable insights both in human and animal evolution, and more spectacular studies are almost certainly on their way. However, there are numerous ways how to obtain and analyse paleogenomes and as in any analysis using extremely large data sets, slight biases in methodology may lead to statistically significant, yet completely artefactual, and thus non-sensical results. I will discuss how to maximize paleogenome yield from ancient samples from selection of the best sampling site on a sample to phylogenetic and population genetic analyses and discuss the various caveats in each step. After this, I will discuss, using examples from hybrid elephants to zombie bears, what insights can be obtained using paleogenome data. Finally, I will give an outlook what can possibly be achieved with paleogenome data in the future in various research fields, including possible forensic applications.

VISUALISING LATENT CELLULAR MATERIAL USING DNA BINDING DYES

Adrian LINACRE

Flinder University, Adelaide, South Australia

Touch DNA is one of the most common sample types submitted for DNA profiling. There is currently no process to visualise the presence of such DNA deposited when a person makes direct contact with items of forensic relevance.
This lecture will demonstrate the effective use of Diamond Dye to bind to DNA and allow visualisation of deposited cellular material using a mini-fluorescence microscope. This screening method has the potential to be a routine step in a forensic laboratory to save costs of processing samples where swabs are devoid of any DNA. This technique is rapid, easy, cheap, non-destructive and safe.

DENTAL CALCULUS MICROBIOME FROM MEDIEVAL POPULATIONS IN PILSEN

Sneberger J.[1], Pospiskova M.[1], Vetrovsky T.[2], Vanek D.[3], Votrubova J.[3], **POSPISEK M.**[1,4]

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Dental calculus becomes a valuable material of choice for retrospective studies aimed to investigate health status and dietary habits of past populations. We aimed to compare dental calculus microbiomes from medieval human remains obtained from burial sites in Pilsen and surrounding settlement. Besides osteological and paleopathological analyses of the human remains, we collected dental calculi for the investigation of microbial DNA. Dental calculi from teeth found freely and without any context in the burial sites were used for the method development. Used methods and results obtained from microbiome analyses as well as analyses of other DNA found in dental calculi will be discussed.

WORLD WARS SOLDIERS IDENTIFICATION : BRING BACK THE HEROES HOME

Alain STEVANOVITCH

Institut National de Police Scientifique, Laboratoire de Marseille, 13004 Marseille, France

World Wars Soldiers identification : Bring back the Heroes home During the two World Wars, more than 20 million of soldiers were killed over Europe battlefields. If most of them were identified, thousands of them are still « missing in assault ». Years have done their job and families have done their mourning, but they still thinking about « where is my grand-father ? ». In collaborative way with anthropologists, historians and archaeologists, we use our experience in forensic bones analysis to try to solve some of these stories. Here we present 4 distinct case reports of identification of a German pilot, a Canadian soldier, a French Resistance fighter killed during WWII and a French soldier killed in WWI. After the study of clues obtained by anthropological analysis, all of the soldiers were identified by DNA analysis of bones remains, using non-autosomal markers (mitochondrial DNA and/or Y Chromosome STR). We performed in some cases comparisons with some of their living relatives and in one case we had to compare with autosomal STR sequenced from the bones of the soldier's mother and sister (died in 1942 and 1957). All of these case reports were technical challenge, but above all they are real human stories.

FACE RECONTRUCTION OF QUEEN JUDITH FROM DURYN USING SKULL 3D PHOTOGRAMMETRY

Jiří ŠINDELÁŘ

Geo-cz s.r.o, Mlada Vozice, Czech Republic

http://geo-cz.com

We will demonstrate 3D scanning of skeletal remains using multi-image photogrammetry. This fast and cheap approach of contact-less data sampling enables to create a skull digital copy, which can be used for face reconstruction. The methods of scanning and subsequent forensic procedures will be demonstrated on a project, where the aim was the face reconstruction of Czech Queen Judith (12th century).

EVALUATION OF THE 2nd COLLABORATIVE EXERCISE ON DNA IDENTIFICATION OF BONE SAMPLES AND GUIDELINES FOR THE 3rd COLLABORATIVE EXERCISE

Daniel VANEK

Forensic DNA Service, Prague, Czech Republic

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The aim of the Collaborative exercise is to make progress on standardization and to identify the technical problems connected with identification of bone samples and difficult DNA templates. The results of the 1st collaborative exercise (which has been published in Croatian Medical journal) has shown some problems in DNA extraction, contamination and evaluation of the CE outputs. Total number of 10 lab submitted the results for the 2nd exercise. The samples selected for the 2nd exercise were grounded in SPEX liquid nitrogen mill and aliquoted. All samples were typed by the organizing laboratories. The success rate of 2nd collaborative exercise was higher than the one of 1st exercise. The samples for the 3rd exercise will comprise bone powder, piece of solid bone and one unknown sample of animal origin.

CEMETERIES OF PRAGUE CASTLE, CENTRAL AND EASTERN BOHEMIA: THE RESULTS OF DNA ANALYSIS

Daniel VANEK

Forensic DNA Service, Prague, Czech Republic

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This talk is connected with the presentation of Dr. FROLIK (Mediaeval population in the centre and country. Archaeology, bioarcheology and genetics of cemeteries of Prague castle, central and eastern bohemia. Results of the project). We will discuss the mtDNA, Y-chromosome and MPS results of DNA analysis of skeletal remains studied within the project.