

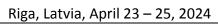
# 11<sup>th</sup> ENFSI APST WORKING GROUP ANNUAL MEETING

VENUE, SCHEDULE, ABSTRACTS, AND PARTICIPANTS



April 23 – 25, 2024 Riga, Latvia







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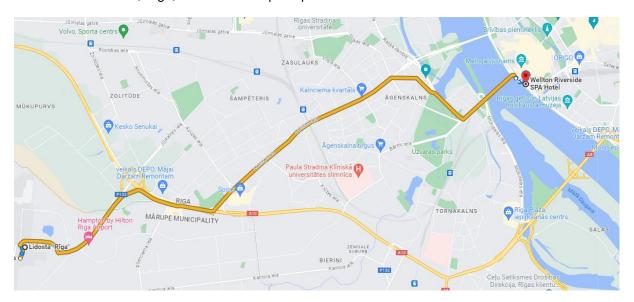


Riga, Latvia, April 23 - 25, 2024



#### **Meeting venue**

The meeting and linked accommodation will take place at the Wellton Riverside SPA Hotel - 33, 11. Novembra krastmala, Riga, LV-1055. Reception phone +371 66777850.



#### TRAVELING WITHIN RIGA:

#### From/to the Airport

**Bus #22** – The bus stop at the Riga Airport to the city centre is in the P1 car park in the middle between arrival exits E and C. If you are staying at Wellton Riverside SPA Hotel, your stop is "11. Novembra krastmala", which is located across the street from the hotel. The bus stop "Grēcinieku iela" from Wellton Riverside SPA Hotel to the Airport is on the hotel's side of the street. The ride takes approximately 25 minutes.

#### Bus tickets

You can use either a 90-minute ticket for 1.50 EUR or a 3-day/5-day ticket for 8.00/10.00 EUR, with the option to travel on any tram, trolleybus, or bus route for 90 minutes or 3/5 days. A ticket must be registered each time another public transport vehicle (tram, trolleybus, bus) is entered.

Tickets can be bought as yellow e-tickets at the Airport:

- in the vending machine at the bus stop,
- in a "Narvesen" kiosk,
- · and, as a code ticket:

(https://www.rigassatiksme.lv/en/tickets-and-e-ticket/code-tickets/)

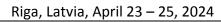
Bus route No. 22 is the only route managed by "Rīgas satiksme", where the ticket can also be bought from the transport vehicle driver. Payment for the ticket is with a bank payment card only, ticket price - 2€.

#### TAXI

Ask the approximate price upfront, a ride to the city centre should not exceed 25 (max 30) EUR. Another option is to buy a taxi voucher to city centre (33.50 EUR) in The RIX Riga Airport Visitor Centre, which is located on the 1st floor of the Airport terminal, in Arrivals Hall E. Vouchers can be used with "Baltic Taxi", "XTaxi", and "TaksiLV. The ride takes approximately 25 minutes. If you have the Bolt app in your country, you can also use it here.

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## **Program**

April 22, 2024

		April 22, 2027
18.30 – 19.30 18.30 – 21.00	Registration (hotel reception area) Get together	
		April 23, 2024
8.30 9.00 – 9.20	Registration (Panorama Hall) Opening (Panorama Hall) Welcome from the Deputy State Secretary on Court Issues Inita Ilgaža Welcome and introduction of new participants	
9.20 – 10.30	Invited speaker: The use of DNA analysis methods for wildlife, environmental and agricultural analyses in Latvia  Vilnis Šķipars Analysis of forensic reports in wildlife crime cases in the Czech Republic Pavla Rihova The Garden Gnome is Innocent! Geologically Derived Building Materials in Criminal Cases  Jodi Webb	Chair: Irene Kuiper
10.30	Coffee break	
11.00 – 12.00	Forensic identification of moss species based on chloroplast DNA sequences  Balazs Egyed Identification of the origin of mineral phases in forensic practice  Marek Kotrly The role of DNA typing in species identification: Case studies  Karolina Mahlerova	Chair: Alejandra Perotti
12.00	Lunch	
13.40 – 15.00	Discrimination of wood fragments in a murder investigation using chemical profiles  Lorna Dawson Forensic investigation of hallucinogenic botanical traces  Arthur Wolterink Far Drowning – Persistence of diatoms in human tissues  Viktor Poór Application of diatom analysis in forensic practice  Kateřina Zubíčková	Chair: Andreas Hellmann
15.00	Coffee break	
15.30 – 16.00	Exhibitor's presentation  Poster presentations Supporting the recovery of canine DNA from livestock attacks through rural community and victim engagement Nick Dawnay Sex detection in Cervidae species using loop-mediated amplification (LAMP) technique and a 3D printed device Petra Zenke The road between a leg and an arm Ana Assis Exploring the impact of environmental variability and clothing type on diatom trace evidence transfer in coastal locations Kristie Scott DNA metabarcoding: can it reliably be used to characterize pollen from surface soils? Kelly Meiklejohn Protein fingerprinting as a tool to authenticate fish products in the food industry Virginia Harvey	Chair: Lorna Dawson
16.00	UFO session	
18.00 – 22.00	Social program and conference dinner	





# Riga, Latvia, April 23 – 25, 2024

# April 24, 2024

9.00 – 11.50	Workshops (Panorama Hall and Mārstaļu Hall)  Soil WS  New developments in forensic soil investigation  Stefan Uitdehaag  Presentation on pollen and non-pollen palynomorphs analyses  Normunds Stivrins	Chair: Stefan Uitdehaag
9.00 – 11.50	nhDNA WS What is growing on my fields? The detection of agricultural hemp by prediction of the chemical phenotype Christina Staginnus Classification, identification, or comparative analysis?	Chair: Nadja Morf & Monique Wesselink
	Monique Wesselink and Nadja Morf	
11.50 – 12.00	Group photo	
12.00	Lunch	
13.00 - 19.00	Field trip	

## April 25, 2024

9.00 – 9.20	Mārstaļu Hall Workshop summaries and outcomes	
9.20 – 10.20	Developing and testing the end user requirements for a novel, field based environmental DNA (eDNA) forensic method  Nick Dawnay  New tool in the belt - analysis of environmental DNA associated with geological evidence  Kelly Meiklejohn  Results of the ENFSI APST Forensic Geology CE 2023  Tamás Stadler	Chair: Frank Reckel
10.20	Coffee break	
10.50 – 12.30	Summary of the ENFSI-APST 2023 Non-human DNA Proficiency Test  Jennifer Kaden  Summary of "APST CE Botany 2023" results	Chair:
. 0.00	leva Gutovska Business meeting Closing session and invitation to the next year's meeting	Irene Kuiper
12.30	Lunch	





#### Riga, Latvia, April 23 – 25, 2024

#### **Abstracts**

V. Harvey

Presentations (in order of appearance) The use of DNA analysis methods for wildlife, environmental and agricultural analyses in p. 7 Latvia V. Škipars Analysis of forensic reports in wildlife crime cases in the Czech Republic p. 8 P. Rihova The Garden Gnome is Innocent! Geologically Derived Building Materials in Criminal Cases p. 9 J. Webb Forensic identification of moss species based on chloroplast DNA sequences p. 10 B. Egyed Identification of the origin of mineral phases in forensic practice p. 11 M. Kotrly The role of DNA typing in species identification: Case studies p. 12 K. Mahlerova Discrimination of wood fragments in a murder investigation using chemical profiles p. 13 Forensic investigation of hallucinogenic botanical traces p. 14 A. Wolterink Far Drowning - Persistence of diatoms in human tissues p. 15 V. Poór Application of diatom analysis in forensic practice p. 16 K. Zubíčková New developments in forensic soil investigation p. 23 S. Uitdehaag What is growing on my fields? The detection of agricultural hemp by prediction of the chemical p. 24 phenotype Ch. Staginnus Classification, identification, or comparative analysis? p. 25 M. Wesselink and N. Morf Developing and testing the end user requirements for a novel, field based environmental DNA (eDNA) p. 26 forensic method N. Dawnay New tool in the belt - analysis of environmental DNA associated with geological evidence p. 27 Kelly Meiklejohn Results of the ENFSI APST Forensic Geology CE 2023 p. 28 Tamás Stadler Summary of the ENFSI-APST 2023 Non-human DNA Proficiency Test p. 29 J. Kaden Summary of "APST CE Botany 2023" results p. 30 I. Gutovska Posters (in order of appearance) Supporting the recovery of canine DNA from livestock attacks through rural community and victim p. 17 engagement N. Dawnay Sex detection in Cervidae species using loop-mediated amplification (LAMP) technique and a 3D p. 18 printed device P. Zenke The road between a leg and an arm p. 19 Exploring the impact of environmental variability and clothing type on diatom trace evidence transfer in p. 20 coastal locations K. Scott DNA metabarcoding: can it reliably be used to characterize pollen from surface soils? p. 21 K. Meikleiohn Protein fingerprinting as a tool to authenticate fish products in the food industry p. 22



Riga, Latvia, April 23 – 25, 2024



# The use of DNS analysis methods for wildlife, environmental and agricultural analyses in Latvia

Dainis Rungis<sup>1</sup> and Vilnis Šķipars<sup>1</sup>

Genetic Resource Centre (GRC), Latvian State Forest Research Institute "Silava"

\*Correspondence: dainis.rungis@silava.lv

Invited speaker

#### Abstract

The Genetic Resource Centre (GRC), located in the Latvian State Forest Research Institute "Silava" (LSFRI Silava), is one of the leading genetic research laboratories in Latvia, specialising in non-model species, including plants, trees, animals and microorganisms. The laboratory conducts genetic analyses in cooperation with all research directions within LSFRI Silava (forest tree breeding, phytopathology, mercury turnover, forest regeneration, entomology, population genetics of plants and animals), as well as other Latvian and international institutions. Nuclear or mitochondrial DNA markers are used for identification of individuals (e.g. large carnivore monitoring with non-invasive samples) and species determination (e.g. livestock predation events, requests from customs or police to identify samples). We use massive parallel sequencing for environmental DNA analysis (e.g. identification of particular species from mixed samples, and microbial community profiling). Satellite DNA analysis is used to fingerprint and identify varieties and clones of agricultural crop and forest tree species.

Non-invasive samples of large carnivore species (bear and lynx) mainly include hair and scats. However, a significant number of saliva samples from bears are collected from destroyed beehives, when the bear has chewed on the wooden frames. It is also possible to collect urine and footprint samples from snow (mainly lynx) and mud (bears). For bears, successful individual identification was highest for hairs and scats (42% and 37%, respectively), while for saliva samples it was 20%, and for footprint samples, the success rate was only 4.5%. Analysis of livestock predation events involves predator species identification using mitochondrial fragment sequencing. Individual identification from these samples is complicated by the fact that DNA from multiple predator individuals may be present in the collected samples. Metabarcoding using high-throughput sequencing has been used to profile microorganism communities in soil, root and leaf samples. Another application of metabarcoding is the identification of the presence of the pseudoscorpion *Anthrenochernes stellae* in substrates collected from tree hollows.



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### Analysis of forensic reports in wildlife crime cases in the Czech Republic

P. Rihova<sup>1,\*</sup>

<sup>1</sup>Charles University in Prague, Faculty of Science, Environmental Forensic Sciences Centre

\*Correspondence: <a href="mailto:pavla.rihova@natur.cuni.cz">pavla.rihova@natur.cuni.cz</a>

Oral presentation

#### Abstract

An analysis of forensic assessments in wildlife crime cases was conducted for the period 2010-2022. The analysis was based on information from enforcement authorities in the Czech Republic. Totally 683 expert opinions were analysed. We were interested in which of the forensic methods were used most often, what questions were asked and what species were involved.

During the analysed period, the number of expert opinions has been increasing. The increasing demand of state authorities for expert support is evident. The most used method was morphology (species identification, assessment of specimens). These expert opinions were usually supplemented by an analysis of legislation, including the conservation status (CITES etc.) and other biological information. A slightly less often used method was genetic analysis. Species identification was the most common request. Verification of identity, parentage or population was rather minor. Veterinary pathology, including necropsy and animal health assessment, was used significantly. Among chemical methods, toxicology (cases of poisoned animals) was the most common. Less frequent was radiocarbon dating or species identification by serology. Very little use was made of ballistic assessments, parasitology, forensic entomology, trichology and stable isotopes.

Mammals and birds were the most frequently judged. In mammals, carnivores were the most represented, followed by elephants (ivory), rhinoceroses and ungulates. For birds, the most frequent species were raptors, followed by parrots and owls. Tortoises were the most common of the reptiles. For invertebrates, species identification was dominated by butterflies and beetles, and for plants by cacti and plants used in traditional Chinese medicine.



Riga, Latvia, April 23 – 25, 2024



# The Garden Gnome is Innocent! Geologically Derived Building Materials in Criminal Cases

Jodi Webb<sup>1,\*</sup> and Ian Saginor<sup>1</sup>

<sup>1</sup>Federal Bureau of Investigation Laboratory, Trace Evidence Unit

\*Correspondence: jbwebb2@fbi.gov

Oral presentation

#### **Abstract**

The analysis of geologically derived materials such as bricks, concrete blocks, and insulating materials can be applied to legal proceedings to 1) determine if two or more materials originate from different sources; 2) identify an unknown material; and 3) determine the origin/end-use of manmade geologic materials. A few examples will be presented of how geologically derived materials analyses have been applied in forensic cases, from homicides and art crime to desecration of human remains.



Riga, Latvia, April 23 – 25, 2024



#### Forensic identification of moss species based on chloroplast DNA sequences

Nora Majerhoffer<sup>1</sup>, Arnika E. Szekely<sup>2</sup>, Judit K. Sandorne<sup>2</sup>, Norbert Matrai<sup>3</sup>, **Balazs Egyed**<sup>1,\*</sup>

- <sup>1</sup> Department of Genetics, Eötvös Loránd University, Budapest, Hungary
- <sup>2</sup> Department of Physics and Chemistry, Hungarian Institute for Forensic Sciences, Budapest, Hungary
- 3 Department of Genetics, Hungarian Institute for Forensic Sciences, Budapest, Hungary

\*Correspondence: egyed.balazs@ttk.elte.hu

Oral presentation

#### **Abstract**

In forensic botany, there is a permanent demand for the identification of different moss species (*Bryophyta*) in plant materials collected at crime scenes or as evidence. Deceased plants are very often difficult, and in many cases almost impossible to be identified by traditional morphology and microscopy. However, DNA in plant cells – especially in the chloroplast – can be preserved intact even in adverse environmental conditions, and hence they can be a source for the identification of plant materials by molecular methods.

In this study, we provide a systematic examination of moss plants collected in rural and urban areas in order to establish a laboratory protocol for forensic identification of *Bryophyta* species. The methods applied include traditional morphology and microscopy, as well as the sequence analysis of variable chloroplast DNA markers. Dried plant materials that were stored over a period of several months have been also involved to test the efficiency of the method adopted. Using the in-house developed cpDNA *rbcL* and *matK* gene-specific PCR-based DNA typing method most of the samples from the collected colonies have been identified. The study highlights the great variety of chloroplast genomes in these taxonomic groups and the lack of available cpDNA sequences in the online DNA databases, that is one of the main objections of molecular identification of moss species.







#### Identification of the origin of mineral phases in forensic practice

Marek Kotrly 1,\*, Ivana Turkova 1

<sup>1</sup> Institute of Criminalistics

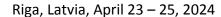
\*Correspondence: <u>marek.kotrly@pcr.cz</u>

Oral presentation

#### Abstract

In the forensic field we guite often see the requirement to determine the genuineness or origin of the mineral material (minerals as well as rocks). The spectrum of materials is quite broad, e.g. building materials (the equivalent of historical material required by the conservation authorities, cheaper substitutes for cladding materials, etc.), precious stones used in the jewellery industry (directly counterfeit or incorrectly declared gemological objects (precious stones), etc., or the location of origin of the material used in the crime is being searched. In forensic gemology we do not meet only easily identifiable cubic zirconia (ZrO2), moissanite (SiC) or synthetic opals. Problems are e.g. synthetic diamonds, the production of HPHT synthetics is growing rapidly in China, the production of CVD synthetics is also growing strongly. These diamonds are often sold as natural. Their identification is possible, but requires instrumentation and the necessary knowledge. In recent years, a large number of synthetic tektites (vltavín, moldavite) have appeared on the market. Chinese-produced synthetics are capable of faithfully replicating color and typical surface morphology (characteristic surface structure called sculpture), these stones are commonly shipped with false certificates of authenticity. Czech garnet counterfeiting frauds are typical for the Central European area. 'Český granát' is a protected regional designation of origin. Czech garnet is a very popular tourist commodity. Currently a massive amount of other types of garnets are imported from other world deposits, or directly jewellery with these garnets and are sold as the Czech garnet.







### The role of DNA typing in species identification: Case studies

Karolina Mahlerová<sup>1,2,3</sup>, Lenka Vaňková<sup>1,2</sup>, Kristýna Hebenstreitová<sup>1,2</sup>, Daniel Vaněk<sup>1,2,4,5,\*</sup>

- <sup>1</sup> Institute for Environmental Sciences, Charles University
- <sup>2</sup> Forensic DNA Service
- <sup>3</sup> Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague
- <sup>4</sup> Bulovka University Hospital
- <sup>5</sup> Charles University, 2nd Faculty of Medicine

\*Correspondence: daniel.vanek@fdnas.cz

Oral presentation

#### **Abstract**

Wildlife crime poses a significant threat to biodiversity and ecosystem stability worldwide, with its profitability and prevalence rising. Combating this illicit trade requires innovative approaches and technologies to keep pace with the evolving tactics of individuals involved in wildlife crime. In this context, genetic tools are crucial in supporting traditional morphological methods for species identification. The presentation aims to show interesting cases involving non-human biological material. The synergistic collaboration between morphological and genetic analyses empowers law enforcement agencies and conservationists in efforts to protect biodiversity.



Riga, Latvia, April 23 – 25, 2024



# Discrimination of wood fragments in a murder investigation using chemical profiles

\*Lorna Dawson<sup>1,3,\*</sup>, Bob Mayes<sup>1</sup>, Rhys Williams<sup>2</sup>, James Periam<sup>2</sup>, Stuart Ramage<sup>1,3</sup> and Emily Hunter<sup>3</sup>

<sup>1</sup>James Hutton Institute, Centre for Forensic Soil Science

<sup>2</sup>Gwent Police

<sup>3</sup>RGU, Pharmacy and Life Sciences

\*Correspondence: lorna.dawson@hutton.ac.uk

Oral presentation

#### **Abstract**

In 2021, a male was killed through a brutal attack. There were no witnesses to this incident. Physical trace evidence was very limited. The suspect murder weapon was a broken fence post. The police force contacted the NCA to help find a wood specialist to compare some very small wooden fragments recovered from the suspect's footwear and from the victim's clothing, with wood from the splintered suspect murder weapon, which was found close to where the incident occurred. The NCA\* approached our laboratory to consider carrying out this work. After clarifying that chemical profiles of the wooden fragments was an acceptable approach, we developed, tested and applied a dual approach of both determining and comparing the inorganic and organic profiles of recovered trace fragments of wood. SEM EDXA (metal composition) and GC MS (hydrocarbons and fatty alcohols) was applied to chemically compare the trace evidence.

Results showed that the wooden fragments from the suspect's footwear contained chemical profiles which were very similar to the murder weapon and were different to the population of fence posts at the scene. The wooden fragments from the victim's clothing were very similar. Evidence was presented that the observations were much more probable if the material in the suspect's footwear originated from the suspect murder weapon rather than if the material in the suspect's footwear originated from the material in the fence post material at or around the locus were true. The accused was found guilty of the murder and given a life sentence.

\*NCA the National Crime Agency of the UK



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### Forensic investigation of hallucinogenic botanical traces

Arthur Wolterink<sup>1,\*</sup>, Monique Wesselink<sup>1</sup>, Stefan Uitdehaag<sup>1</sup>, Irene Kuiper<sup>1</sup>

<sup>1</sup> The Netherlands Forensic Institute

\*Correspondence: a.wolterink@nfi.nl

Oral presentation

#### Abstract

In the Netherlands the Opium Act contains four hallucinogenic plant species. The four plant taxa are: the genera *Erythroxylon* and *Cannabis* and the species *Papaver somniferum* and *Catha edulis*. It is prohibited to bring these taxa within or outside the territory of the Netherlands or perform actions with it (grow, prepare, treat, process, sell, deliver, provide or transport). There is a policy of tolerance for *Cannabis*.

In a forensic investigation the morphological characteristics of plant material can be used/examinated for identification. When plant material cannot be identified morphologically or to verify morphological identification, a DNA-analysis can be carried out.

In recent years, due to changes in the Opium Act and (possible) change in behaviour of drug dealers/producers, questions of investigative authorities have fluctuated considerably. Examples of recent forensic investigations at the department of non-human biological traces of the NFI, techniques and approaches used in order to meet investigative authorities demands, will be presented.



Riga, Latvia, April 23 – 25, 2024



#### Far Drowning – Persistence of diatoms in human tissues

D. Szűcs<sup>1</sup>, V. Fejes<sup>1</sup>, V.S. Poór<sup>1,\*</sup>

Department of Forensic Medicine, Medical School, University of Pécs, Hungary

\*Correspondence: viktor.s.poor@aok.pte.hu

Oral presentation

#### **Abstract**

Diatom testing is still a valuable and widely used ancillary method to confirm the diagnosis of drowning. During the struggle of drowning, waterborne organisms can enter the bloodstream through the alveolar membrane. The circulation then transfers and deposits these algae in various organs. Their presence in the organs of the systemic circulation corroborate the diagnosis of drowning.

But the question lingers: if the victim survives a drowning incident, how long would the silica shells persist? What about the PCR based detection techniques?

We would like to present cases, where the victims passed away following a near drowning accident. Samples were tested after proteinase K digestion. Diatom shells were detected with light microscopy, followed by PCR based detection of Cyanobacteria specific DNA.

While the relatively low sample size does not allow for sweeping conclusions, they can provide interesting insights to the preservation of these algae.



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#### Application of diatom analysis in forensic practice

Tomáš Hauer<sup>1</sup>, Jan Kaštovský<sup>1</sup>, Petr Tomášek<sup>2</sup>, Tomáš Bešta<sup>1</sup>, **Kateřina Zubíčková<sup>3,\*</sup>**, Radek Lefnar<sup>3</sup>, Iva Zagatová<sup>3</sup>, Petra Dohnalová<sup>2</sup>, Jana Markvartová<sup>2</sup>, Hana Hamrová<sup>1</sup>

- <sup>1</sup> Faculty of Science, University of South Bohemia in České Budějovice
- <sup>2</sup> Department of Forensic Medicine, Faculty of Medicine, University Hospital Bulovka, Charles University, 2<sup>nd</sup> Faculty of Medicine
- <sup>3</sup> Institute of Criminalistics

\*Correspondence: katerina.trumhova@pcr.cz

Oral presentation

#### Abstract

Diatoms are widespread microscopic algae with specific frustules. Inhalation of water with diatoms causes them to pass into the bloodstream and deposit their frustules in the internal organs. Their presence reveals the fact of drowning in natural water, and the analysis of the species composition can also help to reveal the exact place where the drowning occurred. Similar analysis can be carried out on the clothing and skin of drowned persons. Diatom analysis (and analysis of other algae) is used in the Czech Republic and abroad in forensic practice, but only to a limited extent and does not exploit the full potential of these organisms. The first results from the experiment showed surprising results and by testing different methods of the diatom isolation was possible to choose the one with the highest yield. The implementation of diatom analysis in criminal practice may lead to the establishment of additional evidence in criminal investigations.



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# Supporting the recovery of canine DNA from livestock attacks through rural community and victim engagement.

Nick Dawnay<sup>1,\*</sup>, Louise Dawnay<sup>2</sup>, S. McColl<sup>3</sup>

- <sup>1</sup> Forensic Research Institute, Liverpool John Moores University.
- <sup>2</sup> International Study Centre, Liverpool John Moores University.
- <sup>3</sup> School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University.

\*Correspondence: n.m.dawnay@ljmu.ac.uk

Poster presentation

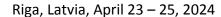
#### Abstract

Data from previous research shows that the number of livestock attack samples submitted by Police underrepresents the true number of livestock attacks in the United Kingdom. This is due to i) the crime being a 'non-recordable offence', ii) police may not be able to attend scene or cannot attend before the carcass is removed, iii) vets may attend scene but not collect a sample, and iv) the farmer may move and dispose the carcass before a sample has been collected.

These issues make it difficult to build a robust dataset to support the transition from current best practice methods to new national recommendations for collecting canine DNA at livestock attacks. Furthermore, it means that potential forensic evidence is going uncollected thereby missing an opportunity to match suspect canine to attacked livestock. The inclusion of vets and farmers in the evidence collection process through participatory research action is the next logical step to increase the number of submitted samples for research and future casework.

Here we present the Livestock And Wildlife Dog DNA Recovery Kit (L.A.W Dog Kit), a single kit containing all necessary components to allow farmer and veterinary sample at attack sites in the absence of Police presence. The kit and collection methods will soon undergo UK field trials with data being collected to explore the future potential of these groups collecting admissible evidence for criminal casework.







# Sex detection in Cervidae species using loop-mediated amplification (LAMP) technique and a 3D printed device

V.S. Poór<sup>1</sup>, O.K. Zorkóczy<sup>2</sup>, P. Lehotzky<sup>3</sup>, and **P. Zenke<sup>2,\*</sup>** 

<sup>1</sup>Department of Forensic Medicine, Medical School, University of Pécs

<sup>2</sup>Department of Animal Breeding and Genetics, University of Veterinary Medicine Budapest

<sup>3</sup>Game Protection and Management Committee of the Pest County of the National Chamber of Hungarian Hunters, Budapest

\*Correspondence: <u>zenke.petra@univet.hu</u>

Poster presentation

#### Abstract

Determining sex in Cervidae species is crucial to both wildlife management and forensic investigations. Wildlife crimes cover many illegal activities, including out-of-season hunting. In certain periods of the year, hunters can only acquire a license to shoot female deer exclusively. The eviscerated and decapitated carcass of the animal delivered to the wild game processing house cold store can be falsely registered as a female based on phenotype. If the hunting association suspects that the animal's sex has been falsely reported, a forensic genetic investigation can be proposed. While existing genetic sexing methods have proven effective, they often involve time-consuming and resource-intensive processes. In response to the growing need for a quick and cost-effective technique, we present the development of a novel LAMP method for sex detection. Primers were specifically designed for Cervidae based on sex chromosome-located genes. Analytical specificity was confirmed using red-, roe-, and fallow deer, and species specificity was also tested in other deer species. Sex determination results were fast and accurate. The advantage of this technique is that it allows researchers to identify the sex of different deer species with the same method. To simplify the tests and make them suitable for field application, we also developed a compact 3D printed device capable of facilitating each step of the workflow, such as vortexing and centrifugation during the preparation of reaction mixtures, and maintaining stable temperatures for amplification. Constant low-speed centrifugation ensures homogeneous heat distribution between samples, and detection is solved with built-in UV and visible blue light.



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#### The road between a leg and an arm

Ricardo Honório<sup>1</sup>, Diogo Gonçalves<sup>1</sup> and **Ana Cristina Assis**<sup>1,\*</sup>

<sup>1</sup> Forensic Science Laboratory of Judiciary Police, Chemistry and Physics Unit

\*Correspondence: ana.assis@pj.pt

Poster presentation

#### Abstract

Soil identification and characterisation plays a valuable role in the field on forensic analysis. Soil samples can be extremely diverse and heterogeneous, with visible differences in their morphology, colour, water and organic content, and anthropogenic contaminants among other defining characteristics, as such, the analytical methods chosen for each case may vary.

In this presentation we will discuss a casework, where we explored the characterisation of a problem soil sample collected from the rear wheel panel of the vehicle of a murder suspect, and compared this sample with some collected from the woody area surrounding each body part found of the dismembered victim.

The chosen approach to characterise all collected samples was a morphological characterisation, in which the particle sizes and mineral colours were observed, a pH analysis, a Energy Dispersive X-Ray Fluorescency (EDXRF) bulk analysis. We also performed a colour comparison via the Munsell scale, identifying the notation colour for the problem sample and the references samples. In order to ease the process of identifying the correct Munsell notation for each sample, we deliberately unfocused a photographic camera lens to blur the edges of the depicted image. When the edge between the Munsell chart chip and the sample placed below was indistinguishable, that colour was considered as the average value for that sample.

Through the previously mentioned analysis, we observed a high degree of similarity between the problem and reference samples.



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# Exploring the impact of environmental variability and clothing type on diatom trace evidence transfer in coastal locations

Alice Stevens<sup>1,2</sup> and Kirstie R Scott<sup>2,3\*</sup>

- <sup>1</sup> School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University
- <sup>2</sup> Forensic Research Institute, Liverpool John Moores University
- <sup>3</sup> School of Biological & Environmental Sciences, Liverpool John Moores University

\*Correspondence: k.r.scott@ljmu.ac.uk

Poster presentation

#### Abstract

Diatoms (*Bacillariophyceae*) are useful trace evidence indicators in forensic investigations. Despite growing research exploring diatom transfer and persistence on clothing/footwear, previous studies typically focus on freshwater locations. Criminal activities may also involve coastal environments: marine diatoms may therefore yield valuable circumstantial evidence, necessitating empirical research within such contexts. This study compared the diatom profile of five coastal locations in north-west England, assessed the extent and similarity of a transferred assemblage, and considered the impact of clothing type on evidential transfer.

Five marine environments were sampled including tidal beaches, marine lakes, and an estuary. Three clothing materials (cotton, polyester, acrylic) were immersed in each, facilitating diatom transfer. Diatoms were extracted using  $H_2O_2$  and analysed microscopically. Valves were counted and identified to genus, supporting quantitative and qualitative data analysis. Results demonstrated environmental differences, with significantly greater diatom abundance and species richness in dynamic tidal locations. This was reflected in the corresponding transfer samples. Clothing type significantly impacted transfer; fewer diatoms were retrieved from cotton than polyester or acrylic. Distinctive genera were identified at each site including *Licmophora*, *Plagiogramma*, *Scoliopleura*, *Biddulphia*, and *Actinocyclus*. Multivariate analysis identified environmental differences in the overall diatom assemblage; this was consistent amongst the corresponding transfer samples. This study presents a novel empirical analysis of marine diatoms in a forensic context. The findings indicate that diatoms offer a useful exclusionary tool to support crime reconstructions in coastal environments; further research is recommended to support the collection, analysis, and interpretation of diatom trace evidence in forensic investigations.







# DNA metabarcoding: can it reliably be used to characterize pollen from surface soils?

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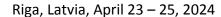
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Poster presentation

#### Abstract

Geologic materials such as surface soils are important types of trace evidence as they are transferred during criminal acts, possess unique characteristics, and are often overlooked by perpetrators. Pollen grains are ubiquitous in the environment year-round and are nearly always present in surface soils. In casework, pollen can provide valuable probative information on sample origin and assist in sample-tosample comparisons. Despite the utility, pollen analysis is not routinely completed on evidence, as it is time consuming, requires specialized expertise and the quantity of material needed varies on sample type. High throughput DNA sequencing approaches, such as DNA metabarcoding, have been successfully applied to characterize pollen in bulk environmental samples, but have yet to be applied in a forensic context. Before DNA metabarcoding could be implemented into casework, a side-by-side comparison with results generated using traditional morphological approaches is needed. This study is focused on completing this comparison using 250 diverse surface soils collected across the continental U.S. For each sample, pollen has been characterized two ways: 1) sequencing two commonly used plant DNA metabarcoding regions (ITS2 and trnL), and 2) using traditional morphological techniques. We will present the results we have generated to date for >75 samples, focusing on whether DNA metabarcoding recovered the same taxa and relative abundance when compared to morphological results. Additionally, we will outline a cost and time effective protocol to purify pollen grains from herbarium material, which provides a straight-forward approach to creating mock standards for proficiency testing or inclusion as positive controls in casework.







# Protein fingerprinting as a tool to authenticate fish products in the food industry

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Poster presentation

#### Abstract

Reliable species identification of specimens in the food chain is crucial for detecting mislabelling fraud. While visual identification and DNA-analyses are the predominant methods for species identification, this research provides an alternative protein-based technique that has been trialled here to identify tissue samples from species within the order Pleuronectiformes (flatfish). Previously, archaeological research has used a mass spectrometry-based technique called 'protein fingerprinting' to identify fish remains to species-level by extracting collagen Type I preserved in bones. As this type of collagen can also be found in the fins, skin and muscle of fishes (types of tissue that can be found throughout the food chain), collagen fingerprinting has been applied here to identify modern fish specimens to species-level. In this study, MALDI-ToF MS protein fingerprints are obtained from enzymatic digestion of collagen Type I from fin (or skin if fin was not available) and muscle tissues from six specimens of Pleuronectiformes. Using diagnostic collagen peptide biomarkers for flatfish, all six specimens could be identified to the correct species for each of the tissue types. We recommend using this rapid approach for future screenings of Actinopterygian fishes in the food chain to detect mislabelling fraud. Additionally, protein fingerprinting is likely to be a valuable tool for other forms of wildlife forensic analysis where DNA preservation is challenged, including both animal and plant derivatives.







#### New developments in forensic soil investigation

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Soil workshop

#### Abstract

This soil workshop is a forum for all the new developments that are either just too small for a full presentation or not quite ready enough to present on a poster. Participants are asked to share their new developments or innovative gadgets with colleagues within the forensic soil community. There is also room for a round table discussion on Soil Collaborative Exercises; what do we want to test or exercise with? We will also discuss data assessments and forensic soil evaluation, including the use of Bayes. In addition, participants are encouraged to bring forensic problems to the workshop that they would like feedback on. Lastly, participants can bring pictures of unknown particles/objects they found in their soil samples and hopefully we can help to identify them. The soil workshop will end with a short verbal survey on what participants would like to hear about or discuss in the next soil workshop.



Riga, Latvia, April 23 – 25, 2024



# What is growing on my fields? The detection of agricultural hemp by prediction of the chemical phenotype

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nhDNA workshop

#### **Abstract**

In Europe, more than 100 approved cultivars of fiber hemp (*Cannabis sativa* L.) are in agricultural production. Their content of psychoactive tetrahydrocannabinol (THC) is legally restricted to < 0.2%. Cannabis strains with much higher THC contents are also grown, illegally or under license for drug production. Differentiation between these two groups relies on biochemical quantification of cannabinoid contents in mature floral material. For nonflowering material or tissue devoid of cannabinoids, the genetic prediction of the chemical phenotype (chemotype) provides a suitable method of distinction. We report about pitfalls of -and helpful tools for this assay.



Riga, Latvia, April 23 – 25, 2024



#### Classification, identification or comparative analysis?

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nhDNA workshop

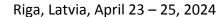
#### **Abstract**

From a biological point of view, species (or taxon) identification is a fairly innocent description of an often occurring task: naming a biological sample that is not recognized to a sufficient level by requesting parties. However the word 'identification' is used in different ways by different scientists, especially within forensic science. Additionally other words can be used to describe comparable processes with different associated values, guidelines or even regulations.

How does the chemical classification of a substance differ from the biological classification of a sample? Does it?

When does classification shift to identification? Could classification of a certain taxonomic level equal identification of higher taxonomic level? And what defines when classification/identification of two samples to the same group should be considered a comparative analysis? Are the ENFSI guidelines for reporting on comparative analyses of added value in such cases? We would like to discuss some alternative perspectives we have encountered in the last years and share our perspective on this topic. And discuss this topic within the APST community to learn from experiences from other institutes, countries and legal systems.







# Developing and testing the end user requirements for a novel, field based environmental DNA (eDNA) forensic method

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Oral presentation

#### **Abstract**

eDNA refers to the DNA found in environmental soil, water or air which can be isolated from environmental samples. Intracellular, intraorganellar, dissolved or particle adsorbed DNA in these samples can originate from skin, mucous, saliva, secretions, sex cells, waste products, blood, plant materials, rotting bodies and entire microorganisms. eDNA has become a rapidly emerging ecological survey method due to its time efficiency and sensitivity.

Freshwater pearl mussels (*Margaritifera margaritifera*) are listed on the IUCN red list as critically endangered. Crimes including habitat destruction, water pollution and pearl harvesting are the most frequently cited illegal activity that threaten the species in the United Kingdom. eDNA analysis methods have the potential to support criminal investigations by demonstrating the presence of the species at the site of the crime. However, operationalizing eDNA as a tool in forensic casework requires end-user feedback and an assessment of the accuracy of the basic approach.

Here we present end-user feedback data that compares three different eDNA collection methods (basic, intermediate, advanced). End-user groups including police, specialist investigators, and eDNA surveyors were asked to watch videos of each method and consider the *ease-of-use* and *associated risk* when sampling. Further data on *operational speed*, *health and safety*, and *data quality* were collected. Together the data demonstrates that methods widely cited in research were not easily transferable to forensic casework with the best data quality observed from the cheapest, easiest and quickest method. The research also shows the potential use of proximity markers for localizing species using eDNA.



Riga, Latvia, April 23 – 25, 2024



# New tool in the belt – analysis of environmental DNA associated with geologic evidence

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Oral presentation

#### **Abstract**

Geologic materials are often submitted to crime laboratories and can be used to link an individual to a crime or to determine provenance. Forensic geologists that analyze these materials aim to characterize physical properties (e.g. color and pH) and inorganic components (e.g. mineral content). However, sample size is often a limiting factor; supplemental methods requiring a small amount of material as input could provide additional evidentiary information. DNA metabarcoding is commonly used to identify biological taxa present in environmental samples by amplifying and sequencing short, yet informative, regions of the genome and is not restricted by sample size. The goal of this research was to determine the utility and stability of environmental DNA associated with mock soil and dust evidence for sampleto-sample comparisons. In this study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a one-year period. DNA metabarcoding and downstream bioinformatic processing was utilized to characterize bacteria (16S), fungi (ITS1), arthropods (COI), and plants (ITS2, trnL) associated with each sample (n, 1026). Important findings from this research include: 1) despite low DNA concentrations of dust samples, it is still possible to characterize biological communities, 2) DNA metabarcoding could successfully recover diverse taxa associated with mock evidence, and 3) changes in the biological communities were observed over time and between locations. This presentation will also include a preliminary assessment of the impact of temporal and spatial variables on the recovery of bacteria, fungi, arthropods, and plants from mock geologic evidence.



Riga, Latvia, April 23 – 25, 2024



### Results of the ENFSI APST Forensic Geology CE 2023

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Oral presentation

#### **Abstract**

The Hungarian Institute for Forensic Sciences, Department of Physics and Chemistry organized the Forensic Geology Collaborative Exercise 2023, for the APST Community. The objective of the CE was to find the different one from three sediment (clay) samples. One of the samples was treated artificially with Pb- and Cu-nitrate solution, to make different it's elemental- és mineralogical composition, that could be identified using instrumental analytical methods. The test was successful, nearly all the laboratories found the matching/different samples.







### Summary of the ENFSI-APST 2023 Non-human DNA Proficiency Test

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<sup>1</sup>Institution of SASA, Wildlife DNA Forensics

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Oral presentation

#### Abstract

The aim of the non-human DNA (nhDNA) proficiency test was to demonstrate that the laboratory taking the test was capable of identifying three unknown non-human samples using molecular methods. To do this, the lab had to identify the samples to as fine a taxonomic level as possible using the molecular tests available in their laboratories. The minimum level of identification accepted was to family.

Fourteen laboratories from eleven countries participated in the test, with results reported to either genus or species level for all three samples. This talk will provide a summary of methods used and results obtained by the test participants.



Riga, Latvia, April 23 – 25, 2024



### Summary of "APST CE Botany 2023" results

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Oral presentation

#### Abstract

This collaborative exercise is designed for a botanical taxon identification via morphological examination, however the samples that were not mounted on glass slides were suitable for DNA extraction and nhDNA plant species identification. The aim is to identify each sample – Item 1 to item 11. Each sample consists of one specimen.

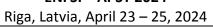
The aim of the test was to carry out routine sample identification of typical forensic botany traces (pollen, spores, seeds, whole plant, wood chips etc.) and to perform morphological examination.

The test consisted of 5 microscopic slides as well as seeds, whole plant, and a wood specimen, in total of 11 samples, due to tight deadline some of the items were optional part of the CE.

There were no case scenario and no interpretation part as CE. Objective of the test was specimen classification and recognition of the limits of morphological examination.

Presentation will cover discussion and methodology for less common types of samples and address the results of the participants.







# **Participants**

FIRST NAME	SURNAME	Institution	COUNTRY
Ana	Assis	FORENSIC SCIENCE LABORATORY, JUDICIARY POLICE	Portugal
BALAZS	EGYED	EÖTVÖS LORAND UNIVERSITY BUDAPEST	Hungary
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RADEK	LEFNAR	Institute of Criminalistics	CZECH REPUBLIC
KAROLINA	Mahlerová	INSTITUTE FOR ENVIRONMENTAL STUDIES, CHARLES UNIVERSITY	CZECH REPUBLIC
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KELLY	MEIKLEJOHN	NORTH CAROLINA STATE UNIVERSITY	USA
Nadja	Morf	Institute of Forensic Medicine Zurich	SWITZERLAND
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Maria Alejandra	PEROTTI	University of Reading	UNITED KINGDOM
VIKTOR	Poór	University of Pécs, Dep. of Forensic Medicine	HUNGARY
FRANK	RECKEL	BAVARIAN STATE CRIMINAL POLICE OFFICE	GERMANY
Māra	RĒPELE	STATE FORENSIC SCIENCE BUREAU	LATVIA
PAVLA	RIHOVA	ENVIRONMENTAL FORENSIC SCIENCES CENTRE	CZECH REPUBLIC
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Jodi	WEBB	FEDERAL BUREAU OF INVESTIGATION LABORATORY	USA
MONIQUE	WESSELINK	NETHERLANDS FORENSIC INSTITUTE	THE NETHERLANDS
ARTHUR	Wolterink	NETHERLANDS FORENSIC INSTITUTE	THE NETHERLANDS
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