83rd International Scientific Conference of the University of Latvia 2025

Innovative and Applied Research in Biology

UNIVERSITY OF LATVIA | FACULTY OF MEDICINE AND LIFE SCIENCES

Thursday, 6 March 2025, 10.00 AM
Riga, Jelgavas street 1, room 702

Program and Abstracts

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Riga, Jelgavas street 1, room 702
and Zoom platform

Program

9:30–10:00		**Registration**
Chair: Prof., Dr. hab. biol. Īzaks Rašals		
10.00–10.05	**Arturs Stalažs** Institute of Biology, Faculty of Medicine and Life Sciences (FMLS), University of Latvia	**Opening**
O1 **10.05–10.25**	**Algimantas Paulauskas** Vytautas Magnus University	Application of molecular methods in zoology in Baltic countries
O2 **10.25–10.40**	**Jana Radzijevskaja** Vytautas Magnus University	Detection and phylogenetic characterization of tick-borne encephalitis virus in Lithuania
O3 **10.40–10.55**	**Asta Aleksandravičienė** Vytautas Magnus University	Genetic variability of American mink (*Neovison vison*) in Lithuania
O4 **10.55–11.10**	**Dalius Butkauskas** Nature Research Centre	3D biotextile manufacturing and assessment of protective properties
11.10–11.45	**Coffee break**	
Chair: Prof., Dr. hab. biol. Dalius Butkauskas		
O5 **11.45–12.00**	**Ieva Ignatavičienė** Nature Research Centre	Impact of amber nanoparticles and its derivatives on *Lemna minor* clone affected by low frequency electromagnetic radiation (50 Hz)
O6 **12.00–12.15**	**Žaneta Maželienė** Faculty of Medicine, Kauno Kolegija Higher Education Institution	Antimicrobial efficacy of different *Rosa rugosa* parts extracted using aqueous and ethanolic extract

O7 **12.15–12.30**	**Laura Āboliņa** Institute of Biology, FMLS, University of Latvia	Shade treatment effect on chlorophyll content and growth of cloudberry *Rubus chamaemorus*
O8 **12.30–12.45**	**Irina Sivicka** Latvia University of Life Sciences and Technologies	Growth and yield performance of carrot (*Daucus carota L.*) influenced by treatments with microalgae extracts
O9 **12.45–13.00**	**Ktristaps Neiberts** Institute of Biology, FMLS, University of Latvia	Utilizing cheese whey for mixotrophic cultivation of microalgae *Graesiella emersonii*: Improving lipid and Omega-3 polyunsaturated fatty acid content in poultry diets
13:00–14:00	**Lunchbreak***	
Chair:	**Prof., Dr. hab. biol. Īzaks Rašals**	
14:00–15:25	**Short poster presentations (3 min), questions (2 min)** https://ej.uz/pcuq	
P1	**Laura Nareckaitė** Vytautas Magnus University	Application of molecular biology methods for the detection and genotyping of *Babesia canis*
P2	**Saulius Bernotas** Research Institute of Natural and Technological Sciences, VMU	Molecular identification of *Borrelia burgdorferi sensu lato* in ticks in Kaunas city, Lithuania
P3	**Martynas Jonkus** Vytautas Magnus University	Molecular screening for tick-borne encephalitis virus in ticks from city environments
P4	**Evelina Genevičiene** Vytautas Magnus University	The role of anthropogenic pressures in shaping the genetic of red deer (*Cervus elaphus*)
P5	**Donata Grikštaitė** Nature Research Centre	Development of cost-effective molecular method to discriminate different *Listeria monocytogenes* strains in food processing sector
P6	**Paulina Maceinaitė** Vytautas Magnus University	Genetic diversity of *Bartonella* spp. in Lithuanian bats based on gltA gene sequences
P7	**Justina Dagytė** Vytautas Magnus University	Application of Enzyme-Linked Immunosorbent Assay for the detection of antibodies against measles virus
P8	**Ugnė Medikaitė** Vytautas Magnus University	Application of molecular methods for the detection of Lyme disease agents
P9	**Ramunė Tamošiūnaitė** Nature Research Centre	Preliminary results on muscle tissues parasites of mustelids in Lithuania
P10	**Eglė Rudaitytė-Lukošienė** Nature Research Centre	*Sarcocystis* species richness in the diaphragm and myocardium of alpine ibex (*Capra ibex*)

P11	**Dovilė Laisvūnė Bagdonaitė** Nature Research Centre	Genetic characterisation of *Sarcocystis* cysts isolated from muscles and brain of voles from Lithuania
P12	**Evelina Slavnova** Institute of Biology, FMLS, University of Latvia	Optimization and challenges in DNA extraction from *Tetrao urogallus* feathers
P13	**Aija Rebāne** Institute of Agriculture, LBTU	Breeding of tetraploid red clover in Latvia
P14	**Dace Grauda** Institute of Biology, FMLS, University of Latvia	Assessment of antimicrobial properties of 3D biotextile with incorporated nanoparticles.
P15	**Irina Sivicka** Latvia University of Life Sciences and Technologies	Evaluation of rust and powdery mildew development in *Mentha ×piperita* and *Mentha spicata* under *ex situ* collection
P16	**Tomass Tumpelis** Institute of Biology, FMLS, University of Latvia	Phenols and flavonoids in microalgal extract for agricultural biostimulants. A comparative study of extraction methods
P17	**Līga Jankevica** Institute of Biology, FMLS, University of Latvia	Research on insect baculoviruses in Latvia: past and future
15:25 – 16:00	**Coffee break**	
Chair: Prof., Dr. biol. Dalius Butkauskas		
O10 **16:00 – 16:15**	**Zoya Tsybouskaya** Vytautas Magnus University	The impact of pyrethroid insecticides on non-target Coleoptera
O11 **16:15 – 16:30**	**Elmīra Boikova** Institute of Biology, FMLS, University of Latvia	The nature reserve of the lake Burtnieku floodplaine meadows
O12 **16:30 – 16:45**	**Oskars Keišs** Institute of Biology, FMLS, University of Latvia	Common starling (*Sturnus vulgaris*)—as a species for citizen science and fundamental research
16:45 - 17:30	**Discussions & Conclusions**	

*Lunch is not provided. There is a cafe on the 1st floor where you can have a hot lunch.

Innovative and Applied Research in Biology

Abstracts

Oral presentations

O1

Application of molecular methods in zoology in Baltic countries

Algimantas Paulauskas

Vytautas Magnus University, K. Donelaičio Str. 58, LT-44248 Kaunas, Lithuania

E-mail: algimantas.paulauskas@vdu.lt

Contemporary tools of molecular biology open new possibilities in zoology and provide important answers to classic problems. Zoological questions of mating strategies, physiological adaptation, identification of life-cycle stages, genetic exchange between populations, analysis of hybridization, population structure in "typical" sexual organisms, measuring inbreeding, species/subspecies identification (a technique for species identification of parasites without the need for separation from the host), determination of geographical origin, and many others are now being powerfully addressed using tools from the molecular arsenal. Molecular genetic techniques are clearly having an impact on what have been traditionally considered to be purely zoological studies. Applications in Baltic countries are discussed.

Key words: molecular methods, zoology, application

O2

Detection and phylogenetic characterization of tick-borne encephalitis virus in Lithuania

Jana Radzijevskaja*[1,] Algimantas Paulauskas[1]

Research Institute of Natural and Technological Sciences; Vytautas Magnus University, Lithuania

E-mail: jana.radzijevskaja@vdu.lt

The Baltic states are the region in Europe where tick-borne encephalitis (TBE) is most prevalent. Lithuania has the highest notification rate of TBE cases, with a notable increase in incidence since 1992. A recent study reported a 0.4% TBE virus (TBEV) prevalence in Lithuania's two most common tick species, *Ixodes ricinus* and *Dermacentor reticulatus*. TBEV was detected in the three developmental stages (adults, nymphs, and larvae) of *I. ricinus* and *D. reticulatus* adults. TBEV-infected ticks were found in 16 locations in seven counties, with prevalence ranging from 0.1 % to 1.0 %. In Lithuania, TBEV was also detected in different rodent species, different breeds of dogs, horses, and goat and sheep milk samples. While TBE is a significant health issue in Lithuania, detailed information about the local virus strains and their genetic variability is still limited. The present study compared nucleotide sequences of the E and NS3 genes of 39 TBEV strains isolated from ticks, rodents, and humans in Lithuania over a period of 20 years. Phylogenetic analysis of the partial E and NS3 gene sequences from the TBEV isolates indicated that these strains were specific to Lithuania and belonged to the European subtype, exhibiting significant regional genetic diversity. The analysis showed that certain TBEV strains were specific to particular regions. In most cases, TBEV strains from the same sampling site were identical; however, genetic differences in the E and NS3 gene sequences were noted among strains from different geographical areas of Lithuania. The detected TBEV genotypes were not specific to the tick species. TBEV has a focal distributional pattern in endemic areas in Lithuania, as the virus is not uniformly present in the tick population. The present study indicates that at least six distinct virus lineages are circulating among ticks and two among rodents in the country. Further research involving whole-genome sequencing is necessary to enhance the understanding of the regional genetic diversity of TBEV strains.

Key words: Tick-borne encephalitis, TBE virus, genetic diversity, Lithuania

O3

Genetic variability of American mink (*Neovison vison*) in Lithuania

Daiva Šakienė, **Asta Aleksandravičienė,** Loreta Griciuvienė, Algimantas Paulauskas*

Faculty of Natural Sciences, Vytautas Magnus University, K. Donelaičio Str. 58, LT-44248 Kaunas, Lithuania

*Correspondence: algimantas.paulauskas@vdu.lt

The American mink (*Neovison vison*), a species native to North America, has been successfully introduced and established in the wild across Europe. As a result, it has largely displaced the native European mink (*Mustela lutreola*). In Lithuania, the American mink has also been observed to negatively impact native wildlife, including small mammals, fish, and amphibians, while contributing to broader ecological imbalances and disrupting local biodiversity.

The aim of this study was to assess the genetic variability of different American mink (*Neovison vison*) subpopulations in Lithuania. The tissue samples of *N. vison* were collected from three Lithuania localities: Alytus, Šilutė, and Zarasai. Genomic DNA was extracted from tissue samples using the “Genomic DNA Purification Kit” (Thermo Fisher Scientific, Lithuania) following the manufacturer's instructions. To assess the genetic diversity of the American mink, six microsatellite markers were used.

The results showed that, within each subpopulation, observed heterozygosity was higher than expected heterozygosity, suggesting possible factors such as genetic admixture, or population bottlenecks. Among the populations studied the Alytus subpopulation displayed the lowest genetic diversity, while the Šilutė subpopulation showed the highest. Principal Coordinates Analysis (PCoA) revealed notable genetic differentiation between the Alytus and Zarasai subpopulations, highlighting significant genetic distances between these geographically separated groups. Additionally, the genetic structure of the Šilutė subpopulation showed similarities to both the Alytus and Zarasai subpopulations. This pattern suggests gene flow or shared ancestry, despite the geographical separation by different river basins (Nemunas River and Dauguva River basins, respectively). These results provide valuable insights into the genetic diversity and population structure of introduced *N. vison* in Lithuania, which may inform future management and conservation strategies aimed at mitigating their ecological impact on native species.

Key words: Lithuania, genetic variability, *Neovison vison*, microsatellite markers

O4

3D biotextile manufacturing and assessment of protective properties

Dalius Butkauskas[1], Dace Grauda[2] , Inga Lashenko[3]

[1]Nature Research Centre, Akademijos Street 2, Vilnius, Lithuania; [2] Institute of Biology, FMLS, University of Latvia, Jelgavas street 1, Riga, Latvia; [3]JLU Technologies Ltd, Ilukstes street, 107/1-16, Riga, Latvia

E-mail: dalius.butkauskas@gamtc.lt

The development of 3D textile models for biological testing began with the production of biotextile material by project partner "A Grupe" (Jonava, Lithuania), a company specializing in linen fabrics and products. To enhance the protective properties of these newly created biotextiles, which consisted of high-quality, 3D fiber composite variants, the materials were impregnated with an aqueous solution. This solution contained 0.5% polyethylene oxide, 0.5% hydroxyethyl cellulose, and 0.5-1% silicone. The resulting polymer structures coating the fabrics also incorporated succinite particles (or succinic acid derivatives) in some versions, along with SiO_2 and Ag nanoparticles in others, creating a range of different polymer matrices.

The impregnation procedures and production of the various biotextile versions were carried out at "JLU Technologies" (Riga, Latvia). Biological testing, focused on the protective properties of the biotextile materials, was conducted at the Laboratory of Environmental Genetics of the FMLS Institute of Biology University of Latvia and the Laboratory of Molecular Ecology of the Nature Research Centre (Lithuania). Common duckweed (*Lemna minor*) and fruit flies (*Drosophila melanogaster*) were chosen as the primary test organisms due to their cost-effectiveness, reproducibility, and reliability in experiments.

To investigate the protective properties of the biotextiles, we examined the effects of low-frequency electromagnetic radiation (LF EMF, 50 Hz, 1.3 mT magnetic flux density) on the physiological and molecular levels of the test organisms. *L. minor* (diploid laboratory line Sta2 axenic culture) plants were grown in Petri dishes wrapped in different biotextile versions and placed within a Helmholtz coil. Growth rate, number of fronds, and total frond area increase per week were measured. After one week, DNA was collected to assess the potential of LF EMF to induce point mutations in genes involved in oxidative stress regulation. Nuclear ascorbate peroxidase (APx) and catalase (Cat) gene fragments, along with selected chloroplast microsatellite loci, were sequenced.

Results showed a reduction in point mutations in *L. minor* Sta2 clones grown with biotextiles containing 0.25%, 0.5%, and 1% succinite nanoparticles compared to the control group (biotextile without succinite). Conversely, biotextiles containing 0.5% succinic acid or Ag nanoparticles showed no positive effect on mutation reduction and significantly suppressed growth intensity, leading to increased point mutations in the APx and Cat gene fragments and microsatellite loci, and decreased frond area.

The positive effect of succinite nanoparticles, particularly at low concentrations, in mitigating point mutations contrasted with the opposite effect of succinic acid. The biotextile containing 0.5% succinite, 0.1% SiO_2, and 0.1% Ag nanoparticles highlighted the importance of nanoparticle origin and concentration, as well as the sensitivity of the *L. minor* Sta2 model, demonstrating its potential for biotesting applications.

In conclusion, 3D biotextile versions incorporating succinite nanoparticles (0.25% to 1%) within the polymer fabric coating demonstrate significant potential as protective biotextiles, mitigating the effects of LF EMF and potentially other stressors.

Acknowledgements: The study was financially supported by the S-M-ERA.NET-22-1 project 3DNano-HPC.

Keywords: succinite nanoparticles, succinic acid, SiO_2, Ag nanoparticles, *L. minor*, low frequency electromagnetic field 50 Hz, ascorbate peroxidase, catalase, microsatellite loci, point mutations

O5

Impact of amber nanoparticles and its derivatives on *Lemna minor* clone affected by low frequency electromagnetic radiation (50 Hz)

Ieva Ignataviciene[1*], Regina Vyšniauskienė[1], Vida Rančelienė[1], Rimantas Petrošius[1], Dace Grauda[2], Dalius Butkauskas[1]

[1] *Nature Research Centre, Akademijos street 2, Vilnius, Lithuania;* [2]*Institute of Biology, FMLS, University of Latvia, Jelgavas street 1, Riga, Latvia*

* E-mail: ieva.ignataviciene@gamtc.lt

Textile technologies have recently attracted great attention as potential tools for biomedical application or protection from stressful environments. Some natural organic compounds with fungicidal and antibacterial properties can be included in the silicon structure covering bio-textile materials to enhance their functional properties. Although it is known that amber or its derivatives have healing properties, the effect on living organisms remains unanswered. In this work the effect of 3D bio-textile with incorporated 0.25%, 0.5%, and 1% concentration of amber, succinic acid, succinic acid with SiO_2 and combining 0.5% amber, 0.1% SiO_2 and 0.1% Ag nanoparticles were investigated. In the study, plants of *L. minor* laboratory clone Sta2 grown in a Petri dishes wrapped within 3D bio-textile specimens embedded with different nanoparticles were exposed to 50 Hz electromagnetic radiation for seven days. After the termination of the experiment, *L. minor* plants were examined the frond area and molecularly by sequencing nuclear ascorbate peroxidase (APx), catalase (Cat) gene fragments and some chloroplast microsatellite loci. In most cases a reduction of point mutations and an increment of frond area was detected in *L. minor* clones, grown coated with bio-textile encompassing 0.25%, 0.5%, and 1% amber nanoparticles compared to control group that was grown coated with the same bio-textile material that doesn't contain amber particles. The opposite effect was observed by adding 0.5% succinic acid or 0.1% Ag nanoparticles into 3D bio-textile, as the number of point mutations significantly increased APx, Cat gene fragments and microsatellite loci, and the frond area decreased in comparison to the control group. Our results demonstrate a positive effect of amber nanoparticles, especially of low concentrations of amber promoting growth and lowering number of point mutations. The opposite negative effect of succinic acid alone and composition of 0.5% amber, 0.1% SiO_2 and 0.1% Ag incorporated into the bio-textile, enhancing the number of point mutations and decreasing growth of the *L. minor* clones exposed to electromagnetic radiation. The 3D bio-textile containing amber nanoparticles have significant potential to be used as a protective tissue in stressful conditions.

Acknowledgements: The study was financially supported by the S-M-ERA.NET-22-1 project 3DNano-HPC..

Keywords: 3D bio-textile, amber nanoparticle, succinic acid nanoparticle, silver nanoparticle, 50 Hz electromagnetic radiation

O6

Antimicrobial efficacy of different *Rosa rugosa* parts extracted using aqueous and ethanolic extract

Žaneta Maželienė [1,3*], Jolita Kirvaitienė [1,2], Giedrė Jarienė [1], Asta Aleksandravičienė [1,4], and Rasa Volskienė [1]

[1] Faculty of Medicine, Kauno Kolegija Higher Education Institution, Pramones pr. 20, LT-50468 Kaunas, Lithuania; [2] Faculty of Public Health, Lithuanian University of Health Sciences, A. Mickevičiaus street. 9, LT-44307 Kaunas, Lithuania; [3] Faculty of Veterinary Medicine, Institute of Microbiology and Virology, Lithuanian University of Health Sciences, A. Mickevičiaus street 9, LT-44307 Kaunas, Lithuania;[4] Faculty of Natural Sciences, Vytautas Magnus University, K. Donelaičio street 58, LT-44248 Kaunas, Lithuania

* E-mail: zaneta.mazeliene@go.kauko.lt

With the increasing prevalence of drug-resistant pathogens, the search for alternative antimicrobial agents has intensified. Bioactive compounds derived from medicinal plants have gained significant attention as they offer a promising natural source of novel antifungal and antibacterial agents with potentially fewer side effects compared to synthetic drugs. *Rosa rugosa* has been traditionally used in herbal medicine for its diverse therapeutic properties.

The aim of this study was to evaluate the antimicrobial potential of aqueous and ethanolic extracts obtained from various parts of *Rosa rugosa*, including rose hips, flowers, petals, leaves, stems, and roots. The antimicrobial efficacy of the extracts was assessed using agar well diffusion method under controlled laboratory conditions. To ensure extract purity and enhance the concentration of active compounds, a rotary evaporator was employed for solvent removal. The antimicrobial activity was tested against a panel of microbial strains, including *Candida albicans* (a common fungal pathogen), four Gram-positive bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Listeria monocytogenes*), and four Gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Klebsiella pneumoniae*). The results indicated that extracts from different plant parts exhibited varying degrees of antimicrobial activity. *Rosa rugosa* leaf extracts demonstrated the highest antimicrobial effectiveness, followed by petal and rose hip extracts, while root extracts showed moderate activity. In contrast, the weakest antimicrobial effects were observed in extracts derived from stems and rose hips. The study also revealed that ethanolic extracts had superior antimicrobial activity compared to aqueous extracts, highlighting the potential of ethanol as a more effective solvent for extracting bioactive compounds. Both aqueous and ethanolic extracts exhibited notable antifungal activity against *Candida albicans*, with ethanolic extracts proving to be more potent in inhibiting fungal growth. Among all tested plant parts, petals exhibited the strongest antifungal properties, suggesting a high concentration of antifungal compounds in these tissues.

In conclusion, this study provides valuable insights into the antimicrobial potential of *Rosa rugosa* extracts and underscores their potential use in developing natural antimicrobial agents. Further research is needed to isolate and characterize the specific bioactive compounds responsible for the observed effects and to explore their possible applications in the pharmaceutical and food industries.

Key words: antimicrobial efficacy; *Rosa rugosa*; aqueous extract, ethanolic extract

O7

Shade treatment effect on chlorophyll content and growth of cloudberry *Rubus chamaemorus*

Laura Āboliņa[1,2*], Andis Karlsons[2], Anita Osvalde[2]

[1]Latvia University of Life Sciences and Technologies, Faculty of Agriculture and Food technology, Jelgava, Latvia; [2]Laboratory of Plant Mineral Nutrition, Institute of Biology, FMLS, University of Latvia, Riga, Latvia

*E-mail: abolina.la@gmail.com

Cloudberry *Rubus chamaemorus* is traditionally harvested in the wild in Latvia. Recently, the development of cloudberry cultivation technology has begun. This study aimed to assess the influence of different shade levels on cloudberry chlorophyll content and overall development under semi-controlled conditions. Propagated cloudberries were planted outdoors and grown under four shading treatments: control (no shade), 30%, 50%, and 80% shade for two years. SPAD measurements were taken weekly from May to August in both years, while leaf size, density, and nutrient concentrations were determined at the end of August 2024. The highest SPAD values, leaf sizes, and nutrient contents were observed in plants under 80% shade in both years. In the second year, plants under 50% shade exhibited similar results to those under 80%. After two years of growth, the highest plant vitality was observed under 50% and 80% shade, as indicated by plant colour and significantly higher chlorophyll content. However, the shaded plants developed larger leaves to increase light capture, a typical adaptation of shade-tolerant species. Leaves under the 80% shade were significantly larger compared to the control and 30% shade treatments, while the 50% shade treatment was similar to all the other treatments. In berry production, increased vegetative growth often occurs at the expense of fruit production. Therefore, 50% shade is considered the most effective and energy-efficient shading level for cloudberries in the Latvian climate, as indicated by our study. Further studies regarding the combined effects of shade and fertilization on cloudberry production are required, as optimal nutrient availability is essential for maximizing berry yield.

Keywords: netting, SPAD, berry cultivation, light capture.

O8

Growth and yield performance of carrot (*Daucus carota L.*) influenced by treatments with microalgae extracts

Irina Sivicka[1]*; Kaspars Kampuss[1]; Pāvels Semjonovs[2].

[1] Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies; [2]Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Biology, FMLS, University of Latvia

*E-mail: Irina.Sivicka@lbtu.lv

The research aimed to the evaluation of growth and yield performance of carrot (*Daucus carota* L.) influenced by treatments with microalgae extracts. In the end of May 2024, carrots (cultivar 'Nantes 2' F1, Vilmorin, vegetation period 110 days after germination) were sown in field conditions of the laboratory of Horticulture and Beekeeping of the Latvia University of Life Sciences and Technologies. From the stage of first leaves (start of June), once per week, total 5 times plants were sprayed weekly with the solution of ethanol extractions of different microalgae species: *Spirulina, Chlorella*, *Tetradesmus* and *Graesiella*. Two concentrations of the extracts were compared with sprays with corresponding ethanol solution as a control.

During experiment, for any microalgae extracts no negative effect to carrots` growth and development was noticed. The influence of microalgae extract`s type as well as concentration to the growth of carrots was not significant ($p > 0.05$). For yield, kg m^{-2}, average result from 3.23 (control variant with drinking water) till 12.03 (*Chlorella,* 10%) was observed. In the case of yield, the significant influence ($p < 0.05$) of concentration was observed, but influence of microalgae extract`s type was not significant.

Acknowledgements: This study was performed within the framework of the project No. 22-00-A01612-000014 co-financed by European Agricultural Fund for Rural Development (EAFRD) and supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

Key words: carrot, microalgae, extracts, yield.

O9

Utilizing cheese whey for mixotrophic cultivation of microalgae *Graesiella emersonii*: improving lipid and omega-3 polyunsaturated fatty acid content in poultry diets

Kristaps Neiberts*, Oto Jēkabs Apse, Pāvels Semjonovs

Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Biology, FMLS, University of Latvia, O. Vācieša iela 4, Rīga, Latvija

*E-mail: kristaps.neiberts@lu.lv

Photoautotrophic cultivation is the most common method for large-scale microalgal growth. However, this process has some limitations, including long cultivation and low cell productivity due to self-shading at the end of the growth (J. Wang et al., 2014). Hence, mixotrophic cultivation method overcomes these drawbacks by combining photosynthesis and organic carbon uptake offering shorter cultivation periods with higher growth rates. This approach minimizes self-shading effects and photo limitation by utilizing both light and organic compounds as energy source. For instance, dairy wastewater has been utilized as both an energy and a carbon source under mixotrophic conditions to support the growth of various microalgae species, such as *Chlorella vulgaris, Chlorella protothecoides, Haematococcu pluvialis, Scenedesmus obliquus, Dunaliella salina* and *Arthrospira platensis* (De Andrade et al., 2022).

Lactose is the main component of CW, contributing to a high chemical oxygen demand (COD) 80–40 g L^{-1} and biological oxygen demand (BOD) 30–50 g L^{-1}. If not properly managed, CW's high organic content can cause environmental pollution. However, its carbon, nitrogen, and phosphorus content make it a potential substrate for microalgal growth. Effective utilization requires microalgae capable of metabolizing lactose as an organic carbon source (De Andrade et al., 2022). As microalgae are rich in pigments, lipids, polyunsaturated fatty acids, polysaccharides, vitamins and other compound it is essential to determine the cost-effective cultivation method (Ibrahim et al., 2023).

Mixotrophic cultivation combines autotrophic and heterotrophic cultivation methods and has a great option to cut cultivation costs. It has been documented that, in contrast to the cultivation under autotrophic conditions, the cultivation of microalgae under mixotrophic conditions generally improved growth rates, biomass, and other high value products (Youssef et al., 2024). For instance, in our study, microalgae *Graesiella emersonii* KM01 grown mixotrophically on cheese whey reached 184,16 ± 5,75 mg/g of lipids compared with autotrophic growth, whereas it was estimated only to 102,10 ± 1,27 mg/g.

Microalgal biomass and metabolites are high-value products that serve as alternative feed supplements for broiler chickens due to their rich lipid and Omega-3 fatty acid content. Feeding microalgal biomass can improve body weight and enrich eggs with Omega-3 fatty acids (Abdel-Wareth et al., 2024).

Acknowledgements: This study was co-financed by European Agricultural Fund for Rural Development (EAFRD) and supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia, grant Nr. 22-00-A01612-000015.

Keywords: microalgae, *Graesiella emersonii*, lipids, biomass, poultry diet

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O10

Impact of pyrethroid insecticides on non-target Coleoptera

Zoya Tsybouskaya*, Austėja Orzekauskaite

Vytautas Magnus University, Kaunas, Lithuania

*E-mail: zoya.tsybouskaya@gmail.com

Insecticides are vital tools for managing pest populations that threaten agricultural productivity and public health by effectively reducing the number of harmful arthropod species. However, their use comes with significant ecological costs. In agricultural ecosystems (agrocenoses) and adjacent natural habitats, insecticides disrupt the delicate biological balance, affecting a broad range of non-target organisms. Pyrethroids, widely used insecticides for their effectiveness and relatively low toxicity to mammals, still pose significant risks to non-target invertebrates. (Pathak VM et al., 2022)

Aim of the study: To explore the impact that pyrethroid insecticides, with using cypermethrin as an example, have on non-target Coleoptera.

We estimated the median lethal dose (LD_{50}) of cypermethrin (used as an example compound for pyrethroid insecticides) for each species of Coleoptera studied to understand their sensitivity to pyrethroid insecticides. Furthermore, we investigated the relationship between body size (body mass and body length) of analyzed species of beetles and their sensitivity to pyrethroid insecticides (also using cypermethrin as a model compound).

Laboratory experiments were performed on 24 species of Coleoptera from six different families (Carabidae, Staphylinidae, Silphidae, Histeridae, Aphodiidae, Cetoniidae). The material was collected from forest ecosystems in the vicinity of Kaunas, Lithuania, using standard entomological methods, including Barber pit-fall traps, manual collection from the litter and soil, usage of butterfly nets. To determine it the invertebrates (4-8 in one) were put in small closable 10 × 6 × 4 cm containers (with micro holes in the lids), made of edible plastic. Onto the bottom of the containers, we put cotton disks to absorb surplus moisture – to prevent death by drowning. Using plastic sprayers, one dose (0.37 ml) of an aqueous solution of cypermethrin of a certain concentration was applied (8 different ones were used). The experiment lasted 24 hours. Then the number of living and dead imagoes of beetles was counted and LD_{50} was determined based on data collected.

As a result of the study, we calculated the average lethal doses of cypermethrin for each studied beetle in order to identify the most sensitive species. In addition, we identified a relationship between the body size (weight and body length) of the analyzed beetle species and their sensitivity to this insecticide.

Among the analyzed beetle groups, Carabidae were found to be the most resistant to cypermethrin. However, exceptions were noted, such as species of the genus *Carabus*, which were very sensitive to cypermethrin. The most sensitive group was Staphylinidae, particularly species of the genus *Philonthus*. This trend was noted for other groups of dung beetles as well. Cattle dung dwellers were more resistant to cypermethrin than forest litter inhabitants with similar body sizes. It was determined that the taxonomic affiliation of insects is related to the average lethal dose of the insecticide. It was also established that for most species of studied beetles there is a relationship between body size and their sensitivity to the insecticide: the shorter the body length and smaller the weight, the higher their sensitivity. However, there are exceptions: coprobionts of the families Histeridae and Aphodiidae were found to be quite resistant to cypermethrin despite their small body size.

Key words: Insecticides, Pyrethroids, Cypermethrin, Coleoptera, non-target organisms

O11

Nature reserve “The Burtnieku lake floodplain meadovs”

Elmīra Boikova, Lelde Eņģele, Oskars Keišs, Uvis Suško

Institute of Biology, FMLS, University of Latvia, Jelgavas str. 1, Riga, Latvia

E-mail: elmira.boikova@lu.lv

The Latvian Nature Foundation (LDF) has designated wetlands as the habitat of 2024. A wetland is an area that is too wet or covered with a shallow layer of water. Wetlands include floodplain meadows, grass marshes, high marshes, also coastal grasslands. To understand the essence of a wetland, it is necessary to remember that life originated in water, and a wetland is a place where land and water meet and alternate. Therefore, the wetland is full of life. It has the conditions to be able to develop both terrestrial and aquatic species, as well as species that need directly flooded areas. The Burtnieku lake floodplain meadows as nature reserve and Natura 2000 site with 432.0 ha area was established in 2004. The reserve stretches in a narrow strip around the Burtnieku coast within the territory of the Burtnieku parish. The new nature protection plan for the next 12 years (2025–2037) is in the final stage of development. In this territory according to the Habitat Directive there are nine biotopes of EU significance, the largest one – Northen boreal alluvial meadows (the type is affected by flooding in spring) – 138.0 ha. Of the nesting 15 specially protected bird species, compared to the previous nature protection plan, the population assessment for 3 species has not changed, for 5 species the population assessment is currently higher: for 3 species in 2024 is lower than before—but 4 species no longer nest in the nature reserve at all: *Gallinago media, Tringa stagnatilis, Larus minutus* and *Asio flammeus. Crex crex and Gallinago media* are of special value for this reserve. Currently, there are only a few suitable places for *Crex crex* in the territory of the reserve. The main negative impact on the entire Burtnieku lake meadow reserve is the overgrowth of meadows with bushes, and in some places – also with reeds. As potential enlargement of this protected area with 178.0 ha Silzemnieku meadows with good conditions for *Crex crex* population development is under discussion. Assessment of this territory also illustrates that *Cnidium dubium* – can be considered as the second largest and richest place of population distribution after Lubana lake floodplain meadows.

Key word: Natura 2000 site, nature protection plan, population assessment

O12

Common starling (*Sturnus vulgaris*)—as a species for citizen science and fundamental research

Oskars Keišs

Institute of Biology, FMLS, University of Latvia, Jelgavas street 1, Riga, Latvia

E-mail: oskars.keiss@lu.lv

The common starling (*Sturnus vulgaris*) is a species seen by the general public on its everyday life. Phenological observations of starling arrival in spring date back to the 19th century in Europe. In Latvia, setting up nest-boxes for starlings has been popular since 20th century. This makes the common starling almost ideal species for citizen–science projects, involving individuals and schools. Common starling in Latvia has been one of the top species ringed between 1925 and 1940—about 20000 ringed, and majority of them were ringed by schoolteachers. Until 2024, more than 80000 common starlings have been ringed in Latvia, and about 1000 recoveries are yielded. During public data gathering on spring arrival dates, common starling has been the most frequent species to be reported due to its synanthropic lifestyle and recognition by the general public. Common starling has also been selected as one of the nest-box monitoring species by Latvian Ornithological Society in 1980ties (Čauns 1987). It is also selected as “Bird of the year” in 2025 by the Latvian Ornithological Society to raise public awareness of the citizen-science contribution to fundamental Science project carried out by the Institute of Biology, University of Latvia.

Key words: Phenological observations, ringing, ringing recoveries

Poster presentations

P1

Application of molecular biology methods for the detection and genotyping of *Babesia canis*

Laura Nareckaitė[1], Miglė Razgūnaitė[1*], Justina Snegiriovaitė[1], Indrė Lipatova[1], Karolina Jankauskaitė[2], Birutė Karvelienė[2], Algimantas Paulauskas[1], Jana Radzijevskaja[1]

[1]Vytautas Magnus University, Faculty of Natural Sciences, Donelaičio str. 52, LT-44243 Kaunas, Lithuania; [2]Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania

*E-mail: migle.razgunaite@vdu.lt

Canine babesiosis is a widespread tick-borne infectious disease caused by intraerythrocytic protozoa *Babesia canis*. Over the past two decades, the number of cases of canine babesiosis in Europe has been increasing significantly, higher number of cases has been observed in Lithuania as well. As the disease spreads rapidly, greater attention is needed for accurate diagnosis and effective treatment. Diagnostic methods commonly used in veterinary clinics, such as blood smears, are not always sensitive enough to identify the infection or do not allow for accurate identification of the pathogen. We aimed to investigate the genetic diversity of *B. canis* strains isolated from infected dogs in Lithuania using sequence analysis of *B. canis* 18S rRNA and Bc28.1 gene fragments. Blood samples were collected from 107 symptomatic dogs in veterinary clinics from spring to autumn of 2024. PCR tests were performed for both 18S rRNA and Bc28.1 genes. *B. canis* DNA was detected in 62 samples (66.34 %) out of 107. Sequence analysis of the 18S rRNA gene fragment showed two variable sites in 81-82 nucleotide positions, dividing *B. canis* into 3 different genotypes. Bc28.1 gene fragment analysis identified 4 gene sequence variants, which were classified into three genotypes based on two nucleotide polymorphisms. The results highlight discrepancies between diagnoses made in veterinary clinics using blood smears and those obtained through molecular methods. Furthermore, they demonstrate the potential of molecular techniques for detecting genetic variability among *B. canis* strains, which could improve diagnostic accuracy and epidemiological surveillance.

Acknowledgements: This study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19).

Key words: canine babesiosis, *Babesia canis*, 18S rRNA gene, Bc28.1 gene.

P2

Molecular identification of *Borrelia burgdorferi sensu lato* in ticks in Kaunas city, Lithuania

Saulius Bernotas*, Justina Snegiriovaitė, Miglė Razgūnaitė, Indrė Lipatova, Algimantas Paulauskas, Jana Radzijevskaja

Research Institute of Natural and Technological Sciences, Vytautas Magnus University

*E-mail: saulius.bernotas@vdu.lt

Lyme disease, caused by *Borrelia burgdorferi sensu lato* (*s. l.*), is one of the most prevalent tick-borne infections in Europe. While the disease is well-documented, data on Borrelia prevalence in urban and peri-urban environments, including Lithuania, remain limited. Since ticks serve as key vectors, monitoring their infection rates is essential for assessing the risk of Lyme disease in these settings. The study aimed to identify *Borrelia burgdorferi* sensu lat*o* in ticks collected in Kaunas city, Lithuania, using molecular methods. A total of 711 questing *Ixodes ricinus* (n = 654) and Dermacentor reticulatus (n = 57) ticks were collected by flagging in various urban and peri-urban areas of Kaunas city in 2023–2024. The presence of Borrelia DNA in ticks was screened using real-time PCR targeting the 23S rRNA gene. For identification of *B. burgdorferi s. l.* species, the partial outer surface protein A (ospA) gene, the chromosomal flagellin gene, and the 16S (rrs)–23S (rrlA) rRNA intergenic spacer (IGS) region were amplified using conventional and nested PCR assays, and sequenced. Overall, Borrelia DNA was detected in 17.02% (121/711) of the collected ticks. Infection was found only in *I. ricinus*. Three Borrelia species belonging to the *B. burgdorferi s. l.* complex were identified: *B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii*. Our results demonstrate the potentially high human risk of exposure to tick-borne infection with *Borrelia burgdorferi* s. l. in cities in Lithuania.

Acknowledgements: This study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19).

Keywords: *Borrelia burgdorferi s. l.*, ticks, urban, peri-urban, Lithuania

P3

Molecular screening for tick-borne encephalitis virus in ticks from city environments

Martynas Jonkus[1], Miglė Razgūnaitė[1], Justina Snegiriovaitė[1], Indrė Lipatova[1], Arūnas Stankevičius[2], Algimantas Paulauskas[1], Jana Radzijevskaja[1]

[1]Vytautas Magnus University, Faculty of Natural Sciences, Donelaičio str. 52, LT-44243 Kaunas, Lithuania; [2]Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania

E-mail: migle.razgunaite@vdu.lt

Climate change has significantly influenced tick ecology, extending their seasonal activity and facilitating their expansion into new habitats. As a result, the incidence of tick-borne diseases is rising in previously low-risk areas, posing increasing public health concerns. Among these, tick-borne encephalitis (TBE) is one of the most significant vector-borne diseases in Europe, with the Baltic States accounting for nearly one-third of reported European cases. This study assessed the prevalence of tick-borne encephalitis virus (TBEV) in questing ticks in Lithuania and key risk factors for transmission. Between 2023 and 2024, 544 ticks (*Ixodes ricinus*, *n* = 525; *Dermacentor reticulatus*, *n* = 19) were collected using the flagging method in urban areas and grouped into 112 pools based on species and developmental stage. TBEV detection was performed using real-time RT-PCR and virus isolation in two cell lines (Neuro-2a, MARC-145). TBEV RNA was detected in 11.39% (62/544) of samples. Virus isolation proved more effective than direct RT-PCR, with a total minimum infection rate (MIR) of 4.41% across both cell lines, compared to 1.47% from direct RT-PCR. The MIR for *I. ricinus* was 1.3% (7/525) and for *D. reticulatus* 5.3% (1/19). Positive samples included six nymphs, nine females, and seven males, highlighting widespread TBEV circulation across developmental stages and sexes. These findings suggest that *D. reticulatus* may play a greater role in TBEV transmission than previously recognized. The study underscores the need for ongoing tick surveillance and public health strategies, as well as the importance of virus isolation for improving detection accuracy.

Acknowledgements: This study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19).

Key words: Tick-borne encephalitis, *Ixodes ricinus*, *Dermacentor reticulatus*, questing ticks

P4

Development of cost-effective molecular method to discriminate different *Listeria monocytogenes* strains in food processing sector

Donata Grikštaitė*, Dalius Butkauskas

Nature Research Centre, Vilnius, Lithuania

*E-mail: grikstaitedonata@gmail.com

Listeria monocytogenes is a common environmental bacterium associated with natural and artificial-human made environments such as soil, water, vegetation, animals, food processing environments. This microorganism is characterized by high genetic diversity, which determines its ability to adapt to various environmental conditions and cause disease in various animal and human populations. Knowledge of the genetic diversity of strains circulating in food processing sectors is essential to assess the risks associated with this pathogen and improve food safety, therefore the genetic diversity of *L. monocytogenes* in the environment plays a very important role in understanding its transmission routes. This study investigates the genetic diversity of *L. monocytogenes* strains in food processing environments. During the study, 27 samples were collected from 2 food processing plants after environmental specimens were propagated in ALOA and OXFORD medias, and their DNA was subsequently isolated. Using the PCR (Polymerase Chain Reaction) method, *L. monocytogenes* detected in 27 cases. Using an advanced sequencing method—multi-locus sequence typing (MLST), we determined the genetic profiles of the isolates. This methodology allows typing of bacteria according to several specific gene loci, providing the possibility to identify different bacterial strains. Our analysis revealed the diversity of isolates from the production environment, i.e. 3 sequence types representing three bacterial strains were identified. It is important to note that 2 types were repeated in both food processing plants, while type 1 was exclusively identified in one food processing environment.

Key words: DNA studies, genetic diversity, listeriosis, PCR

P5

The role of anthropogenic pressures in shaping the genetic of red deer (*Cervus elaphus*)

Evelina Genevičienė, Irma Ražanskė, Loreta Griciuvienė, Kastytis Šimkevičius, Artūras Kibiša, Algimantas Paulauskas

Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

Corresponding author: algimantas.paulauskas@vdu.lt

Red deer, native to Central Europe, have expanded their range over the past thirty years, now inhabiting areas where they coexist with the introduced sika deer. This overlap has led to several ecological challenges, particularly hybridization between the two species and changes to their genetic structure. During the rut, aggressive sika males may attack red deer stags and mate with red deer hinds, raising the chances of hybridization and gene flow between the species. Research
into the genetic diversity of both red and sika deer was conducted in Lithuania using tissue samples from legally hunted animals. Genotyping was performed based on nuclear DNA microsatellite loci (STR), and when combined with statistical analysis, the data provided a clearer understanding of the populations and their genetic differences. Hybridization cases between the two species have been documented in several EU countries, highlighting the growing interaction between red and sika deer. Additionally, the study examined the impact of sika deer on red deer populations, particularly due to human activities, such as hunting and habitat disruption. This research emphasizes the importance of monitoring and managing deer populations to understand the broader ecological implications of these interspecies interactions. The increasing presence of sika deer in regions traditionally inhabited by red deer poses significant challenges for conservation efforts. As hybridization becomes more common, it raises concerns about the potential loss of unique genetic traits in red deer populations

Key words: Red deer, genetic diversity, anthropogenic impact

P6

Genetic diversity of *Bartonella* spp. in Lithuanian bats based on gltA gene sequences

Paulina Maceinaitė; Povilas Sakalauskas; Algimantas Paulauskas

Vytautas Magnus University

E-mail: paulina.maceinaite@stud.vdu.lt

Analyzing the genetic diversity of *Bartonella* in bats is essential to assess the risks associated with its transmission. In addition, our understanding of host specificity and potential pathogenicity is improving with the discovery of new genetic lineages of *Bartonella*. As some *Bartonella* species can cause human diseases such as bartonellosis, this study is important for epidemiological surveillance and prevention of zoonotic diseases. Bats are one of the most diverse and widespread mammalian species. They are important for the ecosystems. They can be a factor in disease transmission due to their ability to adapt to different environments and feeding habits.

To study bats as *Bartonella* reservoirs, samples collected in Lithuania from 2018 to 2020 were analyzed. DNA was extracted from lung, heart, kidney, and spleen tissues using the Genomic DNA Purification Kit. *Bartonella* spp. detection was performed using real-time PCR targeting the gltA gene, followed by agarose gel electrophoresis, purification, and sequencing. Sequences were compared against the GenBank database, and phylogenetic relationships were determined by maximum likelihood using the MEGA 11 software. A total of 53 bats from five species (*Myotis daubentonii, Eptesicus serotinus, Pipistrellus nathusii, Vespertilio murinus,* and *Nyctalus noctula*) were analyzed. Five *Bartonella* samples were subjected to sequencing, yielding sequences with 49 variable nucleotide positions. Comparative analysis with GenBank reference sequences revealed a similarity range of 94% to 100%. The sequences were grouped into two clusters by phylogenetic analysis: one was most closely related to *Bartonella phoceensis*, which was previously detected in rodents from Malaysia, *Bartonella alsatica*, which was previously found in *Apodemus speciosus* rodents, and the other was related to *Bartonella bacilliformis*, which was found in a human from Peru. Phylogenetic analysis showed that there are various species of *Bartonella* in Lithuanian bats, which highlights the role of bats as natural reservoirs.

Key words: bats, *Bartonella*, glta, host specificity

P7

Application of enzyme-linked immunosorbent assay for the detection of antibodies against measles virus

Justina Dagytė, Indrė Lipatova, Algimantas Paulauskas

Vytautas Magnus University, Lithuania

E-mail: justina.dagyte@vdu.lt

Measles is a highly contagious, acute and severe disease that affects people of all ages. It causes fever, maculopapular rash and upper respiratory tract inflammation. The infection is extremely dangerous for older adults with weak immune system and children. Despite universal vaccination programmes, measles outbreaks have been increasing in recent years, suggesting gaps in immunity, potentially due to waning immune memory or insufficient vaccine coverage. This study aimed to evaluate the immune status of adults and to identify individuals at risk of measles infection in Lithuania.

The enzyme-linked immunosorbent assay method applied in this research allowed to detect levels of IgG antibodies in the participants. Serum samples from a total of 88 individuals were included in this study. The results revealed that the majority of individuals with insufficient IgG antibody levels were from the 1976–1981 and 1982–1987 age groups. Participants with negative results were either uncertain about their vaccination status or had received only a single vaccine dose. The distribution of these results was similar between sexes. These findings highlight the importance of improving vaccination coverage, particularly for individuals with incomplete immunization histories, in order to reduce the risk of measles outbreaks.

Key words: measles, IgG antibodies, ELISA, Lithuania

P8

Application of molecular methods for the detection of Lyme disease agents

Ugnė Medikaitė, Indrė Lipatova, Justina Snegiriovaitė, Jana Radzijevskaja, Algimantas Paulauskas

Vytautas Magnus University, Lithuania

ugne.medikaite@vdu.lt

Lyme disease is the most common vector-borne disease in North America and Europe. This infection is caused by the spirochete bacteria complex *Borrelia burgdorferi sensu lato* and is transmitted to humans from the bite of an infected tick. The increased incidence of Lyme disease over recent decades could be linked to factors such as climate and environmental changes, the growing distribution of ticks and their hosts. Understanding the role of potential reservoir hosts in disease transmission is required for effective public health strategies. The red squirrels *Sciurus vulgaris* are commonly found in natural, peri-urban and urban areas, where humans frequently interact with wildlife, may act as a reservoir host for maintaining *Borrelia* spp. In this study, molecular methods were applied for the detection of Borrelia DNA in red squirrels from urban parks in Kaunas city, Lithuania. A total of 45 red squirrel tissue samples were analyzed using real-time PCR with Borrelia-specific primers. Positive samples were further analyzed through PCR amplification targeting the intergenic spacer (IGS) region, ospA, and fla genes, followed by sequencing. The results revealed that 37.7% of the tested samples were positives for *Borrelia* DNA. The detection rates varied among genes, with ospA detected in 17.7% of samples, IGS in 20.0%, and fla in 37.7%. Three pathogenic species were identified by sequencing: *B. afzelii, B. garinii,* and *B. burgdorferi* sensu stricto. These findings indicate that red squirrels could play a role in the spread of Lyme disease by hosting multiple *Borrelia* species. This highlights the need for ongoing monitoring of wildlife and tick populations in areas where the disease is common.

Key words: *Borrelia burgdorferi sensu lato*, spirochete bacteria complex, disease transmission

P9

Preliminary results on muscle tissues parasites of mustelids in Lithuania

Ramunė Tamošiūnaitė*, Evelina Maziliauskaitė, Petras Prakas, Dalius Butkauskas.

Nature Research Centre, Vilnius, Lithuania

*E-mail: ramune.tamosiunaite@gmc.stud.vu.lt

Mustelids are found worldwide and occupy various habitats, including forests, grasslands, wetlands, and urban areas. Their wide variety of food often exposes them to infected wildlife, which makes them reservoirs of zoonotic infections. Mustelids frequently transmit various nematodes, for instance, *Trichinella* spp. To ensure the safety of food it is necessary to investigate these mammals because they can be potential vectors of Trichinella spp. parasites. Furthermore, mustelids carry a variety of trematodes species such as *Alaria* spp., *Neodiplostomum* spp., and *Strigea* spp. Therefore, the objectives of the presence study were to establish parasite infection prevalence and use molecular methods to identify parasite species. Sixty mammals of the Mustelidae family, 26 European pine marten, 19 American mink, 13 European polecats, one European badger, and one stone marten were collected from different regions of Lithuania. The active artificial method has been used to investigate the infection prevalence and intensity of *Trichinell*a spp. in muscle samples. The microscopic compressor method was used to detect different trematodes metacercariae. *Trichinella* species were identified using multiplex-PCR, while trematodes were identified by ITS2 region and Sanger sequencing of amplified fragments. Seven of the 60 mustelids were positive for at least one parasite. Prevalence of *Trichinella* spp. and trematodes were 6.67% and 5.00%, respectively. Overall, 40 larvae were isolated for molecular confirmation of Trichinella species. The thirty larvae isolated from one European polecat and two European pine martens were identified as *T. britovi* being the dominant Trichinella species in wild animals in Lithuania. Also, we found mixed *T. britovi/ T. spiralis* in one positive American mink (ratio 8:2). One of the three trematode-infected animals was examined by molecular analysis. The molecular analysis showed 100% identity to *Neodiplostomum spathula*. In addition, for the first time Trichinella spp. was found in American mink and *Neodiplostomum spathula* has been detected in Lithuania.

Key words: mustelids, *Trichinella* spp., N*eodiplostomum* spp., molecular identification.

P10

Sarcocystis species richness in the diaphragm and myocardium of Alpine ibex (*Capra ibex*)

Eglė Rudaitytė-Lukošienė, Petras Prakas, Dalius Butkauskas

Nature Research Centre, Vilnius, Lithuania

E-mail: egle.rudaityte@gmail.com

The cyst-forming coccidia of the genus *Sarcocystis* (Apicomplexa: Sarcocystidae) are widespread parasites of mammals, particularly domestic and wild ruminants. These protozoan parasites form sarcocysts in muscle tissues of intermediate hosts. Research on *Sarcocystis* in wild members of the subfamily Caprinae is, however, rather limited. A comprehensive investigation of sarcocysts in Alpine ibex (*Capra ibex*) was previously conducted using solely morphological techniques. In the period of 2021–2024, diaphragm and myocardium muscle samples of eight Alpine ibex from Austria were examined for sarcocysts. The detected sarcocysts were morphologically described using light microscopy and transmission electron microscopy. The cysts were classified into three morphological types (I-III), distinguished by their distinct morphometric parameters, including the thickness of the cyst wall and the presence of finger-like or hair-like protrusions, respectively.

Furthermore, the molecular identification and characterization of individual sarcocysts was conducted using the 18S rRNA gene and cox1 sequencing, followed by conducting phylogenetic analyses on the resulting sequences. This study offers a significant advancement in our understanding of *Sarcocystis* species diversity, as it is the first to employ molecular methods to identify this parasite in Alpine ibex. The analysis revealed that type II sarcocysts exhibited the closest phylogenetic relationship with *Sarcocystis* species transmitted by corvid birds, while types I and III showed a closer relationship with species transmitted by canids. However, the 18S rDNA, in contrast to the cox1, was not sufficiently variable to accurately delineate the phylogenetic relationships of numerous *Sarcocystis* species. This study corroborates the role of the Alpine ibex as a host of three *Sarcocystis* species.

Key words: *Sarcocystis*, Alpine ibex, molecular identification, cox1, phylogeny

P11

Genetic characterisation of *Sarcocystis* cysts isolated from muscles and brain of voles from Lithuania

Dovilė Laisvūnė Bagdonaitė*, Eglė Rudaitytė-Lukošienė, Petras Prakas, Dalius Butkauskas

Laboratory of Molecular Ecology, Nature Research Centre, Lithuania

*E-mail: dovile.bagdonaite@gamtc.lt

Sarcocystis (Apicomplexa) is a genus of parasites that employs a variety of hosts—mammals, birds or reptiles to complete their life cycle. These parasites are characterised by the formation of sarcocysts in the muscles or CNS of intermediate hosts and the development of sporocysts in the intestines of definitive hosts. To date, over 40 different *Sarcocystis* species have been identified in rodents. Majority of the investigations have focused on synanthropic rodent species, such as the house mouse (*Mus musculus*) and brown rat (*Rattus norvegicus*), thus data concerning *Sarcocystis* species prevalence and diversity in wild mice and voles remain sparse. Due to the limited amount of available data, the precise effects of these parasites to their hosts remain to be fully determined.

However, it is known that certain *Sarcocystis* species, which utilise rodents as intermediate hosts, are either pathogenic or increase the risk of predation of affected animals. In recent years, molecular techniques have been increasingly used to distinguish even morphologically similar *Sarcocystis* species, and to study animals for which previous studies were too difficult due to technical barriers. In this study we aimed to molecularly characterise *Sarcocystis* parasites isolated from the common vole (Microtus arvalis) and the bank vole (*Clethrionomys glareolus*).

Muscle and brain samples of 52 common voles and bank voles collected in different regions of Lithuania were examined for *Sarcocystis* spp. Parasites were detected and isolated from tissues using light microscopy. *Sarcocystis* species were identified and characterised within 18S rRNA, 28S rRNA, ITS1, ITS2, cox1, cytb and rpoB. Three sarcocysts were found in the muscles of single common vole. These sarcocysts were morphologically similar to those of the recently described *Sarcocystis myodes* species in Lithuania. Molecular analysis of the four gene markers revealed that sarcocysts belong to genetically new *Sarcocystis* species. For the description of a new *Sarcocystis* species a more detailed morphological analysis of sarcocysts involving transmission electron microscopy is needed. Furthermore, *S. glareoli* cysts found in the brain samples of the bank vole were genetically characterised using seven genetic markers located in nuclear, mitochondrial and apicoplast genomes.

The results obtained in this study are important for the development of molecular identification of parasites of the genus *Sarcocystis* and for the overall understanding of the species diversity of *Sarcocystis* parasites in wild small mammals.

Key words: *Sarcocystis*, voles, brain, muscles, molecular characterisation.

P12

Optimization and challenges in DNA extraction from *Tetrao urogallus* feathers

Evelina Slavnova*, Dace Grauda, Dalius Butkauskas.

Institute of Biology, FMLS, University of Latvia.

*E-mail: linamilss28@gmail.com

The western capercaillie (*Tetrao urogallus*) is a key species in boreal forests, serving as a biodiversity indicator in Latvia and Lithuania. However, habitat fragmentation, population decline, and reproductive isolation threaten genetic diversity and survival of capercaillie.

To assess genetic diversity of declining capercaillie populations in Latvia and Lithuania the non-invasive method based on collection of feathers for DNA extraction as the most suitable option was chosen trying not to disturb birds especially during breeding season. To optimise DNA extraction from collected T. urogallus feathers, we used muscle tissue from two dead birds, as control specimens, and down feathers collected in natural habitat or obtained from capercaillie breeding farm, located in Viešvilė Nature Reserve (Lithuania). Initial extractions from feathers and mussles yielded high DNA concentrations but contained impurities that affected PCR performance. To improve quality, we developed a two-step purification protocol. After initial purification, samples were air-dried and subjected to a 70% ethanol wash, significantly enhancing DNA purity. Agarose gel electrophoresis and PCR analysis confirmed successful primer binding after purification, demonstrating the method's effectiveness. Further modifications of the DNA extraction and PCR protocols improved amplification efficiency, leading to the collection of amplified D-loop fragments subjected to sequencing in the Laboratory of Molecular Ecology of Nature Research Centre. The non-invasive genetic studies of T. urogallus are going to be continued including sequencing of informative mtDNA fragments and analysis of microsatellite loci. Our optimized method provides a reliable approach for obtaining high-quality DNA from feathers, supporting future genetic research and conservation efforts. The optimised protocol can also be applied to other bird species requiring non-invasive sampling. Additionally, integrating this method with sequencing of D-loop region or analysis of some nuclear loci could offer deeper insights into genetic diversity, population structure, and adaptive potential. These advancements will aid conservation strategies, including habitat management and genetic rescue efforts.

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Key words: Western Capercaillie, DNA extraction, non-invasive sampling, PCR optimization.

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P13

Breeding of tetraploid red clover in Latvia

Aija Rebane[1*], Dace Grauda[2], Paula Marta Muceniece[2], Sarmite Rancane[1]

[1]Institute of Agriculture, Latvia University of Life Sciences and Technologies Agriculture; Purapuķes 28/ Selekcija, Skriveri, Latvia, LV-5125; [2]Institute of Biology, FMLS, University of Latvia; Jelgava Street 1, Riga, Latvia, LV-1004

*E-mail: aijarebane@inbox.lv

Increasing the sowing area of legumes, including red clover (*Trifolium pratense* L.), is an important task in modern agriculture, not only in Latvia, but in many European and other countries. Growing legumes gives the opportunity to maintain and increase soil fertility, obtain cheap and comprehensive feed by decreasing the use of nitrogen fertilizer, and preserve nature. The ability of legumes, including red clover, to fix atmospheric nitrogen is particularly relevant today, given the drastically rising energy prices. Our feed producers are becoming more demanding, interested in new, more productive varieties. Therefore, breeding work should not be interrupted or stopped due to the rapid changes in growing and harvesting technologies, climatic conditions, diseases, and pests. The breeding goal is to offer consumers a variety with high adaptive potential, able to function effectively in different soil types, which can quickly build a large photosynthetic surface. Competitive struggle with weeds for space and nutrients, in the form of large, stable, and biomass and seed yield, winter-hardy, persistent, and resistant to diseases and pests—they are the main requirements for a new variety. Worldwide, red clover breeding has focused mainly on the creation of genotypes with higher biomass yield and increased adaptability. To meet consumer demands in these changing conditions, breeders need to work continuously using both classic and modern breeding methods.

Since the 1960s red clover breeding programs pay special attention to the development of tetraploid varieties. Tetraploid red clover is characterized by better ecological adaptability, higher biomass, and disease resistance compared to diploid plants. Treatment of diploid sprouts with colchicine is commonly used to obtain red clover polyploids, resulting in a change in the number of chromosomes. However, a relatively small number of stable tetraploids can be obtained using this method. To facilitate the acquisition of tetraploid red clover breeding material, various *in vitro*, molecular, cytometric, and flow cytometric methods were used in collaboration with researchers from the Environmental Genetics Laboratory of the Institute of Biology of the University of Latvia. Seeds of medium-early red clover cultivars 'Dižstende', 'Stendes agrais', and 'Jancis' were used to obtain tetraploid plants.

As part of the red clover breeding program of the Institute of Agriculture of the Latvian University of Life Sciences and Technologies (LBTU ZI), in cooperation with researchers from the Plant Genetics Laboratory of the Institute of Biology of the University of Latvia, from 2013 to 2017, 390 samples of red clover plants were submitted choosed for further evaluation. The plants were planted in open field conditions in the Skriveri Institute (LBTU ZI) experimental fields and evaluated for six generations. From the selection and family nurseries, only the two most promising samples were collected, which will continue to be used in the future breeding program. To make sure that the valuable qualities for which the samples were selected are passed on to future, it is important to re-sow them, establish, and evaluate in progeny test nurseries.

Key words: tetraploid red clover, *in vitro*, molecular methods, flow cytometry

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Assessment of antimicrobial properties of 3D biotextile with incorporated nanoparticles

Elīna Ažēna[1], **Dace Grauda[1*]**, Valters Gobins[1], Inga Lasenko[3], Dalius Butkauskas[2]

[1]Institute of Biology, FMLS, University of Latvia, Jelgavas street 1, Riga, Latvia; [2]Nature Research Centre, Akademijos Street 2, Vilnius, Lithuania; [3]JLU Technologies Ltd, Ilukstes Street, 107/1-16, Riga, Latvia.

*E-mail: dace.grauda@lu.lv

Antimicrobial activity was evaluated for fabrics incorporating succinite, silica, aluminum oxide, silver nanoparticles, and fabrics treated with crystalline succinic acid. For the studies, microorganism cultures (University of Latvia, Microbial Strain Collection of Latvia) representing human microbiota were used, including Gram-positive and Gram-negative bacteria and fungi. *Escherichia coli* (MSCL332) and *Enterococcus faecalis* (MSCL302) served as indicators of fecal contamination, while *Staphylococcus aureus* (MSCL334) and Candida albicans (MSCL378) were included as opportunistic pathogens linked to skin infections. Antimicrobial activity was assessed using the disk diffusion and parallel streak methods, which evaluate microbial growth inhibition on agar. Additional tests determined the impact of fabrics on microbial growth in a liquid medium, analyzed via optical density, colony-forming unit (CFU) counts, and flow cytometry. A BD FACSJazz® cell sorter (BD Biosciences, USA) was used for flow cytometry to measure the relative fluorescence of bacterial cells. A 488 nm blue laser excited cell fluorescence, and the emission was measured at the 585 nm channel. The flow cytometer was calibrated using Sphero™ rainbow calibration particles (3.0 µm, BD Biosciences, USA) in PBS solution before measurements. A coefficient of variation (CV) below 3% was considered a successful calibration. The intensity of bacterial relative fluorescence was expressed in arbitrary logarithmic units. For each sample, events were recorded over a fixed 3-minute period.

The disk diffusion method demonstrated antimicrobial activity in succinic acid-treated fabrics, showing inhibition zones for *S. aureus, E. faecalis*, and *E. coli,* but not for *C. albicans*. The parallel streak method confirmed these results. Further evaluation showed decreasement of bacterial relative fluorescence of *E. coli* and *S. aureus* colonies grown in liquid medium with succinic acid-treated fabrics. Additionally, antimicrobial activity was observed in bacterial colonies grown with fabrics containing succinic nanoparticles.

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Key words: biotextile, succinite nanoparticles, succinic acid, antimicrobial activity, *Escherichia coli, Staphylococcus aureus*

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Evaluation of rust and powdery mildew development in *Mentha ×piperita* and *Mentha spicata* under *ex situ* condition

Irina, Sivicka[1*]; Olga, Sokolova[2].

[1]Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies; [2]Unit Of Plant Pathology and Entomology, Institute of Horticulture.

E-mail: Irina.Sivicka@lbtu.lv

Mint (*Mentha*), belonging to the Lamiaceae family, is widely cultivated aromatic and medicinal plant. However, mint production faces significant threats from fungal diseases. Mint rust, the causal agent *Puccinia menthae*, as well as *Erysiphe cichoracearum*, responsible for powdery mildew, negatively affect both – the quantity and quality—of raw material.

The aim of the work was to evaluate the presence of symptoms of rust and powdery mildew for mint under *ex situ* collection. Study was carried out in 2020-2022, using plant material of *Menta ×piperita* and *Menta spicata* from the *ex situ* collection of aromatic and medicinal plants` genetic resources (N 56º39’ 45.3"; E 23º45’15.2") of the Latvia University of Life Sciences and Technologies. During vegetation period, the severity of infection was determined using a scale from 0 to 4, where 0 indicated no disease symptoms and 4 represented 96–100% leaf surface infection.

The first symptoms of rust and powdery mildew appeared on most mint accessions in early September. By late October, the severity on plants reached 3–4 points. Differences between *M.* x *piperita* and *M. spicata*. All *M. ×piperita* accessions exhibited symptoms of infection by rust and powdery mildew, ranging from 2 to 4 points, while for *M. spicata* – from 0 to 4 points. Accession P4 (*M. ×piperita*) showed no symptoms of powdery mildew but symptoms of rust infection were till 2 points. But accessions P9 and P10 (for both – *M. ×piperita*) showed no signs of rust infection but were affected by powdery mildew. The observed differences in development of rust and powdery mildew for *M. ×piperita* and *M. spicata* highlight the necessity of studying the genetic basis of different *Mentha* accessions` resistance.

Key words: *Erysiphe cichoracearum,* plant health, *Puccinia menthae*

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Phenolic compounds and flavonoids in microalgal extracts for agricultural biostimulants: a comparative study of extraction methods

Tomass Tumpelis*, Santa Sukaruka, Pāvels Semjonovs

Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Biology, FMLS, University of Latvia

***E-mail:** tomass.tumpelis@lu.lv

Microalgae *Chlorella vulgaris* and *Arthrospira platensis* are rich in bioactive compounds which are known to contribute to sustainable plant growth. However, variations in extraction methods significantly influence the concentration of key bioactive compounds, such as phenols and flavonoids, affecting their efficiency as agricultural biostimulants. In this study, varied extraction techniques i.e. heating, sonication and microwave-assisted extraction (MAE) have been studied to improve yield of total phenolic compounds as gallic acid equivalent (mg GAE/L) (Anwer et al. 2022) and total flavonoids as quercetin equivalent (mg QE/L) (Augustini et al. 2015) from microalgal biomass into extracts for plant treatment.

Results indicated that the highest phenolic and flavonoid content was obtained when biomass (25–200 g/L) was treated by heating at 70°C for one hour, followed by MAE for 120 s using an ethanol-water mixture. Specifically, *A. platensis* with high biomass concentration (200 g/L) yielded approximately 400 mg QE/L flavonoids and 240 mg GAE/L phenols under heating conditions, while *C. vulgaris* yielded 100 mg QE/L flavonoids and 150 mg GAE/L phenols. MAE demonstrated greater efficiency, requiring up to $1/60_{th}$ of the time than heating to obtain similar phenols and flavonoids extraction yields from microalgae biomass. Under MAE treatment *A. platensis* flavonoids reached 260 mg QE/L with the lowest biomass concentration (50 g/L). Sonication consistently produced lower phenolic and flavonoid content both in water and water/ethanol (1:1 *v*/*v*) mixture, ranging from 2 to 10 times less than under other methods.

Comparing efficiency and yield, MAE at 120 s with microalgal biomass concentrations of 100 – 200 g/L was the optimal extraction technique for both species. These findings highlight the potential of MAE to enhance bioactive compound extraction, providing a more efficient and faster approach for plant biostimulants development.

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Key words: plant biostimulants, microalgal biomass, microwave-assisted extraction, flavonoids, phenols

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Research on insect baculoviruses in Latvia: past and future

Līga Jankevica*

[1]Department of Experimental Entomology and Microbiology, Institute of Biology, FMLS, University of Latvia, O. Vācieša street 4, Rīga, LV 1004, Latvia

***E-mail:** liga.jankevica@lu.lv

Research on insect viruses in Latvia began at the Institute of Biology in the 60s of the last centuries. The aim was to gain new knowledge on insect viruses and clarify their role in regulating pest populations in Latvia. Only baculoviruses are applicable for biological pest control. The main tasks were to monitor significant forest pest populations; to obtain new isolates and describe their properties; investigate occurrence and natural variability of baculoviruses in pest populations. After 1990s, the focus was on the development of experimental strains with high virulence; development novel viral insecticide preparations and determination their efficacy and assess perspectives for use in pest control. In the beginning of 21st century investigations on interaction of baculoviruses in the plant-pest-pathogen system and the studies of baculoviruses genome was caried out.

Monitoring of outbreaks and natural epizootics of forest pests have been done since 1965 on a regular basis. Living insects were laboratory-reared in isolators under optimal conditions. Viruses were purified using the methods described by Evans & Shapiro (1997). The presence of baculoviruses in larval tissue was detected by direct examination of larval tissue smears. The viral polyhedra or granules were observed in electron microscope. Method of DNA amplification by specific primers were used for identifying latent and persistent viral infections. Biotests were carried out for determining the virulence and efficacy of isolates (Huber, Hughes, 1984). Virulent experimental strains were obtained from wild isolates, using passages through host organisms under stress factors.

The majority of entomopathogenic viruses were isolated from dangerous forest and agricultural pests. Most of the isolated and identified insect virus isolates belong to the family Baculoviridae, 13 of them belong to the genus *Alphabaculoviruses*, 6 to the genus *Betabaculoviruses* and 4 to the genus *Gammabaculoviruses*. On the basis of experimental strains, a viral insecticide VIRIN KS and VIRIN NS was developed. The methods of developing dry and liquid virus insecticide forms were patented. In recent 10 years the role of microorganism associations in development of NPV infection was clarified.

Key words: Baculoviridae, biological control, persistent viral infections, virus insecticide.