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Abstracts

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Programma/Programme

Friday, **5 February 2021, 10.00 AM**, online

Vadītājs/Chair: Dr. Liga Jankevica		
10.00–10.05	***Līga Jankevica*** *Institute of Biology, University of Latvia*	**Atklāšana** **Opening**
10.05–10.20	**Liene Auniņa**	**Vegetation changes in extremely rich fens in Latvia**
10.20–10.35	**Elmīra Boikova**	**Protected water habitats in the landscape area “Augšdaugava”**
10.35–10.50	**Alesia Kruchonok**	**Morphological characteristics of the cloudberry (*Rubus chamaemorus* L.) leaf blade under various conditions in the Belarusian and Latvian populations**
11.05–11.20	**Nikole Krasņevska**	**The comparison of genetic diversity of the white clover (*Trifolium repens* L.) from the population of Europe**
11.20–11.35	**Sandra Dombrovska**	**Genetic diversity of cloudberry *Rubus chamaemorus* L. populations in Latvia and Belarus based on different molecular marker systems**
11.35–11.50	**Sergejs Koļesovs**	**Bacterial cellulose production on whey – an overview of prospects**
Posters (3 min + 2 min questions**)**		
Vadītājs/Chair: Prof., Dr. Isaak Rashal (technical assistance Dr. Uģis Kagainis)		
11.50–11.55	**Eglė Rudaitytė-Lukošienė**	**Identification of *Sarcocystis* spp. in intestine of American mink using molecular *COI* analysis**
11.55–12.00	**Petras Prakas**	**Evidence of the genetic divergence of the European Turtle Dove (*Streptopelia turtur)***
12.00-12.05	**Mihails Pupins**	**An innovative method for the estimation of adult anuran amphibians minimum density from a large-scale audial survey**
12.05-12.10	**Mihails Pupins**	**Invasive fish *Perccottus glenii* in the Protected landscape area "Augsdaugava": triggers of the invasion, threats to rare herpetofauna and proposed control measures**
12.10–12.15	**Mihails Pupins**	**Skin microbiome of released European pond turtles (*Emys orbicularis* (L.)) in Silene nature park NATURA2000, Latvia**
12.15–12.20	**Mihails Pupins**	**Parasites of the invasive Chinese sleeper (*Perccottus glenii*) in Latvia and Ukraine**

12.20-12.25	**Tatsiana Shlapakova**	**Elemental composition of seeds of representatives of the genus *Turbinicarpus* (Backeb.) Buxb. et Backeb.**
12.25-12.30	**Natalia Samokhvalova**	**Genetic diversity of populations of the rare species *Cypripedium calceolus* L. in the Belarus**
12.30-12.35	**Aleksandrs Petjukevics**	**The use of ten pairs of polymorphic microsatellite primers for selection and optimization the most suitable for future evaluation of genetic diversity of local populations of *Elodea canadensis* (Michx.) in Latvia**
12.35–13.00	**Coffee break, discussions**	
Vadītājs/Chair: Dr. Dmitry Telnov		
13.00-13.15	**Evita Rostoka**	**Seasonal changes in nitric oxide production laboratory rats**
13:15-13:30	**Dalius Butkauskas**	**Experimental evidence of the impact of low frequency electromagnetic field on the reproductive success of fruit fly *Drosophila melanogaster* and its potential to generate new point mutations at some candidate genes**
13.15–13.30	**Evelina Juozaitytė-Ngugu**	***Sarcocystis* species identification in muscles of birds from Spain**
13.30–13.45	**Uģis Kagainis**	**Use of a modern SEM imaging towards a complete revision of the checklist of Latvian armoured mites (Acari: Oribatida)**
13.45-14.00	**Zigmunds Orlovskis**	**Insect eggs trigger inter-plant systemic acquired resistance and enhanced insect performance**
14.00-14.15	**Dace Grauda**	**Biotextile protection testing: immature gamete cell flow cytometry and *Drosophfila melanogaster* survival tests**
14.15-14.30	**Ronalds Krams**	**Gut microbiome composition variation of the bumblebees *Bombus terrestris* Linnaeus, 1758 in natural and agricultural environments**
14.30 -14.45	**Ilze Dubova**	**Adaptation of methods for the determination of biodegradation of biotextiles with amber particles**
14.45 -15.30	**Noslēgums, diskusijas Conclusions, discussions**	

Abstracts

Oral presentations

Vegetation changes in extremely rich fens in Latvia

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Extremely rich fens are rare in Latvia as in the rest of Europe due to drainage, cessation of management, and eutrophication. Are there any vegetation changes in extremely rich fens in the last decades? We re-surveyed four extremely rich fens in Natura 2000 areas Ķirbas Mire, Platenes Mire, Ķemeri National Park, and Lake Engure Nature Park in 2020. All sites have been abandoned for many decades and are still unmanaged. Three sites are drainage influenced. The tree cutting was performed in two out of four sites. We studied vegetation changes in 100m^2 vegetation plots established in 2003 in *Schoenus ferrugineus* dominated plant communities. We observed a strong increase of *Molinia caerulea* in all sites. Concurrently, the cover of target species *Sch. ferrugineus* increased in two sites. In another site, its cover decreased close to the fen margin but increased in fen expanse. In one site *Sch. ferrugineus* cover remained the same as in 2003. In sites hosting *Myrica gale*, its cover remarkably increased. We assume that the observed vegetation changes were mainly caused by long-term drainage impact, abandonment as well as climate change. The results cannot be used to draw the decision on conservation status for the whole fen site as it hosts other extremely rich fen communities too or the same plant community in different conservation status.

Key words: extremely rich fens, vegetation changes, *Schoenus ferrugineus*

Protected water habitats in the landscape area “Augšdaugava”

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The “Augšdaugava” landscape area was established already in 1990, but the elaboration of environment protection plan for this largest landscape area in Latvia is in progress from 2018 by creating a new concept of management and protection of biodiversity. The nature park “Daugavas loki” as part of this landscape area also was established in 1990 with protection and management plan (2010-2022). The upper Daugava river valley forms the central axis of the biodiversity and EU protected biotopes in this area. The complex of the Daugava with nine large meanders and small, natural unaltered rivers, crossing the ravins, belongs to the unique EU water protection habitat type 3260. This area is also rich with mineral springs and sprinfens as EU habitat 7160. There are about 30 lakes with different catchment areas and eutrophication impact. The possible threats, nature values and water ecosystem services for the new protection plan are discussed.

Morphological characteristics of the cloudberry (*Rubus chamaemorus* L.) leaf blade under various conditions in the Belarusian and Latvian populations /Lācenes (*Rubus chamaemorus* L.) lapu plātnes morfoloģiskās īpašības dažādos apstākļos Baltkrievijas un Latvijas populācijās/

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Vērtējot augu populāciju dzīvotspējas rādītājus tiek ņemti vērā parametri, kas nosaka sintēzes procesus augā. Primāri uzmanība tiek pievērsta auga lapai, kā galvenajam asimilācijas orgānam. Šajā pētījumā tika novērtēti lāceņu (Rubus chamaemorus L.) lapas morfoloģiskie parametri, lai aprakstītu cenopopulācijas. Lāceņu lapām tika noteikts asimilējošās plātnes laukums un forma, kā arī aprēķināts lapu plātnes indekss. Tika izanalizētas 12 lāceņu cenopopulācijas – četras Baltrievijas un astoņas Latvijas. No Baltkrievijas tika iekļautas četras purvu cenopopulācijas (Lonno, Eļņja, Moh, Žada), kas atrodas lācenes izplatības areāla dienvidu daļā un divas no tām atrodas pēc ugunsgrēkā transformācijas teritorijā. No Latvijas puses tika atlasītas astoņas cenopopulācijas (Zaļezers purvs, Laugas purvs, Ašenieku purvs, Pelečāpes purvs, Lielais un Pemmes purvs, Baltais purvs, Dzelves un Kroņa purvs, Nītaure). Visas analizētās lāceņu cenopopulācijas atrodas optimālos augšanas apstākļos. Augsts lapas plātnes indekss bija raksturīgs lāceņu cenopopulācijām, kas auga ar mežu saistītiem biotopos. Vismazāk attīstītas lapu plātnes tika novērotas uz atklātām, pēc ugunsgrēka teritorijām, bet mazākais lapas izmēra variācijas koeficients bija raksturīgs cenopopulācijām no optimālajiem augšanas apstākļiem

Genetic diversity of cloudberry *Rubus chamaemorus* L. populations in Latvia and Belarus based on different molecular marker systems

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Pētījuma mērķis ir noteikt Latvijas un Baltkrievijas lāceņu (Rubus chamaemorus L.) populāciju ģenētisko daudzveidību, gan starp dažādām populācijām, gan populācijas iekšienē. Lāceņu ģenētiskās daudzveidības analīzei tika izmantotas divas molekulāro marķieru sistēmas: uz retrotranspozoniem balstīta marķieru sistēma iPBS un daļēja sekvenēšana, punktveida mutāciju noteikšanai izmantojot ribosomālo RNS gēna 5.8 subvienību. Izmantojot retrotranspozonu sistēmu, tika veikta praimeru atlase, kopumā pārbaudot 75 iPBS praimerus. Tika atlasīti 12 praimeri, kas uzrādīja augsto polimorfismu. Tālāk veicot kandidātpraimeru paplašināto skrīningu, tika atlasīti 2 praimeri, kurus izmantoja lāceņu populāciju analīzei.

Šajā ziņojumā tiek prezentēti analīžu rezultāti, kas iegūti izmantojot iPBS praimerus 2277 un 2298, un RNS gēna praimerus RChL-Ch1 un RChR-Ch1.

Pētījums tika izpildīts projekta “Latvijas un Baltkrievijas lāceņu (Rubus chamaemorus L.) ģenētisko resursu izvērtēšana sugas saglabāšanai un selekcijai” (LV-BY/2019/2) ietvaros

Key words: *Rubus chamaemorus*, genetic diversity, iPBS, RNA gene, point mutations

The comparison of genetic diversity of the white clover (*Trifolium repens* L.) from the population of Europe /Ložņu āboliņa (*Trifolium repens* L.) Eiropas populāciju ģenētiskās daudzveidības salīdzinājums /

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Zālājiem un ganībām ir būtiska loma ekosistēmu pakalpojumu, piemēram, ūdensapgādes, bioloģiskās daudzveidības nodrošināšanā, oglekļa piesaistīšanā un pārtikas ražošanā (Bartolini et al. 2006). Mērenos pasaules reģionos mūsdienās ir tendence uz augstāku temperatūru, kas var radīt negatīvas veģetācijas produktivitātes un ekosistēmu pakalpojumu izmaiņas. Klimata izmaiņas ietver gaisa temperatūras paaugstināšanos, tai skaitā arī UV-B starojuma palielināšanos (Montiel et al. 2017). Retrotranspozoni ir mobilas ģenētiskās vienības, kas spēj pārvietoties genomā. Ir daudz pētījumu, kas pierāda, ka retrotranspozoni aktivējas, kad augs ir kāda stresora ietekmē. To spēja reaģēt uz vides izmaiņām tos padara par veiksmīgiem funkcionālajiem marķieriem (Casacuberta and Gonzales 2013). *Trifolium repens* ir plaši izplatīts gan savvaļā, gan pilsētu apstādījumos, vietās, ar augstu vides piesārņojuma līmeni, tāpēc tas ir viens no labākajām, ko izmantot pētījumos par sugas adaptāciju vides faktoru maiņas ietekmē (Williams 2014; Fineschi and Loreto 2020).

Darba mērķis ir salīdzināt ložņu āboliņa (*Trifolium repens* L.) Eiropas populāciju daudzveidību. Mērķa sasniegšanai ir izvirzīti šādi uzdevumi: veikt DNS izdalīšanu un iPBS analīzi ievāktajiem materiāliem, kā arī veikt klastera analīzi, lai novērtētu ģenētisko daudzveidību starp darbā analizētajām populācijām. Darba metodikā ietilpst polimerāzes ķēdes reakcija (PCR), uz retrotranspozoniem balstīta iPBS metode un gēla elektroforēze. Paraugi kopumā tika ievākti no 22 lokācijām. Rezultātu apstrāde uz šo brīdi vēl turpinās

Key words: *Trifolium repens*, retrotranspozoni, iPBS, populāciju ģenētika

Bacterial cellulose production on whey – an overview of prospects

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Bacterial cellulose (BC) is a biopolymer with a wide range of potential applications starting from the food and packaging industry to biomedicine and electronics. Despite its high potential, BC large-scale production remains still challenging. High cost of growth media, which can reach up to 30% of production costs is one of them. To decrease production costs, use of industrial and agricultural by-products, including whey, as alternative growth media can be considered. Whey is the main high-volume by-product of dairy industry and can be considered an alternative growth medium for BC production despite its low valorisation opportunities. Currently whey can be recognised as quite a problematic alternative growth substrate for large-scale BC production. However, further extensive studies may improve the prospects in both the search for a cheap alternative growth substrate for industrial BC production and valorisation of whey.

Acknowledgments

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Key words: bacterial cellulose, whey, valorisation, biopolymers

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Seasonal changes in nitric oxide production laboratory rats

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Introduction. Seasonal changes in nitric oxide (NO) production reported up till now appear to reflect reaction to environmental changes, however existence of intrinsic fluctuations of NO production might have many implications in experimental research and clinical use. Aim of the study was to reveal if NO production undergoes seasonal changes in laboratory rats kept in standard conditions throughout the year being isolated from effects of environment factors.

Methods. The study was performed on Wistar male rats. Experiments were performed in 2008 – 2010 during the whole year with interruption for July and August. Intact animals were compared to animals injected intraperitoneally with lipopolysaccharide (10 mg/kg). The NO production in the tissues was performed by means of electron spin resonance spectroscopy of the spin trap diethylthiocarbamate and NO complex.

Results. Seasonal fluctuations of NO concentrations were observed in the cortex, heart, and testes of control and sepsis models however, in the sepsis model it was more pronounced. Statistical data revealed statistically significant differences in NO levels between months, with a particularly high level of NO in April.

Conclusion. The seasonal changes in NO production should be taken into account in pharmacological experiments, especially in evaluation of action of drugs, modifying NO response.

Key words: nitric oxide; Wistar rats; seasonal changes; lipopolysaccharide; ESR spectroscopy

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Sarcocystis species identification in muscles of birds from Spain

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Members of the genus *Sarcocystis* are protozoan parasites characterized by prey-predator two-host life cycle. Birds of prey were mainly examined as possible definitive hosts, while their role as intermediate hosts are poorly studied.

In the period of 2019-2020, muscles tissues of 107 birds collected from Spain were examined for *Sarcocystis* parasites. Fifty-nine samples belonged to birds of prey (Accipitriformes, Falconiformes and Strigiformes) and 48 samples represented birds of the family Corvidae. Morphologically sarcocysts were characterised in fresh squashed samples under a light microscope (LM). *Sarcocystis* species were identified by 18S rRNA, 28S rRNA and ITS1 sequence analysis.

Microcysts were detected in four birds. Under LM, cysts found in birds of prey were characterised by thin (~1 µm) and smooth cyst wall, whereas cysts observed in the Common Raven (*Corvus corax*) had wavy striated cyst wall. Based on sequence comparison, *S. halieti* was identified in the Western Marsh Harrier (*Circus aeruginosus*) and two Black Kites (*Milvus migrans*), whereas *S. kutkienae* was confirmed in the Common Raven. This is the first report of *S. halieti* in muscles of birds of prey. According to current knowledge birds belonging to different orders (Charadriiformes, Suliformes and Accipitriformes) can act as intermediate hosts of this species.

Key words: Birds of prey, Corvidae, parasites, *Sarcocystis*, molecular identification

Use of a modern SEM imaging towards a complete revision of the checklist of Latvian armoured mites (Acari: Oribatida)

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Armoured mites (Acari: Oribatida) represent one of the most species diverse and yet taxonomically complex groups of soil mesofauna. They are often used in various innovative studies of biology science. New species are described as the result of numerous taxonomic investigations on the daily basis. Parallelly, contributions both globally and nationally on describing mite diversity and listing species continues in close relation to morphological analysis, also in Latvia. The previous Oribatida checklist of Latvia was published ten years ago. Taxonomy and species indicating traits need to be revised, species numbers – recalculated by including new records.

In recent years, modern microscopy facilities have become more accessible at the University of Latvia. This has allowed researchers to develop and apply the methodology of microscopic analysis of various taxonomically important yet complex morphological traits significantly.

In this study, a brief analysis on species-specific morphological traits visible exclusively in 3D micrographs of scanning electron microscopy (SEM) is given. In this regard, five model species are selected. Based on this analysis a complete revision of the checklist of Latvian oribatid mites is planned to prepare in the near future.

Key words: SEM, morphological traits, image analysis, Oribatida, revision, checklist, Latvia

Insect Eggs Trigger Inter-plant Systemic Acquired Resistance and Enhanced Insect Performance.

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Recognition of molecular patterns from plant pathogens or herbivores trigger not only responses in local plant tissue but also activate a broad-spectrum plant defence priming in distal leaves against potential future attacks, leading to systemic acquired resistance (SAR). Additionally, attacked plants encode a signal that triggers SAR response in neighbouring plants lacking initial exposure to pathogen elicitors. However, the discrimination between molecular mechanisms involved in the SAR signal generation in local tissue and decoding in distal tissues or neighbouring plants are not fully understood. Here, we demonstrate that insect eggs induce not only intra-plant but also inter-plant SAR against ***Pseudomonas syringae*** via a root-mediated signal in ***Arabidopsis thaliana***. Furthermore, egg-induced activation of SAR in neighbouring plants is coupled with increased insect larval performance on these plants. Thus, these results suggest that insects have evolved strategies to favour optimal development of future larvae by providing a niche of host plants with reduced infection and reduced anti-insect defences

Key words: Inter-plant communication, elicitors, defence signals, systemic acquired resistance, plant-herbivore interactions

Experimental evidence of the impact of low frequency electromagnetic field on the reproductive success of fruit fly *Drosopfilla melanogaster* and its potential to generate new point mutations at some candidate genes

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Up to five generations of *Drosophila melanogaster* of the Oregon line were exposed to low frequency (50 Hz) electromagnetic field (LF-EMF) by growing insects in plastic tubes placed inside a Helmholc coil, producing a region of nearly uniform magnetic field (500-750 µT). All fruit flies *were* placed on a standard sugar-yeast-cornmeal medium at 24±2 °C temperature. Initially 50 larvae's were placed in each tube (10 tubes placed inside the coil represent directly affected group - EM*, the next 10 tubes marked as control group K* are placed 1.5 meters from the coil), two fertilised females were replanted to obtain the next generation. The experiment has been terminated after the fifth generation of fruit flies developed up to imago stage. Revealing negative impact of electromagnetic field on reproductive success based on the current experimental model was found: no alive flies of the fifth generation were obtained in one out of ten tubes at control group and just a few flies of the fifth generation were found in four out of ten tubes exposed to the LF-EMF, in 5 tubes no alive flies were found in the group that was directly exposed to the LF-EMF. No point mutations of Isocitrate dehidrogenaze (*Idh*) 1218 bp fragment were found based on sequencing and alignment of DNA comparing representatives of EM* and K* groups. The study should be extended including experiments devoted to selection of the most informative and sensitive loci in combination with evaluation of the impact of electromagnetic fields of different intensity and frequency.

The study was financially supported by the EUREKA project **E!11170, IFSITEX**

Key words: *D. melanogaster* surviving test, Isocitrate dehidrogenaze (*Idh*) gene, point mutations, LF-EMF

Biotextile protection testing: immature gamete cell flow cytometry and *Drosophila melanogaster* survival tests /Biotekstila aizsargspēju testēšana: augu nenobriedušas gametisko šūnu plūsmas citometrijas un *Drosophlla melanogaster* izdzīvošanas testi/

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Tika veikta EUREKA projektā IFSITEX izstrādāta inovatīva dzintara daļiņu saturoša biotekstila aizsargspēju pret UV-B starojumu un ekstremāli zemas frekvences elektromagnētisko lauku (EZF-EML) testēšana. Testēšanai izmantoja divas dažādas metodes: plūsmas citometrijas metode, kur tika noteiktas augu nenobriedušu gametisko šūnu relatīvās fluorescences izmaiņas (veido, apmēram, 20 šūnu dažādu parametru izmaiņu summa) un *Drosophila melanogaster* izdzīvošanas tests, kas modificēts biotekstila testēšanai. Lai veiktu testēšanu, paraugi (dzīvas augu gametiskās šūnas vai *D. melanogaster* kāpuri) tika apsegti ar dzintara daļiņas saturošu biotekstilu, kā kontroli izmantoja līdzīgu audumu bez dzintara daļiņām un nenosegtus paraugus. Paraugi ar UV-B starojumu tika apstaroti 75 min un ar EZF-EML – 120 min. EZF-EML (50 Hz, 450 μT) ~~re~~ģenerēšanai tika izmantota Helmholca spole, kas veido nemainīgu magnētisko lauku (MF). Tika atrasti dzintara daļiņas saturoši biotekstila paraugi, kas labāk aizturēja UV-B starojumu un EZF-EML nekā audumu paraugi bez dzintara daļiņām.

Key words: Biotekstila aizsargspējas, UV-B, EZF-EML

Gut microbiome composition variation of the bumblebees *Bombus terrestris* Linnaeus, 1758 in natural and agricultural environments

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Composition and stability of the gut microbiome are essential to maintain the bumblebee (*Bombus*) immune system and health. Although bumblebees are key pollinators in agricultural landscapes, little is known about how gut microbial communities respond to anthropogenic changes. We used commercially produced colonies of buff-tailed bumblebees (*Bombus terrestris*) placed in three habitats. Whole guts of *B. terrestris* specimens were dissected from the body and analysed using 16S phylogenetic community analysis. We observed significantly different bacterial community composition between the agricultural landscapes - apple orchards and oilseed rape (*Brassica napus*) fields, and forest meadows. Differences in gut communities between the orchards and oilseed rape fields were nonsignificant. Bee-

specific bacteria like *Lactobacillus*, *Snodgrassella* and *Gilliamella* dominated gut communities of *B. terrestris* specimens. In contrast, the guts of bumblebees from forest meadows were dominated by fructose-associated *Fructobacillus*, which may have been due to high nectar concentrations in cold-climate-associated forest meadow flowers. Bacterial communities of workers were the most diverse, while those of males and young queens were less diverse. Our results suggest that habitat quality, exposure to environmental microbes, nectar quality and land-use significantly affect gut bacterial composition in *B. terrestris*

Key words: Pollinators, bumblebees, *Bombus terrestris*, microbiome, habitats, lactobacteria

Adaptation of methods for the determination of biodegradation of biotextiles with amber particles

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One of the conditions for biotextiles is the potential for biodegradation. Over the last 50 years, global production of synthetic fibers has grown rapidly, which are non-biodegradable and, at best, recyclable and reusable. Biodegradation is the process by which a material is irreversibly broken down into elements physically, chemically or biochemically. During the biofragmentation process, microorganisms develop enzymes as a result of population growth, as well as free radicals, which break down macromolecules into smaller units (oligomers, dimers, monomers). Products containing biopolymers, including cellulose, are more sensitive to microbial growth, which can result in many aesthetic and functional problems, even infections. But on the other hand, this problem can be used as an advantage in the need for short-term materials (Arshad & Mujahid, 2011). It has been studied that cellulose and non-cellulose materials degrade similarly, differing in biodegradation time. Our goal was to adapt the methods for the determination of biodegradation of biotextiles with amber particles. We adapted methods EN ISO 11721-1: 2001 and ISO 11721-2: 2003 developed for determination of resistance of cellulose - containing textiles to microorganisms.

The biotextile burial test, using industrially produced compost mixture (60% compost; 40% high bog peat), with a uniform structure, 60 ± 5% water content from WHC (maximum soil moisture content) was done. Pots (15 cm deep) were used to bury the biotextiles with amber particles and control (cotton fabric) in the soil, in which 750 g of soil was placed and 10 strips of fabric were placed in a U-shape to evenly contact the soil. Incubation at + 29 ± 1 ° C in climatic chambers (95% humidity). Cloth strips were removed at one-day intervals, rinsed under running water and soaked in 70% ethyl alcohol for 30 minutes, dried in a thermostat for 24 hours. To determine the changes in fabric strength, samples were sent for testing to the Scientific Laboratory of Mechanics and Biotextiles of the Institute of Mechanics and Mechanical Engineering, Riga Technical University.

The study was financially supported by the EUREKA project E!11170 “Innovative multifunctional biotextile, integrated with silica dioxide and succinate development, and its impact on biosystems” (**IFSITEX).**

Arshad K., Mujahid M. Biodegradation of Textile Materials: Master Thesis. Boras: The Swedish School of Textiles, 2011. pp. 14-22

ISO 11721-1:2001. Textiles — Determination of resistance of cellulose-containing textiles to micro-organisms — Soil burial test — Part 1: Assessment of rot-retardant finishing. P. 16.

ISO 11721-2:2003. Determination of the resistance of cellulose-containing textiles to micro-organisms — Soil burial test — Part 2: Identification of long-term resistance of a rot retardant finish. P. 7.

Key words: Biodegradation, deterioration, soil burial test

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Posters

Identification of *Sarcocystis* spp. in intestine of American mink using molecular *COI* analysis

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Parasites belonging to the genus *Sarcocystis* have an obligatory two hosts life cycle. Sarcocysts are formed in the muscles of the intermediate host. Development of oocysts and sporulation occur in the intestine of the definitive host by eating cyst-contaminated meat. *Sarcocystis* species do not differ in the morphology of their oocysts/sporocysts, which complicates identification of these parasites in definitive host. Although experimental animal infections are useful in this regard, they are complex and time-consuming, so research on the disclosure of the definitive host of *Sarcocystis* species are scare.

In this study, *Sarcocystis* sporocysts were isolated from intestinal scrapings of 40 American minks (*Neovison vison*). All samples were used for DNA extraction and nested PCR amplification of *COI* sequences by species-specific primers. Eight phylogenetically close *Sarcocystis* species using deer as intermediate hosts and with unknown definitive hosts were chosen for testing. As a result, 80% of animals were infected with at least one parasite species. *Sarcocystis elongata*, *S. entzerothi*, *S. japonica*, *S. silva* and *S. truncata*, were verified in the examined samples. Hence, using molecular analysis, it was demonstrated that American mink is involved in the transmission of five *Sarcocystis* species that forms sarcocysts in the muscles of deer.

Evidence of the genetic divergence of the European Turtle Dove (*Streptopelia turtur*)

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The European Turtle Dove (*Streptopelia turtur*) is a widespread Palearctic species. Due to long-term population decline, it is classified as *Vulnerable* by the IUCN. Previous study based on mtDNA cytb analysis of European Turtle Doves sampled in western and southern Europe suggested that this species is panmictic.

From 2017 to 2020, a total of 258 birds were collected in Ukraine, Spain (6 sites) and Morocco (2 sites). Samples were examined using mtDNA cytb and D-loop sequence analysis. Relatively high genetic diversity was evaluated at both cytb ($Hd=0.905 \pm 0.009$, $\pi=0.00628 \pm 0.00014$) and D-loop ($Hd=0.937 \pm 0.009$, $\pi=0.01502 \pm 0.00034$). Two phylogenetic groups (A and B) were identified based on both analysed mtDNA regions. The representatives of haplogroup A were found with considerably lower frequency in samples from Morocco, Ukraine and at the Gibraltar Strait coast in Spain comparing with other samples from Spain. Various analysis (Φ_{ST}, SAMOVA, PCoA) showed genetic divergence between Turtle Doves sampled in Morocco and Ukraine in comparison with some Spanish samples. Thus, the results indicate that the European population of the Turtle Dove is not panmictic and therefore specific management and conservation plans relevant for the species in various regions should be applied.

Key words: *Streptopelia turtur*, mtDNA, genetic variability, genetic structure, conservation

An innovative method for the estimation of adult anuran amphibians minimum density from a large-scale audial survey

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Knowledge of population size is crucial for evaluating the future prospects of a population, making conservation management decisions, and selecting management priorities for protected areas. Audial surveys of anuran amphibians (frogs, toads and similar) are cost-effective and allow for the coverage of large areas, but they are usually regarded as unsuitable for population size estimations due to imperfect detection.

Our study (Čeirāns et al. 2020) demonstrated a method for obtaining minimum adult population size estimates from vocalising anuran males counts by using sex ratios, life history and vocalising behaviour parameters from other studies. We collected data from 2016 to 2018 for seven taxa on 65 plots (each 25 km2) representing the entirety of Latvia, in 2020 the data were verified in 16 field expeditions in protected territories and aquaculture ponds.

Among taxa, average breeding waterbody audible detection probabilities ranged from 0.56 to 0.88 per plot, minimum adult frog density (MAFD) estimates were from 12.0 to 51.7 individuals per km2, but the estimated fraction of population covered by MAFD varied from 57 to 86%. The least accurate density estimates were in taxa with brief calling activity and quiet mating calls (*Rana temporaria*), and in taxa with a calling activity dependent on the numbers of males in a pond (*Bufo bufo*).

Our study suggests that lek-breeders would be more suitable than explosive-breeding taxa for minimum population size estimates from audial data. The use of MAFD allowed for coarse minimum population size estimates for the entire country from the audial monitoring data, these ranged from 3.7 ± 0.5 thousand (*Bombina bombina*) to 1.64 ± 0.47 million (*B.bufo*) adults. The method can be used for the bioindication of the quality of ecological services of aquaculture ponds ecosystems.

The anuran monitoring survey was financed by the Nature Conservation Agency of Latvia (contract Nr. 7.7/77/2016-P). We thank for cooperation the project “Pond aquaculture production and ecosystem service innovative research with modelling of the climate impact to tackle horizontal challenges and improve aquaculture sustainability governance in Latvia” (Nr. lzp-2020/2-0070), financed by Fundamental and Applied Research Projects (FLPP).

Čeirāns A., Pupins M., Pupina A. (2020): A new method for estimation of the minimum adult frog density from a large-scale audial survey. - *Scientific Reports*: 10:8627. https://doi.org/10.1038/s41598-020-65560-6.

Key words: population density, Anura, vocalizing males, audial survey

Invasive fish *Perccottus glenii* in the Protected landscape area "Augsdaugava": triggers of the invasion, threats to rare herpetofauna and proposed control measures

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The invasive fish Amur sleeper *Perccottus glenii* from the Far East has spread widely across Europe, threatening native species and ecosystems, including protected areas and pond aquaculture. Protected landscape area Augsdaugava Natura2000 (code LV0600400) is located on the border with Belarus (55.863450°N, 27.142637°E).

In 2017-2020, we conducted a study of the distribution of *P.glenii* in the Protected landscape area "Augsdaugava" in the south-east of Latvia. The study used analysis of databases and publications, catching *P.glenii* with a net (mowing method) and interviewing local residents (fishermen, nature friends, and landowners).

We found that *P.glenii* is distributed throughout all the territory of “Augsdaugava” and is found both in different waterbodies and numerous traditional aquaculture ponds, and in the Daugava River and its tributaries. The external triggers of the invasion of *P.glenii* identified in the study are 1) the targeted stocking of *P.glenii* by landowners in the small ponds of traditional aquaculture, 2) the creation of an ameliorative network as ways of its distribution, and 3) the use of *P.glenii* by anglers as bait when fishing for pike and perch.

In the Protected landscape area "Augsdaugava", the *P.glenii* threat is most dangerous for small populations of *Pelobates fuscus*, *Bombina bombina* and *Triturus cristatus*. These main threats to the protected herpetofauna are 1) food and spatial competition, 2) direct predation, 3) the new vector of the spread of parasites, and 4) the complex impact on ecosystems of small ponds. On the basis of the results of the study, measures to combat *P.glenii* are recommended: 1) education of pond owners and fishermen (also by informative boards in the places of fishing), 2) creation of ponds without contact with the surrounding waterways, 3) elimination of *P.glenii* from small amphibian breeding ponds.

We are thankful to the European Commission Cohesion Fund Project “Prieksnosacijumu izveide labakai biologiskas daudzveidibas saglabasanai un ekosistemu aizsardzibai Latvija” Nr. 5.4.2.1/16/I/001 for the support of the study. We thank for cooperation the project “Pond aquaculture production and ecosystem service innovative research with modelling of the climate impact to tackle horizontal challenges and improve aquaculture sustainability governance in Latvia” (Nr. Izp-2020/2-0070), financed by Fundamental and Applied Research Projects (FLPP) and the Joint Ukrainian-Latvian R&D project “The ecological and biological triggers of expansion of the invasive fish, Chinese sleeper (*Perccottus glenii*), in Eastern Europe”.

Key words: Invasive fish, Perccottus glenii, NATURA2000, triggers, herpetofauna

Skin microbiome of released European pond turtles (*Emys orbicularis* (L.)) in Silene nature park NATURA2000, Latvia

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One of the conservation strategies of threatened animal species is housing them in captivity, but many studies had shown decreased or increased microbial diversity in captive hosts compared to free-living animals due to abnormal diets, artificial and sterile environment conditions, antibiotic treatment, stress etc. Lack or imbalance in composition of hosts microbiome in captivity can negatively affect their health and lead to implications for reintroduction schemes. Reintroducing individuals with microbiomes different from wild populations may increase risk of infections, enteric diseases and transmitting abnormal microbiota to wild populations. Thus knowledge of released organisms' microbiome may be important for conservation efforts.

The aim of this preliminary study (Umbrasko et al. 2020) was to identify bacteria of the released in 2010 European pond turtle (*Emys orbicularis* L., 1758) skin microbiome. 26 swab samples were collected. Bacteria were grown on a sterile Petri plates with Plate Count Lab-Agar™. For isolation and differentiation of bacteria CHROMagar™ Orientation agar was used. Individual colonies of bacteria were described based on size, colour, texture and morphology. Gram's staining was performed. Bacteria shape and Gram's reaction was observed under the optical microscope.

The bacterial communities largely consisted of two phyla, the Proteobacteria and Firmicutes. Most common found bacteria were Pseudomonas spp. (40%) and Proteus spp. (27%). This study provides new basic information about the external microbiomes of the released *E.orbicularis* and is the first step in understanding their environmental roles.

We thank for cooperation the project "Pond aquaculture production and ecosystem service innovative research with modelling of the climate impact to tackle horizontal challenges and improve aquaculture sustainability governance in Latvia" (Nr. lzp-2020/2-0070), financed by Fundamental and Applied Research Projects (FLPP).

Umbrasko I., Harlamova N., Pupins M., Skute N. (2020): Skin microbiome of free-living European pond turtle (*Emys orbicularis* (L.)) on the northern border of its range in Silene nature park, Latvia. - *Acta Biologica Universitatis Daugavpiliensis*, 20 (2): 154-162

Key words: Microbiome, population restoration, *Emys orbicularis*, NATURA2000

Parasites of the invasive Chinese sleeper (*Perccottus glenii*) in Latvia and Ukraine

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The Chinese sleeper (*Perccottus glenii* Dybowski, 1877) is a bottom-dwelling invasive freshwater fish with native range in Far-Eastern Asia. Non-indigenous species can be vectors of distribution of new parasite species, which able to infest the local host species. *P.glenii* was introduced to Ukraine in 1972. The first records of *P.glenii* in Latvia come from small natural pond in Daugavpils town. Recent molecular studies showed three different groups of *P.glenii* haplotypes in Central and Eastern Europe, where the Latvian populations are characterized by the presence of specific haplotypes, absent anywhere in Europe, also not confirmed for China (Grabowska et al., 2020).

The aim of the study (Kvach et al. 2020) was to find the relations between the *P.glenii* parasite fauna and populations in Ukraine and Latvia. The *P.glenii* was sampled in August-September 2019 at six bodies of waters in Latvia and Ukraine, three from each country, and was studied for parasites and microparasites (unicellular and myxosporidians) using standard methods.

In the result of the study we registered 17 parasite species in studied *P.glenii*, including of 2 ciliate species, 1 coccidia, 1 monogenean, 1 cestode, 6 trematodes, 3 nematodes, 1 acanthocephalan, 1 parasitic copepod, 1 bivalvia glochidia. Maximal parasite species richness was registered in Ukraine: Lake Kartal (6 species) and Vylkove (8 species), but minimal in Latvia: Ilgas (3 species) and Gailezers (1 species); the microparasites were represented by two ciliates (Trichodina sp., *Ichthyophthirius multifiliis*) and an unidentified coccidian. Among metazoans, only three species are represented by adult stages: monogenean *Gyrodactylus perccotti*, acanthocephalan *Acanthocephalus lucii*, and parasitic copepod *Neoergasilus japonicas*.

We thank for cooperation the project “Pond aquaculture production and ecosystem service innovative research with modelling of the climate impact to tackle horizontal challenges and improve aquaculture sustainability governance in Latvia” (Nr. lzp-2020/2-0070), financed by Fundamental and Applied Research Projects (FLPP) and the Joint Ukrainian-Latvian R&D project “The ecological and biological triggers of expansion of the invasive fish, Chinese sleeper (*Perccottus glenii*), in Eastern Europe”.

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Key words: Invasive fish, *Perccottus glenii*, NATURA2000, parasites

Genetic diversity of populations of the rare species *Cypripedium calceolus* L. in the Belarus

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Studying the level and structure of the genetic diversity of rare species is important for creating an effective strategy for their conservation (Rajorai and Mosseler 2001). Genetic diversity is essential for the development of populations, and its loss often increases the risk of extinction (Frankham 2012). In order to select certain populations for conservation, it is necessary to know the degree to which the populations differ from each other and the level of genetic diversity within them. When choosing priority populations for conservation, it is necessary to give preference to populations with a high level of intrapopulation genetic diversity (Guo et al. 2019).

The lady's-slipper orchid (Cypripedium calceolus L.) is a relict species found on the territory of Belarus in isolated localities of the European fragment of the range. (Red Book of the Republic of Belarus 2015).

To determine the level of genetic diversity of four populations of C. calceolus, we used iPBS (inter-Primer Binding Site amplification) molecular markers (Kalendar et al. 2010).

The iPBS method is a powerful tool for studying genetic diversity of populations due to its high reliability and reproducibility of results, the ability to detect a large number of polymorphic fragments and the relatively low cost of analysis (Coutinho et al. 2018; Kalendar et al. 2010). In addition, since iPBS does not require any prior knowledge of target sequences, this marker is especially suitable for rare orchids for which there has been little previous molecular genetic studies (Nadeem et al. 2018).

Using iPBS markers in our study, it was found that C. calceolus genotypes have a rather low level of genetic diversity. High genetic differentiation was found mainly within populations, which may be caused by the phenomenon of cross-pollination. The genetic distance between populations corresponded to their geographic location

Key words: *Cypripedium calceolus* L., iPBS, genetic diversity, rare species

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Elemental composition of seeds of representatives of the genus *Turbinicarpus* (Backeb.) Buxb. et Backeb.

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Representatives of the genus *Turbinicarpus* (Backeb.) Buxb. et Backeb. in their natural habitat are endangered and are included in CITES Appendix 1. The reason is the illegal extraction of these cacti from nature, as well as the impact of changes in soil cover and overgrazing. The adverse effects of disruption to the cactus population are irreversible, as most populations have very limited opportunities for recovery. *Turbinicarpus* is a genus of small to medium sized cacti that are found in the Sonoran and Highland Mexican provinces of the Holarctic Kingdom (northeastern regions of Mexico, in particular the states of San Luis Potosi, Guanajuato, Nuevo Leon, Queretaro, Hidalgo, Coahuila, Tamaulipas and Sakatekas) (Takhtadzhyan 1978).

The chemical composition of seeds, as a unit of a plant with stable characteristics, can be one of the signs important for taxonomy. The activity of most enzymes depends on the content of activators and inhibitors in the medium, which often include micro- and macroelements. Electron microscopy with electron probe by chemical analysis allows to transport the quantitative and qualitative analysis of the elemental composition of the seeds. Sorokopudov V.N. (Sorokopudov et al. 2012) and Tokhtar L.A. (Tokhtar et al. 2012) noted differences in the percentage of individual chemical elements in different parts of fruits and seeds. Belopukhov S.L. (Belopukhov et al. 2012) divided chemical elements into groups depending on their percentage in plants.

In the course of the study, the weight fraction (%) of the following elements was established: Na, P, Cl, S, Si, Al, Ca, Mg, K. Thus, the seeds have a species-specific elemental composition

Key words: *Turbinicarpus*, seeds, scanning electron microscopy.

The use of ten pairs of polymorphic microsatellite primers for selection and optimization the most suitable for future evaluation of genetic diversity of local populations of Elodea canadensis (Michx.) in Latvia

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Previously, the genetic studies of *Elodea canadensis* (Michx.) were not carried out in Latvia and the Baltics. The aim of this study was the selection of polymorphic nuclear microsatellite loci, available for population structure analysis. This plant is invasive for the Baltic Region and Europe with notable edificatory features. Samples collected in natural ecosystems, in the Basin of Daugava River and nearby lakes from eight locations: Lake Sventes, Lake Ķīšezers, Lake Richu, Lielais Stropu Lake, and Daugava River (four sampling places). In the preliminary study, tested polymorphic microsatellite primers selected from GenBank ® (NIH genetic sequence database) for further investigation of the homogeneity of E. canadensis populations in Southeast Latvia and allow estimating the distribution level of this species. The study identified suitable conditions for amplification. It carried out the PCR optimization and testing of different conditions: primers concentration, a quantity of the cycles, the temperature of denaturation, annealing, and elongation for selected primers. Ten pairs of polymorphic microsatellite primers selected: Ecan5b2; Ecan5c; Ecan16; Ecan45b; Ecan46Lb; Ecan55; Ecan58; Ecan60b; Ecan103; Ecan105, eight pairs of primers were found to be suitable for further genetic analysis, amplification accomplished: Ecan16; Ecan45b; Ecan46Lb; Ecan55; Ecan58; Ecan60b; Ecan103; Ecan105. The future aim is to identify stress-responsive genes and compare the level, structure, and distribution of genetic variation in specific populations based on neutral and adaptive markers.

Key words: *Elodea canadensis*, Canadian water weed, genetic diversity, microsatellites, primers