UNIVERSITY OF PLOVDIV “PAISII HILENDARSKI”, FACULTY OF BIOLOGY • INSTITUTE OF BIODIVERSITY AND ECOSYSTEM RESEARCH - BULGARIAN ACADEMY OF SCIENCES

BOOK OF ABSTRACTS

April 15th-16th, 2021
FOREWORD

Dear Colleagues,

On behalf of the Organizing Committee, we are pleased to welcome you to the 5th Balkan Scientific Conference on Biology, held in Plovdiv, Bulgaria from April 15th to April 16th, 2021.

Plovdiv is one of the oldest cities in Europe. It is claimed that the city is a contemporary of Troy and Mycenae, but it is more ancient than Rome, Athens, and Constantinople. Plovdiv is the second-largest and important city in Bulgaria. Plovdiv is strategically important industrial, commercial, scientific, cultural and transportation-communications center on the Balkans region. The city is famous for the international fair, whose spring, autumn and other specialized exhibitions make it a center of economics and business. Plovdiv is a strategic railway junction and the airport “Plovdiv” recently established itself as an alternative to the airport “Sofia”.

Unfortunately, in these times of the pandemic, even though, we do not have the opportunity to meet in person, we hope that, the Conference will offer a valuable platform for the exchange of ideas and experiences between researchers in the field of biology.

Plovdiv University is one of the leading higher-education institutions in Republic of Bulgaria. It is the largest university in southern Bulgaria as well the second biggest in Bulgaria.

Faculty of Biology at the Plovdiv University is located in the cultural reserve Old Town in Plovdiv, next to the Ancient Theatre. Nowadays the Faculty of Biology has more than 50 years of history. Its development is associated with the changes in modern socio-economic conditions of transition to market economy and the process of democratization of society, the integration of Bulgaria into the European structures and the adoption of the achievements of European and world educational and scientific experience by providing resources for the new economy and society in the spirit of European cultural values.

After the success of the four previous Balkan conferences in Plovdiv (2005, 2010, 2014 and 2017), we hope that this edition will, once again, offer a dynamic and friendly environment for stimulating exchanges and discussions.

Official organizing partner for BalkanBio’2020 is the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.

Yours sincerely,

Sonya Kostadinova (Dean of the Faculty of Biology)

Gana Gecheva (Chair of the Organizing Committee)
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PLENARY LECTURES
Monitoring long-term and large-scale deposition of air pollutants based on moss analysis

Marina Frontasyeva

Joint Institute for Nuclear Research, Dubna, Moscow Region, RUSSIAN FEDERATION

Abstract. A brief historical review is given on the development and milestones of the moss biomonitoring technique used to study atmospheric deposition of trace elements, nitrogen, persistent organic pollutants (POPs); radionuclides of technogenic and natural origin in Europe, as well as cosmic dust. The relevance of these studies to the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP) is shown. Examples of the long-term activity of the ICP Vegetation (International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops) established in 1987 are given to illustrate the tendencies in behavior on a large scale of air pollutants such as heavy metals, nitrogen and persistent organic pollutants. In agreement with the long-term strategy of the LRTAP Convention to enhance participation and improve air quality in Eastern Europe, the Caucasus, Central Asia, and South Eastern Europe, efforts to extend the moss survey to former republics of the USSR such as Armenia, Azerbaijan, Georgia, Moldova, Kazakhstan, and Uzbekistan were successfully undertaken. Around 15 teams were formed in Russia to cover moss sampling in Northern and Central Russia, Western Siberia, and the Far East of Russia (Kamchatka and Sakhalin). To date, 42 countries, including Bulgaria, expressed their desire to participate in the 2020-2021-2022 moss survey. Analytical methods and approaches to data interpretation are reviewed.

Key words: moss biomonitoring, trace elements, nitrogen, POPs, radionuclides, cosmic dust.
**Abstract.** Bryophytes, a second biggest group among terrestrial plants, remain neglected although not completely omitted in conservation initiatives as compared to flowering plants. Additionally, public unawareness and low perception of bryophyte significance lead to have them less presented in conservation policies than they deserve in reality. Thus, bryophyte conservation suffer from unequal approach compared to other plants around the globe, where some countries have solid bryophyte conservancy policy till the others that have no any and do not even pay attention what bryophyte diversity and resources they posses i.e. ignore global responsibility on certain taxa. Europe, as continent with rather huge changes and human pressure on nature has also rather variable approach to bryophyte protection and even within European Union policy and bureaucratic approach is variously represented. In general, passive protection take part over continent and only isolated examples of pro-active and active protection are known. The high time is to start joint pan-European action in pro-active conservation of bryophytes known to be on the edge of survival. The approach should include many different experts apart from the most significant field bryologists and this way we can reach some of the goals of CBD and other relevant international conservancy documents. The initiative from the Bryophyte Biology Group in Belgrade (BBGB, Serbia) will be presented along with the achievement, in so far voluntarily initiative and all colleagues from around the globe interested in bryophyte conservation are welcomed to join, develop and work with us. BBGB are dealing on conservation biology of bryophytes and lately develop among others conservation sub-disciplines - bryophyte conservation physiology. BBGB has already over 260 species in *ex situ* and *in vitro* collection from all over the world with ca. 56% regionally, nationally or globally rare and threatened species. Examples of population straitening, reintroduction and *inter-situ*, with problems, solutions and achievements will be presented.
"The Boiling Stones": prospective and reliable biode corroborators

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Abstract. Zeolite is a collective name for minerals and chemical compounds within the group of aluminium silicates. Clinoptilolite (CLN) is one of the members of the zeolite family and is the most widely used natural zeolite in different studies. Nowadays nature is increasingly exposed to toxic elements, heavy metals, pesticides, different kind of pollutions and toxins. This leads to different health problems like decreased immunity, allergies, respiratory disorders, cancer, cardiovascular diseases, etc. Zeolites are beneficial in many fields because of their grid structure and chelation-like effect in removing heavy metals. They can act as an inorganic cation-exchanger, adsorbent, detergent builder, and active reservoir for metal-catalysed reactions (Sersale 1980). These minerals create a natural buffer in the gastro-intestinal tract by establishing an optimal pH level. Over the last decades numerous studies with CLN were performed in both humans and animals. The basis of interest is its biological effects concerns one or more of their physical and chemical properties. In medicine, especially micronized natural zeolite-clinoptilolite is a relatively novel subject of interest. Animal studies demonstrate that zeolites show great promise for environment protection, capability to detoxication of animal and human organisms, improvement of the nutrition status and immunity of farm animals, separation of various biomolecules and cells, strong antioxidant activity. There is a significant detoxifying effect after CLN supplementation in animal diet because of its ion exchange capacity and effective enterosorption for heavy metals, radionuclides and other inorganic pollutants. Recent findings indicate that CLN applied orally in laboratory mice for 90 days provides direct detoxification and shows significant and effective reduction of the lead accumulation in the intestine by more than 70% (Beltcheva et al., 2012, 2015) and leads to improvements in overall health status. Further research is needed to explore the effects of a zeolite-clinoptilolite to clarify the role of its detoxification mechanisms and auto-bio-regulation in human and animal organisms.
The versatile microRNA world

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Abstract. Since the discovery of microRNAs in 1993, thousands of microRNAs have been identified and characterized in animals, plants, and some animal viruses. In recent years, the application of next-generation deep sequencing (NGS) to small RNA-sequencing projects has revealed an expanding repertoire of diverse sequence variants of mature microRNAs called isomiRs, resulting from alternative post-transcriptional processing and affected by external or internal determinants. During this talk, canonical and non-canonical pathways of microRNA/isomiR biogenesis will be outlined across phyla. Given that biological complexity is determined not by the number of genes encoding proteins, but by the regulation of gene products, the essential role of microRNAs and isomiRs as mediators of flexible gene regulation will be considered. The prospect of using them as genetic markers for plant and animal biology purposes will also not be overlooked. I will also use this framework to provide a brief overview of the microRNA-related research carried out at the Faculty of Biology of the Plovdiv University. Combining bioinformatics analysis of NGS data with wet-laboratory approaches allowed us to identify a variety of conserved and species-specific microRNAs and isomiRs in different biological samples and to assess the dynamics of their expression profiles in response to different stimuli. In order to reveal the function of the identified microRNAs and isomiRs, I will also pay attention to their potential target genes and possible regulatory network in the cases studied.
Ecology, biodiversity and conservation
Various aspects of the distribution and abundance of turbot in Bulgarian Black sea coast

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Abstract. Turbot, *Psetta maxima* (Linnaeus, 1758), is a flatfish of the family Scophthalmidae, which is widespread in the North Atlantic, Mediterranean and Black Seas (Nielsen, 1986). The species inhabits European coastal waters in the North Atlantic from Norway to Morocco, including the Baltic, Mediterranean and the Black Sea (Blanquer, A., Alayse, J.P. Rkhami B.O. and Berrebi, P., 1992). It is a benthic marine species, inhabiting a sandy and muddy bottom, in the shallower part of the continental shelf up to about 110 meters (Karapetkova, 1964). Sahin & Gunes (2011) claim that *Psetta maxima* is widespread in the coastal zone at a depth of 20-60 m. Turbot is a highly valuable commercial species (Faber, 1883) and in the Republic of Bulgaria is fished by GNS - legal gill nets with a 400 mm mesh size. The stock of turbot in the Black Sea is approximately 1000 tons. The present study aims to address various aspects of the distribution and abundance of turbot through landing data. The data for the study is collected from the Bulgarian ports in the Black Sea in the period 2017-2019. Samples of 2062 fish are collected. The average total length and weight of the fish are 52.63 ± 6.12 cm and 2600 ± 910 g respectively. The length-weight ratios are determined by the following equations: 2017 – $W=0.02.L^{2.98}$, $n=566$, $(R^2=0.88)$; 2018 – $W=0.04.L^{2.80}$, $n=783$, $(R^2 = 0.85)$; 2019 – $W=0.34.L^{2.25}$, $n=713$, $(R^2 = 0.71)$; 2017 – 2019: $W=0.08.L^{2.60}$, $n=2062$, $(R^2 = 0.80)$. Based on the data analyzed in the study, *Psetta maxima* has healthy population in the Bulgarian waters of the Black Sea.

Key words: *Psetta maxima*, landings, size-weight relationship.
A study on reproductive capacity in *Primula veris* L. (Primulaceae)

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**Abstract.** Main criteria for evaluation of the reproductive capacity of perennial distylous species *Primula veris* were studied in three natural Bulgarian populations, such as: embryological features, mode of reproduction, pollen and seed (embryo) viability. The anthers are tetrasporangiate. The anther wall developing after Dicotyledonous-type consists of: an epidermis, fibrous endothecium, a middle layer and a secretory tapetum. After simultaneous meiosis in the pollen mother cells predominantly tetrahedral tetrads form in the anthers. The pollen grains are two-celled when shed. In the unilocular ovary many ovules develop. The mature ovules are anatropous, tenuinucellate and bitegmic. The embryo sac develops after *Polygonum* (monosporic)-type from the chalazal cell of the megaspore tetrad in the ovule. The Caryophyllad-type embryo and ab initio nuclear endosperm form after double fertilization. After acetocarmine testing, a high pollen viability in the studied populations more than 95% was established. The tetrazolium test revealed an extremely low seed viability (only just 2%) in the high mountain population, while for the other two studied populations it was more than 65%. No apomixis or even its elements are observed. It was established that *P. veris* is a predominantly amphimictic species corresponding with its diploid status (2n=2x=22) but the vegetative reproduction in this species also occurs. As a results of the present study it was obtained an important information about possibilities of the realization of reproductive capacity in *P. veris* – a valuable medicinal plant, mainly used as an antioxidant and expectorant agent.

**Key words:** embryology, pollen and seed viability, *Primula*, reproduction, reproductive capacity.

**Acknowledgements:** The authors are grateful to the National Science Fund (Grant КП-06-H26/6/13.12.2018) for the financial support provided.
Methodology for satellite control of logging in coniferous forests

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Abstract. Logging is still the predominant utilization of forest ecosystems. The wood obtained by felling is an indispensable natural resource, which is used in a number of economic sectors and this is the reason for the enormous speculative interest in it. This speculative interest is the reason for illegal logging or often prevails in determining the amount of timber envisaged and actually harvested in the legal use of the forest. Despite the institutional and public control of forestry activities, control over illegal logging is difficult to implement due to remoteness, lack of passable roads, corrupt practices, etc. This is the reason for the large share of illegally harvested timber in Bulgarian forests, which is difficult to determine even approximately. This study is aimed at developing a methodology for using multispectral satellite images (with a resolution of 3/3 meters) for quantitative and qualitative control of logging in coniferous forests, by calculating the NDVI (Normalized Difference Vegetation Index) of forest vegetation. The methodology was applied to randomly selected coniferous forests of distinct forestry units in the cadastral parcel of the village of Tishanovo, Nevestino municipality with various age and level of planned logging, in which felling was carried out. To achieve maximum accuracy, different options for comparing NDVI data from satellite images were researched – during the same seasons and the different seasons of the same year (2019) and during different years (2018;2020). For verification, the results were compared with the data obtained by the same methodology from similar forests in which no logging was carried out. A comparative analysis was performed to verify the findings by the satellite images comparing the amount provided for felling under forest management projects and the officially reported quantities of harvested timber after logging.

Key words: logging, coniferous trees, satellite images, remote sensing, NDVI, forest ecosystems.
New remote sensing based methodology for control of logging in coniferous forests

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Abstract. Logging is still the predominant utilization of forest ecosystems. The wood obtained by felling is an indispensable natural resource, which is used in a number of economic sectors and this is the reason for the enormous speculative interest in it. This speculative interest is the reason for illegal logging or often prevails in determining the amount of timber envisaged and actually harvested in the legal use of the forest. Despite the institutional and public control of forestry activities, control over illegal logging is difficult to implement due to remoteness, lack of passable roads, corrupt practices, etc. This is the reason for the large share of illegally harvested timber in Bulgarian forests, which is difficult to determine even approximately. This study is aimed at developing a methodology for using multispectral satellite images (with a resolution of 3/3 meters) for quantitative and qualitative control of logging in coniferous forests, by calculating the NDVI (Normalized Difference Vegetation Index) of forest vegetation. The methodology was applied to randomly selected coniferous forests of distinct forestry units in the cadastral parcel of the village of Tishanovo, Nevestino municipality with various age and level of planned logging, in which felling was carried out. To achieve maximum accuracy, different options for comparing NDVI data from satellite images were researched – during the same seasons and the different seasons of the same year (2019) and during different years (2018; 2020). For verification, the results were compared with the data obtained by the same methodology from similar forests in which no logging was carried out. A comparative analysis was performed to verify the findings by the satellite images comparing the amount provided for felling under forest management projects and the officially reported quantities of harvested timber after logging.
Additions to bryophyte flora of Kosovo from “Bjeshkët e Nemuna” mountains

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Abstract. Bryophyte flora of Kosovo is underexplored. The number of taxa reported is still very low compared with the neighboring countries from one side, and the presence of two important mountain massifs in Kosovo (Bjeshkët e Nemuna/Prokletije and Shar/Šar Mountains) from the other side. Bjeshkët e Nemuna (BN) is made up of many large mountains located in three different countries (Albania, Kosovo and Montenegro) of the Balkan Peninsula. The knowledge of bryoflora of BN is better known for Albania and Montenegro, with recent publications for both countries, whereas there is only one publication for Kosovo. In this context, the results from two field trips (in 2015 and 2019) carried on in various localities (Lagjia e Kaprojve, Reka e Allagës, Liqenat, Bogë and Hajla) in the BN Mountains are presented here. Altogether, 1140 moss specimens were examined, which comprise about 130 taxa. Thirteen species are new records for Kosovo: Calliergonella lindbergii, Campyliadelphus chrysophyllus, Cyrtomnium hymenophylloides, Dialytrichia mucronata, Dicranella varia, Grimmia anodon, G. donniana, Hypnum andoi, Pleuridium acuminatum, Polytrichum strictum, Schistidium helveticum, S. rivulare and Sphagnum centrale. In addition, two taxa are new at the variety level: Hedwigia ciliata var. leucophaea and Syntrichia ruralis var. ruraliformes. Several species have conservation value in the European context; they are red-list candidate species: Brachythecium geheebii, Encalypta affinis, Racomitrium ericoides, Schistidium papillosum, S. helveticum and Sphagnum centrale.

Key words: Mosses, biodiversity, conservation, red-list, Bjeshkët e Nemuna/Prokletije.
Status of conserved local plant biodiversity in Bulgaria –
new challenges and research priorities

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Abstract. Bulgaria is one of the countries with the greatest plant biodiversity in Europe, as well as with a significant number of unique ecosystems. The study aims to present the documented local plant genetic resources, conserved in Bulgarian seed genebank, with a view to correspond to the new challenges, created by the climate changes. The research priorities are focused on expanding the role of biodiversity in agricultural food systems to increase the sustainability of farmers and to return the traditional taste to the consumers. The survey is based on the national register of plant genetic resources and the international databases such as FAO WIEWS, GENESYS, EURISCO and AEGIS. The results show that in the national collection 65,015 accessions were described by passport descriptor. 16,010 accessions are characterized with Bulgarian origin. Through expeditions 10,687 seed samples of local varieties have been collected in the country. Overviews of plant genetic resources originating from Bulgaria is an indication of the wealth of crop plant species and their wild relatives, and they may assist in identifying gaps and need for further collecting. It may also be a good starting point for compiling checklists of cultivated plant species and future research activities. Some weak points in the documentation of the current ex situ collections are pointed out. The results will integrate descriptions of landraces, taking into account consumers’ perceptions and farmer’s needs for creation of thematic networks aimed to promote the durable use of local plant genetic resources. The research was supported by the Bulgarian Ministry of Education and Science under the National Research Programme “Healthy Foods for a Strong Bio-Economy and Quality of Life”, approved by DCM № 577/17.08.2018.

Key words: landraces, ex situ collections, databases, climate changes, sustainability.
Is the marine environment of the Bulgarian Black Sea stressful for organisms: a pilot assessment of oxidative stress in coastal fish species

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Abstract. The present study reports results of the first assessment of oxidative stress (OS) in fish species inhabiting the Bulgarian Black Sea coastal zone. The studied fish species (Platichthys flesus L., Neogobius melanostomus Pallass, 1814, Trachinus draco L., Mullus barbatus L., Merlangius merlangus L.) were caught during trawl selectivity experiments from different localities of the northern and southern coastal regions. The pro/antioxidant status of fish individuals was assessed by measuring standard OS biomarkers (lipid peroxidation, glutathione concentration, activities of superoxide dismutase and catalase) in gills and liver. Differences in OS biomarkers in the studied organs were demonstrated. Lipid peroxidation was higher in gills of all fish species with exception of M. barbatus. The glutathione concentration and superoxide dismutase activity were also higher in gills, except in M. merlangus where the highest activity was present in liver. On the other hand, catalase activity was higher in the liver in all fish species tested. In general, the level of OS in the studied fish differed depending both on the species and on the coastal region they inhabit. In the case of whiting, fish from the northern coast appeared to be more stressed, while along the southern coast mullets had higher level of OS. Our results demonstrated for the first time the presence of OS in fish inhabiting coastal ecosystems with different quality of the marine environment. Obviously, further studies are needed for the assessment of multiple stressor effects on the ecology of Bulgarian Black Sea fish populations.

Key words: oxidative stress, Platichthys flesus, Neogobius melanostomus, Trachinus draco, Mullus barbatus, Merlangius merlangus, Black Sea, Bulgaria

Acknowledgements: This work was supported by grant KPI-06-H41/7 of National Science Fund, Bulgaria.
State of the marine environment along the Bulgarian Black Sea coast as indicated by acetylcholinesterase activity of *Donax trunculus* L.

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Abstract. The aim of the study was to assess changes in the acetylcholinesterase (AChE) activity in wedge clams (*Donax trunculus* L.), gathered in July and September from shallow sublittoral sandy habitats at different localities along the Bulgarian Black Sea coast. It is well established that inhibition of AChE in marine organisms is mainly caused by the neurotoxic effects of organophosphate and carbamate pesticides but recently it was found that a number of other chemical species have similar effects including heavy metals, polycyclic aromatic hydrocarbons, detergents etc. The activity of AChE in the soft body of the clams in our study showed considerable variation among the studied localities. The most significant AChE inhibition was present in clams from locations close to areas with intensive touristic and industrial activities, such as the big resorts Slunchev Bryag, Irakli and Primorsko. Significant seasonal differences in AChE activity were also established. Higher inhibition of AChE was present in the wedge clams gathered in summer than in autumn. Our results demonstrated for the first time the presence of significant ecotoxicological effects of anthropogenic pressures on the shallow sublittoral sandy habitats along the Bulgarian Black Sea coast where *D. trunculus* is a dominant species. Hence, this clam species appears to be a suitable bioindicator of marine environmental quality.

Key words: *Donax trunculus*, acetylcholinesterase activity, Black Sea coast, Bulgaria

Acknowledgements: This work was supported by grant КП-06-Н31/6 of National Science Fund, Bulgaria.
New records and distribution data of stoneflies (Insecta: Plecoptera) from the Western Balkans

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Abstract. The knowledge about stoneflies of the Balkan Peninsula is still not complete with many areas poorly investigated. During this investigation we collected adult stoneflies specimens from Kosovo, Albania, North Macedonia, Serbia and Montenegro during the period 2014-2018. In total we found 43 species belonging to 14 genera and 7 families. Four species are recorded for the first time from Kosovo (Leuctra cingulata Kempny, 1899, Nemoura cf. lucana Nicolai & Fochetti, 1991, Nemoura uncinata Despax, 1934 and Brachyptera macedonica Ikonomov, 1983), one is recorded for the first time from Serbia (Nemoura asceta Murányi, 2007) and one from Albania (Leuctra major Brinck, 1949). This investigation contributes to the distribution patterns of stoneflies species in the Balkan Peninsula.
The spotted orbweaver *Neoscona byzanthina* (Pavesi, 1876) – an enigmatic but a common species within the Balkans (Araneae: Araneidae)

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**Abstract.** The spotted orb-weaver spider *Neoscona byzanthina* was described from the southeastern point of the Balkan Peninsula by Pavesi (1876) and herein is reported from Albania, Bulgaria, Kosovo, and North Macedonia for the first time. Its taxonomy, ecology, and general distribution are summarized and discussed.

**Keywords:** Albania, Bulgaria, Kosovo, North Macedonia.
A preliminary checklist of the spiders of Kosovo (Arachnida: Araneae)

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Abstract. The Republic of Kosovo is the territory with the least known spider fauna within the Balkans. The present list of spiders is based on all published records available to the authors and also includes original data which is in press or unpublished yet. The checklist comprises only 149 species belonging to 26 families and 98 genera. This low number is due to different reasons but mostly because the spiders herein has never been studied in its entirety and because we excluded all the records from Yugoslavia, Serbia, and the border mountain Kopaonik, for which cannot certainly be argued that relate to Kosovo, so we worked only with reports, containing sure data on the spider fauna from there. The aim of this study is to present an annotated preliminary checklist of the spiders of Kosovo with additional taxonomic and faunal data.
Leaf and stem anatomy of Bulgarian endemic *Marrubium frivaldskyanum* Boiss. (Lamiaceae)

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Abstract. In the Bulgarian flora, the genus *Marrubium* L. is represented by four species, of which *M. frivaldskyanum* is a rare species and Bulgarian endemic. The study presents information about the anatomy of leaves and stems of *M. frivaldskyanum*, collected from the floristic region of the Rhodopes Mts (western). The leaves show bifacial structure, with mesophyll which is differentiated as palisade and spongy parenchyma. The stomata are of anomocytic and diacytic types, located primarily on the abaxial epidermis. The leaf and stem surfaces are covered with two types of trichomes: non-glandular (unicellular and multicellular) and glandular. The cross-section of the stem represents the typical anatomy structure of dicotyledons. In terms of the shape, it is quadrangular, and collenchyma tissue can be seen under the epidermis in the area of the edges.
Trends in the change of the ecological condition of the River Mesta for 10 years period of research

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Abstract. Long term trends of the ecological status assessment (classified by biological quality element macrozoobenthos) of the trans-boundary Mesta River (South Bulgaria) during the period 1978-2020 were analyzed. Macrozoobenthos samples were taken and physico-chemical parameters were measured twice at 7 sites situated on the main river and its tributaries during the low waters period (August-September 2019 and 2020). Selected sites represented various ecological situations which are characterized with different types of human impact. Data on the current state of the river were compared with the previously studied periods 1978-1981, 1999-2000 and 2009-2012. The investigation is extension of a long-term research of the Mesta River that associated the river to the European Long Term Ecological Research Network (LTER). During the last decades the self-purification processes and restoration of the bottom invertebrate communities and processes of stabilization of the aquatic ecosystem have been observed. Long-term studies demonstrated there were no significant sources of load on the aquatic ecosystem, which results in improvement of the water quality and the ecological status of the ecosystem Mesta River.
Two new records caddisfly fauna (Insecta: Trichoptera) from the North Macedonia

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Abstract. During 2017 we collected adult caddisfly specimens at the Karadak Mountains, Republic of North Macedonia. In total we found 15 species, two of which are recorded for the first time from North Macedonia: Potamophylax fules Oláh and Ibrahimi, 2013 and Chaetopteryx gonospina Marinkovic-Gospodnetic, 1966. Potamophylax fules belongs to the nigricornis group and is scarcely distributed in Kosovo, Montenegro, Rumania and now Macedonia. Chaetopteryx gonospina has a disjunct distribution in Balkans and Pyrenees. Finding of these two species contributes to the knowledge of their distribution patterns and shows that Balkan Peninsula has still poorly investigated areas.
Benefits and opportunities for using of *Micromeria dalmatica*

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**Abstract.** *Micromeria dalmatica* Benth is a Balkan endemic medicinal and aromatic plant widely used as herb or spice. Methanolic and aqueous extracts of three populations of *M. dalmatica* were evaluated for free radical scavenging activity against DPPH radicals. All studied extracts showed significant activity. No difference was found in the antiradical properties between methanolic and aqueous extracts. Comparative analysis of metabolites composition of plant material of the studied populations was done by GC–MS. The results showed that *M. dalmatica* were rich in essential nutrients as fatty acids (saturated and unsaturated: omega-3), triterpenes (amyrin), sterols, phenolic and organic acids. In short, it is concluded that the contents of these important biologically active substances determine *M. dalmatica* as a valuable spice and medicinal plant with strong antioxidant potential.

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Population status and natural localities of *Rhodiola rosea* in Rila Mts.

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Abstract. *Rhodiola rosea* L., a member of Crassulaceae family, is an alpine plant that grows in specific and sensitive high-mountain habitat and is, therefore, a very suitable for studying the effect of climate change. Although the natural area of distribution of *R. rosea* is wide and includes most of the boreal and temperate parts of the Northern hemisphere, urgent measures for conservation of natural resources of the species are necessary. In Bulgaria, the species occurs on stony and rocky places, often on screes, in habitats near late-melting snowdrifts, which provides sufficient soil moisture (2280 - 2600 m above sea level) in Pirin, Rila and Stara planina Mts. It is protected by the Biodiversity Act of Bulgaria and is listed in the Red List of Plants in Bulgaria. The species is included in the Red Data Book of the Republic of Bulgaria (Peev, ed., 2015). According to the IUCN criteria, its threat status is Critically Endangered. This study aimed to evaluate the population size and the conservation status of six natural localities of *R. rosea* in Rila Mts: 1) Seven Rila Lakes; 2) Skakavitsa's waterfall; 3) Kalin Dam; 4) Nehtenitza; 5) Belmeken; 6) Musala hut. The best characteristics of both population size and conservation status were recorded in the localities situated in remote and hardly accessible areas with limited or no tourists' access. Based on thorough evaluations of trade levels and trends compared to population sizes, *R. rosea* must be considered to face serious threats from overexploitation and growing international trade due to its increasing use in herbal medicine.

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Syntaxonomical diversity of riparian forest vegetation in Bulgaria –
updated information and future perspectives

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Abstract. Riparian forest vegetation is widely distributed on the territory of Bulgaria but still its syntaxonomical diversity is not completely studied. All available reléves published in literature were gathered, as well as new data were sampled from different parts of the country. In total 484 reléves were collected following the Braun-Blanquet approach. All relevés were contributed to the Balkan Vegetation Database (EU-00-013). The nomenclature of the species was standardized according to the Euro+Med PlantBase. PC-ORD hierarchical clustering was used for classification from class to association and community levels. Diagnostic, constant and dominant species were determined for all syntaxa established. Riparian forest data were classified to *Salicetea purpurea* and *Alno glutinosae-Populetea albae* classes and nine associations (*Tamarici-Salicetum purpureae, Tamaricetum smyrnensis, Amorpho fruticosae-Salicetum albae, Salicetum albae, Salicetum fragilis, Stellario nemorum-Alnetum glutinosae, Castaneto sativae-Platanetum, Juglando-Platanetum orientalis, Smilaco excelsae-Fraxinetum oxycarpaceae, Leucojo aestivi-Fraxinetum angustifoliae, Scutellario altissimae-Quercetum roboris*). Two plant communities (*Tamarix tetrandra* and *Alnus incana*) were also identified. Woodlands dominated by *Fraxinus angustifolia* and *Quercus robur* were found in the eastern part of the country at lower altitudes and pronounced Mediterranean influence. *Platanus orientalis* woodlands were distributed in south Bulgaria and their syntaxonomical diversity is presented by two associations. Alliance *Tamaricion parviflorae* was restricted to the rivers in the southern part of the country. Its phytocoenoses were dominated by *Tamarix tetrandra* (incl. *T. parviflora*) and *T. smyrnensis*. *Stellario nemorum-Alnetum glutinosae* and *Salicetum fragilis* floodplain forests occurred from lowlands up to 1000-1300 m a.s.l. *Salix alba* riparian forests are represented by two associations along rivers in lowlands. On the other hand, the data for *Alnus incana* communities is still limited. Its phytocoenoses are distributed in mountainous regions in the beech, coniferous and subalpine belts.

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Integrated assessment of the status of fish fauna reproduction areas (Integrated IcRH Index)

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Abstract. Fish fauna reproduction areas (FRA) are natural habitats that provide conditions for sustainable reproduction of fish species populations. In order to preserve them from anthropogenic pressures, it is necessary to monitor the integrity of both biotic and abiotic environmental components, which ensure prerequisites for sustainable reproduction of fish fauna. In the monitoring studies carried out in September-November 2020 a pilot approach was applied for integrated assessment of the suitability of streams within the catchments of the Iskar and Vit rivers as fish fauna reproduction areas. The studies were performed, adopting the following basic principles for integrated assessment:

1) Applying an ecosystem approach by identifying two main ecosystem components in assessing the status of the FRAs:
   • Biotic Component (1) „Ichthyocenose” (Ic) with 6 criteria, which include the main biotic metrics;
   • Abiotic Component (2) “Reproduction habitat” (HR) with 5 criteria, including basic hydromorphological and physicochemical quality metrics.

2) Equal weight of the two components in the integrated assessment of the state of the potential fish fauna reproduction areas;

3) Application of standardized methodologies and available data in the estimation of the two components and the calculation of the integrated IcRH index.

The development of the integrated approach was carried out with data from the river type R2 Mountain rivers in Ecoregion 12 Pontic province. Its application for assessment to other types of aquatic ecosystems needs to be validated with additional monitoring data.

The integrated approach was developed and tested for the river type R2 Mountain rivers in Ecoregion 12 Pontic province. Its application for other types of surface waters (rivers and lakes) will be further validated with additional data.

Key words: integrated assessment, ecosystem approach, fish reproduction areas (FRA), IcRH Index.
Small mammals as appropriate radioecological monitor species in Alpine ecosystems: total β-activity as an indicator of decreasing consequences of the Chernobyl accident

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Abstract. The importance of Alpine ecosystems increases due to the influence of a wide number of ecological factors. Atmospheric attenuation of solar radiation, particularly in the ultraviolet spectra, is decreasing, leading to higher surface exposure and higher doses in plants and diurnal animals. At the same time, exposure to man-made radionuclides in the high mountains in Europe is decreasing due to the radioactive decay of anthropogenic radioisotopes, as well as the sedimentation and demobilization of heavier elements, which reduce exposure of the Alpine biota to technogenic emitters. The current article is based on biomonitoring studies of wild small mammals, conducted on Rila Mountain in the period 1993-2020. Total β-activity was measured with the use of a low-background beta counter (LAS 3A level activity system with 30% efficiency on 40K). A reduction by a factor of 10 was observed over a two-decade time period, attributable in part to the decay of deposited anthropogenic β-emitters after the Chernobyl accident in 1986. The highest reduction was observed in Apodemus sp., the least – in the herbivorous vole Myodes glareolus, indicating the high potential of the latter species as a zoomonitor of residual radioactive contamination. The comparative utility of several rodent taxa: Apodemus species, and two representatives of the subfamily Arvicolinae: M. glareolus and Chionomys nivalis and insectivorous Sorex araneus is discussed in the context of the radioecological biomonitoring of high mountain ecosystems. Finally, the study is put in the global context of climate change and overall increased anthropogenic pressure, affecting other ecosystems such as tundra and boreal forests.

Key words: biomonitoring, small mammals, total β-activity, alpine ecosystems.
Influence of small run-off HPPs in the Sreden Iskar river section (Iskar River, Bulgaria) on the benthic macroinvertebrate communities – preliminary study results

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Abstract. The effect of three small run-off hydro-power plants situated in the middle part of the Iskar River on the benthic macroinvertebrate communities was studied in 2019 and 2020. The diversity of the main benthic groups and their abundance at five sampling points along the river following the gradient of hydromorphological disturbance were traced. More than 75 taxa of species level, belonging to 16 systematic groups and 45 families were identified. The most diverse groups were Trichoptera (18 taxa), Diptera (17 taxa), Gastropoda (11 taxa) and Ephemeroptera (10 taxa). With a smallest number of species were the orders of Isopoda and Neuroptera, each presented by one species. For the study period the macroinvertebrate communities were very abundant. The groups with the highest number of specimens in total were: Diptera – 7818, Ephemeroptera – 5733, Trichoptera – 5528 and Gastropoda – 1292. The cluster analyse at family taxon level (Bray-Curtis similarity) groups the samples mainly by seasons and according to their geographical proximity. In the most of the groups there was a clear trend for reducing the abundance downstream the river from unaffected to the affected by hydro-power plants areas: Turbellaria, Oligochaeta, Coleoptera, Diptera-diversa, as well as Hydrozoa, Hirudinea, Isopoda and Ephemeroptera. For the mollusks, dragonflies and caddisflies there was a reverse trend to increase the number of specimens for both seasons. As an exception of the above was the family of Chironomidae which increased in abundance in September 2019, and decreased in June 2020 downstream of the river from unaffected to the affected areas.

Key words: macrozoobenthos, run-off HPP, cluster analyse, Sreden Iskar, Bulgaria, hydromorphological impact.
New data about moths fauna (Lepidoptera: Noctuidae) from Kosovo

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Abstract. Noctuid fauna of Kosovo is poorly known with only few registered records until now. Noctuid specimens were collected in seven different localities in Kosovo during 2019 (April to July). We found thirty five species. The distribution of species per families is as follows: 8 subfamilies, 9 tribes, 7 subtribe, 26 genus and 9 subgenus. Five of the found species are recorded for the first time from Kosovo: Hecatera cappa (Hübner, 1809), Simyra nervosa (Denis & Schiffermüller, 1775), Aegle semicana (Esper, 1798), Hecatera bicolorata (Hufnagel, 1766) and Mythimna (Hyphilare) congrua (Hübner, 1817).
Phaeocollybia, a new genus for the Bulgarian mycota, from relict coniferous forests

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Abstract. A new genus, *Phaeocollybia*, and the species *P. festiva* are reported for the first time from Bulgaria. *Phaeocollybia festiva* was found in forests, dominated by the relict pine *Pinus peuce*. Description and illustrations of the species are provided and the biogeographic significance of the finding is discussed.

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Diversity and conservation of Elm (Ulmus) genetic resources in Bulgaria

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Abstract. Elms (Ulmus spp.) are highly valuable and important forest trees facing the risk of decline and genetic erosion due to the Dutch Elm disease. Their share in forest stand is decreasing and the large trees, quite common before, are almost lacking today. In this review we present a survey on the status of the genetic resource of Elm species in Bulgaria – Ulmus glabra Huds., U. minor Mill. and U. laevis Pall. The survey includes a summary of the phenotypic and genetic diversity and conservation of genetic resources. The evaluation of the genetic diversity was done based on phenotypic and isozyme genetic markers. The most abundant species is U. minor, which is the most tolerant to the environmental conditions, while the other two species – U. glabra and U. laevis – occupy particular ecological niches. The assessment of genetic resources was based on a specially developed database. In situ and ex situ conservation measures are presented – natural genetic conservation units, seed orchards, and gene banks. Knowledge gaps were identified, together with the fields of prospective studies.

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Floristic and habitat diversity in representative grassland communities in Bulgaria

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Abstract. Natural and semi-natural grassland communities in Bulgaria are subjected to anthropogenic pressure leading to their reduction and fragmentation. Because they are home of many other groups of living organisms, this poses serious challenges in relation to their conservation and sustainable use. We present results of studies on the floristic and habitat diversity of various grassland communities in the plain areas of Bulgaria. The diversity evaluation was based on 38 experimental plots situated mostly in the eastern and northern parts of the country. More than 400 plant species and 12 habitat types were identified so far. Calculated diversity indices revealed that species richness varies substantially among the different habitats, with no clear trend detected. It is shaped by numerous environmental factors, and to a considerable extent – by the anthropogenic pressure. The threats to plant and habitat diversity are reviewed and the conservation measures are discussed.

Acknowledgements: This work was supported by the Project KP-06-N31/13 of the National Science Fund of Bulgaria.
Macrophyte-based assessment of mountain and semi-mountain rivers in South Bulgaria

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Abstract. Aquatic macrophytes were monitored in 25 river sites from mountain and semi-mountain river types during the main vegetation season in 2020. As basic community metrics species composition (both submerged and at different levels on the banks, where they are occasionally flooded) and abundance were recorded. The applied Reference Index illustrated that the prevailing part of the sites were in high and good status (n=16). These locations were characterized by communities of aquatic mosses *Platyhypnidium riparioides* (Hedw.) Dixon, *Fontinalis antipyretica* Hedw., *Cratoneuron filicinum* (Hedw.), *Leptodictyum riparium* (Hedw.) Warnst. Three sites were assessed in moderate, 5 in poor status, where macrophyte communities were dominated by vascular plants *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. In conclusion, river sites in poor and bad status represented almost 25% of the studied sites and reflected strong anthropogenic pressure.
Contribution to the knowledge of *Pontechium maculatum* (Boraginaceae), a species of high conservation concern in the Bulgarian flora

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Abstract. *Pontechium maculatum* is a species of high conservation concern in the Bulgarian flora. It is legally protected according to the national Biological Diversity Act, and listed in the Habitats Directive. Recent studies in one of the largest populations in the country revealed some new data about the species which are reported here. The population studied is situated in Mt Lozenska, Mt Sredna Gora floristic region. Relevant taxonomic literature states that *P. maculatum* is a biennial plant, however, our observations revealed the plants in Mt Lozenska are perennial (possibly short-lived). A great variation in the corolla-colour was observed, most plants having bluish to violet corolla and only a few – reddish to dark red. This can be explained by either natural variation in the population, not reported in the taxonomic literature so far, or by hybridisation with *Echium vulgare* which co-occur in the same locality. Large fluctuation in the number of flowering plants in two consecutive years was recorded (180 in 2019 and only 25 in 2020). Studies in the genome size of this population revealed the plants have significantly higher DNA-content than plants from the typical populations of the species in Northeast Bulgaria floristic region. Further biosystematics studies (chromosome number, genome size, genetic studies, etc.) are currently in progress to explain the observed phenomena within the Bulgarian populations of *P. maculatum*.

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New records of *Microbotryum* (Microbotryaceae) from the Balkan Peninsula

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**Abstract.** Seven smut fungi belonging to Microbotryum are reported for the first time from the following Balkan countries: *M. heliospermatis*, *M. piperi*, *M. scabiosae*, *M. silenes-dioicae*, and *M. silenes-saxifragae* from Bulgaria, *M. reticulatum*, *M. silenes-saxifragae*, and *M. stygium* from Greece, and *M. silenes-saxifragae* from Bosnia and Herzegovina and Croatia. The finding of *M. piperi* represents the first Balkan record of this smut fungus which, elsewhere in Europe, is only known from the French Pyrenees and the Italian Alps. The finding of *M. stygium* in Crete considerably extends its distribution in Europe. *Rumex tuberosus* subsp. *creticus* is reported as a new host for *M. Stygium*.

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Ground beetles in German oilseed rape fields and extensively used grasslands (Coleoptera: Carabidae)

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Abstract. This study aimed at clarifying species composition and ecological structure of carabids associated with the oilseed rape during its flowering, ripening and after the harvest. The research also encompassed the adjacent pastures. Field work was carried out in 2017. Pitfall traps (5 in each site) were set in 10 sampling sites in different regions in Saxony, Germany. We collected 18633 specimens (14285 in rapeseed fields, 4348 in pastures). Collected beetles belonged to 92 species from 33 genera. The richest tribes were Harpalini (22 species), Amarini (15 species), Pterostichini (12 species), Bembidiini (7 species), Carabini and Sphodrini (6 species each). The most species-rich were genera Amara and Harpalus (15 species each), and Bembidion and Pterostichus (6 species each). The most abundant species in the rape fields were Poecilus cupreus (4014 ex.), Calathus fuscipes (3666 ex.), Pterostichus melanarius (1654 ex.), and Nebria brevicollis (1474 ex.). The most abundant in grasslands was Poecilus versicolor (4014 ex.), being a superdominant with 37% of the total catch in grasslands. It was followed by Calathus fuscipes (420 ex.), and Amara lunicollis (353 ex.). In rape fields we found 68 species, and in grasslands – 76 species. Specific to the rape fields were 16 species, and to pastures – 24 species; 52 species were common for both sites.

Key words: carabids, agrocoenoses, grasslands, Germany, diversity.
Ground beetles in Swiss oilseed rape fields and adjacent mowed meadows (Coleoptera: Carabidae)

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Abstract. This study aimed at clarifying species composition and ecological structure of carabids associated with the oilseed rape during its flowering, ripening and after the harvest. The research also encompassed the adjacent mowed dry grasslands. Field work was carried out in spring and summer 2017. Pitfall traps (5 in each site) were set in 8 sampling sites located in the Swiss Jura (cantons of Argovia and Solothurn). We collected 11549 specimens (11033 in rapeseed fields, 516 in grasslands). Collected beetles belonged to 60 species from 29 genera. The richest tribes were Harpalini (17 species), Pterostichini (8 species), Amarini (7 species), Bembidiini and Carabini (6 species each). The highest species richness was among genera Harpalus (11 species), Amara (7 species), and Carabus (6 species). The most abundant species in the rapeseed fields were *Poecilus cupreus* (7126 ex.), *Anchomenus dorsalis* (842 ex.), *Pterostichus melanarius* (663 ex.), and *Amara ovata* (583 ex.). The most abundant in grasslands were *Carabus auratus* (176 ex.), and *Harpalus dimidiatius* (102 ex.). In rapeseed fields we found 45 species, and in grasslands – 31 species. Specific to the rapeseed fields were 29 species, and to grasslands – 15 species; 16 species were common for both types of habitats.

Key words: carabids, agrocoenoses, grasslands, Switzerland, diversity.

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New and unpublished data about the ground beetles from tribes Amarini and Harpalini
(Coleoptera: Carabidae) of Bulgaria

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Abstract. Bulgarian carabid fauna is relatively well studied but there are still many taxa and regions in the country lacking enough research. This study gives new information and new records on 93 carabid species of the tribes Amarini (28 species) and Harpalini (65 species), and 23 zoogeographical regions and subregions in Bulgaria. The focus is on tribes Amarini and Harpalini, which are among the most abundant and species-rich in Bulgaria. Fifty, or almost the half of the species collected are recorded for the first time in 19 different zoogeographical regions of Bulgaria. Twenty-eight species are reported for the second time about the 14 regions where they were currently collected. Sixty-five species haven’t been reported for more than 20 years from the Eastern Stara Planina Mts., Kraishte region, Osogovska planina Mt., Boboshevo-Simitli valley, Sandanski-Petrich valley, Vitosha Mts., Rila Mts., and Pirin Mts.

Key words: carabids, new records, Bulgaria, distribution, additional data.
Ecology of Carabidae (Coleoptera) from the Sarnena Sredna Gora Mts.

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Abstract. On the basis of material collected during field trips in Sarnena Sredna Gora Mts in 2018–2020, and the available bibliographic data, we completed a list of 175 species of ground beetles (Coleoptera: Carabidae), belonging to 59 genera and 21 tribes. This study aimed at analyzing the ecological structure of the carabid fauna. The dominant structure was characteristic with the presence of 1 eudominant numbering 11% of all specimens (Laemostenus cimmerius), 5 dominants (32%), 6 subdominants (22%), 12 recedents (23%) and 82 subrecedents (12%). Species richness was highest in humid habitats near river banks and ecotones. Analysis of the life forms showed a predominance of the zoophages (107 species, 61%) over the mixophytophages (68 species, 39%). Humidity preferences analysis showed the larger share of the mesophilous and mesoxerophilous carabids. The macropterous carabids were 57% of all species.
Occurrence of haemoparasite of genus Hepatozoon (Adeleorina: Hepatozoidae) in the marsh frog (Pelophylax ridibundus Pallas, 1771) in Bulgaria

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Abstract: The distribution of Hepatozoon from anurans, specifically frogs, and their host-parasite relationship are of great interest. Due to the specifics of frogs’ ontogeny, they form a link between aquatic and terrestrial ecosystems. These amphibians are important in food chains, ensuring the normal functioning of biocenosis. In this study, we present data on Hepatozoon incidence in Pelophylax ridibundus (Pallas, 1771) for the first time in Bulgaria. The blood smears of 137 individuals were investigated. In three of five studied localities, the presence of apicomplexan haemoparasite from the genus Hepatozoon was revealed. The indices Infection extensity (IE) and Infection intensity (II) showed different rate of infection in frogs populations inhabiting Chaya river (IE=27.50%; II=10.96%), Tsalapitsa rice fields (IE=6.38; II=9.91%) and nature wetland Zlato pole (IE=10%; II=8.07%). It was found that the gamonts morphology and morphometric parameters of the parasite are closest to Hepatozoon magna, but molecular tools are required to confirm the species determination. Our findings revealed that acridine orange is appropriate dye for detecting haemoparasites of the gender Hepatozoon.

Key words: amphibia, frog, blood parasite, Hepatozoon, Pelophylax ridibundus.
Chronic exposure to heavy metals induces nuclear abnormalities and micronuclei in erythrocytes of marsh frog (*Pelophylax ridibundus* Pallas, 1771)

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**Abstract.** Amphibians have big potential as bio-indicators based on their combined life cycle as aquatic and terrestrial form. They can play the role of prey or predator, making them a key element in toxic substances transfer between aquatic and terrestrial habitats. The nuclear abnormalities (NAs) in amphibians’ erythrocytes in recent years have been used as a successful biomarker for anthropogenic pollution. The NAs including micronuclei in erythrocytes of the marsh frog (*P. ridibundus*) have been studied to assess the cytotoxic and genotoxic effect in heavy metal polluted area *in situ*. Here we assess the cyto- and genotoxic potential of the polluted waters (Chaya River) close to the lead-zinc smelter near Plovdiv (Bulgaria) situated in an area that has been contaminated with heavy metals for 60 years. Frogs from Strandzha Natural Park were used as a negative control. Peripheral blood smears have been dyed with acridine orange. NAs of the following types: notched nuclei, nuclear buds and blebbled nuclei have shown highest frequency. There is no sexual dependence in the formation of different types of NAs. The significant differences (P≤0.0001) in the mean total NAs (‰) in erythrocytes of marsh frogs from the polluted area compared to the total NAs from the background region “Strandzha” NP demonstrate the presence of *in vivo* active cytotoxic and genotoxic agents in the impacted area. The obtained results for NAs in erythrocytes of *P. ridibundus* are evidence for successful application of NAs as a biomarker in amphibians for the purpose of biomonitoring.

**Key words:** genotoxicity, nuclear abnormalities, micronuclei, heavy metals, *Pelophylax ridibundus*.

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Current status of the upper part of Iskar River

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Abstract. The hydrological characteristics of the upper reaches of the Iskar River are distinguished by significant fluctuations and pronounced seasonality. In these sections of the lotic ecosystem an anthropogenic pressure have been detected, which had an adverse effect on the ecological situation of the aquatic ecosystem. The aim of the research paper was to analyze hydrological characteristics and physico-chemical parameters of the waters at the upper part of the Iskar River. Hydrological data from the conducted own measurements in pre-selected representative sites were collected. An analysis of the water quantity changes for the period 2015-2020 and water balance estimation was made. Water samples from the selected representative points in the upper part of Iskar River were taken. Water temperature, pH, dissolved oxygen/ saturation and conductivity were measured. Ecological status of the studied sites according to the values of the basic physico-chemical parameters of the water was assessed.

Key words: Iskar River, hydrological characteristics, physico-chemical parameters, ecological status.
New taxa for the palaeoflora of Satovcha and Ustren (Rhodope Mts, Bulgaria)

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Abstract. Over the last decade, the Regional Museum of Natural History – Plovdiv has created its palaeobotanical collection and increases it annually. In this presentation new taxa for the palaeoflora of Bulgaria are presented. They are found in the paleobotanical materials obtained during field activities, which were funded by the museum in 2019. The total number of these taxa is six. The taxa Lithocarpus aff. trachycarpus (Hickel et A. Camus) A. Camus and Ficus aff. sarmentosa Buch.-Ham. ex Smith have been identified from the area of the village of Satovcha (Western Rhodopes). Both recent species, which paleotaxa have been compared with, are distributed in areas of SE Asia characterized by a warm and humid climate. It is important to note that the middle Miocene flora from Satovcha is the richest local palaeoflora in Bulgaria. It contains over 120 fossil taxa. The establishment of new taxa in it shows that, despite its diversity, it is possible to enrich its composition with new research. Four new taxa are represented from the area of the village of Ustren (Eastern Rhodopes). These are Bauhinia aff. yunnanensis Franch., Juncus sp., Laurophyllum heeri (Ettings.) Nemejc & Knobloch and Vaccinium acheronticum Unger. The early Oligocene flora of Ustren is poorly studied. It consists of about 30 taxa, but so far only the species Ficus palamarevii Bozukov, Ivanov et Utescher has been published. The nearest living relatives of these four taxa are of different geographical origin. The species Bauhinia yunnanensis Franch. is distributed in SE Asia. Representatives of the Genus Juncus are cosmopolitan, and in the tropics they are found in the mountains. The nearest living relatives of Laurophyllum heeri and Vaccinium acheronticum, respectively Nectandra opositifolia Nees and Vaccinium stamineum L. are distributed in SE North America. The climatic conditions under which these recent species develop are characterized by a warm and humid climate. New research on the paleoflora of Ustren will undoubtedly lead to an increase in its taxonomic composition.
Morphological integration of head capsule in millipede
*Megaphyllum unilineatum* (C. L. Koch, 1838) (Diplopoda: Julida) during ontogeny

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Abstract. Morphological integration is defined as covariation of multiple parts of the morphological traits. Modularity is characterized by the presence of subunits (modules), which have high level of integration within themselves, but weak between themselves. In this respect, differences in function, anatomical structure and embrionical origin of modules can influence development and function of organism. The aim of our study was to test the hypothesis that the distal region of the head capsule is a module in *Megaphyllum unilineatum* using geometric morphometrics. Millipedes belonging to postembryonic stadia VI to XI were collected from Krnjača, Belgrade. The ontogenetic stadia were determined by counting the number of rows of ocelli, the total number of ocelli and rings, and the number of rings with and without walking legs. In data processing, the following statistical programs were used: MakeFan, TpsDig, CoordGen, MorphoJ, and R program. Procrustes ANOVA showed that individual × side interaction was significant. Allometry was significant for the symmetric component (SC), but not for the asymmetric component (FA). Since covariance coefficients (RV) for SC and FA had lower values than 10.4% and 13.8% (respectively) of other RV coefficients obtained by a random contiguous partition of dorsal part of the head capsule, modularity hypothesis is rejected. Further, the scaled variance of the eigenvalues of the SC (EV) was significantly lower before than after allometry removal. These results indicate that distal and proximal regions of the head capsule, due to their strongly morphological integration, develop and function as a module during ontogeny in this species.
Morphological intersexual differences in the Balkan endemite
Apfelbeckia insculpta (L. Koch, 1867) (Diplopoda, Callipodida) during ontogeny

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Abstract. Although sexual dimorphism in morphological traits (SD) has been widely studied in many animal groups (including arthropods), its developmental mechanisms are poorly understood. As ontogenetic allometry describes whether size of a morphological trait significantly affects changes of its shape during ontogeny, comparison of allometric growth trends between sexes can reveal whether and how intersexual differences in developmental pathways influence the presence and patterns of SD in adults. We studied allometric relationships between 10 morphological traits (head length and width, gnathochilarium length and width, antennal length, trunk length, width and height, leg length, body mass) and body length in different ontogenetic stadia of both sexes of the Balkan endemic millipede Apfelbeckia insculpta (L. Koch, 1867) using bivariate and multivariate analyses. Our sample consisted of 61 males and 60 females belonging to two sub-adult groups (postembryonic stadia VIII and IX) and adults (postembryonic stadium X). All individuals were collected from Hadži Prodanova Pećina Cave (Serbia). We detected that all morphological traits share common slope between sub-adults and adults of the same sex. Significant SD in common slope was detected for head and gnathochilarium dimensions and body mass. Also, these traits, together with antennal and leg length, showed different allometric growth trends between sexes. Our data show that SD in morphological traits in adults is achieved through intersexual differences in growth rates, as well as through differences in allometric growth trends. This study suggests allometric scaling as key process in development of adult body plan in A. insculpta.
Invasion of *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in water bodies from the East Aegean River Basin in Bulgaria

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Abstract. Since the first record of the invasive *Corbicula fluminea* (Müller, 1774) in Bulgaria in 2001 (Danube River at Vetren village), the Asian clam spread in a relatively short time to most of the Bulgarian Danube tributaries. For almost a decade after, the mussel was registered in many surface freshwaters in Bulgarian Danube River Basin, including rivers, lakes and reservoirs. The aim of the current study is to report the finding of *C. fluminea* in East Aegean water basin in Bulgaria. As a result of large-scale research of macroinvertebrate communities in different types of water bodies, 6 new localities of this species were registered – two in Tundzha River and four – in Maritsa River, all situated in their lower stretches of the rivers. Considering the current known distribution of the species, we discussed the possible path for invasion of the largest rivers in south-eastern part of Balkan Peninsula and the probability of rapid spread of Corbicula in their tributary systems.

Key words: Asian clam, invasion, dispersal, East Aegean River Basin, Bulgaria.
New approach to control of fasciolosis: Neutralization of *Fasciola hepatica* miracidia with methanol extracts of three *Artemisia* species at preserved vitality of *Galba truncatula* snails

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**Abstract.** *Fasciola hepatica* is a causative agent of fasciolosis, a widespread parasitosis with a significant negative effect on the livestock industry. The disease is controlled through drug treatment of farm animals with synthetic anthelmintics. Two hosts and free-living stages are included in the parasitic life cycle - definitive (grazing animals, humans) and intermediate (freshwater snails), providing the multiplication of the larvae. The infected animals produced the parasite eggs, passed with the faces. Falling into an aquatic environment the eggs hatched the miracidia - the swimming larvae infecting snails after active penetration through the snail body surface.

The targeted miracidial treatment can be an opportunity for the control of fasciolosis. In this study, the effectiveness of the methanolic extracts of *Artemisia absinthium* L., *Artemisia lerchiana* Stechm and *Artemisia santonicum* L. (Asteraceae) were examined for anti-miracidial and molluscicidal action. The chemical composition of the studied extracts and fractions were analyzed by gas chromatography-mass spectrometry (GC-MS) and high-performance thin-layer chromatography (HPTLC). Plant material was collected from the natural localities of the studied species. Phenolic acids (chlorogenic, 4(p)-hydroxybenzoic, vanillic and quinic), flavonoid aglycones (artemetin - quercetagetin 3,6,7,3',4'-pentamethyl ether), fatty acids (linoleic acid, C18:2 as dominant) were identified in the extract of *A. absinthium*. *A. santonicum* and *A. lerchiana* belong to *Artemisia maritima* group and have similar chemical profiles. However, a few differences were found in the extracts. Multi-component flavonoid profiles of both species were detected. Highly methylated flavonoid aglycones derivatives of quercetagetin, apigenin, and luteolin were identified. The high content of unsaturated fatty acids (C18:3, C18:1, C18:2) was found, too. Also chlorogenic, quinic, azelaic, protocatechuic, and caffeic acids as well as polyols, triterpenes were established in the extracts.

*F. hepatica* miracidia and *G. truncatula* snails were obtained from the laboratory maintained life cycles. Three groups of 100 miracidia and 10 snails each were constituted per treatment. Each snail was individually exposed to 10 freshly hatched miracidia in 3 ml water in the absence (control) or presence of *Artemisia* extracts at final concentrations of 50, 100 to 200, and 400 mg/ml, for 2 h (exposure period), at room temperature.

The obtained results clearly showed the effectiveness of used concentrations of *Artemisia* extracts on the viability of invasive larvae of the parasite and its intermediate snail host.

<table>
<thead>
<tr>
<th>Concentration of <em>Artemisia absinthium</em> extract (µg/ml)</th>
<th>No of examined miracidia/snails</th>
<th>No of live miracidia/snails after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>300/30</td>
<td>46/30 30/30 41/30 41/30 37/30</td>
</tr>
<tr>
<td>50.0</td>
<td>300/30</td>
<td>295/30 293/30 12*/30 0/30</td>
</tr>
<tr>
<td>100.0</td>
<td>300/30</td>
<td>297*/30 277*/30 0/30</td>
</tr>
<tr>
<td>200.0</td>
<td>300/30</td>
<td>201*/30 106*/30 0/30</td>
</tr>
<tr>
<td>400.0</td>
<td>200/20</td>
<td>0/20 0/20 0/20</td>
</tr>
</tbody>
</table>

* The larvae with reduced motility.
Here, we presented preliminary data for neutralization of *F. hepatica* miracidia with plant extracts at preserved vitality of *G. truncatula* snails - these results have a potential value for future application and outlined a new approach to the control of fasciolosis.

<table>
<thead>
<tr>
<th>Concentration of <em>Artemisia santonicum</em> extract (µg/ml)</th>
<th>No of examined miracidia/snails</th>
<th>No of live miracidia/snails after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>300/30</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>300/30</td>
<td>46/30</td>
</tr>
<tr>
<td>100.0</td>
<td>300/30</td>
<td>0/30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of <em>Artemisia lerchiana</em> extract (µg/ml)</th>
<th>No of examined miracidia/snails</th>
<th>No of live miracidia/snails after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>300/30</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>300/30</td>
<td>45* /30</td>
</tr>
<tr>
<td>100.0</td>
<td>300/30</td>
<td>0/30</td>
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</tbody>
</table>
Feeding ecology of the Marsh frog (*Pelophylax ridibundus* Pallas, 1771) in areas with different types of anthropogenic pollution

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**Abstract.** In this study, we make an attempt to assess how the food composition of the Marsh Frog (*Pelophylax ridibundus*) populations from anthropogenically polluted areas, is affected and what changes in the diet, can be affiliated with environmental pollution. We examined the trophic spectrum of individuals from three populations – two polluted areas (Site 1 - the Chaya River near the confluence with the Maritza River, where the river receives industrial waste waters and Site 2 – Tsalapitsa Rice Field near Plovdiv City) and one reference site (control) at Vacha River, south of the town of Krichim (located in protected zone that is far from industrial or agricultural human activity). The stomach contents were collected by using the stomach flushing method, stomach contents of 60 (30 males, 30 females) adult individuals were studied.

**Acknowledgments:** This work was supported by Project FP19-BF-013, financed by the Department of Scientific Research, University of Plovdiv, Bulgaria.
Seasonal and sex based differences in the diet of the Green toad 
(*Bufo viridis* Laurenti, 1768) in the city of Plovdiv

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Abstract. Differences in the diet of the green toad (*Bufo viridis*), based on sex and season are
presented. The diet of the green toad consists mainly of insects, predominantly Hymenoptera
(mostly Formicidae) and Coleoptera. When comparing the trophic spectrum between the two sexes,
we did not detect statistically significant differences. The overlapping of food niches between the
sexes, as well as the seasonal differences in the diet of the Green Toad are discussed. The green
toad can be classified as a polyphage (zoophage), which plays an important role in food chains, both
in natural and urban ecosystems.

Acknowledgments: This work was supported by Project MU21-BF-021, financed by the
Department of Scientific Research, University of Plovdiv, Bulgaria.
Overseen ecosystem services of ponds and their insects – their role for supporting terrestrial consumers and biodiversity "EUROPONDS"


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Abstract. Globally there’s an astounding and often underestimated number of ponds (small stagnant water bodies). Ponds, whilst directly providing habitats for a multitude of animals and plants, also contribute to habitat connectivity by enabling organisms to cross the landscape, acting as “blue stepping stones”. The diverse community of insects emerging from such ponds acts as a vector of dietary energy to the surrounding terrestrial environment. By providing highly unsaturated fatty acids, emerging insects constitute a high nutritional food resource for terrestrial (e.g. spiders, lizards) and avian (e.g. bats, birds) consumers. One important dietary resource these insects export is essential fatty acids. These are introduced initially into the local food web and then on into the main terrestrial cycle. However, knowledge about the exact ecological role of ponds, their contribution to beta- and gamma-biodiversity, and dietary energy transfer is limited. Herein, we present preliminary results for the first seasonal sampling (Autumn 2020) of our ongoing “EUROPONDS” project. EUROPONDS aims to fill this gap of knowledge on ponds by analyzing 55 ponds distributed across Europe from Sweden in the north to remote Mediterranean islands in the south, from rural areas to large cities, and including both artificial and natural ponds. EUROPONDS brings together around 80 European early-career limnologists and covers autumn, winter, spring, and summer sampling campaigns through 2020/21. Preliminary results from autumn 2020 indicate differences in emerging insect biomass, trophic status of ponds, and between artificial and natural ponds.

Key words: insect diversity, dietary energy, fatty acids, Europe.

Acknowledgements: This project is funded by the European Federation of Freshwater Sciences (EFFS).
Trophic structure of the macroinvertebrate assemblages of mountainous and semi-mountainous rivers in 7th Ecoregion (R3 and R5 river types)

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Abstract. The current study presents data on the trophic structure of bottom macroinvertebrate communities in mountainous and semi-mountainous river sections from Mesta, Struma and Vardar River catchments all belonging to 7th Ecoregion Eastern Balkans. In total 69 samples were collected at 38 sites - in autumn 2017 and spring 2018. The benthic macroinvertebrate taxa were assigned to seven FFGs (mainly at the genus and family level): shredders, scrapers, collectors, filterers, deposit feeders, predators and parasites. We analyzed both the trophic structure of the bottom macroinvertebrate communities and the dynamics of the functional feeding groups per sampling sites and in different seasons. The similarity between the localities was studied based on the trophic structure of the macroinvertebrate communities. A comparison between Rhithron Feeding Type Index (RETI) and the Index of Trophic Completeness (ITC) bioassessment approach was done in order to analyze the advantages of the application of the two indices for assessment of the ecological condition at the studied sites. We found that due to the human impact, the trophic structure of the benthic macroinvertebrate communities in seemingly similar in typology river sections differs at the sampled sites in both countries with a clear separation of river types R3 and R5. Thus, the predominance of deposit feeders recorded at North Macedonian sites reflects the higher anthropogenic disturbance of the studied rivers there. Long-term anthropogenic pressure leads to changes in microhabitats, which directly affects the functioning of the aquatic ecosystem, and respectively, is reflected on the transformation of the trophic structure of macrozoobenthos.
Improved forest management for plant and fungal diversity conservation under climate change

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Abstract. The contribution elaborate on how effect of forest management on plant and fungal diversity can be evaluated with an example of spruce (P. abies) dominated forests in the Perelik massive in Rhodopes mountain. In this context, the methodological approach used is presented. Evaluation of stand structure and related elements of biodiversity is done in a systematic grid of more than 200 sampled plots. Various stand attributes (as predictors) have been evaluated in each plot, e.g. main canopy tree species composition; DBH distribution of the trees, canopy closure, basal area, quantity and degree of rotting of dead wood of various classes; and management intensity and timing. Plant and fungal biodiversity (as response variables) has been characterized by species richness, cover of each species, total plant cover, number of Red-listed species, and trophic structure in randomly situated plots within the studied representative stands. Additionally, the contribution presents some initial results of forest structure and related plant and fungal diversity as well as principles related to linker functions elaboration.

Acknowledgements: Study is supported by the Bulgarian National Science Fund (BNSF) via the project KP-06-N31/12 or 11.12.2019.
Distribution of *Boeckella poppei* (Copepoda: Calanoida) on the Livingston Island, Antarctica

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**Abstract.** Livingston Island is the second largest island of the South Shetland Archipelago, Maritime Antarctica. Freshwater fauna in the area is scarcely studied. Previous records are limited and include habitats, which are free of permanent ice or snow cover during the austral summer. The calanoid *Boeckella poppei* was firstly recorded from the island in the 1990s, from the Todorina buza Lake. More recently, the species was also recorded form freshwater lakes on the Byers Peninsula, Western Livingston Island. During the 28th Bulgarian Antarctic Expedition, we confirmed the presence of *B. poppei* in the Todorina buza Lake. In addition, we recorded the calanoid in temporary pools (i) situated on the permafrost and formed after the retreat of the Perunika Glacier and (ii) at Hannah Point. In the newly-established habitats the species was very abundant. Our results suggest a considerable expansion in the distribution of *B. poppei* on the Livingston Island, likely owing to recent climate change and the resulting freeing of additional territories of permanent ice cover.

**Acknowledgments:** This study was funded through the National Program for Polar Studies, Polar Research Funding – 2019, projects # 70.25-178/22.11.2019 and 70.25-177/22.11.2019. The authors are grateful to the Bulgarian Antarctic Institute and the staff of the 28th Bulgarian Antarctic Expedition for their logistic support.
Macrozoobenthos from sub-Mediterranean intermittent rivers (R14 national river type) in Bulgaria.

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Abstract. Worldwide, intermittent rivers and ephemeral streams drain more than 50% of the land surface on Earth. Nonetheless, their ecology remains insufficiently understood. In Bulgaria, temporary rivers are typically small- or medium-sized rivers (national type R14) with sub-Mediterranean climate. We present the first data focused explicitly on macrozoobenthos from intermittent rivers in four Bulgarian river basins (of Struma, Arda, Maritsa and Tundzha Rivers) within the drainage of the Aegean Sea. We identified 114 taxa from nine rivers or between five and 33 taxa per site. The absolute abundance varied between 61 and 994 individuals per site. The most common were the mayflies *Baetis rhodani* (recorded at six of the nine sites), *Caenis macrura*, *Ephemerella ignita*, *Ecdyonurus (Ecdyonurus) dispar* and the chironomid *Cricotopus sylvestris*-gr. (all recorded at five of the sites), followed by the crab *Potamon ibericum* (recorded at four sites). Overall, insects dominated in terms of diversity and abundance. Cluster analyses at taxon level indicated considerable differences among macroinvertebrates at different stations. To some extent, important for the grouping of rivers was their affiliation to river basins. However, the distinctness of communities was likely a reflection of the high variability in environmental conditions and local human impacts. According to distance-based linear models, the taxonomic structure of the studied communities could be explained only partially by the measured water parameters. Only pH was associated significantly with the benthic communities and explained circa 19% of the total variation at taxon level and 33% at major-groups level. We could speculate that the taxonomic composition was affected by factors that have not been accounted for in the present study, i.e. by the great annual fluctuations in river flow, characteristic for R14, or by loading with nutrients or other pollutants.

Key words: temporary rivers, benthic invertebrates, Southern Bulgaria, drainage basin of Aegean Sea.

Acknowledgments: The study was funded by the World Bank, project # 71 957 35/17.4.2020, DICON-UBA.
Epigeobiont Carabidae and Pselaphinae beetles (Coleoptera) from high altitude coniferous forests in Bulgaria. Preliminary results

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Abstract. The carabid and pselaphin beetles species distribution at a high altitude depends mostly on climate and soil conditions. In that context, the changes in their distribution could be used as early-warning signal for natural disturbance due to climate change. For that purpose, a detailed study of high altitude communities in Bulgaria needs to be made. There is a lack of information about the pselaphin beetles in the high altitude coniferous forests in Bulgaria. The information about the ground beetles in those habitats is also scarce – the most of the data is from dominated by Picea abies forests of West Rhodope Mts. and Vitosha Mts. The presented preliminary study summarize the available information and add new data about the Carabidae and Pselaphinae species composition in high altitude coniferous forests. The presented detailed study of these beetle groups in the natural and cultural Pinus peuce and Pinus heldreichii communities was made for the first time. The species richness and endemism of both groups is commented.

Acknowledgements: This study was supported by the Bulgarian Science Fund (Grant no. KP-06 PN36/17/2019).
Body nutritional status in Bulgarian preschool children (Smolyan Region, 1996-2019)

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Abstract. Purpose of the present study is to assessment distribution of different categories body nutritional status among children at age between 3-6 year from Smolyan region, Bulgaria in age, gender and time aspects. Material/Methods. A transversal anthropometric study of 330 clinically healthy children (161 boys and 169 girls), aged between 3 to 6 years from Smolyan region, Bulgaria was performed in the period 2017-2019 (Sample A). For the performance of the analysis of the secular changes, earlier anthropometric data, collected by the author, for another 406 children from Smolyan region, aged between 3-6 years, transversally studied in the period 1996-1998 (Sample B) were also used. The anthropometric programme of the study included two directly measured attributes - height (cm) and weight (kg). On the basis of the three directly measured attributes, the body mass index were calculated (BMI, (kg)/(m2). The anthropometric measurements were carried out under the classical methodology of Martin-Saller. For assessment of body nutritional status the investigated children were grouped into four categories of BMI: underweight, normal weight, overweight and obesity in accordance with the international (IOTF) BMI cut-off points for children. Our results show that the growth of the studied children remained relatively unchanged at different ages during the studied 20-year period. On the other hand, weight as well as body mass index show a significant increase, especially well expressed in 3- and 6-year-old girls and 5- and 6-year-old boys. As a result, even in preschool, underweight decreases significantly at the expense of weight gain, and especially obesity. Thus, underweight decreases on average by more than 10% in total for both sexes, while the prevalence of overweight increases - in overweight more than 1-2% on average, and obesity more than 5% on average. The frequency of distribution of obesity is more common among preschool girls, compared with boys. There are two main secular trends in the nutritional status of pre-school Bulgarian children in the Smolyan region in the period 1996-2019: increase of prevalence of overweight and obesity and decrease of prevalence of underweight. These trends are due to increased body weight, against the background of relative stabilization of growth. This facts are connected with increase in the values of the body mass index of the current generation of children and respectively, to an increase in the prevalence of overweight and obesity.

Key words: body nutritional status, secular changes, underweight, overweight, obesity, pre-school children.
Micromorphological leaf characteristics of *Fontinalis antipyretica* Hedw. as biomarkers of water pollution

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**Abstract.** The current study is a part of complex research for establishing new fast and reliable methods for water pollution control. The subject of the study is three anthropogenically affected reservoirs (Kardzhali, Studen Kladenets, and Zhrebchevo), compared with the control - non-polluted one (Vacha Reservoir). *Fontinalis antipyretica* Hedw. was used as a biomonitor and moss bags stayed for 30 days in the dams. The micromorphological characteristics of cells in the stem and twig leaves of the gametophyte of *F. antipyretica* were studied by using a light microscope (CETI, Magnum T Trinocular, magnification 400x). A proven increase in the number of cells was registered (significance level between 99% - 99.9% according to Student t-test), both on the branch leaves (for all three affected reservoirs) and the stem leaves (for Zhrebchevo Reservoir). In parallel, a reduction was recorded in the size of cells, mainly in terms of their width, as a result of the anthropogenic pressure. The twig leaves showed higher sensitivity to water pollution than the stem leaves. Based on Hierarchical cluster analysis, the indicators were compared by using the square Euclidean distance method. The analysis confirmed the differences between the stem and twig leaves and that placed the Zhrebchevo Reservoir in a separate cluster. In conclusion, *Fontinalis antipyretica*, in particular the number of twig leaf cells and their width, can be used as suitable micromorphological markers in biomonitoring studies of water basins.

**Key words:** *Fontinalis antipyretica*, biomonitor, micromorphological characteristics.

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Which one is more toxic? – CYP and CPF effects on different biological tools in Zebra mussel (*Dreissena polymorpha*, Pallas)

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**Abstract.** This study was conducted to deepen our current knowledge on the possible negative effects which chlorpyrifos (CPF) and cypermethrin (CYP) could have on the lysosomal membrane stability (LMS) and enzymatic activity of cholinesterase (ChE), catalase (CAT) and glutathione peroxidase (GPx), using the invasive mollusk zebra mussel (*Dreissena polymorpha*) as bioindicator. The assessment of sentinel species aims to provide valuable information regarding the anthropogenic impact on aquatic ecosystems. The mussels were exposed to different decreasing concentrations of CPF and CYP in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). The applied pesticides are considered as priority substances in surface waters according to Directive 2013/39/EU. Furthermore, lysosomal alterations are recognized as biological tools for determination of the effects of marine contaminants and implementation of the environmental quality standards (EQSs) specified in the Descriptor 8 of the Directive 2008/56/EC (MSFD). Alterations in the specific enzymatic activity could also be used as a sensitive biomarker for the examining the toxic stress in the hydrobionts subjected to various pollutants in water. The results showed changes in the lysosomal membrane destabilization, as well as in the specific enzymatic activity of ChE, CAT and GPx. However, the CYP exposure led to more pronounced alterations in the observed biomarkers.

**Key words:** contamination, pesticides, zebra mussel, lysosomes, enzymatic activity.

**Acknowledgments:** This work was supported by Project MU19-BF-014, financed by the Department of Scientific Research, University of Plovdiv, Bulgaria.
Impaired antioxidant activities in transplanted mussels from contaminated sites in Bulgaria

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Abstract: As bivalves are widely used as bioindicators of pollution in freshwater, marine and estuarine ecosystems, they are known to provide a time integrated indication of environmental contamination as well as reliable information on the ecosystem state under anthropogenic influence. Therefore, transplants with mussels (Sinanodonta woodiana Lea, 1834) were applied to investigate the water quality in standing water bodies by applying sensitive biomarkers. Changes in the activity of the antioxidant enzymes such as cholinesterase (ChE), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) were used to study the oxidative stress in the bioindicators.

Key words: transplanted mussels, contamination, biomarkers, enzymatic activity.

Acknowledgments: This work was supported by Project FP19-BF-013, financed by the Department of Scientific Research, University of Plovdiv, Bulgaria.
Histopathological measures in gills of Common carp, *Cyprinus carpio* (Linnaeus, 1875) after xenobiotics exposure

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**Abstract.** We aimed to determine the negative effects of decreasing concentrations of chlorpyrifos (CPF) and cypermethrin (CYP) based on the EU legislation on common carp (*Cyprinus carpio* Linnaeus, 1758) in laboratory conditions. The fish were exposed to the pesticides for 96 hours and 30 days. Therefore, histological biomarkers in the gills, were investigated. After 96 hours, we found mainly intense proliferative changes and to a lesser extent - degenerative changes and changes in the circulatory system, such as necrosis and vasodilatation. Moreover, after 30 days exposure, we established more intense degenerative changes, as well as changes in the circulatory system along with the observed proliferation. Overall, both tested pesticides impacted the studied biomarkers in common carp, even at lower than the permissible concentrations set by law. However, the results on the analysis showed a relatively higher toxicity of CYP compared to CPF on the fish. To avoid danger or risk, a cautious application of these pesticides must be carried out, especially near water bodies.

**Key words:** pesticides, water, contamination, fish, gills, biomarkers.

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Genetics, cell and molecular biology
First report of *Peach Latent Mosaic Viroid* in symptomatic peach orchards in Southern Bulgaria

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**Abstract.** Viroids are plant infectious agents whose genome is represented by a small (246 to 401 bp), single-stranded, circular, non-coding RNA capable of autonomous replication in susceptible hosts. *Peach latent mosaic viroid* (PLMVd) causes a wide variety of peach symptoms, some of which are barely noticeable, while others such as mosaic or chlorotic spots on leaves or fruit tissue, even more severe like albino phenotype (calico peach) are well distinguishable. Changes in the appearance of the fruit and the reduction in the lifespan of peach trees cause serious economic losses. In the present work, we study the occurrence of PLMVd in peach orchards, located in Plovdiv region. In two successive vegetations (2019-2020) leaf samples from 4-10-years-old peach trees of cvs. ‘Royal Gem’ and ‘Gergana’, manifesting chlorosis or mosaic on leaves and/or pale areas on the skin of ripe fruits, symptoms potentially caused by PLMVd, were selected for RT-PCR analysis. PLMVd specific amplification product of approximately 348 bp was obtained from cv. Royal Gem. The amplicon was directly sequenced and presence of PLMVd was proved. Buds of PLMVd infected peach were grafted on the indicator GF 305, peach cultivar “Summerset” (*Prunus persica*) and myrobalan (*Prunus cerasifera*). Prior to grafting, the experimental plants were serologically tested by ELISA for the presence of ACLSV, PPV, PNRSV and PDV. Four weeks post inoculation, the grafted plants developed a PLMVd-specific phenotype. The presence of PLMVd in the inoculated plants was confirmed. To our knowledge, this is the first report of PLMVd on peach in Bulgaria.

**Acknowledgments:** This study is supported by the Bulgarian National Science Fund under Grant No KP-06-N 36/10 from 2019, “Actual phytopathological challenges in fruit tree species in Bulgaria: Unexplored and invasive alien phytopathogens with potential risk for biodiversity and biosecurity”.

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Hormone- and ROS- signalling genes are involved in the cultivar-specific PSTVd response of pepper

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Abstract. Viroids are unique RNA entities that infect higher plants. Their non-coding genome provides a specific structural signature that withdraws host factors to ensure its replication, movement and pathogenesis. PSTVd is a nuclear localized viroid that uses the cellular RNA pol II to multiply its genome by an asymmetric mode of rolling circle mechanism. PSTVd infected different types of crops, causing disturbances in their growth and development, which leads to reduced yields and economic losses. We study the PSTVd response of two Bulgarian pepper cultivars (Kurtovska kapia and Djulunska shipka) at 43 day post infection (dpi) by applying a next-generation RNA sequencing. At the phenotype level, the two pepper cultivars showed a difference in the severity and timing of symptoms. At molecular level, the comparison of transcriptome data uncovered multiple altered genes, some of which showed opposite expression patterns in the two cultivars. Some of them were associated with hormonal signalling and ROS scavenging action, and selected for further analysis by RT-qPCR. The obtained data might highlight the pepper cultivar-specific defense mechanisms against PSTVd.

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Serological diagnostics of hepatitis E virus infection in pigs and wild boar based on capsid protein (ORF2 genotype 3) expressed in planta – sensitivity and specificity

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Abstract. Hepatitis E virus (HEV) is a lesser-known hepatitis virus, but its worldwide spread is doubtless and has increased recent years. Zoonotic spread of HEV, mainly due to genotype (gt) 3, has emerged in developed countries in the past decade. Furthermore, transmission via contaminated meat from pigs and boars is proven. Detailed analysis of viral dynamic and distribution is needed in order to identify associated risk factors. The current study aims to present better knowledge on the HEV distribution among pigs and for the first time data on wild boar in Bulgaria. This investigation has shown that domestic pigs and wild boar in Bulgaria are widely infected with HEV. There is a need for a method for the cost-effective detection of the HEV infection and production of HEV immunogenic proteins that can be used as diagnostic antigens for the serological tests. Also, in this study we have compared the results from a commercial ELISA and an in house ELISA test based on HEV open reading frame (ORF) 2 protein produced in planta, the sensitivity and the specify of the tests were evaluated. The development of a HEV diagnostic kit based on plant-derived ORF2 capsid protein is our goal and this will help to reduce the cost of such a kit and to enable diagnoses of HEV in developing countries.

Acknowledgments: This research was funded by the Research Fund at the University of Plovdiv, competition “Young Scientists and Doctoral Students” MU21-BF-022.
Evaluation of cytotoxic and genotoxic effects of commonly used food additives on the root meristem cells of *Allium cepa*

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**Abstract.** The effects of the food preservative sodium benzoate, food sweetener aspartame and food colorant carmoazine have been studied on root tips of *Allium cepa* L. Roots of *A. cepa* were treated with a series of concentrations of food additives for 72 h. The results indicate that these food additives reduced values of mitotic index in meristem cells of *A. cepa* compared with the control samples. All concentrations of these chemicals showed an inhibitory effect on cell division. The frequencies of chromosomal aberrations were generally increased with increasing of substances's concentrations. All studied food additives induced c-mitoses, lagging and vagrant chromosomes and fragments and micronucleuses. Sodium benzoate and aspartame were the reason for the formation of anaphase bridges and diagonal anaphases. Only in the root tips were treated with aspartame were established pulverized chromosomes and multipolar metaphases and anaphases.
Response of *Chelidonium majus* in vitro cultures and their alkaloid content under heavy metal stress

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**Abstract.** Heavy metal contamination is becoming an increasing environmental problem worldwide. Heavy metals (HM) retard plant growth, yield and affect the secondary metabolism which results in fluctuations in plant metabolites, such as the alkaloids. The aim of the study was to investigate the influence of HM on the growth of in vitro cultivated plants of *Chelidonium majus* and their alkaloid content. The plants were cultivated on a medium B5 with double the amount of macrosalts, 0.5 g/l activated charcoal and supplemented with different concentrations of HM – Cd$^{2+}$ (0.5; 1 mg/l); Pb$^{2+}$ (100; 250 mg/l); Zn$^{2+}$ (100; 250 mg/l). Plants were obtained in vitro from seeds or were propagated through adventitious shooting (AS). The raw alkaloid mixtures (RAM) were obtained by usual chemical procedure. Plants cultivated at the lower concentration of Cd$^{2+}$ formed roots and developed normally with a gradual reduction in their growth and rooting as the concentration of the supplemented HM increased. There were not any differences in the development of the plants cultivated on Cd$^{2+}$ according to their type of origin. Epinasty and browning of the leaves were observed among the plants cultivated on the medium supplemented with Pb$^{2+}$. These side effects got worse as the concentration of the HM increased, and were also accompanied by a strong reduction in the rooting. The plants which were obtained from seeds developed normally and did not show any of the signs of the ones which were propagated through AS. None of the plants cultivated on a medium supplemented with Zn$^{2+}$ survived, regardless of their origin and HM concentration. A phytochemical study showed that the plants cultivated at the highest concentrations of Cd$^{2+}$ and Pb$^{2+}$ and also obtained through AS had the highest RAM. However, the plants cultivated at the same concentrations but obtained from seeds had lower RAM similar to that of the plants cultivated at lower concentrations of HM and propagated through AS. In conclusion, *Ch. majus* was able to cope with the influence of Cd$^{2+}$ and Pb$^{2+}$ in the culture medium, but not with that of Zn$^{2+}$. Quantity of RAM was influenced by the HM concentration and the origin of the plants.

**Key words:** seeds, adventitious shooting, Cd$^{2+}$, Pb$^{2+}$, Zn$^{2+}$.

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Biochemical-genetic analysis of the effects of Chlorpyrifos and Cypermethrin insecticides on zebra mussels (*Dressena polymorpha* Pallas, 1771)

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**Abstract.** In the present study, a biochemical-genetic analysis was performed to determine the relationship between isoenzyme polymorphism and the zebra mussel *Dressena polymorpha* body’s response to the harmful effects of the pesticides Cypermethrin and Chlorpyrifos. The probable genetic control of four enzyme and protein groups - malate dehydrogenase, malate enzyme, superoxide dismutase and soluble proteins - was determined. Different intensity of expression of allelic products has been reported in individuals exposed to short- and long-term exposure to the pesticides Cypermethrin and Chlorpyrifos. The observed variability in the expression of superoxide dismutases, malate dehydrogenases and soluble proteins in control and experimental samples gives us reason to accept these enzyme and protein systems as markers for reporting the negative impact of the tested pesticides on the zebra mussel.

**Acknowledgments:** This work was supported by Project MU19-BF-014, financed by the Department of Scientific Research, University of Plovdiv, Bulgaria.
Comparative analysis of cytogenetic effects of the neonicotinoid insecticides Nuprid 200 SL and Calypso 480 SC on Allium cepa L. cells

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Abstract. Data from the present study indicate that the neonicotinoid insecticides Nuprid 200 SL and Calypso 480 SC affect mitotic activity and increase chromosomal aberrations in the cells of the root apical meristem of Allium cepa. The tested solutions of pesticides cause a wide range of anomalies associated with disorders in the formation of the cell’s division apparatus and the integrity of chromosomes, which demonstrates their genotoxic potential. A positive relationship was found between the reported frequencies of aberrations and the concentration of pesticides. The conducted study showed that Allium cepa is a sensitive test system that allows determining the main characteristics of the cyto- and genotoxic action of neonicotinoid insecticides.
Occurrence of powdery mildew in Prunus cerasifera caused by Podosphaera sp. in Bulgaria

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Abstract. The rootstock ‘Myrobalan 29C’ (Prunus cerasifera Ehrh.) is one of the widely used rootstocks for production of plum and apricot planting material. The studied ‘Mirobalan 29C’ rootstocks were in vitro propagated, acclimatized to ex vitro conditions and grown in a greenhouse, located in Plovdiv region. In May, 2020, on the adaxial and abaxial leaf surfaces and young stems of the rootstocks, typical symptoms of powdery mildew - white powdery colonies with white superficial mycelium and conidia were observed. Later on chasmothecia appeared in some injured leaf tissues. The microscope findings visualized conidia formed in true chains of erect conidiophores which were ellipsoid, hyaline and measured 24.6 to 33.6 x 10.8 to 20.8 µm (average 29.6 x 15.8 µm) and globose chasmothecia with appendages arising from the upper half of the fruiting bodies. Usually, appendages were in length one to four times long towards the chasmothecial diameter and had two to four dichotomous branches. A single ascus, observed in a chasmothecium, was broadly ellipsoid-ovoid contained six to eight ascospores. The shape of the ascospores was ellipsoid-ovoid too. Pathogenicity tests were carried out by inoculation of young leaves of healthy ‘Myrobalan 29C’, apricot (P. armeniaca L.) and cherry (P. avium L.) plants. The inoculated plants were maintained in a moist chamber at 23°C. Symptoms of powdery mildew on ‘Myrobalan 29C’ and cherry leaves were observed after eight days post-inoculation period. Disease signs on the inoculated apricot leaves appeared 30 days after the artificial inoculation. White powdery coating observed on the inoculated leaves of all experimental species was very similar to the powdery coating on natural infected ‘Myrobalan 29C’. Based on the morphological characteristics of the conidia, chasmothecia and results of the pathogenicity tests, the causal agent of the symptoms observed was presumed to belong to Podosphaera sp. (Ascomycetes). To the best of our knowledge, previously no detection of Podosphaera sp. in P. cerasifera has been reported in Bulgaria.

Key words: Prunus cerasifera, powdery mildew, causal agent, Podosphaera sp.

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Preliminary study on genetic diversity of *Crocus* L.,
Series Biflori from Bulgaria by ISSR markers

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Abstract. The present study was aimed to evaluate the genetic diversity and pattern of variation throughout the ranges of polymorphic aggregate group *C. biflorus* in Bulgaria. For this purpose, samples from five *Crocus* L. species collected from 15 natural populations were assessed using ISSR molecular markers. Cluster dendrogram was created using the Wars’s method (PAST 4.03), then combined into a consensus tree using Consense (PHYLIP 3.69). Our results showed significant levels of genetic variation of the *C. biflorus* group corresponding with morphological diversity. Dendrogram showed that the samples of *C. adamioides* were in the group of *C. biflorus* but cannot be grouped with *C. biflorus* subsp. *adamiii*. The previous analysis based on ITS sequences confirms that the population belongs to *C. adamioides*. The obtained data confirmed that *C. chrysanthus* was a separate taxon. The results also verified the genetic divergence of the *C. cf. biflorus*. The ISSR analysis showed the need for additional DNA-based methods for taxonomic revisions of the genus *Crocus* L.

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A study on reproductive capacity in *Primula veris* L. (Primulaceae)

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**Abstract.** Main criteria for evaluation of the reproductive capacity of perennial distylosous species *Primula veris* were studied in three natural Bulgarian populations, such as: embryological features, mode of reproduction, pollen and seed (embryo) viability. The anthers are tetrassporangiate. The anther wall developing after Dicotyledonous-type consists of: an epidermis, fibrous endothecium, a middle layer and a secretory tapetum. After simultaneous meiosis in the pollen mother cells predominantly tetrahedral tetrads form in the anthers. The pollen grains are two-celled when shed. In the unilocular ovary many ovules develop. The mature ovules are anatropous, tenuinucellate and bitegmic. The embryo sac develops after Polygonum (monosporic)-type from the chalazal cell of the megaspore tetrad in the ovule. The Caryophyllad-type embryo and ab initio nuclear endosperm form after double fertilization. After acetocarmine testing, a high pollen viability in the studied populations more than 95% was established. The tetrazolium test revealed an extremely low seed viability (only just 2%) in the high mountain population, while for the other two studied populations it was more than 65%. No apomixis or even its elements are observed. It was established that *P. veris* is a predominantly amphimictic species corresponding with its diploid status (2n=2x=22) but the vegetative reproduction in this species also occurs. As a results of the present study it was obtained an important information about possibilities of the realization of reproductive capacity in *P. veris* – a valuable medicinal plant, mainly used as an antioxidant and expectorant agent.

**Key words:** embryology, pollen and seed viability, *Primula*, reproduction, reproductive capacity.
Y chromosome microdeletions in men with azoospermia and oligoasthenoteratozoospermia

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Abstract. The causes of male infertility are different and significantly related to both genetic components and a variety of environmental factors. Approximately 5 to 10% of men with azoospermia or severe oligoasthenoteratozoospermia have microdeletions in the area of the azoospermic factor AZF - Yq11, which contains genes that control the processes of spermatogenesis. In the present study totally 48 men with azoospermia and oligoasthenoteratozoospermia were included in the Y-microdeletion analysis by Real time PCR. The set of primers used to detect microdeletions on the Y chromosome included: SRY (sY14) and ZFX / Y (short arm), sY84 and sY86 (for AZFa); sY127 and sY134 (for AZFb); sY254 and sY255 (for AZFc). No microdeletions in the Y chromosome were found in 29.2% of the men studied. Among all others, single microdeletion was found in the AZFa subregion of Yq (2.1%) and in the SRY zone of Yp (22.9%). For all other men included in the study, different combinations of microdeletions were found in two (AZFb + AZFc, AZFa + SRY, AZFb + SRY, AZFc + SRY and SRY + ZFX / Y), three (AZFa + AZFc + SRY, AZFb + AZFc + SRY and AZFb + SRY + ZFX / Y), four (AZFa + AZFb + AZFc + SRY, AZFa + AZFb + SRY + ZFX / Y, AZFa + AZFc + SRY + ZFX / Y and AZFb + AZFc + SRY + ZFX / Y) or five (AZFa + AZFb + AZFc + SRY + ZFX / Y) sections / subsections simultaneously. The obtained results support the opinion that interactions between different deletions in the AZF region of the Y-chromosome with other genes or gene groups are possible, which is a significant factor influencing spermatogenesis.

Key words: male infertility, Y chromosome, microdeletions, semen quality, reproductive health.
Spermatozoa morphology abnormalities in men with reproductive problems influenced by various environmental and lifestyle factors

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Abstract. Totally 1304 men were included in the conventional analysis to determine the spermatozoa morphology. Deviations from the normal morphological status were found in 16.5% of them. The identified morphological abnormalities were analyzed by groups and their frequency of occurrence was determined, both individually and in various combinations. The established anomalies, in descending order according to their frequency, were as follows: tapered heads (95.2%); neck defects (88%); amorphous heads (87.2%); microcephalic – with small heads (79.2%); excess residual cytoplasm (48.8%); macrocephalic – with large heads (42.4%); tail defects (39.2%), double heads (25.6%), acephalic (<0.08%). In the studied individuals there were various complexes of morphological anomalies, which are discussed in detail. The information concerning environmental and lifestyle factors was collected on the base of voluntarily completed questionnaire. The results obtained showed that about 21% of the participants were undergoing occupational hazards, 22% were smokers, 42% alcohol consumers, 13% – drug users, 7% taking anabolic steroids, 31% – taking medications and 5% work or live under stress. Some of the men surveyed were influenced by multiple of the mentioned factors. Statistical analysis demonstrated differences in the clarity of the relationship between the established abnormal spermatozoa morphology and the studied environmental and lifestyle factors. The results of the study showed the presence of statistical significance in the relations "harmfulness – spermatozoa morphology" (P<0.017) and "occupations – spermatozoa morphology" (P<0.003).

Key words: spermatozoa morphology, environment and lifestyle, male reproductive health.
Microsatellite markers reveal genetic diversity among honey bee populations from some Balkan Peninsula regions and distinctive characteristics of the local for Bulgaria *Apis mellifera rodopica*

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**Abstract.** The genetic polymorphism in honey bee populations of the local for Bulgaria *A. m. rodopica* and populations of *A. m. macedonica* and *A. m. carnica*, originating from Greece and Serbia was investigated using microsatellite DNA analysis. Totally, 121 alleles were found for the nine microsatellite loci in the Bulgarian populations studied. The relationships between the local Bulgarian honey bee *A. m. rodopica* and Balkan peninsula populations of *A. m. macedonica* from Greece and *A. m. carnica* from Serbia were analyzed. Twenty private alleles were detected for the local Bulgarian *A. m. rodopica* Petrov, 1991 in comparisons to the other studied European populations. Eight private alleles were detected for the Greek populations of *A. m. macedonica* and 11 – for the *A. m. carnica* populations from Serbia. Clear diagnostic markers, appropriate for distinguishing the local Bulgarian honey bee were found and described. It was concluded that together with the complex of the other molecular, biochemical, morphological and ethological indicators they could be taken into account when conducting activities for conservation of local Bulgarian honey bee *A. m. rodopica* gene pool.

**Key words:** *A. m. rodopica*, microsatellites, polymorphism, genetic distinction.
Study on diversity in some human phenotypic characteristics

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Abstract. The present study aimed to analyze the human phenotypic manifestation of AB0 and Rh blood groups, descriptive features cleft in chin, freckles, hairline, eyebrow shape, dimples, earlobes and the basic characteristics of personality extraversion, agreeableness, consciousness, emotional stability and intellect/imagination and the possible relations between them. About 950 individuals were included in the study. The frequencies of the investigated phenotypic groups were calculated by usage of descriptive statistics. The results obtained were analyzed by SPSS software. Statistically significant relationships were found between some of the studied human immunological, descriptive and behavior features as follows: lower levels of emotional stability in people with Rh+ blood groups (P<0.05); higher levels of agreeableness and consciousness in people without cleft in chin (P=0.003, P=0.001) and in persons with separated eyebrows (P=0.03, P=0.008); higher levels of emotional stability in people with straight line of hair (P<0.05); higher levels of consciousness in people without dimples and higher levels of intellect/imagination in people with dimples (P<0.05); higher levels of agreeableness and intellect/imagination in people with a free ear lobe (P=0.004, P=0.02). No statistically significant differences were detected between blood groups of the AB0 system and the presence of freckles on the face from one side and the mean values of the personality characteristics by the other hand. The present study reveals interesting relationships between various human traits based on a complex approach. It could be used as an appropriate model for other future studies of human phenotypic diversity.

Key words: phenotypic variability, blood groups AB0 and Rh, human descriptive traits, human personality traits.
Histological evaluation of human endometrium at different phases of hCG-primed menstrual cycle

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Abstract. Implantation is a complex process of interactions between the embryo and the endometrium, a layer of mucous membrane on the inner surface of the uterus. The embryo qualities making it fit for embedding, endometrium receptivity, as well as the exchange of multiple molecules between embryo and endometrium, determine whether and when is the best time for pregnancy to occur. In the context of a larger study we are conducting on the dynamics of microRNA expression in endometrium, an objective assessment of the endometrial phases is necessary. Here we present the outcome of endometrial phase assessment by applying a set of criteria in combination with human chorionic gonadotropin (hCG) administration which is considered to cause ovulation within 36-48 hours and allow for more accurate secretory phase dating. Endometrial biopsies were taken from 7 volunteers, outpatient and without anesthesia. Ultrasound measurements of leading follicle and endometrial thickness, together with eestradiol and luteinizing hormone levels were set as criteria for hCG application. It was applied on a strictly individual day for each patient as an inducer of ovulation. Biopsies were planned individually for each woman and conducted at 4 time points – hGC (before hormone application), hGC+2, hGC+7 and hGC+9 (numbers are days after hCG application). Part of each biopsy material was subjected to a classic histological examination by staining with hematoxylin and eosin, and the morphological evaluation was done according to the Noyes criteria. Quality and informative histological preparations for all 4 time points were produced only for 5 of the 7 volunteers. We observed that the histological descriptions at hGC+7 and hGC+9 time points showed no absolute compliance with the Noyes criteria for the corresponding secretory phases for some of the individuals. Thus, we created a new group of endometrial samples according to the Noyes criteria that correspond to the mid-secretory phase which includes 4 samples from LH+9 and 1 sample from LH+7. We consider it as the most representative pool of the most suitable period in receptivity terms to be used to analyze the dynamics of microRNA expression in the next stage of the ongoing project.

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**In vitro** increased wheat stalk growth by highly diluted agitated preparations of a commercial fertilizer

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**Abstract.** Drugs at ultra-high dilutions close to the Avogadro’s number (theoretical 0-molarity is 10-24) and without apparently containing any drug molecule, have been used in homeopathy therapy for about two centuries. Nowadays, homeopathic procedures involving the controversial phenomenon called “water memory”, have regained their use in different application areas. In the present study, 10-7, 10-14, and 10-36 dilutions of a commercial fertilizer were evaluated in a 7-day wheat growth bioassay. Test dilutions were prepared following a standardized protocol, according to the method of stepwise dilution and succession, as derived from traditional homeopathy. Experiments were performed on herbicides- and pesticides-free wheat grains (Triticum durum Rafi C97 variety). Treatments were compared with negative controls including; a) pure bi-distilled water and b) sham-treated pure bi-distilled water, following the same protocol as used for fertilizer dilutions with 200 strokes and the same steps of dilutions. A 2% of commercial fertilizer was used as a positive control. The observed results of three independent bioassays, showed an increased wheat stalk growth after treatment with diluted and agitated fertilizer solutions, as compared with negative controls (p < 0.05). Positive controls showed the highest stalk growth, but it was not significantly different from that of the diluted fertilizer. These results suggest that there was an influence of highly diluted commercial fertilizer on wheat seedling development.

**Key words:** Basic research, Sustainable agriculture, Fertilizers.
Toxic effects of the insecticide Aktara VG investigated on the Allium cepa root meristem

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Abstract. The effect of the insecticide Aktara VG on the cell division rate and on the chromosomal apparatus of Allium cepa root meristem cells was studied. By applying anaphase analysis and micronucleus mutagenicity test, a control sample (tap water) and experimental samples of insecticide solutions with different concentrations - 100%, 50% and 25% (Aktara VG SS; Aktara VG 50; Aktara VG 25) of the recommended by the producer were compared. Approximately 2,000 cells per individual and five individuals per sample were analyzed. Comparative analysis of mitotic indices showed a negative effect of the tested pesticide in solution with the recommended concentration and in 50% solution of it on the rate of the cell division during the root germination for 48 hours. The genotoxic effect of the studied insecticide was analyzed. The chromosomal structural changes observed during the investigation are classified into 7 categories. Chromosomal abnormalities such as pulverized chromosomes, diagonal anaphases, chromosome fragments, anaphase and telophase bridges - alone and in combination with fragments, wandering and lagging chromosomes and micronuclei were detected in meristem cells after treatment with all the tested solutions of Aktara VG, but the highest percentage of aberrations was found after treatment with Aktara VG 50%. Some chromosome aberrations were found in the control sample, but in a significantly lower percentage. It was concluded that the insecticide Aktara VG negatively affects the cell division rate and has a genotoxic effect on the Allium cepa root meristem cells.

Key words: Aktara, insecticide, neonicotinoids, Allium cepa, chromosomal aberration.
Losses of honey bee colonies and risk factors for their mortality in Bulgaria during 2020

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Abstract. The study is based on a comparative analysis of the established losses of bee colonies by regions in Bulgaria and on the characterization of the risk factors for available mortality in 2020. Information for different types of forage sources with potential risk for Apis mellifera due to pesticide treatment, was presented and analyzed. By using the international standardized COLOSS questionnaire for 2020, members of the National Bee Breeding Association and independent beekeepers, owners of a total of 64 apiaries (over 6,800 bee colonies), located in all regions in Bulgaria, were surveyed. Beekeepers were asked to answer questions about the number of wintering honey bee colonies and how many of them after the winter: were alive but had unsolvable queen problems; were lost through natural disaster and were dead or reduced to a few hundred bees. The survey data show that the highest mortality was found for the North Central region (19%), and the lowest – for the Northwest (1%) and Southwest (2%) regions. Among the reasons for the loss of bee colonies, the leading one is the mortality of honey bees or their significant reduction in the colonies, which is also related to the negative impact of the applied pesticides in the studied areas. In this aspect, the most serious problems were reported in the North Central and Southeast (7%) regions. The presented and analyzed data should be taken into account when developing activities to protect the honey bee health status in Bulgaria.

Key words: honey bee, Apis mellifera, reasons for mortality, forage sources, pesticides.
Avoidance of teratogenic effects of ultraviolet radiation in a population of *Boeckella poppei* (Crustacea: Calanoida) from Livingston Island, Maritime Antarctica

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Abstract. Antarctic environment is characterised with potentially high levels of ultraviolet radiation (UVR) damaging to living organisms. Aquatic organisms have different strategies to minimise their negative effects. These strategies might be behavioural (migrating to deeper aquatic layers), biochemical (photoprotection or enzymatic repair of the incurred damages). Recent studies suggest that the genus *Boeckella* has a high level of plasticity in terms of their reaction to UVR, which enables its wide distribution in different regions of Antarctica. Here we present a specific photoprotective reaction in the population of *Boeckella poppei* on the Livingston Island, Maritime Antarctica. In non-ovigerous females, we observed uniform distribution of carotenoids in the body, while these pigments were almost entirely concentrated in the oval sacs of adult females. We consider this a mechanism to protect the eggs from the teratogenic influence of high level of UVR in Antarctic environments. Another mechanism for avoidance of the effects of UVR we observed was the migration of specimens of *B. poppei* towards the deeper zone of the Antarctic glacial lake *Todorina buza*. 
Antibacterial activity of some Cyanobacteria strains

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Abstract. Cyanobacteria are a rich source of biologically active substances with antibacterial, antiviral, immunosuppressive or immunostimulatory activity, which are used mainly in the pharmaceutical industry. Two types of extracts (methanol-water and methanol-chlorophorm) were prepared from 14 cyanobacterial strains of the genera Phormidium, Microcoleus and Phormidesmis, and were evaluated for antimicrobial properties against two bacterial strains – Bacillus cereus (Gram-positive bacterium) and Pseudomonas aeruginosa (Gram-negative bacterium). Only the polar extracts demonstrated antibacterial activity. Five of cyanobacterial strains (Phormidium autumnale PACC 5511, Phormidium autumnale PACC 5527, Phormidium autumnale PACC 5529, Phormidium papyraceum PACC 8600 and Microcoleus vaginatus CCALA 152) showed antimicrobial effects against Bacillus cereus. Maximum antibacterial activity against this Gram-positive bacterium with a zone of inhibition of 30±1 mm showed Phormidium autumnale PACC 5527. Also, 12 from all 14 studed cyanobacteria inhibited the growth of Pseudomonas aeruginosa. Maximum antibacterial activity against this Gram-negative bacterium showed Phormidium autumnale PACC 5517 with a zone of inhibition of 31 mm. Cyanobacterial strains exhibiting antibacterial activity against Bacillus cereus suppressed also the growth of Pseudomonas aeruginosa. These extracts have a broad-spectrum of action against both Gram-positive and Gram-negative bacteria. Our data confirm the presence of promising antibacterial compounds that could be derived from selected cyanobacteria.
Functional and structural features of photosynthetic apparatus of some halophytic and glycophytic representatives from genus *Lactuca* (Asteraceae)

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Abstract. In the present study, chloroplast ultrastructure, PSII functionality and lipid and fatty acids pattern of isolated chloroplasts have been used in order to characterize structural and functional peculiarities of photosynthetic apparatus in some halophytic and glycophytic *Lactuca* species. The comparative studies of *Lactuca tatarica* (L.) C.A. Mey, *Lactuca serriola* (L.) and *Lactuca quercina* (L.) have shown distinctive features of thylakoid membrane system, chlorophyll thermoluminescence emission and kinetic parameters of PSII oxygen-evolving reactions. The analysis of lipid classes and fatty acids composition of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylcholine (PG) show the existence of qualitative and quantitative differences that can contribute in this regard. The results show specific characteristics of photosynthetic membranes in halophytic and glycophytic *Lactuca* species, reflecting different adaptive strategies of the studied species to environmental conditions in their natural habitats.

Key words: *Lactuca tatarica* (L.) C.A. Mey, *Lactuca serriola* (L.), *Lactuca quercina* (L.), chloroplasts, fatty acids, lipid composition, oxygen evolution, photosynthetic activity, thermoluminescence.

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Stimulation of *Primula veris* seeds’ germination under *in vivo* and *in vitro* conditions

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**Abstract.** *Primula veris* L. (Cowslip) is a medicinal plant species with a long history of use, native to Europe and Western Asia. In Bulgaria it is protected by the Biodiversity Act and under special regime of use based on the Medicinal Plants Act. The aim of the present study was to stimulate the extremely low seed germination of *P. veris* by different approaches. Seeds were gathered from one native population in the Golo Bardo Mountain in Bulgaria, and used for in vivo and in vitro experiments in parallel. The effect of different factors was tested: treatment with plant growth regulators (gibberellic acid, kinetin), light quality (white, infrared, red, and blue) or dark, and stratification at low temperature. Combination of factors resulted in 36 variants in vivo and 8 variants in vitro, about 40 seeds per variant. No germination was observed in control variants without seed treatment. Concerning in vivo variants, the highest germination rates were noticed when seeds were cultivated under red or blue light (70% when seeds were soaked into 0.1% GA3 for 5 h before treatment with red light, and 65% when treated with 0.3% GA3 for 5 h and put under blue light); however, red light caused etiolation and death of seedlings, while blue light stimulated development of large cotyledons and leaves. Among in vitro variants, the most successful factors combination was 2-month seeds stratification at 4 °C followed by cultivation on medium containing 0.5 mg/l kinetin and 5 mg/l GA3 – 50% germinated seeds. In all variants significant number of seeds stopped their development at root stage, even the survival rate varied between variants. Seedlings were transferred consecutively to vermiculite and soil mixture, and currently about hundred plants are growing in the phytotron. These results will be used as a base for establishment of a pilot agriculture of the species.

**Key words:** seed germination, gibberellic acid, monochromatic lights, stratification

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Genetic diversity of the Balkan endemic species *Moehringia jankaе* Griseb. ex Janka and *Moehringia grisebachii* Janka (Caryophyllaceae) from Bulgaria using ISSR markers

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**Abstract.** Eleven populations of the endangered plant *Moehringia jankaе* and twenty-eight of the rare plant *Moehringia grisebachii* were collected across its natural range from Bulgaria. Their genetic diversity was investigated through fifteen selected Inter Simple Sequence Repeats (ISSR) primers. The ISSR primers produced a total of 285 bands, of which 275 were polymorphic and 10 – monomorphic. Capability of the primers was assessed through the high mean values for the polymorphic information content (0.78), effective multiplex ratio (14.73), resolving power (27.90) and marker index (11.36). On the based obtained mean values of the molecular data the species *M. grisebachii* (effective number of alleles = 1.39, Shannon’s information index = 0.38, expected heterozygosity = 0.24, Nei's genetic diversity = 0.25, gene flow= 0.65) demonstrated higher genetic diversity than species *M. jankaе* (effective number of alleles = 1.28, Shannon’s information index = 0.26, expected heterozygosity = 0.17, Nei's genetic diversity = 0.23, gene flow=0.52). These results were supported by Analysis of molecular variance (AMOVA), showing higher variability within populations of *M. jankaе* (90%) and *M. grisebachii* (62%), than among populations - 10% and 38%, respectively, and 25% among both species. Neighbor joining and principal coordinate analysis (PCoA) grouped the thirty-nine studied populations by species and region of spread. The data are applicable in conservation programs for protecting and keeping of both species.

**Key words:** *Moehringia jankaе, Moehringia grisebachii*, ISSR markers, endemic plant, genetic diversity.
Influence of biostimulators Regoplant and Charkor on growth and development of micropropagated pear plants at acclimatization stage

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Abstract. In recent years, there has been a growing interest in biostimulants as an alternative to chemicals for safe and sustainable agriculture. The aim of this study was to analyze the effect of biostimulators Regoplant and Charkor (Agrobiotech, Ukraine) on growth and development of micropropagated pear plants (Pyrus communis L. ‘Old Home’ x ‘Farmingdale’) at acclimatization stage. In vitro propagated and rooted plantlets from pear rootstock OHF 333 were acclimatized in a floating system with 100 μl L-1 Regoplant or Charkor. As control plantlets with no additional treatments served. Data on growth parameters, chlorophyll a fluorescence (OJIP test) and antioxidant activity were collected 45 days after transplanting to ex vitro conditions. Enrichment of the nutrient solution with biostimulator Regoplant (100 μl L-1) in floating system led to the highest survival rate (82.5%) of pear plants, the greatest stem length, number, fresh and dry mass of leaves. A positive effect of Regoplant upon the functional activity of the photosynthetic apparatus was also established through chlorophyll a fluorescence parameters. Combining innovative approaches such as a floating system and biostimulators would significantly improve the acclimatization and overall process of micropropagation of fruit plants.
Preliminary study on the effect of LED light and cytokinine on the growth of pear plants in vitro

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Abstract. In the past two decades, light emitting diodes (LED) have become an alternative source of light for plant tissue culture, due to their low energy consumption, low heat emission, specific wavelength irradiation etc. The effect of three LED lights (white, blue and mixed) and two cytokinines (6-benzylaminopurine or meta-Topolin) on the growth of pear (*Pyrus communis* L. OHF 333) in vitro was studied. The plantlets were cultivated in microboxes (SacO2, Belgium) on a modified MS (Murashige and Skoog, 1962) solid medium supplemented with 2.5 µM 6-benzylaminopurine (BAP) or meta-Topolin (mT). The plantlets were grown in controlled room using Philips GreenPower LED research module. Three groups of LEDs emitting in white (W), blue (B), mixed (W:B:far-red=1:1:1) (BR) lights were applied. Biometric parameters, content of photosynthetic pigments and gas-exchange analysis of the plants were measured after three four weeks passages under corresponding light/cytokinine treatment. The results obtained indicated that different LEDs and cytokinine specifically affected the growth and development of in vitro cultured pear plants. The highest fresh and dry mass distinguished the plants grown under white LED light with both cytokinines studied. The maximum values for plant height was achieved in plants grown under white LED light with BAP and blue LED light with mT. The leaf sizes of plants grown on mT enriched medium were larger than those grown on BAP enriched medium, regardless of light and the largest were the leaves of plants grown under white LED light. Also, plants grown with mT in the nutrient medium showed more intensive photosynthesis, with the difference between the white and mixed LED light being insignificant.
Expression of sialyltransferases from the ST3Gal, ST6Gal and ST6GalNAc families in mouse skeletal muscle and mouse C2C12 myotube cell cultures

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Abstract. In skeletal muscles the sialic acids are very important for the functional maintenance of glycoproteins involved in fiber structure and neuromuscular junctions, development and regeneration, muscle excitability and exercise performance. The aim of this work was to investigate the sialylation in mouse skeletal muscle tissue and C2C12 mouse myotube cell culture in the aspect of localization of α-2,3- and α-2,6-sialylated glycoproteins, relative quantification of expressions of ST3Gal, ST6Gal and ST6GalNAc sialyltransferases specific for glycoproteins and comparison of the α-2,3- and α-2,6-sialylated glycoprotein profiles. Expressions of the enzymes ST6Gal2 and ST6GalNAc1 were not found in both experimental groups, and expressions of ST3Gal6 and ST6GalNAc3 were not detected in the myotube cell cultures. The different patterns of enzyme expressions in both experimental groups corresponded with differences between their α-2,3- and α-2,6-sialylated glycoprotein profiles. These results could be a useful addendum to the knowledge concerning the glycosylation of the skeletal muscle tissue. In addition, this report would be helpful and informative for any researches in future where the C2C12 myotube cell cultures will take a place as an experimental model.
Molecular characterization of tomato genotypes (*Solanum lycopersicum* L.) by Simple Sequence Repeat (SSR) markers

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Abstract. Tomato (*Solanum lycopersicum* L.) is widely cultivated, economically important vegetable crops in the world and serves as a preferred food of the population, including in Bulgaria and Turkey. The use of molecular markers provides new opportunities for selection of genotypes in breeding programme. Microsatellites are short tandem repeats scattered throughout the genome of higher organisms. They are highly polymorphic regions containing gene loci represented by multiple alleles of different lengths and nucleotide sequences. The purpose of the present study is to identify reproducible specific molecular profiles of tomato varieties from the collection of Maritsa Vegetable Crops Research Institute, Bulgaria and mutant lines of Nuclear Energy Research Institute, Turkey with microsatellite markers by detecting polymorphic loci. The study include 9 Bulgarian tomato varieties, 5 Turkish mutant lines and control landrace analysed with 19 SSR markers located in different chromosomes of the tomato genome at a distance of 2.0 cM to 115.0 cM. The number of band per microsatellite locus ranged from 1 to 8, with a total of 62 bands being in markers analysed.

Key words: SSR, microsatellite markers, Solanum lycopersicum, tomato.
Overcoming phytoplasma infection in *Paulownia tomentosa* by meristem *in vitro* culture

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**Abstract.** Paulownia witches’ broom (PaWB) is caused by phytoplasma of Aster Yellows group, and resulted in severe damage to the growth and premature death of the infected tree. In vitro cultures of meristems are important tools for solving problems with plant diseases. In recent study meristem cultures from phytoplasma infected *Paulownia tomentosa* have been created in attempt to obtain healthy plants. Faster procedure with longer explants generated 95% regeneration but no healthy plants. The combination of smaller explants and thermotherapy resulted in the generation of plantlets with a healthy phenotype. The PCR analyzes showed the absence of phytoplasma only in one of the produced plants. Despite the low efficiency, expressed in one phytoplasma-free regenerant of a total of 470 explants, these results can serve to continue the work on optimizing the used strategy.
Physiological and agro-biological evaluation of several local grain legumes under climatic condition of Sadovo region

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Abstract. Pea and common vetch are grown on all continents except Antarctica. The aim of the current study was to test few of them under the drought-prone area of Sadovo and to evaluate their physiological and agro-biological traits. The trial included local accessions of white lupin (Lupinus albus L.), chickpea (Cicer arietinum L.) and grass pea (Lathyrus sativum L.) and was carried out at the experimental field of IPGR. The chlorophyll content index of the leaves was used as criteria for drought tolerance and was measured using portable CCM 200 plus Chlorophyll Content Meter. The assessment of agro-biological traits was performed according to the International Descriptor for each crop.

Under the drought stress conditions the highest value of the chlorophyll content index was observed in three lupin accessions (BGR 6341, BGR3080, BGR3085), two from chickpea – variety Balkan and B9E0149 and four from grass pea – BGR 40415, BGR 4835, BGR4847, BGR4834. The highest yield potential was established in two accessions from chickpea (BGR 23151 and B9E0149), one from lupin (BGR 6341) and three from grass pea (BGR4832, BGR4831 and BGR40415).
Assessment of genetic diversity of white lupin (*Lupinus albus* L.) based on agro-morphological traits

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**Abstract.** Large genetic diversity exists in white lupin (*Lupinus albus* L.) expressed by its morphological and agronomical traits. The aim of this study is to assess the genetic diversity of 10 white lupin accessions regarding components of productivity and biological traits. Two years trial was carried out on the experimental field of Institute of plant genetic resources - Sadovo during the period 2018-2019. The traits were evaluated using the International Lupin descriptor; the degree of earliness - by Kuzmova, 2002. Based on morphological and agronomical traits the studied accessions were grouped into seven clusters. They expressed the high genetic diversity within the evaluated lupin accessions. One accession was selected (BGR3086) possessing higher plants compared with the others. This trait influenced on the productivity’s components. According to the degree of earliness, the lupen genotypes were clustered into three groups - ultra-early, early and late. The BGR 3086 accession was selected as ultra-early variety with 1.00 coefficient of earliness. Two other genotypes (BGR 3084 and BGR 6341) were selected to the early group with a coefficient of earliness 1.60. Genetically distant genotypes will be involved in the breeding programs for effective combining the important features into a new genotype.
Chromosome and pollen morphology of *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. in Bulgaria

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**Abstract.** The chromosome and pollen morphology in the Bulgarian populations of *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. was studied. The diploid chromosome number 2*n* = 34 was found. The metacentric type of chromosomes was predominant in all studied populations. In the karyotype of *A. hybridus*, the length of the metaphase chromosome ranged from 0.38 μm to 1.60 μm, and length of the total haploid length of the chromosome set - from 11.44 μm to 13.50 μm. The length of the chromosomes in *A. retroflexus* ranged from 0.18 μm to 2.27 μm, and the total haploid length of the chromosome set from 13.01 μm to 23.32 μm. Pollen morphology was examined using a scanning electron microscope (SEM). Pollen was defined as a spherical, pantoporate type. The pollen diameter in *A. hybridus* varied from 18.93 μm to 22.11 μm, and in *A. retroflexus* - from 16.75 μm to 22.21 μm. Differences in the number and diameter of pores have been found between the two species.

**Key words:** *Amaranthus hybridus* L.; *Amaranthus retroflexus* L.; chromosome number; karyology; pollen morphology; idiograms; Bulgaria.
Microbiology, biochemistry and biotechnologies
Synthesis of glucooligosaccharides by glucansucrases from fructophilic lactic acid bacteria from honey bee *Apis mellifera*

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**Abstract.** In the present work, we report synthesis of glucooligosaccharides (GOSs) by glucansucrase enzymes from fructophilic lactic acid bacteria *Lactobacillus pentosus* AG8, *Lb. kunkeei* AG9, AG10 and AG11 isolated from the gut of honey bees *Apis mellifera*. As an acceptor of glucose units from sucrose was used maltose and maltose to sucrose ratios (M/S) 1, 2, 4, 6, 8, 0.12, 0.16, 0.25 and 0.5. GOSs with degrees of polymerization (DP) ranging from 3 to 7 were obtained. The yields of GOSs and their composition according to DP were strongly influenced by the concentrations of the acceptor and sucrose. The highest yields of GOSs were obtained at M/S=1 – 45 to 53 g/L with predomination of DP3 fraction of oligosaccharides – 47 to 53%. The further increase of M/S ratios led to gradual decrease of the amounts of GOSs and also of their diversity according to DP. At M/S=8 were achieved yields of 13 to 15 g/L, composed only by DP=3 and DP=4 GOSs. In the opposite, the increase of sucrose concentrations at the expense of maltose ones led to more even distribution of the synthesized GOSs according to their DP up to 7, and yields of 40 to 42 g/L at M/S=0.5. Thus by varying the acceptor/donor ratio we were able to produce GOSs preparations with more controlled composition. In addition, GOSs preparations synthesized with glucansucrases from the four strains were subjected to dextranase hydrolysis in order to determine the content of α-(1→6) linkages. The results showed content of α-(1→6) linkages that decreases towards the lower maltose concentrations and is between 71 and 96%. Knowing that the enzymes from the four strains introduce 16 to 22% α-(1→3) linkages in the synthesized glucan polysaccharides such type of linkages could be expected also in the oligosaccharide products and this is promising for their prebiotic potential.

**Key words:** *Apis mellifera*, glucansucrases, glucooligosaccharides, *Lactobacillus*.

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Prebiotic potential of glucooligosaccharides synthesized by glucansucrases from fructophilic lactic acid bacteria

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Abstract. In the present work, we report a cultivation of probiotic strains of Lactobacillus plantarum S26, Lb. brevis S27 and Lb. sakei S16 in media supplemented with 1% of glucooligosacharides (GOSs) synthesized by glucansucrases from fructophilic lactic acid bacteria Lb. pentosus AG8, Lb. kunkeei AG9, AG10 and AG11. GOSs fractions synthesized at maltose/sucrose ratios 0.5 and 1 were chosen for the utilization experiments, determination of specific growth rates (μ) of the probiotic strains and acidification of the media. For the three probiotic lactobacilli were calculated very close μ values to these ones on glucose media. On the other hand, the observed growth of the strains in the GOSs supplemented media showed strain-specific profile. Lb. sakei S16 showed on average 39% lower μ than these ones of Lb. plantarum S26, Lb. brevis S27 independently of the carbohydrate source. The calculated μ values correlated well with the heterofermentative metabolism of the probiotic lactobacilli which is associated with production of lactic, acetic acid and very little of ethanol. At 48 h of the anaerobic cultivation of the strains, strain-specific ratios of the produced lactate to acetate were determined. In case of Lb. plantarum S26 the ratio is 4.1, and for Lb. brevis S27 and Lb. sakei S16 – 1.4 and 0.8, respectively. It seems that the decrease of lactate production at expense of acetate one is associated with the specific metabolism of the given stain and is not directly related to the composition of GOSs preparations tested. In all the cases, the ability of the tested GOSs preparations to support the growth and acids production of the probiotic lactobacilli is a promising sign for their potency as prebiotics.

Key words: glucooligosacharides, prebiotics, probiotics, lactobacilli.

Acknowledgements: This work was supported by Department for Scientific Research – PU, grant № МУ19-БФ-019/2019.
Phytochemical composition of *Pinus nigra* Arn. unripe seeds from Bulgaria

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**Abstract.** Black pine (*Pinus nigra* Arn.) is found in Central and Southern Europe, Southwest Asia, the Balkan Peninsula, including Bulgaria's territory. The present paper aimed to investigate the phytochemical parameters of unripe black pine seeds obtained from Bulgaria. Lipid fraction, cellulose, total carbohydrates, glucose, fructose, and sucrose were evaluated in unripe seeds. The major saturated fatty acid identified in the *P. nigra* seeds was palmitic acid, followed by the unsaturated linoleic, pinolenic and oleic acids. The amino acid composition of the protein fraction of unripe black pine seeds was also determined. The mineral content (N, P, K, Ca, Mg, Cu and Na) of black pine seeds was determined, as the amount of N and P were highest for the sample. These findings emphasized the potential use of the unripe black pine seeds as an alternative source of bioactive components in different areas due to their phytochemical importance and values.
Polycyclic aromatic hydrocarbons in traditionally used medicinal plants from Varna region, Bulgaria

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Abstract. The content of polycyclic aromatic hydrocarbons (PAHs) was determined in Matricaria chamomilla, Thymus serpyllum L., Tilia sp., Sambucus nigra L. and Achillea millefolium L., collected from urban and rural region near Varna. The aim of this study was to compare the levels of PAHs in traditionally used medicinal plants from different regions to assess environmental pollution. Benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene have been identified by EFSA as priority pollutants. The PAHs concentrations were determined in extracts of medicinal plants by GC-MS after purification. Chrysene was registered as most abundant compound in all plant species investigated. Benzo(a)pyrene, identified by the International agency for research on cancer as carcinogenic to humans, was not detected in the samples analyzed. The sum of 4 PAHs in medicinal herbs was found significantly higher in samples from areas with intensive traffic compared to samples from suburban areas. The total content of 4 PAHs in all plant species were found in the range from 0.68 (Sambucus nigra L.) to 6.82 µg/kg d.w. (Tilia sp.) and were below the permissible limit of the European commission.
The muscle phase of trichinellosis in mice is associated with increased ST3Gal-6 sialyltransferase activity in skeletal muscle fibers

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Abstract. In our previous work we showed that the de-differentiation of the occupied portion of muscle fibers toward a Nurse cell after invasion by Trichinella spiralis is associated with increased intracellular accumulation of α-2,3-sialylated glycoproteins. Our current investigation demonstrated up-regulated protein expression of the enzyme ST3Gal-6 in mouse skeletal muscles invaded by T. spiralis. Muscle samples were collected at certain time points after per os invasion. Immunohistochemistry was performed using a rabbit polyclonal antibody against ST3Gal-6 sialyltransferase. We found up-regulation of this enzyme shortly after the occupation of the muscle fibers, which persisted also in the mature Nurse cell. The ST3Gal-6 sialyltransferase is responsible for the transfer of sialic acid to galactose preferably on Gal-β-1,4-GlcNAc lactosamine structure and, to a lesser extent, on Gal-β-1,3-GlcNAc, as acceptors. The substrate specificity of this sialyltransferase and its up-regulation during the muscle phase of trichinellosis are particularly interesting since the lactosamine is one of the anticipated oligosaccharide structures of the α-dystroglycan. Alpha-dystroglycan is the only muscle protein known so far to be sialylated and plays a crucial role in the maintenance of the structural integrity of the skeletal muscle tissue. One of the enigmatic features of the Nurse cell of T. spiralis is its ability to stay integrated within the surrounding unaffected tissue even when the contractile properties of it muscle fiber are irreversibly lost. Our finding raises two important questions: 1. Could be ST3Gal-6 the sialyltransferase that is responsible for the sialylation of the α-dystroglycan? 2. Does the biosynthesis of α-dystroglycan continue also during the de-differentiation of skeletal muscle fiber toward a Nurse cell? Our instant efforts are currently dedicated to elucidate these two matters.
Bioactive compounds in *Camelina sativa* (L.) seeds

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**Abstract.** *Camelina sativa* (L.) is an old oil crop that is gaining interest due to its low impact on the environment impact and wide range of application. Camelina seeds can be used as a food ingredient and raw material for the production of oil. Chemical composition and the content of lipid biologically active components of four varieties *Camelina sativa* (L.) seeds were investigated. The proteins content was from 26.8 % to 29.3% and carbohydrates from 32.0 to 35.4%, respectively. The oil content of the seeds was 26.8%, 27.8%, 29.2% and 29.3%, respectively. Unsaturated fatty acids, which were presented mainly as oleic (21.7 – 26.3%), linolenic (16.4 – 20.4%), linoleic (14.8 – 19.9%) and gadoleic (15.7 – 17.7%) acids, predominated in triacylglycerols. Total content of phospholipids in the oil, mainly phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids was 12.0%, 12.1%, 15.0% and 16.7%, respectively. The amount of sterols was found to be 0.6% in the oil and beta-sitosterol predominated (68.3 – 71.4%) followed by campesterol (25.1 – 27.4%). Total tocopherol content was relatively higher (529 – 738 mg/kg) than those in other seed oils. Gama-tocopherol prevailed in tocopherol fraction in the oils (more than 90.0%).

**Key words:** *Camelina sativa* (L.), fatty acids, phospholipids, sterols, tocopherols.
Herbicide effect of Greek oregano essential oil on metabolite profile of target weeds

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Abstract. Origanum vulgare ssp. hirtum (Link) Ietsw. essential oil has been found to possess a wide range of biological activity. The biocidal properties of the oil are especially relevant in the context of the drive to use natural products in agriculture. In the present study, attention is focused on the metabolic changes that occur when weeds are treated with Greek (white) oregano essential oil as a bioherbicide. Dasyphyrum villosum (L.) Borbás, Matricaria chamomilla L., Sinapis arvensis L., Lolium perenne L., Trifolium repens L. and Trifolium pratense L.) were used as target weeds. The essential oil is applied on weed seedling in the form of an aqueous solution at 5 and 10 µg/mL concentrations by spraying. The experiment was conducted in plastic pots in growth chamber. The effect was reported on the seventh day after treatment and expressed as lethality percentage. The studied Poaceae species were found to be the most resistant, retaining almost 100% of their viability at both tested concentrations, while the other species at the higher tested concentration were destroyed or significantly reduced. The aerial parts of the surviving individuals of each weeds was collected and examined by GC/MS for the content of main metabolites. Organic, phenolic, fatty and amino acids, sterols, polyols as well as mono- and disaccharides were identified. Changes in the accumulation of certain metabolites after treatment with essential oil were observed. However, the trend is not unequivocal - it depends on the concrete weed species and probably on its resistance. The described metabolic changes were noted without large visible morphological changes in resistance species, while different in size and color spots on the surface of the leaves of more sensitive species were found. The results obtained provide data on the use of white oregano essential oil as a herbicide against weeds in post emergence stage and complement knowledge of metabolic response of plant to stress factors. For the first time, data on metabolic changes under the influence of white oregano essential oil were reported.

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Quantitative determination of total triterpenoids in exudates of Thymus species

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Abstract. Exudates consist mainly of lipophilic compounds accumulated on the surface of plant tissue. They are obtained by rapidly rinsed of the whole, not ground plant parts. Previously GC/MS analysis of exudates of Thymus species showed that triterpene acids are the main component of them. Triterpene acids are compounds with important biological and pharmacological activity, among them the antitumor is especially valuable. Simonyan et al., 1972, have described the rapid and easy method for the quantitative determination of triterpenoids. The method is based on the reaction of triterpenes with concentrated sulfuric acid whereupon (whereby) they are oxidized and form colored products that can be measured spectrophotometrically in the 310 nm region. Spectrophotometric analysis of exudates of eight Thymus species was applied to assess quickly and easily the content of triterpenoids. The plant material was collected from their natural localities. Four of the studied species are members of the section Hyphodromi (Thymus atticus, T. perinicus, T. striatus, and T. zygiiodes) and the other four species belong to section Serpyllum (T. longedentatus, T. longicaulis, T. pulegioides, and T. sibthorpii). One of the studied species is a local endemic with distribution in Pirin Mts only (T. perinicus), one is a Balkan endemic (T. longedentatus) and two of them are sub-endemics (T. atticus, T. sibthorpii). Exudates were obtained from dry, not ground plant material by washing with acetone to dissolve the exudate material. Total triterpenoids were determined by sulfuric acid following the principles cited above. The content was expressed as percent (%) in exudate as oleanolic acid. The highest content of triterpenoids was found in T. perinicus, T. pulegioides, T. striatus, T. atticus, T. longicaulis exudates. The present study reports the first data about the content of triterpenoids in four of the studied thyme spices due to their limited and rare distribution. The established high levels of triterpenoids in the exudates define them as promising fractions for the study of antitumor and antimicrobial activities.


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Phytochemical study of Pinus and Juniperus species from the Bulgarian flora and assessment of the potential of their essential oil for biological and biopesticidal activity

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Abstract. The production of sufficient and healthy food for the ever-growing global population while conserving biodiversity and protecting the environment are priority of modern agriculture. Increasingly, consumers prefer foods produced without the use of agricultural chemicals. The biological method for controlling agricultural pests, phytopathogens and weeds is an alternative to the widely used chemical production methods. Plant essential oils (EO) are often used to produce biopesticides. They are volatile, and therefore, the EO are good fumigants, and generally are less or non-toxic to animals, humans and the environment. The production of EO-based biopesticides has several advantages: they are usually effective, some are easy to obtain and relatively inexpensive, and are generally considered much safer for the environment and people. These benefits of the EO inspire the research on underutilized plant species containing EO with potentially significant and desirable biological activity. A research project funded by the National Science Fund was initiated in 2019 on the EO from the genus Pinus (Pinaceae); P. sylvestris (White pine, Scots pine), P. nigra (Black Pine, Austrian pine), P. mugo (Kleck, Mugo pine), P. heldreichii (Black Wall, Bosnian pine), P. peuce (White pine, Balkan pine), and two species from genus Juniperus (junipers); J. excelsa (Greek juniper) and J. sabina (savin). The research includes phytochemical characterization of the EO and assessment for biopesticidal action on (1) fungal pathogens; (2) aphids; (3) weeds
and (4) biological activity. The team includes of scientists from Bulgaria, Slovakia and United States.

**Key words:** *Pinus; Juniperus;* biopesticides; Essential oils; weeds; aphids; pathogens.

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Identification and exopolysaccharide synthesis by Antarctic yeasts

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Abstract. Antarctic is among the most scarcely investigated extreme niches in relation to microorganisms living there and synthesis of unusual molecules for adaptation to extremely low temperatures, lack of water availability and precipitation, numerous freeze–thaw cycles, strong wind levels and high sublimation, evaporation and ultraviolet radiation. Several yeast genera such as Cryptococcus, Mrakia, Candida, Rhodotorula, Leucosporidium, Debaryomyces have been described as part of the biodiversity of the continent. The aim of the present research was to studying yeast variety in the Livingston Island, Antarctica, and yeast potential to synthesize extracellular polysaccharides. Thanks to the cooperation of the Bulgarian Antarctic Expedition, three different strains were isolated and identified. Based on a genetic analysis of ITS1-5.8S-ITS4 regions of rRNA they were related to two genera – Cystobasidium and Vishniacozima. One of the strains belonged to the species Cystobasidium ongulense and two to Vishniacozima victoriae. The morphological differences of the two species included the color of the colonies - red and cream, respectively, while both species were glossy and with a smooth edge. Cystobasidium ongulense cells were larger and elongated. The differences between the species also included the different number of assimilated carbon sources. The ITS domains of the rRNA gene were amplified using the universal primers ITS1 and ITS4. The process of extracellular polymer biosynthesis was performed at a cultivation temperature of 21°C±1°C. The cell growth over 5.5gL⁻¹ and exopolysaccharide production from 1.6 to 3.6gL⁻¹ were registered at 120th h of the fermentation process. Based on sampling of soil, moss or penguin feathers in Bulgarian base, the Livingston Island, new results for yeast diversity were accumulated and they can contribute to the fundamental worldwide knowledge on the biodiversity of the Antarctic continent. Unusual origin of exopolysaccharides synthesized by the isolates and their valuable preliminary characterized properties suggest a possibility for their biotechnological exploration.

Key words: Antarctic yeasts, identification, exopolysaccharides.

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Evaluation of the antineoplastic activity of Antarctic yeast *Sporobolomyces salmonicolor* grown at different culture conditions

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Abstract. Nowadays, deaths from neoplasms are at the top of the World Health Organization list. The interest of scientists is focused on the search for natural substrates perspective for the targeted anticancer chemotherapy. Antarctic yeasts represent an unexplored object regarding their antineoplastic potential. The studies in the present work demonstrated the capacity of *Sporobolomyces salmonicolor* AL 36 for cell growth in different cultivation processes. During the transfer of small-scale flasks cultivation to the bioreactor system, optimal biomass quantities of approximately 6.0 g/L were recorded. A comparative examination of the metabolic profiles of the two extracts tested reveals differences in the synthesized molecules corresponding to different cytotoxicity (antineoplastic activity) *in vitro* on malignant cell lines. The median inhibitory concentration (IC 50) of each extract determined by the MTT test was used as a parameter for evaluating the antiproliferative effects. Most sensitive to the *in vitro* effect of the extract of *Sp. salmonicolor* AL 36 cultivated in flasks was the cell line SKW-3 (T cell leukemia, derivative of KE-37) - IC 50 = 35.3 µg/ml, while the bioreactor biomass extract was most cytotoxic for RPMI-8226 (multiple myeloma) cells - IC 50 = 28.27 µg/ml. The proteome analysis of treated and untreated malignant cells showed that both yeast extracts have a strong potential to inhibit anti-apoptotic proteins which reveals a mode of action related to induction of apoptosis and proliferation inhibition.

Keywords: *Sporobolomyces salmonicolor* extracts, bioreactor, *in vitro* antiproliferative effect, apoptosis.

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Acknowledgements: The antiproliferative activity of the extracts on HD-MY-Z, HuT-78 and MJ (MTT-dye assay) was performed on laboratory equipment donated by the Alexander von Humboldt Foundation to Maya M. Zaharieva, PhD in the frame of the program “Equipment subsidies”.

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Phytochemical analysis of leaves and stems of *Physalis alkekengi* L. (Solanaceae) from Bulgaria

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Abstract. *Physalis alkekengi* L. (Solanaceae) is encountered in different regions of Bulgaria as a wild growing or ornamental plant. All aerial parts of the plant have been used in folk medicine for centuries due to the presence of various bioactive metabolites; the plant is included within the scope of the Medicinal Plants Act of Bulgaria. The objective of this work was to characterize the phytochemical composition of the leaves and stems of two genotypes of *P. alkekengi* collected from natural habitats in South and North Bulgaria (PA-SB and PA-NB), with the view of revealing their use potential in food, feed, cosmetics and other areas. Both aerial parts were analyzed in terms of certain phytochemical indices – moisture, ash, cellulose, protein, amino acids, macro and micro minerals, and data about the respective contents were provided. The results from the study revealed significant differences in the levels of the phytochemical components, both on a plant part and genotype basis. The leaves of the two genotypes were further processed by extraction with n-hexane, for the identification of leaf volatiles (by GC-MS). The results revealed the presence of several aroma-active compounds, belonging to various chemical classes, as well as genotype-related differences. Those findings substantiated the potential of *P. alkekengi* leaves as a new resource for obtaining bioactive extracts for phytopharmacy or cosmetics, although supplementary research focused on that subject is necessary. To the best of our knowledge, the study provides the first data about the content of macro and micro nutrients and volatiles in the leaves and stems of *P. alkekengi* from Bulgaria, in a direct comparison of genotypes from two distinct regions, which could be of further practical interest.
Study of glycosylation of flavonoids using different glycosyltransferases from Leuconostoc mesenteroides

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Abstract. Enzymatic glycosylation is a perspective method for biosynthesis and modification of bio-logically active flavonoids, which can influence the metabolic processes in the human body with different strength. Controllable enzymatic glycosylation of flavonoids from different groups was performed based on the specific action of glycosyltransferases produced by the Leuconostoc mesenteroides strain, such as dextrantransferase LM28, high molecular weight glucansucrase URE13-300 and fructosyltransferase L17. The flavonoids used in the process of glycosylation are rutin and quercetin. During of synthetic reactions are monitored for a wide range ratio between the donor - acceptor in which are set the conditions for the preparation of of new glycosidic products were established, which were proved by HPLC analyzes. During of synthetic reactions are monitored for a wide range ratio between the donor - acceptor in which are set the conditions for the preparation of of new glycosidic products were established, which were proved by HPLC analyzes. At a donor-to-rutine ratio of 10:1 in the presence of the LM28 enzyme, a new product was observed and the acceptor was completely reduced. When quercetin is used as an acceptor in the synthesis reaction with the same enzyme, glycosidic products are again obtained. In this reaction, the donor is 5% sucrose, and the amount of acceptor has no significant effect. Successful glycosylation of anthocyanins occurs with the enzyme L17, with maximum retained activity when the donor is 3% sucrose. In other cases, anthocyanins inhibit the enzyme L17, as there are two possible types of inhibition uncompetitive and mixed. The controlled glycosylation of flavonoids as acceptors with well-defined structures led to the production of new glycosidic molecules.

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Effect of polysaccharides obtained from *Plantago major* L. leaves on *Lactobacillus bulgaricus* L14 in an *iv vitro* model of the gastrointestinal tract

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**Abstract.** There is evidence that prebiotics can impart a range of health benefits if consumed on a regular basis. With a growing market for prebiotic-containing foods there is increasing interest in understanding how prebiotics function at the molecular level. Advances in the genomics of lactobacilli and bifidobacteria have enabled modeling of transport and catabolic pathways for prebiotic utilization. The goal of this study was to develop a physiological model of the upper gastrointestinal tract to investigate the metabolic profile of probiotic strain *Lactobacillus bulgaricus* L14 in present of 0.75% polysaccharides obtained from *Plantago major* L. leaves. The polysaccharide degradation and utilization from probiotic strain L14 using *iv vitro* model including gastric phase and intestinal phase simulated digestion in humans was developed. As a results of hydrolytic processes in gastric phase after 2h, polysaccharides obtained from *Plantago major* L. leaves were partially hydrolyzed to monosaccharides- mainly galactose and oligosaccharides with degree of polymerization higher than 6. In the conditions simulating intestinal phase, the growth of *Lactobacillus bulgaricus* L14 from 1x10\(^5\) CFU/ml on the 0h to 4x10\(^5\) CFU/ml on the 4\(^{th}\) hour was observed. D-lactate (15.69 mmol/L) and L-lactate (3.07 mmol/L) were detected after 4h cultivation in conditions simulating intestinal phase. In addition, an *iv vitro* model induced hyperglycemia (100mM glucose) in human erythrocytes, treated 48h with metabolites simulating intestinal phase from 4\(^{th}\)hour, showed 41% reduction of catalase and 26% incising of superoxide dismutase activity.

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Optimization of enzyme production of glucosyltransferase URE 13-300 by cultivation of recombinant strain Escherichia coli BL21-URE13-300 and enzyme synthesis of insoluble glucan

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Abstract. *Leuconostoc mesenteroides* URE 13 strain produces novel glucosyltransferase enzyme with high molecular mass of about 300 kDa together with dextranucrase and fructosyltransferase. The enzyme URE 13-300 is an α-transglucosylase, part of GH70 family, synthesizing insoluble glucan with α-1,6 and α-1,3 linkages. This enzyme is an object of great interest because of the high prebiotic potential of its oligosaccharide products and branched glucan. The gene encoding the enzyme glucosyltransferase URE 13-300, is cloned and expressed in *Escherichia coli* BL21. Gene expression was optimized in regards to time after induction with isopropyl-b-D-thiogalactopyranoside and change in the temperature from 37 to 16°C. The inductor is added when the optical density (OD) for biomass reaches absorption of 0.7. Enzyme activity and OD were measured once every two hours from 12th hour onwards. It was studied the correlation between OD and enzyme activity to the end of cultivation - 26th hour with a step of 0.1. for OD. The highest enzyme activity of 5.0 (U/mg)^-1 was measured at the 24th hour of induction. This is about twofold increase compared to the activity at the 12th hour, which is 2.7 (U/mg)^-1. At the 26th hour the rate of accumulated biomass is lowered which corresponds with lower enzyme activity. The obtained enzyme was used in enzyme reactions for synthesis of insoluble glucan using sucrose as donor of glucose residues and maltose and low-molecular dextran as acceptors at M/S ratio of 0.5 and D/S ratio of 0.4. Duration of the reactions was 72 hours in bioreactor with agitation of 100rpm. Glucan and oligosaccharide content were analyzed about their structure and rheological properties.
Etiological Structure, Drug Resistance and Biofilm Forming Capabilities of Isolates from Respiratory System from Outpatients

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Abstract. The study is focused on the etiological structure and drug resistance of bacterial strains causing upper respiratory tract infections, which are widely spread amongst children. The aim is to update the data about antimicrobial susceptibility and to establish virulence factors important for infectious process for Staphylococcus spp. isolates in upper respiratory tract. A total of 711 strains were isolated from outpatients with nasopharyngeal and oral infections at Medical Diagnostic Laboratories „Sinevo-Bulgaria“ LTD in Plovdiv for two-year period 2019-2020. The results demonstrate that the etiological structure is highly related to the site of infection. Branhamella catarrhalis was found to dominate in the samples nasopharynx (65%), while the majority of isolates from oral and ear samples were identified as Staphylococcus aureus - 55% and 35% respectively. S. aureus showed higher drug resistance compared to other isolates, reaching up to 70% to penicillins. Over 20% of the Staphylococcus strains were also resistant to macrolides, fluoroquinolones and aminoglycosides. Methicillin resistance was established for 39% strains. The ability of S. aureus to form biofilms was tested as significant virulence factor by cultivation on unmodified and modified (supplemented with 5% human plasma) tryptic Soy Broth (TSB). The analysis shows that only 16,3% St. aureus were capable to form stable biofilm on TSB. The addition of human plasma increases the number up to 87%. The susceptibility profile of the investigated strains in the present study confirms need always to detect resistance before antibiotic prescriptions from physicians.

Key words: antimicrobial susceptibility, biofilm, etiological structure, Staphylococcus aureus.
Taxonomic Composition and Physiological Activity of the Microbial Communities in Heavily Modified Water Bodies in Bulgaria

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Abstract. The present study aims to extend the systematic knowledge on the quantitative and qualitative composition of the aquatic microbiome in large and economically significant Bulgarian reservoirs. Water samples were collected within 4 monitoring campaigns from 9 reservoirs, belonging to 4 national types of standing water bodies. Analyses include physiological profiling (CLPP) and next generation sequencing (NGS) of the V3-V4 hypervariable region of 16S rRNA gene. The surveyed water bodies can be divided into three groups based on their AWCD (average well color development) and specific activity. The communities in Koprinka, Ovcharitsa, Aheloy and Ivaylovgrad showed high steepness of the kinetic curve rapidly reaching a plateau. AWCD indicates that these communities are dominated by a limited number of fast-growing p-strategists. The second group include Dospat and Ogosta, while Studen Kladenetz, Alexander Stamboliyski and Zhrebchevo were consolidated in a separate group with greater biodiversity and conditions suitable for the development of slow-growing k-strategists. The NGS generated 5420 operational taxonomic units (OTUs), affiliated to 1066 genera. The analysis of the taxonomic composition and the abundance showed that communities are formed by similar dominant complex, with > 95% of the total number of sequences affiliated to representatives of only 10 phyla including Proteobacteria, Actinobacteria, Cyanobacteria, Verrucomicrobia, Firmicutes, Acidobacteria, Chloroflexi, Gemmatimonadetes and Fusobacteria in decreasing order. The communities differ mainly in OTUs forming a minor component based on their relative abundance. Cluster analysis of the genera distribution along the longitudinal axis of the water bodies revealed that positioning of net-cage aquaculture farms leads to an increase of the abundance of Cyanobacteria and Bacteroidetes. The second cluster groups the sites located at the walls of water bodies. The river inflow sites and the open aquatory are characterized by a similar composition. CLPP and NGS analysis revealed that water bodies are characterized by good self-purification ability, despite the presence of net-cage farms.

Key words: Metagenomics; 16S rRNA; Community level physiological profile; Microbiome structure; Microbial ecology.
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