Biogeography of the Carpathians

Prague 2022

The Third Interdisciplinary Symposium

12-14 September 2022, Prague, Czech Republic

Book of Abstracts

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Invited talks

Habitat and species monitoring in the Czech part of the Carpathians

Karel Chobot

Nature Conservation Agency of the Czech Republic, Praha, Czech Republic

The Nature Conservation Agency of the Czech Republic is responsible for providing the surveillance of EU Habitats Directive species and habitats, which is in practice translated into the systematic mapping and monitoring. The system covers the entire state territory, including the Czech Carpathians. The principles, methods, collected data and results will be briefly described and presented, with emphasis on the activities in the Czech part of the Carpathians.

Aquatic biodiversity patterns in European mountain ranges and beyond: a molecular perspective

Michał Grabowski

Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Łódź, Poland

In terms of species richness and endemism, Europe was until recently considered the "poor sister" of the tropics. Such judgement was biased mostly by the comparably low number of formally described species, particularly in such emblematic groups as vertebrates, insects, or molluscs and by the believed calamitous role of Pleistocene glaciations in shaping European biodiversity. Only the Mediterranean part of Europe was considered as a noticeable exempt. This now obvious unfair opinion has started to change with the introduction of molecular markers to taxonomy and historical biogeography. The presence of divergent phylogenetic lineages has been documented in a number of conventionally recognised taxa, and soon it has become obvious that a large amount of cryptic diversity remains to be revealed. Even then, most studies have focused on terrestrial taxa, leaving the freshwater biota behind. Nevertheless, evidence already accumulated from the most recent studies on aquatic organisms allows for some generalisation.

As appears, cryptic diversity and divergence of lineages in European aquatic taxa, particularly those without airborne dispersal stages, far exceed what is commonly found in terrestrial ones. Predominantly, such diversity is concentrated in mountain ranges, making them outstanding diversification and endemism hotspots. In several cases, the evolutionary history of European freshwater species reaches far beyond the Pleistocene and dates back to the origins of the continent we know now, questioning the widely accepted views on the historical biogeography of European inland waters. It also has significant implications for planning effective nature conservation strategies.

In my talk, I will present and discuss the most recent findings on diversity patterns for main freshwater taxonomic groups in Europe, using case studies from my team's published and unpublished work, as well as from other research teams.

Species and habitats of the Habitats Directive in the Carpathians: distribution and status

Ľuboš Halada, Juraj Lieskovský, Peter Gajdoš

Institute of Landscape Ecology, Slovak Academy of Sciences, Bratislava, Slovakia

We studied distribution of species and habitats of the Habitats Directive (HD) in the Carpathian Mts. using reports submitted in 2019 by the European Union member states according to the HD Article 17.

The highest number of species of Community importance was registered in the Hungarian part of Carpathians and adjacent part of Slovakia as well as in south-western outcrops of the Carpathians at SK/AT and CZ/AT borders with hotspots in Slovenský kras/Aggtelek and Börzsöny/SW Cserhát. While mammals and partially also invertebrates follow the general species distribution, other groups exhibit different patterns. High numbers of amphibian and reptile species were reported from the Hungarian Carpathians and quite high in the Czech Carpathians, Transylvanian plateau and the Apuseni Mts. The Southern Carpathians along the Danube and Mures rivers in Romania represent hotspots of fish distribution. High numbers of plant species were reported from the Polish Carpathians and scattered through the Slovak and Hungarian Western Carpathians.

The number of habitats per grid cell is generally higher in the Western than in the Eastern and the Southern Carpathians, the highest ones were reported from high parts of the Western Carpathians – the hotspots are the Vysoké and Západné Tatry Mts. (both Poland and Slovakia), Malá Fatra and Veľká Fatra Mts., Malé Karpaty Mts. and parts of the Nízke Tatry Mts., Slovenský raj Mts. and Muránska planina Mts.

We also analysed distribution of endemic species and habitats with restricted distribution. We used our own classification of endemism and found that areas with highest number of local and regional endemic plant species are in the Western Carpathians, more specifically in the Západné Tatry Mts., Veľká Fatra Mts, Slovenský kras/Aggtelek , Börzsöny/SW Cserhát Mts.. In case of animals, the highest number of endemic species were reported from the Southern Carpathians (along Danube), the Mures river valley, Transsylvanian plateau and Aggtelek Mts. The southern part of the Western Carpathians hosts the highest number of habitats with restricted distribution.

Imprints of Holocene history in the modern biota of Western Carpathian spring fens

Michal Horsák

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Spring fens are wetland ecosystems that develop in areas with groundwater supply, forming isolated island-like habitats in the landscape. The permanent waterlogging that favours a continuous accumulation of peat preserves the remains of various organisms (e.g. plants, molluscs, testate amoebae, ostracods, chironomids). Such fossil records, along with radiocarbon dating, allow us to reconstruct the past development and history of these sites and their biota, including data on palaeobiogeography and palaeoclimate.

In this talk, I will present our results from long-term research of spring fen ecosystems in the Western Carpathians. I will relate the modern biogeography, presence of late-glacial relict species, assemblage composition, and species richness of these highly endangered edaphic islands to their age and Holocene history. Vascular plants, bryophytes and molluscs will be used along with stable isotopes and radiocarbon dating to reveal palaeoecological reconstructions, present biogeography and biodiversity patterns.

These insights can be used to identify appropriate conservation measures for these biodiversity hotspots and to maintain the favourable condition of these sites.

Forest molluscs – the key to the Holocene diversity in Central Europe

Lucie Juřičková[1], Jitka Horáčková[2]

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[2]*Department of Landscape and Urban Planning, Faculty of Environmental Sciences, Czech University of Life Sciences, Praha, Czech Republic*

The development of Central European mollusk communities in the Holocene shows local specifics. While forest assemblages have never fully developed in areas inhabited by early farmers, forest fauna has been fully developed in areas immediately adjacent but unsuitable for agricultural use. The rapid development of forest communities started mainly after the cold event of 8.200 BC. In the Late Holocene, forest assemblages are usually gradually declining. An extreme example is the region of sandstone rock cities, where up to 11 species of clausilids were found syntopically in the Holocene forest optimum. A radical transformation of this temporal ecological equilibrium began in the third millennium BC. Over the following millennia, the species-rich canopy forest mollusk assemblages almost completely disappeared, together with calciphilous rock dwellers. Recently, there has been a complete degradation of forest communities due to the decalcification of the landscape. Forest species ranges in Central Europe fluctuated slightly during the Holocene as shown in the data from the Carpathians and Alps, reflecting minor climate changes. Overall, forest species are the most important component of the Central European mollusk fauna during the entire Holocene.

Evolutionary drivers and consequences of parallel alpine evolution

Veronika Konečná

Department of Botany, Charles University, Praha, Czech Republic

Parallel adaptation to similar environmental pressures provides an ideal model system to study the repeatability of evolution in nature. Such replicated natural experiments can also provide important insights into the genomic basis of adaptations. However, well-documented examples are rare, particularly in plants. We brought new evidence of parallel evolution from two plant systems facing one of the most challenging selective environments – alpine stands.

By combining population genetic and experimental approaches, we documented the complex interplay of adaptive, historical, and ecological processes in parallel evolution. The island-like distribution of high-elevation alpine stands promoted independent colonizations by distinct genetic lineages of *Arabidopsis arenosa* and *Primula elatior* separately in each geographic region. Further, we showed how challenging environments structure genetic diversity within a species. For instance, we found higher genetic differentiation among alpine populations than among foothill populations. This suggests that mountain ridges act as migration barriers reducing gene flow among alpine populations. Moreover, colonization of alpine habitats did not result in loss of genetic diversity suggesting rather gradual colonization by large populations than a strong bottleneck. Further, taking advantage of multiple natural replicates of alpine adaptation, we studied the genomic basis of adaptation and quantified the magnitude of phenotypic parallelism and investigated its neutral and adaptive determinants.

In summary, cases of parallel evolution provide important insights into evolutionary drivers and genetic architecture of repeated adaptation and the identification of novel models of parallel evolution is a fruitful approach.

The evolutionary and ecological assembly of the high alpine flora

Sébastien Lavergne

Laboratoire d'Ecologie Alpine, CNRS – Université de Grenoble Alpes, Grenoble, France

The flora of the European Alps is one of the best known floras in the world, due to a long tradition of botanical studies in this region. Yet, the study of the Alpine flora, especially at higher elevations (i.e. in the alpine and nival vegetation belts), still brings new perspectives or even raises unexpected questions about fundamental problems of ecology and evolution.

Here I will present different research projects based on previous collective efforts of mapping, sampling and sequencing of all plant taxa occurring in the Alpine Arc, but also in other European mountain ranges, thanks to companion projects and many collaborators. I will also show how these projects provide new insights into (i) the tempo and drivers of plant macroevolution, (ii) species delimitation and drivers of plant speciation, and (iii) assembly rules 0of natural plant communities in the European high alpine flora.

Contributed talks

Plecoptera (Insecta) from the Carpathians, Romania: barcoding dark taxa in a biodiversity hotspot from Europe

Anna Dénes[1], Avar-Lehel Dénes[2], Lujza Keresztes[1]

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[2]*Institute of Interdisciplinary Research in Bio-Nano-Sciences, Babeş-Bolyai University, Cluj-Napoca, Romania*

Dark taxa represent the overlooked part of biodiversity, which is neglected due to problematic taxonomy, although it includes important cryptic diversity or missing faunistic data. Plecoptera are a very important, yet poorly explored component of aquatic ecosystems due to their difficult species-level taxonomy and cryptic larvae forms. Here we present our DNA barcode initiative, which aims to detect more Plecoptera data from well-known aquatic biodiversity hotspots in the Romanian Carpathians.

In total, 179 specimens were collected between 2020 and 2021 and analyzed using the standard barcode region (mtCOI). The sequencing success was 92.74%, consisting of 101 larvae and 65 adult sequences. These represented 30 known species of which 27 already have BINs assigned and three are yet to receive one. We further identified eight OTUs generated using the REfined Single Linkage algorithm. These results indicate important overlooked diversity, adding new molecular data to known species, and showing the existence of possible cryptic species. We managed to associate 78 larval stage Plecoptera specimens to 21 known species with barcode sequences in the BOLD Systems' database. We generated for the first time DNA barcode data of a newly discovered genus *Zwicknia* Murányi, 2014 and four endemic species of the Carpathians: *Brachyptera starmachi* Sowa, 1966, *Leuctra carpathica* Kis, 1966, *Protonemura aestiva* Kis, 1965 and *Chloroperla kisi* Zwick, 1967.

Our results estimate a more realistic dimension of aquatic biodiversity and encourage the application of molecular methods in conservation and management of water resources in the Carpathian area.

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Phylogenomic data of the beech forest understory species *Euphorbia carniolica* indicate a Pleistocene refugium and pronounced genetic differentiation in the Carpathians

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Deciduous forests are dominant natural vegetation of temperate Europe. During cold stages of the Pleistocene, they were restricted to small areas from where they expanded in the Holocene, but little is known about the refugia, colonization routes and demographic history of deciduous forest understory herbs. It has been suggested that many of them had their main glacial refugium in the western Balkan Peninsula. One of these species is *Euphorbia carniolica*, which is disjunctly distributed from the southern margin of the southern Alps over the northwestern and the central Balkan Peninsula to the Southern and Eastern Carpathians including the Apuseni Mountains.

Using genomic RADseq data in combination with phylogenetic analyses of nuclear and plastid sequences we show that the species originated in the Pliocene and started to diversify at the Pliocene/Pleistocene boundary, when the two main lineages diverged, one restricted to the southern Alps and the northern Balkan Peninsula, and the other to the central Balkan Peninsula and the Carpathians. Whereas the first group did not experience pronounced genetic differentiation, within the second group the central Balkan populations were divergent from the Carpathian populations that likely originated via dispersal from the Balkans. RADseq data indicated a deep split between the Eastern and the Southern Carpathian populations, the latter including the Apuseni Mts. Clear genomic differentiation accompanied with climatic modelling data indicate that the species persisted in different refugia, including the southern Carpathians, at least during the Last Glacial Maximum. Climatic niche modelling indicated a differentiation, but also an overlap between the climatic niches of the two main genomic lineages that were also different in their genome size. Contrary, the two groups overlapped largely in their morphology, which is in line with the observations of slow rates of morphological evolution (morphological stasis) in other forest species.

Characterization of molecular diversity of spring fen macrozoobenthos from two geomorphological regions

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Recent studies on diversity of stream amphipods indicated that the Western Carpathians may have served as an important glacial refugium of freshwater fauna. If this scenario is true, a considerably high molecular diversity can be expected in this biogeographic region also for other aquatic taxa. In our project, we aim to uncover and characterize molecular diversity of benthic macroinvertebrate fauna of calcareous spring fens (a well-studied and hence convenient model community) of the Western Carpathians and for comparison also of the Bohemian Massif, an adjacent region with different geological history.

Using a DNA metabarcoding approach, we sequenced a fragment of the COI gene of pooled spring fen invertebrate communities from 21 localities in the Western Carpathians and 8 localities from Bohemian Massif. Here the up-to-date insights about the comparison of molecular diversity of the same habitats in these two areas with different geological histories will be presented. We observed a considerable lack of reference sequences in public databases (Barcoding of Life Database, GenBank) for a large portion of the detected molecular operational taxonomic units (MOTUs), indicating that the studied regions are not yet sufficiently covered by barcoding efforts, and/or suggesting that there indeed may be a considerable unrecognized diversity of macrozoobenthos. We also aim to compare the MOTU diversity with the morphological diversity, already well studied at most of the localities, and to try to detect the spatial variation in changes of molecular and morphological diversities.

Alpine travelers visiting the Carpathians: story of the land snail *Pyramidula saxatilis* told by its genes

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Several woodland land snails, distributed mainly in the Alps, have disjunct occurrences in the Carpathian Mountains. Fossil evidence suggests that these species colonized the Carpathians primarily during the Holocene forest optimum or later. Since they are now associated with temperate climates, they were probably not able to survive the Last Glacial Maximum north of the Alps. Recently, *Pyramidula saxatilis*, a minute limestone rock-dwelling species was genetically distinguished from a more widespread *P. pusilla*. The distribution of *P. saxatilis* is almost exclusively restricted to the Alps, but a few genetically verified populations were recently recorded also in the Western Carpathians. As the species prefers high elevations and occupies very stable habitats, i.e. exposed limestone rocks, one can assume its survival in the Carpathian refugia over the glacial period(s). To test this hypothesis, we analyzed the genetic diversity of mtDNA and nDNA markers across the populations of both species collected throughout Europe, with particular focus on the Alps and the Carpathians.

The results showed that all known Carpathian populations of *P. saxatilis* are genetically homogenous and similar to those from the Eastern Alps. In contrast, for *P. pusilla*, which prefers warmer climate, we found a unique haplotype in the Slovak Karst (southern Slovakia), suggesting its long-term survival in the Carpathians. All other Carpathian populations were closely related to the Alpine ones. Our results suggest that the Carpathian populations of a cold-climate *P. saxatilis* were unable to survive glacial periods in situ and most likely represent recent Holocene immigrants. We also revealed profound differences in the haplotype structure of both species related to their modern climatic preferences, possibly reflecting past population dynamics affected by the location of their glacial refugia.

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Adaptation of *Campanula serrata* (Kit ex Schult.) Hendrych to habitat conditions along an elevational gradient in the Tatra mountains

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Alpine plant ecosystems, due to strictly defined habitat conditions, are considered as particularly vulnerable to environmental changes, including the more and more pronounced global warming effects. Plants' response under the pressure of mentioned changes may rely on two ways: either shifting the range (e.g. subalpine plants to higher alpine belt) or adaptation the life cycle to new local conditions. The aim of this work was an assessment of morphological and physiological changes of subalpine plant *Campanula serrata* (Kit. ex Schult.) Hendrych in sites at different altitudes (1330-1850 m a.s.l.) in local habitat in the Tatra mountains.

Morphological changes, relative chlorophyll content, photochemical activity as well as ^{13}C and ^{15}N isotope discrimination were assessed. Maximum quantum yield of photosystem II (PSII) (Fv/Fm), being the most known stress indicator as well as performance index (PI) of PSII were unaltered in all analysed sites suggesting the lack of oxidative stress in leaves. In addition, electron transport chain was the most efficient in the lowest analysed site (1330 m a.s.l.), which correlates with morphological leaves analysis as well as ^{15}N isotope discrimination. Despite substantial leaf area increase at 1330 m a.s.l., the dry weight as well as non photochemical quenching and relative chlorophyll content is comparable to the parameters of other sites, indicating relatively best efficiency of photochemical activity in plants from the lowest analysed site. Moreover, observed differences in ^{13}C isotope content do not correlate with elevational gradient. The lowest values of ^{13}C content noticed in the highest analysed site indicate that there are the most unfavourable conditions for plants.

The findings of this research provide valuable information on how a narrow range of elevational gradients may affect alpine plants in the Tatra mountains.

Comparative biogeography and population dynamics of indicator species for riverine habitats – the case of predacious beetles of the Carpathians

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The riverine habitats become threatened in Europe due to human transformation, but rivers still have the potential for natural recovery due to flow dynamics, particularly in the mountains. Species inhabiting riverine habitats are evolutionary adapted and ecologically tolerant to inundations and floods. These phenomena make the distribution and diversity of riverine species variable across space and time. Some riverine species are known as indicators of river habitats' naturalness like predacious beetles Carabidae and Staphylinidae being typical taxa for gravel and cobblestone alluvias. Here we examined phylogeography, niche modelling and population dynamics of the beetle community (four ground beetles of genus *Bembidion*, three rove beetles of genera *Paederidus* and *Paederus*) in order to understand their evolutionary history, contemporary diversity and distribution in the Carpathians. Genetic data revealed complex patterns of their diversity.

There is no clear spatial-genetic division of populations, with many haplotypes being unique to some river systems, which support isolation within drainages, but others found in distant populations indicate rare long-distance migration events. Niche modelling showed congruence of suitable areas for all examined riverine beetles. During the last glacial period, poorly suitable areas were identified only in the Southern Carpathians and the Apuseni Mts. Next, beetles spread across the whole Carpathians finding the most suitable areas in the outer foothill zones. The predicted distribution for 2070 indicates altitudinal shift to higher elevations. There is no clear concordance in yearly population dynamics between species and inundation proved to be a major driver of beetle turnover.

Altogether, the information of this study showed very complex patterns of riverine beetles' diversity and distribution. This study should help in better understanding of meta-population functioning of these indicator species in order for proper planning of river nature conservation and management.

Mitochondrial DNA phylogeography of *Palingenia longicauda* (Olivier, 1791) (Insecta, Ephemeroptera) and its consequent taxonomic implications

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Once widespread across Europe, *Palingenia longicauda* (Olivier, 1791), an iconic species of pristine rivers, has lost almost 98% of its original range, but conservation efforts are greatly hampered by its similar morphology with the sympatric *P. fuliginosa* Georgi, 1902. Based on large sampling of *Palingenia* between 2018 and 2020, 11 different populations, including 142 males were analyzed. Type material of Soldán was investigated as well.

PCA analysis based on penis morphology and eye distances of males detected no significant difference between populations. The closely related sibling species had highly similar morphologies, making the presence of more than one species in Europe questionable. Mitochondrial COI DNA data of 32 mtCOI barcode sequences showed genetically very similar populations, thus confirming the presence of only one species, *P. longicauda*, in Southeastern Europe. Furthermore, we analyzed two additional mitochondrial markers (the mtCOI – different from the barcoding region and the 16S rRNA) of *P. longicauda* individuals from Romanian, Ukrainian and Hungarian populations, and discovered a large number of endemic haplotypes confined to the Danube Delta or the Prut River. The studied populations showed statistically significant differences suggesting independent histories and confirming the survival and recovery of *P. longicauda* populations in several SE European refugia.

The unexpected recovery of the species in recent years should be related to the hydro-morphological conservation of the examined river sections, with near-natural steep clay banks, under the protection of local nature parks, where human activity is limited or absent.

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Development of high diversity beech forest in the Eastern Carpathians

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Over the past decades, a surge in the number of large and uncontrolled wildfires has occurred on all terrestrial ecosystems and global warming may amplify this trend and threaten most ecosystems worldwide for the next decades. These alterations of fire regimes will affect fire-prone systems but also forest ecosystems that have not historically experienced fires, such as the European beech forests. These deciduous forests are characterized by a high plant diversity, and understanding their long-term dynamics is crucial to anticipate changes in these ecosystems in the on-going global warming. The aim of this study is to understand how European beech forests have colonized inner Eastern Carpathians (Slovakia) and what are the main factors explaining their high biodiversity. A multi-proxy approach (charcoals, pollen, macrofossils) has been applied to investigate the development of beech forest and the variation in palynological richness, evenness and turnover has been analyzed.

Low diversity spruce forest was dominant until 5200 cal. BP during a fire prone period mainly due to climatic conditions. The establishment of late-successional, shade tolerant *Fagus sylvatica* was facilitated by fire disturbances, but its expansion coincided with major gaps in fire events from 3900 cal. BP. The palynological richness has increased during the transition from spruce to beech forest highlighting the importance of beech forests in maintaining plant biodiversity. However, the stronger increase of the richness is synchronous with the increase in human activities around 2000 cal. BP, and then 350 cal. BP.

Low frequency fires have been a natural driver of vegetation changes in the Carpathians by promoting the emergence of high diversified beech forest. However, human impact has later also shaped these landscapes.

In the footsteps of Jávorka: systematics and evolution of *Saussurea alpina* (Asteraceae) in the European Alpine System with a focus on the Carpathians

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In the European Alpine System, *Saussurea* is represented by four species, i.e. *S. alpina* (4x), *S. discolor* (2x), *S. porcii* (2x) and *S. pygmaea* (4x). Whereas the taxonomy of *S. discolor*, *S. porcii* and *S. pygmaea* is undisputed, the mostly acidophilic *S. alpina* is a polymorphic species consequently burdened with taxonomic uncertainties. In *S. alpina* three subspecies are currently accepted: subsp. *alpina* (widespread), subsp. *depressa* (the Western Alps), and subsp. *macrophylla* (the Eastern Alps and the Carpathians), the latter described from the Kitzbüheler Alpen (Austria). Additionally, Jávorka (1932) distinguished the Carpathian endemic *S. alpina* subsp. *borbasii* described from the Belianske Tatras (Slovakia), based on leaf morphology and indumentum. This taxon is usually not accepted in literature and has either been treated as *S. alpina* or *S. discolor*. Addressing the taxonomic position of those Alpine and Carpathian *Saussurea* taxa requires clarification of their phylogenetic and phylogeographical relationships. To answer these questions, we obtained nuclear ITS sequences from newly sampled populations and herbarium vouchers. Three RADseq (Restriction Site Associated DNA) libraries were constructed and analysed de novo. DNA ploidy levels and genome sizes were estimated with flow cytometry, accompanied by chromosome counts. The Sanger sequencing data show that ITS is not suitable for inferring a backbone phylogeny, but very useful for barcoding. On the contrary, RADseq sheds light on the systematics and evolution of *S. alpina*. According to these results, *S. alpina* subsp. *depressa* and *S. alpina* subsp. *borbasii* (4x!) are supported, whereas the latter is of hybridogenous origin; *S. alpina* subsp. *macrophylla* is not supported. Therefore, the taxonomy and distribution of Carpathian *S. alpina* and *S. discolor* must be completely revised.

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Diversity and ecology of the *Gammarus fossarum* species complex (Crustacea: Amphipoda) in the Western Carpathian contact zone of divergent lineages

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Discoveries of substantial cryptic diversity in various aquatic invertebrate groups bring new insights into biogeographic patterns, and open questions about potential ecological or evolutionary interactions of cryptic species. Widespread morphospecies of gammarid amphipods, ecologically important benthic macroinvertebrates, often turn out to be diversified species complexes. We studied phylogenetic relationships, distribution, potential hybridization and habitat preferences of divergent lineages of the hyper-diverse *Gammarus fossarum* complex, which shows particularly high phylogenetic diversity in the Western Carpathians, in contrast to adjacent parts of the Bohemian Massif. In the contact zone of multiple *G. fossarum* lineages in eastern Czechia, we initially screened over 60 localities in streams to determine their regional distribution. Syntopy of two to three lineages was detected at almost half of the sites. Subsequently, nine syntopic localities were studied in detail for mesohabitat distribution (fast versus slow water flow), pairing preferences, and temporal stability of the *Gammarus* community

Although a significant fraction of regional spatial variation was explained by position in the dendritic river network, altitude, stream width, and anthropogenic stress, we did not find differences between mesohabitats with contrasting flow rate at the local scale. Mixed precopulatory pairs in syntopy were extremely rare, confirming a strong prezygotic reproductive barrier between studied lineages. This is congruent with the species delimitation analyses on molecular markers, which confirmed the absence of recent gene flow. We may conclude that the studied *G. fossarum* lineages are distinct biological species, which should be characterized both ecologically and morphologically. Their frequent and apparently temporally stable syntopy warrant further research on processes that may facilitate their coexistence.

A prominent regional and local vegetation response to the Older Dryas short-term climatic oscillation in fen deposits of Beskid Makowski Mts. (the Western Carpathians, Poland)

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A short-term climatic oscillation of the Older Dryas Stadial is often problematic in term of distinguishing its imprint in regional vegetation changes and sedimentary records. Study sites with the Older Dryas sedimentary sequences in lacustrine and peatland deposits are relatively rare in the mountainous areas of Europe. They were reported in the Swiss Plateau, the Romanian Carpathians, and the Eifel Mts.

In the territory of Poland, palynological studies of landslide fen deposits of the Klaklowo (472 m a.s.l.) and Kotoń (730 m a.s.l.) landslides, located in the mid-altitude mountain range of the Beskid Makowski Mts. (the Western Carpathians), revealed the exceptionally thick (ca 0.5 m) sequence of mineral deposits attributed to the Older Dryas chronozone. In case of the Klaklowo mire, the currently conducted plant macrofossil analysis highlights some further aspects of local and regional vegetation history. Judging by the abundant macrofossils of aquatic plants (Characeae, *Batrachium* sp., *Potamogeton* sp., *Myriophyllum verticillatum* and *Hippuris vulgaris*), on the course of the Older Dryas Stadial, the Klaklowo basin was filled with water which may have originated from climate moistening and/or permafrost melting. Moreover, climate change of that time is expressed by intensive delivery of mineral matter to the basin at the accumulation rate of 3 mm/year. Shallowing of the water reservoir caused gradual overgrowing with sedges, while *Betula nana*, *Betula pubescens*, and *Larix decidua* started to appear in the vicinity. From the surrounding area some seeds and fruits of heliophilous herbs (Asteraceae, Poaceae, *Dryas octopetala*) were transported to the basin. The identified plant macrofossil taxa along with interpreted climatic implications may confirm the occurrence of shrub steppe-tundra conditions in the Beskid Makowski Mts. during the Older Dryas Stadial.

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First insight into invertebrate diversity of the Carpathian epikarst: an integrative approach

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The uppermost part of the karst landscape, lying at the interface between soil and karstified rocks, is referred to as epikarst. It comprises a complex of fractures retaining water coming from precipitation. Epikarst water drifts therein occurring minute organisms while draining downwards through the adjacent infiltration fissure zone up to underlying cave corridors.

Between 2019 and 2020, we sampled epikarst invertebrates using 27 filtering devices from water permanently dripping into four caves of the Demänová Cave System in northern Slovakia. To evaluate the invertebrate diversity in this understudied subterranean environment in Slovakia, we employed an integrative approach combining both conventional morphological and molecular tools – DNA barcoding. The standard barcoding marker i.e., approximately 650bp long fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene, was amplified, sequenced, and compared with the public sequences in the online Barcode of Life Data system (BOLD) repository.

Morphologically, we identified 26 invertebrate species (four of Copepoda, one of Syncarida, two of Amphipoda, nine of Collembola, and 11 of Diptera) including several subterranean forms such as copepod *Ellaphoidella phreatica*, syncarid *Bathynella* cf. *natans*, amphipod *Niphargus tatrensis*, collembolans *Megalothorax hipmani* and *Megalothorax tatrensis*. So far, we have obtained a total of 744 COI barcode sequences of invertebrates divided into 34 molecular operational taxonomic units (MOTUs, equivalent to species). Copepods displayed a high number of cryptic diversity (8 MOTUs out of 4 morphospecies), while the other taxa each represented at least one putative cryptic species.

High genetic diversity recorded in some invertebrate taxa could indicate the existence of morphologically indistinguishable species which requires further analysis to be confirmed. Our results underline the importance of studying epikarst and its potential hidden diversity.

Biogeography and history of the extremely species-rich peri-Carpathian forest-steppe meadows

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Peri-Carpathian forest-steppe meadows are an outstanding biogeographical phenomenon, deserving active protection and focused research. They are the richest plant communities in the world at the scale of fractions to tens of square metres. They also contain numerous species of conservation concern from different taxonomic and ecological groups, including species with disjunct distribution ranges indicating specific history of these ecosystems.

In the last decade, we have been investigating the variability in their species composition, species richness and historical development on a Holocene time scale. In a broad multi-disciplinary team, we combine classical and less common methodological approaches of vegetation ecology, biogeography, paleoecology and soil science at dozens of sites throughout the peri-Carpathian area.

Our results showed that the main type of these grasslands is the phytosociological association *Brachypodio pinnati-Molinietum arundinaceae* described from the Bílé Karpaty Mts. (the Czech Republic), but distributed over a wide area between Brno, Vienna, Chernivtsi in western Ukraine and Buzau in Romania. Local hotspots of fine-scale species richness are situated in the Bílé Karpaty Mts., Prut-Siret interfluve (Ukraine) and Transylvanian Basin (Romania). The results of our paleoecological investigations so far suggest a great age of these ecosystems and Holocene continuity of open grasslands or open-canopy woodlands at least at some of their sites across the peri-Carpathian space. A major challenge now is to link off-site and on-site proxies of their historical development and to better understand the long-term dynamics of woody and herbaceous components of these ecosystems and their reflection in the soil properties.

Changes in species composition and diversity of a montane beetle community over the last millennium in the High Tatras, Slovakia: implications for conservation and management

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Temperate mountain forests are niche ecosystems with a high number of endemic species, which are under pressure by climate change and human activities. UNESCO nature reserves such as the Tatra National Park (TANAP) in Slovakia were established to preserve local wildlife and floristic biodiversity. In recent decades, windstorms and insect outbreaks have caused widespread forest disturbances resulting in the implementation of extensive forest management practices such as salvage logging to subside insect outbreaks in the TANAP. To predict future developments of disturbance regimes in central Europe, palaeoecological data can be utilized to create long-term records (e.g., changes in vegetation, fire history and insect community composition), which can be used as baseline to compare the more recent changes in temperate forest ecosystems.

Peat sediment cores were collected from a small forest hollow from the TANAP to make a quantitative reconstruction of the local community of beetles (Coleoptera), through time. From 19 sediment samples, 313 beetle taxa were identified from 38 different families which were living on and around the peat bog. The sub-fossil beetle record provided detailed information about how the forest insect community responded to changes in the landscape over the past 1000 years. The beetle record complemented the vegetation and fire history reconstructed from pollen and charcoal records, allowing comparison between local and regional changes. Based on a Shannon index, beetle diversity followed pollen diversity until ca. AD 1600, when the beetle diversity steadily starts declining. Additional information from historical records suggests that human activity in the region had a significant influence on this decline in beetle diversity.

Relevant for forest management of this region, this study highlights the importance of long-term records and the preservation of mountain peat bogs to preserve insect biodiversity.

The creative role of hybridization and introgression in the evolution of the European mountain genus *Soldanella* L. (Primulaceae)

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Hybridization is one of the pivotal mechanisms contributing to the processes of lineage diversification, particularly in ecosystems exposed to environmental fluctuations. Recently radiated plant species that have evolved in mountain environments influenced by past climate change serve as an excellent model system for examining how gene flow can affect diversification processes. We examined the effect of hybridization and introgression on the diversification and speciation of the European Alpine System-dwelling genus *Soldanella* (snowbells, Primulaceae) using organellar and nuclear genomic data combined with a cytogenetic technique. We further investigated whether Carpathian and Balkan species with large ecological amplitudes were more affected by hybridization than widespread exclusively alpine dwelling snowbell species. Finally, we tested the hypothesis that hybridization resulted in the speciation of *S. alpina* while causing the dissolution of species boundaries in several Carpathian taxa.

We found that extensive introgression occurred among the ancestral lineages of snowbells and persisted throughout the evolutionary history of the genus, regardless of the ecology or size of distribution area of the impacted species. The highest extent of introgression has been detected in the Carpathian species, which is also reflected in their extensive karyotype variation. *Soldanella alpina* has been implicated in numerous hybridization events, particularly with alpine-dwelling species, but its hybidogenous origin remains uncertain. Our findings highlight the creative role of hybridization and its evolutionary significance as both a facilitator of speciation and a barrier to the processes of diversification.

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What does endemism say about diversification of mountain biota?

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Understanding the relationships between species rarity and its evolutionary past and future is of great importance for both conservation planning and ecological theory. In European mountains, the relationship between local endemism and species diversification has been discussed since the times of de Candolle. It is typically assumed that concentration of range restricted species in certain mountain areas is an indication of slower extinction (paleoendemism in refugia) or faster speciation (neoendemism in cradles). These interpretations are however at odds with ecological theory. Macroecological theories, such as Hubbell's neutral model, often imply that diversification is driven by common species, and rare species are evolutionary dead ends with low probability of speciation and high probability of extinction.

Here we explore the relationship between range size and the rates of diversification (speciation and extinction) using a combination of global phylogenetic datasets and local phylogenomic data on European mountain plants (PhyloAlps).

We show that, globally, large-ranged species and lineages diversify faster, as predicted by the macroecological theory. This pattern is however partly reverted in mountains, where diversification is often driven by small-ranged species and lineages, confirming thus the intuition of mountain biogeographers about the hotspots of endemism. Our results stress the importance of protection of local endemic hotspots in European mountains. Mountain endemics are not only rare and thus potentially vulnerable, but often also have high cladogenetic potential, i.e. high probability to diversify in future.

Lighter and less structured primeval forests host more understory species

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Recently, nature conservation in the Carpathians has focused on old-grown and primary forests, which are believed to be important biodiversity hotspots. They are often recognized by complex structural characteristics such as tree size heterogeneity, presence of dead wood, dense and multilayered canopy, or occurrence of ancient trees. Nevertheless, the effect of these structural characteristics on the herb layer's richness and composition is still unclear. From the studies in managed or abandoned forests, it could be expected that more complex forest structure leads to higher diversity also in the herb layer. However, it is not clear whether such a relationship takes place also in primeval forests, where the structural complexity is not driven by the time since anthropogenic impacts. We have tested the effect of structural complexity, light conditions and canopy continuity on the herb vegetation composition and richness on 150 plots in primary beech forests across the Western Carpathians.

The overall species richness is declining with the decrease of available light in the understory, which can be expressed as tree and shrub cover, tree layer structural complexity, or continuity of canopy cover. Importantly, this decline is true also for the absolute richness of beech forest specialists, although their proportion to the other species is growing. Interestingly, we did not find any effect of canopy continuity or time since the last disturbance on the plot on the forest specialist's proportion.

Our results thus suggest that, in contrast to managed forests, the richest stands of primary forests are characterized by relatively low structural complexity and a lot of available light, and this is true also if we consider only specialist species of beech forests. The positive relationship between structural complexity and herb richness in managed forests may thus reflect particular development towards more natural states, rather than general ecological rule.

Comparative phylogeography of two *Arabidopsis* species in the Carpathians

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The biogeography of Europe was influenced mainly by Pleistocene climatic oscillations and the traces of the evolutionary history of plant species can be inferred from the genetic structure of their populations. Important insights into the spatio-temporal evolution of flora and fauna of the European mountains (especially the Alps) have been provided using (comparative) phylogeography, but we still lack information on plants inhabiting the area below the timberline and/or plants with a wider elevation span, especially from the Carpathians. In our contribution, we will present new findings from the reconstruction of the evolutionary history of two *Arabidopsis* species (Brassicaceae), which have a centre of their diversity in the Carpathians, mainly in the lower (below timberline) mountain ranges, but in several areas, they also reach the subalpine positions.

Investigations on the evolutionary history of *Arabidopsis arenosa* and *A. halleri* populations by means of multilocus markers (AFLP or SNPs) revealed surprising parallels in the evolution of both species. In particular, the results confirmed the presence of a main genetic barrier situated between the Eastern and Western Carpathians, the barrier which has already been observed in several alpine and subalpine species. Our results, however, point also to the repeated evolution of alpine morphotypes from geographically close foothill populations. In the case of *A. arenosa*, the Carpathians are not only a refugium of a rare genetic diversity but also a source of the populations that recolonized the northern parts of Europe.

Morphometric evidence does not support the differentiation of the Carpathian endemic taxon *Eritrichium nanum* subsp. *jankae* from *Eritrichium nanum* subsp. *nanum*

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Eritrichium nanum (L.) Gaudin subsp. *nanum* (EN) is a European endemic taxon, emblematic of the high-alpine ecosystems of the Alps and the South-eastern Carpathians. Within the Carpathians another taxon endemic for this range was described: *E. nanum* subsp. *jankae* (Simonk) Jáv. (EJ). The discriminant characters between the two infrataxa are mostly quantitative, based on minute differences in indumentum, calyx length, stem height and leaves width. This light morphological differentiation leads to equivocal chorology for these subspecies in the Carpathians, often being reported both infrataxa from the same massifs, in identical habitats. The aim of the present study was the validation of stable phenotypic patterns within the Carpathian *Eritrichium nanum* s.l. populations, resulting in an unquestionable identification.

We performed a morphometric analysis of five random selected individuals from 24 populations sampled throughout the Carpathian distribution range, in addition to 15 Alpine populations. We measured and analysed thirteen anatomical traits including the ones based on which EJ was described (mentioned above). We also searched for correlations between the traits and ecological factors (e.g. elevation, climatic water deficit and growing degree days).

Though most of the Carpathian populations exhibited a larger number of trichomes and a higher length of the floral stem comparative to the Alpine populations, none of the analysed traits presented any consistent phenotypic pattern between the populations prior identified as EN or EJ. The indumentum revealed the sole positive correlation with the environmental factors, respectively with growing degree days, suggesting that this trait merely reflects an adaptation to the microclimate conditions. In conclusion, surprisingly, the differentiation of EJ was not supported by the morphometric analyses, but it cannot be entirely ruled out in absence of genetic studies, even if our preliminary genotyping and sequencing data seem to point out the same hypothesis.

Palynological record of the Grofetskij stav, Transcarpathia, Ukraine

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The Grofetsky stav is situated in the Transcarpathian Mountains, in the protected Grofa landscape area (1100 m a.s.l), in the mountainous part of Ukraine. Nowadays, this region is covered by beech forests, while spruce and fir are less represented. Samples were taken from a drilled core 245 cm deep. The pollen dataset was analyzed in a 2 cm interval and records mountain forest vegetation at the age of about 5000 years.

Peat accumulation ranged from 64 years / 1cm^3 in the lower part of the profile to 110 years / 1cm^3 in the middle part, when the peat grew more slowly, so a large amount of pollen was stored in 1cm^3 for a longer time span. According to the pollen concentration, the original coniferous forests with the dominant spruce turned into the dominant beeches about 4000 years ago. In the Middle Ages, fir penetrated beech forests, probably as a result of human activity. Anthropogenic indicators, weeds and crops, have been recorded since about the 14th century, when, also based on literature sources, the territory was colonized according to Wallachian law. Various non-pollen objects and microscopic charcoal document local development on the peat bog.

Population-level functional diversity in peripheral and ecologically marginal situations: insights from the alpine sedge *Carex curvula*

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Alpine species offer great opportunities to discuss functional biogeography hypothesis. In this research, we focused on *Carex curvula*, a dominant alpine sedge in the Alps, Carpathians, Pyrenees and in some Balkan mountain ranges. In comparison to the central part of its range (the Alps and the Carpathians) where *C. curvula* forms alpine grasslands, at its southernmost range limits the Mediterranean climatic influence leads to drier summers, therefore the species appears in more diffused and scarce communities. Thus, we hypothesised that these peripheral zones constitute marginal conditions for *C. curvula*, occupied by populations that are characterized by particular functional trait values. We measured plant size (height) and leaf traits (including anatomical traits) that link to nutrient economy and drought resistance. Using population trait means, we did trait-based hierarchical clustering to find emergent functional groups of populations showing related suites of trait values.

The resulting three functional groups displayed a geographical structure. The southernmost populations were grouped in two clusters (Pyrenees and Balkans) that had particular suites of conservative trait values associated with drought resistance. In contrast, the phenotypic coordination across populations was unconnected to marginality. Moreover, within-population intraspecific trait variability (ITV) was not lower, as expected, in the groups of marginal populations that were genetically depauperate (i.e., poor neutral genetic diversity) due to the legacy of Quaternary history of this alpine sedge.

Our research at biogeographical scale reveals that ecological marginality is depicted in the functional structure of populations and to some extent mirrored in the genetic structure at range margins, in contradiction to within-population ITV.

Posters

Conservation genetics of the Eastern Carpathian endemic *Saussurea porcii* (Asteraceae)

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A good knowledge of range-restricted species is important for conservation biologists, because accurate information is necessary for prioritizing conservation efforts. The Carpathian Mountain range is the second longest mountain range in Europe and hosts one third of all plant species in Europe, of which 5% are endemic. As the Carpathians were largely unglaciated in Last Glacial Maximum and due to their microclimates and different geological substrates, levels of biodiversity, rarity and endemism are high in this mountain range. The perennial *Saussurea porcii*, a glacial relict related to the Siberian *S. parviflora*, is a rare, critically endangered endemic of the Eastern Carpathians in Romania and Ukraine. It occurs in a few rather small and isolated populations in wet calciferous mountain grasslands, fen meadows and edges of rivulets between 1300 and 1800 m a.s.l.. To characterize these populations with respect to genetic diversity, we generated SNP markers via RAD sequencing from 68 individuals from the five still extant populations. Genetic differentiation among and between populations, genetic clustering of individuals, F-statistics, allelic richness, degree of clonality will be presented. For the first time in this species, the chromosome number was determined and the absolute genome size measured. This conservation genetic study will allow better conservation guidelines for this species to be devised.

Variation and correlation of leaf anatomical traits: preliminary results in the alpine sedge *Carex curvula* across the European Alpine System

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Due to the spatial isolation of mountain ranges, habitat fragmentation and sharp topoclimatic gradients over short distances, the temperate mountains of the European Alpine System (EAS) offer great opportunities to investigate intraspecific trait variability (ITV) in widely distributed species. We focused on *Carex curvula* (Cyperaceae), a graminoid species dominating the late-successional alpine communities of the EAS. Specifically, we aimed to uncover the sources of variation and better understand the relationship among intraspecific leaf anatomical traits, in relation to plant height.

We sampled five random individuals from 38 populations distributed across the EAS. Fourteen anatomical traits were analysed based on standardized images of leaf cross-sections performed through the middle part of the lamina. We manually marked specific areas from the leaf cross-sections (e.g., whole area, the bulliform cells) and automatically calculated the number of pixels. We counted the number of stomata and measured the heights of cuticula and of epidermal cells on both abaxial and adaxial sides of lamina. We tested how much of the ITV was due to within- versus between-population variation, and examined the correlation between traits.

All analysed traits showed large within-population variability and a much lower amount of between-population variability. Traits such as the total leaf cross-sectional area and surface of sclerenchyma showed positive allometric relationships with the plant height. In contrast, traits that were normalized to the total leaf cross-sectional area were less coordinated. We showed that while not normalized leaf anatomical traits are allometrically related, normalized traits could show different dimensions of ITV. While we are currently investigating the specific functional roles of some individual traits of *C. curvula*, particularly their implication in drought adaptation, our methodological framework opens new avenues to explore anatomic ITV at large scales.

Threatened species of Lycopodiaceae in the Ukrainian Carpathians: distribution and spatial patterns

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There are six threatened species of *Lycopodiaceae* (sensu Pterydophyte Phylogeny Group I, 2016) within the Carpathians in Ukraine: *Diphasiastrum alpinum* (L.) Holub, *D. complanatum* (L.) Holub, *D. issleri* (Rouy) Holub, *Lycopodiella inundata* (L.) Holub, *Huperzia selago* (L.) Bernh. ex Schrank et Mart. and *Lycopodium annotinum* L. The two last species are quite common throughout the region. *L. annotinum* prefers wet conifer forests in the montane belt of the highest mountain ranges, but also could rarely be found in the subalpine belt among *Vaccinium* heaths or rock fields. *Huperzia selago* is distributed less frequently but occupies more different habitats: from beech forests of the lower montane belt up to rock fields of the alpine belt. In general, it tends to occur in higher elevations and less competitive phytocoenoses. *Diphasiastrum alpinum* has a much narrower ecological niche and is found predominantly on hilltops among *Vaccinium* heaths or rock fields and outcrops of subalpine and alpine belts of most mountain ridges. Although it has fewer known localities, it is abundant within suitable habitats. Localities of *D. complanatum* in the Ukrainian Carpathians are situated in the lower montane belt in beech forests. There are only six known localities and most of them were discovered in the previous century. The two last species, *D. issleri* and *Lycopodiella inundata* are known only from four localities each (Felbaba-Klushina, Votkalchuk, 2015). Most of them are outdated as well and need new research.

Due to the ecological preferences of considered species, most localities are concentrated in the south-eastern part of the Ukrainian Carpathians where there are the highest ridges within the region. A major number of localities are known from the Chornogora ridge as it is both one of the most floristically rich territories and historically popular for botanical investigations. The lower parts of the Ukrainian Carpathians are less studied and need more attention henceforth.

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Lobaria pulmonaria as an umbrella species for the protection of lichen diversity in the Polish Western Carpathians

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The use of indicators of natural forest habitats (old-growth forests, primeval forests) as stenotopic umbrella species for the protection of accompanying biodiversity is one of the concepts of nature protection in the world. This concept was implemented in Polish law for the protection of 11 species of lichenized fungi, including *Lobaria pulmonaria*, currently persisting in the Polish Western Carpathians at a few sites. The aim of the study was to verify the hypothesis on the rightness of the legal reduction of the protection circle zone for *L. pulmonaria* in Poland from R=100 m to R=50 m, in the context of the need to protect the accompanying lichen biota. A total of 234 beeches were investigated along three lines at azimuths 0°, 120° and 240°, in three concentric zones with radii of 50 m, 100 m and 150 m around 10 known sites of this species in the Gorce Mts. Only the corticolous lichen species diversity was estimated.

A total of 139 species were found, including 110, 90 and 105 respectively in zones R≤50 m, R=50–100 m, R=100–150 m and 110, 121 and 137 respectively in zones R≤50 m, R≤100 m, R≤150 m. In both cases of zoning, the differences in the number of species were not statistically significant, in contrast to the diversity between sites. Between 9 and 19 lichen species were found on trees inhabited by *L. pulmonaria* (14–36% of the whole investigated biota). In the R≤50 m zone, their contribution was 60–86%, and 77–98% in the R≤100 m zone. The values of the H Shannon index in all zones were similar, as were the values of J Pielou's equality index. The contribution of old-growth forest, endangered and legally protected lichen species in Poland in individual zones was also analyzed.

For more effective protection of lichen diversity under the *L. pulmonaria* umbrella and preservation of the natural features of the habitat, it would be reasonable to apply in the Polish Western Carpathians the protection of this species in the zone with a radius of 100 m.

Functional traits variation in the Carpathian endemic *Campanula serrata* along altitudinal gradients

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Plants' traits variation is the outcome of adaptive ecological processes and is often used to evaluate ecosystem functioning. Trait variation can arise from either biotic interactions or in response to differing environmental conditions. Mountainous regions offer a great opportunity to study the effect of ecological conditions on plants by analysing trait variation along environmental gradients determined by altitude. Here, we studied intraspecific trait variation in *Campanula serrata* to understand how differences in altitude and associated ecological specificity may trigger different functional responses of the plant.

We measured traits referring to: i) leaves (SLA, leaf width and length, stomatal density); ii) plant size (height, number of leaves, number of tillers); and iii) flowers (flower width and length, calyx length, number of flowers). Specimens were sampled from eight populations along altitudinal gradients in three mountains of the Southern Carpathians (Romania). To address the relations between local site conditions and trait variation, we used linear mixed models, with altitude and a set of abiotic variables (soil parameters, mean annual temperature and solar radiation) as predictors.

We found plant size and number of flowers to decrease with altitude while the size of flowers increased. No overall trend was found for leaf traits. Soil fertility was positively related to many of the measured traits. Conversely, temperature and solar radiation had a weaker and sometimes contrasting effect. We interpreted the reduction of plant size with increasing altitude as a strategy to cope with the harsher abiotic condition, while the increase of flower size is likely to be an adaptation to pollinators' characteristics in high altitude environments. The lack of a clear pattern for leaf traits was probably caused by the complexity of drivers occurring along altitudinal gradients, as shown by multiple studies carried out in other mountainous regions from the temperate world.

Lichens of the Carpathians

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Mountains are species-rich systems. Diverse topography, microclimate, geology, habitat heterogeneity or latitudinal extent enhance biodiversity. Altitudinal range and high elevations also support the presence of unique species, including lichens. One of the largest European mountains – the Alps, belong to globally lichenologically best investigated areas. The lichen checklist of the Alps includes more than 3100 taxa. In contrast, there is a gap in knowledge of lichen diversity in other European orobioms, including the Carpathians. The first steps to fill this gap were the lists of the two subunits – the Eastern (2003) and the Western Carpathians (2004). Further important contributions represent monographs on the lichens of the Tatry Mts (2005), and national or regional checklists (Slovakia 2013; Polish Carpathians 2020; Ukraine 2021; Romania 2020). The Carpathians, rich in geodiversity, unlike the Alps, feature high afforestation, high representation of primeval beech, spruce and fir forests. Lichen diversity richness is affected by meso-climatic, geomorphological and vegetation cover differences between the Western, the Eastern and the Southern Carpathians. Higher precipitation in the Eastern Carpathians support surprising occurrences of oceanic epiphytes (e.g. *Pyxine sorediata*, *Thelotrema suecicum*, *Wadeana dendrographa*). Contrary to vascular plants, no endemic lichens are in the Carpathians. However, the majority of known localities of some rare species occur here, e. g. *Belonia herculina*, *Ramalina carpatica*, *Thelopsis lojkana*. In relatively small territory we can find Mediterranean and boreal elements, sometimes within different aspects of the same mountain. Interesting records of biogeographically significant elements include e.g. *Glypholecia scabra*, *Leptogium hildenbrandii* or *Nephroma arcticum*. In 2017 we established an informal regional working group. Our long-term aims are to summarize verified published data on lichen diversity across the Carpathians and to elaborate a comprehensive overview of the Carpathian lichen diversity.

Collembola endemism centres and biodiversity hotspots in the Romanian Carpathians Mountains

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The Carpathians are one of the key biodiversity hotspots in Europe, containing a high amount of natural and cultural heritage. Areas of endemism are conservation priorities and indicators of biogeographical processes, representing concentrations of range-restricted biodiversity.

In the present study, we analyzed species distribution patterns based on a dataset with 8,226 records for 350 species of invertebrates (Collembola) to identify hotspots using two criteria: i) species richness (SR); ii) weighted and corrected weighted endemism (WE and CWE). We also provided a first evaluation of the conservation status of biodiversity hotspots based on these criteria considering the protected areas covered by Natura 2000.

Analyses were made in ArcGIS 10.7.1. In order to perform the optimized hotspot analysis, we created a 2 km square grid covering the entire Carpathian Mountains in Romania similar to the standard grid used by IUCN for calculating area of occupancy (AOO). Species richness was calculated on a 5 km square grid for the Carpathians, created based on the clustering distance from the hot spot analysis, and also individually for each physio-geographic unit. Weighted endemicity (WE) and corrected weighted endemicity (CWE) calculations were also performed using the 5 km square grid and ENMTools, available as an add-on for ArcGIS.

The richest mountains in Collembola species are the Retezat, Giumalău, Rarău, Călimani, Rodnei and Bucegi Mountains. The most important hotspots (considering endemism criterium) are located in the southwestern area of the Romanian Carpathians. The Natura 2000 network can potentially constitute an important baseline for protecting invertebrate diversity in the Romanian Carpathians although further improvements are needed.

Sexual reproduction biology of the West Carpathian narrow endemic species *Daphne arbuscula* Čelak. (Thymelaeaceae)

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Reduced reproductive success of species with a restricted range may be one of the factors contributing to their limited dispersal abilities and, consequently, their threat. Our research is focused on the sexual reproduction of *Daphne arbuscula* (Thymelaeaceae), a narrow endemic species that inhabits rocky limestone habitats in the Muránska Planina Mts. of the Western Carpathians. We investigated various aspects of its sexual reproduction, such as flower and fruit production, pollen viability, seed viability, and germination success.

Self-compatibility tests indicated strict allogamy in this species, and high pollen viability was observed (more than 95%). Fruit production varied between 10% and 50% depending on the season, individual and population level. The germination tests revealed moderate to high seed viability, ranging from 58 to 94%. However, this endemic has a low germination rate, ranging from 11 to 63%. In order to break physiological dormancy and allow the majority of *D. arbuscula* seeds to germinate, long-term cold stratification is required. At both the population and individual levels, the relationship between genetic diversity and fruit production was analyzed and discussed. Sexual reproduction of this endemic species is obviously not optimal, which in consequence might lead to shifts to asexual reproduction, especially to clonal growth. We hypothesize that this behaviour might be associated with extreme environmental conditions in the localities where it occurs. More research is needed to find out how sexual reproduction affects the fitness and survival of this Western Carpathians flag endemic.

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Molecular study and MaxEnt modelling approach revealed continuous distribution of Swiss stone pine extending towards East

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The history of forests and the process of formation of the current vegetation has always been in the focus of the scientific community. However, the rapid climate change that is taking place today calls for urgent action and the preservation of forest ecosystems requires understanding and knowledge of historical processes.

The European *Pinus* species underwent a significant rearrangement during the Holocene vegetation development, and the range of the species changed greatly compared to their Pleistocene status. Population sizes of the species have decreased, and the species' ranges have become highly disjunct. Climate warming restricted most *Pinus* populations into ecologically marginal positions, and this was due also to the spread of broadleaf tree species. Scots pine (*Pinus sylvestris* L.) colonized both extremes of its ecological tolerance and survived in edaphically determined extreme habitats. Dwarf pine (*Pinus mugo* Turra) and Swiss stone pine (*Pinus cembra* L.) were forced to survive on the higher elevations, occupying the timber line and the subalpine region. Although historical reconstruction of the species' distribution were highly altered by the human intervention and afforestation works, and so our molecular studies detected in several sites allochthonous plant material, the molecular diversity and the most important genetic lineages in natural *Pinus* populations of the Carpathians could have been revealed. Our earlier studies based on microsatellite markers in the case of Scots pine in the Carpathians and the Pannonian region revealed two genetic lineages and the historical reconstruction was also tested by MaxEnt species' distribution modelling. In the case of *P. cembra*, results based on microsatellites markedly separated the Carpathian populations from the Alps but MaxEnt climate based reconstruction approach detected a significant widening of the species' potential distribution area in the LGM towards East, modelling a single genetic lineage and continuous distribution area during the Pleistocene.

Glacial relict stone pine (*Pinus cembra*) as the "sentinel of change" in the Tatras cliff forests

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Although we are still far from the Holocene climate optimum, further progress towards higher temperatures and less precipitation is foreseen. This raises questions about forest dynamics and species distributions. The high-elevation cliff forests with glacial relict stone pine (*Pinus cembra*) growing in the Alps and the Carpathians are valuable types of ecosystems, where trees are exposed to multiple site-specific environmental factors.

We used a dendroecological approach based on tree-ring to determine how temperature and precipitation, as well as disturbance regimes, influence the radial growth of stone pine in interannual and intra-annual scales over the last century. The 449-year long chronology was constructed based on 104 tree-ring series from stone pine growing on the cliffs of the Western Carpathians (Tatras). The quality of tree-ring measurements was verified with COFECHA, the climatic analyses were done using DendroClim2002 and the disturbance regime was detected with the TRADER package in R software.

Mean annual temperature in the Tatras has risen over the last 100 years (+2.0°C), which is lower than that observed in the Alps (+2.5°C). We showed that a pattern of climate/growth relationship of stone pine has been changing over the last century, and is strongly related to summer temperature over the whole studied period. In the last decades, the negative influence of summer precipitation on tree growth has abated which is contrary to observation from some parts of the Alps. The temperature of the previous autumn, winter, and early spring had a significant influence on stone pine growth in the past, but nowadays it is not an important factor. Numerous releases in tree rings show a small-scale disturbance pattern, which is responsible for the gradual mortality of trees in cliffs.

The understanding of the climate/growth relationship of trees in extreme environments (i.e., above timberline) may give guidance to practitioners about the proper management of such ecologically valuable areas.

Dryas octopetala and its ectomycorrhizal communities — How changing environmental conditions may influence them in the future?

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Dryas octopetala L. is an important element of the Carpathian arctic-alpine flora. Its habitats are expected to decline in the near future due to global warming. Small size and island-like distribution makes Western Carpathian populations of this plant especially vulnerable. *Dryas octopetala* forms symbiosis with ectomycorrhizal fungi (EcMF), which are known to play a major role in host plant resistance to environmental stress. Understanding of *D. octopetala* EcMF communities dynamics is crucial in the assessment of plant vulnerability to environmental changes. Continuing earlier study of *D. octopetala* EcMF communities in the Western Carpathians, we now looked for specific environmental factors influencing their richness, taxonomic structure and composition. To achieve this, assemblages of EcMF associated with this plant were compared in a setup of 6 localities at the environmental gradient (alpine to montane zone), and correlated with environmental data. EcM root tips were morphotyped and identified based on rDNA sequences.

We revealed high species richness of all analysed communities. No significant correlation between communities' richness and environmental conditions was found. Species composition of the communities in different localities poorly overlapped, which may suggest low specificity in plant-fungal relations. Despite this, the lack or very low abundance of EcMF of key arctic-alpine genera *Russula* and *Lactarius* indicate, however, some degree of selectiveness of this symbiosis. *Dryas octopetala* root system was colonized mainly by ecological generalists (e.g. *Cenococcum geophilum*). Some correlations of taxa abundance and environmental factors were found.

Our results suggest that *D. octopetala* has the ability of involving various symbionts from the local pools to suit its needs in different habitats. The lack of specialists among plant's fungal symbionts and high species richness and diversity along environmental gradients seem to favour its adaptive potential.

Effect of the wildlife supplementary feeding on alien vascular plants spreading: evidence from the Western Carpathians

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Ecological relationships between supplementary feeding and baiting in situ and vegetation patterns were studied in the Western Carpathians to test the role of wildlife management practices in alien plants spreading. Presence of vascular plant species was recorded at 82 localities with diverse hunting facilities stretched throughout Slovakia.

Altogether 208 taxa (144 native and 64 aliens) were found. Among aliens, 44 archaeophytes and 20 neophytes were recognized, including eight invasive species (*Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Bidens frondosa*, *Conyza canadensis*, *Echinochloa crus-galli*, *Galinsoga urticifolia*, *Helianthus tuberosus*, *Stenactis annua*). Vertical maxima of the distribution range in the Western Carpathians were found for *Abutilon theophrastii*, *Amaranthus powellii*, *Datura stramonium*, *Echinochloa crus-galli*, and *Iva xanthiifolia* (1097 m a. s. l.). In addition, 33 phytosociological relevés were sampled to cover main vegetation types using methods of the Zürich-Montpelière approach. Six plant communities and one group of stands with weak phytosociological relations were identified. Plant species diversity and relationships between particular hunting facilities, supplementary feed and bait presence and composition versus species assembling of plant stands were analysed using CCA analyses and GLM. Bait site, elevation, coarse fodder (hay), grain feed and fleshy feed were identified as the most important predictors. The proportion of aliens increased with the occurrence of bait sites and with the use of grain feed and corn silage. The neophyte frequency was positively associated with the presence of high shooting stands and crushed corn.

Concerning risk of alien and/or invasive plant species spreading, wildlife supplementary feeding, baiting and whatever fodder application (except hay of local provenience) seem to be undesirable, especially in natural ecosystems and strictly protected areas.

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Molecular markers and ecological niche modeling uncover migration routes of the Carpathian endemic round leaved ox-eye daisy (*Leucanthemum rotundifolium*)

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The Carpathian Mts. are located in central Europe and are important as a biodiversity hotspot and as a link between mountain ranges in other parts of the continent. Their endemic flora consists of ca. 420 plant taxa. A small but interesting part of that number are subendemics (ca. 39 species) – taxa which typically occur in one to a few isolated populations outside the Carpathians. Here we study one of them, round-leaved oxeye daisy [*Leucanthemum rotundifolium* (Willd.) DC.] – a Carpathian subendemic plant linked to montane and subalpine climatic belts distributed throughout the whole Carpathian Mts and in one isolated stand in Dinaric Alps in the Balkans. Using a range-wide sample collection, we examined the phylogeographic patterns within this species to gain new information about its origin and evolutionary history. We applied molecular methods (sequencing of cpDNA markers and genome-wide SNPs) and ecological niche modeling. We also examined links with other species through phylogeny and documented a possible ongoing hybridization with sympatric alpine and lowland species [*L. gaudinii* Dalla Torre (2x) and *L. ircutianum* DC. (4x)]. We hope that this study will also contribute data to analyze more general patterns in the Carpathian region.

Relationship between recent and Holocene mollusc species diversity of the Veľká Fatra Mts.

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Quaternary molluscs assemblages are one of the most important sources of information used for paleoecology and biogeography reconstructions, yet little attention has been paid to the informative value of quaternary profiles on a large spatial scale. In the area of the Veľká Fatra Mts., large amount of existing records concerning both recent and quaternary land snails enable us to compare Holocene and recent species diversity in order to estimate how many profiles are needed to evaluate species richness of such geomorphologicaly diverse area. In this study, available quaternary data are supplemented with results of a recent land snails survey.

All seven quaternary profiles from the Veľká Fatra Mountains covered more than 85 % of recent species diversity, while only about 50 % of recent land snail species were recorded in a single profile, thus highlighting the importance of multiple profiles evaluation in paleoecological studies.

Trait evolution in the genus *Soldanella* L. (Primulaceae): true parallel evolution or the result of historical hybridization?

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High mountain ranges serve as an excellent natural laboratory for studying plant evolution phenomena such as adaptation to extreme environments and/or trait parallel evolution. Hybridization, and especially adaptive introgression, may, however, result in the reappearance of phenotypes and ecotypes that closely mimic genuine evolutionary parallelisms. For decades, the mountain-dwelling genus *Soldanella* (Primulaceae) has been one of the model systems for the study of the diversification of mountain plants in the European Alpine system. The evolution of specific parallelisms among its members was assumed, but recent studies have revealed extensive hybridization throughout the genus' evolutionary history, indicating gene flow mediated biases in trait reconstruction. Thus, we study here the extent to which introgression affected ancestral state reconstruction of selected intrinsic and extrinsic traits in *Soldanella* phylogeny.

Evolutionary relationships within the genus were found to be strongly influenced by gene flow, which also had a substantial effect on resulting trait reconstructions. To distinguish between the emergence of genuine parallelism and traits that were introgressed from relatives, we excluded taxa that were significantly affected by introgression from the phylogeny. Ancestral state reconstruction on reduced phylogeny indicates that traits such as the evolution of dysploid cytotypes, arose in parallel in several lineages. On the other hand, the supposed independent origins of traits, like dwarf phenotypes in alpine-dwelling taxa and colonization of arctic-alpine environments that had been inferred from all-species phylogeny, are artifacts of extensive historical introgression or/and represent ancestral polymorphisms that disappeared in some species.

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Biodiversity of Bieszczady Mountains' streams

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The biodiversity of aquatic insects in the streams of the Bieszczady National Park's buffer zone (BPN b.z.) has not been studied extensively yet. The Bieszczady Mountains are an attractive tourist destination, and thus its streams' fauna is potentially exposed for habitat loss and water pollution if appropriate steps will not be implemented. Here we present the project aimed for flowing waters' biodiversity inventory in the BPN b.z. DNA barcoding following morphological identification was used for cryptic diversity estimation and extending Polish barcode databases (PolBOL) in the Carpathians. The invertebrates and chemical parameters were collected from four dominant mesohabitats – stones, gravel, wood and detritus to estimate which one is most important for protection of benthic communities in the region. The final results will give us an insight into the state of the diversity of aquatic insects in the region, dispel discussion on the desirability of expanding the BPN b.z. to Bieszczady northern foothills and establishment of a Turnicki National Park.

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Morphological differentiation of *Rhododendron myrtifolium* Schott et Kotschy in the Carpathians

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Rhododendron myrtifolium occurs in the Carpathians and mountain systems of the Balkan Peninsula on siliceous substrates in the subalpine vegetation. Disjunctive geographical range raises the hypothesis that Eastern Carpathians populations vary in terms of the morphology of the leaves and fruits of the Southern Carpathians ones. Hence the aim of the study was the verification of this hypothesis.

The natural populations of *R. myrtifolium* were sampled in 11 localities, eight from the Eastern and three from the Southern Carpathians. Each population was represented by (22–)26–30 individuals well separated in the field and each individual by ten 2-year-old leaves taken from the central parts of well-insolated shoots. For one population from the Eastern Carpathians and one population from the Southern Carpathians of *R. myrtifolium*, one ripe and uninjured capsule was taken from each of the same individual. The materials were pressed and dried after sampling. In total, 3100 leaves from 311 individuals and 60 capsules were analysed and compared. Measurements were performed on the scanned images with the implementation of digiShape software. Differences between populations were specified analysing 14 characteristics of leaves and 12 characteristics of capsules. The multivariate statistical analyses: the analysis of variance, the analysis of discrimination, the principal component analysis, the agglomeration analysis according to the Ward method and the Student's test were performed, with the use of package STATISTICA 12.

The populations of *R. myrtifolium* from these two regions differ at statistically significant levels in leaf blade length and perimeter, leaf blade width at its quarter and at its half-length, stalk length and ratio of capsule valve length to stalk length. The analyses confirmed our hypothesis, that the geographic isolation between the Eastern Carpathians and Southern Carpathians was the most probable reason for morphological differentiation *R. myrtifolium.*

Fuzzy species borders of glacial survivalists in the Carpathian biodiversity hotspot revealed using a multimarker approach

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The Carpathians are one of the key biodiversity hotspots in Europe. The mountain chain uplifted during the Alpine orogenesis and is characterised by a complex geological history. Its current biodiversity was highly influenced by Pleistocene glaciations. The goal of the current study was to examine the phylogenetic and demographic history of *Gammarus balcanicus* species complex in the Carpathians using multiple markers as well as to delimit, using an integrative approach, and describe new species hidden so far under the name *G. balcanicus*.

Results showed that divergence of the studied lineages reaches back to the Miocene, which supports the hypothesis of their survival in multiple micro refugia. Moreover, the increase of their diversification rate in the Pleistocene suggests that glaciation was the driving force of their speciation. The climatic changes during and after the Pleistocene also played a major role in the demography of the local Carpathian lineages. Comparison of diversity patterns and phylogenetic relationships of both, the mitochondrial and nuclear markers, provide evidence of putative hybridisation and retention of ancient polymorphism (i.e., incomplete lineage sorting). The morphological examination supported the existence of two morphological types; one we describe as a *G. stasiuki* species nova and another we redescribe as *G. tatrensis* (S. Karaman, 1931).

Genetic diversity of the Carpathian populations of *Juniperus sabina* L. (Cupressaceae)

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Juniperus sabina L. has a very wide range but its sites are sparse and scattered over the mountain ranges. Based on genetic studies, two varieties of the species were distinguished. The presence of the diploid *J. sabina* var. *sabina* was reported so far from the Alps, the mountains of the Iberian Peninsula and of northern Turkey, Crimea, South-West Asia, and from the Western Carpathians (Pieniny). The tetraploid *J. sabina* var. *balkanensis*, hybrid of *J. sabina* and *J. thurifera*, was found in the mountains of the Balkans and in central and southern Italy. *Juniperus sabina* from the Eastern and Southern Carpathians has not been studied before.

We analysed *J. sabina* from the Southern (RO1 population) and Eastern (RO3) Carpathians and the Apuseni Mountains (RO2) in respect of three molecular marker systems: codominant nuclear microsatellites, haploid non-coding cpDNA fragments and dominant SilicoDArT markers. In each population, the ploidy level of the samples and genetic diversity were assessed. Next, genetic differentiation between populations as well as genetic and phylogenetic relationships between individuals were tested.

Microsatellite analysis revealed tetraploid *J. sabina* individuals in the RO1 and RO2 populations (= var. *balkanensis*), diploid genotypes were found in RO3 (= var. *sabina*). Two varieties were also distinct in the phylogenetic tree. Genetic diversity in particular populations was average, but pairwise genetic differentiation was significant. Significant differentiation of all populations was also confirmed by Bayesian clustering (optimal K = 3) and PCoA investigation.

The intra-species diversity of *J. sabina* fits well with biogeography of the Carpathians. The massif is indicated as the link between Asia and the mountains of Europe, which may explain the appearance of the typical *J. sabina* variety in the Eastern Carpathians. The variety '*balkanensis*' could arise in the Balkans in the time when *J. thurifera* occurred here and migrated to the Carpathians.

Mean annual temperature reconstruction in a *Fagus sylvatica* forest during the last 2,600 cal yr BP

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Understanding climatic variability and how it affects the long-term vegetation dynamics is essential to face the uncertainties of the current global change. For this, we performed a mean annual temperature reconstruction for the last 2,600 cal yr BP at Morské oko, a lake located in the Vihorlat Mountain, Inner Eastern Carpathian Mountains, Slovakia. This lake is surrounded by *Fagus sylvatica* forest, and the sediment, a total of 9 meters, is abundant, highly organic, and depleted in oxygen content. The proxy used for the temperature reconstruction is branched glycerol dialkyl glycerol tetraethers (brGDGTs), which are membrane lipids of some soil bacteria. Also, we used geochemistry data to assess changes in the catchment area and pollen analysis to reconstruct the vegetation dynamics.

There is considerable temperature stability in the lake and the range of the temperatures oscillates only about 2°C. The lake, which originated by a landslide, presents the greatest changes in erosion rates and climatic oscillations during its onset. There was a fast increase in the temperatures followed by several cooling episodes. In contrast, we found relatively climatic stability during the last millennia and more minor changes in the catchment area.

This study helps us to understand the forest dynamics and how they relate to temperature changes in this primaeval beech forest in the Vihorlat Mountains, proclaimed by UNESCO as a World Heritage Site.

Morphological differentiation of the Carpathian *Juniperus sabina* L. populations against the species variability

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Juniperus sabina L. is a mountain species with a wide range, from the Iberian Peninsula in the west to China and Mongolia in the east. Its highly dispersed range consists of small, separated populations, which suggests their refugial character and could be reflected in the morphological differentiation between distant sites.

On the basis of 18 features of cones, seeds and shoots, the morphological differentiation of 18 European isolated populations was determined. Three samples from the Carpathian Mountains in Romania (from the Southern Carpathians, the Apuseni Mountains and the Eastern Carpathians) and 15 others from the Alps (Italy, Switzerland, Austria), the Apennine Mountains, the mountains of the Balkan Peninsula (Bulgaria, Croatia, Greece, North Macedonia), the Iberian Peninsula (Spain), and mountains of western Turkey and Crimea, were measured biometrically and statistically analysed.

All the examined morphological features statistically significantly differentiated the populations. In the discrimination analysis results, the population from the Eastern Carpathians differed from the other Carpathian populations, and was most similar to these from the Apennines and the Iberian Peninsula. Populations from the Southern Carpathians and from the Apuseni Mountains were most similar to the Balkan Peninsula populations, especially the one from Greece. Thickness of shoot, thickness of cone and length of seed differentiated them at the highest level.

The differences between the population from the Eastern Carpathians on one side and populations from the Southern Carpathians and the Apuseni mountains on the other, may suggest their migrations to the Carpathians from the different sources and probably their relation to different taxonomic groups/varieties.

Cytological variation of *Fallopia* sect. *Reynoutria* taxa in the Western Carpathians

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Fallopia sect. *Reynoutria* members (knotweeds) represent one of the most dangerous invasive plants in Europe. However, several aspects of their biology remain little known, including the cytological variation, which is notable for the fact that some taxa have multiple ploidy levels and that the frequency of cytotypes in seedlings does not correspond to that found in adult plants.

In this study we used flow cytometry and determined the relative genome size in 1111 adult individuals (933 populations) of knotweeds collected in the Western Carpathians and adjacent areas, and the results were combined with our previous data from the Krivánska Malá Fatra Mts. In total, 644 individuals from Slovakia, 167 from Hungary, 75 from Poland, 63 from Czechia and 27 from Austria were evaluated.

We found that *F.* sect. *Reynoutria* is represented in the studied area mainly by hexaploid (2*n* = 66) cytotype of *F.* ×*bohemica* (683 populations) and octoploid (2*n* = 88) cytotype of *F. japonica* (276 populations). To a small extent we found tetraploid (2*n* = 44) and hexaploid (2n = 66) cytotypes of *F. sachalinensis* (41 and 8 populations, respectively). In addition, for the first time, we found in adult plants also aneuploid cytotypes, namely 2*n* = 65 and 2*n* = 107 (both in *F.* ×*bohemica*). On the contrary, we were unable to confirm the occurrence of tetraploid (2*n* = 44) cytotype in *F. japonica* reported by older authors from the Western Carpathians and we consider this information to be incorrect. In comparison with the neighbouring Hercynian part of the Czech Republic, the cytological variation in the Western Carpathians is slightly lower and the following cytotypes/taxa are absent in the studied area: tetraploid *F. compacta*, tetraploid plants assigned by other authors to *F.* ×*bohemica*, octoploid *F.* ×*bohemica* and octoploid *F. sachalinensis*.

The "steppe" *Gammarus tatrensis* (S. Karaman, 1931) lineage in Podillia region (north-western Ukraine)

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The species-level taxonomic status of *Gammarus tatrensis* (S. Karaman, 1931) was restored recently (Mamos et al. 2021). The species consists of five distinct genetic lineages, two of which are widespread in Ukraine: the first is in the Upper Dniester catchment, and the second is along the north-western Black Sea coast (karst springs of the Dniester Estuary and the Southern Bug River).

In 2018-2019, we collected individuals of *G. tatrensis* in the Podillya region: from a spring in the Khmelnytsky region (Southern Bug river basin) and Nemiya River in the Vinnytsia region (middle Dniester basin). They were DNA barcoded and the sequences were uploaded to the BOLD public repository, and each was assigned to a Barcode Index Number (BIN).

As a result, new haplotypes were revealed (p-distance between them 0.9%). All of them belong to one BIN (BOLD: ADD2205), known from a previous study as the "steppe"; lineage of *G. tatrensis* from the Southern Bug River. Additional BIN of *G. tatrenis* was already reported from the Dniester River basin however further south from our current study area.

The "steppe"; lineage of *G. tatrensis* sensu Mamos et al. (2021) appears to occur also in the northern part of Ukraine i.e., in the Podillia region. It is the only lineage in the region. Other, "western"; lineages of this and other species of the *G. balcanicus* complex are distributed in the Upper Dniester catchment (e.g. *G. stasiuki*). The results indicate that the endemic amphipod fauna of the Black Sea region is formed not only by Ponto-Caspian relics of marine origin but also by local freshwater taxa.

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Deep phylogeographic structure in one of the rarest plants in the world: effect of extremely low seed production, limited seed dispersal and clonality

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Over the two past decades, considerable attention has been paid to evolutionary histories of high mountain species with medium to large size ranges. These studies frequently revealed genetically structured populations with phylogeographical breaks corresponding to natural barriers. However, little is known about molecular variation of species with very narrow ranges and therefore without obvious geographical barriers.

Using three plastid and one nuclear (ITS) loci, we studied genetic variation of *Andryala laevitomentosa* (Asteraceae), an extremely rare species consisting of plants growing in six patches, each of several square meters large, distributed over a 3 km long mountain ridge in the Romanian Eastern Carpathians. In order to understand such a narrow distribution of this species, we also quantified its seed production.

Plastid markers which are inherited maternally, i.e. through seeds, revealed strong genetic structure largely corresponding to spatially isolated patches. In contrast, biparentally inherited ITS locus was invariable. Our results indicate very limited seed dispersal which can be directly linked to specific seed phenotype where pappus – morphological structure allowing effective wind dispersal – detaches in mature seeds which consequently fall close to a seed plant. Although this phenomenon certainly contributes to the observed pattern, even more surprising was finding that this species is almost seed-sterile. Average seed sets from several tens of plants surveyed in three years was 0.4% only. The extremely low seed production is not only a straightforward explanation for strong genetic structure in this species but at the same time also for its rareness. Survival of this species thus almost entirely relies on vegetative reproduction by root sprouts.

Our results imply that even narrow endemics can exhibit strong phylogeographic structure caused by intrinsic species traits involved in limited gene flow.

Assessing forest habitats connectivity for terrestrial species in the Romanian Carpathians

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In the European Union, reducing landscape fragmentation and improving connectivity are some of the most important means to ensure the conservation of habitats and species of community interest. Maintaining and enhancing connectivity between valuable habitats and protected areas in Romania represents one of the priorities for biodiversity conservation. Also, connectivity is a very important aspect in maintaining the resilience of ecosystems and ensuring the well balanced use of ecosystem services. The effectiveness of the protected area network is efficiently assessed by calculating the structural and functional connectivity of protected areas. Our study aims to evaluate the structural and functional connectivity of the protected forest patches for terrestrial species in the Romanian Carpathians.

We used the graph theory approach and the CONEFOR 2.6 software to measure forest landscape connectivity and the importance of forest patches in maintaining landscape connectivity. The analysis used a wide range of median dispersal distance (1 km, 10 km and 25 km).

Our results show that the forest patches are less connected for some groups of species with short distance dispersal (in protected areas). At the same time, the connectivity of the protected forest patches needs to be improved, in order to achieve one of the main objective - maintaining and improving the connectivity for protected forest patches respectively. Furthermore, new protected areas are needed for increasing landscape connectivity for some groups of species.

For future studies, we consider that it is necessary to take into account the heterogeneity and friction effect of the non-habitat areas that might need to be crossed by the species in order to move between forest patches.

Assessment of the ecosystem services in the national parks: examples from Slovakia – the first results

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National parks and their hinterland are under pressure from increasing housing development and tourism infrastructure. To support the conservation of the natural areas it is important to identify the ecosystem values and services of these areas in a spatially-explicit way. The aim of this study is to evaluate selected ecosystem services in national parks of Slovakia.

We focused on 7 national parks (Tatry, Nízke Tatry, Slovenský Raj, Veľká Fatra, Malá Fatra, Muránska Planina and Pieniny) and three specific ecosystem services: carbon storage, crop pollination and habitat quality. The evaluation was performed by using the Invest model, capable of synthesizing multiple biophysical and socio-economic variables.

The distribution of the ecosystem services revealed several areas of conflicts between the planned development and provision of the ecosystem values. Presented outcomes are further evaluated with stakeholders and decision makers to support the role of “trade-off” analysis in the spatial planning of the future development of the natural areas that are under increasing risk of disappearance.

Tanacetum corymbosum agg. (Asteraceae) in the Slovak Carpathians: karyological and morphological variability

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Tanacetum corymbosum group is in the Slovak part of the Carpathians represented by the native species *Tanacetum corymbosum* Sch. Bip. and *Tanacetum clusii* (Fisch. ex Rchb.) Soják. The biosystematics of the polyploid complex has not been prefomed yet. Previous karyological studies indicated mainly the diploid cytotype of *T. clusii* (*2n* = 18) and the tetraploid cytotype of *T. corymbosum* (*2n* = 36). We provide the overview of the karyological and morphological variability of the species, based on the population sampling in the Slovak part of their sympathetic area.

The results of cytotype screening by DAPI flow cytometry (45 populations, 530 individuals) showed, in addition to a predominance of known cytotypes, the existence (only 1.3% of whole dataset) of presumably pentaploid, as well as triploid cytotypes in single species populations. The estimation size of the haploid genome (Cx) of the individuals did not differ significantly from the individuals with the major cytotypes. The exception was a triploid individual collected in the species contact zone, whose Cx value was more than 3% lower than the size of diploids Cx (Cx of pentaploids is more than 5% lower than diploids). The uni- and multivariate morphometric analyses of 10 traits (39 populations, 351 individuals) showed a statistically significant separation of individuals corresponding to the diploid and tetraploid cytotypes. The traits that contributed the most significantly to this separation were the colour of the margin of the involucral bracts and the number and size of capitula. With a combination of studied traits, it is possible to assign individuals to a species with more than 91% confidence. The triploid individual from the contact zone was morphologically a transitional type between the studied taxa. This fact, together with the results of karyological analysis suggests its hybrid origin.

Bryophytes of the Ukrainian Carpathians (Ivano-Frankivsk region): peculiarities of regionally rare species

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An inventory of regionally rare species of bryophytes was conducted on the basis of critical analysis of literature sources, processing of herbarium materials and field survey of the territory.

On the territory of the Ivano-Frankivsk region were found 66 species of regionally rare bryophytes. Among them, moosses dominated, but the proportion of liverworts was also significant. Based on the analysis of the taxonomic structure, it was found that liverworts belonged to two classes, four orders, 13 families, 16 genera, 19 species; mosses – to three classes, 12 orders, 20 families, 38 genera, 47 species. According to the number of species, the species rich bryophyte families are as follows: Pottiaceae – 11 species (16.8%); Brachytheciaceae – 5 (7.6%); Cephaloziaceae and Splachnaceae – 3 (4,6%), the rest of the families are oligo- and monospecies. Among rare species, the boreal geographical element predominated (27 species, 41.0%), followed by arid (11 species, 16.7%), and nemoral (10 species, 15.1%) species. The analysis of ecological groups of bryophytes by type, chemistry, humidity, trophic and illuminance of the substrate was carried out. We found that most rare species belong to epigeous heliophytes. As for pH substrate preferences, acidoneutrophilous species growing on substrates with pH values ranging from 3 to 7 predominated. Quantitatively, species that grow on nutrient-rich substrates (mesotrophs, mesoeutrophs, eutrophs) predominate. According to the humidity of the locations, the ecological group of mesophytes prevails, but a significant share of hygrophytes was found. Features of the biomorphological structure of bryophytes were established. Sexual types of bryophytes and the presence of specialized organs of asexual reproduction have been determined. Most rare species with life forms of mats, tall turfs and wefts are confined to wetlands (swamps and shores of reservoirs), a significant part mat and short turfs – to rocky outcrops. Rare species, confined to wet and humid habitats, are particularly sensitive to any changes in hydrothermal conditions.

Structure of soil nematode communities in managed and unmanaged forest of the Poľana Mts., the Western Carpathian Mountains in central Slovakia

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Forest management and the stand age play key roles in determining the composition of soil biota, including nematodes. We analyzed the effect of the interaction between stands of natural forest and stands influenced by human activity on nematode communities, necessary for realistically assessing the specific potentials of forest soils, plant protection, forest management, and land use management. This study was carried out on Mount Poľana (48°37´ N, 19°30´ E) in the Western Carpathian Mountains in central Slovakia (Europe). Mount Poľana is one of the highest European former volcanoes, with an altitude of 1458 m a.s.l., formed mainly of andesite and andesite tuffs rich in soil nutrients.

Nematode communities were evaluated and compared in managed beech and spruce forests in three age classes (0–20, 40–60, and 100–120 years old) and an unmanaged old-growth temperate forest.

A total of 51 nematode genera were found in the forests. The number of nematode genera was the highest (46) in European beech forests, dominated by *Rhabditis* and *Filenchus*. In contrast, the number of nematode genera was the lowest (37) in a Norway spruce forest, where nematode abundance was, however, the highest due to high abundance of bacterivorous nematodes such as *Acrobeloides*, *Plectus*, and *Rhabditis*. The unmanaged old-growth forest had the lowest nematode abundance and total biomass but the highest abundance of herbivorous nematodes of the order Tylenchida, especially *Filenchus*, *Malenchus*, and *Paratylenchus*, and a high abundance of identified genera of predators. The number of identified nematode genera, abundance, total biomass, and diversity index were the highest in young 0–20-year-old stands, and the lowest in 100–120-year-old stands. Enrichment, structure, and basal indices were influenced by both the stands and the ages of the forests.

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The research biodiversity and biogeography of the Ukrainian Carpathians to use the web resource "Biodiversity of Ukraine"

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The web source Data Center "Biodiversity of Ukraine" (DCBU) was established at the State Museum of Natural History, National Academy of Sciences of Ukraine (Lviv), for all users it became available on internet on May 25, 2017 The resource is based on a database containing information about the biota of Ukraine: scientific and vernacular names, dates of registration of species, their geotagged geographical distribution, value of species, conservation categories, representation in physical-geographical regions, and objects of nature reserves of Ukraine. Currently, the database includes 10,885 species and 48,518 records, of which 2,856 and 17,310, respectively represent the region of the Ukrainian Carpathians. In total, the data to 97 endemic species confined to the Ukrainian Carpathians are currently included in this online source.

Thanks to the web source, it is possible to trace the distribution of endemic species by physical-geographical oblasts (mesoregions) and districts in the Eastern Carpathians in Ukraine. The great number of endemic species is registered in the Polonynsko-Chornohirska – 82, the Outercarpathians and Vododilno-Verkhovynska – 49 and the Marmarosko-Chyvchynska – 36 oblasts. In the Ciscarpathians Upland – 21, Vulkanichno-mizhhirnoulohovynna – 13 and Trancarpathian Lowland – 12 endemic species. Within the physical-geographical districts of Polonynsko-Chornohirska oblast, the number of Carpathian endemics decreases from south-east to north-west: Svydovets – 54, Chornohora – 51, Borzhava – 26, Krasna – 25, Rivna – 9 species. In the Outercarpathians and Vododilno-Verkhovynska oblasts, the largest number of endemic species was found in physical-geographical districs of the Outer Gorgany – 22, Skolivski Beskydy – 20 and Stryisko-Siansko Verkhovynskyi and Verkhovynskyi watershed range – 16. Due to the web source DCBU, it is possible to visualize the boundaries of the range of south-eastern Carpathian and western-eastern Carpathian endemic species in the Ukrainian Carpathians.

Nivicolous myxomycetes of the Carpathians – new data from Poland, Slovakia and Ukraine

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Myxomycetes are soil protists that occur in terrestrial ecosystems worldwide. The group associated with snow that occurs in mountainous environments is called nivicolous myxomycetes. Species from this ecological group undergo their life cycle under snow and occur in the form of spore bearing structures (sporophores) just after snowmelt in spring to early summer. Nearly 100 species are recognized in this group, most of them can be found in the mountains of the temperate zone. The Carpathians are poorly investigated with 34 species reported so far: 28 from Poland, one from Romania, one from Slovakia and 25 from Ukraine.

Here we investigated several Carpathian massifs in Poland (Beskid Niski Mts., Beskid Sądecki Mts, Beskid Śląski Mts., Beskid Wyspowy Mts., Beskid Żywiecki Mts., Gorce Mts., Pieniny Mts., Western Bieszczady Mts., Tatra Mts.), Slovakia (Malá Fatra Mts., Muránska planina Mts., Tatra Mts. and Veľká Fatra Mts.) and Ukraine (Chornohora Mts., Eastern Beskyds Mts., Gorgany Mts., Maramures Mts., Svydovets Mts.), and collected ca. 1200 specimens. They represent 37 species, 11 of them are reported for the first time in Poland, eight are new to Slovakia and four are new for the Ukrainian Carpathians. The following species are recorded by us for the first time in the Carpathians: *Badhamia alpina*, *Lamproderma cristatum*, *L. cucumer*, *L. piriforme*, *L. retirugisporum*, *L. zonatum*, *Lepidoderma granuliferum* and *L. neoperforatum*.

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Palaeogenomics of the Central European refugia: spatio-temporal dynamics of the arctic-alpine flora between northern and temperate latitudes – a new project

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Our poster introduces a new project focused on the role of the Central European mountains (Carpathians, Sudetes) as cold- and warm period refugia and as postglacial colonization sources for cold-adapted plants in the late Quaternary. The project relies on exploring both the contemporary phylogeographical structure of selected species and the ancient genetic data acquired from mountain lake sediments in the Tatra Mts. and Karkonosze Mts. We aim to explore the role of the Central European (CE) mountains by two main approaches: (i) using metabarcoding of DNA in ancient lake sediments to gain insight into Last Glacial to Holocene changes in plant communities and assess to what extent the CE mountains efficiently ensured a long-term resilience of the arctic-alpine biota; (ii) using DNA capture techniques applied on both modern samples and a chronoseries of ancient sediments, to assess the dynamics of intraspecific genetic lineages of arctic-alpine species in time and space, and to test the role of the CE mountains in the maintenance of rare, relic lineages, and as sources of postglacial colonization.

First contemporary phylogeographical data from *Dryas octopetala*, one of our model species, show a key importance of the Carpathians for the species' lineage diversity in Europe as this range harbored two distinct lineages absent from western Europe albeit with a limited role in postglacial recolonization of the North. First sediment cores were obtained in winter 2022 in mountain lakes of the Tatra Mts. and preliminary insight into the past communities and population genetic composition of selected species, to confront the contemporary patterns, is expected in 2023. Intraspecific data from the CE mountains will be explored in a larger geographical context through international collaboration.

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Mapping the molecular variability of the amphipods crustaceans in the Bieszczady biodiversity hotspot – a student project

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The Carpathians are known as one of the most important European biodiversity hotspots. Their northern part was thought to contain lower biodiversity because of Pleistocene glaciations. However, recent research focused on diversity of amphipods – Gammaridae (Crustacea, Amphipoda) proved that this hypothesis needs a critical re-assessment. The *Gammarus* genus shows great variability in this region due to its allopatric speciation in glacial refugia in the northern Carpathians. The Bieszczady Mountains reveal high diversity of Gammaridae. In 2021, a new endemic species was described – *Gammarus stasiuki* Jazdzewki, Mamos et Grabowski, 2011. According to the recent data, the northern range limits of *G. stasiuki*, *G. tatrensis*, *G. leopoliensis*, and *G. fossarum* can be found in the region, while the detailed range limits and the directions of local migrations remain unknown. Latest studies about range limits of *Gammarus* spp. in the region are based on information from the 1970s and 1990s.

The presented new project aims to study molecular variability of *Gammarus* spp. the in the Bieszczady Mountains and the Przemyskie Foothills. To define molecular biodiversity hotspots, molecular variability analyses, and Geographic Information System will be used. Finally, new studies will contribute to better protection of local crustacean populations. The proposals of extending the surface area of the Bieszczady National Park and the establishment of a new Turnicki National Park will be considered.

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Karyological variation of invasive alien *Solidago canadensis* and *S. gigantea* in the Central Europe: generally boring but locally noteworthy

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Nowadays two alien species *S. canadensis* and *S. gigantea*, both of North American origin, are considered among the most aggressive plant invaders in almost all of Europe (except southernmost and northernmost parts). Only diploid plants of *S. canadensis* (2n=18) and tetraploid plants of *S. gigantea* (2n=36) have been found in previous cytogeographic studies within their European invasive range.

Our findings based on karyological and flow cytometric analyses of more than 4,800 plants from almost 800 sites sampled across until now poorly investigated Central Europe are in accord with previous reports. Furthermore, we observed only negligible variation (up to 6%) in relative as well as absolute DNA content within the datasets of studied European populations of *S. canadensis* and *S. gigantea*. However, our extensive research also uncovered the presence of rare cytotypes unknown for the invasive range: triploids (*2n* = 27) of *S. canadensis* and pentaploids (*2n* = 45) of *S. gigantea*. While triploids of *S. canadensis* are represented only by scattered plants within diploid populations and are most likely a result of occasional fusion of reduced and unreduced gametes, pentaploids of *S. gigantea* surprisingly occur in hundreds of plants in a few Slovak populations although so far only one pentaploid individual has been reported from its native range. As genome duplication could promote invasiveness, the origin of European newly discovered polyploids as well as their invasive potential need to be investigated in order to estimate their potentially negative impact to native European flora.

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Can we learn a lesson from the past and present distribution of *Solidago canadensis* and *S. gigantea* to mitigate their potentially negative impact in the future?

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Today, alien invasive *Solidago canadensis* and *S. gigantea* are distributed across almost the whole Europe, where they are fully established and sustain self-replacing populations without direct human intervention. Increasing number of records of these species induced the need to monitor their occurrence and spread. In the present study we aim to reveal the past and current distribution, the course of the invasion, habitat preferences, ecological demands, and to assess potential and further spreading of *S. canadensis* and *S. gigantea* in Slovakia and adjacent Carpathian and Pannonian regions. Our study is based on revised herbarium specimens and own field data (745 sites) accompanied by abundance and habitat data from the field as well as climatic data from GIS layers.

Analyses of occurrence in the period of 100 years after the first records in the wild (1854-1953) in Slovakia showed an exponentially increasing number of localities of *S. gigantea* while the number of localities of *S. canadensis* was low and did not increase noteworthy. However, *Solidago canadensis* is nowadays more widespread in Slovakia, being widely distributed mainly in the Carpathian region. On the other hand, *S. gigantea* prevails throughout lowlands in SW and SE Slovakia. Data on habitat type show that species have similar habitat preferences, both occur mainly on the disturbed places affected by human activities, but are common also in natural (alluvial and riparian vegetation) and semi-natural (grasslands, margins of fields) habitats. However, the two species differ in their ecological preferences and, consequently, in the extent of their current potential distribution areas. The differences are mirrored in distinct expected magnitude and direction of potential distribution shifts under climate change scenarios in upcoming decades for them.

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Resource limitation and soil toxicity shape species dominance and diversity in wetland forests across biogeographical regions

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A pronounced hierarchy of above- and belowground resources in forest ecosystems underlines an importance of resource availability for diversity and dominance of plant species. According to the soil chemistry-biogeographical hypothesis, variability in resource availability along climatic gradients could essentially shape patterns of species diversity among biogeographical regions. Here, we asked how the resource availability, soil toxicity and interactions with dominant herb-layer species affect the plant species richness of wetland forests in a broad biogeographical context.

We used vegetation plots sampled in temperate *Alnus* swamp forests evenly distributed along latitudinal gradients in Central Europe (Hungary, Slovakia, Poland). Path analysis (structural equation modelling) was used to quantify and visualize the effects of temperature, key productivity drivers, toxic elements and plant dominants on species diversity.

Species diversity ranged from 21 to 69 species per plot (mean 44). Species diversity increased along an increasing latitudinal gradient, which controlled belowground resources (water availability, soil N/P ratio), but also the concentrations of toxic elements limiting nutrient availability (soil manganese). Local site productivity determined species diversity either directly or through the effect of the dominant herb-layer plants. Soil manganese and calcium positively affected plant dominants, but negatively affected species diversity, while soil C/N ratio and light conditions affected only plant dominants that reduced the number of plant species.

Our results indicate that resource availability but also its interaction with toxic soil elements affect dominance and diversity in wetland forests. Increasing concentrations of soil manganese in regions with higher temperature support dominance of graminoids with negative effect on diversity of other species and as such it could be potential threats for wetland diversity in northern regions under climatic change.

Distribution features of rare nematode species *Longidorus piceicola* Liskova, Robbins et Brown, 1997 in European mountain systems

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Nematodes of the genus *Longidorus* Micoletzky, 1922 are ectoparasites of vascular plants inhabiting terrestrial biotops and are considered as economically important pests. This genus consists of more than 150 valid species, but currently only ten have been reported from Ukraine and only two of them were detected in the Ukrainian Carpathians.

During current nematological research, *Longidorus piceicola* Liskova, Robbins et Brown, 1997 was found for the first time in Ukraine. *L. piceicola* is a rare longidorid species which was described from the Muránska planina Mts. in central Slovakia in association with Norway spruce (*Picea abies*). Subsequently *L. piceicola* was reported from several localities in Serbia, Montenegro, Bosnia and Herzegovina, Romania and Poland. Most populations were collected in mountainous forests from the rhizosphere of coniferous trees. All three Ukrainian populations of this species were detected in 2015-2020 in the western region of the country. One of them was collected in the typical biotope in the Gorgany mountain range in the Ukrainian Carpathians from the rhizosphere of *P. abies*. Two other populations were detected in urban biotopes in the city park and botanical garden in Lviv in the rhizosphere of *Acer pseudoplatanus* and *Magnolia* sp., respectively. We hypothesize that modern distribution of *L. piceicola* is caused by its persistence in the refuge areas in European mountain systems during Last Glacial Maximum and postglacial recolonization. This typically mountainous species could arrive in urban biotopes due to human activity with soil or planting materials, in the same way as in the arboretum in Rogów, Poland (Kornobis, Peneva, 2011).

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An insight into the crenic diatom communities from several karstic springs located in the Apuseni Mountains

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The unique geomorphological and hydrological characteristics along with the constant abiotic parameters, have shaped springs into being a suitable aquatic habitat for a high number of microorganisms. Furthermore, based on these characteristics, springs can be classified in: limnocrene, rheocrene, rh. cave, helocrene and rheohelocrene. Increasing anthropological impact, such as groundwater pollution, alteration or destruction of the eucrenal spring area and high demand of pristine water, are affecting the high crenic biodiversity. Being at the beginning of the food chain, diatoms are one of the key bioindicators of water quality. Thus, the present study includes qualitative and quantitative diatom samples taken from 30 karstic springs located in the Apuseni Mountains. Often displaying distinct preferences for a specific available substrate, 15 diatom samples were taken for each spring, from five different spots in the eucrenal area for each of the three substrate types: bryophytes, stones and sand.

Two groups of dominant taxa, according to the type of substrate they prefer (present in at least 25 springs) have been observed: *Caloneis fontinalis*, *Cocconeis lineata* and *Meridion circulare* in epibryon, followed by *Gomphonema parvulum* and *Planothidium dubium* in epipsammic; three taxa *Achnanthidium minutissimum*, *Amphora pediculus* and *Cocconeis placentula* stand out as a dominant group in the all three types of substrates, but in epilithon samples these were the only dominant taxa. A total of 217 taxa were identified in the analyzed samples, with 25% taxa showing a preference for sand and 12% having a preference for bryophytes. More than half of the studied springs are currently used as main water sources; thus, the diatom communities indicate a medium level of saprobity and trophicity of the water because of the anthropogenic impact upon the eucrenal area. *Gomphonema elegantissimum* and *C. fontinalis*, along with other 13 taxa have been identified for the first time in Romania.

Chaoboridae of Romania (Diptera, Chaoboridae): integrative data detects a new genus for the Carpathians: *Mochlonyx* Loew, 1844

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Chaoboridae (Diptera) was largely overlooked in the Romanian aquatic habitats, having only sporadic records from a single literature data. The genus *Mochlonyx* Loew, 1844 is recorded for the first time from the Carpathian area, based on DNA Barcode sequences and morphology. Recent field samplings along different lentic water bodies revealed altogether new distribution data of four species, from which *Chaoborus obscuripes* (Wulp, 1859) (detected from an oligotrophic marsh, Molhașurile de la Izbuce, Ic Ponor), *Mochlonyx fuliginosus* (Felt, 1905) (collected in almost every small ponds in woods in the spring sector of the River Someșul Cald, the Apuseni Mountains) and *M. velutinus* De Geer, 1776 (recorded from Făgetul Noroios, Feleacului Hills, Cluj-Napoca) are recorded for the first time from Romania. The Chaoboridae fauna from here is now represented by six different species. Beside the aforementioned species, *Chaoborus flavicans* Meigen, 1830 is mentioned for the second time from Romania (Știucilor Lake near Sic, Transylvania), while *Ch. crystallinus* (De Geer, 1794) and *Ch. pallidus* (Fabricius, 1794) were not found during our faunistic investigations. The observed genetic distances based on the mtCOI barcode sequences are low, the Romanian population of *M. fuliginosus* show a p-distances of 1.55 and the *M. velutinus* population one of 1.11 when compared to the conspecific sequences available in the BOLD System database. Our data shows that the identified species have a general distribution in late spring to early summer, right after snow melts in mountainous areas and are crucial to control Culicidae larvae populations.

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Some rare and endangered species of the subalpine zone of the Ukrainian Carpathians

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In this contribution, we present new data on the occurrence of the species *Allium schoenoprasum* L., *Carex capillaris* L., *C. limosa* L., *C. pauciflora* Lightf., *Heracleum carpaticum* Porcius, *Juncus castaneus* Smith, *Juncus triglumis* L., *Salix rhaetica* Andersson, *Scheuchzeria palustris* L. and *Swertia punctata* Baumg. all recorded in 2021, primarily in the vicinity of subalpine lakes of glacial origin in the Svydovets Mts and Chornohora Mts (Ukrainian Carpathians), which are included in the Red Book of Ukraine (Didukh 2009) or are assessed as rare (Malinovskyi et al. 2002), endangered (Kricsfalusy & Budnikov 2007), endemic (Malinovskyi et al. 2002, Kliment et al. 2016), or species with an insufficiently known area of distribution.

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An ecological network of large carnivores in the Carpathians: a key tool for protecting landscape connectivity

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Increasing fragmentation as a result of the constant development of the transport network is a threat to a landscape across the European continent. One of the last relatively unfragmented European regions with extensive natural habitats without significant human impact are the Carpathians, which are home for a large population of large carnivores. However, large projects of transport infrastructure development are planned here as well and threaten unfragmented forest habitats important for maintenance of the Carpathian population. This could be a problem especially in the most preserved eastern and southern part, where currently there are not sufficient and efficient tools to protect ecological connectivity.

Within the ConnectGREEN project, using a unified methodology, we identified and delineated a cross-border and coherent network of suitable habitats connected by migration corridors. Using fragmentation geometry composed of road network and settlement we identified critical connectivity zones where animals are forced to cross the barriers. When delineating the ecological network, we considered the heterogeneous character of the Carpathian landscape and its dynamics, and designed an innovative basis for nature and landscape connectivity protection across the Carpathians. All the components of the ecological network will be implemented into spatial planning processes and documentation in the Carpathian countries.

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