



Ecological and evolutionary facets of biodiversity

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Biogeography of the Carpathians: Ecological and evolutionary facets of biodiversity

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=== KEYNOTE ABSTRACT ===

The Millenium Seed Bank and its role of *ex situ* plant conservation in meeting global challenges on biodiversity conservation: current status and perspectives

Elinor Breman^{1,⊠}

The need for plant conservation has never been greater. Of the 393,000 vascular plants known to science, 1 in 5 if faced with extinction. Yet plants underpin our very existence and offer solutions to the global environmental challenges facing humanity.

The Millennium Seed Bank (MSB) of the Royal Botanic Gardens, Kew has been working to conserve the seeds of orthodox seed bearing plants to help address this issue. Working in partnership with over 95 countries and territories since 2000, it is the largest ex situ conservation programme in the world. There are currently 54 countries involved in active seed conservation projects. The work of the MSB includes: ensuring seed conservation standards are adhered to across the partnership; capacity building and technology transfer; and increasing the profile and availability of data from seed collections across the partnership.

The MSB's most recent programme focuses on conserving the flora of the Carpathian region together with partners in Romania, Ukraine and Slovakia. This project will ensure the long-term *ex situ* conservation of 500 plant species (notably endemics and sub-endemics), deliver research on biogeographic, taxonomic and genetic diversity of five model genera (*Carex, Daphne, Draba, Silene zawadzkii* and *Soldanella*), and establish a Carpathian research network and associated database. Additional outputs include research papers, a monograph on Carpathian endemics, and red listing of Carpathian species.

¹ Kew Royal Botanical Gardens.

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=== KEYNOTE ABSTRACT ===

The biogeography of soil diversity: insights from European mountains

Philippe Choler^{1, \boxtimes} and the ODYSSEE Consortium

My presentation will focus on the distribution patterns of soil biodiversity across the European Alpine System, with a special emphasis on the Carpathians. I will address two questions: are there biogeographical patterns for soil micro-organisms and soil mesofauna? and, are these patterns consistent with those described for macro-organisms? I will briefly review past literature before showing recent results obtained in the framework of the ODYSSEE project. This project aims at integrating ecology and evolution to understand multi-trophic species assemblages in two high-elevation ecosystems: subalpine grasslands dominated by *Nardus stricta* and alpine meadows dominated by *Carex curvula*. I will discuss the main findings of the project in light of postglacial history, spatial connectivity, soil climate and disturbance regimes of mountain grasslands. I will conclude by some perspectives pertaining to multitrophic comparative phylogeography in the European Alpine System and to the implemntation of meta-community models able to track the response of species assemblages to environmental changes in a dynamic landscape.

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=== KEYNOTE ABSTRACT ===

Past responses of Carpathian vegetation to a warmer world and anthropogenic impacts

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Long-term ecological (palaeoecological) research provided by various indirect measurements, the so-called proxies, can provide fundamental ecological and biogeographic understanding of ecosystems under a range of environmental conditions and disturbance regimes. ETo illustrate this, I will show examples from long-term research (pollen, plant macrofossil, micro-and macro-charcoal, dung fungi) from forests and open grassy systems of the Carpathians in order to determine biotic responses (range shifts, turnover, biodiversity) during time intervals when the magnitude and rate of climate change were comparable to those predicted to occur in the next century as well as the effect of increasing anthropogenic impact on resilience of these systems. One important outcome is that the mechanisms responsible for these past biotic changes were different in time (i.e., natural processes vs. anthropogenic), but the magnitude of biotic changes can be large in both cases. Finally, I stress the importance of long-term palaeoecological data in understanding contemporary and future biotic responses.

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=== KEYNOTE ABSTRACT ===

Central European and Carpathian phylogeography: evidence for cryptic refugia?

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The origins of phylogeography date back to the pioneering works of Avise and his collaborators, which resulted in their classical 1987 paper, where the term "phylogeography" was used for the first time. The following years saw the publication of a considerable number of papers dealing with the phylogeography of European terrestrial species, both of animals and plants. Their results were later synthesized particularly by Hewitt (1999 and subsequent papers), who proposed three main paradigm patterns of postglacial recolonization of Central and Northern Europe from the three Mediterranean peninsulas (Iberian, Italian and Balkan), each of them representing a single refugium. This was, however, a rather simplistic approach that did not reflect the wide range of evolutionary histories of species composing the European biota. Already in 2007 the "refugia-within-refugia" model was introduced by Gómez and Lunt, reflecting the complex glacial and postglacial histories of species in the Mediterranean peninsular refugia. With the increasing amount of phylogeographic evidence amassed over the last decade or so, the possibility that plant populations survived the Last Glacial Maximum in Central Europe is a matter of ongoing discussion. Theories concerning the survival of some species in so-called "northern refugia" are supported also by plant macrofossil evidence and by snail fossils. However, especially the survival of comparatively thermophilous species in Central Europe during the Last Glacial Maximum is still not commonly accepted and may be considered doubtful (cf. Tzedakis et al., 2013). Nevertheless, for a number of species, the existence of "northern" refugia in Central Europe is the most likely explanation of the discovered phylogeographic patterns.

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=== KEYNOTE ABSTRACT ===

The zoogeography of the Carpathians and their links to the adjoining high mountain systems

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Although the Carpathians represent the most extended high mountain region of south-eastern Europe, the zoogeography of this area is still relatively poorly studied, especially if focusing on phylogeographic analyses. However, some recent studies reveal the biogeographic structuring within the Carpathians and their links with adjoining mountains systems, i.e. the eastern Alps and the eastern Balkan high mountain systems (Schmitt, 2009).

For most of the wide-spread mountain species, several phylogeographic lineages have been detected within the Carpathians, most likely resulting from independent differentiation in geographically isolated glacial refugia in different regions along this mountain chain in many of these cases. One example for two lineages (one in the Tatra region and the other in the eastern and southern Carpathians) is the caddisfly Drusus discolor (Pauls et al., 2006). A relatively similar pattern but with a third lineage endemic to the Apuseni Mountains is known for the stonefly Arcynopteryx dichroa (Theisinger et al., 2012). Even more complicated is the phylogeographic structuring of the tipulid *Pedicia occulta* with one wide-spread lineage in many regions of Europe and another one restricted to the Romanian Carpathians. While the first shows almost no differentiation all over the Carpathians, the latter has two well defined lineages, one in the southwestern Carpathians and the other in the Apuseni Mountains and regions in the eastern Carpathians (i.e. Rodna) (Ujvárosi et al., 2010). An even more complex phylogeographic pattern is known for the butterfly Erebia *medusa*, in Romania mostly found in hilly and mountainous areas. This species has several genetic lineages in the southern Carpathians, one in common for the Apuseni Mountains and eastern Carpathians and a completely different lineage in the Tatras (Schmitt et al., 2007, Besold and Schmitt, 2015).

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Distribution patterns of many species demonstrate a close zoogeographic cohesiveness between the Tatras and the northeastern Alps. This pattern is also supported by phylogeographic analyses. Thus, the jumping spider *Pardosa saltuaria* has the same genetic linage all over the Alps and Carpathians (Muster and Berendonk, 2006). Also one of the seven genetic lineages of the leave beetle species complex *Oreina alpestris/speciosa* is wide-spread all over the Carpathians and in the Alps north of their main chain (Triponez *et al.*, 2011). Furthermore, the butterfly *Erebia manto* shows an identical genetic make-up in the Tatras and the north-eastern Alps, but a different genetic lineage in the southern Carpathians (Schmitt *et al.*, 2014). These examples demonstrate frequent exchanges between Tatras and north-eastern Alps along the Pleistocene for many species.

The Danube valley separating the Carpathian and the eastern Balkan region apparently is an important obstacle for dispersal for many mountain species. Nevertheless, occurrences of several mountain butterfly species on either side (e.g. *Erebia melas, E. neleus, Coenonympha rhodopensis*) demonstrate its permeability (Varga and Schmitt, 2008). Stricing similarities of gential structures of the butterfly *Erebia pandrose* (Cupedo, 2007) and of allozyme patterns of *Erebia euryale* (Schmitt and Haubrich, 2008) support late Pleistocene exchanges. Introgression of a Carpathian haplotype of the stonefly *Arcynopteryx dichroa* into the Balkan phylogroup shows that secondary exchange has taken place recently after a longer periode of separation between both regions for this species (Theissinger *et al.*, 2012).

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=== ORAL PRESENTATION ABSTRACT ===

Endemic rodent species in the Ukrainian Carpathians and their spatial distribution

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Mountain systems are characterized by a significant habitat diversity not only in geographic dimension (massifs, ridges, valleys), but also elevational zones, which leads to a high level of biodiversity and a complex set of geographical ranges. The altitudinal zonation in the Ukrainian Carpathians has its own features on both the northern and southern megaslopes related mainly to the ratio of mountain biotopes with their corresponding analogues on adjacent plains.

Accordingly, the peculiarities of the region's fauna and the uniqueness of assemblages are determined by two groups of species: (i) species typical for certain altitudinal zones (or interzonal biotopes as well), and (ii) species distributed exclusively within these zones or only in the Carpathians (endemics, regional endemics). In most cases, these two groups are represented by the same set of species meaning that they should be analysed together.

Our study focuses on the order Rodentia as model object, being the largest group of mammals in the region's fauna. The distribution of rodents is closely related to the type of biotopes and altitudinal zones. Such connection is more evident in endemic species having a strict specialization and limited adaptive capability.

In the rodent fauna of the Ukrainian Carpathians, two groups of endemics can be distinguished: (i) actual endemics (species considered endemic for the entire Carpathians), and (ii) regional endemics (species that within Ukraine occur only in the Carpathians). The sole endemic and autochthonous rodent species in the Carpathians is *Terricola tatricus* (Mitchel-Jones *et al.*, 1999) represented in the Ukrainian Carpathians by a subspecies *T. t. zykovi* (Zagorodniuk, 1989). Such species as *Chionomys nivalis*,

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Sicista betulina, and *Arvicola scherman* are regional endemics, the two latter are also represented by separate subspecies *S. b. montana* (Zagorodniuk and Kondratenko, 2000) and *A. s. gutsulius* (Zagorodniuk, 2001).

Analysis shows that the distribution of these species is related to the elevational zones with corresponding types of assemblages. In particular, both *C. nivalis* and *S. betulina* are part of the montane mammalian assemblage having insular range in this region. The first species, as a glacial relict (Mitchel-Jones *et al.*, 1999), is common for the subalpine zone of Chornohora massif, where it lives among scree and krummholz thickets (mainly of *Pinus mugo*). The second one inhabits subalpine meadows and krummholz of the Chornohora and Gorgany Mts. As a boreal species, *S. betulina* "tends" to the northern megaslope, which is evidenced by its records from the Skole Beskids.

The Tatra pine vole (*T. tatricus*) has a distribution restricted to the forest zone where it occupies habitats on early stages of succession both natural (windthrows, glades) and anthropogenic (clearcuttings) origin.

The montane water vole (*A. scherman*) is distributed from the foothills up to the subalpine zone, and its range is actually continuous in the region up to the valley of the Dniester River. However, this vole expanses its range further north to Roztochia (Zagorodniuk and Zatushevsky, 2012).

Projections of ranges of these species in the Ukrainian Carpathians concentrically overlap. Such pattern is common for the largest massifs such as Chornohora, Svydovets, and Gorgany, which are characterized by a high diversity of local biotopes and clearly expressed elevational zones. Obviously, with the increase of elevation the absolute area of biotopes decreases, so does their carrying capacity. It means that higher elevations have not only less species diversity (McCain and Grytnes, 2010), but also less effective population size of species. Therefore, mountain species and assemblages (in particular montane ones) are considered highly rare and they need prior conservational attention.

Endemic rodent species of the Ukrainian Carpathians deserve priority protection that is particularly provided by their inclusion (except for *A. scherman*) into the Red Data Books of Ukraine and of the Ukrainian Carpathians.

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=== ORAL PRESENTATION ABSTRACT ===

Long-term effects of different management practices on soil nematode communities in European mountain spruce forests after a windstorm

Andrea Čerevková^{1,⊠}, Marek Renčo¹ and Erika Gömöryová²

Windstorms are significant and key disturbances in most natural (unmanaged) forests and affect not only trees and stands but can also change soil biota and initiate ground-layer successions (Peterson and Pickett, 1991). Disturbances are a natural part of all ecosystems and should not be regarded as unexpected, unusual, or annoving events. More than 12000 ha of a forest of Norway spruce (*Picea abies*) in the Tatra National Park, Slovakia, were laid down by a strong windstorm on 19 November 2004. As much as 2500000 m³ of timber were broken or uprooted. Most of the broken and uprooted trees were completely removed from the area in the following months, and the remaining fallen trees were left in their natural successional state. We analysed the impact of these two strategies of forest management on the structure of the soil-nematode community and its relationships with basic soil physicochemical properties nine years after the windstorm. The relationships were investigated in a cleared windstorm plot (EXT), a non-extracted windstorm plot (NEX), and an undamaged forest plot (REF) as a control. All plots were sampled twice, in June and October 2013. We hypothesised that changes in soil-nematode communities will not persist beyond the first few years after a windstorm and that different management strategies implemented in damaged areas could have considerable effects on the long-term rate of rehabilitation of nematode communities.

Results showed that EXT and NEX had a significantly higher mean nematode abundance at both sampling dates than REF. REF had the mean number of species, similar to the mean number in NEX. Analysis of variance showed significant main interaction among sampling time, plots and number of species. Spearman's correlations identified positive correlations between the number of species and soil carbon and

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nitrogen contents and between the number of fungivores and soil nitrogen content. Predators and root-fungal feeders were significantly less abundant in EXT and NEX, and omnivores were most abundant in EXT and NEX.

The analysis of the soil-nematode communities of the spruce-forest ecosystem indicated that they re-established relatively well over time after a damaging windstorm, also indicated by the abundance of omnivorous nematodes and by the community indices and metabolic footprint.

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=== ORAL PRESENTATION ABSTRACT ===

The footprints of the geological and climatic history of the Carpathians on the biogeography of their freshwater amphipods

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The Carpathian Mountains had a complex geological past with episodes of landmass uplift and subsidence, and dramatic sea level fluctuations of the surrounding Paratethys Sea. They are also considered as one of the most important extra-Mediterranean glacial refugia for terrestrial and aquatic taxa and are consequently regarded as a major European biodiversity hotspot. Freshwater amphipods of the genera Gammarus and Niphargus are ancient groups with poor dispersal abilities, being highly diverse and ubiquitous throughout the Carpathians and their basins. Therefore, these taxa can be regarded as models for the study of historical biogeography. Moreover, the relatively well known geological and climatic history of the Carpathians allows for testing of explicit historical biogeographic hypotheses. Our fine-scale, multilocus phylogeographic analyses strongly support the existence of relict diversity, with dozens of microendemic lineages dating back to the Miocene and Pliocene being detected in all the Carpathian gammarid species complexes (G. balcanicus, G. fossarum and G. leopoliensis). These mountainous taxa bear strong signatures of ancient geological and climatic events such as different timings of orogenic episodes in G. balcanicus, geological subsidence in G. fossarum and longterm survival in high latitude glacial refugia in both G. fossarum and G. leopoliensis. Contrastingly, the phylogeographical patterns of the two lowland epigean niphargids (N. hrabei and N. valachicus) point out to Middle Pleistocene dispersal events across vast distances via the Danube River. Phylogenetic and ancestral state reconstruction indicates that these two species have independently colonized hypoxic epigean habitats from subterranean ancestors, an ecological shift likely facilitated by the scarcity of rheophilic gammarid competitors in such environments. We conclude that the turbulent geological and climatic history of the Carpathians and the surrounding basins has left deep genetic signatures on the contemporaneous diversity patterns of their amphipod fauna, promoting ancient diversification of lineages to recent ecological shifts.

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=== ORAL PRESENTATION ABSTRACT ===

Genetic diversity of circum-mediterranean lichen Solenopsora candicans with special focus on the marginal Carpatho-Pannonian populations

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Marginal populations may exhibit certain level of differentiation from those in the distributional centre since the range edges may harbour limiting conditions for the species occurrence which may promote their differentiation. We analyzed mycobiont of the species Solenopsora candicans, a saxicolous sexually reproducing lichen with circum-mediterranean distribution, in order to assess if marginal populations laving in the Western Carpathian Mts. show differentiation patterns from those in the range centre, i.e. in the Mediterranean Basin. The genetic variation of two nuclear DNA markers (ITS, ß tubulin) was investigated from 315 samples across eight broad geographical regions (Alpine, Apennine, Atlantic, Balkan, Carpatho-Pannonian, Black Sea coast, East and West Mediterranean). The Eastern and Western Mediterranean and the Balkan Peninsula have been detected as the main diversity centres of the species. The Western Carpathian populations show only low genetic diversity in comparison with them indicating potential genetic drift operating at the range margin. They predominantly consist of the common ribotypes/haplotypes, which are widely spread also in the other parts of its distributional range and one private haplotype which has been observed only in Malé Karpaty Mts. The presence of the haplotype shared with the Balkan populations, as well as lower differentiation between Balkan and Carpatho-Pannonian region suggest connection between these two regions. These patterns point out potential migration event from the Balkans to the Western Carpathians.

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The obtained genetic variation in the Western Carpathian samples was not sufficient to consider that these samples represent a specific genetic lineage. Actually, maximum likelihood and neighbour joining trees showed polytomy across whole datasets with only one ancestral accession significantly delineated from the rest comprising few individuals from Turkey and Morocco. This might indicate cryptic speciation. However, to confirm this hypothesis, further investigations and more extensive sampling are required.

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=== ORAL PRESENTATION ABSTRACT ===

Linking vegetation dynamics and stability in the forests of the Eastern Romanian Carpathians

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The Carpathian Mountains are rich in biodiversity and host Europe's largest continuous temperate forest ecosystems (UNEP, 2007). We used a fossil record (Tăul Muced) from a montane coniferous forest of the Eastern Carpathians to explore patterns of change in vegetation composition, turnover and diversity in response to various forcing factors (e.g., climate, wildfire, species interactions, natural and anthopogenic disturbances) during the Holocene, and to investigate the plant communities associated with periods of high landscape stability.

Results from the palynological and numerical analyses reveal three apparent periods in the dynamics of past plant communities: 8700–5500, 5500–1750 and 1750 cal BP – present. Low to moderate compositional changes were recorded during the early to mid Holocene (8700-5500 cal BP) when dense forests of *Picea abies-Corylus avellana* and mixed oaks (*Ulmus, Quercus, Tilia*) developed in the region. Evaluation of drivers of these changes leads to the outcome of climate conditions and natural disturbances (e.g., fire) as the main drivers of change at the time. Low compositional change that indicate great landscape stability was noted between 5500 and 1750 cal BP. A high amount of compositional change at 5500 cal BP is concurrent with *Carpinus betulus*, soon followed by *Fagus sylvatica*, becoming the main deciduous trees, and a noticeable increase in anthropogenic indicators. These compositional changes are most likely linked to the onset of human influence on the otherwise climate-driven forest ecosystem. The period of highest compositional change is shown between 1750 cal BP and present, but in particular over the last 40 years. Main vegetation characteristics include the declining

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abundance of primary tree taxa and marked increased abundance and diversity of herbaceous taxa and anthropogenic indicators. These changes become significantly greater through the last four decades with the deforestation of the *Picea abies* and *Abies alba* forests.

We conclude that the long term forest dynamics in the study area were primarily influenced by climatic conditions, natural disturbance and species interaction. However, human activities imprinted changes on forest dynamics over the last 1750 years with a strong effect in the last decades. Our study reveals that up to a few decades ago these forests were one of the most pristine in the Romanian Carpathians and most likely in Eastern Europe, however, they have undergone accelerating changes due to human impact over recent years. Our study supports recent investigations that we are now losing one the last old-growth forests of Europe at rapid rates.

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=== ORAL PRESENTATION ABSTRACT ===

Ecological or historical biogeography of calcareous fens? Differences and similarities between the Western and Eastern Carpathians in the European context

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Calcareous fens represent the habitats mostly fed by springs of bicarbonaterich groundwater, characterised by the occurrence of calcicole brown mosses and small sedges; peat mosses (Sphagnum) occur rarely and belong among so called calciumtolerant species. Calcareous fens are usually quite waterlogged, otherwise they change into broadleaved fen grasslands or poor Sphagnum fens. Despite the ecological homogeneity they display great variability in both plant and snail assemblages. including a number of rare and threatened species such as fen specialists and relics. In the previous studies from the Western Carpathians (presented in the talk on the first Biogeography of the Carpathians meeting in Kraków) we demonstrated an important role of Holocene history on such variability. Here in Clui-Napoca we confront these results to the pattern observed in the Eastern Carpathians (Romania, Ukraine, marginally also Slovakia and Poland). Again, developmentally young sloping spring fens of the flysch bedrock or mountain summits differed from relict fens located mostly in basins or on more consolidated bedrock, and their variability followed water chemistry, hydrology and hemeroby level (see the poster of Hájková et al.). Relict fens, located mostly in the Harghita county and Rodna Mts., showed quite a complicated variability. All these fens had been classified into a single alliance, the Caricion davallianae, in previous vegetation surveys of Romania. A comparison with all available data on European fen vegetation showed the occurrence of some

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ORAL PRESENTATION ABSTRACT

previously unrecognised major vegetation types in few unique sites of the Romanian Eastern Carpathians: boreal brown-moss quaking fens (the Stygio-Caricion limosae alliance), rich fens with calcium-tolerant peat mosses (the Sphagno warnstorfii-Tomentypnion alliance) and boreal-continental N-limited fens (with low N:P ratio in plant biomass) of the Saxifrago hirculi-Tomentypnion alliance. The latter type represents a unique case when enhanced phosphorus concentration does not annihilate fen vegetation, and instead determines a specific species composition which is rare but compositionally similar across Eurasia. Romanian occurrences are very isolated and associated with phosphorus-rich volcanic bedrock where high iron concentrations probably maintain ancient fen vegetation. According to the macrofossil records from the Czech and Slovak Republic and according to the ecological theory (increasing N:P ratio during the Iversen cycle), boreal brown-moss quaking fens and borealcontinental N-limited fens may represent relicts from glacial and early postglacial times. During our research in Romanian fens we further enlarged substantially the knowledge about distribution and ecology of rare and relict fen species such as Vertigo geyeri, Hamatocaulis vernicosus and Carex vaginata.

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Disentangling historical and ecological processes driving alpine species assemblages through an analysis of *Carex curvula* phylogenetic community structure across the European Alpine System

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Past events in Earth's history, including orogenesis processes and major climatic oscillations, have shaped both the evolution and spatial distribution of plants. In order to better understand these processes and how they contributed to the currently observed diversity across different scales, the most appropriate evolutionary systems to be chosen, besides the island archipelagos, are the mountains (or "sky islands"), characterised by alpine areas with a similar, insular-type, spatial configuration. In Europe, one of the most prominent evolutionary imprints is preserved by the European Alpine System (EAS), which encompasses several major ranges developed along longitude: the Pyrenees, the Alps, the Carpathians and the Northern Balkan Peninsula Mts.

Current developments in DNA sequencing and analytical tools allow us to obtain better inferences about the evolutionary relationships between species. Among such developments, the existence of well-resolved phylogenies became a major asset for ecological (Tucker *et al.*, 2017) and biogeographical studies (Laffan *et al.*, 2016), allowing more complex questions to be raised. Through the use of a genus-level phylogeny (Roquet *et al.*, 2013; Thuiller *et al.*, 2014) and different phylogenetic metrics (SES-PD, MPD, MNTD), we hereby aim to explore the main phylogenetic patterns found within the late-successional alpine communities dominated by *Carex*

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curvula, in the spatial context of the EAS. Our aims are to investigate and explain the (1) influence of regional species pool on the phylogenetic diversity within communities (alpha-diversity), (2) relationship between phylogenetic alpha- and beta-diversity among communities across the EAS, by applying range-weighted metrics (3) the influence of yearly snow coverage and distance between known glacial refugia and *C. curvula* communities' on their phylogenetic diversity, and (4) the role of environmental diversity and environmental filtering in shaping the phylogenetic structure of species assemblages.

Previous studies on C. curvula (Puscas et al., 2008a) focusing on its phylogeography and genetic diversity have shown contrasting patterns between the Alps and Carpathians, highlighting a recent history of postglacial recolonization in the Alps and Pyrenees and a long-term resilience of the species in the Carpathians and Balkans. Conversely, no relationship was found between community species diversity and genetic diversity of C. curvula across the EAS (Puscas et al., 2008b), possibly indicating a range of different assembly processes acting in the alpine system, in direct connection with the available regional species pool. However, these studies did not account for the total amount of evolutionary history encompassed by these communities. Consequently, phylogenetic diversity analyses within and between communities could potentially highlight different signals for C. curvula communities across the EAS than previously found, mainly influenced by range-restriction processes acting at regional scales and promoting phylogenetic endemism and by differential horizontal / vertical migration processes. Finally, this new perspective on C. curvula communities might strengthen previous findings or highlight new aspects of the postglacial history of the alpine siliceous vegetation within the studied geographical range.

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Genetic variation in *Isophya* species from the *Isophya pyrenaea* complex (Insecta: Orthoptera) in the Carpathians

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Genus *Isophyais* is one of the most species-rich genera from the European Orthoptera, comprising 97 taxons described so far, commonly known as plump bush-crickets.

Based on their morphology, most of these taxa are grouped in several species complexes. One of these is "*Isophya pyrenaea*" species complex with 14 species, some of them recently described (Warchałowska–Śliwa *et al.*, 2008, Iorgu *et al.*, 2017). All taxa from this group share a series of morphological traits such as: fastigium half as wide as scapus, male elytra narrow, as long as pronotum. The cubital vein forms an obtuse angle with the right margin of the right tegmen. The cerci are slender, incurved in the distal quarter and with an apical denticle. The female ovipositor is usually short and upcurved. Due to these similarities, the best way to identify each species is by analyzing the male species-specific song structure and the female's acoustic response (Heller *et al.*, 2004).

The species from this group have AN Eurasian distribution, mainly in the mountain ranges of the Pyrenees, the Alps, the Carpathians, the Balkans and the Altai Mts. They usually inhabit subalpine mesophytic meadows, dominated by dicotyledonous broadleaf plants.

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In this study we analyzed the genetic diversity of ten species within the *I. pyrenaea* group, using two mitochondrial and two nuclear gene fragments: cytochrome C oxidase subunit I (COI), 16s ribosomal gene (16s) and the internal transcribed spacers (ITS1 and ITS 2). A total of 170 specimens were sampled from 71 populations across the Carpathians. The specimens were later recorded, identified by morphology and song pattern and then genetically analyzed.

Our results revealed a high level of genetic variability between and within the analyzed taxa. We used different phylogenetic reconstruction methods, with *Isophya modesta* as outgroup and the resulted trees had similar topologies. Several major clades were revealed within the Carpathian populations, with sympatric lineages. Our data suggest that several species were formed by independent sympatric or parapatric speciation events from isolated populations of *Isophya camptoxypha*.

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Biogeographical patterns of *Carex humilis*-dominated rocky steppes in the Carpathian-Pannonian region

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Post-glacial fate of Central-European steppe grasslands is debated by paleoecologists and phytosociologists for decades. The fundamental question concerns the continuity of open habitats throughout the Holocene and especially during its critical period of maximum afforestation. In our study we used large phytosociological data to analyze recent vegetation patterns in rocky steppes dominated by *Carex humilis*, which are considered as relic vegetation of the cold Pleistocene steppes. We suppose that this vegetation was more widespread in Central Europe in the past and its development during the Holocene was strongly affected by forest expansion on the one hand and Neolithic farming on the other hand. While the high-altitude rocky steppes became isolated by gradual expansion of forests to higher altitudes in Middle Holocene, low-altitude rocky steppes remained interconnected within dry

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lowland regions throughout the Holocene until rather recent fragmentation by intensive agriculture and loss of habitats. We asked whether the current structure and composition of rocky steppes along an altitudinal gradient reflect their evolutionary history. The study area included four biogeographical regions: NW Pannonian Basin, West-Carpathian Mountains, Transdanubian Mountains and Transvlvanian Basin. The large compositional variation in both low- and high-altitude rocky steppes was better explained by geographical distance than by the environment. It might be the result of long-term retreat of rocky steppe vegetation, fragmentation and isolation of their habitats during the Holocene. We conclude that some of the indicated differences in floristic composition, chorological structure and diversity between the low- and high-altitude rocky steppes dominated by Carex humilis reflect their distinct evolutionary history rather than their current habitat conditions; i) set of constant common species with similar ranges and niches indicating common origin of this vegetation; ii) existence of local types regardless of altitude indicating temporal divergences during the history of this vegetation; iii) largest species pool and highest beta-diversity at middle altitudes reflecting climatic fluctuations during the Ouaternary Period; iv) decreasing proportion of archaeophytes at higher altitudes confirming strong role of isolation. The low- and high-altitude rocky steppes did not differ in species richness, species mean niche breadth and proportion of habitat specialists. These features might be controlled by recent habitat conditions.

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Phylogeography and landscape genetics of western capercaillie (*Tetrao urogallus*) in the Carpathians

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Population structure and barriers to gene flow are important components for understanding the evolutionary history of a species. Here we studied population structure and differentiation in the western capercaillie in the Carpathian Mountains, we compared them with the Balkans (Bulgaria) and the boreal forest (Russia and Sweden) in order to reveal past and current processes which may influence population structure. Analyses of mtDNA sequences revealed a Southern subclade within the Northern clade. Within the Northern clade, microsatellite data distinguished two groups: (1) Western Carpathian populations and (2) Eastern Carpathian and boreal forest populations. Bulgarian populations constituted a third cluster corresponding to the Southern phylogenetic subclade. The Western Carpathian populations showed a heterozygote deficiency, that might indicate historical processes of long term isolation of the Western Carpathians birds or limited geneflow within the island Western Carpathian birds. Therefore in the second part of study we focuse on geneflow and connectivity in the Western Carpathians. The mountain spruce forests of the Western Carpathians have experienced a dramatic deterioration in the last decades increasing the landscape fragmentation. This considerably affected western capercaillie population recently surviving within small habitat patches surrounded by unfavourable habitats. We use landscape genetic methods to investigate the effect of isolation by environment and Euclidean distance to gene flow among patchily distributed population of western capercaillie (*Tetrao urogallus*). We address the question of Euclidean

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distance threshold for gene flow in the fragmented landscape. The main objective of this study is to uncover the spatial pattern reflecting the landscape features in the genetic distances among individuals using microsatellite markers. We study elusive, umbrella, cryptic, sedentary grouse species currently persisting in highly fragmented landscape. Our study takes an advantage of the largest sample size ever included in capercaillie ecology and their landscape genetic structure in the Western Carpathians. Landscape genetic analysis confirmed that the isolation by human-dominated environment explains observed genetic patterns better than a linear geographic distance. We highlight urgent need for an active conservation management in the critical habitats where migration might be constrained or "bottlenecked" in order to ensure gene flow within the fragmented capercaillie metapopulation of the Western Carpathian mountain forests.

Role of high- and low-elevation postglacial refugia in preserving plant diversity: case of central European *Galium pusillum agg*.

Adam Knotek^{1,⊠} and Filip Kolář¹

Species preferring open heliophilous habitats with low competition in Central Europe are considered as relicts of open landscapes of Pleistocene/early Holocene while nowadays they contracted to small 'warming stage refugia' scattered across the landscape. Such habitats, spanning from lowland rocky outcrops up to subalpine grasslands, likely provide varying chances to survive, however, their relative contribution for preserving diversity is unclear. Central European members of *Galium pusillum* species complex provide suitable system for addressing this questions as they encompass six polymorphic taxa and several cytotypes distributed over various low-competitive grassland, rocky and open forest habitats over approx. 1700 m of altitudinal range. We would like to reconstruct evolutionary history of the *G. pusillum* members in Central Europe, infer origin of higher polyploids and estimate levels of overall and rare genetic and cytological diversity over different types of habitats.

DNA flow cytometry was used to estimate relative genome size/ploidy level in 70 populations. Sequencing of two non-coding cpDNA regions (212 inds. of 61 pops.) and AFLP genotyping (364 inds. of 58 pops.) have been employed to recognize major genetic groups. To test scenarios of polyploid origin we used R script *InSilicoHybridisation* from Winkler *et al.*, 2017.

Our results showed that both isolated lowland and more connected alpine habitats hold similar diversity which is high over the whole studied area and no true refugial regions were found, however serpentine habitats are slightly more diverse than limestone habitats. Analysis of AFLP profiles showed clear separation of diploid species, on the other hand two admixed groups were found for tetraploids species that are represented mainly by *G. valdepilosum* and *G. anisophyllon*. The level of admixture was noticeably higher in hercynian mountain ranges, e.g. in the Krkonoše

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Mts. where the populations are treated as a *G. sudeticum*. Best fitting scenario of polyploid origin suggest allopolyploidy, e.g. hybridization of diploid *G. valdepilosum* and *G. anisophyllon*.

Our results demonstrate that both lowland and alpine habitats are able to preserve diversity in a similar extent and deserves same priority in nature conservation. The Krkonoše Mts. endemic *G. sudeticum* is at least holocene relict, an unique evidence of hybridization where one of the parrents (*G. anisophyllon*) went extinct in the Czech Republic and its nearest occurrence is in the Slovak Carpathians. High genetic diversity in whole studied area makes *G. pusillum* agg. a good candidate for periglacial cryptic refugia, however further study will be necessary.

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Phylogeography of Carpathian plants above and below the timberline – case study of two *Arabidopsis* species with pronounced altitudinal ecotypic variation

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The evolutionary history of plant species, as can be inferred from the genetic structure of their populations, is still poorly known for the Carpathian flora. While (comparative) phylogeography has provided important insights into the spatio-temporal evolution of flora and fauna of other European mountains (especially the Alps), we still have fragmentary information from the Carpathians, that is largely based on investigations of plants inhabiting the highest, (sub)alpine habitats. In contrast, we lack information on plants inhabiting the area below the timberline and/or plants with a wider elevation span. 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 tomberline) mountain ranges, but in several areas they also reach the subalpine positions. By investigating evolutionary history of Arabidopsis arenosa and A. halleri populations by means of multilocus markers (AFLP or SNP) reveals surprising parallels in the evolution of both species. In particular, they confirm the main genetic barrier, already observed in the subalpine species, at the East and West Carpathian borders. but they also point 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 rare genetic diversity, but also a source of the populations that recolonized the northern parts of Europe.

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Hybridization as the pivotal source of variation and taxonomic confusion in Carpathian *Centaurea*

Petr Koutecký^{1,⊠}

The genus *Centaurea* is notorious for hybridization and taxonomic intricacy. Among infrageneric groups present in Central Europe, the section *Jacea* is most problematic. It comprises about 50 taxa (of a species or subspecies level, based on the taxonomic concept applied) in Europe and the Caucasus, of which about 20 taxa are present in Central Europe. The section is a polyploid complex with two dominant ploidy levels, diploids and tetraploids, and three minority cytotypes (tri-, penta- and hexaploids) that are found within populations of the dominant cytotypes due to occurrence of unreduced gametes and/or hybridization.

Previous as well as ongoing studies using crossing experiments and/or sampling natural mixed populations, flow cytometry and molecular methods have shown that the extent of hybridization strongly depends on ploidy levels of hybridizing taxa. Homoploid hybridization (both at the diploid and tetraploid level) is frequent and the hybrids are usually fertile and capable of backcrossing. In contrast, heteroploid hybridization is very rare. Heteroploid hybrids are either triploid or tetraploid. Triploid hybrid seeds arise more frequently that tetraploid hybrid seeds but the triploid hybrids are extremely rarely established in natural populations due to reduced growth and are sterile. In contrast, tetraploid hybrids are vigorous and fertile. Thus, if heteroploid hybrids are found in the wild, they are usually tetraploid. Their fertility may allow unidirectional gene flow from diploids to tetraploids and even formation of triple hybrids with other tetraploid species.

Individual taxa of the sect. *Jacea* has usually small to medium-sized distributions and are often allo- or parapatric, except for the widespread tetraploid *C. jacea* sensu lato. The Carpathians are one of the few "melting pots" where several taxa of the same ploidy level come into contact and may form extensive hybrid zones.

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Phylogeography of common primrose (*Primula acaulis* Huds.) and the role of the Carpathian Basin in the colonisation of Europe

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Primula acaulis Huds. (syn. P. vulgaris Hill) has a distribution from the Caucasus to the British Isles and from Norway to North Africa. We investigated the postglacial colonisation routes of this Atlantic-temperate species using a phylogeographical approach on the whole species area by paying a special attention to the Carpathian Basin, where the distribution pattern were previously interpreted based on the hypothesis of 'pincer migration', i.e. the postglacial colonisation occurred along a western, the 'Illiric-Noric', and an eastern, 'Dacian' migration routes (Hendrych, 1996; Varga, 1964). To test this – which implies the existence of refugia on the S European peninsulas - we used a molecular phylogenetic approach as outlined by Volkova et al. (2013). Nuclear ribosomal ITS and plastid IGS (trnL-trnF, rpl32-trnL) were sequenced then ribo- and haplotype networks were reconstructed. In case of populations located in the Carpathian Basin ("local dataset"), we supplemented the previous dataset with SSR analysis (14 loci specifically designed for this species) and geometric morphometry (Hangle-Fourier morphometric outline analysis). Finally, we assessed migration rates between the main geographic areas. Surprisingly, the Black Sea Coastal region (i.e. SW Caucasus) and the Carpathian Basin shared the same, basal ribotype. Populations located in W Europe and in the Carpathian Basin share the same haplogroup, while the basal haplotype is situated in NE Black Sea Coast. The local dataset did not

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bring any evidence of the existence of the 'pincer-migration' routes in case of *P. acaulis*, partly because of apparent inbreeding within many populations. The (postglacial?) colonisation did not take place from S Europe. A 'secondary' centre of Caucasian ribotypes is in the Carpathian Basin, which corresponds to previous results of Bartha *et al.* (2015). Our results support that the colonisation of Europe occurred from the Carpathian Basin, while the primary refugium was in the Colchis region of the Caucasus Mts.

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Conservation status of plant species and habitats of community importance on the Romanian Carpathians

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The conservative status of the plant species and habitat types which occur in the Alpine biogeographic region (ALP) were presented, based on available data that were resulted from the first monitoring of conservative status of the species and habitats of Community interest from Romania. This has been achieved during the 2007-2012 monitoring period as an obligation arising from Article 11 of Habitats Directive (HD) in order to report in 2013. The results were summarized, analysed and reported to the European Commission according to Article 17 of the directive that requires Member States to report every six years the progress made with the implementation of the HD.

The ALP overlaps all mountain ranges from the Carpathian range and occurs in 13 EU countries (31.1% of total terrestrial area of EU) extends over 20% of the Romanian territory, which represents 8.6% of EU territory and around 6% of entire surface of ALP at the Europe continent level.

According to latest reference list of ALP from Romania are found 21 plant species and 49 habitat types, as following: freshwater habitats (5), temperate heath and scrub habitats (5), grasslands habitats (11), bogs and fens habitats (7), screes and rocky habitats (6), cave habitat (1), and forests habitats (14). From all these habitat types 17 occur only in the ALP as follows: freshwaters (2), temperate heath and scrub habitats (4), grasslands (4), bogs and fens (3), screes (1), forests (3). Another

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24 habitat types occur both in ALP and Continental biogeographical region (CON) from Romania, but several of them are located only in Carpathian System – mountains range and highlands.

The assessment of conservative status of all plant species and habitat types was carried out following the methodology agreed by the European Commission and the Member States that is based on separate evaluation of four parameters which define the "Favourable Conservation Status" given in the HD.

For the plant species, the results show that the conservative status is "Favourable" for 13 species, "Unfavourable inadequate" for 15 species, "Unfavourable bad" for one species, and "Unknown" for none.

For the habitats, the results show that the conservative status is "Favourable" for 35 habitat types, "Unfavourable inadequate" for 10 habitat types, "Unfavourable bad" for 2, and "Unknown" for 2 other. For all seven bogs and fens habitat types the conservative status is unfavourable but is favourable for all 10 grassland habitat types. Regarding habitats that occur only in ALP the conservative status is "Favourable" for 12 habitat types (1 freshwater, 4 heat and scrub, 4 grasslands, 1 rocky habitat, 2 forest habitats), "Unfavourable inadequate" for 3 habitat types (1 freshwater, 1 bog and fen, 1 forest), "Unfavourable bad" for one bog and fen habitat type, and "Unknown" for another bog and fen habitat type.

Due to this biogeographical junction and the great heterogeneity of the landscape within its territory, Romania has a great diversity of plant species and natural habitats. A lot of these were included in Natura 2000 European ecological network.

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Diversity and distribution of Carpathian subterranean fauna

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Eastern Europe is one of the richest regions in the world in subterranean (caves and associated habitats) endemic species. Although considered as one of the main refugial area for several groups of surface fauna during the climate changes of the Pleistocene and the Holocene, the Carpathian Mountains subterranean fauna importance is underestimated especially due to dispersed information on its diversity and the scarcity of molecular studies in the area. Here, we present a first general view of the cave fauna hotspot represented by the Carpathians and the geological and historical processes that shaped the patterns of subterranean distribution and diversity at regional scale. Phylogeography of Coleoptera and environmental parameters are adding to the general view at regional scale and offer additional explanation for this exceptional subterranean diversification in a non-Mediterranean region. The Carpathians are an amalgam of various geological units with different paleogeographical evolution that is reflected in completely different species assemblages dominated by unit specific fauna groups.

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The importance of the Carpathians for understanding of evolutionary processes and biodiversity patterns in the genus *Hieracium* L. s.str. (Asteraceae)

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Hieracium L. belongs to the most diverse angiosperm genera in the world due to a large amount of morphological and molecular variation likely caused by massive interspecific hybridization in the past (Zahn 1921-1923, Fehrer *et al.*, 2009). This process has been tightly coupled with polyploidization and shift to apomixis – asexual seed reproduction – which has assured the reproduction and thus persistence of otherwise sterile interspecific hybrids. Sexually reproducing diploid taxa have undoubtedly played a crucial role in creating new apomictic polyploid lineages of hybridogeneous origin. It has been suggested that apomixis is obligate or nearly obligate in polyploid *Hieracium* taxa.

In our talk we will show that based on our exhaustive search of published and unpublished chromosome counts / ploidy level estimations, the overwhelming majority of *Hieracium* taxa are tri- and tetraploid, whereas diploidy has been found only in a few species. Except for the widespread *H. umbellatum*, diploid taxa have restricted distribution and are usually geographically and / or ecologically allopatric. This efficiently prevents, or at least severely limits, current interspecific gene flow. The geographical distribution of diploids is very uneven, being concentrated in the mountain ranges of Central and Southern Europe including the South-Eastern Carpathians. This pattern thus represents a nice example of so-called geographical parthenogenesis – a pattern where sexual diploid taxa occupy more restricted ranges,

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which are shifted towards southern latitudes when compared to more widespread polyploid asexuals occurring also at high latitudes.

Specifically, the South-Eastern Carpathians are an important evolutionary center of the genus. In this mountain range, several diploid taxa have evolved or found their refugium during the Pleistocene. This pattern has recently been corroborated by a discovery of a new diploid species for science. Karyological and molecular analyses not only confirmed its species status, but furthermore revealed that this narrow endemic taxon has been involved in the origin of at least one allopolyloid taxon with very peculiar morphology. In addition, in this part of the Carpathians, some co-occurring populations of diploid species can be found where interspecific gene flow is still ongoing (Mráz *et al.*, 2011). Furthermore, we have an evidence for recent natural heteroploid hybridization and presence of apomictic populations which are still able to produce some progeny by sexual pathway. These findings thus stress the importance of the South-Eastern Carpathians for studying evolutionary mechanisms involved in the evolution of the genus *Hieracium* in particular, and of apomixis in general.

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Evolution of high mountain plant species in the Alps and Carpathians – the "hairy" case of the *Doronicum clusii* aggregate (Asteraceae)

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With its high degree of endemism in both animals and plants, the European Alpine system is an excellent region to study speciation modes related to Pleistocene glaciations. A well-suited system to address such questions is the monophyletic *Doronicum clusii* aggregate (Asteraceae). It comprises four taxa endemic to the Alps and the Carpathians, which are differentiated geographically, ecologically (basiphilous versus silicicolous), by their ploidy levels (diploid versus tetraploid) and can be morphologically distinguished by hair characters. The silicicolous diploid *D. clusii* is distributed in the western and central Alps and the silicicolous tetraploid *D. stiriacum* (= *D. clusii* subsp. *villosum*) in the eastern central Alps (outside the range of *D. clusii*) and the eastern and western Carpathians. The basiphilous diploid *D. glaciale* subsp. *glaciale* is known from the eastern Alps and *D. glaciale* subsp. *calcareum* (= *D. calcareum*) from the northeastern Alps (Pachschwöll *et al.*, 2015).

In order to infer phylogenetic relationships, origin of polyploids and phylogeographic history, three plastid DNA regions (*ndhF-rpl32*, *rpl32-trnL*, *rps16-trnK*) and one nuclear DNA region (ITS) were sequenced, including molecular cloning of ITS. AFLP fingerprinting data were generated from 58 populations. Furthermore, DNA ploidy levels were estimated, and for selected individuals, absolute genome sizes were measured and chromosome numbers counted (Pachschwöll *et al.*, 2015).

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Molecular data identified three lineages corresponding to *D. clusii*, *D. stiriacum* and *D. glaciale* sensu lato. *Doronicum clusii* and *D. stiriacum* were genetically more strongly separated than expected from morphology. *Doronicum stiriacum* is exclusively tetraploid. The ploidy level only known from the Carpathians was confirmed for the Eastern Alps (Pachschwöll *et al.*, 2015).

A novel method to infer the origin of polyploids with AFLP data was used to clarify the polyploidisation mode of *D. stiriacum* (Winkler *et al.*, in press) as Pachschwöll *et al.* (2015) could neither exclude auto- (from *D. clusii*) nor allopolyploidy (*D. clusii* \times *D. glaciale*). The new results suggested a preglacial (based on macrofossils of the western Carpathians foothills), allopolyploid origin of *D. stiriacum* from *D. clusii* and *D. glaciale* in the Alps with subsequent migration to the Carpathians, although one would intuitively assume autopolyploidy based on overall morphological similarity. The parental species *D. clusii* and *D. glaciale* hybridize in their overlapping distribution ranges in the central Alps, especially on intermediate bedrock. The existence of such hybrids (*D. ×bauhini*) was confirmed by AFLPs and (cloned) ITS sequences (Pachschwöll *et al.*, 2015).

Doronicum glaciale subsp. calcareum is morphologically and genetically only weakly separated from *D. glaciale* subsp. glaciale but exhibited significantly higher genetic diversities and rarities (both derived from AFLP data). It likely survived Pleistocene glaciations in the northeastern-most Calcareous Alps, a prominent refugial area. The genetically depauperate *D. glaciale* subsp. glaciale might be the result of progenitor-derivative differentiation from D. glaciale subsp. calcareum followed by westward expansion. In the Hochschwab mountain range in Styria, transitional forms between these two taxa are common. Taxonomically, the Austrian endemic *D. glaciale* subsp. calcareum is best treated as subspecies (Pachschwöll et al., 2015).

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Vegetation and landscape variation of Western Carpathians during Late Glacial and Holocene

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Current distributions of species and habitats at large geographic scales is a result of ecological conditions and historical processes. We have investigated more than 100 paleoecological localities through the Western Carpathians (Jamrichová et al., 2017) covering environmental diversity and time span from Late Glacial to modern time. Analysis of such big dataset revealed that since the Late Glacial, the landscape has been differentiated into temperate, continental and cold regions. Pollen of temperate trees occurred predominantly in humid but relatively warm mountains during the Late Glacial, which might act as glacial refugia of temperate forest species. Lowlands and leeward basins might act as postglacial refugia of steppe grasslands (Petr et al., 2013). The beginning of the Holocene showed a great contrast between landscapes encroached by deciduous temperate forests and more open steppe-tundra landscapes. While in the Pannonian Lowland, the Holocene vegetation openness was largest at the Holocene onset, some Carpathian regions experienced well-developed deciduous forests. Since ca 8600-8000 BP, climate moistened in Central-Eastern Europe resulting in forest spread (Hájková et al., 2016). Neolithic colonisation that was particularly abundant just in Pannonian lowland and Spiš Basin largely utilised the landscape pattern that existed before and maintained light-demanding species pool. We demonstrate that in the Western Carpathian-Pannonian interface, the contrast between deciduous-forest landscapes and grassland landscapes (Hájek et al., 2017) was established at onset of the Holocene. Hence, landscape diversification during this period would be a meaningful historical factor for explaining recent species distributions. Spruce

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forests in most parts of central Slovakia resisted from beech expansion during 5000-4000 BP. It could be caused by harsh mountain climate, which prevented human settlements. Recently, these areas display short and cold summers what could determine the boundary between taiga and temperate forest biomes in late Holocene. Since 750 BP, strong colonisation waves affected the Western Carpathians. Here we demonstrate that their effects were indeed more pronounced in the Carpathian mountains. Geographically, (post)medieval colonisation wave disrupted totally the past gradient and through deforestation formed more patterned landscape than macroclimate or tree migrations did previously. The inter-regional differences in Holocene development may hence support some species to survive in geographically constrained refugia and create uncommon distributional pattern that is otherwise difficult to explain by recent environmental conditions.

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Changes in vascular plant diversity within the alpine zone of the Eastern Carpathians: 15 years of continuous survey of the GLORIA summits in the Rodna Mountains (2001-2015)

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In recent years, an increasing number of studies showed that the flora and vegetation of high mountains is reacting in a very sensitive way to climate change. The severity of such changes in the alpine ecosystems can be documented only by *in situ* long-term monitoring. The GLORIA Multi-Summit Approach Project (www.gloria.ac.at) provides the framework of a world-wide long-term observation network with standardised, user-friendly and cost-efficient monitoring scheme to track the influence of climate change on local plant diversity (Pauli *et al.*, 2015).

In this study we focus on four summits above the treeline in the Rodna Mountains (Eastern Carpathians, Romania), which were comprehensively surveyed following the standardized GLORIA protocol, from the highest elevation point down to the 10-m-horizontal contour line around the summit. The first floristic survey was done in 2001. During the first monitoring cycle (2001–2008), there has been observed a 'thermophilization' and increase in species richness (Gottfried *et al.*, 2012; Pauli *et al.*, 2012; Puşcaş *et al.*, 2013) as a response to temperature changes for the study area (+0.8 °C for multi-annual Tmean).

To further expand the research in the area with new monitoring periods, our present discussion aims to look at the last cycle between 2008 and 2015. This corresponded to the warmest period ever recorded for this part of the Rodna Mountains (data from the lezer Weather Station, 1798 msm): Tmean + 1.43 °C compared to 1961–2007, with absolute historical maximum in 2014 for annual Tmean (3.9°C).

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The percentage of 'mountainous', 'treeline' or 'treeline-low-alpine' species categories found in 2015 increased even more from 2008, on the Buhăiescu (2221 msm, + 7.3%), Golgota (2116 msm, + 1.2%) and Gropile peaks (2063 msm, + 4%). This trend was also revealed within the first GLORIA monitoring cycle (2001–2008), translated into a 'thermophilization' phenomenon. However, no such changes were detected on the Rebra summit (2268 msm, the highest GLORIA summit in the Rodna Mts.) during 2008–2015. Remarkably, on the lowest two summits, we observed an increased number of juvenile trees and saplings of *Pinus mugo*, *Picea abies* and *Juniperus communis* subsp. *nana* on different summit area sections than recorded before. In conclusion, our results bring even stronger support to the 'thermophilization' processes observed for the previous survey cycles. Although vegetation changes in alpine zones would require even longer monitoring periods, our results might also already indicate an upward movement of the forest boundary within the Rodna Mountains.

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A database and atlas of endemic vascular plants of the Carpathian Region

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The study of endemism, which reflect both a biogeographic and an evolutionary phenomenon, can help to reveal the important historical and ecological aspects of the flora of a region (Hurdu et al., 2016). Based on a critical revision of published and unpublished data, a survey of vascular plants endemics of the Carpathian Mountains has recently been published (Kliment et al., 2016). To bring together this recent taxonomic knowledge with spatial distribution information, we have developed a relational database. It contains the taxonomic catalogue of the species and subspecies considered in the survey as endemic for the Carpathian Region (e.g., scientific name, vernacular name, and family name) together with links to digitised herbarium collections, photographs of in situ specimens and their representative habitats, relevant literature sources, habitat preferences, and functional traits data. It also holds the spatial distribution information for all the taxa across the operational geographic units (OGUs) delimited in the Carpathians. Finally, in the attempt to communicate both to the scientific community but also to the general public, a web-platform is being developed. Based on the development principles of a former project (www.cushionplants.eu/), the aim of this web-application is to valorise the

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exhaustive knowledge over both historical and ecological data encompassed in the database, with the final aim to deliver this information to people interested in endemism, ecology or, more generally, in the Carpathians' flora and enhance the interdisciplinary character of future biogeographic studies in the region. Further developments would also allow contributors to enter different types of data (e.g., occurrences, traits data or pictures).

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Diversity and distribution of nivicolous myxomycetes (Amoebozoa) in the Carpathians in the larger geographical context

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Myxomycetes (plasmodial slime moulds) are phagotrophic eucaryotic organisms that commonly occur in associations with decaying plant material in terrestrial ecosystems. They belong to the supergroup (kingdom) Amoebozoa (Baldauf et al., 2000) where they form a monophyletic group (Fiore-Donno et al., 2010b) characterized by the highest level of organization within the Amoebozoa. One of most spectacular ecological groups of slime moulds is formed by nivicolous myxomycetes. They occur, often abundantly, at the edge of spring-melting snow in the mountainous areas (Ronikier and Ronikier, 2009). The diversity and taxonomical assessment of nivicolous slime moulds is a recently well developing field and data from various areas become available. The Carpathians are, however, still poorly investigated compared to other main European mountain ranges. At present, there are nearly 80 species of nivicolous myxomycetes described worldwide (Lado, 2005-2017), while less than 30 species have been reported from the Carpathians so far. This figure however clearly results mainly from the underinvestigation of the area. Some data suggest that the Carpathians may be an important diveristy hotspot for nivicolous myxomycetes. The discovery of a possibly undescribed species closely related to Lamproderma retirugisporum from the Ukrainian Carpathians (Krivomaz et al., 2005) and report of a rare species, Lamproderma argenteobrunneum (Ronikier et al., 2010) may hold as examples. The aims of the presentation are to: (i) summarize available data on diversity and distribution of nivicolous myxomycetes in the Carpathians in the context of the global diveristy of the group, (ii) present recent results of investigation of nivicolus myxomycetes in the Polish part of the Carpathian range (Janik and Ronikier, 2016, unpbl. data).

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Evolutionary history of a high-mountain plant *Hypochaeris uniflora* (Asteraceae): the Carpathians as ancestral area and colonization source of the Alps and the Sudetes

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The Carpathians constitute the main mountain range of Central Europe and one of major elements of the European Alpine System, of high importance for extant biodiversity of Europe and its evolutionary history (Mráz and Ronikier, 2016). Phylogeographical studies focused on this area, intensified in recent years, allowed revealing some general patterns of intraspecific divergence and diversity and main barriers, along with a range of more idiosyncratic patterns (reviewed by Ronikier, 2011, Mráz and Ronikier, 2016). Current efforts should focus, apart from accumulating further data on large-scale patterns, on more detailed testing of barriers and contact zones and on attempts to better understand the spatial and temporal history that led to biogeographical patterns observed today.

In this study, we focus on *Hypochaeris uniflora* as a suitable model that fulfills several important prerequisites: it has a continuous distribution over the Carpathians, a wide altitudinal range, and a significant phylogeographical structure revealed in previous analyses (Mráz *et al.*, 2007); it can also profit from relatively large knowledge on time-calibrated phylogeny of the *Cichoriae* tribe (Tremetsberger *et al.*, 2013). We aim to further advance our knowledge on biogeographical history of the Carpathian mountain flora and in particular: (i) to attempt identification of the ancestral area of the species and (ii) spatial and temporal dynamics of its intraspecific divergence; (iii) to test the consistency of previously detected main phylogeographical breaks in the Carpathians and (iv) to refine knowledge on phylogeographical transitions between mountains of

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Central Europe. We based our study on genome-wide AFLP fingerprinting and sequencing of nuclear and plastid DNA loci, applied on a population sampling representing previously detected main phylogeographical groups, complemented by samples from biogeographically important areas not included in the previous study and by samples of several closely related taxa for phylogeny calibration.

All three data sets provided largely congruent results although likely reflecting differing temporal perspectives. Our results, especially the nuclear ribosomal DNA variation (ITS data), confirm an earlier hypothesis (Mráz et al., 2007) that the Carpathian populations are more variable and likely represent the ancestral area and source of colonization of other mountain ranges – the Alps and the Sudety Mts. Samples representing the latter range were included within the Western Carpathian phylogeographical group. Preliminary analyses of the sequencing data point at the South-Eastern Carpathians as the likely cradle of the species. Furthermore, ITS data revealed an admixture pattern in the contact zone between two deeply divergent lineages from the Eastern and Southern Carpathians. The main phylogeographical break located at the border between the Eastern and Western Carpathians remains fully supported by data including close populations (Bieszczady Mts. and Volovské Vrchy Mts.), which further demonstrates its biogeographical relevance. Additionally, our data do not support a hypothesis on anthropogenic origin (driven by historical Valachian migrations) of the lower-altitude populations at the eastern edge of the Western Carpathians but rather their natural establishment from nearby high-altitude mountain ranges.

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Notes on the evolution and biogeography of Carpathian members of the genus *Soldanella* (Primulaceae)

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Snowbells, the genus Soldanella (Primulaceae), belong to one of the most attractive mountain plants in the European continent. Members of this genus are characterized by an outstanding and complex morphological diversity accompanied by large ecological amplitude. Despite the long-standing interests of botanists in this genus, evolutionary relationships among species, taxonomic status of numerous taxa, and their genetic diversity were left unresolved. We focused here on the bio-ecological diversity of members of the genus Soldanella originated predominantly from the Carpathians and closely adjacent mountain ranges, namely, the Eastern Alps and the Hercynian Massif. We employed a combination of various methodological approaches including karvological, morphological, and genetic analyses. Comprehensive karvological investigations confirmed existence of two cytotypes in studied snowbells, namely dysploid 2n = 38 and euploid 2n = 40, and a minute absolute genome size variation. Obviously, cytotype and AGS diversification did not played important role in evolutionary history and speciation process of the genus. The RADseq technique (restriction site associated sequencing) was used to reveal phylogenetic relationships and genetic diversity of studied snowbells. Phylogenetic analyses revealed that taxa

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from the Carpathians and adjacent regions are segregated into two discrete, well delimited groups. Soldanella carpatica formed its own cluster unrelated to all other taxa from studied region. The rest of the species were co-clustered with south-eastern Balkan and south Apennine species. Carpathian taxa form several evolutionary lineages with various level of statistical support. Several species appeared to be polyphyletic. Using multivariate morphometric analyses we identified suits of morphological characters out of which at least a part can serve for clear delimitation of species. Preliminary morphometric analyses indicated that some of snowbells species are morphologically evidently distinguishable from other related congeners (e.g. S. *carnatica* or *S. montana*). However, there are also taxa with continual variation (e.g., S. major and S. hungarica) and their precise delimitation is more complex. It seems that our data do not support recognition of several, especially newly described taxa of snowbells (S. angusta, S. rugosa, S. montana subsp. gubalowkae, and S. tatricola). Results of our investigation clearly indicate that the real taxonomic identity of at least the Carpathian snowbell taxa needs to be critically revised and a new taxonomic concept needs to be proposed.

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Disentangling drivers of plant endemism and diversification in the Alps – a phylogenetic and spatially explicit approach

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The Alps have rich endemic flora and large portion of this endemic diversity is clustered in particular geographic areas. These hotspots of endemism have traditionally been explained in two ways: (i) these areas are "museums" of biodiversity in glacial refugia, and current floristic patterns are linked to survival-recolonisation dynamics during the Quaternary (Tribsch and Schönswetter, 2003), (ii) these areas are "craddles" of biodiversity in high elevations, and endemism is caused by increased speciation rate due to steep environmental gradients and free niches (Roquet *et al.*, 2013), or by specific reproductive and dispersal strategies of high mountain plants (Körner, 2003).

Using a spatially explicit model of endemism and "community phylogenetics" dealing with data uncertainty, we show that some areas of high endemism can be explained by presence of Quaternary refugia while others by high elevation. These different types of hotspots of endemism carry different signature in phylogenetic structure of local plant assemblages: Species assemblages of glacial refugia are phylogenetically overdispersed, suggesting that they constitute "museums" of biodiversity conserving pre-glacial flora. Species assemblages in high elevation hotspots are on the other hand phylogenetically clustered, suggesting that they constitute "craddles" of biodiversity shaped by higher speciation rate in high elevation flora combined with ecological filtering for dispersal capacities.

To shed more light on processes forming endemism in high elevation hotspots, we related endemism to species altitudinal optimum, ecological and functional characteristics. We also compared diversification dynamics of seven plant groups exhibiting high degree of endemism in European mountains, using phylogenies estimated from genome-skimming data. Our results suggest that some of high

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elevation lineages indeed speciate faster than their relatives. On the other hand, endemism in high elevation areas is formed by species dispersal characteristics, rather than higher speciation rates, suggesting that endemism-speciation relationship was erased by migration processes acting on shorter timescales.

Our results suggest that glacial survival on one hand, and the interplay of migration and speciation in high elevation areas on the other hand, generate similar amount of endemism and strong phylogenetic structures in local plant assemblages in the Alps. This stresses out that future studies of endemism or diversification in temperate mountain ranges should take in account glacial survival, species migration and speciation dynamics alltogether, as these three processes may all have comparable influence on floristic patterns. Interesting step forward would be confronting our results with similar study in the Carpathians, that were much less glaciated than the Alps, but were exposed to severe changes of humidity and temperature during the ice ages.

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=== ORAL PRESENTATION ABSTRACT ===

Genetic structure of *Doronicum austriacum* Jacq. (Asteraceae) in the Carpathians and adjacent areas: towards a comparative phylogeographical pattern of tall-herb communities

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Tall-herb communities in the Carpathians are formed by tall perennial plant species that create a distinct element of the mountainous vegetation. They develop in humid habitats with relatively nutrient rich soil from sub-mountain to subalpine zones. As the contemporary flora of the tall-herb communities consists of different geographical elements, there is a question of its formation and changes, especially in the connection to the climatic oscillations throughout the Pleistocene. Our previous phylogeographical studies from this kind of vegetation (*Cicerbita alpina, Ranunculus platanifolius*) unraveled a roughly consistent pattern pointing at existence of two main genetic groups within the European mountains (Stachurska-Swakoń *et al.*, 2011, 2012, 2013).

In the next step of our research heading toward a comparative phylogeography of the European tall-herb communities, we approach *Doronicum austriacum* (Asteraceae), another key species building tall-herb vegetation. It is a Central-European species with the centre of its distribution in the European mountains. In Poland it has also some localities in the lowland area beyond the Carpathians. Population samples from the Carpathians, Alps, Sudetes, mountains of the Balkan Peninsula and from the Polish lowland were used for a genetic structure analysis using AFLP fingerprinting and DNA sequencing. Results emphasize the significance of the history and biological features on the genetic diversity and differentiation within *D. austriacum*. They confirm the phylogeographical break between the Western and South-Eastern Carpathians

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as a major regional biogeographical feature but also indicate (in contrast to the phylogeographical structure found in other tall-herb species) a divergence of populations from the Balkan Peninsula.

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=== ORAL PRESENTATION ABSTRACT ===

Population demographic inferences of Scots pine along the Carpathians and the Pannonian Basin based on bioclimatic and molecular genetic data

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Quaternary climatic fluctuations caused dramatic changes in species distribution, with large-scale range shifts, population contractions, expansions and extinctions, as well as aggregation and disassociation of forest communities. Highly fragmented and isolated Carpathian Scots pine (Pinus sylvestris L.) populations experienced such events, which induced the modern-day genetic structure and phylogeographic pattern of the species. By applying combined approaches, including independent climatic and molecular data, we aimed to highlight historical demographic features of Scots pine along the Carpathians and the Pannonian Basin. Approximate Bayesian Computation (ABC) approach based on nSSRs (nuclear microsatellite markers) revealed congruencies with the bioclimatic data. MaxEnt historical distribution model analysis of temperature- and precipitation-related bioclimatic variables confirmed Late Glacial and early Holocene widespread distribution of Scots pine in accordance with palynological proxies. The detected increased probability of presence indicates that mid-Pleistocene geographically segregated genetic groups could have merged allowing gene flow and admixture. Modelling also highlighted large reduction in distribution and low probability of occurrence from the mid-Holocene period, leading to disjunction and fragmentation of nowadays isolated Scots pine populations.

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=== ORAL PRESENTATION ABSTRACT ===

Intraspecific functional trait variation and structure at a biogeographical scale: comparative analysis of two high-mountain graminoids co-distributed over the European Alpine System

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Functional traits have been used to demonstrate the linkages between plant resource uptake strategies and performance across environmental gradients. While general patterns of trait values, variation and structure have been largely examined across a wide range of plant species, using single mean trait values per species, evidence has been proposed that intraspecific trait variation can affect various ecological processes. Moreover, most studies accounting for intraspecific trait variation were focused on particular regions. Our study aimed to capture intraspecific trait variation across a large geographical area. We sampled populations of two widespread, dominant high-mountain graminoids: Nardus stricta from anthropogenic subalpine pastures and *Carex curvula* from alpine natural grasslands across the European Alpine System (the Alps, Carpathians, Pyrenees, Balkans and Sudetes). Due to their contrasting niche, we hypothesize that N. stricta and C. curvula show distinct mean trait values, structure and responses to environmental variables. In each population we measured vegetative height (Hveg), specific leaf area (SLA), leaf nitrogen and carbon content (LNC, LCC) and tensile strength (TS) from different individuals. Our results showed that N. stricta had significantly higher Hveg, TS and C:N, indicating taller individuals with tougher leaves and stronger mechanical

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resistance to grazing pressure. The higher SLA and LNC of *C. curvula* indicate adaptation to short growing season and low temperatures in alpine habitats. Despite divergence in trait means, individual trait values partially overlapped between species due to high intraspecific variability. The divergent trait responses to environmental parameters were presumably due to different ranges of environmental conditions in the subalpine vs. alpine belts. Both species exhibited the expected positive SLA – LNC relationship (structuring the first axis of variability). Correlation among other traits did not align so well with the leaf economics spectrum, *N. stricta* showing stronger trait coordination. We discuss these findings in light of the divergent ecology and distribution of *N. stricta* vs. *C. curvula*. Our results confirm the underlying importance of investigating intraspecific trait patterns and structure to provide insight into the processes that link species and ecosystems across large spatial scales.

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=== ORAL PRESENTATION ABSTRACT ===

Bog woodlands of the Western Carpathians – A unique ecological phenomenon in the transition of phytogeographical regions

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This study has been inspired by very close syngenetic relationships between raised bogs communities of the class Oxycocco-Sphagnetea, extrazonal Pinus mugo scrubs on peaty soils and slightly wooded bogs dominated by Pinus sylvestris and/or Picea abies, belonging to the class Vaccinio uliginosi-Pinetea sylvestris. The most important localities of the Western Carpathians (in Slovakia) with well-preserved relict fragments of the unique raised bogs complexes are situated in the northern part of Slovakia (the Upper Orava region near Slovak-Polish border and the Podtatranská brázda Furrow). Bog vegetation create mosaic of various ecological successional stages covering whole gradient of environmental conditions. The localities represent hotspots of specific mires communities which are valuable not only floristically by the presence of many relict species, but also from the geo-historic, biogeographic and landscape point of view. Where possible, a comparison with the past conditions (in the horizon of about fifty years) was made; and up to now knowledge about taxonomy, ecology, evolution and management has been summarized. Syntaxonomical position of extrazonal dwarf pine stands and bog woodlands dominated by Pinus sylvestris and Picea abies was sketched and discussed, as well. Our main goal was to find a satisfactory syntaxonomical solution for the unique stands with a different presence of various hybrids originating from the parental combination of *Pinus mugo* s. str. and P. sylvestris. We distinguished 5 associations within the class Vaccinio uliginosi-Pinetea sylvestris that represent woodland bog vegetation. Our syntaxonomical scheme reflects not only floristic criteria of individual plant communities but also the evolution of studied hybrid complexes and the sites' history.

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=== ORAL PRESENTATION ABSTRACT ===

Biogeographical limitations of alpine and arctic-alpine species in the Carpathians and Balkans

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Less mobile Alpine species (e.g. flightless females) are much more represented in the Carpathians than in the Balkanic high mountains (e.g. genera of the tribe Gnophini: *Charissa, Elophos, Glacies*). Species of humid subalpine-alpine grasslands are also much more represented in the Carpathians (examples of *Erebia*). The more mobile Alpine and Arctic-Alpine species (*Erebia* spp., Noctuidae) are, however, more represented in the highest Balkanic mountains with extended alpine zone. Thus, they may be seriously threatened by climate warming. The southern limit of such species is nearly without exception the Adamovic-line, i.e. the southern limit of the vertical zonation of Alpine type. Some Balkanic oreal species show close connections with parts of the Southern and/or Eastern Carpathians. While the Alpine and Arctic-Alpine species were connected also during the glaciations to the higher elevations (see: West-East subspecific differentiation), the Balkanic oreal species were more widely "downslope" distributed during the cold phases.

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Validation of the taxonomic status of *Onobrychis transsilvanica* Simk. (Fabaceae) through genomic SSR fingerprinting

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Onobrychis transsilvanica (syn. *Onobrychis montana* DC. var. *transsilvanica* (Simk.) Beck syn. *Onobrychis montana* DC. subsp. *transsilvanica* (Simk.) Jáv.) (Fabaceae) is an endemic high-mountain plant, confined to the Southeastern Carpathians. It shares close, yet controversial, taxonomic relationships and a strong morphological resemblance with the alpine allopatric species *Onobrychis montana* DC. Its unclear taxonomic status derives from the fact that, so far, many authors have considered, based solely on the morphological traits, this taxon to be either a valid species or a subspecies of *O. montana*. Our previous study (Băcilă *et al.*, 2015) represented the first attempt based on molecular markers to provide both phylogeographic and phylogenetic insights for this Carpathian controversial endemic species.

The present study employs seven SSR markers (initially developed for other legume species, but later successfully used for species from *Onobrychis* genus) to in-depth explore the taxonomic boundaries between the *O. transsilvanica* and *O. montana* populations. When compared to the cpDNA and AFLP previously performed analysis, the SSR markers conferred a higher resolution of taxa groupings, adding two more clusters to the previous assorting of *O. transsilvanica* populations. Another element of novelty resides in the intriguing grouping of *O. montana* populations from the Western Carpathians together with *O. transsilvanica* in the Neighbour-joining analysis. Nevertheless, despite those new uncoverings, the results led essentially to the same general conclusions.

The taxonomic split between *O. transsilvanica* and *O. montana* does not concur with the major break in the genetic structure of the studied populations. Thus, the main differentiation was attributed to the geographical defined groups of

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populations. The SSR genetic data may suggest either recent postglacial speciation with incomplete lineage sorting of ancestral polymorphisms, or a genetic divergence followed by a continuous glacial gene flow that ceased in the postglacial period.

Altogether, these data do not support species recognition for *O. transsilvanica* apart from *O. montana*. Therefore, and also in agreement with the most recent classifications, we consider appropriate its taxonomic rank as a subspecies of *O. montana*.

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Ecological aspects and genetic diversity of *Cypripedium calceolus* L. populations from Transylvania, Romania

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The spectacular orchid *Cypripedium calceolus* L. once widespread through Eurasia, has suffered important decline with countries where it completely disappeared (Luxembourg) or with only one (UK) or two (Netherlands) populations still present. In Romania, many populations of *C. calceolus* L., previously described within the Romanian Flora (1972), are no longer found. Recently, a population of *C. calceolus* near the city of Cluj-Napoca was rediscovered by the authors (Balazs *et al.*, 2016).

With the aim of contributing to the conservation of this rare and endangered orchid species, four populations of *C. calceolus* L. identified within Transylvania, Romania (Sovata – Mureş County and Vălul Miresei, Făget 1 and Făget 2 Cluj county) were investigated under ecological and genetic diversity aspects. Individuals within Făget 1 and 2 populations are spread as group of clumps, whereas within Vălul Miresei and Sovata they are randomized.

Molecular diversity was evaluated at eleven microsatellite loci (Minasiewicz and Znaniecka, 2014), final dataset being composed of 76 individuals.

The four analysed populations of *C. calceolus* from Transylvania showed a high level of genetic diversity, both in terms of heterozygosity and allelic diversity, compatibly with all the previous studies on *C. calceolus* (Brzosko *et al.*, 2002; Fay *et al.*, 2009). Differentiation among populations is not very high, as found in Poland using allozymes (Brzosko *et al.*, 2002) and even on wider geographical scales (Fay *et al.*, 2009). In general, low differentiation might be due to effective gene flow

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and/or dispersal among populations, or to persistence of ancestral variation. However, it is worth noting that when heterozygosity is very high, F_{ST} is biased downwards. Admixture in Romania is quite high in comparison to other European populations (data not shown), suggesting mixed ancestry, possibly from different post-glacial colonisation routes.

In order to infer the dynamics within *C. calceolus* populations more precisely, it is important to consider demographic factors and the complex ecology of clonal reproduction. Consequently, perspective studies on Romanian populations should be focused on combining genetic data with demographic observations.

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Comparative plastid phylogeography of two deciduous forest geophytes (Scilla bifolia and Galanthus nivalis): implications to their glacial survival in the Carpathian Basin

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We attempted to explore the plastid DNA-based phylogeography of two widespread plant taxa (Scilla bifolia and Galanthus nivalis) in the context of the growing body of evidence for extra Mediterranean glacial refugia of deciduous forest species. One sample was analysed from more than 150 populations of both taxa by sequencing the *ndh*F-*rpl*32 and *rpl*32-*trn*L noncoding plastid DNA regions. The combined analysis of sequences under a Maximum Likelihood criterion provided moderately resolved but biogeographycally rather meaningful phylogenies. In case of Scilla bifolia the phylogenies recovered the so-called Anatolian. Caucasian. Transylvanian and 'non-Transyilvanian' lineages whereas in case of Galanthus nivalis the so-called Transvlvanian, 'non-Transvilvanian' and 'northern Italian' clades could be circumscribed. Geographic distribution of lineages is more clear-cut in Scilla when compared with Galanthus. The Transvlvanian Scilla clade is restricted almost exclusively to the northeastern part of the Carpathian Basin whereas the Transylvanian Galanthus clade is present westward of the Alps, in the Balkan Peninsula and also eastward of the Carpathian Basin. Our results highlight the barrier role of the Carpathians that should have been more emphasized during the Quaternary glaciations as well as the importance of the Eastern Carpathian Basin as a 'general' refugium for deciduous forest species.

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Towards clarifying the phylogenetic position and taxonomy of *Pedicularis baumgartenii* Simonk., a rare endemic species of the Southern Carpathians (Romania)

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Pedicularis L. (louseworts) represents the largest genus of the family *Orobanchaceae*. It comprises 600-800 species distributed mainly in the alpine, subalpine and arctic habitats of the Northern Hemisphere. Ten species are mentioned as occurring in the South-Eastern Carpathians, with *P. baumgartenii* being recognized as endemic.

P. baumgartenii is a high mountain species and was described by Lajos Simonkai in the late XIXth century (Simonkai, 1886) based on a herbarium specimen collected by Johann C. Baumgarten (1765-1843) in the Retezat Mountains (Mts.). The species was traditionally regarded as endemic of the South-Eastern Carpathians with the following massifs mentioned as places of occurrences: Ceahlău, Postăvaru, Făgăraş, Retezat, Țarcu Mts. (Sârbu *et al.*, 2013) and Căpăţânii Mts. (Pócs, 1963). Intensive floristic surveys of the past years confirmed the presence of the species in the Căpăţânii and Retezat Mts. and we seriously question its occurrence in the remaining massifs from where floristic data within the past century or any herbarium records are lacking.

We have sequenced the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA in two samples of *P. baumgartenii* originating from the Retezat and Căpăţânii Mts., respectively. The newly generated sequences were placed in the publicly available, ITS-based broad phylogenetic context of Tkach *et al.* (2014). Beyond finding unprecedented evidence for the phylogenetic position of P. *baumgartenii*, we

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also identified a relatively high, eight-nucleotide difference between the two samples analysed, although they were resolved as sister with maximal statistical support in our maximum likelihood phylogeny. The surprising genetic difference between populations of "*P. baumgartenii*" from the Retezat and Căpăţânii Mts. are apparently followed by morphological differences. Ongoing analysis of them will help to ultimately resolve the potential taxonomic differentiation within this species.

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Patterns of invertebrate diversity in several saline lakes from the Transylvanian Basin

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Numerous saline lakes formed by salt dissolution of halite (NaCl) deposits are found in the Transylvanian Basin. These lakes are dispersed inside the Eastern Carpathian arc and clustered in three different groups: (i) in the east: lakes located in Sovata (lakes Ursu, Aluniş, Verde, Roşu, Mierlei); (ii) in the south, in the Ocna Sibiu area; and (iii) in the west: lakes located in Coştiui, Ocna Şugatag, Ocna Dej, Cojocna, Sic, Turda and Ocna Mureş. Their origin can be either natural ("karstosaline") or as a result of salt mining activities ("anthroposaline") (Bulgăreanu, 1996).

The Transylvanian salt lakes do not exhibit homogeneous morphometric and abiotic characteristics. Even if the majority has small lake areas and great depths (Alexe, 2010), they differ in salinity, from brackish (total dissolved solids - TDS: $0.5 - 30 \text{ g L}^{-1}$) to moderately saline (TDS: $30 - 40 \text{ g L}^{-1}$) and hypersaline waters (TDS > 40 g L⁻¹). Lakes with significant depths develop stable density stratifications with steady vertical gradients of water temperature, dissolved oxygen, salinity etc. Overall, most permanently stratified Transylvanian salt lakes are best known for their heliothermal characteristic and for massive deposits of sapropelic mud with therapeutic value.

The invertebrate diversity of these salt lakes was poorly studied. Several species characteristic for these habitats were first cited in the taxonomical keys of the Romanian invertebrate fauna (Botnariuc and Orghidan, 1953; Damian-Georgescu, 1963, 1966, 1970; Negrea, 1962, 1983). More recent literature refers to a review on biota of several saline lakes from Romania, including invertebrates (Ionescu *et al.*, 1998) and a list of microcrustaceans from Maramureş (Forró and Kovács, 2008).

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The purpose of the present study was to analyze the invertebrate diversity in pelagic and benthic areas of several salt lakes from the Transylvanian Basin and to depict the patterns of diversity variance between these lakes. The following factors were hypothesized to influence the diversity differences in the lakes: (1) geomorphological factors such as lake surface, depth, origin, bank sinuosity; (2) abiotic factors (physical and chemical gradients, stratification); (3) biotic factors: presence of bank vegetation, total chlorophyll concentration; (4) time: seasonal dynamics and lake age; and (5) human impact: bathing, therapeutic mud exploitation, tourism.

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Vulnerability of *Pinus cembra L*. in the Carpathian Mountains under the Impact of Climate Change

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Climate change and human influence are the two most important aspects that can have evident impacts on the distribution of plant species. The purpose of this study is to investigate the effects of climate change and human intervention on *Pinus cembra L*., in order to identify strategies that can encourage natural regeneration process.

This study was conducted in the Southern Carpathians, the actual species distribution range and the measurements data were collected during 3 field trips, from the records kept by Parks administration and from prior research papers on the subject. The Species Distribution Model (SDM) and linear regression models were used to identify a suitable habitat and to predict a possible present, past and future distribution (Elith and Leathwick, 2009).

The results suggested that the *Pinus cembra L*. grows mainly on the eastern versant of the Southern Carpathians, on a slope between 11.3-16.7 degrees and a temperature of 2°C. The predicted suitable habitat is bigger than the actual distribution range and the climate predictions indicate an upslope shift of the optimal environment. The predicted paleoclimatic distribution for mid-Holocene reveals larger and continuous areas along the Southern Carpathians. Also, the relationships that are established between the diameter at breast height (dbh), tree height and elevation showed that most seedlings and saplings are growing at higher elevations of lower temperature and higher precipitation.

This study provides new information about the distribution of *Pinus cembra L.*, the vulnerability under climate changes, the possibility to be identified new locations where the species can spread. Furthermore, there is the need to focus on the elaboration of strategies that can support a better protection and regeneration.

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Notes on the ecological preferences of the rare root hemiparasitic plant Tozzia carpathica Woł. from the Romanian Carpathians

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Tozzia carpathica Woł. (Scrophulariaceae) is a rare root hemiparasite (Săvulescu, 1960), found in the Carpathians and in the southern Balkans, and whose populations are the most abundant on Romanian territory (IUCN, 2017). The ecology of this particular taxon has been very sparsely studied until recent years, when finding and mapping out its populations became a necessity given the fact that Romania became part of the Nature 2000 network in 2006 (Brînzan, 2013). With most of the data regarding this species being an adaptation of the data existing for its close ally from the Alps, *Tozzia alpina* L., who prefers Petasites, Adenostyles and/or Cicerbita plant communities (Mered'a and Hodálová. 2011). searches for it in such communities were unfruitful.

We presumed that there were three main causes for our insuccess, first being the rarity of the plant (EUNIS, 2017), second the biological cycle of the species (being a root hemiparasite it can be possible it has a short vegetation period) and third being the possibility of a different preference towards the plant community it grows in.

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Rare arctic-alpine plant species of the Ukrainian Carpathians: ecological aspects

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Flora of the Carpathians includes 121 arctic-alpine taxa of species and subspecies rank. The arctic-alpine elements of Ukrainian flora consist of 67 species, or 7,4% of the Ukrainian Carpathians highland flora. Out of those, 55 species have Holarctic distribution, 3 species – Eurasian, 6 species – Euro-American and 3 species are European. Arctic-alpine plants are confined mainly to the high mountains landscape, which include glacial landforms – cirques and rocky ridges of mountain ranges. Subalpine and alpine belts of Chornohora, Svydovets, Marmarosh and Chyvchyny massifs are the basic refugia for arctic-alpine plant species in Ukraine.

Among the arctic-alpine plants occuring in the Ukrainian Carpathians, there is a large part of rare species. For instance, 28 species are listed in the Red Book of Ukraine (2009). Out of them, 16 species are rare, 5 species are vulnerable, 5 are endangered, one is not evaluated and one is extinct in the wild in Ukraine. A lot of populations among rare arctic-alpine plants are represented by small number of individuals or small areas of their habitats. Such species are particularly vulnerable to exogenous disturbances and stochastic environmental changes.

The rare arctic-alpine plant species in the Ukrainian Carpathians are confined to the following types of habitats, in accordance with Annex 1 of the Habitats Directive: siliceous scree of the montane to nival belts; calcareous and calcschist screes of the montane to alpine belts; alpine and subalpine calcareous grasslands; calcareous fens; active raised bogs; petrifying springs; mineral-rich springs and spring fens.

Ecotopes transformation and changes in habitat properties are the most threatening factors for rare arctic-alpine species. Drying up of mesophytic meadows and highland bogs determine the reduction of area of the last *Pedicularis oederi* Vahl

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population in Ukraine, as well as of *Carex pauciflora* Light populations. Narrow ecological-coenotic amplitude of *Lloydia serotina* (L.) Reichenb. and overgrowing of its ecotopes by *Pinus mugo* Turra and *Alnus viridis* (Chaix.) D.C. due to rising of upper forest limit, leads to decrease the number of individuals in populations and reduction the habitats area. Shrub invasion into high mountain grasslands is also threatening for the last *Saxifraga aizoides* L. population in Ukraine. Therefore, it is important to control demutation processes in ecosystems to conserve endangered plant species and if it is necessary to take active protection measures.

It was determined decrease of some species' population viability due to intensive trampling. For instance, the habitat of *Saussurea alpina* (L.) DC. on Petros Mountain is exposed to grazing that causes a decrease of the population, breaks in flowering and reduction of individuals' vitality. *Anemone narcissiflora* L. and *Bartsia alpina* L. show lower generative reproduction coefficient, density, seeds production, and more active vegetative reproduction. Damages of grass cover and denudation of soil surface due to moderate anthropogenic influence stimulates overgrowth of *Loiseleuria procumbens* (L.) Desv.

Hiking trail which passes through the habitat of *Saussurea alpina* on Brebeneskul Mountain decreases the cover of the species and recovery index in population. Taking into account the exceptional value of this habitat where the single Ukrainian population of *Callianthemum coriandrifolium* Reichenb. exists, and rare *Rhodiola rosea* L. and *Ranunculus thora* L. grow, recreational impact on this territory need to be significantly reduced. This can be achieved by preparing the tourist route through the roundabout path, which runs below the habitat. It is important to eliminate human impact on populations with low vitality and on rare species.

Population genetic structure of Cochlearia tatrae Borbás (Brassicaceae) – a narrow endemic species of the Tatra Mts.

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In the mountain system of Europe, the Carpathians are an important centre of biodiversity. This results, among others, from their geographical position, extend, landscape heterogeneity, well-preserved environment and relatively low direct impact of Quaternary glaciations. For understanding the flora history of this area, contribution from phylogeography and population genetics provides an important insight. However, while knowledge on mountainous species with a wider distribution has been advanced during last years, little data are available regarding strictly endemic species of the Carpathians. Such species are important components of regional biodiversity and have spatially restricted, well delimited and often stable in time geographical ranges.

In this work, we focus on an alpine species *Cochlearia tatrae*, a narrow endemic species occurring within the Tatra Mountains (Western Carpathians). The main goal was to study its fine-scale phylogeographical structure in order to establish the genetic relationship between populations in a high-mountain landscape and attempt to estimate potential impact of past isolation in microrefugia. Furthermore, by placing the genetic variation of *Cochlearia tatrae* in a broader context, we aimed to verify the differentiation level of this endemic species. To this end, we applied genome-wide AFLP fingerprinting and sequence screening of non-coding DNA regions to a whole range sampling of *Cochlearia tarae*.

Our study showed no clear genetic structure in ranges of this species, which on the other hand the later was characterized by the high within-population genetic variability. However, a significant lineage differentiation was observed between

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populations from the Western and the High (Eastern) Tatras, which could indicate a past isolation in the two areas (presently divided by a gap in the species' distribution). Apart from this, the main factors influencing genetic variation of *Cochlearia tatrae* seem to be of biological nature, e.g. hybrid origin, outcrossing, long-lived individuals or overlapping generations. Lack of stronger genetic differentiation may support a relatively recent (neoendemic) origin of *Cochlearia tatrae* in the Carpathian flora. This additionally goes in line with the results of sequencing analysis, which did not reveal any haplotype diversity within the species and also a very weak differentiation between several Central European *Cochlearia* species indicating that the whole group of local polyploid taxa is evolutionarily young, likely formed in Pleistocene.

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Floristic analysis for the plant community growing on gypsum from the area of Sfăraş-Jebucu

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The gypsum from Sfăraş-Jebucu area is situated in the south-eastern part of Sălaj County and is considered one of the most important biological hotspots in Transylvania. The floristic richness of this area is due to the calcareous and gypsum substrate. The landscape is mostly natural, archaic and represents a refugium for some Carpathian and Transylvanian endemic species (*Thymus comosus* Heuff. ex Griseb. & Schenk., *Sesleria heufleriana* Schur., *Jurinea transylvanica* (Spreng.) Simonk., *Cephalaria radiata* Griseb. & Schenk, *Onosma pseudoarenaria* Schur.) and also for rare species encountered in Romania (*Gypsophila collina* Steven ex Ser., *Daphne cneorum* L, *Artemisia alba* Turra, *Salvia nutans* L, *Plantago argentea* Chaix, *Echinops ritro* subsp. *ruthenicus* (M.Bieb.) Nyman, *Astragalus monspessulanus* L., *Seseli gracile* Waldst. & Kit.). The most representative species for this area is *Gypsophylla collina*, which, according to Flora Europaea, is present in only a few spots in Romania, Moldavia, and Crimea.

In this area, we recorded until this moment 293 species, belonging to 46 plant families according to APG III classification system. A large number of these species belong to six families: Asteraceae, Poaceae, Fabaceae, Lamiaceae, Apiaceae, and Rosaceae.

The floristic analysis for the xerothermic flora growing in this area revealed some interesting aspects. From the bioforms analysis we found out that the most prevalent species are hemicryptophytes (53.58%), followed by annual (17.40%) and biannual therophytes (8.53%). The predominance of hemicryptophyte species suggest that the studied area is situated in a temperate climate.

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The geo-element analysis reveals the predominance of Eurasian species (44.48%), followed by European species (12.41%) and Central European species (10%), indicating the Central Europe geographic character of the studied area. Taken together, about 22% of the species encountered here are coming from southern regions and the presence of these geo-element categories highlights the particularity of gypsum rendzina soil which offers a favorable microclimate for these species.

The analysis of the ecological indices reveals that 47% of the species are xeromesophyle followed by mesophyle species (24.23%) and xerophyle species (19.11%). According to the temperature preferences, 58.7% of the species are mesotherms suggesting that they are better adapted to low temperature during the cold season. According to the soil pH reaction, the flora in this region has a low-acid neutrophilous character (56.65%).

Due to the richness and the peculiarity of the vascular flora encountered on the gypsum sediment from Sfăraș-Jebucu it would be justified to promote its official conservation.

Important drivers for lignicolous fungal diversity in beech and oak forests in North-Eastern Romania

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Lignicolous fungi are critical players in nutrient cycles in temperate forests (Juutilainen *et al.*, 2014; Küffer *et al.*, 2004; Lonsdale *et al.*, 2008; Zhou and Dai, 2012).

In North-Eastern Romania, as well as in temperate Europe (Kűffer *et al.*, 2004), beech and oak forests are one of the most important ecosystems as they support a great fungal diversity.

Fungal diversity is a key component of forest total diversity (Blaser *et al.*, 2013; Sefidi and Etemad, 2015).

Many researches highlighted different factors that influence the fungal lignicolous diversity, factors that varies from wood characteristics (Bîrsan *et al.*, 2014; Heilmann-Clausen *et al.*, 2005; Junninen and Komonen, 2004; Lassauce *et al.*, 2011; Sefidi and Etemad, 2015; Shi *et al.*, 2014) and climate (Salerni *et al.*, 2002; Shi *et al.*, 2014) to forest management history (Juutilainen *et al.*, 2014; Kűffer *et al.*, 2004; Zhou and Dai, 2012).

The purpose of our research was to identify the main drivers of fungal lignicolous diversity.

Therefore, we studied the relation between some biotic and abiotic drivers for lignicolous fungi from beech and oak dominated forests in plots of 2000 m^2 area.

In order to identify the most influential drivers for fungal diversity we used generalized linear models.

The most important factors influencing the diversity of lignicolous fungi were related with dominant trees, stumps and wood dimensions.

Therefore, in order to keep and enhance the diversity of this trophic fungal group, it is important to apply good silvicultural measurements (Lonsdale *et al.*, 2014), focused on deadwood and old trees management.

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Culturable diversity of heterotrophic bacteria isolated from Transylvanian salt lakes

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More than forty salt lakes with natural or anthropic origin are found at the periphery of the Transylvanian Basin (Central Romania). Despite their extreme salinity, these aquatic ecosystems appear to harbor diverse microbial communities that may drive full biogeochemical cycling of main elements (C, N, P, S). The aim of this research was to explore the diversity of chemoorganoheterotrophic, aerobic bacteria isolated from surface water or sediment of five Transylvanian salt lakes.

Water and sediment samples collected from salt lakes located in Ocna Sibiului (Fără Fund, Brâncoveanu) and Turda (Ocnei, Rotund, Tarzan) were used for the bacterial strain isolation. Sampling and strain identification followed the steps described by Baricz *et al.* (2015) for the analogous archaeal strain isolation.

Thirty two bacterial strains belonging to 8 genera (*Salicola* sp., *Salinivibrio* sp., *Halomonas* sp., *Chromohalobacter* sp., *Salimicrobium* sp., *Halobacillus* sp., *Staphylococcus* sp., *Marinococcus* sp.) were isolated from the sampled lakes. Highest number of bacterial isolates were retrieved from Fără Fund and Tarzan lakes and assigned to *Halomonas* sp., whereas *Marinococcus* spp. – the second most often isolated genus was obtained from Rotund Lake. Overall, the isolated bacterial strains were able to grow at 10% w/v NaCl on organic substrate under aerobic conditions. It was inferred that members of microbial communities dwelling the surface and sediments of the sampled lakes possess heterotrophic metabolism with implication in the aerobic step of biogeochemical C cycling in these saline systems.

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Cultivation of einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*) in the Carpathian Basin

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We examine history and spread, current use, breeding and cultivation of einkorn wheat (*Tirticum monococcum* L. subsp. *monococcum*), furthermore we would like to compare nutritional values of traditional and modern varieties of grain as well. The experiments have been taken in microparcels, in Nagygombos-Hungary. On the experimental area were sown 4 types of einkorn, 3 types of winter wheat, 1 type of spelt and 2 types of emmer. For measurement of nutritional values was used Mininfra-ScanT NIT Analyzer spectrometer in Szent István Univesity, Gödöllő.

Triticum monococcum species is subdivided into a wild (ssp. *aegilopoides*) and cultivated (spp. *monococcum*) subspecies (Hanelt, 2001). The demographic expansion has started with the warming, followed the last ice age about 13000 BP. The Old World agriculture was probably born in the Fertile Crescent between 12000 and 9500 BP because the natural resources were rare as a result of climate change and demographic pressure (Charmet, 2011). According to grain remains, T. monococcum subsp. monococcum was domesticated approx. from 10600 to 9900 BP during the Pre-Pottery Neolithic A or B periods in South-East Turkey – Karcadag Montains (Salamini et al., 2002). Einkorn spread from Middle-East to Caucasus, Balkans, Central and Mediteranean Europe, North-Africa and finally to Western and Northern Europe throughout the Middle Ages until the early part of the 20th century. In Romania, historical sources document significant einkorn cultivation during the 15th century (Péntek and Szabó, 1981). As a consequences of replacement by free-threshing wheat einkorn was completely disappeared in the Carpathian Basin during the 17th century except for mountainous and isolated regions in Transvlvania where its cultivation was maintained until the 20th century (Szabó, 1976).

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Nowadays the re-introduction of its cultivation has been promoted by organic farming among due to its high adaptation to low-input agriculture. Although it has a lower yield, but thanks to rusticity and adaptation to harsh climate can survive on poor, dry, marginal soils where other varieties of modern wheat can not. The species has a good tolerance to abiotic and biotic stress factors, being resistant to diseases and pests (Zaharieva and Monneveux, 2014). It was re-discovered as a healthy food because its nutritional benefits. The wild ancestor and landraces are valuable potential reservoir sources of genetic diversity, being useful for modern breeding methods. In Hungary, thanks to breeding programs, several cultivars ('Mv Alkor' and 'Mv Menket') have been developed by the Agricultural Research Institute in Martonvásár. We need to preserve the reservoir of wild species and landraces by gene banks and by in situ conservation as well as by recultivating the historical local varieties.

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Fire regime dynamics in south-eastern European grasslands (Romania)

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Fire drives significant changes in ecosystems structure, diversity and functions. While disturbances by fire are widely acknowledge to benefit tropical and North America temperate grasslands and dry woodlands, the effect of fire on temperate European grasslands and tree-grass dynamic is poorly understood. Prescribed burning has been proposed as a tool to manage European grasslands that are in markedly decline due to the afforestation of abandoned farmlands. However, little is known about the past fire regime, as this is limited to a few decades of observational studies derived from remote sensing. Moreover, in place with long human history, humans have greatly impacted global land cover through biomass burning and deforestation, contributing considerably to the grassland extension.

To better understand the effect of fire on temperate European open grassy systems and land cover changes we performed macrocharcoal (counts and morphologies) and pollen analysis on a-10 meter long core profile extracted in Lake Oltina, the southeast Romania, and present the first record of Holocene variability in fire regime, fuel sources and fire types in grasslands from south-eastern Europe. We also aim to determine which grassland / vegetation types in this region are more resilient to fire and how traits relate to fire resistance and regeneration affect species persistence. Our study aims to advance the knowledge of ecosystems behavior to fire dynamics in south-eastern Europe, a poorly studied region.

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The impact of climate change on vegetation cover in the Ukrainian Carpathians

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Biota is a sensitive indicator of climate change and it simultaneously suffers from these changes. It is important for ecologists to search correlative links between climate forming factors and other ones that determine ecosystem development. By applying of synphytoindication methodology (Didukh, 2011; 2012) and ordination analysis we conducted the evaluation of non-linear dependencies between changes of ecological factor values; among them the role of climate factors was reflected. We determined that indirect impact of climate through the change of hydrothermal soil regime and their chemical properties is stronger that direct one. Therefore, climate changes act as a trigger, which affects different areas of structure and function of ecosystems. In this context, it is more correct to talk about the effect of climatogenic changes.

The objects of research were the rare and adventive plant species, plant communities and habitats. Analysis showed that reducing of populations' size and extinction of rare species (the first category) are most probable in high-altitude habitats (*Anthemis carpatica, Antennaria carpatica, Astragalus krajinae, Carex rupestris, Oreochloa disticha, Saussurea alpina, Silene zawadskii, Trifolium badium, Veronica bellidioides* etc.).

The second category is represented by the species and habitats, whose development is connected to the change of hydrological regime determined by various kinds of economic activity. Nevertheless, climate change influences indirectly these

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processes (Anacamptis palustris, Carex bicolor, C. buxbaumii, Gentiana verna, Oxycoccus microcarpus, Saussurea porcii, Tofieldia calyculata etc.).

The third category includes low competitive species, which are related to unstable communities, intermediate succession stages; these species disappear when competition in the community increases (*Anacamptis pyramidalis, Dianthus speciosus, Erigeron atticus, Gentiana utriculosa, Nigritella carpatica, Poa rehmannii, Saussurea discolor, Saxifraga bulbifera* etc.).

At the same time, the river valleys become the ways of penetration for adventive species, when their functional regime is transformed. In riverside and meadow habitats there were noticed 12 invasive species, in forests (especially in willow-and-poplar forests) -11 species. A big number of agrio-epoecophytes indicates the increase of invasive level of natural plant communities.

By evaluating the threats' influence, the degree and rate of restoring of coenosis, its position in succession range, properties and ecological conditions of hemerobic indices, presence of invasive and rare species, correlation between types of strategy of floristic composition and other indicators, we calculated sozological significance, the influence of threat factors and the assessment of habitats loss. To the most threatened cathegory belong the high-altitude habitats, which are directly connected with hydrological regime, forest habitats under the pressure of unfavorable conditions, and the ones on the boundary of their area of distribution.

Fast-growth rate of spruce forests drying in the Ukrainian Carpathians since the beginning of the 21st century is assessed on the level of the national disaster. It was determined by a complex of natural and anthropogenic factors, of which interaction has synergistic effect (Didukh *et al.*, 2016).

The presented data are important for the development of measures for biodiversity conservation on the various levels of existence and prediction of possible changes.

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New records of *Tuber* species (Pezizales, Ascomycota) in the Ukrainian Carpathians

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Truffles (*Tuber* spp.) are ascomycetous hypogeous fungi belonging to Pezizales. They form ectomycorrhiza with a wide range of vascular plant species.

Some species of truffles have unique aromas and excellent flavours, and they are highly appreciated as delicacies and commercialized throughout the world. The most valuable and economically important species are *T. magnatum* Pico (the Piemont white truffle), *T. melanosporum* Vittad. (the Perigord black truffle), *T. aestivum* (the summer or Burgundy truffle), *T. borchii* (the bianchetto truffle) and *T. brumale* Vittad. (the winter truffle), but other *Tuber* species are edible and locally appreciated as well. Nowadays, most of these species have a flourishing market, are routinely and successfully cultivated and has diversified crops and incomes for local farmers.

The geographic distribution of truffle species mainly covers the temperate zones of the Northern hemisphere, with at least three areas of genetic differentiation – Europe, South East Asia and North America. In Europe, around 32 species are considered to be valid (Jeandroz *et al.*, 2008).

In Ukraine, there is only one truffle species known – T. *aestivum* Vittad. It forms symbiotic associations with *Quercus robur* and *Carpinus betulus* in Zakarpattya region. This truffle is listed in the Red Book of Ukraine under the category of "endangered". *T. aestivum* is distributed all over Europe and occurs in habitats over broad ecological amplitude.

During field sampling trips to montane forests in the Ukrainian Carpathians in 2014-2016, the second author found an ascomata of hypogeous fungi, which we later identified as *T. maculatum* Vittad. and *T. rufum* Picco. They belong to the light coloured truffles group and are recorded in Ukraine for the first time. Both of them are widely distributed throughout Europe, grow in different types of and form mycorrhizal associations with numerous species of broadleaf and coniferous trees.

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T. maculatum was found in valley of Chorna Tysa river (Rakhiv District) (N: 48.32119°, E: 24.2238°) in a forest where the species *Picea abies, Abies alba* and *Fagus sylvatica* grow together. This species is characterized by yellow ascomata with clearly labelled brownish spots on the surface, hyphal structure of peridium (prosenchymatous type), ellipsoid, thin-walled (1-4) spored asci and ellipsoid ascospores, ornamented with a regular reticulum formed by mostly hexagonal meshes numbering 5-12 along the spore length and 3-8 across the spore width. *T. maculatum* is among the more common *Tuberspecies* of Europe and North America and has been introduced to South America, New Zealand and Australia with *Quercus* seedlings brought from Europe.

T. rufum grows in Synevyr National Nature Park (N: 48.5313°, E: 23.644619°) in ectomycorrhizal symbiosis with *Picea abies, Abies alba* and *Fagus sylvatica*. Ascomata can readily be identified by their reddish to rufous peridium that is smooth to scabrous, and saccate to globose asci with elongated stalk, containing (1-5) elliptical to subglobose ascospores, ornamented with pointed spines 2-3 μ long. *T. rufum* is a truffle species that is common in countries where truffles are commercially important. Although *T. rufum* is commonly found alongside with other valuable species, it is considered to be a poor flavoured species with no commercial value and is deemed a 'contaminant' in cultivated truffles (Zambonelli *et al.*, 2016).

The finding of *T. rufum* and *T. maculatum* widen the list of the truffle species that occur in Ukraine. But a further revision of hypogeous specimens and more collections are clearly necessary before a full picture can be obtained.

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Full-glacial and Late-glacial forest dynamics in the Carpathian area

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The study of vegetation dynamics based on fossil records has long been and still remains a constant subject for specialists, in order to clarify certain aspects from the past but also to build scenarios for the future. It is known that the Carpathian Mountains are a major biodiversity area, with "hotspots", serving also as a refugium for various taxa during the Pleistocene glaciations (Fărcaș *et al.*, 2006, Feurdean and Tanțău, 2016, Jamrichová *et al.*, 2017).

In the current study we have attempted an exhaustive approach, both in what regards the study area (comprising the whole Carpathian mountain chain from Romania to the Czech Republic) and the time frame (the last glaciation, namely the interval Middle-Upper Pleniglacial – Preboreal). We have analyzed the records of pollen, plant macrofossils and charcoal from the sequences available within the European Pollen Database (EPD) and from literature.

During Pleniglacial cold stadials, the amplitude of glacial phenomena in the Carpathians was confined mainly to higher altitudes, and lower compared to the extent of the glacial ice sheet in the Northern and North-Western Europe. This has facilitated the survival of plant taxa in refugial areas, at middle altitudes, that offered tolerable climatic and micro-topographic conditions.

The appropriate sites from the Carpathian area were selected within a buffer zone of 200 km. In addition, selection criteria included the age of sites, supported by ¹⁴C datings, or at least mentioned by authors on the basis of scientific evidence.

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The accomplished database comprises site names, location (country, geographical coordinates, and altitude), also time frame of sequences as well as literature references. It also contains the main woody taxa mentioned from the selected periods, namely *Abies alba, Betula* sp., *Carpinus betulus, Corylus avellana, Fagus sylvatica, Fraxinus* sp., *Juniperus* sp., *Larix decidua, Picea abies, Pinus sylvestris, Quercus* sp., *Salix* sp., *Tilia* sp., *Ulmus* sp.

The data were used to generate distribution maps for these taxa in the selected time intervals. Their analysis revealed features connected with the glacial refugia of woody taxa, in relation to climate and glacier dynamics. The periods were selected in order to exclude human impact and allow the investigation of natural vegetation dynamics.

Both similarities and discrepancies have been noticed between the different units of the Carpathians, regarding the dynamics of the woody taxa, under the influence of latitude, longitude and regional climate particularities. This study strengthens the importance of the Carpathians as a refugial area for the woody taxa and for their postglacial migration to other areas of Europe.

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The effects of Holocene land use on habitat diversity and slope erosion in the subalpine landscapes of Northern Carpathians, Romania

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Land use in the subalpine areas, particularly grazing and fire, influences vegetation structure and composition parallel to the climate. Designated as biologically outstanding ecosystems in the Global 200 Initiative for their species endemism and habitat diversity, the Carpathian Mountains are among the terrestrial ecoregions most critically endangered by the impacts of human activities and climate change (KEO, 2007). Such threats are likely to result in a dramatic loss of biodiversity and habitat change, particularly affecting the highly diverse, endemic species rich subalpine pastures (Pauli *et al.*, 2012).

Here we employ a high resolution, multi-proxy palaeoenvironmental reconstruction (pollen, dung fungal spores, micro and macro-charcoal, sediment mineral magnetic properties and geochemistry) in two mid to late Holocene sedimentary sequences located in the present subalpine belt in the Rodna and Maramureş Mts, Northern Carpathians (Romania). We aim to: i) determine what aspects of human activity (e.g. burning, clearing, grazing) have shaped these subalpine landscapes; and ii) use this information to facilitate their environmental management and optimize ecosystem restoration strategies.

Results show that throughout the last 6000 years the current subalpine belt was subjected to anthropogenically - induced change, characterised by: i) enhanced catchment erosion induced by natural and anthropogenic fire between 5000 and 3000 cal yr BP, and over the last 1000 years; ii) increases in landscape openness after ca. 3000 cal yr BP, and particularly over the last millennium; iii) decrease in pollen richness/diversity during intervals with maximum grazing pressure and/or moderate to

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high biomass burning. Our results also suggest that low-intensity land-use practices (fire, grazing) on mountain pastures appear to be beneficial for subalpine grasslands resulting in the formation of rich mountain communities. Fire activity was further identified as a key driver of vegetation change at high elevations, mainly due to its use as a tool to enlarge and maintain subalpine grassland areas used for grazing.

Our reconstruction offers a greater understanding of the legacy of traditional land use management for vegetation and habitat change to improve our predictive capacity of future environmental changes in the subalpine and alpine areas of the Carpathian Mountains. Based on our findings, we argue that an effective strategy to maintain grassland openness and likely diversity in the subalpine areas of the Northern Carpathians is to promote low intensity grazing and burning.

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Subalpine species *Oreojuncus trifidus* (L.) Záveská Drábková & Kirschner in the Ukrainian Carpathians

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The genus *Oreojuncus* was described not long ago (Záveská Drábková and Kirschner, 2013). This genus includes two species: *Oreojuncus trifidus* (L.) Záveská Drábková & Kirschner (= *Juncus trifidus* L.) and *Oreojuncus monanthos* (Jacq.) Záveská Drábková & Kirschner (= *Juncus monanthos* Jacq.). The first of them grows in Europe, Western Siberia and eastern part of North America. In the Carpathians only *Oreojuncus trifidus* is widespread. *Oreojuncus monanthos* grows in Central Europe (Alps, the Balkans and the Apennines). In Ukrainian Carpathians *Oreojuncus trifidus* occurs in the subalpine zone of both Chornohora and Maramureş (Hutsulski Alpy).

At the same time, researchers pay a significant attention to features of the micromorphology of leaf and stem as a diagnostic criterion for distinguishing between taxons, identifying ecological characteristics of species. For the first time the anatomy structure of the leaf and stem of species of *Oreojuncus trifidus* in the flora of Ukrainian Carpathians was studied. Herbarium material collected during expeditions, samples from herbaria of the M.G. Kholodny Institute of Botany (KW) were used for the research. We have identified common features of leaf and stem for the studied species (types leaves, stomata paracitic regularly located, upper and lower epidermis of leaves and stems have well-developed cuticle, a common type of relief, single-cell papillae that are formed by outer periclinal walls are present on adaxial epidermis etc.). Thus, the anatomical structure of the stem is characterized by the well-developed

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core, chlorenchyma and thick-walled epidermal cells. Following anatomical features of leaf are of considerable interest: unimmersed stomata, compact arrangement of mesophyll cells, small cells of all tissues.

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Eastern Carpathians - a host for the red listed bryophytes

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Romanian red list of bryophytes comprises 374 species: 2 hornworts, 71 liverworts and 301 mosses (Stefanut and Goia, 2012). A large part of these species (87.70%) are occuring in the Carpathians: 128 species are Critical Endangered, 104 species are Endangered and 96 species are Vulnerable. Liverworts and hornworts represent 21% of the Carpathian red listed bryophytes and 79% are represented by mosses.

We expected a higher number of red listed species in the southern Carpathians, due to higher elevation and variety of microclimatic condition, but they host 59.15% of the red listed bryophytes. A higher value (63.41%) was found in the Eastern Romanian Carpathians. Apuseni Mountains host 33.54% of the red listed bryophytes, almost half comparing with eastern part of this mountain chain. This finding can be explained by their lower elevation, the lack of the alpine belt and the presece of small islands of subalpine vegetation only on two summits (Biharia and Vlădeasa Peak). Southern Carpathians host 78.26% of the hepatics. As expected, most of the species prefer habitats with a low human impact (74.69% ahemerobous, 80.31% oligohemerobous). This dependence of undisturbed habitats is higher for hepatics (82.61% ahemerobous, 86.96% oligohemerobous) than for mosses (73.68% ahemerobous, 79.75% oligohemerobous), perhaps as a consequence of their higher sensitivity. A percentage of 33.3% of the Romanian red listed hepatics and 28.57% of the red listed mosses are distributed in alpine and subalpine area, and cca. 4% of them are dependent of snow beds. Most of the red list species are recorded from the saxicolous substrate, followed by the terricolous species. Short lasting substrates (decaying wood, dung, dead bodies), usually display a lower species richness, with few stenotopic species.

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The species number is dynamic since new species are recorded for Romania every year and many areas are still white spots for the bryological inventories. On the other hand, the climate changes will impact alpine species, especially those dependent of late snow-beds, by decreasing the area of their suitable habitats. The monitoring of such habitats should bring new information about the bryophyte species strategies and will allow development of species - climate models as a tool for their conservation.

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Impact of climate change on the biodiversity of rare and protected vascular plants occurring in the high mountain areas of the Ukrainian Carpathians

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Biodiversity protection is an issue of particular relevance in the Ukrainian Carpathians (UC), where the high mountain areas have a limited extent.

During the years 2003-2016 the average daily temperature rose about 1,3 °C in the high mountain area of the UC. According to the altitudinal temperature coefficient (0.5-0.8°C per 100m) summer isotherms moved upward for approximately 200 m. As a direct effect, the air temperature increased, reducing the depth and duration of snow cover, increasing the length of the growing season and reducing precipitation during the year as well as the growing season. All these effects provide evidence of the climate change influence in the high-mountain areas of the UC. As climate warming causes accelerated vegetation zones shifts, the treeline and the limits of subalpine and alpine communities move upward. Consequently, low-competitive rare plant species are displaced by trees, shrubs and high-competitive common herbaceous species at the subalpine and the lower limit of alpine zones.

We found that in the high-mountain areas of the UC the trees, shrubs and dwarf shrubs significantly increased their covered area, numbers and population density. Among the investigated species were the following: *Picea abies, Pinus mugo, Alnus viridis, Juniperus sibirica, Rhododendron myrtifolium* and several *Vaccinium* species.

Under the influence of climate change, populations of many rare and endemic Red List plants are threatened degradation and extinction: *Aconitum jaquinii, Astragalus krajinae, Dichodon cerastoides, Erigeron alpinus, E. atticus, Festuca porcii, Gentiana laciniata, Leontopodium alpinum, Minuartia pauciflora, Primula halleri, Saxifraga*

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androsacea, S. carpatica, Saussurea alpina, Ptarmica lingulata, P. tenuifolia and others. In addition, negative population dynamics were recorded for petrophytes, heliophytes, hygrophilous and chionophilous species.

A high variety of rare and endemic herbaceous species in the subalpine zone occurs in rocky habitats and on abrupt slopes. These habitats were recently overgrown by shrub vegetation between 1400-1800 m asl in all high mountain ranges of the UC. In addition, in meadow openings, an increase of plant density and swarding of high-competitive common herbaceous species was observed. Moreover, a significant increase in shrub projective cover in the subalpine and lower alpine zones may have led to a massive reduction of populations of rare meadow species: *Anemone narcissiflora*, *Gentiana acaulis*, *G. punctata*, *Pulsatilla alba*. Similarly, there was a decrease in population density and covered area of several endemic species: *Heracleum carpaticum*, *Dactylis glomerata* subsp. *slovenica*, *Festuca carpatica*, *F. porcii*.

Coverage increase and altitude shift of *Alnus viridis* (green alder) presumably led to fragmentation of *D. glomerata* subsp. *slovenica* populations and isolation among groups of individuals. Conversely, *Pulmonaria filarszkyana* populations in the green alder community were putatively facilitated by this process, increasing their density and occupied area.

When vegetation recovers and competition between species increases, *Galium album* subsp. *suberectum*, an endemic of the Eastern Carpathians, occurred only in the most suitable habitats, while the number of populations decreased. Another endemic, *Galium pawlowskii*, is found in the Chyvchyny Mts. at the margin of its range, thus being more sensitive and vulnerable to rapid changes. Since the years 1995–1997 grazing in the subalpine meadows of the Chyvchyny Mts. was stopped and as a result of restoration of natural vegetation this species population regained their initial coverage and density.

Due to climate changes and vegetation dynamics, the amount of suitable habitats for many rare and endemic species in the high mountain areas of the UC is expected to gradually decrease as a result of grass displacement, tall forbs overgrowth and treeline upshift.

Chloroplast *trnL-F* region reveals several diversity hot spots for the arctic-alpine *Rhodiola rosea*

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Our study describes genetic lineages and historical biogeography of *Rhodiola* rosea, a widely distributed arctic-alpine perennial species of the Northern Hemisphere based on sequence analysis of the chloroplast *trnL-F* region, which is frequently used in phylogeography (Taberlet et al., 1991). Specimens of 44 localities from the Northern Hemisphere including the Alps, the Carpathians, the Pyrenees, Scandinavia. the British Isles, Central-and Eastern-Asia, have been sequenced and compared with those available in the Genebank. Altogether five indels were identified at 4 sites of the *trnL-F* region. Indels of 23 and 19 bp detected in this study were already known from this region (Cuerrier et al., 2015). Furthermore an indel of 12 bp was found close to the 3' end, which is a duplication and an insertion of 67 bp was detected alone or duplicated at the 5' end of this region. Our results support the migration of the species into Europe via the Central Asian highland corridor, reaching the European Alpine System (EAS) and also the western European edge, the British Isles. The EAS proved to be an important diversification centre of high genetic variation, specially the region of the Eastern Carpathians, the Eastern Alps and the Dolomites where glacial refugia might have had existed. Apart from those of the EAS, a common lineage was detected along the Atlantic coast from the British Isles towards Scandinavia as well as Iceland and the eastern parts of North America. Accordingly, the British Isles represent a main link between the northern Atlantic and southern EAS lineages.

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Vegetation variability of calcareous fens in the Eastern Carpathians

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Calcareous fens, critically endangered habitats harbouring rare and threatened species, form clear ecological and biogeographical gradients. Their typology, although important for conservation planning and referencing of the ecological studies, is complicated. In Romania, most of them have been assigned to the single association Carici flavae-Eriophoretum latifolii, contrary to other Eastern-Carpathian countries. During 1996-2014 we recorded vegetation data in the Eastern Carpathians (Romania, Ukraine, Slovakia, and Poland). All these data have been already utilised in the pan-European synthesis of fen vegetation (Peterka et al. 2017). Here we analysed only our own data to guarantee the same sampling protocol and sampling effort across all four countries. The unsupervised classification (Hierarchical Isopam) formed 8 clusters. In line with Peterka et al. (2017), the vegetation alliances previously unrecognised in Romania were delimited: Stygio-Caricion limosae in the Rodnei Mts and Saxifrago hirculi-Tomentypnion in Vlăhița, Tușnadu Nou and Sâncrăieni. Other clusters represented the *Caricion davallianae* alliance and its transitions towards grasslands (Molinio-Arrhenatheretea) and springs (Montio-Cardaminetea). Two of them have been identified as associations already reported from the Carpathians and two others corresponded with transitions to grasslands. In the next step we fixed the identified vegetation types as the cores in the subsequent semi-supervised PAM classification, with a possibility to create new clusters kept open. Five vegetation types of Caricion davallianae fens were finally delimited: (1) Schoenus nigricans fens (Hărman and Prejmer in Romania); (2) Tufa-forming or initial spring fens with Cratoneuron and Palustriella species, Carici flavae-Cratoneuretum filicini,

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occurring in Romania (with low-mountain and high-mountain subassociation), and rarely in Ukraine, and previously reported from the Western Carpathians and Bulgaria; (3) Peat-forming fens, Valeriano simplicifoliae-Caricetum flavae, dominated usually by Scorpidium cossonii, previously reported from Poland and Slovakia, occurring in northern parts of the Eastern Carpathians (Poland, Slovakia, Ukraine, and northernmost Romania); (4) Transitions to grasslands that may be either classified within Calthion (the Molinio-Arrhenatheretea class, Cirsietum rivularis eriophoretosum latifolii) or interpreted as Carici flavae-Eriophoretum latifolii s.s. (perhaps also according to quite dubious nomenclatural type), but contain no clear diagnostic fen species. (5) Ancient fens recorded close to Sânsimion, Vrabia, Botus and Bilbor (Romania), differentiated against all other Eastern-Carpathian fens by Hamatocaulis vernicosus, Carex dioica, Eleocharis auinqueflora and Carex diandra. They are dominated by Homalothecium nitens, S. cossonii, H. vernicosus and Campylium stellatum and contain rare relic species (Ligularia sibirica, Pedicularis sceptrum-carolinum, Primula farinosa, Carex *limosa*, and *Vertigo geveri*). Similar vegetation has been reported from northern Europe under the name Campylio-Caricetum dioicae, but Romanian vegetation is usually taller.

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Cryopreservation of endemic and rare Dianthus species

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The Carpathian Region (CR) and eastern Pontic Region (ePR) harbour a rich biodiversity with a remarkable percentage of endemic taxa from various taxonomic groups. Among them, the *Dianthus* genus (fam. *Caryophylaceae*) detains a high evolutionary and implicitly conservation importance value due to the large number of endemic and subendemic species occurring in the CR and ePR, but presents also a relevant economic importance due to its ornamental use (Jarda *et al.*, 2011).

Currently, beyond the general measures involving in situ conservation strategies through the network of protected areas, ex situ conservation strategies are feasible methods for saving plants from extinction, and for certain species the only possible conservation way (Ramsay *et al.*, 2000). Mainly, in vitro plant tissue cultures and conservation in botanical gardens have been successfully applied for the ex-situ conservation of some species belonging to this genus (Jarda *et al.*, 2011; Cristea *et al.*, 2013).

Within the current context of declining biodiversity, cryopreservation plays an essential role for the secure, long-term storage of rare and endangered species. More specifically, two main categories of elements are higly prone to be affected by rapid range shifts driven by climate change: (1) endemics and (2) populations found at the limit of their range. Therefore, the aim of our study was to develop cryopreservation protocols for seven representatives of this genus that fall within these two categories, taxa that were preserved in our active in vitro gene-bank collection: *D. callizonus*, *D. dobrogensis*, *D. glacialis* ssp. gelidus, *D. henteri*, *D. nardiformis*, *D. spiculifolius*, and *D. tenuifolius*.

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In vitro grown plants micropropagated for 2 years were used in cryopreservation studies. Shoot tips (apical dome with 2-4 leaf primordia) with 2-3 mm in length were excised from 2 months old in vitro plants and served as explants for cryopreservation studies. The excised shoot tips were precultured (for osmotic dehydration) for 24 h in sucrose (0.1, 0.25, 0.5, 0.75, 1.0 M) enriched liquid basal MS medium on sterilized filter paper placed in Petri dishes (5 cm diameter) in the same environmental conditions as mentioned for plant multiplication. For dehydration shoot tips were incubated in PVS2 (Sakai et al., 1991) solution for 20 min, at room temperature. For freezing shoot tips were individually transferred to a drop (6 µl) of PVS2 on sterilized aluminium foil strips (0.5 cm x 2.0 cm) and were transferred to 2 ml cryovials which were immersed in a 25 l Dewar flask. Samples remained in liquid nitrogen for 24 h. After storage in liquid nitrogen rewarming of samples was performed in liquid MS basal medium by transfer of the aluminium strips into the liquid medium at room temperature. For plant recovery after cryopreservation a semisolid (with 6 g l^{-1} agar) basal MS medium was used. The samples were maintained at 24±1°C under 16 h light/8h dark photoperiod. The regrowth after cryopreservation was recorded as percentages of shoot apices producing shoots 3 weeks after rewarming and transfer to regeneration medium.

The highest regeneration rate after cryopreservation was obtained for 0.25 and 0.5 M sucrose solution treatments (up to 66% regenerated apices). The high survival rate registered for these cryoprotective treatments, validate them as reliable and effective for long-term conservation of these unique species.

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Calcicolous subalpine vegetation of the Chyvchyny Mountains (Ukraine)

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In the Ukrainian part of the Eastern Carpathians, carbonate bedrocks with specific flora and vegetation are restricted only to few regions (Malinovsky, 1991). The Chyvchyny Mountains is a region with carbonate deposits and outcrops, so their vegetation cover differs in details from the other parts of Ukrainian Carpathians. The first vegetation survey of the study area according to the Braun-Blanquet approach was conducted in the 3rd decade of the XX century (Pawłowski and Walas, 1949). After that, subalpine calcicolous vegetation of the region has been studied only scarcely (Malinovsky and Kricsfalusy, 2000).

The aim of our research is to develop a classification scheme of the calcicolous herbal communities from the montane and subalpine belts of the Chyvchyny Mountains. A data set of about 850 phytosociological relevés was assembled, comprising either previously published surveys or performed by the authors during 2009-2013 in various parts of the study area. The data set was analyzed using JUICE 7.0 software package (Tichý, 2002).

As a result, the classification scheme of the calcicolous grassland communities in the Chyvchyny Mountains was developed and the characteristics of the distinguished syntaxa are hereby presented. The differentiation of the studied communities reflects several ecological gradients. The grassland communities belong to the class *Elyno-Seslerietea* Br.-Bl. 1948, while the submontane tall-forb communities – to the class *Mulgedio-Aconitetea* Hadač et Klika in Klika et Hadač 1944. Also, the communities

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of calcareous springs (*Montio-Cardaminetea* Br.-Bl. et Tx. ex Klika et Hadač 1944) and fens (*Scheuchzerio palustris-Caricetea fuscae* Tx. 1937) are distinguished. New association within the alliance *Delphinion elati* Hadač in Hadač et al. 1969 is preliminary described. Finally, the studied vegetation is compared with the calcicolous communities from the other parts of the Carpathians.

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Centipede species diversity and distribution in the Romanian Carpathians – state of knowledge

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The first record of a centipede species (*Clinopodes flavidus*) from the Romanian Carpathians was mentioned in the Banat Mountains, in the 19th century. However, records number rose to the highest only after the middle of the 20th century, when a few Romanian zoologists started to study this group of invertebrates in this area.

In order to understand the factors influencing centipedes' occurrence and dispersal, gathering knowledge on their presence, distribution and taxonomic status is of first importance. National georeferenced database, summing up previous literature and own records, indicates that the centipede fauna of the Romanian Carpathians comprises 99 species, representing roughly 83% of the species in Romania, and more than 70% of all entries. Out of these, 29 species are reported only in the mountain area of the country, but just 11 species are strictly endemic.

Our study revealed that the species richness is significantly higher in the Southern Carpathians (70 species) and in Banat (58 sp.). The distribution of endemics among different areas seems to be also uneven. Areas with high endemic species richness are located in the Retezat-Godeanu Mountains group and in the Anina Mountains, but these findings could be biased by the historical sampling effort. While some of the most frequent endemics are restricted to the Southern Carpathians (*Lithobius decapolitus*, with 80 localities), others have extended distribution also to the Apuseni Mountains (*Harpolithobius banaticus*), or even to the whole South-Eastern Carpathian range in Romania (*Clinopodes rodnaensis*).

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Combining resource selection functions, home range data, and systematic conservation planning to identify conservation priorities for brown bears (*Ursus arctos*) in the Romanian Carpathians

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The recovery of large carnivores in the human-dominated landscapes of Europe has sparked a debate regarding the optimal landscape conditions in which carnivores can thrive and coexist with humans, with conservation planning as a key component for broad scale management of large carnivore populations. Here, we use brown bears (Ursus arctos) in the Romanian Carpathians as a test case to develop a framework for identifying habitat conservation priorities based on a novel integration of resource selection functions, home range data, and systematic conservation planning. We used a comprehensive GPS telemetry dataset from 18 individuals in the Eastern Carpathians to (1) calculate seasonal home ranges using Brownian Bridge Movement Models, and (2) characterize seasonal population-level habitat selection using Manly's selection ratios. We then used the systematic conservation planning software Zonation to identify habitat conservation priorities combining Manly's selection ratios as weights for their respective habitat layers, and seasonal home range information as a smoothing parameter for habitat connectivity, and identified contiguous areas of high conservation value. Seasonal home ranges were smallest during winter (November-February: $18.5 \pm 4.6 \text{ km}^2$), and largest during the Intense-feeding season (September-November: $102.5 \pm 28.4 \text{ km}^2$). Bears selected for mixed forest during winter and intense-feeding seasons, and for transitional woods and shrubs during Low-feeding / reproduction and Wild berries seasons. We identified large tracts of relatively undisturbed habitat selected across seasons as key habitats for brown bear conservation

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in the Carpathians (~15% of the landscape). Spatially, high-value winter habitat was the most dissimilar, suggesting that conservation actions should focus on protecting contiguous denning habitat. We developed a framework that integrates basic knowledge on habitat selection and movement ecology with systematic conservation planning to identify biologically meaningful spatial conservation priorities. Our novel approach can be readily applied in any management system, including those particularly characterised by low resource allocation for wildlife research. Lastly, our findings can enable transboundary management of the Carpathian brown bear population, and contribute to maintaining Favourable Conservation Status, an important target of European Union Strategy for Biodiversity.

Comparison of microfungal diversity on the *Salix herbacea* and *Juncus trifidus* in the isolated localities of the Carpathians and Sudetes

Brayan Jacewski^{1,⊠}, Jacek Urbaniak¹, Wojciech Pusz² and Paweł Kwiatkowski³

Results of phylogeographical research on current distribution and molecular differentiation of arctic-alpine plant species usually pointed on strong impact of quaternary climatic fluctuations. During the cold periods many plants, called presently as "relict species" or "glacial relicts", migrated from the boreal – arctic area (Scandinavia, Siberia) into the South and inhabited different mountain ranges in Europe. However, together with the relict plants, also some microfungi migrated and inhabited the new non-colonized areas. Therefore, we decided to compare the diversity and distribution of microfungi present on two relict plants: *Salix herbacea* and *Juncus trifidus*.

Fungi were collected in 2017 in Karkonosze Mts, Śnieżnik Mts (Sudetes), Pilsko Mt., Babia Góra Mt. and Tatry Mts. (Carpathians). Field observations included all parts of the plants (except roots). Fungi were isolated and identified with the percentage of infected plants at selected locations, as well as the percentage of infected foliage. In total, *c*. 30 microfungi species were found in examined localities, that represented several genera. Summarized results were presented in tables and on a map. Our study shows, that natural alpine communities offer relatively stable conditions for microfungi inhabiting communities of *S. herbacea (Salicetum herbaceae)* and *J. trifidus (Oreochloo distichae-Juncetum trifidi)* which seems to be similar to results obtained previously (Pusz and Urbaniak, 2017; Chlebicki, 2002). Although the study of plant – fungi relations are not novel, the presented results are part of further phylogeographical studies, that should explain history and migrations of not only

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plants, but also microfungi connected with them. We hope that this will allow for a better understanding of influences in boreal – arctic areas on the flora of European mountains.

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Ecology and chorology of *Pulmonaria mollis* Wulfen ex Kern. s.s. – spying migration routes?

Maria Janicka^{1,⊠}

The aim of the study was to find out if vegetation type and specific local conditions characterizing known stands of *Pulmonaria mollis* may explain contemporary range of this taxon and bring information about its possible migration routes during Holocene.

P. mollis s.s. from the Boraginaceae family is a relatively young taxon with chromosome number 2n=18 (dyploid). Its main range is limited to C Europe, but *P. mollis* s.l. is dispersed in a larger area of the Euro-Asian continent (Sauer 1987, Janicka & Kasjaniuk 2013). *P. mollis* s.s. has probably evolved from aggregate ancestors during the Pleistocene and might have migrated with oak woodland communities from SE to C Europe (Sauer 1987). Precise routes and directions are unknown.

Analyze of 210 phytosociological relevés made in C and SE Europe, and Russia in the years 1929–2017, shows that *P. mollis* s.s. occurrs in many different types of plant communities, similar to *P. mollis* s.l. Data analyze suggests relatively wide ecological amplitude of *P. mollis* s.s., but its presence in particular place is connected rather with special type of habitat than with a type of plant community. Irrespectively of vegetation type, stands of *P. mollis* s.s. are always characterized by specific combination of local/microsite conditions, as it occurres in thermo-privileged places on nutrient rich, fresh/wet soils with CaCO₃. *P. mollis* s.s. could be consider as an indicator of such habitats. It is observed only in unmanaged or extensively used places, since it does not prefer regular mowing. Ecology of *P. mollis* s.s may explain its contemporary dispersed range, but it brings only indirect information about migration routes. It seems that mild and wet climate of Atlantic period has facilitated occupying the new areas of C Europe, but agriculture management has stopped or modified this process.

Analyze of chorological data (272 herbarium specimens: KRA, WA, LOD, KTC, BSG, BIL) from C Europe suggests the existence of a few migration routes. The main are: (1) from White Carpathians through Moravian Karst and Moravian

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Gate to Silesia (Pszczyna Forests) and Kraków-Częstochowa Upland, (2) from Slovakia through Dunajec valley to Beskid Wyspowy Mts. and Kraków-Częstochowa Upland, then to Sandomierz Basin and Świętokrzyskie Mts., (3) from Slovakia through Dukla Pass to Foothills macroregion, then to Ukraine in the east, Niepołomice Primeval Forest in the west, Lublin Upland, Bielany Primeval Forest (remnants of the Mazowiecka Primeval Forest), Inowrocław environs, and Białowieża Primeval Forest in the north, then to Belarus Upland. Distribution of *P. mollis* s.s. is strightly connected with presence of large river valleys, so migration from upland to upland along rivers and streams on the north of Carpathians is possible.

To set migration routes, especially their directions, molecular analyzes are needed. Further studies could also precise genesis (e.g. specific habitat requirements, climate changes during Holocene, effect of agriculture management during last 8,000 years) and age of contemporary dispersed *P. mollis* s.s. range.

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New and rare species of lichen-forming and lichenicolous fungi for Ukraine from the Carpathian Mountains

Nadiia Kapets^{1,⊠}

The first data about lichen-forming fungi from the Ukrainian Carpathians (UC) were mentioned in the late 19th century by Fr. Hazslinszky. Further information on this subject was published by famous lihenologists such as M. Makarevych, J. Nadvornik, A. Hilitzer, J. Hruby, A. Oxner, J. Suza, O. Szatala, and A. Vezda et al. Further valuable data in the study of lichen-forming fungi from the UC were presented by A. Khodosovtsev, S. Kondratyuk, S. S. Postoyalkin, O. Roms, and Zelenko et al. (Makarevich et al., 1982; Oxner, 2010). Our investigation focused on the lichenforming and lichenicolous fungi form this region were conducted in 2014-2016 using classical methods of field sampling and light microscopy. As results, information on three species of lichen-forming fungi (Arctoparmelia incurva (Pers.) Hale, Lichenomphalia hudsoniana (H.S. Jenn.) Redhead, Lutzoni, Moncalvo & Vilgalys, Lichenomphalia umbellifera (L.) Redhead, Lutzoni, Moncalyo & Vilgalys) and two species of lichenicolous fungi (Phaeopyxis punctum (A. Massal.) Rambold, Triebel & Coppins, Marchandiomyces corallinus (Roberge) Diederich & D. Hawksw.) occurring in the UC are given. A. incurva and P. punctum are hereby firstly mentioned from Ukraine, while *M. corallinus* is first mentioned from the UC. Moreover, the genus Phaeopyxis Rambold & Triebel is reported for the first time from Ukraine. New habitats of some rare species for Ukraine of basidial lichenized fungi such as L. hudsoniana and L. umbellifera (L.) Redhead, Lutzoni, Moncalvo & Vilgalys Hale are hereby reported too. Finally, details on their localities are given.

Arctoparmelia incurva – Ivano-Frankivsk Region, Kosiv District, Pokutsko-Bukovynski Carpathians, National Nature Park Hutsulshchyna, at the top of mount Lysyna Kosmatska, 48°18'07"N / 24°43'53"E, 1466 m alt., on rocks, 17.08.2016, O.O. Barsukov, N.V. Kapets.

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Lichenomphalia hudsoniana (H.S. Jenn.) Redhead, Lutzoni, Moncalvo & Vilgalys – Ivano-Frankivsk Region, Verkhovyna District, *Chornohora Mountains,* Carpathian National Nature Park, on top of Mt. Pip Ivan, 48°02'52"N / 24°37'40"E, 2018 m alt., on rocks, 19.08.2016, N.V. Kapets.

Lichenomphalia umbellifera (L.) Redhead, Lutzoni, Moncalvo & Vilgalys – Ivano-Frankivsk Region, Kosiv District, Pokutsko-Bukovynski Carpathians, National Nature Park Hutsulshchyna, on the trail to the top of Mt. Lysyna Kosmatska, on moss growing between rocky outcrops, 17.08.2016, N.V. Kapets; Ivano-Frankivsk Region, Verkhovyna District, Chornohora Mts., Carpathian National Nature Park, Mt. Vykhatyi Kamin, on rocks, 18.08.2016, N.V. Kapets, T.I. Brynda.

Marchandiomyces corallinus (Roberge) Diederich & D. Hawksw. – Ivano-Frankivsk Region, Kosiv District, Pokutsko-Bukovynski Carpathians, National Nature Park Hutsulshchyna, Kamianystyi Khrebet, 48°18'50"N / 25°02'48"E, on thallus *Parmelia saxatilis* (L.) Ach., 16.08.2016, N.V. Kapets; Ivano-Frankivsk Region, Kosiv District, Pokutsko-Bukovynski Carpathians, National Nature Park Hutsulshchyna, top of Mt. Lysyna Kosmatska, 48°18'07"N / 24°43'53"E, 1466 m alt., on thallus of *Melanelia stygia* (L.) Essl., 17.08.2016, N.V. Kapets.

Phaeopyxis punctum (A. Massal.) Rambold, Triebel & Coppins – Ivano-Frankivsk Region, Kosiv District, Pokutsko-Bukovynski Carpathians, National Nature Park Hutsulshchyna, at the foothills of Mt. Lysyna Kosmatska, on thallus of *Cladonia sp.*, growing on rotten stump, 17.08.2016, N.V. Kapets.

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Unused genetic resources – The genetic potential of genus of wild fruits

Viktor Kerényi-Nagy^{1,⊠} and Károly Penksza²

It may be a fundamental issue for everyone: why is it necessary to expand fruit, ornamental and herbaceous crops which are still existing and rich in varieties? We believe that XXI century will bring many new challenges to the everyday life of horticultural profession, therefore we have determined 6 main direction of possible innovations which may lead to improvements:

- 1. Production technology;
- 2. Instead of quantitative cultivation, it is necessary to produce quality nutrition or special medicinal products;
- 3. New pomology research is needed due to adapting to the developing industry;
- 4. These new species could be grown in depleted sites;
- 5. Providing seasonal work to reduce social inequalities
- 6. Nature conservation functions.

In this presentation we would like to point out religious and mythological bonds of Roses (*Rosa*, Kerényi-Nagy, 2012, Margittai – Kerényi-Nagy 2017) and hawthorns (*Crataegus*, Kerényi-Nagy, 2015). These species pervade our everyday lives from ancient times. Nomenclature and taxonomy questions are often referred to as self-intended. However these characterization become more understandable during biodiversity and evolutionary pathways, since well-known and named taxa can already be collected into a living collection such as gene bank and further phenological and nutritional content examinations ca be made. These may create

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the basis for nature conservation and cultivation. There is already a significant tradition in the cultivation of these species, especially in Turkey, Poland and Germany. Not to mention Chinese medicine where hawthorns have been applied for thousands of years.

In addition to basic and practical research:

- Gene bank was created from 85 rose and 25 hawthorn taxa on Botanical Garden of Szent István University which was solemnly handed over to the 1st International conference of Rose and Hawthorn Research in the Carpathian basin. About 70 experts from 7 countries participated with numerous lectures and posters (Kerényi-Nagy *et al.*, 2015).
- The 2nd Rose- and hawthorn research conference in the Carpathian Basin at Budatétény, organized and supported by National Agricultural Research and Innovation Centre, Museum and Library of Hungarian Agriculture, Szent István University Faculty of Agricultural and Environmental Sciences and Herman Ottó Institute. About 65 professionals from 6 countries participated with several lectures and posters (Kerényi-Nagy *et al.*, 2017, Kerényi-Nagy, 2017).

Further research routes includes where we see gene exploration reserves are: sorb (*Sorbus*), mulberry (*Rubus*), pear (*Pyrus*), plum (*Prunus*) genera, cornelian cherry (*Cornus mas*), juniperus (*Juniperus communis* and *J. oxycedrus*), black strawberry (*Morus nigra*) and figs (*Ficus carica*).

Connection of this actual work and the work of the Hungarian Agricultural Museum and Library are to the development of fruit core stock, its morphometrical determination and monographic expansion/procession of the aforementioned generas.

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Vegetation changes along Czchów Reservoir in the Carpathian Foothills (Poland) after 40 years – initial results

Sabina Klich^{1,\infty} and Alina Stachurska-Swakoń¹

Construction of a water reservoir is connected with a degradation of the natural valley and influences on various habitat conditions and local landscape. This comes under particular attention when it concerns mountain areas (Trzcińska-Tacik and Stachurska-Swakoń, 2002). Czchów Reservoir was built on the Dunajec river in 1938-1948. It is the left tributary of the Vistula river and one of most important rivers in the southern Poland flowing through the Western Carpathians. Czchów Reservoir is the fourth (lowest) water reservoir on the Dunajec.

First studies of vegetation and flora along the reservoir were conducted during 1970-1974 (Loster, 1976). They showed more than 200 vascular plant species growing on the reservoir shore. Many of them were associated with willow riparian forests or characteristic for *Molinio-Arrhenatheretea*. Thirteen plant communities connected with the water reservoir were also recognized and documented, eg. *Leersio-Bidentetum, Scirpo-Phragmitetum, Phalaridetum arundinaceae, Acoretum calami* and *Salicetum triandro-viminalis*.

The studies of contemporary flora and vegetation along the reservoir started this year. The presentation shows the results of the inventory and gives some remarks of changes of flora and vegetation.

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Biodiversity assessment of the polyploid species group Onosma arenaria – O. pseudoarenaria in the Carpathians

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The genus *Onosma* (vascular plants, Boraginaceae) comprises ca. 150 species distributed mainly in the Mediterranean area, in Asia Minor and in eastern and central Asia. The European group of species (ca. 30) is probably isolated at the margin of the genus distribution range and overrun independent evolutionary trajectories, which were presumably shaped by climatic, orographic, as well as anthropogenic factors. Traditionally, the genus is divided into three informal groups (originally described as sections), Asterotricha Boiss., Haplotricha Boiss. and Heterotricha Boiss. based on morphological data, particularly the type of indumentum. Ancient taxa of morphologically well defined groups Asterotricha and Haplotricha in the European part of the genus range are probably ancestors of the hybridogenous group Heterotricha. This hypothesis is supported mainly by karyological and molecular data.

The hybridogenous group Heterotricha comprises 2 groups of taxa, mostly recognized at species level: *O. arenaria* s.l. with triploid (3x) 2n=20 and *O. pseudoarenaria* s.l. with tetraploid (4x) 2n=26 chromosome composition. Morphological variation among and within taxa is considerable and several characters overlap across taxa, therefore no general taxonomic concept broadly accepted for the species group was established so far, but three and nine taxa (at specific or subspecific level) are recognized within *O. arenaria* s.l. (3x) and *O. pseudoarenaria* s.l. (4x) respectively by the majority of authors. Geographic range of all taxa is quite well defined and most of them are isolated, with few exceptions. Two geographically well defined phylo-groups occur in Central Europe. *Onosma arenaria* subsp. *arenaria* (3x) and *O. pseudoarenaria* subsp. *tuberculata* (4x) are broadly distributed on sands in Pannonia and further extend to middle altitudes of the Western (W) Carpathians while *O. pseudoarenaria* subsp. *pseudoarenaria* is confined to loess hills of middle altitudes in the Eastern (E) Carpathians.

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Present contribution aims to summarize recent published and unpublished molecular, karyological, morphological and reproduction data to assess diversity of *O. arenaria* – *O. pseudoarenaria* species group in the Carpathians. Recent molecular studies have revealed considerable genetic variation among populations from the Carpatho-Pannonian region and adjacent mountain territories in the Balkan peninsula with isolated position of *O. pseudoarenaria* subsp. *pseudoarenaria* (4x, E Carpathians). Slight to significant differences have been revealed in several characters between populations from the W and E Carpathians. Genome size data only negligibly differentiate particular populations within *O. pseudoarenaria* (4x) with higher values measured in samples originated from the E Carpathians. Flow cytometry seed screen suggests hemisexuality and sexuality in *O. arenaria* (3x) and *O. pseudoarenaria* (4x), which is attributed to genome reduplication in sexualy derived endosperm cells. This feature does not allow to distinguish the W and E Carpathian populations.

Evolutionary and taxonomic consequences of present data have been taken into consideration and are discussed.

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Evaluation of hypogeous fungi diversity in the Western Carpathians based on the analysis of rodent and carnivore faeces

Patryk Komur^{1,⊠}, Izabela Wierzbowska², Piotr Chachuła³, Marcin Matysek⁴ and Piotr Mleczko¹

Hypogeous fungi are the members of Ascomycota, Basidiomycota and Mucoromycota characterised by gasteroid sporocarps that form and mature underground. The main means of dispersal of their spores are vector organisms such as invertebrates and mammals, i.e. rodents, for which fungal sporocarps are often important supplements of the diet. Most hypogeous fungi form ectomycorrhizal symbiosis mainly with trees. Close relationships between hypogeous fungi, plants and animals create a complex net of ecological connections.

The aim of the research was to investigate the diversity of hypogeous fungi consumed by small mammals in the three contrasting Western Carpathian mountain ranges: the Gorce, the Pieniny and the Tatra Mts. Additionally, we examined the possibility of spore dispersal by carnivores feeding on the rodents.

We collected droppings from rodents during live trapping sessions in 36 line transects set in forests and meadows in three study areas. The trapping was performed twice a season, in the years 2014 (the Gorce and the Tatra Mts.) and 2016 (the Pieniny). The faeces were analysed microscopically for the presence and identity of fungal spores.

In total, during trapping sessions we identified 11 animal species including rodents and soricomorphs, with bank vole, yellow-necked mouse and the common vole being most commonly trapped.

In the Gorce Mts. spores of hypogeous fungi were noticed in 75% of bank vole and 55% of yellow-necked mouse dropping samples. In the Tatra Mts. spores were present in 72% of bank vole and 60% of yellow-necked mouse, but also in 50% of

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field vole and 80% of European pine vole samples. In the Pieniny Mts. spores were identified in faeces of 85% of bank voles, 73% of yellow-necked mice, 50% of field voles, 16% of wooden mice, 10% of common voles and 40% of shrews.

Altogether spores of 22 hypogeous fungal taxa (species or genera) were identified, with 14, 17 and 20 taxa present in the Gorce, the Tatra and the Pieniny Mts., respectively. The most commonly encountered genera were *Chamonixia, Genea, Hymenogaster, Hysterangium, Melanogaster* and *Octaviania,* however, the differences in fungal diversity was noted between mountain ranges. In the Tatra and the Pieniny Mts. higher numbers of fungal taxa were recorded in autumn, whereas in the Gorce Mts. in summer. The concentration of spores and the number of taxa per sample was higher in autumn than in summer in all study areas.

In all studied sites the spores of hypogeous fungi were identified in samples collected both in forests and meadows. This proves the role of small mammals in dispersal of ectomycorrhizal inoculum to non-forest habitats.

The analysis of faeces of carnivores from the Gorce Mts. revealed the presence of spores of 8 hypogeous fungal taxa in 45% of the examined samples from red foxes and martens, and from one individual of badger. The most probable sources of spores were consumed rodents. This finding indicates the possible role of carnivores in a long-distance transport of spores.

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A transfer zone between Yellow-bellied and Fire-bellied Toads in Hungary and Slovakia

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The up till now known distribution areas of Fire-bellied (*Bombina bombina*, Bb) and Yellow-bellied Toads (*Bombina variegata*, Bv) meet in the Carpathian Basin. Bv occurs mainly in higher elevation with mountainous or submountainous climate while Bb prefers lowlands. The contact zones, where hybrids often appear, are established usually around the foothills of larger mounts or mountains. However, studies in two neighbouring mountains, the Börzsöny Hills in Hungary and Krupinska Planina in Slovakia show a different picture which may indicate the spreading of Bb to the North and at the same time the colonisation of Bv habitats in these areas.

We collected specimens from several locations in both areas. In order to apply the most certain identification of these species we used two methods. Tissue samples were taken for further molecular analysis and also the abdominal patterns were photographed of each individual. We found an unexpected result in the geographical distribution pattern of these two species. Bb almost completely colonised the Börzsöny Hills including habitats which can be classified as originally Bv habitats (ie ponds and pools above 400 m asl). No animals with Bv abdominal patterns nor with Bv genetical markers were found here. In Krupinska Planina large numbers of locations were colonised by Bb, even ponds located above 400 m asl. However, the most remote sites still harbour Bv populations and in a few cases we found hybrid individuals in Slovakia.

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Given these results, it can be assumed that Bb might push the transfer zone of the two species towards the North and the higher and cooler areas.

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Variation in leaf morphology of *Ribes petraeum* (Grossulariaceae) in the West Carpathians and the Sudetes

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Ribes petraeum Wulfen is an European species, limited in its occurrence to mountain ranges. It occurs from the Pyrenees through the Vosges, Alps, Dinarides, Sudetes, Carpathians, to the mountains of the Balkan Peninsula, where it grows in sycamore forests and subalpine scrub and tall-forbs. It was also noted in North Africa (the Atlas Mts.). Localities reported from the Caucasus and Asia Minor (Anatolia) presumably refer to another taxon and require separate studies. The purpose of the present study was to determine the intraspecific variability of selected populations located in the Western Carpathians and the Sudetes.

Leaf morphology was chosen for the investigations since they are the most easily available plant organs, in which many of the specific traits can be distinguished. For detailed biometric analysis, samples were collected over several years between the end of September and the end of October, which guaranteed that the leaves were fully grown. The study was conducted on herbarium material collected by the authors, consisting of leaves from long shoots and short shoots. From each locality 40-50 large leaves from randomly selected shoots of different types were collected. Each leaf was imaged and characterized by a set of features (e.g. petiole length, midrib length, middle lobe length, middle lobe width at the base, angle between midrib and lateral nerve, number of lobes). The results obtained were analyzed using ANOVA with Tukey's post hoc test (p = 0.05) and Principal Component Analysis (PCA) correlation. The material originated from 15 localities in Poland, the Czech Republic and Slovakia.

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Quantitative data on variation of *Ribes petraeum* concerns mainly differences between the leaves of short and long shoots, and the diversity within samples from individual populations. The most variable are the two features: petiole length and the length of lateral nerve of short shoot leaves; the smallest variation is in the angle between the main and lateral nerves. The largest leaf blades and the longest petioles are those from the long shoot leaves. Within the investigated populations, local morphotypes (A, B, C) were distinguished, which are related to particular mountain ranges. Each of them is characterized by a specific combination of morphological traits. In the Sudetes (Karkonosze/Krkonose Mts., Śnieżnik Massive, Hruby Jesenik Mts. - morphotype A) the samples are characterized by a relatively short leaf petiole and generally small blade. In the Western Carpathians (Beskidy Mts, Pilsko and Babia Góra Massives, Velka Fatra Mts. - morphotype B) the leaves have long lateral nerves and dense venation. In the remaining mountain ranges (Tatras, Nizke Tatry Mts., Chocz Mts. - morphotype C) the leaves are distinguished by prominent middle lobes.

The differences between populations from the Western Carpathians and the Sudetes, as well as within the Carpathian and Sudetes populations, result also from habitat and phytocoenoses variation. The present studies have confirmed the intraspecific variation within the *Ribes petraeum*. The alpine variety (subsp. *alpinum*) is represented by Sudetic populations, while other population are taxonomically associated with the Carpathian unit (subsp. *carpaticum*).

Green algae (Chlorophyta) in the lakes of the highest Carpathian range – 150 years of phycological studies in the Tatra National Park (Poland)

Joanna Lenarczyk^{1,⊠}

The current knowledge about Chlorophyta, one of the most diverse algal group in the water bodies of the Tatra mountains, is still poor in this area despite a relatively long period of studies. Data on green algae are to be found in sixteen publications, including the most comprehensive one which dates back more than 100 years. Usually, floristic composition and taxonomy of the algae were described. Rarely, vertical distribution and seasonal changes of biomass were analysed. Twenty water bodies were sampled so far, especially in the two largest valleys, Gasienicowa Valley and Five Polish Ponds Valley. Desmids (Desmidaceae), one-celled algae preferring low trophy habitats, were mainly identified. More than thirty taxa new for science were described from the water bodies. Pediastrum (Hydrodictyaceae) is the only green algal genus whose specimens were isolated from the Tatra lakes and analysed molecularly. Future research plans include a taxonomic revision and a critical list of taxa, similar to that already prepared for the desmid genera Euastrum and *Micrasterias*, as well as laboratory experiments on phenotypic plasticity of monoclonal strains. There is also a need for intensive molecular analyses to examine phylogenetic relationships between mountain and lowland populations, as well as migration history of the green algal flora.

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Cosmarium species (Desmidiaceae) in the lakes of the Western and Eastern Carpathians – the Tatra Mountains (Poland) and the Chornohora Mountains (Ukraine)

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The microscopic green alga Cosmarium, forming cells composed of two symmetric halves usually prominently ornamented, is one of the most common desmid genera. Many species are typical of strongly acidic peat bogs, but the genus is to be found in neutral to light alkaline ponds and lakes, as well as in polluted waters. In order to describe floristic composition and distribution of the Cosmarium species in the mountain lakes of the Western and Eastern Carpathians, 8 water bodies in the Tatra Mountains (Długi Staw, Dwoisty Staw Wschodni, Czarny Staw Gąsienicowy, Kurtkowiec, Litworowy Staw, Troiśniak Pośredni, Wyżni Czerwony Stawek, Zmarzły Staw in the Gasienicowa Valley) and 4 in the Chornohora Mountains (Cyclops, Nesamowyte, Nyzne Ozerne, Verchnie Ozerne) were sampled in 2011 and 2013, respectively. The highest number of species was found in Litworowy Staw (16), Kurtkowiec (14) and Wyżni Czerwony Stawek (11), which are relatively shallow oligo/mesotrophic water bodies situated at 1600-170 m a.s.l., having neutral or slightly alkaline pH and low conductivity. Single species were reported from Cyclops, Nesamowyte, Troiśniak Pośredni and Verchnie Ozerne lakes. In most studied lakes, only a few Cosmarium species were identified. Most species were recorded from 1-2 water bodies. Only 7 species, including C. bioculatum var. bioculatum, C. caelatum, C. dentiferum var. alpinum, C. difficile, C. pygmaeum var. pygmaeum, C. quadratum var. quadratum and C. subcrenatum, were found at 3 localities. Altogether, 32 species (36 taxa) were identified. About a half of them are common in both lowland, upland and mountain habitats and about one third is characteristic for upland and

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mountain ones, including 7 rarely occurring taxa (*C. norimbergense* var. *depressum*, *C. plicatum*, *C. pygmaeum* var. *pygmaeum*, *C. subspeciosum* var. *subspeciosum*, *C. undulatum* var. *minutum*, *C. variolatum* var. *variolatum* and *C. ventusum* var. *minus*). Most species are mesotrophic and are to be found in acidic habitats, a few of them are also considered as oligotrophic and acidophilous (*C. margaritiferum* var. *margaritiferum*, *C. obliquum*, *C. pseudoamoenum*, *C. regnesii*, *C. subcrenatum* and *C. subtumidum* var. *subtumidum*), and almost a third is known from eutrophic water bodies. The obtained data will further help to revise the desmid flora of the studied region in spatial and temporal gradients.

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Contributions to the biodiversity of lichen-forming and lichenicolous fungi of the Călimani Mountains (Eastern Carpathians, Romania)

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Exploration of the lichen flora of the Călimani Mts received less attention from lichenologists, comparing with other high mountain areas. Paul Cretzoiu published the first lichen records of 44 species in several papers between 1930 and 1950 from various localities in the Călimani Mts. Vasile Codoreanu visited the main peaks and the valley "Drâglele" in July 1949 and 1950, and reported 74 species in 1952 and 1954 (55 new to the lichen flora of the mountains among them). Another considerable study was done in 1998 by Stoie and Crişan who made a publication on 51 foliose and fruticose lichen species from some western areas of the Călimani Mts (with 33 new floristical records). Unfortunately, these latter results were not included in Ciurchea's Romanian lichen flora (2004). Several species should be excluded due to erroneous citations, nomenclatural mistakes or misidentifications, therefore the number of species published from the Călimani Mts is 128 altogether. Further revisions on the available old specimens are also necessary and it is in progress.

Our preliminary investigations in 2009 contributed further 30 species to the lichen flora of the Călimani Mts, increasing the number of the known species to 158. However, our knowledge of lichens and their lichenicolous fungi in the Călimani Mts is still limited. Further systematic and regular, fresh collections should be done to obtain new floristical results, which are essential also for the study of molecular genetic diversity. Nevertheless, ecological and evolutionary aspects can be studied

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later, only after we had full and more precise knowledge of the biodiversity of the lichens. Now we have an opportunity to continue our preliminary studies due to a project support.

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Changes in the vertical distribution of *Ixodes ricinus* ticks in Veľká Fatra Mts. (Western Carpathians, Slovakia)

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Ixodes ricinus, one of the 900 worldwide tick species, is among the 20 tick species known from Slovakia. Approximately 10% of currently known species act as vectors for viruses, bacteria and protozoa responsible for human and animal diseases. The most common diseases in Northern Hemisphere are Lyme borreliosis, tick borne encephalitis, babesiosis and anaplasmosis. In the context of global changes of climate, there are subsequent affected abiotic and biotic factors, which, on turn, influence changes in distribution of vectors. In the last few decades of the 20th century the ticks occurred very rarely in northern part of Slovakia. Optimal biotope for all development stages were 600-800 m a.s.l (Řeháček *et al.*, 1996). By 2008, the occurrence of ticks has shifted to 1000-1200 m a.s.l. (Bullová *et al.*, 2008). Nowadays ticks are spreading to new areas within the site, but also to new areas in the original area of distribution.

The aim of this study was to observe submountain and mountains localities, along two vertical transects in Veľká Fatra Mts. (Smrekovica 680- 1450 m a.s.l. and Malinô Brdo 600- 1200 m a.s.l.) and to present up-to date changes of vertical distribution of ixodid ticks in terms of microclimatic condition. The ticks were recorded by flagging the vegetation during one hour, which represented an area of 700 m² (Peťko *et al.*, 1996). The average density of flagging ticks on sampling sites was 15 ticks per hour of flagging. According to our data different stage of ticks biology require different biotope. Thus, nymphs were dominant in mixed forest with significant litter. Unimodal pattern of activity was observed during spring in May 2016 (61 ticks per hour on Malinô Brdo sampling sites and 90 ticks per hour on Smrekovica sampling site).

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In terms of vertical distribution of ticks, we recorded the highest relative density of ixodid ticks in two sampling sites: Malinô Brdo, 1100 m a.s.l. 40-62 ticks per hour flagging, and in Smrekovica, sampling sites 680 m a.s.l., 90 ticks per hour flagging. The highest altitude where all the developmental stages of *Ixodes ricinus* were confirmed is Skalná Alpa (1450 m a. s. l.). The knowledge gained through this study suggests a change in the geographical distribution of tick in Slovakia and its shift to higher altitudes.

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Dusk of sexual reproduction? Endless possibilities of plant breeding systems – insight into the genus *Cotoneaster*

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Polyploidization plays an important role in the formation of plant diversity. However, newly arisen polyploids usually have problems with sexual reproduction and therefore their fate could be dependent on their ability to switch to another breeding system. Especially apomixis (asexual reproduction through the seeds) could be very advantageous for the newly formed lineages. Both microevolutionary processes together with hybridization generate great variation in the family Rosaceae which include taxonomically complex groups such as *Potentilla*, *Alchemilla*, *Crataegus* or *Sorbus*.

Diversity of the genus *Cotoneaster* is also supposed to be affected by these mechanisms, although cytotype and breeding system variation has never been proven. The section *Cotoneaster* is represented by two autochthonous widespread taxa in Central Europe. *Cotoneaster tomentosus* is a well differentiated taxon, whereas *C. integerrimus* aggregate includes numerous microspecies occurring especially in the Western Carpathians, but their taxonomic value is uncertain. Here we reveal cytotype distribution and variation in reproduction modes of the genus *Cotoneaster* in the Western Carpathians compared to the Bohemian Massif.

In contrast to previously reported data, only two ploidy levels (tetraploid and pentaploid) were detected among more than 350 individuals using flow cytometry. While tetraploids were widespread, pentaploids were restricted only for the Western Carpathians. All pentaploids were represented by *C. tomentosus* which was supposed to be tetraploid. Four modes of reproduction were revealed by flow cytometric seed

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screen. Majority of seeds were apomictic (pseudogamous), whereas sexuality was only residual (around 7 %). Haploid parthenogenesis (production of reduced seed without fertilization) and BIII hybrids (fertilization of an unreduced egg) were detected sporadically and preferentially in the Western Carpathians.

Polyploidization and variation in breeding systems are the driving force for microevolution within the genus *Cotoneaster*. Thanks to rare types of reproduction, new cytotypes can arise. Still only minor part of the variation is probably maintained under natural conditions. The detection of highly variable reproductive pattern is inspiring for further study of other regions (incl. the Alps).

Population genetics of Gladiolus palustris in the Carpathian Basin

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The Sword Lily (Gladiolus palustris Gaudin, 1882) is a strictly protected species in Hungary and is also a species of high conservation importance according to the Habitats Directive of the EU, present in the Red List of several countries (eg. Király, 2007; Towpasz et al., 2014). Although it is an endemic species in Europe, it is not included in the IUCN's Red List. Here, we present the genetic structure of *Gladiolus palustris* populations mainly in the Carpathian Basin. We sampled a total of 12 populations in Germany, Hungary and Slovakia and performed SSR-based (simple sequence repeats) population genetics analyses (eg. Polysat v.1.7; Clark and Jasieniuk, 2011). Our results indicate that specimens found several hundred kilometers apart form distinct populations, while relatively distant specimens (~20 km) are capable of forming a metapopulation. Mostly, large populations show high genetic diversity, while some of these units show excess of heterozygotes inferring to a recent population bottleneck event. This is evident in small populations as well, although in the latter case this is also paired with low genetic diversity. Our results also show relatively low inbreeding within the sampled populations, except one situated within the Mátra mountain range which represented a very small,

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receding population fragment. Genetic differentiation between populations was also determined. Populations displayed significant genetic differentiation, except the ones found in relatively close geographic proximity to each other, for example in the Balaton-felvidék in Hungary. We discuss the significance of our results in relation to the European conservation status of the species and provide suggestions for future conservation efforts.

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Plastid DNA variation in *Cicerbita alpina* (Asteraceae) populations across the European mountains

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Cicerbita alpina (L.) Wallr. (Asteraceae, Lactucoideae, Lactuceae) is one of the four species of the genus *Cicerbita* native to the European mountains. It is distributed in the Alps, Pyrenees, northern part of the Balkan Peninsula mountains, Carpathians, Sudetes, some lower mountain ranges of central Europe and in Scandinavia. The species usually grows in the tall-herb communities and it is considered a diagnostic species for the *Adenostylion alliariae* alliance.

Range-wide phylogeography of *Cicerbita alpina* was assessed based on AFLP fingerprinting (Michl *et al.*, 2010, Stachurska-Swakoń *et al.*, 2011, 2012). In general, these studies revealed a lack of a strong phylogeographical structure except for the distinct population from the Pyrenees. However, genetic differentiation of a lineage including the Balkan and South-Eastern Carpathian populations was observed, along with population groups in the Western Carpathians, Eastern Alps, Western Alps, and affinity of the Sudetes populations to those from the Eastern Alps and of Scandinavian populations to the Central European mountains. Potential importance of cryptic glacial refugia for the species persistence in Central Europe was also hypothesized based on these data.

Here, we aim to extend the view on the Pleistocene phylogeography of *Cicerbita alpina* based on exploration of plastid DNA sequence data. Variation in several non-coding DNA regions was analysed in 26 populations across the species' range (Eastern and Western Alps, Eastern and North-Western Balkan Mountains, Western, Eastern and Southern Carpathians, Scandinavia and Sudetes). Preliminary results

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indicate a lack of a strong genetic structure in the data set. All three groups of haplotypes detected have a wide distribution across mountain ranges and some populations were characterized by a high haplotype diversity (sometimes with presence of all main haplotype groups). However, distribution of specific haplotypes supports, e.g., linking Eastern Balkan and Southern Carpathian populations and affinity of the Sudetes populations to those of the Eastern Alps. They also confirm the hypothesis of cryptic glacial refugia for the species. The results will be discussed on a broad background of the phylogeographical patterns in the European mountains.

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Biogeographical distribution and ecological demands of mite species from genus Veigaia Oudemans, 1905 (Mesostigmata: Veigaiidae), Romania

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Soil mites (Acari) represents one of the most abundant invertebrate group which lives in terrestrial ecosystems. They have an essential role in pedogenesis, participating on decomposing processes, directly or indirectly-regulating the population of other soil invertebrates (Garcia-Palacios *et al.*, 2013; Zhang *et al.*, 2015).

Genus *Veigaia* of the Veigaiidae family (Acari, Mesostigmata) is cosmopolite. Veigaiids are hemiedaphic species, very mobile hunters, inhabiting predominantly litter-fermentation and humus layers. Veigaiids are predators, feeding with nematods, collembolans, proturans, pauropods and soft-bodied mites (Walter and Proctor, 2013). Their habitats are very variate: decaying organic matter, moss, litter, humus layer, grass roots or even caves (troglophilic habitats). Specialists from Europe studied their taxonomy, biology and ecology from different types of terrestrial ecosystems: forests, bogs, agroecosystems, cliffs, shrub ecosystems, rarely pastures and meadows (Masan and Madej, 2011).

In Europe, twenty nine species from Veigaia genus were described (Masan *et al.*, 2008; Kaczmarek *et al.*, 2009; Acs and Kontschan, 2015). In Romania, ten species were recorded. In this research, we present the national distribution data of all Romanian Veigaia species: *Veigaia cerva*, *V. exigua*, *V. kochi*, *V. nemorensis*,

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V. propinqua, V. paradoxa, V. planicola, V. transisalae, V. transylvanica and *V. uncata.* Taking into consideration the overlap with Natura 2000 protected areas from Romania, the Veigaia species were identified in 23 special areas of conservation (SCI), and in 15 special protected areas (SPA). From 224 published records and new collection sites, 32.12% of them were situated in Natura 2000 protected areas. These results demonstrated the conservative role of the protected areas, even for the soil fauna, which contain proper habitats for mites.

Describing the ecological requirements for the veigaiids, we discovered that each species had specific demands. The majority of them preferred natural forests, with developed layers of litter, humus, moss or bryophytes and with soil rich in organic matter.

Further studies are requested, in order to cover a larger surface from Romania, and to investigate more detailed the ecological requirements for Veigaia species.

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Changes in the Carpathian fauna of Dasytidae and Malachiidae beetles in the context of climate change

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The beetles of Dasytidae and Malachiidae families are characterized by similar biological traits and ecological requirements. The flying period of these species mainly depends of several habitat particularities: altitude, temperature, and humidity.

The analysis of changes in the phenology of species was performed for the Ukrainian part of the Carpathians and was based on the study of museum collections. First recordings for this species from the Transcarpathian Lowland date back to the third decade of April during the 1950's. Furthermore, the highest number of specimens was recorded through an extended period, spanning from the end of May until July. Since the beginning of the 1960's until the 1980's the first recordings were dated by mid-May, and the period with highest density of individuals shifted to the second half of June – mid July. Since the mid-1980's to the beginning of 2010's the first recordings for these species came in the second decade of April, while the highest number of beetles were registered between mid-May and mid-June. In this last period of recordins, the end of the flight occurred 10-20 days earlier, comparable to the first recordings dating back 30-50 years.

On the south-western foothills, during the 1950's, the first records were dated by mid-April, and since the 1960's to mid-1980's by the beginning of May. Since mid-1980's to the late 2000's the period of the flight shifted by ten days (in average) comparable with the middle of last century, and it begun in early April. The periods with highest number of recorded individuals were shifted from mid-May to late June, shifting in 1960-80's with one-two weeks later.

In the 1950's, in the mountainous areas, the beginning of flight was recorded in the second decade of May. From the 1960's - to mid 1980's adults were recorded from late May to the second half of August. Since the mid-1990's to the late 2000's

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adults were active from early May to late July. The highest number of beetles was recorded in the early - mid June, comparable to the mid-century period and to the recent years. In the 1960-70's, the period of peak recordings shifted to the end of June – July, which was a significant change.

Also, it should be noted that over the past decades, some species of Southern European and Mediterranean origins have expanded the boundaries of their ranges reaching new regions, including the Carpathians. Standing proof is *Clanoptilus spinipennis* (Germ. 1824), a species that had a South European-Asia Minor distribution in the beginning of the 20th century, in present days extended its range to Central and Eastern Europe, including the Carpathian region. Another species, *Anthomalachius strangulates* (Ab. de Perrin 1885), spread from the Balkans and Central Europe to Northern and Eastern Europe. Conversely, *Clanoptilus falcifer* (Ab. de Perrin 1882) expanded its areal from Central-East Europe and Asia Minor to the Balkan Peninsula and the Carpathians.

Climate in the Carpathian Region had a high dynamic during the last century. In the 1960's – early 1980's period temperatures contributed to more later appearance of beetles, with a comparable phenology in the 1950's. But since the late 1980's to the present time an opposite tendency has been observed. Adults appear in present period more early than they were 40-50 years ago.

Similar phenological shifts and changes in faunal structure are typical for all insects trophicly related to vegetation. As a result of change in temperature conditions the insects expanded their areals in an accelerated manner. This last process can probably lead to the emergence of secondary generations during the same year, this effect posing real threats to vegetation in the case of plant pests.

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A synopsis of the genus Aconitum subgen. Aconitum in Europe

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Aconitum L. subgen. Aconitum, a monophyletic group, contains some 250 species with the major center of diversity in Eastern Asia. Approximately 22 native species (plus 26 natural hybrid species, including 10 intersectional hybrids) of the subgenus occur in Europe. Diploid sect. Cammarum DC. in Europe, as a rule, consists of montane/lowland species. Tetraploid sect. Aconitum and sect. Catenata, and allopolyploid sect. Aconitum × sect. Cammarum) consists of 10 nothospecies.

Taxonomic structure of subgen. Aconitum in Europe is as following: 1. Sect. Aconitum L. (syn. sect. Napellus (Wolf.) DC.) – Type species: A. napellus L. em. Skalický. 1A. – subsect. Aconitum. Species: A. bucovinese Zapał., A. corsicum Gáyer, A. firmum Rchb., A. moravicum (Skalický) Mitka (stat. nov.), A. napellus, A. plicatum Rchb., A. superbum Fritsch, A. tauricum Wulfen. Nothospecies: A. ×bavaricum Starm. (napellus × plicatum), A. ×czarnohorense (Zapał.) Mitka (firmum × nanum), A. ×nanum (Baumg.) Simonk. (bucovinese × firmum), A. ×paxii (Starm.) Mitka (stat. nov.) (maninense × moravicum), A. ×teppneri Mucher ex Starm. (napellus × tauricum), A. ×zapalowiczii (Starm.) Mitka (stat. nov.) (firmum × paxii), A. firmum × maninense, A. firmum × A. moravicum. 1B. – subsect. Burnatii Rottensteiner subsect. nov. – Type species: A. burnatii Gáyer. Species: A. burnatii, A. maninense (Skalický) Mitka (stat. nov.), A. nevadense Uetrch. ex Gáyer, A. pentheri Hayek. 1C. – subsect. Catenata (Steinb. ex H.Riedl) Luferov. – Type species: A. soongaricum Stapf. Species: A. cochleare Vorosch., A. cymbulatum (Schmalh.) Lipsky, A. nasutum Fisch. ex Rchb. 2. – Sect. Cammarum DC. (syn. sect. Aconitum). – Type species: A. variegatum L.

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2A. – subsect. *Cammarum* (DC.) Rapaics. Species: *A. degenii* Gáyer, *A. lasiocarpum* (Rchb.) Gáyer, *A. pilipes* (Rchb.) Gáyer, *A. toxicum* (Rchb.) Gáyer, *A. vitosanum* Gáyer, *A. variegatum*. Nothospecies: *A. ×austriacum* Starm. (*pilipes × variegatum*), *A. ×aquilonare* A.Kerner ex Gáyer (*variegatum × nasutum*), *A. ×bartokianum* Starm. (*toxicum × variegatum*), *A. ×dragulescuanum* Mucher (*degenii × toxicum*), *A. ×gayeri* Starm. (*degenii × lasiocarpum*), *A. ×hebegynum* DC. (*degenii × variegatum*), *A. ×pawlowskii* Mitka & Starm. (*lasiocarpum × variegatum*), *A. ×pilosiusculum* (Sér.) Gáyer (*degenii × pilipes*). 3. – Nothosect. *Acomarum* Starm. (syn. nothsect. *Acopellus* Starm.). Nothospecies: *A. ×acuminatum* Rchb. (*degenii × napellus*), *A. ×acutum* Rchb. (*tauricum × variegatum*), *A. ×cammarum* L. em. Fries (*napellus × variegatum*), *A. ×berdaui* Zapał. (*firmum × variegatum*) *A. ×exaltatum* Bernh. ex Rchb. (*plicatum × variegatum*), *A. mielichhoferi* Rchb. (*degenii × tauricum*), *A. ×schneebergense* Gáyer (*napellus × variegatum*), *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum × variegatum*, *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum × variegatum*, *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum* × variegatum, *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum* × variegatum), *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum* × variegatum, *A. napellus × A. pilipes*, *A. pilipes × A. tauricum*, *A. tauricum* × variegatum, *A. –* Sect. *Angustifolium* (Seitz) Rottensteiner. – Type species: *A. angustifolium*.

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Signs of Holocene Thermal Maximum in horizontal and vertical distribution patterns of vascular plant species in the Carpathians and the Pannon region

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Based on the absence of forest-dwelling conifers in the North Hungarian Mountains (and some other isolated mountains in Transylvania), and their presence in the surrounding Carpathians on the same altitude, we provide a method to reconstruct the mean annual temperature (MAT) of the Pannon region and the Carpathians at the early Holocene Thermal Maximum (HTM, the warmest period of the Holocene until recently). We suppose that the HTM was enough warm to drive conifers to extinction from elevations between 900 and 1100 m above sea level in the mentioned, relatively isolated mountains. Conversely, HTM still allowed the survival of residual dwarf pine (*Pinus mugo*) stands on the isolated peaks of the West Transylvanian Mountains between 1600 and 1800 m a.s.l. Our study provides an estimate for the value of MAT of HTM of the region with an interval of 0.4° C, relying on macroecological considerations and counting with the value of adiabatic laps rate, 0.65° C / 1000 m (Gabler *et al.*, 2008). We calculate the temperature of the HTM 1.3–1.7° C warmer than the present temperature.

This method can be used in a general sense, even in horizontal cases, with area isolates of climate-sensitive species. To do it, we suppose that a minimal MAT difference (MATD) of the HTM compared to the recent climate allowed for some locally relict vascular plant species to expand continuously northwards, at least to the recent Pannonian-Transylvanian isolated occurrences (northernmost occurrences of these taxa). We investigated the latitudinal distances between these relict occurrences and those of the main distribution further south. Considering reliable estimates for HTM difference from recent time (Feurdean *et al.*, 2014; Magyari *et al.*, 2012; Molnár and Végvári, 2016; Tóth *et al.*, 2015), we restricted the study only to species

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with gap-distances of maximum 300-350 km from the southern larger distribution of those given species to the isolated occurrences further north, since a latitudinally directed distance can be translated into temperature, showing altitudinal cooling trend polewards, 0.69° C/100 km, (Chen *et al.*, 2013). Out of 15 selected species, 11 indicate 1.0-1.4° C MATD, and 4 indicate 1.7-2.2° C. It is suggested that these four species (or at least some of them) are presumably interglacial relicts. All the 15 relicts are species of xerotherm grasslands and woody vegetation, two of them, *Hypericum umbellatum* and *Saponaria bellidifolia* are mostly inhabitants of higher mountains, while *Aethionema saxatile* prefers higher altitudes in Transylvania while occuring on lower hills further west.

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Distribution of the Carpathian-Balkan species *Centaurea calocephala* Willd. (Asteraceae)

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Centaurea calocephala Willd. (*Centaurea atropurpurea* Waldst. & Kit.) belongs to subgenus *Acrocentron*, subtribe Cardueae (Asteraceae). *C. calocephala* inhabits rocky places, calcareous rocks and soils rich in CaCO₃ and with pH 7.5 - 8.5 from the hilly areas in the mountains (Gajić, 1975; Vonica *et al.*, 2012). Also, it can be found in mesoxeric meadows with southern exposure (Prodan and Nyarady, 1964; Vonica *et al.*, 2012) and as well as on serpentinite.

This Carpatho-Balkan species is a hemicryptophyte, robust plants with large capitulas and pinnate-leaf side (Prodan and Nyarady, 1964; Gajić, 1975; Dostál, 1976; Vonica *et al.*, 2012).

The data on the distribution of *C. calocephala* were obtained from the literature and herbaria (BEOU, BEO, BUNS, MKNH, SO, SOA, SOM, ZA, ZAHO, SARA). The literature and herbaria data were also georeferenced using software OziExplorer 3.95 4s.

According to review of available data, *C.calocephala* is mostly distributed in the central parts of the Balkan Peninsula (Romania, Serbia, Bulgaria and Macedonia), wherein, only a few isolated populations are recorded in Croatia, Albania, Bosnia-Herzegovina, Montenegro and Albania.

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Geomorphologic division of the Ukrainian Carpathians for routine use in biogeography

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The Ukrainian Carpathians (UC), part of the Eastern Carpathians (EC), span for 280 km on a NW to SE axis of folding and cover cca. 24, 000 sq. km. (cca. 10% of the entire Carpathians).

The most widely accepted among Ukrainian scientists is the geomorphologic division of UC proposed by Tsys (1962), which was later adopted and further developed in several other publications (e.g., Herenchuk, 1968; Slyvka, 2001; Kravchuk, 2008). According to this concept, the UC are subdivided into 7 main operational geographic units (OGUs) called 'oblasts', which follow the main mountain ranges: a) Ciscarpathia; b) the Outer Carpathians (incl. subprovinces of the Beskydy-Gorgany and Pokutia-Bukovyna Mts.); c) the Central Waterdivided-Verkhovyna Carpathians; d) the Polonynas-Chornohora Mts.; e) the Maramureş (Marmarosh) Mts.; f) the Volcanic Carpathians and adjacent lowlands (incl. Khust-Solotvyno Depression); g) Transcarpathia.

The division of the UC by Tsys (1962) was also used as geomorphologic basis for the floristic regionalisation (Chopyk and Fedoronchuk, 2015). According to this, the UC are divided in two subprovinces. The Pannonian subprovince includes only the Transcarpathian lowland, while EC subprovince has 9 OGUs: a) Ciscarpathia; b) the Eastern Beskyds and Low Polonynas; c) the Gorgany Mts.; d) the Svydovets Mts.; e) the Chorhohora Mts.; f) the Chyvchyny-Gryniava Mts.; g) the Maramureş Mts.; h) the Volcanic Carpathians; i) the Khust-Solotvyno Depression. This regionalization taims to reach a comprehensive outline of the phytogeographical districts within the UC. However, the author does not fully and consistently present all the arguments for delimitation of some units (e.g., the merging of the Chyvchyny with Gryniava and its splitting from the Maramureş). Nevertheless, this regionalization is commonly used for biogeographic purposes in Ukraine and in most cases the labels of biological

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samples follow it. This was the main reasons why this division was used, with some corrections, in the latest list of Carpathian endemics from Ukraine (Novikoff and Hurdu, 2015).

Although highly debated, the proposed divisions of the UC fail to obtain consensus. One example is the regionalization scheme proposed by Kondracki (1989), which is often applied for biogeographic analyses of Carpathians (Mráz and Ronikier, 2016). In particular, Tasenkevich (2004) used this scheme for phytogeographic regionalization of the Carpathians. The biogeographic classifications of the UC by Kruhlov (2008) included 33 'morphogenic meso-ecoregions'. Later, Kruhlov (2012) recognized 44 OGUs, which were grouped in 15 main classes. However in some cases, the delimitation of OGUs (e.g. Hutsulska Verkhovyna etc.) and hierarchical classification of OGUs in Kruhlov (2008, 2012) are questionable.

Therefore, we present here a new scheme for the geomorphological division of the UC, following a correct hierarchical and nomenclatural use of biogeographic units (Cox, 2001), which includes comprehensive data from all the referenced sources and a clear statement over the applied classification criteria. It includes 3 hierarchical levels, with the lowest encompassing 53 OGUs. For the majority of OGUs, equivalence with the previous relevant studies is provided.

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Phylogeographical structure of the Carpathian endemic plant Campanula serrata (Kit.) Hendrych (Campanulaceae)

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The Carpathian Region is one of the main centers of endemism and biodiversity hotspots in Europe. Regional endemics represent a unique contribution to the European biodiversity and at the same time excellent, well defined spatially models for phylogeographical analyses (Kliment *et al.*, 2016; Mráz and Ronikier, 2016).

In this study we address the fine-scale phylogeographical analysis of *Campanula serrata* (Kit.) Hendrych, a Pan-Carpathian endemic occurring in a relatively wide altitudinal range (centered in the subalpine belt) across various tall-grass, grassland and meadow communities. Due to its habitat requirement and low competitiveness, it is locally a vulnerable species (e.g. in Poland). Most available research on *C. serrata* focuses on habitat requirements or conservation and genetic data are lacking. Applying molecular tools (DNA sequencing and AFLP genotyping) we analyze the genetic structure of *C. serrata* populations in a comprehensive latitudinal and altitudinal context. We aim to test whether genetic differentiation of this species' Carpathian range. We assess the level of genetic affinity between the populations of different parts of the Carpathians. Here, we focus in particular on the areas adjoining main known biogeographical borders (e.g., the Bieszczady Mts.). Furthermore, we plan to analyze the genetic variation and divergence of populations in relation to their altitudinal location, character of habitats and plant communities and regional abundance of populations.

At the current stage, a pilot sequencing analysis of the nuclear ITS and three selected chloroplast regions has been conducted. First results show that populations from the Southern Carpathians are characterized by the highest genetic variability

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and that there is a significant genetic differentiation between the Eastern and Western Carpathian populations, which fits trends detected in earlier studies. At the moment, our results suggest that genetic diversity of *Campanula serrata* (Kit.) Hendrych populations may be correlated with elevation and also that the Bieszczady region (Eastern Carpathians) has a specific phylogeographical position, but it has to be verified in further analyses.

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Patterns of plant species diversity and communities of grasslands from the Ampoi River catchment (the Apuseni Mountains, Romanian Carpathians)

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In the Carpathians the limit between the Metaliferi and Trascău Mountains is drawn by the middle and inferior course of the Ampoi River (Arăboaei and Arăboaei, 2002). The anthropic impact in the area (changes in land use, air pollution, intensity of grazing, etc.) it is high and variable. Following the idea of Dixon et al. (2014), our local and regional grasslands may serve as a tool for convincing policy makers and land managers about the conservation values of grasslands ecosystems, especially grasslands with high plant species diversity and high rate of conversion. For tracking the changes in land use we interviewed local people. Our *in situ* fine-scale study focused on 182 altitudinal gradients' investigation plots (100 sq.m.) inventorying the plant species composition and coverage, grazing intensity and heavy metals concentration of the soil. DCA and PCA multivariate statistical analysis highlighted that the plant species composition and distribution is shaped by altitude, soil type, slope, exposition, while the intensity of grazing mainly by sheep and soil pollution. Because of all of these synergic anthropic impacts it is difficult to frame the phytocoenoses in a particularly plant association. There are relevees with more than 35% coverage of *Poa angustifolia* suggesting the framing of this plant community in Cynodonti-Poetum angustifoliae association from Festuco-Brometea class but with numerous inclusions of the species from Molinio-Arrhenatheretea class. From Festuco rubrae-Agrostetum capillaris association, the most palatable Festuca rubra species is not recorded being totally grazed or still absent. A good indicator of grassland degradation is the presence of species from Stellarietea mediae and Artemisietea

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classes. *Rubus caesius* with more than 40% abundance-dominance is characteristic to *Galio-Urticetea* class grouping nitrophilous vegetation. Inside *Artemisietea* class there are some elements specific to *Potentillion anserinae* alliance and *Potentillo-Polygonetalia* class illustrating the existence of temporary wetlands intrusions or with humidity excess. In other plots is remarkable the existence of *Festuco rubrae-Agrostetum capillaris nardetosum strictae* sub-association with high coverage of *Nardus stricta*.

The complex pattern of plant species diversity and communities of grasslands from the Ampoi river catchment show a pronounced differentiation in biogeographic patterns, related to the natural but mainly anthropic impact in the region. Most of the grasslands here follow an abandonment of agricultural fields. For increasing or at least not decreasing the ecosystem services provided by these grasslands, the knowledge provided by us may be found valuable by policy makers and conservation managers.

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Comparative analysis of vegetation on grasslands abandoned, grazed and mowed after shrub cuttings in the Matra Mountains from Hungary

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Antropogenous meadows are among the habitats with the highest biodiversity from Hungary. In the past few decades, the intensive growth of shrubs has triggered the need for artificial interventions. These tasks had been carried out in 2012 by the Bükk National Park Directorate, but on some of the spots there has been no management afterwards. On all shrub-cut areas and on mowed areas (as control) we made coenological surveys using 2×2 m quadrats in every year from 2013 to 2017, where the cover values of species had been assigned. The vegetation data had been evaluated employing cluster analysis and detrended correspondence analysis (DCA).

The results also clearly showed the positive effect of pasturage. On area I, which had been shrub-cut in 2011, and mowed on the next two years, but remained unmanaged afterwards, the number of species decreased, amount of dead leaves increased, competitor species came into prominence and shrub increased. In aspect of nature conservation, the proportion of natural species has increased.

Differences emerged between the vegetations of two halves of area II, which had been separated in 2014. On the untreated part, the proportion of weeds, arboreal and disturbance-tolerant species increased and biodiversity decreased, while on managed part it was just the opposite. Area IV, which had been also halved in 2014, showed similar results. Here, the two parts are grazed with lower and higher intensity respectively. The more intensively grazed part showed more similarity with the vegetation of the area considered for many years as control. The latter have higher number of species, highest diversity values and dominancy of natural vegetation elements. This denotes that long-term mowing is the appropriate

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management method, which has maintained a species-rich vegetation. During intensive grazing the proportion of rosulate and reptant annual species has increased, but diversity too.

Despite of lack of management, vegetation with a natural distribution of species have developed on area III within six years. During this period of time, arboreal species have not become dominant; their proportion stagnated, even decreased on some spots. On this untreated grassland, on plant communities threatened by shrubs' increase in coverage, big game potentially controlled the cover of arboreal species, and can play a large role in sustaining natural states.

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Pedological study on grasslands dominated by *Festuca vaginata* and *F. pseudovaginata* in the center of the Carpathian Basin

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The aim of our study was to reveal the differences the soil parameters in open sandy grasslands dominated by *Festuca vaginata* and *F. pseudovaginata*. Based on previous coenological studies we hypothesised that the grasslands characterized by the endemic *Festuca vaginata* are more species-rich than ones with *Festuca pseudovaginata*. Coenological sampling was carried out in June 2009 and 2017 in two study sites (Tatárszentgyörgy, Imrehegy), in quadrats of 2×2 m. Analyses of the relevés were based on the following data: values of seven soil properties (pH[KCL]; pH[H₂O]; humus; total-N; Ca; P₂O₅ and K₂O) measured in the 0-15 and 15-30 cm soil layers. Soil properties of the grasslands dominated by *F. pseudovaginata* and *F. vaginata* were compared by linear mixed models, where 'grassland type' was the fixed factor and 'site' was a random factor nested in 'grassland type'. Data were analysed by cluster analysis, fusion algorithm was a combinatorial method (minimizing increase of variance) and the correlation was used as comparative function.

Based on the cluster analyses using plant coverand only upper 0-15 cm soil layer data, we can state that *F. pseudovaginata* and *F. vaginata* groups were well separated. Linear mixed models revealed that *F. vaginata* grasslands were typical on soils with higher pH, nitrogen, phosphorous and potassium contents compared to *F. pseudovaginata* grasslands, which refers to a tight connection between the properties of the upper soil layer and the vegetation in sandy grasslands.

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Three major vegetation turnovers since the Last Glacial Maximum recorded in calcareous tufa deposit in the Danubian Lowland (Slovakia)

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The long-term development of European vegetation is determined particularly by climatic changes and, since the arrival of the first Neolithic farmers, also by human impact. After the Last Glacial Maximum (ca. 21,000 cal. yrs BP) the most pronounced climatic amelioration occurred ca. 14,600 cal. yrs BP (short time fluctuation) and at the onset of the Holocene around 11,700 cal. yrs BP. During the Holocene period several climatic oscillations occurred, but since ca. 8,000 cal. yrs BP the main driver of the vegetation change were people.

To detect past vegetation changes pollen, macrofossil, mollusc and geochemical analyses were processed in the travertine deposit near the Santovka village (south Slovakia). Palaeoecological profile was radiocarbon dated back to 17,000 cal. yrs BP and thus, it represents one of the few palaeoecological records dated back to the Pleniglacial in this region.

At the end of the Pleniglacial, climate became more humid and warmer, which led to expansion of *Pinus sylvestris* at the expanse of grasses. Local environment underwent complete turnover from vegetation dominated by *Betula nana*, *B. humilis* and *Selaginella selaginoides* to species-rich wetland vegetation with *Schoenoplectus tabernaemontani*, *Sparganium erectum* and *Zannichellia palustris*. During the Early Holocene (around 10,000 cal. yrs BP) steppe-tundra vegetation dominated by *Pinus sylvestris*, *Betula* spp., herbs and grasses was replaced by temperate forest with *Quercus*, *Corylus* and *Ulmus*. Around 6,500 cal. yrs BP soil runoffs, most probably initiated by human activities (e.g. deforestation), overlaid relatively species-rich wetland with Cladium *mariscus* and *Schoenoplectus tabernaemontani*, which resulted in disappearance of this biotope and final creation of reed marsh. Surrounding landscape became again more open due to agricultural activities of people and among trees Pinus and Picea started to prevail.

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The response of soil nematodes and microbes to windstorm beech forest devastation

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Forests are species-rich terrestrial ecosystems supporting a wide range of taxa from numerous groups ranging from plants and vertebrates to canopy arthropods, soil microorganisms etc. This biological diversity is essential for forest ecosystem dynamics; unfortunately, it is constantly affected by various harmful factors. Windstorms are considered as a significant phenomenon and key disturbance factor in the most natural (unmanaged) and production forests, which affects not only trees and stands but can also change soil biota and initiate ground layer successions.

The windstorm that appeared in Slovakia in May 2014 damaged the productive beech deciduous forests in many areas throughout the country. Our study has been carried out in a selected area with all fallen trees removed; as a control, the close standing vital beech forest un damaged by windstorm has been chosen. The main aim of the study was to investigate how soil nematode communities and microbes react to dramatic changes in their living environment (soil) caused by windstorm trees devastation and their removals. Soil samples for analysis were collected in Year 1 and 3 after the windstorm event. Among the soil physical-chemical properties, soil acidity, N, C, S and K content were slightly lower at the windstorm affected plot, while Mg and Ca content increased in soil samples of clearing. The nematode communities negatively responded to fallen trees removing (clearing) on the windstorm damaged plot by decreasing their total abundance as well as population densities of all functional and feeding groups in comparison to control. Microbial soil characteristics showed a variable trend. While basal respiration, catalase activity, species richness and functional diversity were not significantly affected by trees removal, microbial biomass carbon and N-mineralization considerably decreased, indicating degraded living condition for microorganisms compared with the vital

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forest soil. Our study showed that differences occur in the nematode communities, microbial activity and biomass at the disturbed plot in comparison to an intact forest. Future long-term observations could reveal the changes of ground vegetation in the abundance, richness and diversity of functional guilds of soil nematodes and microorganisms in the ecosystem recovery processes.

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Robinia pseudoacacia plantations as the factor of homogenization of the Carpathian forest vegetation

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Species composition of forest vegetation is changing in past decades due to changes in land use, fragmentation, and synanthrophisation of forest communities. All these processes cause extinction of some typical (including rare) forest species and invasion of aliens and lead to the homogenization of forest vegetation. Planting of alien trees and intensive forest management play a key role in the homogenisation process. Alien forests are often the hotspots of non-native species diversity supporting spreading of non-native species to the surrounding environment. In the Carpathian Region one of the most commonly planted alien trees is *Robinia pseudoacacia*. Many types of native forests (hardwood floodplain forests, oak forests, Carpinion forests) were replaced by *Robinia* plantations. The diversity of broadleaved deciduous forests is huge in the Carpathian Region with many local differences, geographical variability and endemic species occurrence. We hypothesize that (1) areas where these native forests were replaced by *Robinia* forests show lower diversity on a geographical gradient along the Carpathians than native communities do, (2) total species pool of native broadleaved deciduous forests is higher than the species pool of alternative/ substitute/replaced Robinia and (3) plantations of Robinia pseudoacacia are a factor that unifies species composition of forests, limiting endemic species occurrence and restraining local differences in forest diversity. We used more than 100 paired relevés of *Robinia* forests and native forests sampled during 2014 - 2017 vegetation seasons in the area of Slovakia, Hungary, Poland, Ukraine and Romania. Relevés were sampled following standard methodology of Zurich-Montpelier school using the new

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Braun-Blanquet cover-abundance scale. Each pair of relevés was sampled in native and adjacent *Robinia* forest patches with less than 250 meters distance among them and in the same environmental conditions (slope, orientation, soil type) to avoid the influence of local environmental conditions' variability on the forest undergrowth. Thus, the difference in species composition can be interpreted as an effect of alien trees. The dataset of native forest relevés and dataset of corresponding *Robinia* relevés were analysed using SYN-TAX and Canoco software and the differences in betadiversity and dissimilarity within native and *Robinia* forests were compared using Wilcoxon paired test (R-software).

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Is Tephroseris longifolia subsp. moravica a West Carpathian endemic?

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Tephroseris longifolia subsp. *moravica* was recognized as a separate taxon relatively late (Holub, 1979) and soon after it was considered a West Carpathian endemic (Maglocký, 1983). Over the last decades, a decline in abundance was recorded for the Carpathian populations. Nowadays, *T. l.* subsp. *moravica* is known just from nine localities in Slovakia and the Czech Republic and it is treated as an endangered taxon of European importance (NATURA 2000). *Tephroseris longifolia* subsp. *moravica* is a member of *T. longifolia* agg. - an intricate complex of perennial outcrossing herbs distributed in the Western Carpathians, the Eastern and Central Alps, and reaching also the Apennines and Pannonia. Five subspecies with rather diverging distribution patterns are distinguished within this aggregate. While *T. longifolia* subsp. *moravica* and subsp. *brachychaeta* are endemic taxa with narrow ranges, *T. longifolia* subsp. *gaudinii*, subsp. *pseudocrispa* and subsp. *longifolia* are more widely distributed.

In the last years, the *T. longifolia* aggregate is subject of biosystematic research aimed to evaluate the taxonomic position of *T. l.* subsp. *moravica*. Experimental hybridizations showed that the subspecies of the aggregate hybridize easily and produce viable progeny (Šingliarová *et al.*, 2013). Further, we confirmed morphological and genome size differentiation of the subspecies (Olšavská *et al.*, 2015). Similarly, evaluation of selected topographic (geographical location, altitude), climatic (temperature and precipitation) and soil parameters as well as analyses of co-occurring plants, displayed a remarkable differentiation in ecological requirements (Janišová *et al.*, in prep.). However, we found that Pannonian populations of nominated subspecies are morphologically and ecologically closer to the populations of the Carpathian endemic *T. l.* subsp. *moravica* than to Alpine populations of the same subspecies. Thus, the

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endemic position of the Carpathian and Pannonian populations remained controversial and require further study. Therefore, in ongoing study we aim to detect genetic variability and evolutionary relationships within the *Tephroseris longifolia* agg. using nuclear ITS sequences and highly variable AFLPs on 32 populations covering the whole distribution range of the aggregate. We analyze also other closely related taxa such as *T. aurantiaca, T. capitata, T. crispa, T. integrifolia* and *T. papossa*. This study represents an inevitable step to validate the endemic status of the Western Carpathian *T. l.* subsp. *moravica*.

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Preliminary notes from a population study for the local endemic Lychnis nivalis in the Rodna Mountains

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Lychnis nivalis Kit ex. Schultes has been the focus of many studies in the Austrian, Hungarian and Romanian botanical literature, mostly due to its uncertain taxonomy and its restricted distribution in the alpine and subalpine belts of the Rodna Mountains (in the Romanian Carpathians).

Currently, the species faces an unknown future in the predicted warming of the climate, as its ecology is linked to cold habitats from high altitudes and northern slopes. Our study explores the roles of topography, competition and dispersal in the distribution of the species *Lychnis nivalis* and represents a first step in understanding the potential effects of climate change on its distribution.

Distribution data for the species have been collected from several locations in the field during the months of July and August 2016 and 2017 (Lala valley, Gărgălău glacial cirque, Galați peak, Iezerul glacial cirque, Iezerul peak, Rebra peak, Buhăiescu peak).

The 2017 survey will be focused on the Iezerul glacial cirque, where all nonwoody vegetation types will be surveyed, with the exception of extended scree (with no vegetation or soil) and steep rock formations. Visual inspection for species presence in all non-investigated areas will be conducted from accesible points where possible.

Lychnis nivalis individuals will be inventoried using a grid of 4 m^2 , with 40 cm subgrids. In order to optimize the data collection in habitats where the species is frequent, the minimum distance between plots will be at least 20-30 meters. All the

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vegetation will be recorded per subgrid. For *Lychnis nivalis*, the number of shoots will be recorded, with number of flowers per individual. Shoot height will also be recorded, as well as the height of the dominant vegetation. Portions of basal leafs will be collected (1-5 per plot, depending on the number of individuals). Inventoried individuals will be tagged for future seed collection (in late August). At the end of the floristic inventory, each collection site will be sampled for soil (1-200 g), in the vicinity of the *Lychnis nivalis* shoots, at root depth. GPS location will be registered for the middle of the grid. Photos will be taken of each subgrid, and of the entire grid from above, as well as from each corner facing towards the grid. Additional photos will be taken for all the *Lychnis* individuals and of the overall position of the grid in the landscape. Samples of dominant vegetation as well as from plants which could not be identified in the field will be collected from the plot, for determination in the laboratory.

The location of *Lychnis nivalis* individuals observed between sampling grids will be marked using a GPS, and a photo will be taken.

The preliminary analysis will be focused on a description of the samples location, a first estimation of vegetation cover in the plots, the number of *Lychnis nivalis* shoots recorded inside the plots, and the average number of flowers per shoot. Some conclusions may be drawn related to the species preference to vegetation types, and topographical conditions (e.g. slope, aspect, potential solar radiation, insolation time, irradiance, topographic position index, topographic wetness index, roughness index, derived from a Digital Elevation Model with 12 meters resolution).

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Comparative coenological study on grasslands dominated by *Festuca* vaginata and *F. pseudovaginata* in the center of the Carpathian Basin

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The aim of our study was to reveal the differences in open sandy grasslands dominated by *Festuca vaginata* and *F. pseudovaginata*. Due to the arid conditions, sandy grasslands are generally covered by xerothermic vegetation where *Festuca vaginata* is a typical dominant species. *Festuca pseudovaginata*, a species newly described by the authors, can also gain dominance in sandy grasslands. Based on previous coenological studies we hypothesised that the grasslands characterized by the endemic *Festuca vaginata* are more species-rich than ones with *Festuca pseudovaginata*. Coenological sampling was carried out in June 2009 and 2017 in two study sites (Tatárszentgyörgy, Imrehegy), in quadrats of 2×2 m. Analyses of the relevés were based on the following data: cover scores of vascular plant species and cryptogam crust. Data were analysed by cluster analysis, fusion algorithm was a combinatorial method (minimizing increase of variance) and the correlation was used as a comparative function.

Statistical analyses of the vegetation data showed that *F. pseudovaginata* and *F. vaginata* samples were well separated and grasslands dominated by *F. pseudovaginata* had nearly two times more species than the ones dominated by *F. vaginata*.

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Alpine plants at the margin: a case of alpine plants survival in a warm climate massif of the Southern Carpathians (the Cozia Massif)

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This preliminary study is based on several floristic surveys carried out by the authors from 2013 to 2015 and aims to address to the question of glacial refugia in the Carpathians, focusing on the Cozia Massif. Located in the southern part of the Făgăras Mountains, this massif detains one of the most diverse flora within the Carpathians. The Cozia Massif is known to harbour several narrow (e.g. Centaurea coziensis, Rosa × argesana, Rosa coziae, Stipa crassiculmis subsp. heterotricha,) and more widespread Carpathian endemics (e.g. Dianthus henteri, Draba simonkaiana, Galium baillonii, Galium kitaibelianum, Genista tinctoria subsp. oligosperma, Scabiosa lucida subsp. barbata, Silene nutans subsp. dubia, Thlaspi dacicum or Thymus comosus), rare and protected species at national or European level (e.g. Arabis procurrens, Campanula wanneri, Carex brachystachys, Daphne blagayana, Drymocallis rupestris, Lilium jankae or Plantago holosteum) and numerous termophilous species of Balkan and Mediterranean origin that are reaching here their northern distribution range limit (e.g. Alyssoides utriculata var. graeca, Erysimum comatum, Ophrys fusca, Potentilla haynaldiana, Sorbus cretica or Veronica bachofenii) (Dimitriu - Tătăranu, 1949; Nyárády, 1955; Coldea and Pop, 1988). Several highly interesting floristic elements are represented by the subalpine and alpine species, found here at the margin of their known to be preferred habitat and optimal ecological conditions, at elevations below 1500 m a.s.l. Among them, Alnus viridis, Cerastium alpinum subsp. lanatum, Draba simonkaiana, Hieracium alpinum, Jacobaea abrotanifolia subsp. carpathica,

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Leontopodium nivale subsp. alpinum, Myosotis alpestris, Phyteuma nanum, Polygonum alpinum, Primula minima, Saxifraga oppositifolia, Saxifraga pedemontana subsp. cymosa or Sedum alpestre are just several examples of high mountain taxa that occur in narrow and remote areas of the Cozia Mountains like the northern slopes of the Bulzu peak.

The diversity of microclimates and the varied geological bedrock characterizing the Cozia Mountains, together with the historical climatic oscillations during the Ouaternary period are presumed to be among the factors that contributed to the survival of alpine species in this warm climate massif. The high number of highmountain taxa and their occurrence here at lower than typicalaltitudes may support the hypothesized relict character of these populations. They are presumed to have originated here during the Ouaternary glaciations through a vertical migration process in the colder climate periods. According to the classification of glacial refugia types (Holderegger et al., 2009), the Cozia Mountains would enlist as a peripheral refugia, which is characterized by lower elevations and localization at the periphery of high mountain ranges, which in this case would be represented by the Făgăraş Mountains. It is likely that these taxa migrated from the highly-glaciated Făgăras Mountains to the lower Cozia Mountains in the south where, due to favorable microclimatic conditions, they established and survived until today. Currently, these taxa can be more frequently encountered in the Cozia Mts. on the north facets of cliffs and steep slopes with gravels, characterized by more shady and humid conditions.

Our results will not only help to better understand the processes behind the survival of different plant species in the Carpathians during the Quaternary glaciations, but will also increase the knowledge over the entire flora of Cozia Massif, which will contribute to a better aimed protection and conservation of its valuable biodiversity assets.

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Morphological delimitation of *Sphagnum angustifolium*, S. fallax and S. flexuosum

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The moss genus *Sphagnum* L. (peat mosses) is an ancient plant group and counts approximately 300 species with a widespread distribution across the northern hemisphere. Delimitation of species in the genus *Sphagnum* has been notoriously difficult and gave rise to many controversies during the last decade. High degree of morphological similarity of species and extreme phenotypic plasticity of distinctive characters has been invoked to explain controversial taxonomic assessments based on morphology.

Here we investigate morphological and genetic variability within a well-delimited but taxonomically contentious species complex, named *S. recurvum* group, in order to reveal the ultimate causes of discordance between morphology and molecular patterns. Three most distributed species of this group were involved in this study: *S. angustifolium*, *S. fallax* and *S. flexuosum*.

A total of 351 specimens collected in 22 European countries were included in this study covering a significant part of the distribution of the group in Europe. In order to investigate the influence of latitude on the morphological differentiation of the three genetic groups, accessions were split into five arbitrarily defined groups: all Europe, North, Central and South Europe and Hungary.

Eleven microsatellite loci were utilized to evaluate the genetic diversity. Morphological measurements are held on spreading and hanging branches, stem leaves, pendent and spreading branch leaves, altogether 12 variables were used for analyses.

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On the basis of microsatellite variation the three investigated species were separated from each other on the base of STRUCTURE analysis. On the base of Principal Coordinate Analysis (PCoA), *S. fallax* specimens were clearly distinct from the two other species. *S. angustifolium* and *S. flexuosum* were clearly separated only in North European and Hungarian groups along the first two axes of the PCoA. In other groups (all, Central and South Europe) they formed separated but contiguous clouds.

In order to look for the morphological characters best distinguishing the three genetic clusters, Linear Discriminant Function Analysis (LDA) was conducted with eight morphological variables that showed significant differences among the genetic clusters. Separation of the genetic clusters based on their measured morphological characters was far from perfect in all five geographic groups. The power of discriminant functions was tested using a leave-one-out cross validation procedure. The LDA misallocated 12-21 % of samples on the base of leaf morphology in different geographic groups. The proportion of misclassified accessions showed an increasing trend towards the south.

To test the dissimilarities between genetic clusters on the base of morphological characters, multivariate ANOVA were calculated. The results of multivariate ANOVA confirmed the results of LDA, where the three genetic clusters were primarily restricted to the three edges of the point cloud, but they showed considerable overlap. The R^2 values of multivariate ANOVA ranged from 0.14 (Hungary) to 0.35 (North Europe).

Based on these analyses we can conclude that the three genetically different species were morphologically well separated in North Europe, but they had a considerable overlap in other parts of Europe. This result can be related to the much higher fragmentation in Central and South Europe.

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Endemic and rare weevils (Coleoptera, Curculionoidea) in northern part of the Eastern Carpathians, Romania

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Weevils were studied and collected in 2008- 2015 from the Bârgău, Călimani, Gutâi and Rodna Mountains area (northern part of the Eastern Carpathians), in several characteristic ecosystems: spruce forests, mixted forest of coniferous and decidous trees, *Telekio speciosae - Petasitetun hybridi association* along the mountain valleys, beech forests, coppices, mountain meadows (hayfields) and subalpine meadows.

We recorded 10 species or subspecies endemic for the Carpathian area: *Bryodaemon hanakii hanakii* (Friv.), *Otiorhynchus (Magnanotius) deubeli* Ganglb., *O. (Magnanotius) kollari* Gyll., *O. (Magnanotius) schaumi* Stierl., *O. (Nihus) proximus depauperatus* Penecke, *O. (Prilisvanus) asplenii* Miller, *O. (Prilisvanus) obsidianus* Boh., *O. (Prilisvanus) opulentus* Germ., *Phyllobius (s. str.) transsylvanicus* Stierl. and *Liophloeus (Liophloeodes) liptoviensis* (Weise). Some of these species are spread in all Carpathians, but *B. hanakii hanakii* (Friv.) and *O. (Magnanotius) deubeli* Ganglb. are spread only in the eastern and northern parts of the Carpathians (Holdhaus and Deubel, 1910; Podlussány, 1998; Mazur, 2002; Teodor and Vlad Antonie, 2007; Magnano and Alonso-Zarazaga, 2013).

We found 9 rare species or subspecies: Rutidosoma (Scleropteridius) monticola (Otto), Otiorhynchus (Magnanotius) equestris (Rich.), O. (Magnanotius) schaumi Stierl., O. (Prilisvanus) rugosus krattereri Boh., Onyxacalles pyrenaeus Boh., Tychius sharpi Tourn., Stomodes gyrasicollis Boh., Adexius scrobipennis Gyll. and Plinthus (s. str.) illigeri Germ;

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The high number of endemic and rare weevil species found in the studied ecosystems proves the rich biodiversity of the northern part of the Eastern Carpathians.

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Biogeography and molecular diversity of *Swertia perennis* (Gentianaceae) from Europe

Jacek Urbaniak^{1,,,} Paweł Kwiatkowski² and Paweł Pawlikowski³

Swertia perennis (Gentianaceae) is one of the perennial diploid plants and clonal plants inhabiting both lowland and mountain peat bogs. The species is discontinuously distributed from northern Asia (Siberia), through the European lowlands and mountains such as the Carpathians, the Alps, and the Pyrenees to North America. The current geographical dispersion of *S. perennis* is probably an effect of climatic changes that happened in the Quaternary and were the most important factor that determined the present-day distribution of numerous plants and especially *S. perennis*. Here, we present a study based on chloroplast DNA markers (*trnL-trnF* and *trnH-psbA*) that was conducted to compare and explain the phylogeography of *S. perennis* from both lowland and mountain localities in Europe. Plants were collected from different localities in the Polish lowland, the Sudetes, the Carpathians, the Bohemian Forest and the Alps.

During the study, we identified about 20 haplotypes that characterized a high level of genetic variability in populations of *S. perennis*, however we identified lack of phylogeographical structure. We mean, that it can be a result of slow successive colonization, confirmed by multi-directional gene flow between plants from investigated populations and expansion from several areas. This genetic differentiation may also have been due to the relatively long-term isolation of *S. perennis* in Pleistocene refugia in Europe, that resulted in independent separation cpDNA lineages.

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The lack of phylogeographical structure makes it impossible to indicate the centre of haplotype diversity. On the other side, plant refugia located in peat bogs between the ice sheets in the lowlands, the Carpathians, the Sudetes or the Alps were the most probable sites, where *S. perennis* survived. The lack of evidence for phylogeographic structure can also indicate a high level of gene flow in the recent past. The variation in nucleotide composition of cpDNA may also reflect the genetic variability from the not fragmented landscape in the Pleistocene.

The threats and conservation measures for communities of Narcissus angustifolius Curt in the natural reserve area "The Narcissus Valley" (Ukraine)

Pavlo Ustymenko^{1,⊠} and Dmytro Dubyna¹

In the geographical center of Europe (at the end of Khust, Transcarpathia) there is a unique and extremely valuable floristic and phytocoenotic natural reserve -"The Narcissus Valley" (256.2 hectares), which is part of the Carpathian Biosphere Reserve. This is the only area in Europe where a large population of *Narcissus angustifolius* Curt occurs at such low altitudes, respectively 180-200 m above sea level. In Ukraine, this species occurs only in Transcarpathia - in several locations along the ridge of the Svydovets and the Marmarosky Alps and in the piedmont and lowland parts of these regions. The presence of this species in "The Narcissus Valley" protected area represents, thus, the population found at the lowest altitude within the entire range of the species. In the Tertiary period, *N. angustifolius* presumably grew only in the highlands, while during the Ice Age a downward vertical migration process towards the piedmonts and lowlands has occurred.

Currently, the priority is to identify and assess the threats and negative trends in order to identify the most suitable conservation strategies for the protection of habitats dominated by *N. angustifolius*. This assessment should take into consideration the insular nature of the protected area and the threat deriving from the vegetation changes.

Nowadays, among the major threats identified for this protected area are vegetation succession caused by the introduction of the absolute protection regime in the natural reserve. In these conditions, herbal communities transform, their cenostructure changes, and as a consequence the rare species might be excluded. A process of phytosystems simplification might reduce their structural complexity due to inadequate regulatory measures. As result of non-mowing or irregular mowing, we observed an

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increase in number of grass species, together with the expansion of shrub vegetation dominated by *Prunus spinosa* at high altitudes and by *Salix cinerea* in wetland depressions.

Restoration channels regulated surface drain, but were slowly overgrown with trees and shrub species and finaly did not attain their initially intended purpose. This caused three to five times annual flooding of "The Narcissus Valley" and water saturated the soils from the surrounding areas. Due to the absence of rapid drainage, siltation occurred in the natural reserve. This subsequently led to an edaphotope change and to the formation of communities dominated by *Molinia caerulea*. Furthermore, the presence of external phytocoenotic perturbation sources generated threats caused by phytopollution through colonization of ruderal and invasive species (e.g., *Solidago* sp., *Heracleum* sp., *Echinocystis lobata*, etc.).

To preserve the rare plant communities present in the natural reserve, a different management regime should be applied through regular mowing of grass stands after *N. angustifolius* attains its seeding stage. As stabilizing factors for these plant communities, management plans should include elements that would prevent trees and shrubs overgrowth, regular arsoning and the standing grazing of ungulates together with the impact of other components of the heterotrophic block of ecosystems. Also, on the territory of the natural reserve, any kind of transformation of meadow communities and the use of fertilizers should be forbidden, because these factors can lead to significant structural changes in these plant communities and change their floral component.

Strict implementation of these recommendations for optimizing the protection regime in "The Narcissus Valley" will ensure stability of most of its ecosystems and assure its main purpose – to preserve and restore populations of *Narcissus angustifolius* and plant communities harbouring them. Evaluating the efficiency of these measures should be done by monitoring the plant communities and ecotopes.

Distribution of xeromontane and steppic Noctuidae in Southeastern Central Europe

Zoltán Varga^{1,⊠}

While the distribution of alpine and boreo-montane species is connected with the vertical zonation of the vegetation, the xeromontane species are restricted to special geomorphological (physical weathering) and/or edaphic (rupicolous habitats) conditions. As a consequence, they mostly occur in the Carpathians in calcareous mountains but also in karstic areas of medium altitudes (examples: Slovakian and North Hungarian Karst, Mt. Apuseni). Species of Mediterraneanxeromontane (in Ornithology: Palaeomediterran-xeromontan) faunal type often reach a northern boundary of range in karstic areas of the Carpathian basin. The most diverse representation of this faunal type is present in the southern Balcanic (mostly calcareous) mountains, south of the "Adamovic-line". Western and Central Asiatic xeromontane species occur more sporadically. Some of them show also zonal steppic occurrences North and East of the Carpathians. Historically they are connected with the kryoxerotic late glacial and earliest post-glacial climatic phases and they could survive the re-forestation in habitats with high resilience against macro-climatic succession.

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Vegetation changes and fire history during the last 17,000 years at Lake St. Anne, Eastern Carpathians (Romania)

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For reconstructing local vegetation changes and fire history around Lake St Anne (950 m a.s.l., the Eastern Carpathians, Romania) during the last 17,000 years, plant macrofossil and macrocharcoal analysis were performed on the sediment at a high resolution. Previous studies (Magyari *et al.*, 2009, 2014) were successful in using multiproxy methods on the same core (SZA-2010) from the lake, which focused on the Holocene and the Late Pleniglacial part. Our results revealed that the first plant macrofossils of *Pinus* sp. appeared around 15,900 cal yr BP, which was closely followed by *Picea abies* remains at 15,600 cal yr BP. *Picea* became dominant around the lake from 12,000 cal yr BP, while many wetland species were also abundant. *Sphagnum* sp. floating mat started to develop from 13,000 cal yr BP and became dominant with *Carex* sp. species probably on the northeastern corner of the lake from 10,500 cal yr BP. Chironomidae remains suddenly disappeared form the sediment at 10,500 cal yr BP implying significant changes in temperature and lake-level occurred.

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Macrofossil analysis revealed high charcoal concentration between 16,000 and 14,600 cal yr BP suggesting high local fire activity, although the high resolution analysis of the SZA-2013 core does not support their early presence. The time-lag between these records could be the result of a strongly mosaicked environment or due to a hiatus in the sediment.

Lake St. Anne is considered to be a vulnerable ecosystem, the changes of hydrological, biological and chemical processes are strongly influenced by the vegetation of the lakeshore and by the nearby soil. Understanding these changes in the past could provide more information for the future preservation in the area.

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Species delimitation and phylogeography of the genus Liophloeodes

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Liophloeus is a genus of weevils that occurs mostly in the eastern and central Europe. Traditional taxonomy of this group, based only on male genitalia, is rather poor. The genus currently consists of two subgenera: Liophloeus sensu stricto (including sexual and parthenogenetic taxa with wide range of ecological preferences) and Liophloeodes (including only sexual taxa, connected with wet and cold biotopes, occurs mostly in mountains). The latter is claimed to be Carpathian subendemic, though its range is much wider and includes also regions such as the Alps, the Sudetes, the Dinaric Alps and the Pannonian Basin. However, it seems that the Carpathians are the centre of its range. Liophloeodes species share similar ecological preferences and almost identical morphology, apart from the shape of aedeagus (part of male genitalia), which is the traditional diagnostic trait for species delimitation; also their distribution range is overlapping (Smreczynski, 1958). Therefore, they could be young species or populations under speciation, and their evolution may have been shaped by processes connected to the last glaciation and interglaciation events. It is quite possible, that such species are currently in the "warm-stage" refugia, and the Carpathians are one of them

The aim of this study was twofold: (1) delimiting species within the genus using an integrative approach based on molecular evidence [by sequencing a nuclear (*28S-D2*) and mitochondrial (*COI*) marker] and morphometric evidence (by measuring several morphological traits); (2) assessing the phylogeographic distribution of *Liophloeodes* in order to get insights on the biogeography of these weevils.

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COI data show a fairly strong phylogeographic pattern suggesting also the existence of new cryptic taxa. Some sympatric species are not closely related, which suggests long range dispersal of *Liophloeodes*. On the other hand, the *28S-D2* phylogeny is incongruent with the one based on *COI*, which suggests hybridization between species in the contact zones. This hypothesis seems to be supported also by heterozygosity detected at this locus. Morphometric analyses were less informative, only indicating a clear differentiation between *Liophloeus sensu stricto* and *Liophloeodes*.

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The effect of habitat conditions on number and traits of individuals of rare plant species *Arum alpinum* in the Góra Chełm reserve (the Western Carpathians, Southern Poland)

Tomasz Wójcik^{1,⊠} and Kinga Kostrakiewicz-Gierałt²

Arum alpinum is a rare mountain plant species and represents the Europeantemperate sub-element. The aforementioned taxon is distibuted in the central and southern Europe, Asia Minor, as well as the Caucasus and it reaches the northern limit of its range in Poland (Zając and Zając, 2009).

The aim of the presented investigations was to evaluate the abundance and traits of individuals of *Arum alpinum* under different habitat conditions. Observations were carried out in the Góra Chełm reserve (Strzyżowskie Foothills, the Western Carpathians) in a plant community from the alliance *Fagion sylvaticae*. Studies were conducted on a habitat with steep slope and N-NE aspect (Patch I) and in a broad depression formed in a wide gorge with a S-SW aspect (Patch II). In the herb layer in Patch I, the species with oblong or cordate leaves were dominant (*Dentaria glandulosa, Galium odoratum*), whereas species with broad leaves (*Impatiens parviflora, Mercurialis perennis, Salvia glutinosa*) abundantly occurred in Patch II. The detailed characteristics of habitat conditions in both study sites is given in the publication of Wójcik and Ziaja (2015). In order to detail the observations, permanent study plots measuring 100 m² were established within each of the above described Patches.

After the assessment of number of all vegetative and generative individuals occuring within the study plots, 30 vegetative and 30 generative individuals were selected and marked for further studies. The investigations focused on selected traits of leaf rosettes (number of leaves, size of the longest leaf) and the traits of flowering stems (height of stems, length of infructescence, number of fruits per infructescence).

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The traits of leaf rosettes were surveyed in May, while traits of generative stems were examined in August in the year 2016.

The performed investigations showed, that the abundance of both populations of *Arum alpinum* was similar. Altogether, 62 vegetative and 99 generative individuals occurred within Patch I, whereas 67 vegetative and 114 generative individuals were found within Patch II. The number of leaves per rosette, the length of leaf stalks and width of leaf blade in vegetative and generative individuals were substantially greater in Patch I. Also generative individuals growing in Patch I produced longer leaf blades than in Patch II, while length of the leaf blades in vegetative individuals did not differ among Patches. The subsequent observations showed that 30 generative individuals marked within Patch I and only 6 generative individuals marked within Patch II created completely developed stems with infructescences. The height of generative stems was remarkably greater in Patch II, while length of infructescences and number of fruits per infructescence in both study sites were similar.

Summarizing, it might be stated that better condition of population *Arum alpinum* occurring in Patch I might be an effect of absence of strong competitors in the close vicinity. The worse state of individuals occurring within Patch II might be caused by over-shading by abundantly occurring broad-leaved species (especially *Impatiens parviflora*). Moreover, the periodic waterlogging observed in local depressions presumably contributed to the rotting of majority of generative individuals.

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Euglenophytes of the Tatra Mountains (Central Western Carpathians)

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Research on algae and cyanobacteria occurring in streams and water bodies of the Polish areas of the Tatra Mountains were initiated by Kalchbrenner and Schumann in the second half of the nineteenth century. Euglenophytes were not frequent components in those studies. Currently, these organisms are not often reported from the Tatra aquatic or peat bog ecosystems. This probably results from both atmospheric and habitat conditions of the region, which is fraught with clean, deep, oligotrophic lakes and ponds, turbulent streams and peat bogs surrounding bodies of water. Euglenophytes have specific requirements with reference to habitat conditions, e.g., content of dissolved or particulate organic matter, water temperature, water reaction, and water motion and circulation. We believe that the number of water habitats in the Tatra Mountains providing optimal conditions for the development of euglenophytes is limited. It does not mean, however, that euglenophytes are absent in these habitats. Our studies present the occurrence of euglenophytes in several permanent and short-term, astatic water bodies of the Tatra Mountains. This group of organisms was represented mainly by widespread cosmopolitan taxa such as: Lepicinclis acus, Phacus acuminatus, Trachelomonas volvocina, T. volvocinopsis, T. hispida. Interesting and rare taxa were also observed including Trachelomonas armata var. longispina and Phacus wettsteinii that were both found more than fifty vears ago. In recent studies, apart from chlorophyllous autotrophic taxa, we have also found colourless species - Menoidium distractum and Petalomonas klebsii, both represented euglenophyte flora in Toporowy Staw Wyżni.

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The seed's ultrastructure of genus *Iris* L. (Iridaceae) species from the Ukrainian Carpathians

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We have studied the ultrastructure of five species of genus *Iris* L., which grow in the Ukrainian Carpathians – *I. aphylla* L., *I.graminea* L., *I. pseudacorus* L., *I. sibirica* L. and *I. sintenisii* Janka. *Iris sibirica* and *I. pseudacorus* belong to subgenus *Limniris* (Tausch) Spach, *I. aphylla* – to subgenus *Iris*, *I. graminea* and *I. sintenisii* – to subgenus *Xyridion* (Tausch) Spach. The above species grow in the Carpathian and Transcarpathian regions.

We have found that, regarding the shape, the seeds are rounded-fusiform (*I. aphylla*), D-shaped (*I. pseudacorus, I. sibirica, I.graminea*), and pyriform (*I. sintenisii*). Hilum is rounded or drop-shaped, small, by position – basal (*I. graminea*), with well-seen roll (*I. graminea, I. aphylla*). The seeds of *I. sibirica* and *I. pseudacorus* have a wing that surrounds all the seed (for seeds of *I. pseudacorus,* the wing is smaller). The wings are absent in seeds of others species.

<u>Primary sculpture.</u> The relief of anticlinal cell walls (straight) is common for all studied species. The seeds of all examined species are characterized by polygonal cells of testa, they are isodiametric (*I. aphylla*, *I. sintenisii*, *I. graminea*), or prolonged (*I. sibirica*, *I. pseudacorus*) – mostly on the wing. The boundaries of cells of testa are practically not visible, anticlinal cell walls are tightly merged only in *I. sintenisii* seeds, while in other species' seeds they are clearly seen. The anticlinal sell walls of adjacent cells of testa are divided along the middle lamella. The distal and proximal anticlinal cell walls are raised in relation to surface of periclinal cell walls. The external periclinal cell walls are concave (*I. sintenisii*, *I. aphylla*), flat (*I. graminea*), or convex (*I. pseudacorus*, *I. sibirica*).

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<u>Secondary sculpture</u>. As a result of the study, we established that the sculpture of external periclinal sell walls is smooth (*I. graminea*, *I. sintenisii*, *I. aphylla*), or furrowed (*I. sibirica*, *I. pseudacorus*). Considering all the above features, we concluded that seeds of studied species are characterized by coliculate (*I. sibirica*, *I. pseudacorus*), reticulate (*I. aphylla*) or indistinct-coliculate with slightly convex periclinal cell walls (*I. graminea*, *I. sintenisii*) relief.

Thus, we can conclude that the ultrastructure of seed surface belonging to the studied species from genus *Iris* shows that species from definite subgenus are similar in complex of seed features.

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