



& **BalkBioDiv**

**International Symposium on  
“Evolution of Balkan Biodiversity”**

Book of Abstracts

*Editors*

Ivana Rešetnik  
Sandro Bogdanović  
Antun Alegro

BalkBioDiv Consortium and Croatian Botanical Society  
Zagreb, Croatia  
June 28<sup>th</sup> - 30<sup>th</sup> 2012

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**Layout & Print**

mtg-topgraf d.o.o., Velika Gorica

**Symposium organization**

**International Symposium on "Evolution of Balkan Biodiversity"** is organized by BalkBioDiv Consortium and Croatian Botanical Society.

**Members of the BalkBioDiv Consortium:** Peter Schönswetter, Božo Frajman, Antun Alegro, Sandro Bogdanović, Ivana Rešetnik, DMITAR Lakušić, Marjan Niketić, Gordana Tomović, Maja Lazarević, Nevena Kuzmanović  
URL: <http://www.uibk.ac.at/botany/balkbiodiv/?BALKBIODIV>

**Croatian Botanical Society**

Marulićev trg 20/2, HR-10000 Zagreb, Croatia  
URL: <http://hirc.botanic.hr/HBoD/HBoD-home.htm>

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**Under the auspices of:**

State Institute for Nature Protection  
Ministry of Agriculture  
Ministry of Science, Education and Sports

**Sponsors:**

Botanical Garden of Faculty of Science, University of Zagreb  
Institute Ruđer Bošković  
Zagreb Tourist Board

ISBN 978-953-99774-5-8

A CIP catalogue record for this book is available from the National and University Library in Zagreb under 809952.

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## Preface

Dear Colleagues,

The BalkBioDiv Consortium and the Croatian Botanical Society welcome you to the **International Symposium on "Evolution of Balkan Biodiversity"** hosted by the Botanical garden of the Faculty of Science, University of Zagreb.

This Symposium arose from the trilateral SEE-ERA.NET Plus project "Evolution, biodiversity and conservation of indigenous plant species of the Balkan Peninsula" held by the Institute of Botany of the University of Innsbruck, Institute of Botany of the University of Belgrade and the Department of Botany of the University of Zagreb.

The Balkan Peninsula has long been recognised as one of the hotspots of European biodiversity and endemism. The reasons for this diversity are the geographic position of the Balkans at the transition of different floral provinces, topographic, climatic and geological complexity, and the relatively high environmental stability through geologic history. The important role of the Balkans as one of the main Pleistocene refugia for the postglacial colonisation of Europe is revealed in many recent phylogenetic and phylogeographic studies. Therefore this symposium encompasses all groups of organisms and present novel perspectives on Balkan biogeography across spatial and temporal scales. However, socioeconomic changes in last decades lead to loss and degradation of many habitats making conservation biology one of the most important themes. This give new dimension to the research of evolution and spatiotemporal diversification of biota, making it fundamental for the biodiversity conservation and protection.

All these themes are presented in 27 oral and 28 poster presentations and their abstracts are collected in this booklet.

And last but not list, the Organizing Committee greatly appreciates support by the auspices: the State Institute for Nature Protection, Ministry of Science, Education and Sports and Ministry of Agriculture, as well as sponsors: the Botanical Garden of University of Zagreb, Institute "Ruđer Bošković" and Zagreb Tourist Board.

Editors



## Symposium Programme

Thursday, 28.6.2012

- 11.00-13.00 Registration**
- 13.00-13.15 Opening**
- 13.15-15.05 Session 1**
- 13.15-13.45 Peter Schönswetter\*, Antun Alegro, Božo Frajman: Spatiotemporal diversification of the Balkan flora: What do we know?
- 13.45-14.05 Božo Frajman\*, Antun Alegro, Sandro Bogdanović, Nevena Kuzmanović, Dmitar Lakušić, Maja Lazarević, Marjan Niketić, Ivana Rešetnik, Gordana Tomović, Peter Schönswetter: BalkBioDiv - Insights into the plant diversity of the Balkan Peninsula. Polyploid evolution of *Cerastium*, *Knautia*, and *Sesleria*.
- 14.05-14.25 Ivana Rešetnik\*, Sandro Bogdanović, Friedrich Ehrendorfer, Peter Schönswetter, Božo Frajman: Phylogenetic relationships among diploid members of genus *Knautia* (Dipsacaceae)
- 14.25-14.45 Denis Kutnjak\*, Marjan Niketić, Peter Schönswetter, Božo Frajman: Genetic structure of the disjunctly distributed Dinaric mountain plant *Cerastium dinaricum* (Caryophyllaceae)
- 14.45-15.05 Maja Lazarević\*, Antun Alegro, Dmitar Lakušić, Nevena Kuzmanović, Božo Frajman, Peter Schönswetter: Genome size and chromosome numbers in genus *Sesleria* Scop. (Poaceae)
- 15.05-15.30 Coffee break**
- 15.30-17.10 Session 2**
- 15.30-15.50 Emilio Padoa-Schioppa\*, Anna Bonardi, Massimo Cagnetta, Edoardo Razzetti, Roberto Sindaco, Gentile Francesco Ficetola: Island Biogeography in Anthropocene era: perspectives for reptiles conservation in the Mediterranean basin and Balkan archipelagos
- 15.50-16.10 Ana Previšić\*, Jan Schnitzler, Mladen Kučinić, Wolfram Graf, Halil Ibrahim, Mladen Kerovec, Steffen U. Pauls:

- Karstification and micro-scale vicariance - diversification of stream insects in the Dinaric western Balkan
- 16.10-16.30 Ildikó Szivák\*, Steffen Pauls, Mladen Kučinić, Ivan Vučković, Bálint Szalontai, Edit Vadkerti, Tamás Mikes, Miklós Bálint: Adaptive radiation of *Chaetopteryx rugulosa* group (Trichoptera) induced by climate and geology in the Western Balkans
- 16.30-16.50 M. Jelić\*, G.I.V. Klobučar, F. Grandjean, I. Maguire: Genetic structure of threatened freshwater crayfish *Austropotamobius italicus* from the eastern Adriatic coast
- 16.50-17.10 Roman Ozimec, Davorin Marković\*: Agrobiodiversity and vulnerability of autochthonous domestic animal breeds of Croatia
- 17.10-19.00 Poster presentation and coffee break**

**20:00 Symposium dinner**

**Friday, 29.6.2012**

**9.00-11.10 Session 3**

- 9.00-9.30 Valerija Zakšek: Comparative phylogeography of some subterranean taxa on the Dinaric Karst
- 9.30-9.50 Petra Kutleša: The Dinaric karst cave biodiversity - advances in understanding of a highly diverse and endangered fauna
- 9.50-10.10 Maja Zgmajster: Subterranean Biodiversity Database and studies of subterranean fauna distribution patterns in the Dinarides
- 10.10-10.30 Roman Ozimec: Biodiversity, endemism and endangerment of Dinaric cave fauna: Example for Croatia
- 10.30-10.50 Dajana Hmura\*, Helena Bilandžija, Branko Jalžić, Helena Četković: Phylogeography of the Ogulin cave sponge *Eunapius subterraneus* Sket i Velikonja, 1984 from the Dinaric karst
- 10.50-11.10 Lada Lukić-Bilela\*, Roman Ozimec, Naris Pojskić, Helena Četković, Bruna Pleše: Diversity and ecological approach of bacterial mats/biofilms in Dinaric range caves

**11.10-11.40 Coffee break**



**11.40-13.00 Session 4**

- 11.40-12.00 Angelica Feurdean\*, Ioan Tanțău, Sorina Fărcaș: The relevance of palaeoecological data in understanding contemporary and future biotic responses in the Carpathian region
- 12.00-12.20 Nevena Kuzmanović\*, Petronela Comanescu, Ovidiu Paun, Božo Frajman, Peter Schönswetter, Maja Lazarević, Dmtar Lakušić: Genetic and morphological differentiation within the Balkan-Carpathian *Sesleria rigida* sensu Fl. Eur. (Poaceae), a taxonomically intricate tetraploid-octoploid complex
- 12.20-12.40 Michal Ronikier\*, Elzbieta Cieslak: Northern component to the southern richness: genetic diversity of the arctic-alpine *Dryas octopetala* at its Balkan range limits
- 12.40-13.00 Toni Nikolić\*, Boris Milašinović : Distribution pattern, range, ecology, hotspots and conservation of Croatian endemic plants

**13.00-14.30 Lunch break****14.30-15.50 Session 5**

- 14.30-14.50 Sandro Bogdanović\*, Zlatko Liber, Zlatko Šatović, Toni Nikolić: Phylogeography of the Adriatic *Limonium cancellatum* complex (Plumbaginaceae) – evidence from AFLP
- 14.50-15.10 M. Temunović\*, J. Franjić, Z. Šatović, N. Frascaria-Lacoste, J. F. Fernández-Manjarrés: Range-wide patterns of genetic variation in *Fraxinus angustifolia* and the potential effects of climate change on its genetic diversity
- 15.10-15.30 H. Mayrhofer\*, P. O. Bilovitz: Towards a checklist of the lichenized fungi from the Balkan Peninsula
- 15.30-15.50 P.O. Bilovitz\*, C. Scheidegger, S. Werth, I. Widmer, H. Mayrhofer: Population genetics of the epiphytic lichen *Lobaria pulmonaria* in primeval and managed forests in Southeast Europe

**15.50-16.20 Coffee break****16.20-17.20 Session 6**

- 16.20-16.40 Jelena M. Aleksić\*, Thomas Geburek: Recent admixture of three gene pools in a small natural population of an endemic and relict conifer *Picea omorika* (Panč.) Purk.

- 16.40-17.00 Vedran Šegota\*, Vladimir Hršak, Sandro Bogdanović, Antun Alegro, Višnja Besendorfer: Hybridogenous origin of *Serapias x todaroi* Tineo: morphological and karyological evidences
- 17.00-17.20 Željko Zgrablić: Rare mycorrhizal fungi of the genus *Tricholoma* in Austrian pine (*Pinus nigra*) stands in Istria, Croatia – preliminary results
- 17.20-17.40 Closing**

### Poster Presentations

- Atanassova, A. & Mayrhofer, H.: Diversity and Distribution of the Foliose Genera of the Lichen Family Physciaceae in Bulgaria
- Cieślak, E.: Does the genetic structure of *Carlina acanthifolia* subsp. *utzka* reflect distinctions of Balkans and Pontic phylogeographical areas?
- Clementi, M. & Miola, A.: The De Visiani Plant Collection in Padova
- Flatscher, R., Novak, Š., Schönswetter, P. & Frajman, B.: Patterns of morphological, genetic and ecological differentiation within the *Heliosperma pusillum* complex (Caryophyllaceae)
- Glasnović, P. & Temunović, M.: Present and past distribution of *Edraianthus* species based on ecological niche modelling
- Greguraš, D., Šatović, Z., Pruša, M., Radosavljević, I. & Liber, Z.: Genetic diversity of Dalmatian and short-tooth sage populations on the Pelješac peninsula
- Hajrudinović, A., Siljak-Yakovlev, S., Brown, S.C., Pustahija, F., Bourge, M. & Bogunić, F.: Cytotype and morphological diversity of *Sorbus aria* and *S. austriaca* from Bosnia and Herzegovina
- Iliadou, E. & Panitsa, M.: Plant diversity and phytogeography of "open sea" islets (Ionian area) vs. islets of a "semi-closed" gulf and a lagoon (Western Greece)
- Ivković, M., Previšić, A., Horvat, B. & Kerovec, M.: Ancient divergence of dance flies (Empididae, Diptera): first insights from the mtDNA phylogeny with special emphasis on the Balkans
- Jug-Dujaković, M., Liber, Z., Carović-Stanko, K., Grdiša, M., Hančević, K., Radić, T. & Šatović, Z.: High genetic diversity and low genetic differentiation of Dalmatian sage (*Salvia officinalis* L.) populations

- Karalija, E., Neimarlija, D., Muratović, E. & Parić, A.: Micropropagation and *in vitro* preservation of the endemic species *Symphyandra hofmannii* Pant.
- Kilikowska, A., Wysocka, A., Rychlińska, J., Kozłowska, K. & Sell, J.: The patterns of diversification of endemic *Proasellus* isopods from relict Lake Ohrid revealed by molecular data
- Lelo, S. & Lukić-Bilela, L.: Biodiversity of Ground Beetles (Coleoptera, Carabidae) in Bosnia and Herzegovina
- Magauer, M., Frajman, B. & Schönswetter, P.: Tales of the Unexpected: revising the disjunct distribution pattern of *Alyssum ovirense* (Brassicaceae) at different geographical scales
- Maxhuni, Q., Morina, I., Morina, R., Ferizi, R. & Hashani Z.: The state of Kosovo biodiversity 2006 – 2011
- Niketić, M., Gratl, V., Lazarević, M., Schönswetter, P., Tomović, G. & Frajman, B.: Ploidy levels and relative genome size in (sub)endemic taxa of *Cerastium* (Caryophyllaceae) from the Balkan Peninsula
- Pojškić, N., Kalamujić, B., Mašović, M. & Škrijelj, R.: Possible methods for detecting hybrid: a case study on *Abramis brama* (L.) x *Rutilus rutilus* (L.)
- Pustahija, F., Bogunić, F., Bašić, N., Muratović, E., Bourge, M., Brown, S.C., Stevanović, V. & Siljak-Yakovlev, S.: Relationships between genome size and some life history traits of serpentinophytes from northwest Balkans
- Radosavljević, I., Bogdanović, S., Šatovic, Z. & Liber, Z.: Natural hybridization between *Salvia officinalis* L. and *Salvia fruticosa* Mill. (Lamiaceae) as revealed by microsatellite markers
- Reich, D., Berger, A., Flatscher, R., Gilli, C., Hofbauer, M., Lachmayer, M., Prehsler, D., Sonnleitner, M. & Gutermann, W.: A Greek Odyssey: Constructing/Developing an online Flora of the Ionian Islands
- Sabovljević, M., Dragačević, L., Vujičić, M. & Sabovljević, A.: Bryophyte diversity of the Balkans: an insight from molecular and biochemical markers
- Sikorski, P., Wierzba, M. & Sikorska, D.: Land use management and location of rare plant species in suburban landscape (Poland)

- Stojanović, D. L., Aleksić, J. M., Jančić, I. R., & Jančić, R. B.: The indigeneity of a Mediterranean herb in the continental Balkans: A plastid-based phylogeographic survey of *Salvia officinalis* L.
- Szczepaniak, M.: Differentiation center and glacial refugia for the postglacial colonization routes of *Melica ciliata* complex (Poaceae) in Europe
- Vesnić, A. & Jugo, A.: The qualitative and quantitative composition of ant fauna (Hymenoptera, Formicidae) of Popovo Polje
- Wysocka, A., Grabowski, M., Łukaszewska, L., Kilikowska, A. & Sell, J.: Incongruence between morphological and molecular diversity of endemic gammarids in ancient Lake Ohrid
- Zebec, M., Idžojtić, M., Šatović, Z., Poljak, I. & Liber, Z.: Genetic variability and population structure in natural populations of the European field elm (*Ulmus minor* Mill. sensu latissimo) from Croatia as revealed by SSR markers
- Žujo Zekić, D., Kašić-Lelo, M. & Lelo, S.: Sex ratio in local population of species *Chondrostoma phoxinus* Heckel, 1843 (Teleostei: Ostaryophysi, Cyprinidae) from Buško Lake

# **ORAL PRESENTATIONS**



## **Spatiotemporal diversification of the Balkan flora: What do we know?**

Schönschwetter, P.\*<sup>1</sup>, Alegro, A.<sup>2</sup> & Frajman, B.<sup>1</sup>

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Surveys of regional patterns of biodiversity confirm that the Balkans, alongside Iberia, harbour the richest flora in Europe, possessing not only the largest number of species but also hosting most endemics. Phylogenetic research on plants in the past ten years has established a fairly stable system of evolutionary relationships. The advent of modern molecular techniques has enabled researchers not only to construct the “tree-of-life” depicting ties among major groups of living beings, but also to unravel relationships among closely related species. Molecular tools allow for studying genetic diversity and divergence of populations and have thus provided significant insights into intraspecific lineages, migration routes and locations of Pleistocene refugia. The Balkan and the Iberian Peninsulas were the main Pleistocene refugia for the postglacial colonisation of Europe. Whereas in Central and Northern Europe life was either entirely extirpated by the advance of ice sheets, or vegetation was transformed into tundras or cold steppes, southern and western parts of the Balkans offered buffered conditions, allowing even temperate tree species to survive the cold stages of the Pleistocene. Due to the rugged orography of the Balkans, populations isolated by glaciated mountains gradually diverged, potentially forming new taxa through allopatric speciation. When the climate ameliorated, secondary contacts enabled hybridisation, including the formation of polyploid lineages, some of which were highly successful in the colonisation of Central and Northern Europe. In contrast to the good coverage of the Western, Northern and Central European floras by molecular studies, the biota of southeastern Europe, and especially that of the western Balkan countries remains neglected in this respect. This presentation summarises the few available phylogenetic and phylogeographic studies of plants from the Balkans that have revealed complex patterns in the geographic distribution of genetic diversity and challenged traditional taxonomic concepts.

## **BalkBioDiv - Insights into the plant diversity of the Balkan Peninsula. Polyploid evolution of *Cerastium*, *Knautia*, and *Sesleria***

Frajman, B.\*<sup>1</sup>, Alegro, A.<sup>2</sup>, Bogdanović, S.<sup>3</sup>, Kuzmanović, N.<sup>4</sup>, Lakušić, D.<sup>4</sup>, Lazarević, M.<sup>4</sup>, Niketić, M.<sup>5</sup>, Rešetnik, I.<sup>2</sup>, Tomović, G.<sup>4</sup> & Schönswetter, P.<sup>1</sup>

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An important source of biodiversity is polyploidy (multiplication of chromosome sets), which is an important mechanism allowing sympatric speciation. In the frame of an EU-funded See-Era.Net Plus project we have studied the spatiotemporal diversification of three polyploid plant genera, which have their centres of diversity on the Balkan Peninsula: *Cerastium* (Caryophyllaceae), *Knautia* (Dipsacaceae) and *Sesleria* (Poaceae).

We sampled 360 populations of *Cerastium*, 375 of *Knautia* and 450 of *Sesleria*. Measurements of the genome size using flow cytometry show striking similarity of genome sizes across different taxa belonging of the same ploidy in *Knautia* and *Sesleria*, whereas in *Cerastium* the monoploid genome size differs considerably among different taxa of the same ploidy. Also divergence of nuclear and chloroplast DNA sequences is much higher in *Cerastium*, often following the taxonomic boundaries. On the contrary, the lineages revealed in *Knautia* and *Sesleria* are mostly geographically correlated. Preliminary analyses of the DNA sequences as well as the AFLP fingerprinting methods indicate that polyploidisation has taken place in many different lineages of the three genera, but the timescale was likely different, older in *Cerastium* and younger in *Knautia* and *Sesleria*. Further analyses to comprehensively disentangle the evolutionary history of the three study groups are underway.



## Phylogenetic relationships among diploid members of the genus *Knautia* (Dipsacaceae)

Rešetnik, I.\*<sup>1</sup>, Bogdanović, S.<sup>2</sup>, Ehrendorfer, F.<sup>3</sup>, Schönswetter, P.<sup>4</sup> & Frajman, B.<sup>4</sup>

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The genus *Knautia* L. (Dipsacaceae) comprises ca. 40–60 species and is distributed in western Eurasia and North Africa with highest species diversity in southern and southeastern Europe, especially the Alps and the Balkan Peninsula. Traditionally *Knautia* has always been known for its taxonomic complexity due to the widespread occurrence of polyploidy and the high incidence of recurrent hybridization, which blur species boundaries. The aim of this study was to provide insights into spatiotemporal diversification of the genus by assessing relationships among diploid accessions and to test previous infrageneric taxonomic hypotheses based on morphological and karyological traits. Here, using the nuclear ribosomal internal transcribed spacer (ITS) and the plastid *petN(ycf6)-psbM* region as well as the amplified fragment length polymorphisms (AFLPs) we provide the first phylogeny of *Knautia* and its infrageneric circumscription. Our molecular data unambiguously support the monophyly of *Knautia* and the overall shallow structure in ITS and cpDNA data suggest the relatively young age of the genus. Furthermore the distribution of haplotype groups and the occurrence of the same haplotypes on large geographical scales imply recent and fast range expansion. Our results show that infrageneric classification and circumscription of traditional groups are not congruent with molecular data. In addition our work provides the basis for a future study of the origin of the polyploids and the overall phylogeny of the genus.

## Genetic structure of the disjunctly distributed Dinaric mountain plant *Cerastium dinaricum* (Caryophyllaceae)

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*Cerastium dinaricum* is an endemic western Balkan mountain plant species. Its distribution range consists of several disjunct populations, ranging from Slovenia in the north-west to Montenegro in the south-east. It is a species of a high conservation concern in Europe, listed in Annex II of the Habitats Directive (Natura 2000). Using amplified fragment length polymorphism (AFLP), sequences of plastid *ndhJ-trnT* and nuclear ITS regions, as well as relative genome size estimations, we investigated genetic differentiation within *C. dinaricum*. No clear genetic structure was indicated by the nuclear ITS sequence data. Six closely related plastid DNA haplotypes were found, one of them occurring in all south-eastern populations from Montenegro, whereas all other populations were characterised by own haplotypes. A deep phylogeographic split, coinciding with the lowermost Neretva valley, was indicated by AFLPs and relative genome size measurements. Results of different marker systems indicate that the disjunct distribution within the north-western group could be a result of survival in two glacial refugia (Snežnik, Dinara) and subsequent range expansion from one of them, later followed by range fragmentation. The disjunct distribution within the south-eastern group likely results from survival in multiple refugia. Intraspecific variation of genome size indicates that both groups represent distinct biological species, but further studies are needed to confirm this. In any case, the two distinct lineages represent evolutionarily significant units important for conservation management purposes.

## Genome size and chromosome numbers in the genus *Sesleria* Scop. (Poaceae)

Lazarević, M.\*<sup>1</sup>, Alegro, A.<sup>2</sup>, Lakušić, D.<sup>1</sup>, Kuzmanović, N.<sup>1</sup>, Frajman, B.<sup>3</sup> & Schönschwetter, P.<sup>3</sup>

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Polyploidy is considered as a major force in plant evolution and diversification, which may foster adaptation to new ecological niches and often confers reproductive isolation, ultimately leading to speciation. Our aim was to explore ploidy levels of different sections and subsections of the genus *Sesleria* using flow cytometry and standard karyological techniques. The genus *Sesleria* Scop. (Poaceae) comprises around 30 species distributed mainly in Europe with extensions in West Asia and North Africa. A few diploid species ( $2n=2x=14$ ) are known from the Alps, leading to the suggestion that this region is a primary centre of this genus' development. On the other hand, the Balkan Peninsula with its tetra- to dodecaploid taxa ( $2n=4x=28$ ,  $2n=8x=56$ ,  $2n=2x=84$ ) is a centre of diversity and secondary evolution of the genus. About 80% of the known *Sesleria* species inhabit this region, half of which are endemic. In order to investigate as many populations as possible, we used silica-dried as well as fresh plant material and compared these results with chromosome numbers. Around 440 populations collected throughout Europe were analysed. Results confirmed the diploid status of the Alpine sections *Psilathera* and *Sesleriella* and also of closely related genus *Oreochloa*. Different ploidy levels are present in sections *Argenteae* and *Calcariae*. There is a strong correlation between ploidy levels and genome size. Decreasing Cx-value with increasing ploidy level suggests substantial genome downsizing after polyploidization.

## **Island Biogeography in Antropocene era: perspectives for reptiles conservation in the Mediterranean basin and Balkan archipelagos**

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The theory of island biogeography predicts species richness based on geographical factors that influence the extinction–colonization balance, such as area and isolation. Although the relationship between island area and species richness is considered general law of ecology, new patterns are emerging when we consider the human presence. The knowledge of biodiversity distribution may be biased by incomplete knowledge. As an example in the most wild areas of Western Palaearctic we found a very strong relationship between accessibility and known biodiversity of reptiles. An independent validation dataset confirmed that combining accessibility measures with eco-geographical variables allows to correctly predict the actual species richness of reptiles, even in remote areas that have received limited monitoring so far. Integrating accessibility measures into ecological models allows to identify areas where current knowledge underestimates the actual biodiversity. At a finer scale we analysed a large data set describing reptile distribution in Mediterranean islands, to evaluate the effect of human activities on the species richness of reptiles on islands. The richness of both native and alien species was best explained by models combining geographical and human factors. The richness of native species was negatively related to human influence, while that of alien species was positively related, with the overall balance being negative. In models that did not take into account human factors, the relationship between island area and species richness was not linear. Large islands hosted fewer native species than expected from a linear (on log–log axes) species–area relationship, because they were more strongly affected by human influence than were

small islands. Anthropogenic factors can strongly modify the biogeographical pattern of islands, probably because they are major drivers of present-day extinctions and colonisations and can displace island biodiversity from the equilibrium points expected by theory on the basis of geographical features. The results of our studies may open new perspectives for the conservation of reptiles, allowing to identify the areas where biodiversity information is limited but new protected areas are required. In the archipelagos of Balkan peninsula, our approach can allow to identify areas where the anthropic pressure is higher and what are human impact on known biodiversity.

## **Karstification and micro-scale vicariance - diversification of stream insects in the Dinaric western Balkan**

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The Dinaric western Balkan karst area is a European biodiversity hotspot with particularly high degrees of endemism in the freshwater and subterranean fauna. For animals inhabiting surface waters karst development results in increased fragmentation of habitats. The reduction of surface waters and parallel development of higher energy relief may drive small scale vicariance of aquatic insect populations. We hypothesise that karstification mediated fragmentation of suitable surface water habitats for caddisflies and drove small scale vicariant diversification in the region. Using mtDNA sequence data, we apply a comparative phylogeographic approach to analyse population genetic structure of four ecologically similar *Drusus* species (Trichoptera, Limnephilidae) with different distribution ranges, date intra- and interspecific divergence of our target species, and link our findings with geological events. We observed marked differences in population genetic structure of our target species: three range-restricted endemics exhibited extremely high intraregional population differentiation dating to the late Pliocene/Pleistocene; the widespread species had shallower divergence (Pleistocene) among regional clades of polyphyletic origin. These results suggest that the population histories of the endemic species differ from those of the more widespread species: populations of the endemic species differentiated within the study area due to regional fragmentation, potentially resulting from intensified karstification; whereas the more widespread species repeatedly colonised the region from different sources.

## Adaptive radiation of *Chaetopteryx rugulosa* group (Trichoptera) induced by climate and geology in the Western Balkans

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The *Chaetopteryx rugulosa* group is composed of 9 closely related taxa and endemics to the western Balkan. It is suspected that these are currently under formation, by undergoing radiation into adjacent habitats. Several contact zones are also known, where hybridization occurs among some of the group members. Given the climatic and geologic heterogeneity of the Western Balkans, we hypothesized that the formation of these taxa might be mediated by adaptation to abiotic conditions. We intended to clarify the phylogenetic relationships within members of the species group. To test if the adaptation to abiotic conditions played a role in the formation of the group, we compared the phylogenetic structures of the group with climatic and geological conditions characterizing their habitats. We used 37 specimens from 22 populations and generated multilocus sequence (mtCOI, wingless, EF-1 $\alpha$ ) data from 3-4 specimens per each species and subspecies. The phylogeny of the group does not reflect the relationships inferred from the current taxonomy. We also show further cryptic diversity within the species group. Climatic and geologic factors strongly discriminate among the previously defined taxa. We showed that importance of adaptation to environmental conditions might be comparable to physical isolation in the formation of Mediterranean endemisms.

## **Genetic structure of threatened freshwater crayfish *Austropotamobius italicus* from the eastern Adriatic coast**

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Within species *Austropotamobius italicus* four subspecies are recognized based on molecular phylogeny analyses: *A. i. carinthiacus*, *A. i. italicus*, *A. i. meridionalis* and *A. i. carsicus*. The two latter are distributed in the Adriatic Sea drainage. *A. i. meridionalis* is distributed from Venezia Friuli Giulia region (Italy) to Montenegro and *A. i. carsicus* only in Istria (Croatia) and Slovenian Littoral. The aim of our research was to examine genetic and geographical distribution pattern of these subspecies. 172 specimens from 51 localities were examined and different phylogenetic and phylogeographical analyses on 16s and COI mitochondrial genes were performed. The most important result was the finding of fine genetic structure within *A. i. meridionalis*. Altogether, 6 subgroups inside *A. i. meridionalis* were found. Five subgroups are distributed on the eastern Adriatic coast and only one subgroup is widely distributed in the Central and Southern Italy. The result of molecular clock analysis showed that the oldest split within subspecies occurred during Late Pleistocene. Our results support the hypothesis that the complex distribution of these subgroups was caused by sea level oscillations and Paleo-Po River drainage that probably had a crucial role for the observed distribution pattern.



## **Agrobiodiversity and vulnerability of autochthonous domestic animal breeds of Croatia**

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During 20th century begin agriculture industrialization, caused decrease of agrobiodiversity and separation of agriculture's from natural ecosystems, followed by degradation and disappearing of some ecosystems, habitats and taxa. Croatian State Institute for Natural protection, following Red books experience, prepared Green book of autochthonous breeds, using four methods for defining endangerment: FAO, IUCN, EU and National program for preserving domestic animals breeds. In total 12 species of domestic animals are recognised, belong to 2 phyla, 3 class, 6 orders and 7 families, with 62 traditional breeds: 10 sheep's; 8 pigs, chickens and dogs; 7 horse and cattle; 3 ass, goats, goose and turkey and one for duck and bees. According to IUCN methodology, 12 breeds are probably extinct (?EX), 9 globally and 3 regionally, 3 are critically endangered (CR), 13 are endangered (EN), 12 are vulnerable (VU) and not threatened are 5 breeds only. For 16 breeds status is not established due to data deficient (DD), indicated necessity for further researches. With evolution of methodology, agrobiodiversity analyses should amplify on other cultures, but also conducted on Balkan region and whole SE Europe, with establishing of protection and preservation measures, but also evaluating of economical use for autochthonous domestic animal breeds.

## Comparative phylogeography of some subterranean taxa on the Dinaric Karst

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The relative role of the history, geography and ecological factors that lead to differentiation and speciation represent an important research focus in evolutionary biology. Concordant phylogeographical patterns of large number of organisms in the same geographic region are a strong evidence for the common biogeographical history. High biodiversity and a large number of specialized subterranean species are two of the main characteristics of the Dinaric Karst. We used taxa with so called holodinaric distribution, distribution along the Dinaric Karst from Italy to southern Herzegovina, as a basis for a comparative phylogeographic study of selected subterranean taxa: the cave shrimps (*Troglocaris* s. str.), the cave tube worm (*Marifugia cavatica*), the cave amphipod (*Niphargus steueri*) and the European cave salamander (*Proteus anguinus*). All four, although taxonomically so distant from each other, similarly show a very high levels of genetic differentiation. Ranges of phylogroups are usually small and rarely exceed 200 kilometers. Furthermore, there is substantial agreement between the geographic extent of the phylogroups, most markedly between *Troglocaris* and *Proteus*, while the cave tube worm shows a somewhat different pattern in the inner part of Dinarides. Common phylogeographic breaks between different taxa and their differences will be discussed as a possible consequence of their different life history and dispersal abilities.

## **The Dinaric karst cave biodiversity - advances in understanding of a highly diverse and endangered fauna**

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The Dinaric karst is the world's hotspot of cave fauna biodiversity with a large number of subterranean species, high abundance of their populations and specific taxa found nowhere else in the world (cave bivalve, sponge and serpulid). However, the subterranean fauna of the Dinarides remains largely unexplored, with new taxa being frequently discovered. Karst habitats are extremely vulnerable and anthropological pressures could soon eradicate much of the subterranean biodiversity, even before it's full discovery and recognition. Hence, in 2000 the Croatian Biospeleological Society launched a project aiming to collect all the data on Croatian cave fauna, enhance further biospeleological research and to raise public awareness about this valuable natural heritage. Caves that are type localities were chosen as a starting point to achieve these long term goals. In 2010 The Cave Type Localities Atlas of Croatian Fauna was published listing all Croatian cave type localities and species described from them. The Atlas approaches the cave fauna biodiversity in a popular and illustrative way bringing this issue to a wider audience. It also attempts to demonstrate the scientific value of cave type localities and to induce rethinking about proper legislative and management actions for conservation of the entire Dinaric karst area.

## **Subterranean Biodiversity Database and studies of subterranean fauna distribution patterns in the Dinarides**

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Subterranean habitats harbor highly specialised animal species, that are living in unusual environmental conditions, most prominent being constant darkness. Dinarides in the Western Balkans are a world hotspot in subterranean biodiversity, with about 1000 obligate subterranean (troglobiotic) species described. A large amount of data on their distribution are scattered in the numerous published sources. Subterranean Biodiversity Database managed at the Department of Biology, Biotechnical Faculty, University of Ljubljana contains such records in one place. In recent years the database has been upgraded into a relational format, containing uniquely defined taxa and georeferenced localities. Records have been used to prepare point distribution maps, but also deliverables like maps of species (or other taxonomic levels) richness or of areas of high endemism. Implementation of spatial analytical techniques and modelling presents a new approach in investigating distribution patterns of subterranean fauna in the Dinarides, in predicting species richness in unsampled areas, evaluating differences in sampling intensity etc. Examples of such analyses will be presented using the dataset of troglobiotic beetles, which comprise about half of all terrestrial troglobiotic animals in the region.

## **Biodiversity, endemism and endangerment of Dinaric cave fauna: Example for Croatia**

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Highest world biodiversity of cave fauna is confirmed for Dinaric range. Due to fact that Croatia occupied all Dinarid biogeographic regions: north, middle and south, same as three biggest macro-tectonic units: Dinaric, Supradinaric and Adriatic with 1185 islands and almost 6000 km of coastal line, biodiversity is highly rated with more than 550 cave taxa. All troglobionts are endemic for Dinarides, frequently stenoendemic, confirmed with more than 400 taxa described from Croatia. Some of them are paleoendems, living fossils; relicts from some geological period, without living related taxa and other are neoendems, become due to geographic isolation of populations in cave habitats. Croatia is first state which have published Red book of cave fauna according to the IUCN criteria. Majority, 70 taxa (37%) are vulnerable, 65 (35%) critically endangered, 49 (26%) endangered and two taxa are with data deficient. Due to many devastations and pollutions, underground water habitats with fauna are especially endangered. In collection of Croatian Biospeleological Society (CBSS) are many new taxa for science, so biodiversity of cave fauna will increase in following decades. Researches, protection and promotion of Dinarid cave fauna are impossible without integral cooperation between experts and society from all Dinarid states.

## Phylogeography of the Ogulin cave sponge *Eunapius subterraneus* Sket i Velikonja, 1984 from the Dinaric karst

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The Ogulin cave sponge *Eunapius subterraneus*, Sket & Velikonja, 1984 (Porifera, Spongillidae), is thus far the only known stygobitic (obligatory subterranean) sponge in the world. It is found only in a small region near the town of Ogulin and, remarkably, in a sump 1392 meters below ground in the Lukina jama – Trojama cave system on Mt. Velebit. Two subspecies are known: the more widely distributed *E. s. subterraneus* and *E. s. mollisparspanis*, which is described from a single cave (Rudnica VI), within the range of *E. s. subterraneus*. We used an intron region of ATP Synthetase Subunit  $\beta$  (ATPS  $\beta$ ) gene as a molecular marker. The sponges were sampled throughout the entire range in order to clarify systematic relationships and infer phylogeographical structure of this species. We were interested in (i) the phylogenetic relationship of the Rudnica VI subspecies to populations of the other subspecies, (ii) the phylogenetic relationship of a disjunct population from Lukina jama - Trojama system to the sponges from the main range and (iii) the overall phylogeographic structure and haplotype diversity of this endangered karst species.

## Diversity and ecological approach of bacterial mats/biofilms in Dinaric range caves

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Bacterial mats/biofilms from three caves in Dinaric range were assessed with regard to bacterial diversity and community structure using a 16S rDNA-based phylogenetic approach combined with phylogenetic analysis of universally conserved genes/proteins. Bacterial diversity, in "anthozoan tentacles-like" and "hair-like tendrils" biofilms, was high with predominance of *Proteobacteria* more specifically *Delta*- and *Gammaproteobacteria*. The recovered protein sequences from bacterial phylotypes, generally acknowledged results obtained with 16S rRNA results on the class/division level. In addition, the presence of *Nitrospirae* was revealed in all analysed localities. An overview of fauna associated with bacterial biofilms was implemented with a special emphasis on ecological concepts and their connection to microbial ecosystems as a nutrition resource. Complex interspecies interactions should be considered among the cave-dwelling animal taxa and microbial (bacterial, archaeal, fungal and protists) communities in the mats/biofilm. The cave ecosystems susceptibility is outcome of their inhabitants' adaptation to constant conditions in the natural environment. Thus, the acceptance of idea that interspecies interactions in extreme ecosystems, among the members of the three domains of life, incline to the mutual influences achievement rather than to competition, could elucidate many new aspects in evolution of animal taxa, adjacent to their energy suppliers in microbial communities and biofilms.

## The relevance of palaeoecological data in understanding contemporary and future biotic responses in the Carpathian region

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In the context of current and future climate change and land-use pressure numerous studies aim to identify changes in tree species range distribution. Here, a synthesis of palaeoecological records from the Carpathian region are reviewed highlighting the importance of long-term data in understanding contemporary and future biotic responses. Key questions addressed are: i) glacial refugia: identifying species that persisted in the region and the implication for postglacial species range shifts; ii) the temporal and spatial variability in the range distribution and abundance of few economically valuable tree taxa in Eastern Europe (*Pinus diploxylon* type i.e., *P. sylvestris* and *P. mugo*, *P. cembra*, *Picea abies*, and *Quercus*), during the Holocene. The conditions under which these taxa arose, the timing and processes responsible for their variability and ultimately for their decline, all are essential for understanding their current status in the forests of Eastern Europe; and iii) how the long-term records can be used in the biodiversity management and conservation in this region.



## Genetic and morphological differentiation within the Balkan-Carpathian *Sesleria rigida* sensu Fl. Eur. (Poaceae), a taxonomically intricate tetraploid-octoploid complex

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Although the Balkan Peninsula and adjacent areas such as the Romanian Carpathians are a centre of European biodiversity, little is known about diversification processes and their spatio-temporal patterns on the Balkan Peninsula, especially at lower taxonomic levels. One of the genera with centre of diversity on the Balkan Peninsula is *Sesleria* comprising ca. 28 species. It is taxonomically intricate, what may relate to the high incidence of polyploidy. A few diploid species are known from the Alps, leading to their suggestion as centre of primary development, while the majority of taxa are tetra- to octoploid. Our study taxon, *S. rigida* s. Fl. Eur. belongs to section *Calcariae*, sub-section ("turma") *Rigida*. Taxonomic value, delimitation and distribution of most intraspecific taxa are not clear and in the modern floristic literature they are most often considered synonymous with *S. rigida*. A reconstruction of the relationships among populations based on Amplified Fragment Polymorphisms (AFLPs) revealed four clearly differentiated genetic groups that did only partly follow actual taxonomic concepts, but were strictly allopatric. While some of the taxa constitute distinct genetic entities, others have no taxonomic value. Intersecting our AFLP data with ploidy-level information obtained from all genetically investigated individuals as well as with several chromosome counts revealed that octoploid individuals originated solely by autopolyploidy. The genetic data set and a morphometric data set were congruent, allowing for a taxonomic revision of the constituents of *S. rigida* s. Fl. Eur.

## **Northern component to the southern richness: genetic diversity of the arctic-alpine *Dryas octopetala* at its Balkan range limits**

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The arctic-alpine element, although not originating from the area, plays a considerable role in high-mountain plant diversity of the Balkan Peninsula (especially in its central and northern part). In many cases, the Balkan mountains outline southernmost reaches of such species in Europe, making these regional ranges especially interesting. Here, we performed an AFLP analysis of *Dryas octopetala* to assess the genetic diversity and phylogeographical structure of this key circumpolar arctic-alpine species across the Balkan Peninsula. We also analysed the Balkan populations in a larger context of the European range to unravel their biogeographical relationships with other mountain areas (especially the adjacent Alps and Carpathians) and estimate phylogeographical divergence of the southernmost populations. No important genetic breaks were found within the Balkan populations but they displayed a clear phylogeographical East–West gradient with Bulgarian (Rila, Stara Planina, Rhodopes) and Montenegro/Albanian mountains (Durmitor, Komovi, Prokletije) forming two most distant groups while Suva Planina and Shar Planina having an intermediate situation. At a European scale, Balkan populations did not form a divergent phylogeographical group. A strong influence of two fundamental gene pools: South-Eastern Carpathian and Alpic, on the Eastern Balkan and (North-) Western Balkan populations, respectively, was found to shape the Balkan genetic structure.

## **Distribution pattern, range, ecology, hotspots and conservation of Croatian endemic plants**

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The Balkan Peninsula is recognized as an important centre of floral diversity and endemism and as an important European wildlife refuge area. However, although Croatia hosts more than half of all of the floral species found on the Balkan Peninsula, the endemic taxa in this country have never been evaluated or used to define conservation priorities. Based on recent inventory data, we analyse the qualitative composition of the endemic flora, spatial distribution, centres of endemism, and patterns of range sizes; their correlations with altitude, climate and habitats; and the relationship between floral distribution and conservation efforts. A total of 377 endemic taxa (7.56% of the total flora), with an average of 7.47 taxa/grid cell, are found over 46.3% of the national territory. The range size is strongly skewed toward very narrow distributions. The analysis of the areas with the highest numbers of taxa (up to 89/grid cell) indicate two notable centres of endemism – the mountainous Dinaric Alps and the SE Adriatic coast, including the islands. Endemics exhibit modest bimodality with respect to altitudinal distribution. A positive correlation between the number of endemic species and the latitude/longitude ratio indicates that the number of endemic species gradually increases from the NW to the SE. Considering the mean annual air temperature and the duration of insolation, the endemic taxa distribution is highly asymmetric to the right, with a higher incidence in warmer areas with longer insolation. With respect to the mean annual rainfall, the endemic taxa distribution is extremely asymmetric to the left, with a higher incidence in areas with less rainfall. The ecological range of a given endemic taxon, expressed as the number of habitats colonized, is strongly positively correlated with the area of occupancy. Narrow endemic species largely occur within the dry grasslands, forest habitats, and especially in the alpine-Carpathian-Balkan and the Tyrrhenian and Adriatic limestone rocks. In Croatia, 13 endemic taxa are endangered, and 71 are almost threatened. For 39 endemic taxa, there are insufficient data to assess the degree of the

threat. However, spatial analysis shows that approximately 50% of endemic plants do not grow within the network of protected areas that are actively managed. The regions with high overall richness tend to be different from those areas with high endemism. Changes in conservation practice are proposed.

## Phylogeography of the Adriatic *Limonium cancellatum* complex (Plumbaginaceae) – evidence from AFLP

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Adriatic basin is relatively isolated phylogeographical region within Mediterranean area with numerous endemic taxa. Molecular analysis based on AFLP markers was used to resolve relationships between endemic taxa belonging to the Adriatic *Limonium cancellatum* complex. The AFLP data indicate that *L. cancellatum*, *L. dictyophorum*, *L. liburnicum*, *L. apulum*, *L. diomedea*, *L. vestitum*, *L. brusnicense* and *L. subanfractum* belong to the *L. cancellatum* complex. While Ionian taxa from Greek islands together with south Italian *L. japygicum* does not belong to the *L. cancellatum* complex. They form independent Mediterranean group of *L. bocconeii*. Current taxonomical status and division of endemic *Limonium* taxa is not congruent with AFLP data. Our results revealed the presence of three genetically and morphologically differentiated groups within *L. cancellatum* complex along the eastern Adriatic coast where *L. cancellatum* and *L. liburnicum* forms one group (Istria, Kvarner, north Dalmatia), *L. apulum* and *L. dictyophorum* forms the second group (southern Adriatic) and the third group is represented by *L. vestitum*, *L. brusnicense* and *L. subanfractum* (central Dalmatian islands). The third group could be the result of secondary contacts after post-glacial range expansion. Hybridogen origin of some populations of *L. subanfractum*, *L. dictyophorum* and *L. cancellatum* on remote islands was detected. Highly significant correlation between genetic and geographical distance was established.

## **Range-wide patterns of genetic variation in *Fraxinus angustifolia* and the potential effects of climate change on its genetic diversity**

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The potential for adaptation to changing environment is largely dependent on current genetic variation across species' ranges. To identify spatial patterns of genetic variation in Mediterranean tree *Fraxinus angustifolia* and to investigate the effects of potential range shifts under climate change on species genetic diversity, we applied a multidisciplinary approach by combining classic population genetics based on nuclear microsatellite markers, Bayesian clustering and Ecological niche modelling (ENM) methods. We used 38 natural populations across species' European range. We identified two main genetic groups in Europe with a clear West - East pattern of population structure. We revealed centres of highest genetic diversity in West and North Mediterranean populations compared to East and South Mediterranean populations as well as significant longitudinal West - East geographical cline of decreasing genetic diversity. As expected, ENM predicted northwards range shift of *F. angustifolia* in response to warming climate, while detected areas of habitat stability may be considered as in situ refugia from 21st century climate change. Our results suggest that predicted climate changes, which are expected to be most pronounced in the south Mediterranean, combined with low migration rates in trees, may cause a partial loss of the overall species' genetic diversity.

## **Towards a checklist of the lichenized fungi from the Balkan Peninsula**

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The present knowledge of the biodiversity of lichenized fungi from the Balkan Peninsula is still rather incomplete. During the last twenty years H. Mayrhofer, his students and co-workers intensified lichenological activities by establishing projects with local scientists. Publications started with floristic papers, followed by catalogues based on a thorough evaluation of the floristic and taxonomic literature for Slovenia (2000), Crete (2001), Bulgaria (2005), Montenegro (2009), Bosnia & Herzegovina (2011) and Macedonia (in prep.). Printed checklists by other authors are available for Serbia (2006), Albania (2007) and Greece (2009) and a preliminary online version for Croatia (2010). The catalogues and checklists stimulated other scientists to contribute and to improve the knowledge with several floristic papers. The last extensive virgin forest areas in Europe (e.g. Biogradska gora in Montenegro and Perućica in Bosnia & Herzegovina) are hot spots of lichen biodiversity. Today Greece (without Crete) has a known diversity of c. 1170 lichen species (Crete c. 670, most of them also known from mainland Greece), Slovenia (including the Southern Alps) and Bulgaria each c. 1010, Croatia c. 950, Montenegro c. 770, Serbia (incl. Kosovo) c. 640, Bosnia & Herzegovina c. 625, Macedonia c. 570, Thracia c. 400, and Albania, the least known, only 187.

## **Population genetics of the epiphytic lichen *Lobaria pulmonaria* in primeval and managed forests in Southeast Europe**

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24 populations of the tree-colonizing lichen *Lobaria pulmonaria*, collected from primeval and managed forests, were analysed with 8 fungus-specific microsatellites. Populations belonged to either of two gene pools or they were admixed. Gene pool A showed a broad geographic distribution in the Eastern Alps, in canyons in Montenegro, and in Bulgaria, whereas gene pool B was concentrated in Greece, Montenegro and Bosnia and Herzegovina. Gene diversity was higher in populations of gene pool B than of gene pool A, and the highest values were reached in admixed populations. Gene diversity was significantly higher in primeval than in managed forests. At small distances (up to 170 meters), genotype diversity was lower in managed compared to primeval forests. We found significant correlations between groups of potential phorophyte species and the two *Lobaria pulmonaria* gene pools, which may indicate that *Lobaria pulmonaria* was depending on certain forest communities during its postglacial migration. Gene pool B of *Lobaria pulmonaria* was associated with *Fagus sylvatica*, and we can hypothesize that gene pool B survived the last glaciation associated within the refuge area of *Fagus sylvatica* on the Coastal and Central Dinarides. The allelic richness of gene pool A was highest in the Alps, which suggests an additional northern refuge area of *Lobaria pulmonaria*.



## Recent admixture of three gene pools in a small natural population of an endemic and relict conifer *Picea omorika* (Panč.) Purk.

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Spatial genetic structure (SGS) in a single natural population of *Picea omorika* (Panč.) Purk., occupying 0.35 ha and comprising 419 trees, was analyzed by five nuclear EST-SSRs and a second intron of the NADH dehydrogenase subunit 1 gene (*nad1* intron 2). Nuclear markers, displaying neutrality and lack of linkage disequilibrium in previously studied populations of this species, were found to be non neutral and linked in studied population. Upon exclusion of typing errors and Wahlund effect, it was assumed that this population was exposed either to inbreeding or recent admixture causing the observed deviations from neutrality and linkage equilibrium. Model based clustering method in InStruct revealed inbreeding coefficients and selfing rates close to zero, and both InStruct and STRUCTURE analyses revealed recent admixture of three different gene pools within this population. The comparison of spatial locations of genotypes strongly assigned (>90%) to each of the three gene pools detected at nuclear loci and four haplotypes found at maternally inherited mtDNA locus has enabled delineation of a resident sub-population and two immigrant sub-populations of which one immigrated by seeds and the other by pollen. Our results demonstrate very complex history of populations (and species) within the refugial Balkan region.

## Hybridogenous origin of *Serapias x todaroi* Tineo: morphological and karyological evidences

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The genus *Serapias* is monophyletic genus with Mediterranean distribution. While consisting of morphologically similar taxa, the number of recognised species varies greatly between authors. 26 species and 23 hybrids are recognised by lumpers, of which nine are found in Croatia. *S. x todaroi* Tineo was described from Sicily by Tineo (1846). Later on, Camus (1908) established the name *S. semilingua*, indicating its hybridogenous origin between *S. lingua* and *S. parviflora*. In Croatian flora, *S. x todaroi* was discovered for the first time on Dalmatian island of Dugi otok in 2008 and after on the island of Mljet in 2010. On both localities *S. x todaroi* was found coexisting with hypothetical parental species. Twenty seven morphological (15 floral and 12 vegetative) characters were measured in populations of *S. x todaroi*, *S. lingua* and *S. parviflora* from Dugi otok and Mljet. Petal width, labelum length, epichile length, maximum epichile width, basal epichile width, plant height, inflorescence length and length of the longest stem leaves showed statistically significant differences among studied taxa. In respect to revealed characters, *S. x todaroi* evidently shows intermediate features between parental species. Intermediate chromosome number ( $2n=54$ ) for *S. x todaroi* was detected, while parental species *S. lingua* possessed  $2n=72$  and *S. parviflora* 36 chromosomes.

## **Rare mycorrhizal fungi of the genus *Tricholoma* in Austrian pine (*Pinus nigra*) stands in Istria, Croatia – preliminary results**

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Rare mycorrhizal fungi *Tricholoma caligatum*, *T. focale*, and *T. stans* have been recorded in the Austrian pine forest (*Pinus nigra*) in fall of 2011 on permanent mycorrhizal fungi research plots in central Istria, Croatia. Investigated Austrian pine forest is 11-21 years old stand on the flysch bedrock with eastern aspect and 11-20° slope. Characteristic woody species beside the Austrian pine are elements of the natural downy oak (*Quercus pubescens*) and hop hornbeam (*Ostrya carpinifolia*) forest community. Species *T. focale* and *T. stans* are new records for Croatian mycobiota, while *T. caligatum* is rare in Croatia and protected by law.



# **POSTER PRESENTATIONS**



## Diversity and Distribution of the Foliose Genera of the Lichen Family Physciaceae in Bulgaria

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Within the framework of the “Fungi of Bulgaria” project several months lasting field works took place in order to present an overview of the diversity and distribution of the foliose genera of the family Physciaceae in Bulgaria. The field works were mainly carried out in 2009 and 2010 with special emphasis on less explored floristic regions of the country. The study is based on 144 older herbarium specimens kept in Bulgarian institutions and 962 new collected samples including 439 specimens of family Physciaceae, the bigger part of which was classified into the following foliose genera (number of species in brackets): *Anaptychia* (4), *Heterodermia* (2), *Hyperphyscia* (1), *Phaeophyscia* (9), *Physcia* (15), *Physciella* (2) and *Physconia* (9). Two species are new for Bulgaria: *Physciella nepalensis* and *Physconia thorstennii*. Distribution, special characteristics illustrated by images, ecology and common associating taxa of selected species are provided.

## **Does the genetic structure of *Carlina acanthifolia* subsp. *utzka* reflect distinctions of Balkans and Pontic phylogeographical areas?**

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*Carlina acanthifolia* subsp. *utzka* (including *Carlina onopordifolia* Szafer ex Besser) mainly occurs in the Balkans and Pontic area. Its range comprises several isolated populations in south-central Europe. The aim of the study is to determine, if a genetic differentiation correlated with geographical disjunction, Balkans versus Pontic area, does exist within the subspecies. This work is a part of research on the genetic structure of *C. acanthifolia* s.l., which is based on AFLP and DNA sequencing analyses. The genetic structure study was conducted by determination of the genetic interrelationships both within and between isolated groups of the populations using the following statistical methods: NJ, PCO, AMOVA and Bayesian analyses. Results showed no specific markers for any population, balanced and low levels of genetic variation within populations, which may be due to an asexual type of reproduction - apomixis. Such a pattern of genetic structure confirms the common origin of the populations from the Balkans and Pontic area. Disjunctive range of subsp. *utzka* is probably a remnant of the larger range that may have been created by the quaternary colonization of these areas. Grant of National Science Centre (Poland), no NN304300940.



## **The De Visiani Plant Collection in Padova**

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Roberto De Visiani (1800-1878) was a Dalmatian botanist who served as Praefectus of the Botanical Garden of Padova from 1836 to 1878. His main interest was the vascular flora of his homeland, to which he dedicated numerous publications. His *Flora Dalmatica* is a milestone in the knowledge of the Balkan biodiversity. The collection on which this work was based is held in our Herbarium and was never thoroughly restudied. In 2011 a project was started by the Department of Biology to catalogue it and share this information with colleagues who presently work on the Illyric flora to put it back to work and to spur the development and testing of a working database solution. High resolution images of the specimens will be made available thanks to a specially designed scanner (HerbScan), starting from type material. A geocoding system is also being developed. Up to now nearly 5000 of the estimated 8000 specimens have been catalogued, 130 of which are probably types. The catalogue is expected to be available online in the next few months.

## Patterns of morphological, genetic and ecological differentiation within the *Heliosperma pusillum* complex (Caryophyllaceae)

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Although the Balkan Peninsula is probably the “hottest biodiversity hotspot” on the European subcontinent, characterized by high habitat and (micro)climate diversity and a complex biogeographic history as one of the main Pleistocene refugial areas in Europe, little work has been done on phylogeography, radiation and speciation processes in plants of that area. One of the few well-studied groups is the *Heliosperma pusillum* complex, consisting of widespread *H. pusillum* distributed from the Sierra Cantabrica to the Carpathians, and several narrowly endemic species of the southeastern Alps and the Balkan Peninsula, where the genus has its centre of diversity.

Outcomes of a detailed morphometric survey of all described species of the group are illustrated with regard to traditional taxonomy, and compared to patterns of genetic and ecological diversification within the *H. pusillum* complex. Our findings suggest that morphology does not reflect the (weak) phylogenetic structure within the genus but rather seems to be a product of local adaptation and variability between populations and individuals, which makes this group an ideal study system for examining the interplay of environmental and (epi)genetic factors in shaping morphological diversity.

## **Present and past distribution of *Edraianthus* species based on ecological niche modelling**

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The Balkan Peninsula is recognized as one of the south European Pleistocene refugia for temperate species, a biodiversity hotspot harbouring numerous endemic species within its flora. The genus *Edraianthus* (Campanulaceae) has the centre of its distribution in western Balkans. In recent years it has been extensively studied. Special efforts have been given to the study of phylogenetic relations within the group and to the study of quaternary range shift patterns of some representatives, based on molecular data. Some of the published studies were accompanied by accurate data of species occurrences. Using such data together with environmental data in ecological niche modelling (ENM) we can predict species distribution in a geographic space according to a mathematical prediction of their environmental space. Models projected to past environmental conditions can help us understand late quaternary range shifts and to locate potential Pleistocene refugia. Here we present the results of the potential present and past (Last Glacial Maximum) distribution patterns of selected *Edraianthus* species based on ENM. Our aim is to complete the knowledge of their current distribution and to contribute to the understanding of biogeographical patterns as a response to late quaternary climatic oscillations in the Balkan Peninsula.

## Genetic diversity of Dalmatian and short-tooth sage populations on the Pelješac peninsula

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Dalmatian sage (*Salvia officinalis* L.) and short-tooth sage (*Salvia brachyodon* Vandas) are closely related but differently distributed species. Dalmatian sage is naturally widespread throughout the Mediterranean region, while short-tooth sage is a steno-endemic species of the Dinaric Karst. The Pelješac peninsula and the Mt Orjen are only known localities of short-tooth sage today. In the Red book of vascular flora of Croatia this species has been categorized as Near Threatened (NT). Genetic diversity of populations of Dalmatian and short-tooth sage on the Pelješac peninsula was analyzed using five microsatellite markers. The population of short-tooth sage showed half the number of alleles and a significantly smaller genetic distance among individuals than population of Dalmatian sage. Moreover, in the population of Dalmatian sage Hardy-Weinberg equilibrium was noticed, while in the population of short-tooth sage a surplus of heterozygosity and a recent bottleneck were detected. The assumption is that the short-tooth sage population strongly reduced its size in the recent past, and thus lost a considerable portion of alleles, especially those with low frequency. The results imply the necessity of a new risk assessment for short-tooth sage in Croatia.

## **Cytotype and morphological diversity of *Sorbus aria* and *S. austriaca* from Bosnia and Herzegovina**

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Diversification of the genus *Sorbus* is strongly affected by hybridization, polyploidy and apomixis. *Sorbus aria* represents an extremely variable species, taking part in interspecific hybridization. In sympatry with *S. austriaca*, a series of specific morphological forms are generated. Thus, we have explored patterns and relationships between the two species using genome size, ploidy level, flow cytometric seed screening (FCSS) and morphological characterisation. *Sorbus aria* and *S. austriaca* were sampled in 16 natural populations from Bosnia and Herzegovina. Genome size, ploidy levels and FCSS were determined using flow cytometry while discriminant analysis used 21 quantitative morphological characters of leaf, fruit and flower. Variation of 2C DNA nuclear content of *S. aria* was related to the presence of three different cytotypes: di- ( $1.376 \pm 0.033$  pg), tri- ( $2.005 \pm 0.062$  pg) and tetraploid ( $2.691 \pm 0.095$  pg). *Sorbus austriaca* had  $2.732 \pm 0.086$  2C DNA/pg which corresponded to tetraploid level. 47% of *S. aria* s.l. individuals were polyploids. Discriminant analysis revealed morphological discrimination of di-, tri- and tetraploid *S. aria* cytotypes and morphological homogeneity of *S. austriaca*. Apomictic mode of reproduction appears to be the major factor maintaining specific morphological traits for distinction of cytotypes.

## **Plant diversity and phytogeography of "open sea" islets (Ionian area) vs. islets of a "semi-closed" gulf and a lagoon (Western Greece)**

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The studied islets are part of two protected areas under "Ramsar Convention" and consequently, part of "Natura 2000" sites. A total number of 29 islets are included in this study: nineteen "open sea" limestone islets, belonging to the Echinades islets' group situated in the Ionian area, as well as six conglomerate islets in the "semi-closed" Amvrakikos Gulf and four sandy islets in the Mesologgi lagoon. The aim of the present study is to focus on the plant diversity of the islets and to examine the b-diversity between "open sea" islets and the ones of the gulf and the lagoon. Factors affecting plant diversity as well as phytogeographic patterns are also examined and discussed. This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

## **Ancient divergence of dance flies (Empididae, Diptera): first insights from the mtDNA phylogeny with special emphasis on the Balkans**

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Family Empididae consists of three subfamilies out of which Clinocerinae and Hemerodromiinae are related to aquatic habitats and are predators in all of their life stages. These two subfamilies are rich in endemic species, specifically the genus *Wiedemannia* (Clinocerinae). Most of the endemics inhabit mountain areas of Europe including the Balkan Peninsula. The aim of this study was to provide insights into phylogeny and estimate timing of divergence of dance flies of the subfamily Clinocerinae, particularly the genus *Wiedemannia* in the Balkans. Preliminary phylogeny based on mitochondrial DNA sequences (COI gene) of 37 species indicates strong radiation within the subfamily Clinocerinae and is only to some degree in accordance with morphologically based systematics of this group. Subgenera of the genus *Wiedemannia* are mixed in the phylogeny tree, thus further studies based on nuclear gene sequences should be executed to verify or discard these preliminary findings. Estimation of divergence times reveals very old splits within dance flies and ancient age of Balkans' endemic species. Oldest divergence among aquatic dance flies subfamilies was estimated to approximately 80 Mya and divergence of Balkan endemics within the period of 40 and 11 Mya.

## High genetic diversity and low genetic differentiation of Dalmatian sage (*Salvia officinalis* L.) populations

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Genetic diversity of 25 Dalmatian sage populations from Croatia and Bosnia and Herzegovina, each consisting of 20 to 25 plants, was assessed using AFLP markers. Four AFLP primer combinations yielded 559 polymorphic markers. Allelic frequencies at AFLP marker loci in each population were calculated from the observed fragment frequencies assuming Hardy-Weinberg equilibrium. Gene diversity ranged from 0.162 to 0.199 with an average value of 0.178. The overall genetic differentiation among populations was very low,  $F_{ST} = 0.042$ , ranging from 0.006 to 0.081. The analysis of molecular variance indicated that most of the genetic diversity was attributable to differences among individuals within populations (92%). The Fitch-Margoliash tree based on Nei's genetic distance matrix showed that most of the populations tended to group together in accordance to geographical position of their collecting sites. Spatial analysis of genetic diversity revealed a typical pattern of isolation-by-distance.



## **Micropropagation and in vitro preservation of the endemic species *Symphyandra hofmannii* Pant.**

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*Symphyandra hofmannii* Pant., member of the Campanulaceae family, is an species endemic to the Western Balkans. In Bosnia and Herzegovina it is mainly distributed along the central regions of river Vrbas' and river Bosna's basins, and has a horticultural value. Micropropagation protocol of this endemic and decorative plant was developed in order to protect these dwindling natural populations. Surface-sterilised seeds were cultivated on a Murashige and Skoog (MS) medium supplemented with 0.15 mg/L Gibberellic acid (GA3). Shoot multiplication from seedlings was obtained using MS medium containing 1.00 mg/L 6-benzyladenine (BA) or kinetin (Kin). BA treatment enhanced shoot proliferation. Shoots were rooted successfully when placed on MS medium containing different concentration of indole-3-butyric acid (IBA). Plantlets with developed roots were successfully acclimatized in greenhouse.

## The patterns of diversification of endemic *Proasellus* isopods from relict Lake Ohrid revealed by molecular data

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The Balkan Lake Ohrid, is considered as the oldest ancient lake in Europe. Fauna of ancient lakes is characterized by exceptionally high level of endemism and biodiversity and the presence of species flocks, especially closely related subspecies and morphological forms, evolving through intralacustrine speciation. Lake Ohrid is inhabited by three endemic species from genus *Proasellus*. According to previous taxonomic studies based on morphology, *P. arnautovici* is subdivided into subspecies *elongatus* and *arnautovici*. Within *P. remyi*, three distinct forms were recognised: *remyi*, *acutangulus* and *nudus* whereas within *P. gjorgjevici* two forms: *typicus* and *litoralis* were described. Each taxa is characterized by a complex of distinctive morphological features and is limited in occurrence to the particular vertical zone of the lake with specific conditions of life. Here, on the basis of mitochondrial DNA sequencing data (COI), we provide a picture of patterns and processes of intralacustrine diversification of endemic isopods in Lake Ohrid. Analyses of phylogenetic and genealogical relationships of populations sampled from different bathymetric zones and localities within the lake suggest the crucial role of vertical barriers for promoting diversification in Lake Ohrid. Also, the horizontal distribution of populations from the southern feeder springs and lake seems to contribute to cladogenesis events in endemic *Proasellus* taxa. On the basis of haplotype network analyses it can be concluded that the radiation of Ohrid endemic isopods had started from the sublittoral/profundal population inhabiting southern part of the Lake, near St. Naum.

## **Biodiversity of Ground Beetles (Coleoptera, Carabidae) in Bosnia and Herzegovina**

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In this study a present biosystematic and taxonomical data of family Carabidae in Bosnia and Herzegovina was analysed. Total of 626 species divided in 20 families and 126 genera, including two endemic genera, 51 endemic species and 56 endemic subspecies have been noticed in the researched area. Based on obtained data for the biodiversity of the particular groups, an overview of species concept in biology was implemented with a special emphasis on urgent need to clarify adaptive type and the adaptive zone when describing new taxa. A concept of avoiding the orthodox typological access during the trophile, troglone or troglobitic species description was critically considered. Finally, the initiative for the protection of a type localities has been supported, respectively the typical habitats of already described taxa. Solely on the basis of those taxa, an obvious scientific revision of biosystematic relations among the tightly closed species and higher taxonomic categories could be performed.

## **Tales of the Unexpected: revising the disjunct distribution pattern of *Alyssum ovirense* (Brassicaceae) at different geographical scales**

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The southern European mountain ranges contribute over proportionally to the continent's biodiversity. While the flora of the Alps is well known and its spatiotemporal diversification is relatively well understood, there is fairly limited knowledge of biogeographical links between separate mountain ranges. This is especially true for connections between temperate and sub-mediterranean ranges such as the Alps and the Dinaric Mountains. In order to contribute filling this gap, we analysed a comprehensive sampling of populations of *Alyssum ovirense*, which is disjunctly distributed in the Eastern Alps (predominantly in the Southern Limestone Alps plus one population in the Northern Limestone Alps) and the southern Dinaric Mountains (e.g., Prenj, Durmitor). Preliminary molecular genetic data (AFLPs; plastid and nuclear DNA sequences) suggest that the Dinaric populations of *A. ovirense* in fact do not belong to this species, but are rather closely related to *A. scardicum*. The situation within the Alps strongly contradicts the current taxonomy as well: the population from the Northern Limestone Alps was shown to be hexaploid (all other Alpine populations of *A. ovirense* are diploid) and to be more closely related to the Dinaric populations than to those from the Southern Limestone Alps. In the same line, *A. wulfenianum*, a rare and endangered endemic of the valleys of the rivers Gailitz and Jezernica is in fact nested within *A. ovirense* and apparently does not deserve taxonomic recognition.

## **The state of Kosovo biodiversity 2006 - 2011**

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Kosovo even a small country, it is distinguished with rich biodiversity. Its geographical position, geological factors, hydrology and climate are some of factors that enabled Kosovo to have rich biological diversity, as well as presence relict, endemic and other important species.

Based on up-to-date floristic research, over 2.000 species of vascular plant flora have been identified. Within explored species, about 200 of them are endemic, endemic-relict and sub-endemic. Especially important is the local endemic group, whose number is still not fully defined, some of which have quite limited spread. Kosovo vegetation is represented by 139 plant associations grouped in 63 alliances, 35 orders and 20 classes.

In terms of fauna Kosovo is characterized by a large variety of species, although research in this aspect has not been completed. It is estimated that about 250 vertebrate species live in Kosovo, 200 species of butterflies and over 500 macrozoobenthos water taxon.

Kosovo like many countries is facing with the problem of loss of biodiversity. The main factor that has caused serious threat to the existence of many plant and animal species undoubtedly is anthropogenic factor, meanwhile the massive deforestation, intensive exploitation of land, air and water pollution and uncontrolled hunting are just some of the other factors that continue to threaten biodiversity.

## Ploidy levels and relative genome size in (sub)endemic taxa of *Cerastium* (Caryophyllaceae) from the Balkan Peninsula

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A large-scale screening of ploidy level using flow cytometry was performed for 280 populations from 35 perennial species of *Cerastium*, mostly from the Balkan Peninsula. The genome size varies strongly among different species within the same ploidy levels. Here, we concentrate on six (sub)endemic species from the Balkan Peninsula. The highest values of intracytotypic variation were recorded in tetraploid and octoploid *C. decalvans* and *C. banaticum*. New ploidy levels were recorded in otherwise hexadecaploid *C. malyi* ( $2n=12x$ ), as well as in *C. banaticum* subsp. *kosaninii* ( $2n=8x$ ). Whereas in *C. banaticum* the geographic structure in distribution of cytotypes is not so clear (octoploids are scattered in the central and southern parts of the distribution range, tetraploids are the most widespread in the northern and western parts, and decaploids and hexadecaploids are restricted to *C. b.* subsp. *speciosum* in the southwest), in *C. decalvans* only tetraploids can be found in the western part of the distribution range, the octoploids in the central part and the hexadecaploids in the eastern part. In *C. grandiflorum* only the population from Velebit (Croatia) is octoploid, whereas all other populations are tetraploid. No strong correlation was found between the cytotypic and taxonomic variability in *C. decalvans* and *C. banaticum*.

## Possible methods for detecting hybrid: a case study on *Abramis brama* (L.) x *Rutilus rutilus* (L.)

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Hybridization represents a worldwide burning issue due to extensive habitat alterations and anthropogenic introgression. Intra- and intergeneric cross-breeding is particularly well documented in the family Cyprinidae. Even though molecular studies suggest that bream *Abramis brama* (L.) and roach *Rutilus rutilus* (L.) do not belong to sister taxa, the natural hybrids between these two species are reported throughout of Europe. Microsatellites are common markers-of-choice in genetic diversity estimation. Being co-dominantly inherited and highly polymorphic, they are particularly useful for investigation of levels of hybridization and introgression. The importance of chosen genetic marker were described by Epifanio and Philipp (1997) who noted that error rates when classifying individuals to hybrid categories may be quite high if few loci are available. Lately, using SNPs in the detection of hybrids has gained significance. Numerous methods have been proposed for identifying hybrid individuals (Campton et Utter 1985; Nason et Ellstrand 1993; Barton 2000; Miller 2000; Young et al. 2001; Anderson et Thompson 2002). Some methods rely on the use alleles that are unique to each species. Other suggested Maximum-likelihood, Bayesian method and Markov chain Monte Carlo (MCMC) or principal coordinate analysis (a general multivariate statistical technique) to cluster pure individuals of two species and their hybrids. In this study, we tested the sensitivity and informativeness of microsatellites against species-specific ITS1 markers in determining bream x roach hybrids. For this purpose, we have applied some of abovementioned models of hybrid detection, and their results have been compared.

## Relationships between genome size and some life history traits of serpentinophytes from northwest Balkans

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Serpentine substrata present hostile environment for plant life and are characterized by a small number of species but higher level of endemism. For the first time, the specialized flora of north-western region of serpentine zone in the Balkans is analyzed in order to assess relationships between plant genome size and chromosome number, ploidy level, affinity to the substrate, plant life cycle and growth form. The sample includes 308 taxa belonging to 213 genera, with new values recorded for 28 genera and 99 species. According to Leitch's criteria, more than half of estimated taxa (56%) belong to the group of very small genomes, 22% small, 19% intermediary, 3% large and only 0.31% to very large genomes. In relation to the affinity to the substrate, the majority of species (56%) were indifferent or facultative serpentinophytes (33%). Concerning the life cycle, annuals represent about 4% of analyzed set, while 88% of species were perennials, mainly with very small genomes (57%). In our sample, hemicryptophytes represent a dominant life form (48%). The results indicate that the water stress, high temperatures and presence of heavy metals in serpentine habitats most likely express a high selective pressure favouring perennial species with very small genomes.



## Natural hybridization between *Salvia officinalis* L. and *Salvia fruticosa* Mill. (Lamiaceae) as revealed by microsatellite markers

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Dalmatian sage (*Salvia officinalis* L.) and Greek sage (*S. fruticosa* Mill.) are two closely related species. In Croatia, the island of Vis is the only known locality where these two species grow sympatrically. Dalmatian sage is naturally widespread in Croatia, whereas the origin of Greek sage in this location is probably related with the ancient Greek colony. Besides sharing same habitat on the island, these two species have same chromosome number ( $2n=14$ ) as well as the same flowering time. All these features together with the facts that plant breeders managed to produce their commercial hybrid, that botanists reported morphologically intermediate individuals in the wild and that several microsatellite loci identified for *S. officinalis* were successfully amplified in *S. fruticosa*, encouraged us to study putative natural hybridization events on the island of Vis using microsatellite markers. A total of 79 plant samples were analyzed (16 individuals of *S. officinalis*, 38 of *S. fruticosa* and 25 of intermediate hybrids). Hybrid origin of morphologically intermediate individuals has been genetically strongly supported. Microsatellite genotyping showed that the putative hybrids possessed alleles in common with the proposed parental species and intermediate values of genetic differentiation. Evolutionary, conservation and commercial implications of this hybridization were discussed.

## **A Greek Odyssey: Constructing/Developing an online Flora of the Ionian Islands**

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The flora of the Ionian Islands (W Greece) is characterized by an extraordinary diversity, exhibiting more than 2000 species of ferns and flowering plants in an area of 2300 km<sup>2</sup>. A systematic mapping of the islands started with fieldtrips on Mediterranean plant biodiversity by the University of Vienna, supervised by W. Gutermann. Floristic and phytosociological mapping was intensified in the years 1994-1999. Since 2011 detailed mapping activities, based on a 1x1 km UTM grid have been continued. At present, more than 100000 floristic records are available, most of which are stored in an appropriate database.

For an online representation of the floristic project, data will be transferred into a web-compatible database, which will be the precondition for an online catalogue of all vascular plants of the Ionian Islands supplemented with critical comments and brief information on the occurrences on the individual islands. The checklist will be complemented by distribution maps and progressively enriched by existing in situ photographs and scanned herbarium material as well as determination keys for difficult groups. The continuously updated online flora will thus offer comprehensive information on the rich plant life of the Ionian Islands, addressing plant scientists well as all botanically interested persons.

## Bryophyte diversity of the Balkans: an insight from molecular and biochemical markers

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Bryophytes are among the first land plants, and the second biggest terrestrial plant group by the recent species number. However, significantly less is known on their biology comparing to tracheophytes. In general, bryophyte species are slowly evolving group and the one that has huge ranges, often spreading over few continents. The phenomena such as strict endemism, rarity can be considered as rather different as those in vascular plants. Here, an insight into genetic diversity (inferred from isozymes (peroxidases, superoxyddismutases, catalases) and various DNA markers (ITS, *trnL*, *trnF*, *trnG*, *psbT*, *rpb2*) of selected moss and hepatic species from the bryologically rich Balkan peninsula (over 1200 bryophyte taxa, 899 moss and 272 hepatic species) is given. A circum-tethyan moss *Campylopus oerstedianus*, with only 12 small disjunct populations in Europe seems to have the oldest one in Greece, while temperate-boreal moss *Rhytidium rugosum*, scatterly spread in Europe, seems to survive Balkan glaciations in various places around the Balkan peninsula. Also, the xerotherm relic moss *Hypertia velenovsky* showed interesting genetic pattern in Europe, and this in the Balkans. Namely, the Vojvodina populations show relationships among themselves but some population distinct genetically significantly giving to the inference that long-distance dispersal occurs even nowadays. The similar patterns were documented in *Rhodobryum ontariense*, *Atrichum undulatum*. The population from western Serbia of the liverwort *Metzgeria conjugata* seems to be the oldest one in Europe, indicting its relictiness, while in *M. furcata* at least two distinct lineages are known from the Balkans. The first one comprises Serbian populations genetically similar to Central European and Italian counterparts, and the second one consist of Serbian and Greek populations closely related to Canary Island and Southern France.

## **Land use management and location of rare plant species in suburban landscape (Poland)**

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One of the main concerns in plant species preservation is to distinguish the areas of high rare taxa concentration. The sites abundant in non-synanthropic species, especially of high ecological importance, consist mostly of natural areas of continuous land use management. A simple method based on overlapping aerial photographs from three different periods was used to identify locations with rare plant species. The aim of this study was to determine if rare plant species often occur in locations where the land use hasn't changed for decades and if so, whether the method we used for their identification is effective. We also assessed the range of land use changes in the natural and semi-natural sites of Milanówek commune beginning from year 1987 to 2004. Without undertaking protective measures, observed general tendencies in the landscape structure may cause to the rare plant species impoverishment in Milanówek commune. The number of semi-natural locations and their border line length decreases. The area of natural populations slightly increases but their border line length shortens. The presence of rare species is associated with areas of continuous land use type dating back to 1987. Preservation of such locations is crucial for the preservation of rare species, including those strictly protected by law.

## **The indigeneity of a Mediterranean herb in the continental Balkans: A plastid-based phylogeographic survey of *Salvia officinalis* L.**

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The main natural range of *Salvia officinalis* is continuous along the eastern Adriatic coast. The indigeneity of its continental disjunctions in the central Balkans, however, is questionable, since they may have been established post-glacially via anthropogenic mediation. Utilizing two chloroplast intergenic spacers, 3' *rps16*-5' *trnK* and *rpl32-trnL*, we found seven haplotypes non-randomly distributed among eight studied populations of this species and revealed that their divergence dates back to the Pliocene (3.267 MYA). Two lineages, a more heterogeneous and less diverse continental-Adriatic lineage (CAL, Hd = 0.426,  $\pi$  = 0.00106) and a more diverse, purely Adriatic lineage (PAL, Hd = 0.403,  $\pi$  = 0.00257) do not exhibit signs of recent demographic expansions and currently overlap on the southeastern Adriatic coast, a region designated as the main refugium of *S. officinalis*. All four continental populations comprising the continental sub-lineage of CAL were almost fixed for a distinct haplotype which was rare in the Adriatic sub-lineage of CAL and absent in PAL. Given the close genealogical relation of that haplotype to the ancestral haplotype as inferred from a statistical parsimony analysis, we characterized all continental populations as relict and refugial.

## Differentiation center and glacial refugia for the postglacial colonization routes of *Melica ciliata* complex (Poaceae) in Europe

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*Melica ciliata* (Poaceae) is a complex of sub-Mediterranean-continental species. Its range comprises also disjunct areas in the southern part of the Scandinavian Peninsula and in north-western Africa. The phylogeographic patterns of European taxa of *M. ciliata* complex were determined in order to reconstruct its glacial history, including identifying potential glacial refugia. Haplotype analysis, based on non-coding regions of chloroplast DNA sequences variability, and AFLP analysis were also used to define the hypothetical differentiation center for the species. One hundred three populations within Euro-Asiatic distribution area were studied. Based on the AFLPs study of *M. ciliata* s.l. a low level of genetic diversity and a clear genetic structure of species was showed. The results indicate a discrete genetic reservoir and genetic isolations of populations mainly due to asexual reproduction. The contemporary scattered distribution of suitable habitats and the predominant selfing breeding system of the species strengthen the effect of selection pressure on fixation of unique loci in individual populations. Patterns of phylogeographic diversity were more associated with historical than contemporary connections, indicating that major change in the patterns of the region played an important role in the generation of complex diversity. The shared occurrence of rare AFLP markers between distant populations may indicate more effective gene flow in the Holocene, when steppe communities occupied vast and continuous areas. The greatest haplotype variation of cpDNA and divergence were revealed on Balkan and in eastern Mediterranean area, implying possible glacial refugia in these regions. Phylogeographic and population genetic diversity and differentiation parameters indicated a divergence of five main lineages within *M. ciliata* s.l.: eastern European, central and western European, Balkan, Iberian with western Mediterranean and Scandinavian. Three lineages correspond with morphologically unambiguous species: *Melica transsilvanica* Schur, *M. ciliata* L. and *M. magnolii* Gren. et Godron. This research was supported by the National Science Centre (Poland), grant no. N N303 373436.

## The qualitative and quantitative composition of ant fauna (Hymenoptera, Formicidae) of Popovo Polje

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A faunistic study on ants of Popovo Polje was conducted in order to analyse the biodiversity of ants inhabiting karst poljes in the Mediterranean part of Bosnia and Herzegovina. Field studies were conducted during the four seasons between 2006 and 2009, and have employed active methods of collection of specimens. The results show that the fauna of ants of Popovo Polje is composed of species belonging to three subfamilies. Subfamily Myrmicinae Lepeletier de Saint Fargeau, 1835 is represented with 10 genera and 16 species. Subfamily Formicinae Latreille is represented with six genera and 14 species, while only two genera and two species belonging to the subfamily Dolichoderinae Forel were recorded. In the entire sample, the most dominant subfamily Myrmicinae Lepeletier de Saint Fargeau is represented with 3.655 individuals of the worker caste which amounts to 59,90%. Subfamilies Formicinae Latreille and Dolichoderinae Forel, 1878 represent 35,00% and 12,18% of the entire sample respectively. Genus *Messor* Forel, 1890, dominates the sample with its three species and 1.664 individuals or 24,05%, while the species *Pheidole pallidula* (Nylander, 1849) is the most abundant species in the sample and is represented with 875 individuals which comprises 12,38% of all the worker caste individuals collected.

## **Incongruence between morphological and molecular diversity of endemic gammarids in ancient Lake Ohrid**

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Tentatively dated Plio-/Pleistocene origin of ancient Lake Ohrid on the Balkan Peninsula makes it the oldest ancient lake in Europe. Given the surface area of the lake and the adjusted endemism rate, it may be defined also as the most diverse of all the ancient lakes in the world. From all the animal groups endemic to this lake, gammarids are among the most scarcely known in terms of their diversity and taxonomic/phylogenetic relationships. Partial DNA sequence data of two mitochondrial genes, cytochrome oxidase subunit I (*cox1*) and 16S rRNA allowed to determine the taxonomic/phylogenetic status of all the known endemic *Gammarus* species from Lake Ohrid and verify the currently established taxonomy based on morphological characters. According to our data the Ohridian endemic *Gammarus* species represent a monophyletic ancient lake species flock.



## **Genetic variability and population structure in natural populations of the European field elm (*Ulmus minor* Mill. sensu latissimo) from Croatia as revealed by SSR markers**

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This study, conducted in Croatia, encompassed for the first time ever populations of the field elm that are solely natural. We studied the genetic diversity and assessed recent bottleneck events and the population structure of 5 natural field elm populations from continental and Mediterranean Croatia, using 5 microsatellite markers. The study results suggest that the observed populations are characterized by a satisfactory amount of heterozygosity, and that the impact of the Dutch elm disease on the amount of genetic diversity in sampled populations is currently negligible. As genetic diversity in forest tree species is slowly diminishing, in the long term there is a danger of the depletion of genetic resources. The danger of inbreeding within populations is low, and the majority of the populations are in Hardy-Weinberg equilibrium. The appearance of bottleneck, initiated by anthropogenic habitat destruction, has been established in only one population. The values of Nei's standard genetic distance, as well as the values of the genetic differentiation index between pairs of observed populations are indicative and suggest, together with the performed factor correspondence analysis and unrooted neighbor-joining tree, the existence of a very clear genetic differentiation between the continental and the Mediterranean populations of the field elm in Croatia.

## **Sex ratio in local population of species *Chondrostoma phoxinus* Heckel, 1843 (Teleostei: Ostaryophysi, Cyprinidae) from Buško Lake**

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Within species with separated male and female sex, the sex ratio is one of the significant features of the population. The specified aspect of the sexual differentiation is represented in the development of needs for fertilization and realization of total population size and minimal presentation of genetic drift and inbreeding effect.

The species *Chondrostoma phoxinus* Heckel, 1843 (known as The Minnow Nase) is described based on samples from Sinj (Croatia), and later it has been found in the territory of Bosnia and Herzegovina, more precisely in Buško Lake.

Material, individuals *Chondrostoma phoxinus* Heckel, 1843, has been collected periodically from April 2003 September 2004 during and during 2008 and 2009 with standard ichthyology methods. The noticed differences are analyzed with chi-square test.

In the summed sample from 2003 and 2004 (marked as the first subsample) there are 71 units of the minnow nase: 51 males and 20 females (0,7183:0,2817). Summed sample from 2008 and 2009. (marked as the second subsample) included 252 units of minnow nase, 87 males and 118 females (0,4244:0,5756), while for 47 units we cannot determine the sex. From the specified data it is possible to determine imbalance in the subsamples (first subsample: 2,55:1,00 with chi-square 13,56 and probability of random finding of the noticed differences  $P < 0,001$ ; second subsample: 0,74: 1,00 with chi-square 2,50 and probability of random finding of the noticed differences  $0,2 < p < 0,01$ ) between males and females in samples. However, total sample of 276 individuals have been represented with perfect ratio of 138 males and 138 females and ideal sex ratio 1:1.

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