

BOOK OF ABSTRACTS

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OF THE REPUBLIC OF NORTH MACEDONIA,
WITH INTERNATIONAL PARTICIPATION**

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AIR, WATER, AND SOIL POLLUTION
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Chemistry of ultramafic soils and hyperaccumulation in the genus *Odontarrhena*

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Ultramafic soils represent one of the most chemically extreme terrestrial environments, characterized by high concentrations of potentially toxic metals such as nickel (Ni), cobalt (Co), chromium (Cr), and manganese (Mn), low levels of essential nutrients, and unfavourable Ca/Mg ratios. These edaphic conditions exert strong selective pressure on plants and have promoted the evolution of specialized physiological and biochemical adaptations. Among the most remarkable adaptations is metal hyperaccumulation, a phenomenon in which plants actively absorb and tolerate exceptionally high concentrations of metals in their aboveground tissues without suffering toxic effects. The genus *Odontarrhena* (Brassicaceae), widely distributed in the Mediterranean and Balkan regions, represents one of the most important groups of nickel hyperaccumulator plants associated with ultramafic substrates. Numerous species within the genus have evolved the capacity to accumulate nickel at concentrations exceeding the threshold for hyperaccumulation, while other closely related taxa occurring on non-ultramafic soils exhibit exclusion strategies and limited metal uptake. This diversity makes *Odontarrhena* an excellent model for studying the evolution of metal tolerance, accumulation mechanisms, and plant adaptation to metalliferous environments. The chemistry of ultramafic soils strongly influences metal bioavailability and plant uptake. Soil properties such as pH, mineral composition, organic matter content, and the ratio between calcium and magnesium control the mobility of trace metals and their transfer into plant tissues. Hyperaccumulator species possess highly efficient uptake, transport, detoxification, and storage mechanisms that enable preferential translocation of nickel from roots to shoots and leaves. In contrast, non-hyperaccumulator species generally restrict metal uptake or retain metals in roots as part of an exclusion strategy. Research on *Odontarrhena* species has significantly expanded current understanding of plant–soil interactions under metal stress conditions. Comparative studies among obligate hyperaccumulators, facultative accumulators, and excluders reveal substantial interspecific variation in metal tolerance and accumulation capacity. These differences are closely related to ecological specialization, evolutionary history, and adaptation to ultramafic habitats. In addition to their ecological importance, nickel hyperaccumulators have attracted increasing scientific interest because of their potential applications in phytoremediation, phytomining, and restoration of metal-contaminated soils. Understanding the relationships between ultramafic soil chemistry and metal accumulation strategies in *Odontarrhena* contributes not only to the fields of plant ecology and geochemistry but also to the development of sustainable technologies for the management of metalliferous environments. Further investigations integrating physiological, ecological, and molecular approaches are essential for clarifying the evolutionary origins and functional mechanisms of hyperaccumulation within this diverse genus.

Key words: Ultramafic Soils, Hyperaccumulation, Genus *Odontarrhena*, bioavailability

Validation of a phosphovanadomolybdate UV-VIS spectrophotometric method for the determination of plant-available phosphorus in agricultural soils and evaluation of dissolved silicon interference

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Reliable determination of plant-available phosphorus is essential for soil fertility evaluation and sustainable nutrient management. In the present study, a UV–Visible spectrophotometric method based on the phosphovanadomolybdate reaction was validated for the determination of plant-available phosphorus in agricultural soils following Egner–Riehm ammonium lactate (AL) extraction. In addition, the influence of dissolved silicon on the analytical performance of the method was investigated. Validation included assessment of linearity, sensitivity, selectivity, trueness, accuracy and precision. Excellent linearity was obtained over the concentration range of 1–20 mg P L⁻¹ ($R^2 > 0.999$), while the calculated limits of detection and quantification confirmed adequate sensitivity for routine soil analysis. The influence of dissolved silicon was negligible, with a calculated matrix effect of only 0.9%, indicating no significant interference under the selected analytical conditions. Trueness was verified using a BIPEA certified reference soil material, whereas accuracy was assessed by the standard addition procedure. The obtained z-score (<2), recoveries (82.1–93.3%) and repeatability (RSD = 0.09–0.67%) confirmed satisfactory analytical performance. The validated method provides a reliable and robust analytical approach for routine determination of plant-available phosphorus in agricultural soils.

Key words: agricultural soils, plant-available phosphorus, phosphovanadomolybdate reaction, UV–Vis spectrophotometry; method validation, silicon interference; Egner–Riehm ammonium lactate extraction.

***Glomeris hexasticha* Brandt, 1833 (Myriapoda, Diplopoda, Glomerida) as a model organism for monitoring seasonal shifts of potentially toxic elements accumulation in urban areas**

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Millipedes (Diplopoda) represent a significant group of saprophagous arthropods that provide ecosystem services, including decomposition and nutrient cycling, thereby enhancing soil health. Given their roles, millipedes may serve as effective bioindicators for potentially toxic elements (PTE) pollution. This study involved monitoring the concentrations of selected elements in both sexes of the millipede *Glomeris hexasticha* Brandt, 1833, as well as in their surrounding environment, to identify potential patterns of accumulation. Sampling was performed in spring and autumn 2023, autumn 2024 and spring 2025 in the urban Belgrade area at 3 localities (Hajd Park, Makiš Forest and Banjica Forest). In total, we collected 630 females and 592 males for the analysis. Adults were kept in plastic boxes in laboratory for a month on the soil and leaf litter from the sampling site. For sample preparation, animals were frozen for an hour in the freezer, rinsed with tap and distilled water, and air dried. Following the immersion in liquid nitrogen, the specimens were ground in ceramic mortars, and the resulting paste was dried to constant weight at 65 °C. The concentrations of nine elements (Fe, Zn, Cu, Mn, Co, Pb, Cd, Cr, Ni) were analyzed in the whole body of millipedes, soil and leaf litter from sampling sites using atomic absorption spectrophotometry (Shimadzu AA 7000). Statistical analyses, NMDS (non-metric multidimensional scaling), and calculation of BAF (bioaccumulation factor) and MPI (metal pollution index) were conducted in R (version 4.5.2). The most abundant elements in millipede tissues were Fe, Zn and Mn, and Fe and Mn in both soil and leaf litter at all localities. The BAF values, calculated as the ratio of element concentration in an organism to that in the surrounding soil and litter, suggested a stronger contribution of litter than soil to element accumulation, and greater accumulation in autumn than in spring. The MPI values were considerably higher in autumn than in spring at all localities. NMDS analysis using Bray-Curtis distance (stress = 0.072) revealed a distinct seasonal separation in the elemental profiles obtained from millipede whole body samples, whereas the effects of locality and sex were less pronounced. Environmental vector fitting (envfit) identified soil Cd, Mn and Zn, together with litter Pb, as the strongest drivers of the observed patterns. PERMANOVA confirmed significant multivariate differences among samples ($R^2 = 0.32$, $p = 0.034$), driven primarily by season (pairwise comparison spring vs. autumn: $p = 0.001$). Locality and sex had no significant effects. Mann-Whitney tests further showed significantly elevated concentrations of Pb ($p = 0.0004$), Co ($p = 0.007$) and Ni ($p = 0.039$) in autumn compared with spring animal samples. The observed seasonal variation may stem from various factors, including differences in leaf litter decay processes across seasons and tree species, the bioavailability of elements, and the physiological requirements and responses of the millipede. These results highlight the potential application of *G. hexasticha* as a bioindicator for PTE pollution in urban ecosystems, warranting further exploration of the diplopod internal mechanisms that are triggered under stressful conditions.

Key words: atomic absorption spectrophotometry; bioindicator; millipede; pollution; soil; urban ecosystems

Moss biomonitoring as a tool for assessing atmospheric microplastic deposition in North Macedonia

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Abstract Atmospheric microplastics are increasingly recognized as emerging contaminants, yet data on their atmospheric deposition remain limited, particularly in Southeast Europe. Moss biomonitoring has been successfully applied within the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) to assess atmospheric deposition of heavy metals, nitrogen, and persistent organic pollutants across Europe. More recently, mosses have also been proposed as suitable biomonitors for atmospheric microplastics, offering a cost-effective and standardized approach for large-scale environmental monitoring. Building upon the extensive European moss monitoring network originally established for assessing atmospheric deposition of trace elements and other pollutants, international initiatives have recently begun incorporating microplastics into biomonitoring programmes, including the MADAME (Microplastic Atmospheric Deposition Assessment using Moss in Europe) project, involving approximately 30 countries and around 90 sampling locations. In this study, moss biomonitoring was used to provide a preliminary assessment of atmospheric microplastic deposition at three locations in North Macedonia characterized by different degrees of anthropogenic influence. Moss samples representing different species were collected from Vodno (Skopje), Majdan, and Bojančište and analyzed using micro-Fourier transform infrared (μ -FTIR) spectroscopy. Microplastics were detected in all investigated samples, with concentrations ranging from 3.29 to 16.72 particles g^{-1} dry weight. The highest concentration was recorded at the urban Vodno site, while the greatest polymer diversity was also observed there, including polypropylene, polystyrene, polyvinyl chloride, polylactic acid, and cellulose acetate. These findings provide the first evidence of atmospheric microplastic deposition in North Macedonia and demonstrate the potential of mosses as passive biomonitors of atmospheric microplastic contamination. Although the preliminary dataset does not allow definitive attribution of observed patterns to specific emission sources, the results support the need for expanded spatial and temporal monitoring using standardized sampling and analytical approaches.

Key words: atmospheric deposition; microplastics; moss biomonitoring; μ -FTIR; North Macedonia;

Microclimate characteristics of the Leskoečka Peštera cave in Galičica National Park (North Macedonia)

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Although cave climate used to be considered relatively stable, recent studies demonstrated significant spatial and temporal thermal variability, highlighting the influence of various natural and human-induced factors on the microclimate in caves. High-resolution quantitative microclimatic data provide valuable insights into the physical processes that determine the climate inside caves and their interactions with external environmental conditions. Among the various microclimatic parameters, thermal conditions are among the most frequently recorded and monitored variables, as they provide information for cave ventilation, thermal buffering, and energy exchange with the surface environment. The cave Leskoečka Peštera is located on the eastern slopes of the Galičica mountain within Galičica National Park, North Macedonia. The entrance is situated near the village of Leskoec, at an altitude of 1070 m. asl. The cave is formed in Triassic limestone. It is a single-entrance cave, 48 m long and morphologically consists of three cave expansions connected by narrow passages. The objectives of this study were: (i) to characterize the cave's seasonal microclimate through continuous monitoring of air temperature, relative humidity, and dew point; and (ii) to investigate the effects of external climatic conditions on the cave's microclimate by comparing external and cave air temperatures. Measurements in the cave were conducted from March 2023 to July 2024. Air temperature, relative air humidity, and dew point were measured using an Onset HOBO MX2302 External temperature/RH type logger. A data logger was installed in the third cave hall, 1 m above ground and 45 m from the entrance. In March 2024, an Onset HOBOconnect MX2303 data logger, which measures the outdoor air temperature, was installed near the cave entrance at a height of 2 m above the ground. Data were continuously recorded at 15-minute intervals. Descriptive statistical analyses and linear correlation were used to evaluate seasonal microclimatic variations and the relationship between external and cave air temperatures. The mean annual cave air temperature was 11.11 °C, with the highest air temperature recorded at 13.12 °C (October 2023) and the lowest at 9.95 °C (April 2023). The annual temperature amplitude was 3.17 °C, indicating a relatively stable thermal regime. The air temperature exhibited a clearly defined seasonal pattern that followed the seasonal trend of the external air temperature, although with a delayed response. Mean monthly air temperatures increased at regular intervals from April (10.04 °C) through October (12.48 °C) and then gradually decreased until March (10.38 °C). For the period between March 2024 and July 2024, the correlation between the mean diurnal inside air temperature and the mean diurnal external air temperature was 0.80, indicating that external climatic conditions had a significant influence on the cave thermal regime. Despite this strong correlation, the air temperature inside the cave exhibited a delayed seasonal response compared to the outdoor air temperature, reflecting the cave's thermal buffering capacity. The mean difference between the external air temperature and the cave air temperature (ΔT) was 6.10 °C, which illustrates the cave's ability to buffer external temperature fluctuations and maintain a relatively stable thermal environment. Relative humidity remained consistently high, approximately 98%. Small differences between the air temperature and the dew point were measured, indicating that the cave atmosphere remained close to saturation. The cave Leskoečka Peštera showed a typical cave microclimate, in which the narrow cave entrance and morphology limited the direct inflow of external air, while the surrounding rock encouraged slow heat transfer, thereby maintaining a thermally buffered, nearly saturated, and remarkably stable microclimatic environment.

Key words: cave microclimate, air temperature, relative humidity, dew point, cave Leskoečka Peštera

Chromium-tolerant bacteria from Jugohrom: Comparative Cr(III)/Cr(VI) assessment

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Chromium contamination resulting from metallurgical activities represents a serious environmental concern, particularly due to the high mobility, bioavailability, and toxicity of hexavalent chromium [Cr(VI)]. In contrast, trivalent chromium [Cr(III)] is generally less soluble and less toxic. Bacteria originating from chronically contaminated environments may develop mechanisms that enable them to tolerate elevated chromium concentrations, making them promising candidates for environmental bioremediation. Despite their potential importance, chromium-tolerant bacteria from industrially contaminated soils in North Macedonia remain insufficiently investigated. This study focused on the characterization of eight bacterial isolates recovered from contaminated soil collected at the former Jugohrom metallurgical complex near Tetovo, North Macedonia, and the evaluation of their tolerance to Cr(III) and Cr(VI). The isolates were examined using macroscopic, microscopic, and biochemical tests. Chromium tolerance was evaluated in a resazurin-based broth microdilution assay with $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{K}_2\text{Cr}_2\text{O}_7$, using twofold serial concentrations from 1000 to 7.81 $\mu\text{g/mL}$. A colour change from purple to pink indicated metabolic activity, and growth from positive wells was confirmed on agar. The isolates differed in their colony appearance and biochemical profiles, showing that the collection was phenotypically diverse. Their response to chromium also varied clearly. With Cr(III), isolates J1 and J2 retained metabolic activity at 1000 $\mu\text{g/mL}$, the highest concentration tested, while J3–J8 remained active up to 500 $\mu\text{g/mL}$. Cr(VI) was considerably more inhibitory. Isolates J1, J2, and J5–J8 remained active up to 250 $\mu\text{g/mL}$, whereas J3 and J4 remained active only up to 125 $\mu\text{g/mL}$. No isolate showed detectable metabolic activity at 500 or 1000 $\mu\text{g/mL}$ Cr(VI). Taken together, J1 and J2 showed the strongest tolerance to both chromium forms, while J3 and J4 were the most sensitive to Cr(VI). The lower tolerance limits observed for Cr(VI) reflect its greater toxicity compared with Cr(III). These results show that Jugohrom soil contains bacteria adapted to chromium stress, with J1 and J2 standing out as the most promising candidates for further study. Molecular identification and direct measurements of Cr(VI) reduction, biosorption, and chromium removal will be needed to determine whether these isolates may contribute to sustainable strategies for chromium detoxification in contaminated soils and industrial effluents.

Key words: chromium tolerance, bacterial isolates, bioremediation, environmental microbiology, heavy metal contamination

Heavy metal accumulation in tobacco plants (*Nicotiana tabacum* L.) infected with *Agrobacterium rhizogenes* A4: Implications for phytoremediation of contaminated soils

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Tobacco is one of the most important crops grown in the Republic of North Macedonia. The infection of tobacco (*Nicotiana tabacum*) type "Prilep" variety P12-2/1 with a fresh culture of *Agrobacterium rhizogenes* strain A4, under in situ conditions, induces the appearance of "Hairy Roots-HR" and morphological modifications of seedlings. Infected tobacco plants showed enhanced capability to absorb and accumulate heavy metals from the soil. The aim of the current study was to investigate the levels of uptake and distribution of heavy metals from soils of the Prilep and Veles regions in different parts of infected and uninfected tobacco. Soil and tobacco samples, after appropriate preparation, were analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) Varian, 715-ES. Infection induced a 1.8-fold increase in root length and a 2.6-fold increase in lateral root number compared to controls. The content of heavy metals tested in the soil and tobacco near the smelter showed extreme values compared to other samples. In summary, the highest content of Fe, As, Cu, Mn, Pb and Zn was accumulated in the root of the infected tobacco plant, while the highest content of Cd was accumulated in the leaves. Infected plants demonstrated significantly greater metal accumulation capacity than non-infected controls, resulting in substantial reduction of soil metal content after cultivation (Pb by 82.5%, Cd by 86.4%, Zn by 67.7% in Veles soil). Therefore, at sites where there is a higher content of heavy metals, we recommend phytoremediation with plants such as tobacco infected with *Agrobacterium rhizogenes* A4 that have the ability to extract and bind heavy metals in their organs. However, cultivation on Cd-contaminated soils requires caution due to leaf accumulation and potential risks of trophic transfer.

Key words: phytoremediation, *Agrobacterium rhizogenes*, hairy roots, heavy metal accumulation, soil contamination

Regulation of zinc content in *Glomeris hexasticha* Brandt, 1833 (Myriapoda, Diplopoda, Glomerida) through fecal excretion after consumption of zinc oxide treated leaf litter

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Zinc (Zn) is a trace metal and an essential micronutrient, with a range of important functions. Nevertheless, high concentrations of this element can cause different physiological, biochemical, and behavioural changes. In response, arthropods can utilize several mitigating mechanisms, including reduced intake, reduced nutrient assimilation, sequestration, excretion, and excretion during molting. Here, we exposed adult millipedes of the species *Glomeris hexasticha* Brandt, 1833 to zinc oxide (ZnO) suspension to evaluate the efficiency of the excretion pathway. The animals were collected from Hajd Park, urban Belgrade forest, in October 2025. In total, 90 females and 72 males were used (30 females and 24 males per treatment), arranged in three independent experimental replicates, each set up in triplicate. Millipedes were kept in plastic boxes with 300 g of soil and 3 g of leaf litter from the sampling site. The leaves were treated with 4.5 mL of distilled water (as control), 2 mg/mL and 5 mg/mL ZnO suspension. Following the four-day fasting period and subsequent four weeks of exposure, the zinc levels in the entire body of the animals, as well as in the soil, leaves, and feces, were assessed using atomic absorption spectrophotometry (Shimadzu AA 7000). Fecal pellets were collected at the end of each week. Statistical analyses and calculation of BAF (bioaccumulation factor) were conducted in R (version 4.5.2). The results showed that zinc concentrations in millipedes increased significantly with ZnO suspension concentration (ANOVA, $F = 30.46$, $p < 0.001$). Post-hoc tests showed the strongest difference between control group and 5 mg/mL ($p < 0.001$). No significant effect of sex or treatment $\times$ sex interaction was found. BAF values relative to leaf litter were high in the control group (means ≈ 2.8 – 3.1) but dropped sharply in both ZnO treatments (means < 0.10). The effect of treatment on bioaccumulation was highly significant ($F = 54.68$, $p < 0.001$), while sex had no significant influence. Zinc concentrations in feces increased with treatment concentrations (ANOVA, $p < 0.001$) and were significantly higher in males than in females ($p < 0.001$). The highest fecal Zn levels were recorded after week 1 of the exposure in the 5 mg/mL group, while the 2 mg/mL group also showed significantly elevated values compared with the control. This pattern remained consistent during the experiment. All pairwise comparisons between treatments were significant in both sexes. The results suggest that millipedes exposed to ZnO-treated leaves accumulated moderate amounts of zinc in their tissues. Hence, fecal excretion could serve as a potentially major mechanism for regulating zinc content in millipedes. Tissue Zn increased only moderately relative to the much higher external exposure, while fecal Zn increased substantially, suggesting effective regulation of internal Zn concentrations. The potential for reduced feeding may have additionally influenced the observed pattern. Notable sex-specific variations in fecal zinc levels could arise from differing rates of uptake, allocation, and/or gut processing. Further research should focus on elucidating the differences between sexes regarding detoxification pathways, histological localizations of ingested zinc and its effects on growth, reproduction and development.

Key words: atomic absorption spectrophotometry; bioaccumulation factor; millipede; pollution; trace metal

Validation of a modified Egner–Riehm–Domingo (AL) method for determination of plant-available phosphorus in soil

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Determination of plant-available phosphorus in soil is fundamental to agrochemical soil testing, fertilization planning, and high yields in all crops. The Egner–Riehm–Domingo (AL) method, extracting soil with ammonium lactate–acetic acid solution, is widely used for this purpose. However, because a modified version of the AL procedure was adopted for routine use rather than the original standardized method, its analytical performance required formal validation before implementation. The objective of this work was to validate the modified AL method for determining plant-available phosphorus measured by UV-Vis spectrophotometry in soil samples, in order to confirm its suitability for routine laboratory use. Validation followed a standard analytical-quality framework, evaluating limit of detection (LOD), limit of quantification (LOQ), linear and working range, precision (repeatability and reproducibility), recovery, accuracy, and measurement uncertainty (MU) using BIPEA reference soil material and certified reference standards. Calibration curves were constructed from at least five KH_2PO_4 standard solutions, each measured in replicate, with correlation coefficients exceeding 0.995. LOD and LOQ were derived from blank measurements combined with the calibration equation. Repeatability and reproducibility were assessed from ten parallel measurements of control samples and periodic re-preparations, respectively. Recovery was tested using certified reference standards and accuracy was verified through interlaboratory proficiency testing (z-score). Measurement uncertainty was calculated at a 95% confidence level, incorporating precision, bias, and instrument/reagent uncertainty components. The LOD and LOQ were 0.2 and 1.0 mg/100 g P_2O_5 , respectively, with a linear/working range of 1.0–100 mg/100 g P_2O_5 (extendable to 400 mg/100 g via tenfold dilution), repeatability and reproducibility RSD below 10%, recovery within 90–110%, z-score ≤ 2.0 , and a relative measurement uncertainty of $\pm 2.74\%$. The method satisfied all pre-defined acceptance criteria for accuracy, precision, working range, and measurement uncertainty, indicating that the modified AL procedure, combined with UV-Vis spectrophotometry, provides reliable and reproducible results across a practically relevant concentration range, with the dilution step effectively extending applicability to higher-content soils without compromising linearity. In conclusion, the modified AL method for plant-available phosphorus in soils was successfully validated according to internal laboratory quality criteria and satisfies the requirements for accuracy, precision, and measurement uncertainty.

Key words: plant-available phosphorus, modified AL method, soil analysis, reference soil material

Environmental assessment of macro- and potentially toxic elements in agricultural soils of the Bitola region

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The chemical status of agricultural soils is an important indicator of soil quality and sustainable crop production, particularly in regions where tobacco cultivation is an important agricultural activity. This study aimed to assess the elemental composition of soils from the Bitola tobacco-producing region in North Macedonia, focusing on selected macroelements and potentially toxic elements. Soil samples were collected from tobacco-growing areas and analysed by inductively coupled plasma mass spectrometry (ICP-MS). The contents of K, Mg, Fe, Na, As, Cd, Cr, Cu, Ni, Pb and Zn were determined and compared with regional geochemical data and reference values for agricultural soils. The mean contents of Fe, K and Mg were lower than the corresponding values reported for uncultivated soils in the Pelagonian region, possibly due to long-term agricultural use and crop uptake. The Na content was within the range previously reported for tobacco-growing soils in the region. Most potentially toxic elements had contents below the corresponding regional geochemical values and within the permissible limits for agricultural soils. Higher mean arsenic content was observed in soil from the area of the village of Novaci, likely due to the proximity of the REK Bitola mining and thermal power complex. Overall, the investigated soils showed lower contents of several essential macroelements compared with regional geochemical values, while the contents of potentially toxic elements did not indicate significant environmental risk. These findings provide baseline information for soil quality assessment and highlight the importance of continued monitoring of elemental contents in tobacco-growing areas.

Key words: agricultural soils; soil quality; potentially toxic elements; ICP-MS

Low-cost passive approaches for monitoring organic air pollutants in Skopje: personal PDMS samplers and pine needles

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Monitoring organic air pollutants in urban environments commonly relies on active sampling techniques, which require specialized equipment and sampling infrastructure. Low-cost passive approaches can provide a simpler alternative for characterizing atmospheric contamination and exposure. In this study, two complementary passive sampling approaches were investigated for monitoring organic pollutants in the urban atmosphere of Skopje: personal samplers based on porous polydimethylsiloxane (PDMS) foam and plant leaves as natural biomonitors. After preparation, PDMS foam samplers were worn by seven participants for nine days. Following exposure, the samplers were extracted with ethyl acetate, and the extracts were analyzed by gas chromatography with electron-capture detection and mass spectrometry (GC-ECD and GC-MS). They captured a broad suite of organochlorines and polycyclic aromatic hydrocarbons (PAHs), reflecting compounds encountered during daily activities and personal exposure pathways. Dominant compounds were chlorobenzenes (e.g., 1,3,5-trichlorobenzene, tetrachlorobenzenes, pentachlorobenzene), PCB 180, and PAHs such as fluorene, phenanthrene, chrysene and benzo[k]fluoranthene. In parallel, pine needles were collected from several urban locations in Skopje, including sites near the former OHIS industrial facility. Additional plant species from the OHIS area were analyzed to investigate the influence of plant species on pollutant accumulation. The leaves were extracted with a dichloromethane–hexane mixture according to an optimized procedure, and the extracts were analyzed by GC-ECD and GC-MS. This survey revealed spatial patterns that overlapped with, but also extended, the information obtained from personal samplers. As in the personal samplers, chlorobenzenes and organochlorine pesticides (OCPs) were prominent, but their spatial distribution highlighted source-related hotspots: 1,2,4-trichlorobenzene and endrin showed consistently elevated concentrations at several sites, whereas HCH isomers and HCB exhibited greater spatial variability. Samples collected near the former OHIS industrial site showed higher concentrations of δ - and ϵ - HCH, tetrachlorobenzenes than samples from other locations, indicating a localized influence of historical industrial emissions. In contrast, the relatively low HCH concentrations observed in personal samplers suggest that OHIS-related pollutants contribute modestly to exposure during routine activities. PCBs were generally low or non-detectable in pine needles across the city, whereas PCB 180 was occasionally detected in the personal samplers suggesting that exposure to higher-chlorinated PCBs is not due to a widespread urban background. Among PAHs, phenanthrene was relatively uniform across locations, consistent with its frequent detection in personal samples, while fluorene, anthracene and pyrene were frequently detected in needles, mirroring the dominance of low–medium molecular weight PAHs in personal exposure profiles. Overall, pine needles accumulated OCPs efficiently, while PAH accumulation varied according to compound properties and plant species. The combined application of personal PDMS samplers and pine needles provides complementary information and together these low-cost passive approaches represent a practical strategy for screening and comparing organic air pollution in urban environments.

Key words: passive air sampling; personal exposure; organochlorines; polycyclic aromatic hydrocarbons; urban pollution

Screening of volatile and semi-volatile organic contaminants in Macedonian surface waters: Occurrence, spatial profiles, and ecological risk assessment

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Volatile and semi-volatile organic compounds (VOCs/SVOCs) represent a diverse group of organic contaminants in aquatic environments with inputs from wastewater, petroleum-related activities, industrial processes, and anthropogenic sources. Despite their potential ecological relevance, they are not routinely monitored in the existing water-quality monitoring framework in Macedonia, resulting in a substantial gap in information on their occurrence and distribution in Macedonian rivers. This study provides a first exploratory assessment of GC-amenable organic contaminants in selected surface waters of Macedonia and evaluates their potential ecological risk. Surface-water samples were collected from five locations: three sites along the Vardar River, one site on the Treska River, and one wastewater treatment plant (WWTP) effluent site discharging into the Treska River. A complementary passive-sampling experiment was conducted to compare the chemical profiles obtained by time-integrated passive accumulation and conventional grab sampling. After extraction with dichloromethane, samples were analyzed by gas chromatography–electron ionization mass spectrometry (GC-EI-MS). Both targeted and non-targeted screening approaches were applied. Ecological risk was assessed using toxic units (TUs) for algae, crustaceans, and fish, with individual and mixture toxicities compared against established acute and chronic thresholds. Across the five sampling locations, 32–84 chromatographic features were detected, with 8–23 unique compounds identified per site. The identified compounds represented diverse chemical groups, including aliphatic hydrocarbons, BTEX (benzene, toluene, ethylbenzene, and xylenes) and alkylbenzenes, cyclic siloxanes and oxygenated compounds, phthalates and related additives, and PAH-type (polycyclic aromatic hydrocarbons) compounds. Total identified concentrations ranged from 726 ng/L at Taor to 4300 ng/L at the WWTP site. Taor and Treska were characterized predominantly by aliphatic hydrocarbons, whereas Zhelino showed a pronounced BTEX contribution. Bashino Selo exhibited a strong contribution from musks, while the WWTP site displayed the highest chemical complexity and a mixed profile comprising hydrocarbons, siloxanes and phthalate-related compounds. At Zhelino, passive sampling detected 39 unique compounds compared with 15 identified in grab samples, demonstrating the greater chemical coverage achievable through time-integrated sampling. Risk assessment indicated potential ecological effects across all three trophic groups, with the chronic threshold exceeded for algae only at the WWTP, for crustaceans at all sites, and for fish at Zhelino, Bashino Selo, Treska, and WWTP. The acute threshold was exceeded only for crustaceans and fish at the WWTP. These findings provide baseline evidence of a diverse mixture of anthropogenic and wastewater-associated GC-amenable organic contaminants in Macedonian surface waters, addressing an important gap in current monitoring. The observed potential ecological risks, particularly for crustaceans and at the WWTP site, emphasize the need for systematic monitoring of these contaminants. Passive sampling further enables time-integrated screening and detection of a broader range of contaminants than conventional grab sampling.

Key words: VOCs/SVOCs; passive water sampling; surface water; Macedonia; ecological risk assessment

Menthol-based deep eutectic solvent as a greener extraction medium for organochlorine pesticides from human hair

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The replacement of volatile and hazardous organic solvents is an important aspect of greener analytical sample preparation. Deep eutectic solvents (DESs) offer an alternative through their low volatility, simple preparation, and adjustable physicochemical properties. Human hair is a promising non-invasive matrix for assessment of exposure to persistent contaminants, including organochlorine pesticides (OCPs). Due to the incorporation and retention of lipophilic compounds within the matrix, hair can provide information of long-term exposure. Still, the efficient extraction of hydrophobic contaminants from the complex hair matrix remains an important analytical challenge. Strong interactions between these compounds and the hair matrix, together with the dense, chemically heterogeneous structure of keratin, can hinder their release and limit extraction efficiency. In this study, a hydrophobic DES (HDES) composed of menthol and levulinic acid was studied as a greener extraction medium for organochlorine pesticides from human hair. The hydrophobic DES was prepared by mixing menthol and levulinic acid at a 1:1 molar ratio, followed by magnetic stirring until a stable homogeneous liquid was obtained. Formation of the solvent was confirmed using Fourier-transform infrared (FTIR) spectroscopy by comparing its spectrum with the spectra of the individual components. Changes in the FTIR spectra included broadening and shifts in the O-H stretching region (3200-3600 cm⁻¹), reflecting enhanced hydrogen-bonding interactions between the components. Changes in the C=O stretching region (1680-1750 cm⁻¹) are attributed to interactions involving the carbonyl group of levulinic acid. The HDES was further characterized by determining its density, refractive index and viscosity to assess its physicochemical properties and their relevance to extraction performance. The extraction efficiency of DESs depends on several experimental parameters. Extraction conditions were optimized using response surface methodology (RSM) based on a Box-Behnken design. HDES volume (3, 5 and 10 mL), incubation temperature (30, 40 and 50 °C) and incubation time (30, 60 and 90 min) were varied simultaneously to assess their influence on extraction efficiency. The experimental results showed that extraction depended on the combined effect of these parameters rather than on a single factor. Further refinement of the experimental conditions established 10 mL of DES, incubation at 40 °C for 45 min, followed by 45 min of mechanical agitation, as the optimized extraction conditions. The optimized menthol-based HDES provided efficient extraction of OCPs from human hair, demonstrating its potential as an alternative to conventional organic solvent-based extraction. The hydrophobic character of the synthesized HDES may promote partitioning of lipophilic OCPs from the keratin-rich hair matrix, while its hydrogen-bonding capacity and tunable physicochemical properties may facilitate analyte release and extraction. These findings support the application of hydrophobic DESs as promising extraction media within more sustainable analytical workflows for OCP exposure assessment.

Key words: green chemistry, biomonitoring, persistent contaminants, human hair

Heavy metals in the Vardar River: Influence of Skopje and its tributaries

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This study assesses the impact of the urban area of Skopje and its tributaries on the water quality of the Vardar River. The assessment is based on monitoring data collected in 2024 from four sites: Radusha and Taor, located directly on the Vardar River, the Treska River at Saraj on the right bank, and the Lepenec River upstream of its confluence with the Vardar River on the left bank, representing its tributaries. The results show clear differences in heavy metal concentrations among the four sites. Taor recorded the highest average concentrations of Fe, Mn, Zn and Cu, while Pb was highest at the Treska River. The highest average concentration of Cd was found in the Lepenec River, whereas the concentration of Cr was highest at Radusha. The higher levels of several metals at Taor, downstream of Skopje, indicate a possible influence of urban activities on the water quality of the Vardar River. The comparison of the four sites shows how water quality and metal concentrations change along the river system. These findings highlight the importance of regular monitoring of the Vardar River and its tributaries to better understand pollution sources and support effective water management and protection.

Key words: Vardar River, water quality, heavy metals, tributaries

HYDROBIOLOGY, WATER MANAGEMENT, AND MONITORING

Implications for monitoring design of high-mountain mires in North Macedonia based on ecohydrological differentiation

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High-mountain mires are sustained by complex interactions among water supply, hydrological dynamics, biogeochemical processes, peat properties and vegetation. However, their ecohydrological functioning remains very poorly documented in North Macedonia and the wider Balkan Peninsula. This study examined variability among selected mires and evaluated the suitability of different parameters and sampling strategies for condition assessment and long-term monitoring. Seven mires from three protected areas including Shar Planina National Park (Ceripashina, Studena Reka, Turchinski Livadi), Osogovski Planini Protected Landscape (Aramiska Cheshma, Babina Cheshma, Sasa) and Maleshevski Planini Protected Landscape (Elensko Blato) were studied. Piezometer transects were used to sample surface and groundwater across several depth categories, while automated loggers recorded water-level and water-temperature dynamics. Water samples were analysed for pH, electrical conductivity, inorganic nitrogen compounds, and total and dissolved major and trace elements with monthly dynamics during the vegetation season. Soil water retention capacity and bulk density were determined, alongside vegetation biomass, abundance and diversity. The resulting patterns were evaluated using mixed models, principal component analysis, PERMANOVA, hierarchical clustering and correlation analyses. Continuous records revealed substantial differences in hydrological behavior. Ceripashina and Studena Reka maintained comparatively stable water levels, whereas Turchinski Livadi and Aramiska Cheshma exhibited greater seasonal variability and more pronounced summer recession. Hydrochemical composition provided the clearest differentiation among mires. The first three principal components explained 75.6% of hydrochemical variability and mire identity accounted for 52% of the multivariate variation. Mn, Mg, Fe, K, electrical conductivity, inorganic nitrogen, pH and Ca contributed most strongly to the principal gradients. Ceripashina, Studena Reka and Turchinski Livadi formed a relatively coherent hydrochemical group, while Sasa, Aramiska Cheshma and particularly Elensko Blato displayed more distinct profiles. Depth-related sampling revealed significant differences in nitrate concentration and electrical conductivity primarily between surface and groundwater. In contrast, separation among individual subsurface depths was weak for most parameters. Plant biomass, but not the measured soil properties or other vegetation metrics, was significantly associated with the principal hydrochemical gradient. The limited number of sites constrains broader inference across the heterogeneous high-mountain mire systems of North Macedonia. Nevertheless, the results indicate that expanding spatial coverage may be more informative than intensive sampling at multiple subsurface depths. A feasible monitoring framework should combine continuous and long-term metrics of water-level variability with a reduced set of hydrochemical indicators, selected according to their discriminatory power. Interpretation should additionally incorporate water sources and flow pathways, surface-groundwater connectivity, peat depth and extent and geomorphological evidence of disturbance, particularly erosion channels, drainage features and redirected flow. Integrating these variables across a broader network of ecologically comparable mires would support the development of reference ranges and improve detection of directional ecological change.

Key words: groundwater connectivity; hydrochemistry; peatlands; water-level dynamics;

Impact of urban area and tributaries on the ecological status of the Vardar River in Skopje Region

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This study examines the impact of the urban area of Skopje and its tributaries on the ecological status of the Vardar River. The assessment was based on biological quality elements, including diatoms and macroinvertebrates, and supporting physico-chemical elements, using monitoring data from 2022. Four monitoring sites were considered: Radusha and Taor, located directly on the Vardar River, and the Treska River at Saraj on the right bank and the Lepenec River upstream of its confluence with the Vardar River on the left bank, representing its tributaries. The results showed clear differences in ecological status among the monitoring sites. Radusha was found to have poor ecological status, Taor to have bad ecological status, Treska to have moderate ecological status, and Lepenec to have poor ecological status. The results indicate that urban pressures and inputs from tributaries influence the ecological condition of the Vardar River in the Skopje Region. The study demonstrates the importance of combining biological and supporting physico-chemical elements to obtain a more comprehensive assessment of the ecological status of the river.

Key words: Vardar River, ecological status, biological quality elements, diatoms and macroinvertebrates, supporting physico-chemical elements, tributaries.

Assessment of leachate quality and treatment feasibility at Drisla landfill, Skopje: A Multi-parameter approach

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Sanitary landfills generate leachate with complex physicochemical and microbiological characteristics that pose significant risks to surface and groundwater quality if left untreated. The Drisla landfill, serving the Skopje region, requires comprehensive characterization of its leachate to inform appropriate treatment solutions aligned with EU environmental standards. This study aimed to evaluate the physicochemical, microbiological, and biotechnological properties of leachate from Drisla landfill and assess the applicability of various treatment technologies. Composite leachate samples were collected from the collection basin and subjected to comprehensive analyses including physicochemical parameters (COD, BOD₅, nitrogen species, heavy metals, conductivity, TDS), microbiological indicators (total heterotrophic bacteria, *E. coli*, coliforms, *Pseudomonas* spp., enterococci), and biotechnological testing of primary coagulation, biological treatment with nitrifying/denitrifying granules, and tertiary membrane filtration. Results revealed exceptionally high organic and inorganic loads: COD 3,960 mg/L, BOD₅ 1,372.6 mg/L, ammonium nitrogen 2,004.4 mg/L, sulfates 8,190 mg/L, chlorides 5,680 mg/L, and electrical conductivity 24,800 µS/cm. Microbiological analysis confirmed fecal contamination with *E. coli* (50 CFU/100 mL), coliforms (>300 CFU/100 mL), and total heterotrophs (>2,460 CFU/mL). Coagulation tests demonstrated optimal suspended solids removal (0.72 g/L sludge) at pH 5.5 using 3 mL/L BIO-TAL coagulant. Biological treatment achieved only 18% COD reduction during the initial adaptation phase, indicating limited biodegradability. Tertiary treatment combining microfiltration, activated carbon adsorption, and reverse osmosis reduced COD by 61% and achieved 74% decolorization. The findings demonstrate that Drisla landfill leachate represents a complex wastewater stream characterized by high mineralization, elevated ammonium concentrations, and significant microbiological contamination. Biological treatment alone proved insufficient for achieving discharge standards due to high salinity and recalcitrant organic compounds. An integrated modular treatment system combining mechanical pre-treatment with two-stage Disc Tube Reverse Osmosis (DTRO) membrane technology is proposed as the optimal conceptual solution. This approach offers operational flexibility, adaptability to seasonal variations, and staged implementation potential, ensuring compliance with environmental requirements while minimizing initial capital investment and enabling future capacity expansion.

Key words: landfill leachate, membrane filtration, wastewater treatment, DTRO technology, water quality monitoring

Dragonfly and damselfly assemblages along the river Bregalnica: responses to longitudinal habitat variation and hydromorphological disturbance

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Dragonflies and damselflies (Odonata) are closely associated with the structural and hydrological characteristics of freshwater habitats and can provide valuable information on ecological changes in river systems. However, ecological studies of Odonata assemblages in running waters remain scarce in North Macedonia. This study assessed spatial and seasonal changes in Odonata assemblages along the main course of the river Bregalnica, from its upper reaches to the confluence with the river Vardar. Surveys were conducted from April to October 2015 at 12 sites representing the longitudinal gradient of the river. Adult Odonata were surveyed qualitatively and quantitatively using standardized transects, while larval sampling provided complementary information on local development within riverine habitats. Species composition and community structure were assessed using indices of richness – d , diversity – H' and evenness – $J(e)$. A total of 26 species were recorded in the adult stage and nine species in the larval stage. Species richness and abundance showed seasonal variation and generally increased downstream, although this pattern was interrupted by pronounced local declines. Low richness and abundance were recorded in the upper river reaches, immediately downstream of the Kalimanci reservoir and at the heavily disturbed site near Kezovica. Downstream of the Kalimanci reservoir, five adult species were recorded, mean abundance decreased to 6.7 individuals/100 m/h, diversity and richness indices were low ($H' = 0.63$; $d = 0.61$), and no Odonata larvae were detected. At Kezovica, only three species were recorded and the lowest values of diversity and richness were observed ($H' = 0.23$; $d = 0.12$). In contrast, assemblage richness and abundance increased again further downstream, reaching 13 species and 71 individuals/100 m/h near the confluence with Vardar. High Odonata diversity in parts of the middle and lower river occurred despite anthropogenic pressures and was associated with a heterogeneous mosaic of riverine and riparian microhabitats, including slow-flowing sections, pools, shallow marginal waters, temporary wetlands, sedimentary banks and riparian vegetation. The results indicate a general downstream increase in dragonfly and damselfly richness and abundance along the river Bregalnica, with distinct local declines associated with hydromorphological alteration, habitat degradation and pollution. These spatial patterns suggest that habitat heterogeneity and hydromorphological conditions strongly influence community composition and demonstrate the sensitivity of Odonata to alterations in river hydrology and habitat structure. The pronounced declines in assemblage structure and larval occurrence at disturbed sites further highlight the value of combining adult and larval data as a useful approach for ecological assessment and future monitoring of rivers and associated riverine and riparian habitats in North Macedonia.

Key words: Odonata, river Bregalnica, longitudinal gradient, habitat heterogeneity, hydromorphological alteration, freshwater monitoring, ecological assessment

Remote sensing-based assessment of lake surface and phytoplankton dynamics in transboundary Lake Prespa

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Lake Prespa, one of Europe's oldest transboundary lakes shared by North Macedonia, Albania, and Greece, has undergone substantial hydrological changes over recent decades, including a persistent decline in water level and lake surface area. These changes may significantly influence water quality, aquatic ecosystems, and trophic conditions, highlighting the need for continuous monitoring using satellite-based observations. This study presents a cloud-based remote sensing approach for assessing the recent spatio-temporal dynamics of Lake Prespa using the Google Earth Engine (GEE) platform and Sentinel-2 multispectral imagery. Monthly cloud-free composites for the April–October growing season were generated for the 2021–2026 period using Sentinel-2 Surface Reflectance Harmonized imagery combined with cloud probability masking. A dynamic water mask was developed to delineate the actual lake extent for each monthly composite, enabling consistent estimation of lake surface area despite pronounced shoreline fluctuations. Chlorophyll-sensitive spectral indicators, including the Normalized Difference Chlorophyll Index (NDCI) and Floating Algae Index (FAI), were calculated to characterize the spatial and seasonal variability of optically active constituents in open-water areas while minimizing the influence of emergent vegetation and mixed shoreline pixels. The preliminary results reveal a gradual reduction in lake surface area during the analysed period, accompanied by pronounced seasonal variability in chlorophyll-sensitive spectral responses. Elevated NDCI values were primarily detected during early and late summer, whereas lower values generally occurred during spring and autumn. Spatial analysis indicates that enhanced spectral signals are concentrated mainly within shallow nearshore zones, suggesting localized environmental controls associated with reduced water depth, shoreline processes, and potential nutrient inputs. The generated monthly database provides a consistent framework for analysing temporal trends in lake extent and spectral water-quality indicators. The presented methodology demonstrates the capability of Google Earth Engine to efficiently process long-term Sentinel-2 archives and generate reproducible indicators for monitoring transboundary lakes. The developed workflow provides a robust basis for future integration with in situ limnological observations, climatic variables, hydrological data, and machine-learning approaches for quantitative estimation of chlorophyll concentration and trophic status. The proposed approach offers an effective tool for supporting long-term environmental monitoring and sustainable management of Lake Prespa and other vulnerable freshwater ecosystems.

Key words: Remote Sensing; Water Quality; NDCI; Floating Algae Index; environmental monitoring

Characterising inflows to Prespa Lake using hydrochemical and isotopic tracers

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Prespa Lake, a transboundary lake shared by North Macedonia, Albania and Greece, has experienced a substantial decline in water level, raising concerns about its water balance and the factors controlling its hydrological dynamics. While previous studies have focused largely on the hydrological connection between Prespa and Ohrid Lakes, significant knowledge gaps remain regarding the inflows contributing to the lake. This study aimed to provide a reconnaissance assessment of the main surface and subsurface inflows to Prespa Lake and to evaluate their contributions using hydrochemical and stable isotope analyses. A total of 72 samples were collected from rivers, springs, groundwater sources and lake water within the Prespa basin during two sampling campaigns conducted in April and May 2024. The samples were analysed for hydrochemical parameters and water stable isotopes of oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$). Hierarchical cluster analysis was used to group the sources with similar hydrochemical and isotopic characteristics. Based on the isotope data, the Local Meteoric Water Line and an evaporation line were derived. Both isotopic and hydrochemical data were then integrated into a mixing model to estimate the contribution of the examined water sources to the lake. Four main groups were identified, broadly corresponding to river water, springs, deep groundwater and lake water. River, spring and groundwater samples showed little evidence of evaporation, whereas lake water displayed clear evaporative enrichment in $\delta^{18}\text{O}$ and $\delta^2\text{H}$. The mixing model estimated rivers as the dominant contributor to the lake, accounting for 75.8% of the total inflow, followed by karstic springs with 17.5% and mineralised groundwater with 6.7%. The results indicate that river inflows may play a greater role in the water balance of Prespa Lake than the analysed groundwater sources, highlighting the potential importance of changes in precipitation, snowmelt and river runoff for water inputs to the lake. These findings highlight the importance of protecting surface water, efficient agricultural water use and transboundary monitoring of river flows and abstractions. The study provides an integrated baseline characterisation of the main water sources contributing to Prespa Lake and establishes a baseline for long-term transboundary hydrological, hydrochemical and isotope monitoring to track changes in the lake's water balance.

Key words: Prespa Lake, stable isotopes, hydrochemistry, water balance, mixing model

Integrated monitoring of the ecological condition of the Belica River

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Integrated ecological monitoring supports evidence-based river conservation by providing information on ecosystem conditions, environmental pressures, and management needs. The Belica River is a right-bank tributary of the Treska River in western North Macedonia. It originates from the Belica Springs on the eastern slopes of Ilinska Mountain (1,580 m a.s.l.), flows through the proposed Protected Landscape Ilinska–Plakenska Mountain, and joins the Treska River near Dolno Popolžani. The river corridor is an important biodiversity hotspot and ecological corridor, supporting priority Habitat 91E0* (Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*) and species of European conservation importance. However, inadequate municipal waste management and illegal dumping threaten its ecological integrity. The objective of this study was to characterize the ecological condition of the Belica River through an integrated monitoring approach informed by the principles and quality elements of the EU Water Framework Directive (WFD; 2000/60/EC) and to identify principal anthropogenic pressures affecting the river ecosystem. The assessment combined physico-chemical analyses with biological monitoring. Monitoring was conducted at five representative sites along the river continuum, including upstream reaches and downstream sections exposed to increasing human pressures. Physicochemical analyses included pH, dissolved oxygen, water temperature, nutrient concentrations, and other relevant water-quality parameters. Biological assessment focused on two species of European conservation importance protected under the EU Habitats Directive: the dragonfly *Cordulegaster heros* and the Eurasian otter (*Lutra lutra*). *Cordulegaster heros* is associated with well-preserved running-water habitats, with larval development dependent on suitable aquatic and microhabitat conditions, while *L. lutra*, as a top freshwater predator, provides complementary information on broader river ecosystem integrity and connectivity. Preliminary monitoring indicated generally low nutrient concentrations in the Belica River, while nutrient concentrations showed a slow increasing trend towards the downstream sections, suggesting higher anthropogenic influence or erosion. Biological monitoring confirmed the presence of *Cordulegaster heros* along the river, with both larvae and adults recorded. The occurrence of larvae confirms local development and indicates suitable habitat conditions in particular river sections. *Lutra lutra* was recorded at all five monitoring sites along the Belica River through camera-trap footage and field evidence, including spraints and tracks. Its presence indicates that the river provides suitable habitat and sufficient food resources. The integration of physico-chemical measurements and selected biological indicators provides a valuable baseline framework for evaluating river ecosystem condition. The study provides a basis for developing a more comprehensive long-term monitoring system incorporating additional biological quality elements, hydromorphological characteristics, supporting physico-chemical parameters, and relevant pollutants. Such an integrated approach can strengthen evidence-based conservation, restoration, pollution prevention and adaptive management of the Belica River.

Key words: Integrated ecological monitoring; water quality; *Cordulegaster heros*; *Lutra lutra*; freshwater ecosystems; EU Water Framework Directive.

Anthropogenic influence on the water quality of Lake Ohrid

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The quality of surface waters is a matter of extremely great importance. Bacterial indicators may be considered as highly sensitive indicators for the status of anthropogenic influence in aquatic systems. The sensitivity of rotifers to a range of physicochemical parameters allows their use as biological indicators of the saprobity of aquatic ecosystems. Aim of this work is to determine status of the water quality and assessment of the anthropogenic impact in the littoral zone of the Lake Ohrid (at Evrohotel, Sateska, Grasnica, Mazija, Konsko and Pestani), as well as from the pelagial in a vertical profile from the surface to the depth of 150 m, during the period summer 2025- summer 2026. Biological and chemical investigations was conducted seasonally. For all analyses were used standard limnological methods. Received results support the well-known phenomena that cooperation of the biotic and abiotic factors of in the environment that impact the life, dynamics and distribution of the microorganisms in the water. According to the microbiological parameters, the littoral water of the Lake Ohrid in the investigated localities belongs from I to IV class. The poorest water quality was found at Grasnica and Mazija, followed by Eurohotel and Sateska. At these locations, the presence of the indicator of recent fecal contamination - *Escherichia coli*, was recorded across all seasons, with relatively high values even in winter. The number of heterotrophic bacteria (indicators for organic pollution) increases with the trophic state and contamination of water with organic matter. The results indicate organic and fecal pollution of the lake water near the settlements, river mouths and tourist localities. The littoral is still under the impact from the sewerage, industrial and drain waters reach in pollutants and nutrients that lead to increased eutrophication as it is in the Grasnica area. Furthermore, numerous touristic objects located near the shore are potential polluters. Unfortunately, anthropogenic impact has also been recorded in the water of the lake pelagic (in the vertical profile at 150 m depth) where indicators of fecal pollution have been registered, and according to organic pollution the water is predominantly class II, even III in the summer period. The highest number of bacteria were found in the region of thermocline and near the bottom. The registered rotifera species belong to waters of I, II and II categories. The numerical values of the saprobity index (1.61 to 1.97 at Grasnica) indicates waters with oligo-β-mesosaprobic to β-mesosaprobic character at Grasnica. The results obtained from these studies indicate negative anthropogenic impact and accelerated eutrophication in some littoral localities. A negative impact is also evident on the water in the pelagic zone, specifically the area of the lake in the immediate vicinity of settlements (Ohrid Bay), which is also influenced by tributaries (Velgoska and Koselska Rivers). Therefore, continuous research on the water and sediment of the Lake is necessary, which will contribute to the adoption of measures for constant quality control and protection of Lake Ohrid.

Key words: water quality, bacteria, rotifers, pollutants

Assessment of anthropogenic impacts on water quality in the littoral zones of Lakes Ohrid and Prespa

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Pollution from river catchments represents a major threat to aquatic ecosystems, biodiversity, and water quality. Industrial discharges, agricultural runoff, and urban wastewater contribute to organic and nutrient loading, eutrophication and ecological degradation. Lakes Ohrid and Prespa are among the most important natural freshwater ecosystems in North Macedonia and are globally recognized as ancient lakes supporting exceptionally diverse and endemic biota. Despite their high ecological value, both lakes are increasingly exposed to anthropogenic pressures, with Lake Prespa being particularly vulnerable to environmental degradation. Localized impacts along the shoreline may create pollution hotspots and contribute to deterioration of littoral water quality. The study aimed to assess water quality and trophic status in the littoral zones of Lakes Ohrid and Prespa, with particular emphasis on the influence of anthropogenic activities. Seasonal sampling was conducted during 2025 and 2026 at six littoral sites in Lake Ohrid and five selected sites in Lake Prespa. Physico-chemical parameters, including dissolved oxygen, biodegradable organic matter, total phosphorus (TP), and total nitrogen (TN), were analysed together with biological indicators, including heterotrophic bacteria, total coliforms, chlorophyll a and rotifers. Trophic status was evaluated using Carlson's Trophic State Index (TSI), the OECD classification, and the national Directive for Classification of Surface Waters. Rotifer-based saprobic conditions were assessed using the Pantle–Buck saprobity index. Results The results revealed pronounced spatial differences in the degree of anthropogenic influence among the investigated littoral sites. In Lake Ohrid, the highest TP concentrations were recorded in the Grashnica littoral ($18.29 \mu\text{g L}^{-1}$) and Sateska River littoral ($20.15 \mu\text{g L}^{-1}$), indicating a strong influence of inflowing rivers. In Lake Prespa, elevated TP concentrations were particularly pronounced at the Ezerani north-eastern ($44.95 \mu\text{g L}^{-1}$) and Ezerani central ($62.5 \mu\text{g L}^{-1}$) sites, while the influence of the Golema River was also evident. Microbiological parameters were consistent with the physicochemical results and indicated localized organic and fecal pollution. Rotifer-based saprobiological assessment revealed saprobity index values of 1.61–1.74 in the Lake Ohrid littoral and 1.62–1.82 in the Lake Prespa littoral, indicating predominantly oligo- β -mesosaprobic to β -mesosaprobic conditions. Based on Carlson's TSI calculated from total phosphorus and chlorophyll a, the littoral zone of Lake Prespa was characterized predominantly by mesotrophic to mesotrophic–eutrophic conditions, whereas Lake Ohrid remained within the oligotrophic to oligo-mesotrophic range. Microbiological indicators further supported the presence of localized anthropogenic pressures, with the Lake Ohrid littoral generally corresponding to water-quality classes II–III and the Lake Prespa littoral to classes III–IV. The rotifer-based assessment was consistent with these findings, indicating oligo- β -mesosaprobic to β -mesosaprobic conditions in both lakes, with higher saprobity values occurring in parts of the Lake Prespa littoral. Overall, the findings demonstrate that river mouths, agricultural areas, and intensively used tourist zones represent important local sources of anthropogenic pressure. The results emphasize the need for continued integrated physicochemical, microbiological and biological monitoring, together with targeted management measures, to prevent further deterioration and safeguard the ecological integrity of these unique lake ecosystems.

Key words: anthropogenic pressure; water quality; eutrophication; trophic status; littoral zone; saprobity; rotifers

Hepatic lesions in Black barbel (*Barbus balcanicus*) as indicators of anthropogenic pollution in the Lepenec River

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The fish liver, as the primary site for the accumulation and metabolism of xenobiotics, represents a sensitive bioindicator for assessing the status of river ecosystems. Histopathological analysis enables a detailed evaluation of structural and cellular damage, providing insight into the acute and chronic effects of pollution in the aquatic ecosystem. These alterations often occur as early responses of the organism before the appearance of visible morphological or population-level disruptions, which increases their diagnostic value. The focus of this research is the histopathological evaluation of cellular changes in the liver of the Black barbel (*Barbus balcanicus*) from the River Lepenec. This is the first study to assess the health status of fish from this river, examining a total of 176 fish from three localities along the course of the River Lepenec, starting from its headwaters in the Republic of Kosovo down to its confluence into the Vardar River in the Republic of North Macedonia, during two seasons (spring-summer and autumn-winter). The analysis of hepatic tissue showed the presence of multiple lesions: leukocyte infiltration, hepatocyte dissociation, hemorrhage, parasitic granuloma, necrosis, bile duct proliferation, and regressive changes of the biliary epithelium, as well as isolated cases of vacuolated hepatocytes, fibrosis, a clear focus of histiocytes, and the presence of clusters of macrophage aggregates. These lesions belong to four categories: circulatory, inflammatory, regressive, and progressive lesions, with the highest prevalence found in the latter two categories. Furthermore, based on their relevance, these lesions are classified as pathological changes with an importance factor of 1 or 2. Lesions were also observed at the reference site, indicating that this locality is not entirely exempt from anthropogenic pressure. However, the prevalence of lesions in individuals from the middle and lower reaches of the river, which enters our country, is significantly higher. The results of this study underscore the need to introduce integrated monitoring in our country, where histopathological biomarkers would serve as a key biological component for tracking the health status of fish in aquatic ecosystems.

Key words: liver, histopathology, biomarkers, pollution, River Lepenec

Nuclear abnormalities as bioindicators to assess the effects of pollution in *Barbus balcanicus* from Lepenec River

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The exposure of the Lepenec River to industrial and urban wastewater in the transboundary region between the R. Kosovo and the R. North Macedonia necessitates an urgent assessment of the ecogenotoxic status of this ecosystem. This study focuses on the registration of micronuclei and specific nuclear abnormalities in the mature erythrocytes of the Black barbel (*Barbus balcanicus*), serving as highly sensitive biomarkers for the detection of DNA damage. Through seasonal (summer and autumn) and spatial analysis, the research identifies ecological risks and the impact of both natural and anthropogenic factors on the ichthyofauna within the river basin. Sampling localities were selected based on the gradient of anthropogenic pressure and transboundary impact, encompassing three key points: a reference site (the headwaters of the river in the Republic of Kosovo), a border site (direct impact from the upper reaches), and Novo Selo (before the confluence into the Vardar River, acting as a zone of cumulative pollution). By monitoring the frequency of micronuclei (MN), binucleated cells (BN), vacuolated nuclei (VN), and irregularly shaped nuclei (ISN), the mutagenic and cytotoxic status of the aquatic ecosystem was evaluated. The obtained results reveal a pronounced seasonal trend, with a significant increase in nuclear aberration values during autumn, particularly at localities exposed to intense anthropogenic pressure. Namely, the frequencies of MN and BN exhibited a similar variation trend, showing a statistically significant increase from summer to autumn at the Border and Novo Selo localities. During autumn, the values at Novo Selo were statistically significantly higher (MN=0.21 ‰, BN=0.29 ‰) compared to the Reference site (MN=0.10 ‰, BN=0.09 ‰), and for BN, this significant elevation was also observed in summer. Regarding ISN and VN, the only statistical difference was noted in relation to the season, with significantly higher values recorded in autumn for ISN at the Border and Novo Selo sites, and for VN at the Reference site. Although increased values were calculated for both abnormalities at the Border locality, no statistical differences were evident. This profile of nuclear and cytoplasmic alterations (ISN and VN), correlated with the previously established high frequencies of MN and BN, definitively confirms the pronounced genotoxic and cytotoxic potential of pollution in the Lepenec River, which severely compromises the structural and functional integrity of the hematopoietic system in the studied fish species.

Key words: genotoxicity, micronuclei, nuclear abnormalities, pollution, Lepenec River

Distribution and current status of the freshwater shrimp *Atyaephyra thymisensis* (Decapoda: Atyidae) in Lake Prespa: New findings

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The freshwater decapod shrimp *Atyaephyra thymisensis* was documented on the Macedonian side of Lake Prespa for the first time in early 2018. However, drastic fluctuations in the lake level during the recent period and intense anthropogenic pressures imposed a serious need to explore the current status of this species population. The aim of this study is to confirm the presence of the species through field investigations conducted during the intensive hydrobiological investigations in the period October 2025 - August 2026. Samples were collected using a standard Kick-net method in the littoral zone of the coastline near the village of Stenje (at the locality below the "Saint Ilija" church and below the monitoring station), as well as at two new locations in Pretor and Oteshevo. The results of the study definitively confirm the continuous presence of *A. thymisensis* in Lake Prespa. Specimens were isolated in shallow areas with a rocky-sandy substrate characterized by well-developed submerged macrophyte vegetation. Finding these populations along these specific coastlines indicates that the terrain configuration at these localities offers more stable ecological niches and refugia for this species. This zone protects the shrimp compared to the shallower and open parts of the lake where water recession is more drastic. This rediscovery highlights the role of *A. thymisensis* as an exceptionally important, but ecologically vulnerable bioindicator. The data from this study point to an urgent need for targeted monitoring and protection of littoral habitats to prevent further degradation of the relict biodiversity in Lake Prespa.

Key words: Lake Prespa, R. North Macedonia, decapods, conservation

Ecological and reproductive characteristics of *Cobitis vardarensis* Karaman, 1928 from Lake Dojran

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Although the ichthyocenosis of Lake Dojran has been the subject of numerous ichthyological studies, the biological and reproductive traits of the Vardar spined loach (*Cobitis vardarensis*) within this ecosystem remain critically understudied, particularly regarding its vulnerable littoral habitats. To address these critical knowledge gaps, the present study aimed to determine the species' spatial distribution and provide baseline data on its reproductive biology. Understanding the ecology of this benthic species is paramount for developing guidelines for habitat management and protection, especially considering its status as a target Natura 2000 species within Lake Dojran as a proposed Natura 2000 site. Seasonal ichthyological surveys, conducted via electrofishing supplemented by hand-netting, across nine coastal locations along the Macedonian side of the lake, revealed a restricted spatial distribution. The species was confirmed at only four sites, yielding a cumulative catch of 108 individuals (comprising both sexually mature and juvenile specimens). While the 'Nikolich' and 'St. Nicholas' sites exhibited a good conservation status, characterized by intact natural shorelines, low tourist pressure, and dense belts of native macrophyte vegetation that provide stable spawning grounds, conversely the 'Mrdaja' and 'Toplec' habitats displayed severe anthropogenic degradation. This degradation was evident through intense shoreline modification and artificial beach development at Mrdaja, alongside organic nutrient enrichment and wastewater pressures at the Toplec stream catchment, rendering both habitats unfavorable for population maintenance in the lake. Histological examination of the gonads allowed for the determination of reproductive status and specific stages of gonadal development. The analysis of ovaries from females sampled in June revealed the presence of multiple developmental stages of oocytes, indicating asynchronous gonadal development, which is characteristic of cyprinid fish representatives. Specifically, previtellogenic oocytes, cortical alveoli oocytes, early vitellogenic oocytes, and late vitellogenic oocytes were observed within the ovaries, while postovulatory follicles were absent, indicating that the first batch of oocytes had not yet been spawned in these individuals. The histological structure of the testes in male individuals demonstrated a cystic type of spermatogenesis, with the presence of spermatogonia, spermatocytes, and spermatids within cystic formations, as well as mature spermatozoa, particularly within the efferent ductules. This histological profile of both ovaries and testes indicates that the individuals during the June season were in the spawning phase of their reproductive cycle. Additionally, quantification of fecundity parameters was performed for females during the spawning season, revealing an absolute fecundity (mean value of 4,376 eggs), batch fecundity (mean value of 2,070 eggs), and relative fecundity (mean value of 511 eggs per gram of body mass), which indicates an optimal reproductive potential. The findings of this study provide valuable insights into the distribution of *C. vardarensis* in Lake Dojran and offer baseline data on its reproductive potential, thereby establishing a useful foundation for future monitoring of the conservation status of this target Natura 2000 species.

Key words: *Cobitis vardarensis*, Lake Dojran, ecology, reproduction, fecundity, Natura 2000

***Melanoides tuberculata* (O. F. Müller, 1774) in Serbia and the Balkans: Distribution, ecological preferences and potential risk for spring ecosystems**

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Springs represent unique and fragile freshwater ecosystems, often characterized by high biodiversity and the presence of ecologically specialized, relict, and endemic species. Due to their spatial isolation, they are particularly vulnerable to anthropogenic pressures, including habitat degradation, pollution, and the spread of invasive non-native species. The introduction of invasive species may significantly affect the structure of native communities and lead to the displacement of indigenous species into suboptimal ecological niches. Thermal springs may be particularly susceptible to colonization by thermophilic invasive species, as their elevated water temperatures may facilitate their establishment. *Melanoides tuberculata* (O. F. Müller, 1774) (Gastropoda: Thiaridae) is a thermophilic invasive species native to Africa and South Asia, which has been successfully introduced into numerous regions outside its native range due to its broad ecological tolerance and high reproductive potential. Following the first published record of the species in Serbia in 2012, *M. tuberculata* had been recorded at three localities in the country. Our recent field surveys revealed two additional localities, both representing new records from spring ecosystems: one at a thermal spring in the Niška Banja area and another at the Vidrište spring. These findings prompted us to systematize the available knowledge on the species' distribution and habitat characteristics. The aim of this study was to review and systematize published data on *M. tuberculata* across the Balkan Peninsula and to complement current distributional knowledge with habitat characteristics of the newly recorded populations. At Niška Banja, water temperature was 35.2 °C and conductivity was 471 $\mu\text{S cm}^{-1}$. The substrate was heterogeneous, with coarse mineral fractions (cobbles, stones and bedrock) accounting for 60%, while mud, detritus, sand and gravel constituted the remainder. *M. tuberculata* reached a density of 43 ind. m^{-2} , and no other gastropod species were recorded. At Vidrište, water temperature was 32.4 °C and conductivity was 517 $\mu\text{S cm}^{-1}$. In contrast, the substrate was predominantly muddy (95%), with 5% detritus. *M. tuberculata* reached a density of 34 ind. m^{-2} and co-occurred with *Gyraulus albus* (2 ind. m^{-2}) and *Peregriana peregra* (1 ind. m^{-2}). Available data indicate that knowledge of the species' distribution in the Balkans remains fragmented. The species has also been recorded in Romania, Bulgaria and Greece, whereas data on its occurrence in other countries of the Balkan Peninsula are lacking. However, the apparent distribution pattern may partly reflect uneven sampling effort, particularly in spring and thermal habitats. The two new records from the Niška Banja and Vidrište springs highlight the importance of systematic surveys of spring ecosystems, which may represent previously overlooked habitats for this thermophilic invasive species. The relatively high densities of *M. tuberculata*, accompanied by the absence or low abundance of other gastropods at the investigated sites, warrant further investigation of its potential interactions with native mollusc communities. Future research should focus on systematic surveys of thermal springs, potential introduction and dispersal pathways, and assessment of the ecological consequences of the species' further spread.

Key words: biological invasions; thermophilic species; range expansion; freshwater gastropods; biodiversity conservation

Reproductive health and intersex occurrence in Black barbell population of the River Lepenec

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The Lepenec River, as a significant transboundary water resource with a prominent role in industrial and agricultural activities, is exposed to continuous anthropogenic pressure, while the effects on its biological components remain insufficiently explored. Due to their ecological role and ability to bioaccumulate pollutants, fish represent important bioindicator for assessing the state of river ecosystems. The primary objective of this study is to evaluate the effects of anthropogenic pressure in the River Lepenec on potential reproductive health disruptions of the ichthyofauna. *Barbus balcanicus*, as a benthophagous species, is particularly suitable for such research due to its exposure to contaminants from both the water and the sediment. In the study, which took place over two seasons (spring-summer and autumn-winter), a total of 177 fish were caught from three localities: a reference site located upstream of all major sources of pollution in the Republic of Kosovo, a second site immediately after the border with the Republic of North Macedonia and a third locality before the confluence of the Lepenec into the River Vardar. The gonadosomatic index and histopathological analysis of the gonads were analyzed as biomarkers of reproductive health. Histopathological analysis showed the presence of inflammatory lesions: hemorrhage; circulatory lesions: macrophage accumulation, leukocyte infiltration, parasitic granuloma; regressive lesions: necrosis, disorganization of the seminiferous tubules in males, atretic follicles in females; and progressive lesions-fibrosis. Additionally, the occurrence of intersex was recorded in six individuals, namely ovotestis in five of them where oocytes from the previtellogenic as well as the more advanced early vitellogenic stage were observed within the testicular tissue and a less common occurrence of testis-ova registered in one individual, i.e., the presence of testicular tissue within an ovary, which represents the first case ever recorded in our country. Statistical analysis showed a more severe clinical picture in the localities under anthropogenic pressure, which increases ecological concern as it indicates pollution with the probable presence of xenoestrogenic substances in the river, which could, among other things, cause long-term negative impacts on the reproductive success of the fish.

Key words: gonads, histopathology, intersex, pollution, River Lepenec

Cyanobacterial community of Sava Lake during a two-year period

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Sava Lake is an artificial reservoir located in the urban area of Belgrade, representing an important resource for recreation, tourism and various human activities. Considering its ecological importance, intensive recreational use and exposure to anthropogenic pressures, continuous assessment of its condition is essential for maintaining water quality and ensuring its safe use. Cyanobacteria are an important component of freshwater phytoplankton communities, contributing to primary production and nutrient cycling. However, under favorable environmental conditions, they may proliferate rapidly and form blooms that can adversely affect aquatic ecosystems. Additionally, some bloom-forming cyanobacteria are capable of producing cyanotoxins, posing a potential risk to aquatic organisms and human health, particularly in ecosystems intensively used for recreation. Therefore, regular monitoring of cyanobacterial diversity and seasonal dynamics is essential for the detection of bloom-forming and potentially toxic taxa and for assessing potential ecological and health risks. Accordingly, the aim of this study was to characterize the cyanobacterial community of Sava Lake, with emphasis on its diversity, seasonal dynamics, contribution to phytoplankton biomass and the occurrence of potentially toxic taxa. Phytoplankton samples were collected monthly from April to December 2023 and March to December 2024 at two sampling sites. Qualitative analysis was performed using a Zeiss Axiolmager M1 light microscope, followed by the determination of phytoplankton abundance using the Utermöhl method. Cyanobacterial contribution to phytoplankton biomass was estimated based on cell biovolume obtained through geometric approximations of individual taxa, integrating information on cell abundance and size. During the two-year investigation, a total of 55 cyanobacterial taxa were identified, of which 21 were classified as potentially toxic according to relevant literature. Cyanobacteria were present throughout the whole investigated period, but their contribution to total phytoplankton biomass was substantial mainly during the summer months (June–August). Their biomass was generally higher in June and July 2024 compared with the same months in 2023, whereas the opposite pattern was observed in August. During the summer of 2023, the potentially toxic species *Microcystis aeruginosa* represented the dominant cyanobacterial taxon in all samples. In contrast, during 2024, non-toxic taxa were more frequently dominant, while *M. aeruginosa* and *Snowella lacustris* occurred mainly as subdominant taxa. These differences indicate that, although seasonal patterns of cyanobacterial development were similar between the two years, variations in environmental conditions influenced species composition and dominance relationships within the community. The results obtained at the investigated sites highlight the value of continuous cyanobacterial monitoring for the early detection of bloom-forming and potentially toxic taxa as well as for assessing potential risks associated with their occurrence. Such monitoring provides valuable information for detecting changes in cyanobacterial community structure, identifying periods of increased potential risk and supporting further assessment and management of Sava Lake.

Key words: biomonitoring; cyanotoxins; cyanobacterial bloom; ecosystem health; freshwater ecosystem

Diatom community structure and increasing trophic values in the limnocrene spring Zelenci

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Zelenci is a limnocrene spring located at the foothills of the Julian Alps in Slovenia, known for its unique structure and high diatom diversity. This study aimed to assess the seasonal dynamics of diatom communities, determine how the underwater springs and abiotic factors influence their structure in different habitats and compare changes in the trophic status in over 10 years of investigation. The research was conducted between November 2023 and October 2024 at four sampling sites, two representing tychoplankton and two epipelon. Tychoplankton was collected from the open-water part of the spring using a plankton net, while epipelon samples were obtained from the soft, silty substrate in shallow littoral areas. Selected abiotic parameters were measured at all sampling sites and water samples were collected for chemical analysis. A total of 167 diatom species were identified, with species richness peaking in June 2024. *Achnantheidium minutissimum* was the most abundant species in both mesohabitats, while *Navicula* was the most species-rich genus, followed by *Gomphonema* and *Nitzschia*. Planktonic guild species dominated the tychoplanktonic samples, while only occasional taxa occurred in the epipelon, possibly due to resuspension or transport. Motile and low-profile ecological types dominated the epipelon, whereas high-profile species were mostly recorded in epipellic samples, likely due to their size and fragility. Tychoplanktonic communities showed greater internal similarity among samples, while epipellic communities were characterized by high and relatively consistent Shannon–Wiener diversity. Water level and temperature reflected seasonal patterns, being higher and colder in winter and lower and warmer in summer, while conductivity was relatively high due to the limestone substrate. The Shannon–Wiener diversity index was strongly influenced by water level, temperature, and NH_4^+ concentration. Increasing water levels were associated with higher tychoplankton diversity, likely reflecting increased activity of underwater springs that transport diatoms into the water column. The most important factors influencing the tychoplanktonic community were temperature and NH_4^+ , whereas water level, NH_4^+ , pH and dissolved oxygen concentration were the most important factors affecting epipellic community structure. Occasional increases in NH_4^+ , NO_3^- and PO_4^{3-} concentrations were possibly related to anthropogenic activity in the catchment area, although PO_4^{3-} concentrations were mostly below the detection limit. The trophic index indicated eutrophic conditions and revealed an increase in nutrient inflow compared with previous research conducted 10 years earlier, when the spring was oligotrophic to oligo-mesotrophic. Compared with 2014 and 2015, the trophic index was considerably higher in certain months, suggesting increased nutrient enrichment from the catchment area and growing human pressure. The high diversity and structural complexity of the diatom communities, particularly in the epipelon, emphasize the ecological value of Zelenci and the importance of preserving this valuable ecosystem and preventing further nutrient enrichment associated with mass tourism and agriculture.

Key words: limnocrene spring; diatoms; epipelon; tychoplankton; underwater spring

From diatom data in the Sava River Basin of Bosnia and Herzegovina to biological metrics: The Oskova River as a case study of municipal and mining-related pressures

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Diatoms are widely used components of phytobenthos in ecological status assessment due to their sensitivity to changes in water quality. Although phytobenthos is recognized within the regulatory framework of Bosnia and Herzegovina as a biological quality element for ecological status assessment in accordance with the EU Water Framework Directive, phytobenthos based metrics have not yet been fully established for routine monitoring. This highlights the importance of integrating existing data on diatom taxa across the Sava River Basin in Bosnia and Herzegovina, both to elucidate the ecological characteristics of diatom taxa and to evaluate how specific anthropogenic pressures influence community composition and structure. The systematization of available data from the Sava River Basin resulted in the identification of 468 diatom taxa and highlighted the fragmented and insufficiently integrated nature of previous research. Despite this limited level of synthesis, the available studies consistently indicate a predominance of alkaliphilous, oligohalobous, N-autotrophic tolerant, β -mesosaprobic and eutrophic taxa. To assess the combined effects of municipal and mining-related industrial wastewater, the Oskova River was investigated as a case study. Sampling at 16 sites along the Oskova River and its tributaries was conducted twice in 2018. Water chemistry, diatom composition, diversity and several diatom-based metrics listed in Omnidia software were analysed (Specific Pollution Sensitivity Index, IPS; TDI, Trophic Diatom Index; IBD, Biological Diatom Index; EPI – D, Diatom eutrophication/pollution index; TI, Trophic index, and SI, Saprobic index. A total of 106 diatom taxa were identified. The upper reaches supported communities with higher taxon richness and diversity, mainly characterized by representatives of the genera *Achnanthes* and *Gomphonema*. In the lower reaches, increased municipal pressure and high (occasionally exceeding 1000 mg L^{-1}), and strongly variable concentrations of suspended solids, associated with mining-related wastewater coincided with a marked shift in diatom composition, with *Amphora pediculus*, *Nitzschia amphibia*, *N. inconspicua* and *N. dissipata* becoming more prominent. The biological metrics applied in this study did not respond uniformly to the combined effects of municipal and mining-related pollution. This variability highlights the importance of considering multiple biological metrics rather than relying on a single index when assessing rivers exposed to multiple pressures. The results also support the need for further testing and regional calibration of diatom-based metrics under the specific environmental conditions of the Sava River Basin. Funding: The conference presentation was supported through the project Analysis of Selected Biological Methods for Assessing the Ecological Status of Waters in the Sava River Basin, funded by the Ministry of Education and Science of Tuzla Canton (Bosnia and Herzegovina).

Key words: diatoms, metrics, mining impact, suspended solids, nutrient enrichment, ecological assessment

Functional organization of phytoplankton communities in three Serbian lowland rivers: insights from long-term monitoring

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Phytoplankton represents a key component of river ecosystems, playing a crucial role in ecosystem functioning and serving as a sensitive indicator of environmental change due to its rapid response to variations in environmental conditions. Although taxonomic approaches provide essential information on biodiversity patterns, functional group classification provides additional insight into the ecological strategies of phytoplankton. Long-term assessments of functional composition are particularly valuable for understanding spatio-temporal patterns in community organization and ecological processes in large rivers exposed to multiple environmental pressures. Phytoplankton monitoring was conducted in three major Serbian lowland rivers from 2016 to 2025. The Danube was surveyed at 12 sites along its Serbian course between 2018 and 2020, whereas the Sava and the Tisza were monitored over longer periods (2017–2025 and 2016–2025, respectively) at two sampling sites each. Phytoplankton sampling was performed in accordance with the standard method EN 16698:2016. Species contributing $\geq 5\%$ of the total phytoplankton biomass were considered dominant and assigned to functional groups (FGs, codons) according to their shared morphological, physiological and ecological characteristics. The biomass contribution of species assigned to the same codon was summed and used for comparative analysis. The dominance patterns of functional groups differed among the investigated rivers, indicating distinct spatial and temporal dynamics of phytoplankton communities. In the Tisza River, codon C was consistently dominant throughout the study period, with an increasing contribution of codon Y in recent years, while codons P and D occurred occasionally and MP was regularly present but rarely dominant. The Sava River showed a more variable functional structure, characterized by frequent shifts among codons Y, D and C, whereas other groups appeared only sporadically. Along the Danube, functional composition varied considerably among sampling sites, reflecting longitudinal heterogeneity and the occurrence of codons C, MP and TB as dominant components at different river sections. The observed differences in functional composition reflect contrasting ecological strategies of phytoplankton among the investigated rivers. The prevalence of codon C in the Tisza suggests a recurrent functional pattern associated with productive conditions, while the increased contribution of codon Y may indicate the ability of the community to persist under changing environmental conditions, including variations in thermal and hydrological regimes. In contrast, the pronounced shifts among codons Y, D and C in the Sava highlight a more dynamic functional organization likely driven by temporal variability and changing hydrological conditions. The spatial variability observed along the Danube emphasizes the importance of longitudinal gradients and hydromorphological characteristics in shaping phytoplankton functional structure. Overall, the observed functional shifts demonstrate that river-specific hydromorphological characteristics and temporal variability are important drivers of phytoplankton community organization. The functional group approach enhances our understanding of long-term changes in phytoplankton community structure, providing a more integrative interpretation of community responses to environmental variability in large lowland river ecosystems.

Key words: biomonitoring; codons; ecological strategies; hydromorphological variability; community organization

Pelagic Rotifera in Lakes Ohrid and Prespa: Seasonal shifts and vertical patterns during 2025–2026

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Rotifera are an important component of freshwater zooplankton communities and are characterized by rapid population responses to environmental changes. Their taxonomic composition, abundance and vertical distribution can therefore provide valuable information on seasonal processes and environmental changes in pelagic ecosystems. Lakes Ohrid and Prespa are geographically close, but differ substantially in depth, morphology and pelagic habitat structure. The aim of this study was to comparatively assess the seasonal composition, observed abundance and vertical distribution of pelagic Rotifera in the two lakes during 2025–2026, using data obtained through seasonal monitoring programmes of the PSI Hydrobiological Institute – Ohrid. The analysis was based on seasonal zooplankton monitoring data collected from pelagic stations in Lakes Ohrid and Prespa. For the purposes of this study, the same seasonal periods were analyzed in both lakes over the two-year investigation period, comprising summer and autumn 2025, and winter and spring 2026. In Lake Ohrid, samples were collected at depths of 0, 10, 20, 30, 40, 50, 100 and 150 m, while in Lake Prespa, sampling was conducted in the upper pelagic water column at 0, 5 and 15 m. The use of the same seasonal periods in both lakes provided a consistent temporal framework for comparative analysis of their zooplankton communities. Rotifers were identified to the lowest taxonomic level supported by the monitoring data, and abundance was expressed as individuals m^{-3} . Seasonal richness was calculated as the number of recorded taxa or taxonomic units. For comparative purposes, density values represent the sum of positive depth-specific observations and not lake-wide mean abundance. The available Lake Ohrid dataset contained two rotifer taxa, *Kellicottia longispina* and *Conochilus hippocrepis*. Their occurrence was predominantly associated with deeper pelagic layers, particularly 30–50 m. The highest observed density was recorded in spring 2026, reaching 6,500 ind. m^{-3} across positive depth records, with *K. longispina* occurring from the surface to 50 m. Lake Prespa showed substantially greater taxonomic turnover. Eight taxonomic units/forms were recorded: *Keratella cochlearis*, *Kellicottia longispina*, *Filinia longiseta*, *Asplanchna priodonta*, *Polyarthra* sp., *Trichocerca similis*, *Brachionus* sp. and *Trichocerca* sp. The highest richness was observed in autumn 2025, when six taxa/forms were recorded, with a summed observed density of 3,250 ind. m^{-3} . In contrast to Ohrid, rotifer records in Prespa were concentrated within the shallow 0–15 m pelagic zone. The results demonstrate distinct rotifer community patterns in the two lakes. Lake Ohrid was characterized by lower recorded richness and a predominantly deeper vertical distribution, whereas Lake Prespa exhibited greater seasonal taxonomic turnover and concentration of rotifers in the upper water column. The contrasting patterns are consistent with differences in lake morphology and pelagic habitat structure, although the non-identical sampling designs prevent direct standardized estimates of lake-wide abundance. The observed data also demonstrate that taxonomic richness and abundance may vary independently, emphasizing the importance of retaining both parameters in long-term monitoring. The 2025–2026 monitoring data provide a contemporary baseline for pelagic Rotifera in Lakes Ohrid and Prespa. The marked differences in taxonomic composition, seasonal turnover and vertical distribution highlight the ecological distinctiveness of the two lake systems. Continued standardized seasonal monitoring, combined with physicochemical measurements and longer time series, is recommended to determine whether these patterns represent persistent ecological differences or components of longer-term environmental change.

Key words: zooplankton; taxonomic composition; vertical distribution; ecological variability; ecological monitoring

BIODIVERSITY AND CONSERVATION BIOLOGY

From evo-devo to conservation: Twenty years of crested newt biology, one solar plant, a baseline for conservation practice

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How do closely related species evolve? Why do some species survive for millions of years, even with traits that seem to work against them? We have studied these questions for two decades in crested newts (*Triturus*), combining developmental biology, life history, and evolutionary morphology. Most of this work was basic, curiosity-driven research, with no conservation goal in mind. This spring, we stood at Duboka pond, near the village of Brebevnica in eastern Serbia. Its surroundings had just been cleared. Vegetation and topsoil were removed to prepare the site for a new solar power plant. An environmental impact assessment had judged the damage to be “negligible”. Yet the pond is home to *Triturus ivanbureschi*, a species strictly protected under national law and the Bern Convention, together with five other amphibian species. This particular population turned out to have special value. It lies at the edge of the species’ range, right next to a hybrid zone. This genetically distinct, largely unadmixed population is one of the few “pure” populations left, in a landscape where many others show more mixing with *T. macedonicus*. Years of developmental and physiological research had already shown how these species cope with stress. We could apply this accumulated knowledge directly to this case. However, information on where and how far these newts travel once they leave the water is still missing. Both that knowledge and the official guidance for protecting crested newts come mostly from studies on a different species, in western Europe. This same *T. ivanbureschi* × *T. macedonicus* hybrid zone runs along North Macedonia, where the situation is even more complex. In some *T. macedonicus* populations, mitochondrial and nuclear DNA tell the same story. In others, the mitochondrial DNA actually comes from *T. ivanbureschi*, a trace of past hybridisation and species displacement. Hybrid populations occupy the centre of the zone, while small, genetically pure populations of *T. ivanbureschi* survive only at the edges, much like the population at Duboka pond. Baseline studies of selected North Macedonian populations have already begun, but more data and basic research are still needed. Conservation is especially challenging in hybrid zones and the basic research is crucial to it. It is part of the knowledge infrastructure that conservation decisions ultimately depend on. Teams in Serbia and North Macedonia now share one task: turning decades of curiosity into an action plan to save populations that we are still fortunate to have.

Key words: *Triturus*, marginal population, hybrid zones

A preliminary survey of pathogens in blood samples from the Critically Endangered Balkan Lynx (*Lynx lynx balcanicus*)

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The Balkan lynx (*Lynx lynx balcanicus*) is one of Europe's most endangered felids, and understanding its pathogen profile is essential for effective conservation and health monitoring. Blood samples collected during capture and GPS-collaring activities provided an opportunity to investigate haemopathogens using both targeted and untargeted molecular approaches. Fourteen blood samples from live-captured and dead individuals were analysed by PCR and Sanger sequencing, while eleven samples underwent host-read removal, de novo metagenomic assembly and taxonomic identification. Metagenomic analysis revealed the presence of *Cytauxzoon* sp. in seven samples, with mitochondrial genome contigs showing 99.57–99.95% nucleotide identity to previously described European isolates. Variation in contig coverage (8.3×–93.6×) indicated differences in parasite abundance among individuals. Additional contigs with lower similarity to other piroplasmid genomes were consistent with nuclear and apicoplast genomic regions of *C. europaeus*, for which a complete reference genome is currently unavailable. Samples without detectable *Cytauxzoon* sequences either lacked sufficient assembly data or were dominated by other bacterial taxa. PCR and Sanger sequencing further confirmed the presence of *Cytauxzoon* sp., *Candidatus Mycoplasma haemominutum*, and *Hepatozoon felis*. This study provides the first molecular assessment of blood-associated pathogens in the Balkan lynx and demonstrates the value of combining metagenomic sequencing with targeted diagnostics for wildlife disease surveillance and conservation management.

Key words: *Cytauxzoon* sp., *Hepatozoon felis*, haemotropic *Mycoplasma*, metagenomics, wildlife health, Balkan lynx

Strengthening transboundary Brown Bear conservation in the Southwestern Balkans

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Brown bears (*Ursus arctos*) in the southwestern Balkans are part of the wider Dinaric-Pindos population, with a range divided by the borders between Albania, North Macedonia and Greece. Contrary to bears, monitoring data, management decisions and operational context historically did not cross these borders when it came to bear conservation and management. The Transboundary Brown Bear Conservation in the Southwestern Balkans (TBBC, 2024–2026), implemented by EuroNatur, with the participation of PPNEA (Albania), Callisto (Greece), MES and Eko-svest (North Macedonia), was designed to strengthen cross-border cooperation, by addressing both strategic and practical conservation needs across the trinational brown bear population range. The project's transboundary architecture has produced results on both levels. Partners implemented a joint-camera trap survey under a common monitoring protocol, with 38 stations and 6,004 trapping days in 2025, producing a harmonized dataset of 130 bear occurrences. Data confirmed bear presence across the three cross-border areas and an evident corridor structure across the Greek–Albanian border. GPS tracking of a rescued female from the poacher's snare in Albania provided additional insights into habitat use and landscape permeability, both in Albania and North Macedonia. Combined with GIS layers of pressure/conflict areas, including roads, settlements and hydropower infrastructure, tracking data indicated that these landscapes faced seasonally rising presence, with the strongest overlap in late spring. Traditional orchards were mapped with drones in both Albania and Greece, providing a baseline for assessing natural food resources for bears and for the subsequent management measures proposed, on both sides of the border. Alongside monitoring, the project supported the establishment of Bear Intervention Teams in Albania and North Macedonia, where such structures were not yet formally operational. Through joint field expeditions, coordinated rescue operations and cross-border exchange with experienced teams, emerging intervention structures have responded to wildlife crime incidents and strengthened capacities for rapid response and human–bear conflict mitigation. At the strategic level, the Regional Bear Action Plan for the Prespa Lakes' Watershed was revised through trilateral consultation, providing a shared framework for conservation priorities across national boundaries. Communication and outreach activities complemented all actions by engaging relevant stakeholders and increasing public awareness of brown bear conservation, connectivity and coexistence across borders. By integrating ecological monitoring, capacity-building, stakeholder engagement, along with bear conflict intervention and wildlife crime response, TBBC highlighted transferable elements and demonstrated a practical approach to transboundary large carnivore conservation, serving as a working example for similar initiatives in the wider Balkan region and beyond.

Key words: Brown bear; Dinaric–Pindos population; transboundary conservation; ecological connectivity; conservation management

Towards a more complete picture of Balkan Lynx spatial ecology

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The Balkan lynx (*Lynx lynx balcanicus*) is a Critically Endangered subspecies of the Eurasian lynx (*Lynx lynx*) whose remaining population is centred in western North Macedonia. Radio-telemetry is an important tool for assessing the spatial ecology of wildlife populations, yet sex-biased capture probabilities can result in male-dominated datasets and potentially biased ecological inferences. This is particularly relevant for the planned genetic rescue of the Balkan lynx, where selecting an ecologically appropriate source population is essential. We assessed sex-specific home-range characteristics of the Balkan lynx using a sex-balanced telemetry dataset of 16 individuals (compared to 7 males and 1 female before) and compared seven home-range estimators: Minimum Convex Polygon (MCP), Kernel Density Estimator using a reduced reference bandwidth (KDE_{href}*0.7), conventional non-weighted and weighted KDEs with algorithmically selected bandwidths (KDE_c and wKDE_c), autocorrelated non-weighted and weighted KDEs (AKDE_c and wAKDE_c), and the dynamic Brownian Bridge Movement Model (dBBMM). Estimator performance was evaluated using area-under-the-curve (AUC) comparisons and visual inspection of utilization distributions. KDE_{href}*0.7 provided the most suitable representation of home ranges and core areas and was selected for subsequent analyses. Mean home-range and core-area sizes were 144.88 km² (93.11 - 196.65 km²) and 36.82 km² (22.02 - 51.62 km²) respectively, for females, and 404.28 km² (243.49 - 539.55 km²) and 104.83 km² (68.70 - 140.97 km²) for males. Males occupied larger home ranges than females, demonstrating clear sex-specific differences in space use. By incorporating equal numbers of males and females, our study provides a more balanced assessment of Balkan lynx spatial ecology and highlights the importance of considering sex composition when interpreting telemetry-derived home-range estimates. Home-range sizes of both sexes were more similar to those reported for the neighbouring Carpathian lynx (*Lynx lynx carpathicus*) than to those of the Anatolian population of the Caucasian lynx (*Lynx lynx dinniki*). These findings suggest that the Carpathian lynx represents the more ecologically suitable candidate for the planned genetic rescue of the Balkan lynx. More broadly, our results demonstrate how sex-balanced sampling and careful selection of home-range estimators can improve ecological assessments and inform conservation planning for small, threatened felid populations.

Key words: Balkan lynx; Spatial Ecology; sex-bias; Radio Telemetry

Modelling genetic reinforcement strategies for the Balkan Lynx

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The Balkan lynx (*Lynx lynx balcanicus*) is restricted to a small and fragmented range in the mountainous regions between North Macedonia, Albania and Kosovo. Fewer than 50 mature individuals are estimated to remain in the wild, making the Balkan lynx one of Europe's most vulnerable mammalian populations. The subspecies is exposed to multiple interacting threats, including habitat loss and fragmentation, illegal killing, depletion of prey, and increasing isolation. These pressures are compounded by severe genetic erosion resulting from prolonged small population size and isolation. Recent whole-genome analyses revealed the lowest genetic diversity and highest inbreeding among the Eurasian lynx populations examined, with inbreeding accumulated predominantly over the last 32 generations. The Balkan lynx also shows significantly elevated genetic drift and genetic load, including an excess of putatively deleterious mutations, with no evidence of genetic purging. Together, these findings indicate a substantial risk of inbreeding depression and highlight the urgent need for human-assisted gene flow. Genetic rescue through targeted reinforcement therefore represents a potentially essential component of the Balkan lynx recovery strategy. The Carpathian lynx (*L. l. carpathicus*) was selected as the most promising source population because of its ecological similarity and recent genetic admixture with the Balkan lynx and the precedent of successful Carpathian-to-Dinaric lynx reinforcement. However, the outcome of genetic rescue will depend on how many individuals are translocated, the frequency of the releases, as well as the sex, age and genetic composition of the selected candidates. To address these questions, we are developing an individual-based model in SLiM that integrates Balkan lynx demography and population genetics to evaluate reinforcement scenarios using Carpathian lynx. The model will investigate how the number, frequency, and genetic composition of translocated individuals influence long-term population persistence, genetic diversity, inbreeding, and the dynamics of genetic load. By linking demographic and genetic outcomes, this framework will allow us to identify reinforcement strategies that maximize genetic and demographic benefits while remaining realistic and feasible for conservation implementation.

Key words: Genetic rescue, simulation, critically endangered, mammal, conservation genetics

From conspecific hibernation to multispecies nursery colonies: Cross-border Insights into the seasonal ecology and conservation of the Schreibers' Bent-winged Bat (*Miniopterus schreibersii*) in Bulgaria and North Macedonia

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The Schreibers' Bent-Winged Bat (*Miniopterus schreibersii*) is a cave-dwelling species with a broad Mediterranean distribution and one of the most distinctive migratory ecologies among European cave-dwelling bats. The southern Balkans, including Bulgaria and North Macedonia, harbor some of the largest known colonies of this species, yet cross-border ecological assessments remain scarce. Unlike most cave-roosting bats, *M. schreibersii* occupies separate sites for winter hibernation and summer parturition, where it mixes with nursery colonies of other cave-dwelling species. The species exhibits a pronounced seasonal shift in spatial organization and social structure. It has also been identified as a reservoir host for several viral pathogens, including filoviruses and lyssaviruses. Despite its ecological and epidemiological significance, knowledge of its seasonal roosting behavior, population dynamics, and interspecific interactions in the region remains limited, constraining our understanding of the mechanisms governing pathogen transmission. This study investigated the seasonal biology, ecology, and social organization of *M. schreibersii* during both winter hibernation and summer nursery periods across the southern Balkan region. Specific objectives included characterizing underground microclimatic conditions at occupied roosts, estimating population sizes across seasonal phases, and documenting interspecific associations with sympatric bat communities at monitored sites in Bulgaria and North Macedonia. Between 2024 and 2026, four underground roosts in Bulgaria and one in North Macedonia were surveyed across seasonal periods, encompassing both winter hibernation and summer maternity roost assessments. Temperature and relative humidity were recorded continuously at each site using data loggers. Population size was estimated through direct counting of small clusters and photographic analysis of large aggregations. Selected individuals were captured using hand nets for age-class and sex determination. Interspecific associations were systematically documented and spatially referenced within roost microhabitats during both the hibernation and nursery phases. During the summer nursery period, *M. schreibersii* formed large mixed-species colonies concentrated primarily within the core areas of maternity clusters. Age-class and sex assessments documented subadult males early in the nursery period, whereas non-volant juveniles appeared later in the season, consistent with the seasonal timing of reproduction within the monitored maternity colonies. In Bulgarian sites, individuals were frequently associated with the Greater Horseshoe Bat (*Rhinolophus ferrumequinum*), the Mediterranean Horseshoe Bat (*Rhinolophus euryale*), and the Long-fingered Bat (*Myotis capaccinii*). However, in North Macedonia, associations were documented with the cryptic Mouse-eared/Lesser Mouse-eared Bat complex (*Myotis myotis/blythii*). Nursery colonies occupied the warmest and most humid cave sections, with temperatures ranging from 28 to 40°C and relative humidity near 80%. In contrast, during winter hibernation, *M. schreibersii* formed species-specific aggregations that were clearly segregated from those of other bat taxa. Hibernacula were characterized by markedly lower temperatures (4.8–11.6°C) and persistently high humidity levels (67–100%), reflecting distinct ecological requirements during the passive season. These findings demonstrate that underground microclimatic conditions strongly govern the seasonal activity patterns and social organization of *M. schreibersii*. The species exhibits a pronounced transition from species-specific winter aggregations to complex interspecific summer associations, generating markedly different ecological contexts that may modulate pathogen circulation and interspecific transmission dynamics. Understanding these seasonal shifts provides critical baseline information for evaluating the role of *M. schreibersii* as a

viral reservoir host and for designing effective, cross-border One Health surveillance frameworks targeting cave-dwelling bat communities across the southern Balkans.

Key words: One Health, microclimate, roost selection, interspecific interactions, ecology

From pilot monitoring to a standardized wildlife monitoring system in Shar Planina National Park: Experiences, challenges, and perspectives (2025–2030)

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Establishing long-term wildlife monitoring is a prerequisite for evidence-based biodiversity management. Shar Mountain National Park is developing an integrated monitoring system for multiple mammal species, including roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), wolf (*Canis lupus*), brown bear (*Ursus arctos*), red fox (*Vulpes vulpes*), wild boar (*Sus scrofa*), Balkan lynx (*Lynx lynx balcanicus*), and European wildcat (*Felis silvestris*). The objective is to establish a standardized methodology for long-term monitoring of relative abundance, spatial distribution, and population parameters to support natural resource management. With support from the Macedonian Ecological Society (MES), an approach involving park rangers in camera-trap deployment, field data collection, and the development of a shared database was tested. During 2025 and 2026, lessons learned were applied to improve fieldwork and implement more intensive monitoring. Researchers from the Faculty of Veterinary Medicine – Skopje also contributed to the analysis of data collected during both years. In 2025, 35 camera traps were deployed, generating 1,025 camera-trap days and 610 detections of 12 species. In 2026, 28 camera traps generated 1,327 camera-trap days and 747 detections of 12 species. The Relative Abundance Index (RAI) was calculated for five species. RAI values for 2025 and 2026, respectively, were 0.68 and 0.98 for wildcat; 1.27 and 1.13 for wolf; 1.95 and 4.67 for chamois; 1.95 and 3.54 for brown bear; and 2.44 and 3.46 for wild boar. These differences indicate variations in recorded activity but cannot be directly interpreted as changes in population size due to unequal spatial and temporal sampling coverage. Beyond species detection, camera traps provide information on reproduction, visible indicators of health status, the presence of golden jackals and free-ranging dogs, and potentially illegal activities. Camera-trap data are complemented by opportunistic visual observations recorded during routine ranger patrols and a planned chamois census at selected locations in cooperation with MES in 2026. For 2027, the establishment of a standardized monitoring network based on an 8 × 8 km spatial grid is planned, alongside expanded seasonal coverage, harmonized data processing and analysis protocols, and publication of suitable data through the Global Biodiversity Information Facility (GBIF). Potential transboundary cooperation with Sharri National Park in Kosovo is also being explored. This experience demonstrates that monitoring based on internal institutional capacities, complemented by scientific collaboration, can gradually evolve into a long-term system for tracking population trends and supporting adaptive management of protected areas.

Key words: camera trapping, wildlife monitoring, relative abundance index (RAI), Shar Mountain, adaptive management.

Traditional knowledge in agricultural practices and specific uses of crop landraces in North Macedonia

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Traditional knowledge is an integral component of agrobiodiversity conservation because crop landraces are maintained not only as genetic resources but also through practices, preferences and culturally defined uses. This study aimed to identify and systematize traditional knowledge associated with crop landraces collected during one 10-day collecting mission in 2003 and 52 one-day missions conducted throughout North Macedonia during 2014–2025, resulting in 6079 samples and collecting activities conducted by students during 2014–2017 resulting in 3500 samples. Knowledge was recorded through direct conversations with seed donors during seed collection. Archived accession-linked descriptions and field notes were subjected to secondary qualitative content analysis. Records containing only basic morphological descriptions were excluded unless these traits were explicitly linked to use, management, preference or cultural meaning. The final analytical dataset comprised 132 high-confidence traditional-knowledge records, which were coded into non-exclusive categories and synthesized into five broader domains: use and processing; seed and crop management; cultural transmission and history; reasons for maintenance; and threats and erosion. Traditional knowledge was frequently linked to highly specific functions that explained continued cultivation. Examples included chickpea used for the ritual bread *nautlija*; maize prepared with ash for St Barbara; winter melon and watermelon landraces selected for long storage; old rye maintained for soft straw used in traditional saddles; and pepper landraces maintained for *ajvar*, dried spice and other locally specific products. Seed-management knowledge included selection of particular fruits, cobs or plants for seed, maintenance of reserve seed for two seasons, mixed cropping of wheat and rye, alfalfa and oats, and crop-specific cultivation practices like perforating copper wire in tomato stem. Many landraces were maintained through family inheritance or migration histories, while farmers commonly emphasized taste, processing quality, adaptation, perceived resistance, reliability of farmer-saved seed and seed autonomy rather than yield alone. At the same time, rural depopulation, ageing, replacement by commercial cultivars, changing farming conditions and wildlife damage were repeatedly reported as threats to continued cultivation and knowledge transmission. Folk-medicinal statements were treated as ethnobotanical records rather than evidence of therapeutic efficacy. The findings show that the persistence of crop landraces is closely linked to their continuing functional, cultural and household relevance. Conservation of plant genetic resources should therefore preserve not only germplasm but also the knowledge required to select, cultivate, process, use and transmit it. Future collecting and database work should integrate standardized but open-ended documentation of associated traditional knowledge.

Key words: Ethnobotany; biocultural heritage; food processing; seed management; on-farm conservation

Phenotypic biodiversity of indigenous halotolerant bacteria associated with traditional Macedonian beaten sheep cheese from the Shar Mountain ecosystem

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Traditional Macedonian beaten cheese is an autochthonous fermented sheep milk product manufactured using artisanal practices and preserved with high concentrations of salt. The combination of traditional production methods, prolonged ripening, and the unique environmental conditions of the Shar Mountain region creates a distinctive ecological niche supporting diverse indigenous microbial communities. Despite its ecological and cultural significance, the biodiversity of cultivable bacteria associated with this traditional dairy ecosystem remains insufficiently explored. The objective of this study was to evaluate the phenotypic biodiversity of cultivable bacteria isolated from traditional Macedonian beaten sheep cheese and to assess their ecological adaptation. Cheese samples were collected from a traditional sheep farm located in the Shar Mountain region of North Macedonia. Three groups of bacterial isolates were recovered using different enrichment conditions: BST isolates after incubation in nutrient medium at 45°C for 24 h, BS isolates after incubation in nutrient medium at 30°C, and BSA isolates after cultivation in MRS medium under anaerobic conditions at 37°C. The isolates were characterized according to colony morphology, microscopic characteristics following Gram staining, growth at different temperatures (10, 25, 37, and 45°C), tolerance to increasing NaCl concentrations (3%, 5%, and 7%), and physiological and biochemical properties, including catalase and oxidase activities, gas production, lactose fermentation, and carbohydrate utilization. The obtained isolates exhibited pronounced phenotypic diversity, reflecting the complexity of the indigenous microbiota associated with traditional beaten cheese. Distinct physiological groups were recovered under the different cultivation regimes, including thermotolerant populations enriched at 45°C, mesophilic bacteria isolated at 30°C, and facultatively anaerobic fermentative microorganisms recovered on MRS medium. Most isolates demonstrated growth at all tested NaCl concentrations, including 7% NaCl, confirming strong halotolerance and adaptation to the highly saline environment characteristic of beaten cheese. Morphological characterization revealed considerable variability in colony form, elevation, margin, pigmentation, and texture, while microscopic examination demonstrated both Gram-positive and Gram-negative bacteria with coccoid and bacillary morphologies. Biochemical characterization revealed broad metabolic variability among the isolates. A large proportion of the isolates fermented lactose, indicating strong adaptation to the milk environment and suggesting an important ecological role in cheese fermentation and ripening. Differences in physiological, morphological, and biochemical characteristics among the BST, BS, and BSA groups demonstrate that cultivation conditions selectively recovered distinct fractions of the indigenous microbiota, thereby increasing the observed bacterial diversity. The results demonstrate that traditional Macedonian beaten sheep cheese represents a valuable reservoir of indigenous halotolerant bacteria exhibiting high phenotypic, morphological, and physiological diversity. The coexistence of thermotolerant, mesophilic, and facultatively anaerobic microorganisms with diverse metabolic capabilities reflects long-term adaptation to harsh environmental conditions, high salt concentrations, and traditional cheese production practices. These indigenous isolates may have potential applications in biotechnology, controlled fermentation, and the development or preservation of traditional fermented foods. These findings contribute to the understanding of microbial biodiversity associated with traditional fermented foods and emphasize the importance of preserving autochthonous dairy products as reservoirs of unique microbial genetic resources for future ecological, taxonomic, and functional investigations.

Key words: halotolerance; indigenous microbiota; mountain ecosystems; phenotypic diversity; lactose fermentation

Influence of plant species and ecological factors on the diversity of endophytic and epiphytic bacteria associated with medicinal plants in North Macedonia

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Endophytic and epiphytic microorganisms play essential roles in plant health, nutrient acquisition, and adaptation to environmental conditions. Despite increasing interest in plant-associated microbiomes, information on the bacterial diversity of native medicinal plants in North Macedonia remains limited. This study investigated how plant species and ecological factors influence the diversity and distribution of bacterial communities associated with medicinal plants collected from two ecologically preserved mountain ecosystems. The objective of this study was to characterize the endophytic and epiphytic bacterial communities associated with *Verbascum pulverulentum*, *Onopordum acanthium*, and *Cichorium intybus*, and to evaluate the effects of altitude, geographic location (Galichica and Shar Mountain), and plant species on microbial diversity. Plant samples were collected from three altitudinal zones within each mountain. Epiphytic and endophytic bacteria were isolated using standard microbiological procedures, including surface sterilization, serial dilution, cultivation on selective media, and purification of isolates. Bacterial characterization was performed using colony morphology, Gram staining, physiological and biochemical identification to the genus level. The results demonstrated that plant species, altitude, and geographic location significantly influenced bacterial community composition. Epiphytic communities generally exhibited greater taxonomic diversity than endophytic communities. High-altitude samples were predominantly associated with *Bacillus*, whereas *Pseudomonas* were more frequently isolated from lower elevations. Distinct bacterial genera were associated with specific plant species and mountain ecosystems, reflecting the influence of local environmental conditions and host characteristics. Physiological analyses further revealed that many isolates possessed amylolytic, cellulolytic, phosphate-solubilizing, and nitrogen-fixing activities, indicating their potential ecological importance. These findings demonstrate that both host plant characteristics and environmental gradients shape the composition of medicinal plant microbiomes. The study provides the first systematic overview of cultivable endophytic and epiphytic bacterial communities associated with these native medicinal plants in North Macedonia and contributes valuable baseline data for future ecological, microbiological, and biotechnological research. These isolates may have potential applications in biofertilizer development, improvement of plant nutrient acquisition, sustainable medicinal plant cultivation and organic matter decomposition.

Key words: altitude gradient; plant microbiome; bacterial diversity; mountain ecosystems; medicinal flora

Floristic monitoring of the population of *Ramonda nathaliae* Pančić & Petrović (Gesneriaceae) in the Zhelina Region

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Continuous monitoring of endemic and threatened plant species is essential for the development of effective conservation strategies. *Ramonda nathaliae* Pančić & Petrović is an endemic and Tertiary relict species of the Balkans from the Gesneriaceae family, known as the "resurrection plant" for its ability to survive complete desiccation and revive upon rehydration. In North Macedonia, it has been documented in several localities along the Vardar River basin. In the Zhelina region, the distribution sites of this species were discovered several years ago, near the village of Cerovo/Cerovë 557 m, 41°59'N, 21°9'E and in Karpalak/Karpallëk 425 m 41°58'N, 21°10'E, (Abdullai K., Haziri A., Millaku F. and Krasniqi E, May 2008), as well as the village of Laskartsi/Llaskarcë (Municipality of Saraj) 364 m, 41° 58' N, 21° 11' E, (Haziri A., Memishi Sh., Abdii N., May 2022). Another new locality in the Zhelina region, which represents a new record of the distribution of this species in the flora of North Macedonia, on steep rocky terrain was discovered near the village of Sedlarevo/Sallarevë 1338 m, 41° 52' N, 21° 8' E, May 2026. This study focuses on assessing the population status of this plant species in the Zhelina region. During May 2026, intensive field research was undertaken using the quadrat sampling method. The geographical positioning of individuals was recorded with GPS. The results showed a fragmented distribution of the species according to localities: Cerovo/Cerovë with an average density of 10 to 20 individuals/m²; Karpalak/Karpallëk 15 to 25 individuals/m²; Laskartsi/Llaskarcë 20-35 individuals/m²; Sedlarevo/Sallarevë 2-10 individuals/m². This study provides a new database that facilitates further monitoring and suggests a re-evaluation of the conservation status of this species at the level of the Zhelina region.

Key words: *Ramonda nathaliae*, endemic species, monitoring, conservation, Zhelina region

Red books of flora of Serbia 1 (1999, 2026): changes, challenges and experiences

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The Red Book of the Flora of Serbia 1 published in 1999 was the first publication on threatened plants on the territory of Serbia. Within this monograph, 171 plant species and subspecies are covered, of which 50 are estimated as extinct from the territory of Serbia, and 151 taxa were critically endangered. After more than 27 years, a new edition of the Red Book of the Flora of Serbia 1 is being prepared. In this volume of the Red Book, 181 regionally extinct (RE) and critically endangered (CR) taxa are processed, which amounts to about 4% of the total flora of Serbia. Of that number, 23 taxa (12.7%) were assessed as regionally extinct, while 158 taxa (87.3%) were assessed as critically endangered. In the new edition of the Red Book of the Flora of Serbia 1, the changes in the endangered statuses of plant taxa compared to the previous edition are particularly highlighted, while a special review is also given to the challenges and experiences in the application of the new versions of the IUCN criteria for assessing the endangered taxa. Compared to the previous edition, 87 new species and subspecies were included, while in the meantime 80 taxa were moved to lower IUCN categories – EN or VU (due to new established subpopulations and localities) or their current taxonomic status is unresolved. As in the previous edition, an analysis of the centers of diversity of threatened flora of Serbia has been carried out, as well as a chorological and ecological analyses, based on regionally extinct and critically endangered taxa. Analysis of the distribution of regionally extinct and critically endangered taxa on the territory of Serbia proved that the highest concentration of RE and CR taxa is in Vojvodina and in the area of the extreme south and east of Serbia. Chorological analysis of RE and CR taxa showed that the most numerous is the subendemic-endemic areal type, the second most abundant is the submediterranean-Mediterranean, while the third is the Eurasian mountain areal type. The ecological analysis of the regionally extinct and critically endangered taxa of the flora of Serbia is based on the determination of the occurrence of the 181 most endangered species and subspecies of Serbia in relation to the altitude, the geological substrate and the habitat type they inhabit. Thus, in relation to the altitude zones, the largest number of RE and CR taxa was registered in the hilly and mountainous zones, and the smallest in the subalpine and alpine zones. In relation to the type of geological substrate, by far the largest number of RE and CR species and subspecies is present on carbonates, and the smallest on loess and sand. Observed in relation to the basic type of habitat inhabited, the largest number of RE and CR taxa was recorded in grasslands, followed by forests, rocks and screes, as well as various types of shrub formations. The analysis of factors endangering the flora of Serbia, as well as the review of protection measures for extinct and critically endangered taxa and their habitats on the territory of Serbia, are of special conservation importance. Acknowledgment: This study was supported by the Ministry of Environmental Protection of the Republic of Serbia.

Key words: red book, vascular flora, Serbia

Diversity, host associations, and ecological preferences of the genus *Tuber* in Turkey

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Turkey possesses a remarkably diverse mycota owing to its rich topographical, climatic, and phytogeographical variation. This study synthesises extensive research conducted across various regions of Turkey between 1997 and 2024 to provide an up-to-date overview of the country's hypogeous fungal diversity. The primary objective of this work is to evaluate the taxonomic, ecological, and phytogeographical characteristics of the genus *Tuber* (Tuberaceae) within Turkey, highlighting its host preferences, altitudinal distribution, and economic potential. Data spanning a 27-year period (1997–2024) were systematically compiled and analysed to assess 13 recorded *Tuber* taxa. The synthesis involved evaluating their spatial and altitudinal distributions across distinct phytogeographical regions, identifying associated mycorrhizal host plant species (both angiosperms and gymnosperms), and characterising their preferred substrate conditions and ascocarp maturation periods. A total of 13 taxa were identified, including 12 defined species (*Tuber aestivum*, *T. borchii*, *T. brumale*, *T. excavatum*, *T. ferrugineum*, *T. fulgens*, *T. macrosporum*, *T. magnatum*, *T. mesentericum*, *T. nitidum*, *T. puberulum*, *T. rufum*) and one unidentified taxon (*Tuber* sp.). These taxa occur across the Mediterranean, Aegean, Black Sea, Marmara, Thrace, Central Anatolia, and Eastern Anatolia regions, spanning an altitudinal gradient from 122 m (Sakarya-Hendek) to 1,680 m (Trabzon-Tonya). Major angiosperm hosts belong to Fagaceae (*Quercus* spp., *Fagus orientalis*, *Castanea sativa*) and Betulaceae (*Corylus* spp., *Carpinus betulus*, *Alnus glutinosa*), alongside members of Salicaceae and Tiliaceae, while gymnosperm hosts include Pinaceae (*Pinus* spp., *Abies nordmanniana*, *Picea orientalis*) and Cupressaceae (*Juniperus excelsa*, *J. foetidissima*). Habitat analyses reveal a strong affinity for calcium-rich (calcareous), well-drained sandy, and humic soils, with ascocarp maturation extending from March to January. The broad altitudinal and geographical range of *Tuber* species highlights their high ecological plasticity and strong symbiotic adaptability to diverse forest ecosystems across Anatolia. The presence of globally renowned, high-value species underlines the ecological significance of Turkey's subterranean fungal genetic resources. Turkey hosts a taxonomically and ecologically diverse population of *Tuber* species. The presence of bio-economically high-value species such as *Tuber magnatum* and *Tuber macrosporum*, alongside commercially harvested black (*Tuber aestivum*, *T. uncinatum*) and white (*Tuber borchii*) truffles, demonstrates that these hypogeous fungi are not only vital components of forest ecosystems but also crucial genetic resources capable of generating substantial economic revenue.

Key words: Tuberaceae, *Tuber*, Hypogeous Fungi, Ecosystem, Mycorrhiza, Fungal Diversity of Turkey.

Phylogeny and phylogeography of *Tuber brumale* based on new molecular data from North Macedonia

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The *Melanosporum* clade is one of the most numerous within the genus *Tuber*, comprising sixteen recognized species. It includes commercially important species, such as *T. melanosporum* and *T. brumale*, the latter commonly known in Europe as the winter black truffle. Given the high morphological variability of *T. brumale*, the pseudocryptic species *T. cryptobrumale* was recently described. Distinguishing these species is difficult since it is based on a small number of highly variable micromorphological characteristics. To resolve these diagnostic challenges, we revised *T. brumale* agg. samples collected in North Macedonia using a molecular phylogenetic approach. Additionally, we conducted large-scale phylogeographic analyses, extending the sampling areas of previous studies. The ITS region of the nuclear DNA was sequenced from 71 samples from North Macedonia and the dataset was expanded to include all publicly available sequences of species within the *Melanosporum* clade, yielding a dataset of 228 sequences. The phylogram obtained with Maximum Likelihood analysis and fast bootstrap support confirmed the presence of *T. brumale* haplotype A1, *T. brumale* haplotype A2, *T. cryptobrumale* and *T. thracicum* in North Macedonia. These findings provide the first records of *T. brumale* haplotype A1 and *T. cryptobrumale* on the Balkan Peninsula alongside the first record of *T. thracicum* in North Macedonia. Statistical analyses are currently underway to test the possible presence of a phylogeographic structure among populations. Expanding knowledge on the distribution of these species provides insights into the impacts of glaciations and postglacial periods on species migrations and their survival in different refugia.

Key words: biodiversity, biogeography, Europe, population structure, refugia, truffles

Diversity and distribution of macromycetes in the riparian vegetation of Lake Prespa, North Macedonia

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Riparian zones along Lake Prespa represent ecologically vital ecosystems characterized by a mosaic of wetland, shrub, woodland, and shoreline reed communities. These habitats generate a wide spectrum of microhabitats and organic substrates—ranging from decaying woody debris and leaf litter to mycorrhizal associations—that support diverse fungal assemblages. Despite their ecological significance in nutrient cycling, the mycobiota of these lowland riparian zones in North Macedonia remains poorly documented. To address this critical knowledge gap, this study provides a comprehensive preliminary inventory of macromycetes inhabiting the riparian vegetation of Lake Prespa. The research integrates empirical data collected during targeted field surveys in autumn (October 2025) and spring (May 2026) with a thorough synthesis of historical literature and herbarium records. Sampling efforts focused on representative plant associations, specifically targeting mature alder stands, mixed willow and poplar woodlands, and extensive lakeside reed belts dominated by *Phragmites australis*. Diagnostic macroscopic features were recorded immediately from fresh basidiomata and ascomata in the field. Detailed microscopic analyses were subsequently conducted on fresh and dried material at the Mycological Laboratory at the Faculty of Natural Sciences and Mathematics in Skopje, employing standard taxonomic literature, specialized monographs, and updated identification keys. A total of 155 species-level fungi were documented. The recorded mycobiota is dominated by the phylum Basidiomycota, alongside a smaller representation of Ascomycota across different ecological niches, including wood-decay saprotrophs, litter decomposers, and ectomycorrhizal species. The results expand the knowledge on mycological diversity for the region: 130 taxa represent new records for the Lake Prespa basin, 13 of which are reported for the first time on the territory of North Macedonia. All voucher specimens examined during this study are deposited in the Macedonian Collection of Fungi (MCF), while associated ecological, host/substrate preference, and geo-referenced distributional data have been recorded into the MACFUNG database. Ultimately, this work provides essential baseline empirical data that enhances our understanding of fungal biodiversity and spatial patterns in North Macedonia, establishing a foundation to support ongoing conservation initiatives, long-term ecosystem monitoring, and future revisions of the National Red List of Fungi.

Key words: Fungal diversity, Floodplain habitats, National new records, Conservation.

Composition, diversity and relationships of the subterranean terrestrial isopod fauna (Crustacea: Isopoda: Oniscidea) of North Macedonia

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This study presents published and unpublished data related to an interesting old group of terrestrial isopods in North Macedonia. Like most of the Balkans, this area is characterized by relative species richness, a high degree of endemism and pronounced heterogeneity within its subterranean terrestrial isopod fauna. The peculiarity of the karst areas of North Macedonia is that they represent a border zone of the penetration of two faunas, the Aegeid fauna (predominantly) and the Dinaric fauna (to a much lesser extent). The Aegeid fauna of North Macedonia is characterized by the subgenus *Alpioniscus* Racovitza, 1908 (with 7 taxa) and the genus *Graeconiscus* Strouhal, 1940 (with one taxon) which are present from the western parts to the extreme northwest. Elements that are of Dinaric origin or have penetrated from that area are represented in the far north of North Macedonia by a single taxon from each of the genera *Cyphonethes* Verhoeff, 1926 and *Cyphoniscellus* Verhoeff, 1901. The high degree of endemism is reflected in the fact that, with the exception of two species, all other subterranean terrestrial isopods are stenoendemic. The endemic, monotypic genus *Mladenoniscus* I. Karaman, 2008 with its morpho-anatomical characteristics calls into question the validity of the current division of the family Trichoniscidae. It combines the characteristics that define the two most numerous subfamilies Trichoniscinae and Haplophthalminae, leaving the phylogenetic position of the genus *Mladenoniscus* within the family unclear. The genus *Macedonethes* Buturović, 1955, currently comprising two species, stands out as the most interesting element of the Macedonian troglobite fauna. The third known species of this genus is also present in a cave in eastern Spain. The species *Macedonethes skopjensis* Buturović, 1955 seems to have become extinct due to submergence of Matka Canyon. The secondary aquatic *Macedonethes stankoi* I. Karaman, 2003, discovered in the source of Babuna River, as a rhithral species represents a unique life form among all terrestrial isopods and most probably among all *Isopoda*. Adapting to a completely new environment, this species underwent significant morpho-anatomical changes and evolved a completely new body structures. Therefore, this genus is of exceptional evolutionary interest and should be the focus of research into the troglobite fauna of North Macedonia.

Key words: distribution, endemism, troglobites, zoogeography

Fifteen years later – a brief review of the distribution of recently established invasive alien true bugs in Bulgaria

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New and interesting records of alien true bugs species (*Corythucha arcuata* (Say, 1832), *Deraeocoris flavilinea* (A. Costa, 1862), *Empicoris tabellarius* Ribes & P.V. Putshkov, 1992, *Zelus renardii* Kolenati, 1857, *Belonochilus numenius* (Say, 1832), *Oxycarenus lavaterae* (Fabricius, 1787), *Halyomorpha halys* (Stål, 1855), *Perillus bioculatus* (Fabricius, 1775), *Nezara viridula* (Linnaeus, 1758)) in Bulgaria are reported after summarizing data from citizen science, social media and our observation over the last 15 years. The pace of spread of these species in Bulgaria, some of their biological peculiarities, and their potential threat to the native fauna and biodiversity in the country as a whole are briefly discussed. New data on the distribution of several native species with expanding range (*Lethocerus patruelis* (Stål, 1854), *Anisops sardeus* Herrich-Schaeffer, 1849, *Nagusta goedeli* Kolenati, 1857; *Caenocoris nerii* (Germar, 1847), *Arocatus longiceps* Stål, 1872, *Acrosternum heegeri* Fieber, 1861) are presented and the role of climate change (milder winters in the last years) is discussed. Funding for this study was provided by the Bulgarian National Science Fund (project KP-06-N91/11/, BG-175467353-2025-08-0082 “Comprehensive studies on alien representatives of the order Hemiptera in urbanized areas and their impact on natural and anthropogenic habitats”).

Key words: Heteroptera, fauna, invasions, spread

Scuttle fly (Diptera: Phoridae) communities on Vitosha Mountain (Bulgaria): Structure and dynamics in different habitats

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This study is based on material collected using pitfall traps (placed both on the ground and in tree crowns) across the main types of plant communities (vegetation belts) on Vitosha Mountain. The species distribution in relation to altitude and vegetation belts, as well as their phenology are discussed. In five model habitats (mixed forest, beech forest, spruce forest, dwarf mountain pine in subalpine zone and subalpine meadow), the dynamics of the species composition and the structure of the scuttle fly communities were established. These communities are characterised based on the number of the species, specimens and dominance structure. Furthermore, ecological indices were calculated and compared among communities. As a result, several conclusions regarding the species composition across the different habitats have been reached.

Key words: Diptera, Phoridae, Vitosha Mt, ecological structure

Unraveling the identity of Bulgarian *Osmoderma* (Coleoptera: Cetoniidae): Species delimitation using museomics and DNA barcoding

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The hermit beetle, *Osmoderma eremita* s.l. (Scopoli 1763), is a species complex of scarab saproxylic beetles listed in the IUCN Red List of Threatened Species and protected in Bulgaria by Bern Convention, the Habitats Directive, and the Bulgarian Biodiversity Act. The taxonomic status of the species complex in Bulgaria is not fully known. It is believed that *Osmoderma barnabita* Motschulsky, 1845 is the main species present in the country, but it is also possible that *Osmoderma lassallei* Baraud and Tauzin, 1991 exists in the southernmost parts, where is the northern limit of its distribution. Determining the species affiliation of the population found in Bulgaria is of great importance, as the two species are classified as having different levels of threat according to IUCN— *O. barnabita* (Near Threatened), *O. lassallei* (Vulnerable). For this reason, the material available in the National Museum of Natural History–Bulgarian Academy of Sciences collection proved to be of significant value and to hold great potential for determining the species identity of the specimens using molecular methods. A total of 17 newly generated COI sequences obtained from museum specimens from Bulgaria and Greece were analysed and compared with reference barcode sequences from GenBank. The material was collected between 1899 and 2008. Oxford Nanopore sequencing from variously preserved specimens, some over a century old, through non-destructive protocols, was used. This study forms part of a project pioneering the large-scale application of museomics and DNA barcoding to Bulgarian invertebrates. The project represents the first mass effort to sequence historical type material and link it to newly collected samples across diverse taxonomic groups.

Key words: museomics, DNA, saproxylic, beetle

New taxonomic and molecular data on the terrestrial gastropods of Vietnam obtained during Bulgarian expeditions in 2018, 2023 and 2025

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This study presents unpublished and published data on the terrestrial gastropods of Vietnam, obtained during three Bulgarian expeditions to Southeast Asia in 2018, 2023 and 2025. A new locality of the rare species *Plectostoma tonkinianum* (Dautzenberg & Fisher, 1905) is reported. The first record of the palearctic species *Deroceras laeve* (O. F. Mueller, 1774) in Southeast Asia, as well as the first finding of the micro-snail species *Bensonella boettgeri* (Möllerndorff, 1897) in Vietnam, are documented. Furthermore, two new genera, *Fansipanica* Páll-Gergely & Dedov 2026 and *Rethiculete* Dedov, Bobeva & Manh (submitted), along with seven new species for the country are described. These species include: *Laocaia simovi* Dedov and Schnepapat, 2019; *Muangnua vumanhi* Dedov, 2024; *Ostracolethe penevi* Dedov & Vu, 2025; *Cambodiparmarion bekchievi* Dedov & Vu 2025; *Bensonella didoi* Gojšina, Dedov, Páll-Gergely 2026; *Fansipanica milae* Páll-Gergely & Dedov, 2026; and *Ostracolethe lei* sp. nov. (submitted). Molecular data have also been obtained for a number of rare and newly described species.

Key words: DNA, new taxa, terrestrial gastropods, Vietnam

Cryptic pollinators? Assessing plant-visitation patterns of *Polistes dominula* and *Vespula germanica* across contrasting European climatic zones using open-access biodiversity data

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Social wasps are frequently overlooked in pollination ecology despite growing evidence that they visit flowers regularly, potentially contributing to plant reproduction as cryptic pollinators. This study leverages open-access citizen science and occurrence data platforms such as iNaturalist and the Global Biodiversity Information Facility (GBIF) to characterize plant-visitation patterns of two common eusocial wasp species, *Polistes dominula* and *Vespula germanica*, across two climatically distinct European regions: Bavaria (Germany, temperate continental/alpine) and Andalusia (Spain, mediterranean/arid). Georeferenced occurrence records, including associated photographic observations documenting wasp-flower interactions, were extracted and filtered to distinguish putative visiting and foraging events from incidental sightings. Records were cross-referenced with plant taxa to identify visited flowering species and to assess the temporal overlap between wasp activity and host-plant blooming periods. Phenological trends were reconstructed by aggregating observation dates to model seasonal activity windows, colony life-cycle stages, and their alignment with regional flowering phenology. Preliminary comparisons reveal marked regional asynchrony: wasp activity in Andalusia begins earlier and extends later in the year relative to Bavaria, tracking the extended Mediterranean flowering season, while both regions show peak visitation coinciding with late-summer resource demands linked to colony reproductive investment. Both species were associated with a broader range of flowering plant taxa than commonly assumed, suggesting underrecognized floral resource use. These findings highlight the utility of aggregated open-access biodiversity data for reconstructing fine-scale ecological interactions at a continental scale and underscore the need for targeted field validation of pollination effectiveness. By mapping spatial and temporal patterns of wasp-flower interactions across divergent climatic contexts, this study contributes to a growing case for reconsidering social wasps as functionally relevant components of European pollinator communities.

Key words: pollination, social wasps, open-access data.

Microplastic pollution in Serbian freshwater ecosystems: FTIR-Based analysis of *Rana temporaria* tadpoles

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Microplastics (MPs) represent a significant threat to freshwater ecosystems, while amphibians are particularly vulnerable because of their biphasic life cycle, encompassing both aquatic and terrestrial environments. The polymers most extensively used in plastic production are also among those most frequently detected in natural environments. This study investigated the occurrence and chemical composition of MPs in *Rana temporaria* tadpoles collected from seven localities in Serbia. Microplastics were detected in tadpoles from all sampled localities, regardless of habitat type, altitude, or proximity to urban areas. Fibers and fragments constituted the predominant MP morphotypes, both of which are readily ingested by tadpoles. Fourier-transform infrared (FTIR) spectroscopy identified these particles as predominantly anthropogenic polymers, including polyethylene, polypropylene, polyvinyl chloride (PVC), and polystyrene. The widespread occurrence of these polymers across diverse geographic and ecological settings demonstrates the extensive dispersal and long-term persistence of synthetic materials in freshwater ecosystems. These findings underscore the need for long-term biomonitoring in both protected and human-impacted freshwater ecosystems and further support the use of amphibians as valuable bioindicators in ecotoxicological assessments of microplastic pollution.

Key words: MPs, pollution, polymers, amphibians, FTIR

Amphibian and reptile real estate: Habitat associations and spatial patterns in North Macedonia

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This study quantifies spatial habitat associations of amphibians and reptiles in North Macedonia, assesses their representation within the national protected-area network and the working network of identified Natura 2000 sites, and identifies ecologically significant habitat complexes located outside these spatial frameworks. A total of 38,253 database rows were analyzed and aggregated into 16,166 unique source records and 15,384 analytical species–coordinate combinations distributed across 6,653 locations. The dataset comprised 46 species, including 14 amphibians and 32 reptiles. Given the presence-only nature of the data, the results are interpreted as relative habitat associations against a target-group background rather than as estimates of absolute occurrence probability or evidence of causal habitat selection. Spatial dependence and multiple testing were addressed using 20-km spatial blocks, Cochran–Mantel–Haenszel common odds ratios, false discovery rate correction, and spatially blocked cross-validation. In the record-weighted analysis, amphibians exhibited stronger associations with inland surface waters, wetlands, and artificial habitat mosaics, and a weaker association with cultivated agricultural habitats. However, the sensitivity analysis assigning equal weight to each species did not confirm significant class-level differences, indicating that the most frequently recorded species influenced the pooled effects. The multivariable model retained independent positive effects for inland waters and artificial habitats and showed moderate spatial discrimination (AUC = 0.634). Pronounced species-specific associations were identified with high-mountain grasslands and rocky habitats, oligotrophic water bodies, reedbeds, riparian forests, and gallery woodlands. The combined conservation network encompassed 22.12% of the analyzed records, whereas 77.88% occurred outside the protected areas and identified Natura 2000 sites. Of these, 6,981 records were associated with seven priority screening complexes, particularly thermophilous oak forests and dry grassland systems. The findings support a complementary conservation strategy that combines strengthening and ecological connectivity of the national protected-area network with scientifically justified additions to the Natura 2000 network, following field-based and legal verification, and also encouraging cross-border habitat protection activities.

Key words: Amphibia; Reptilia; habitat associations; protected areas; Natura 2000 sites;

Overview of twenty years of herpetological research on Golem Grad Island

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Research on reptiles on the Golem Grad Island started in the spring of 2007. Back then, four herpetologists came to the Island, with the idea of researching the nose-horned viper's population. From that almost "pioneering" visit, until 2026, the research grew into 20 years long professional "adventure". Over the years, in addition to the nose-horned vipers, research continued on two other species of reptiles – dice snakes and Hermann's tortoises. Several other reptile species were found on the island – wall lizards and grass snakes; as isolated incidents, a pond turtle, a four-lined snake and a smooth snake were also recorded. As concerned to amphibians, we found a large green frog and a green toad. By August 2026, the total research effort included 50 field sessions and 336 field days. 65 scientists and students from 10 countries were included in the research on the Island, of which over 90% were undergraduate and postgraduate students. The greatest contribution of this "laboratory in nature" was that it enabled international students to gain knowledge about the complexity of nature, reptiles populations and their mutual interactions, directly in the field. It could be said that for many of us, this was the professional "school of life". When it comes to scientific production, over 30 scientific papers were published in international scientific journals. The research resulted in five doctoral dissertations, as well as six master and bachelor theses. Over the years, fascinating (and sometimes bizarre) phenomena related to morphology, ecology, behaviour, reproduction, nutrition, as well as other biological characteristics of nose-horned vipers, dice snakes and Hermann's tortoises were recorded for the first time. As for the quantitative indicators, a total of over 680 nose-horned vipers, 7100 dice snakes and 2060 Hermann's tortoises were processed. Long-term population-ecological research showed the sensitivity of reptile populations, both to the effects of human activities and to climate change. The dice snakes' population is particularly sensitive to the negative effects of net fishing close to the Island shore, while the nose-horned vipers' population is most likely affected by climatic fluctuations. For the unique ecosystem of Golem Grad, the impact of intensive tourist visits to the Island, as well as insufficient control by the border police and National park authorities, is particularly significant.

Key words: Golem Grad Island, population-ecological study, reptiles

The White Stork (*Ciconia ciconia*) population in North Macedonia – results from the 2024 national census

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The White Stork (*Ciconia ciconia*) is a widespread breeding species in Macedonia[U1.1], closely connected with agricultural landscapes and various human settlements. In 2024, the second National White Stork Census was conducted in Macedonia, as part of the 8th European White Stork Census. The main aim was to assess the current population size and distribution, breeding success and nest characteristics, and to compare the results with the previous national census from 2014/15. The field survey was conducted between 20 June and 10 July 2024, by 23 observers, covering more than 400 populated places across the country. All known and potential breeding areas were surveyed, mainly by car, while settlements with high density of nests were surveyed on foot. For each nest, GPS location, number of adults and juveniles, nest position and other nest characteristics were recorded. In total, 1,765 White Stork nests were recorded, of which 1,388 were classified as active and 377 as inactive. A total of 3,934 juveniles were recorded, with an average of 2.83 juveniles per active nest. Most nests were located on human-made structures, with 1,152 nests on electricity pylons, 221 on roofs and chimneys, and only 15 on trees. The highest concentration was recorded in Pelagonia region, with 541 nests, followed by Ovche Pole region with 326 nests. At settlement level, the highest numbers were recorded in Stojakovo (53 nests), Bogorodica (45) and Rosoman (41). Compared with the previous national census, the results show a strong increase of the White Stork population in Macedonia. The number of active nests increased by approximately 65.83%, from 837 in 2014 to 1,388 in 2024, while the number of juveniles increased from 1,964 to 3,934 which represents increase of app. 100%. The results show that the White Stork population in Macedonia is currently increasing, with lowland agricultural areas remaining the most important breeding areas. At the same time, the strong dependence on electricity pylons and other human-made structures shows the importance of maintaining safe nesting sites and continuing regular population monitoring.

Key words: breeding population, breeding success

On the road to recovery: How an Egyptian Vulture reinforcement programme is successfully supplementing the Balkan breeding population

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The Balkan population of the Egyptian Vulture (*Neophron percnopterus*) has undergone a severe decline during the last 40 years and currently comprises only around 50 breeding pairs distributed across Bulgaria, North Macedonia, Albania, and Greece. Population viability analyses have shown that reinforcement through the release of captive-bred birds can markedly decrease the probability of extinction. To support recovery efforts, a reinforcement programme was initiated in Bulgaria in 2016, evaluating three release techniques, namely delayed release, hacking, and fostering, to determine their relative effectiveness in promoting long-term population restoration. Between 2016 and 2025, a total of 46 individuals were released, including 34 captive-bred birds and 12 wild-sourced juveniles. Survival until completion of the first autumn migration varied significantly among release methods. Delayed release achieved the highest survival rate (69%, $n = 19$), followed by fostering (50%, $n = 4$), whereas hacking resulted in the lowest survival (22%, $n = 9$). Juveniles originating from the wild exhibited an intermediate survival rate of 59% ($n = 17$). Owing to these results, delayed release has been adopted as the sole release strategy since 2021. Survival of reinforced birds to breeding age reached 46%, substantially exceeding the corresponding rate recorded for wild-fledged juveniles (14%). Recruitment of released individuals into the breeding population has been documented since 2022. By 2025, seven released birds had established breeding territories, and three breeding pairs involving released individuals had successfully fledged seven young. At present, released birds are represented in 21% of breeding pairs in the Eastern Rhodopes, indicating a substantial contribution of the reinforcement programme to the regional breeding population. Combined with extensive conservation actions implemented along the species' migratory flyway, these reinforcement efforts have coincided with recent stabilization and a slight increase in the Balkan Egyptian Vulture population.

Key words: *Neophron percnopterus*; Population reinforcement; Release methods; Post-release survival; Population recovery

Long-term breeding population trends of six herons, pygmy cormorant, spoonbill and glossy ibis in lakes Prespa: 1968-2025

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We report on the population trends of nine waterbird species breeding in Prespa lakes for the period 1968-2025. This is the longest, uninterrupted series of similar data in any major Balkan wetland whatsoever. Species included in this report are the pygmy cormorant *Microcarbo pygmaeus*, the great white egret *Ardea alba*, the grey heron *Ardea cinerea*, the purple heron *Ardea purpurea*, the little egret *Egretta garzetta*, the black-crowned night-heron *Nycticorax nycticorax*, the squacco heron *Ardeola ralloides*, the spoonbill *Platalea leucorodia*, and the glossy ibis *Plegadis falcinellus*. The summed breeding populations of the six herons rose from 100-150 in the mid-1980s to 400-450 in the mid-2020s, while the population of pygmy cormorants rose from 150-200 in the 1980s to 2000-2500 in the mid-2020s. After being analysed with a GLM model, the mean annual population growth rate (expressed as AONs -Apparently Occupied Nests) (1983/4-2025) was 6.4% for the pygmy cormorant, 6.6% for the great white egret, 3.1% for the grey heron, 2.8% for the little egret, 4% for the night-heron, 1.8% for the squacco heron, while for the purple heron was -1.3%. Spoonbills ceased nesting in the early '90s, while glossy ibises had nested up to the '70s and then, after 2005, they resumed breeding sporadically with less than 10 pairs. Only the breeding success of the great white egret was monitored after 2012. It varied proportionately to the water level of the Lesser Lake Prespa from 1 to 2.3 fledged chicks per nest. There is no single factor that could be used to explain the changes in population trends, but we discuss hydrological variation, nesting and feeding habitat shrinkage and degradation, distance between feeding and nesting grounds, reedbed management, predation, wildfires, emigration and conditions prevailing in winter quarters, which all have documented contributions. Locations of colonies change more frequently than in the past as these waterbirds face difficulties in finding flooded reedbeds to nest safely and there is also a resulting fragmentation of colonies into smaller parts owed to suitable habitat shrinkage due to dropping water level and recession of the waterline. In the last 15 years the feeding grounds of herons, shifted gradually from the shrinking wet meadows of Lesser Lake Prespa to the open shallow waters of the Greater Lake Prespa's continuously receding coastline. Furthermore, predation and wildfires' negative effects have increased dramatically in the last five years, while noteworthy numbers of grey herons and little egrets are considered to increasingly relocate from Lesser Lake Prespa to the mixed great cormorant-heron colony on the juniper trees of Golem Grad Island. It is anticipated that, due to climate change, in less than ten years there will be no flooded reedbeds suitable for nesting and these species will have to shift their nesting colonies elsewhere, possibly in the riparian forest belts along the lakeshores or/and the lake islands, and therefore these habitats should be effectively protected and accordingly managed in advance.

Key words: waterbirds, transboundary, population growth

GPS tracking reveals that nocturnal feeding is common in Dalmatian pelicans *Pelecanus crispus*: first results

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Several waterbirds forage both day and night, adapting their activity to food availability and other environmental factors. The Dalmatian pelican (*Pelecanus crispus*) is a large piscivorous waterbird, feeding in a variety of habitats, mainly freshwater. Although previous studies have investigated diet, diurnal feeding habitats and foraging behaviour, its nocturnal feeding activity, although empirically known to occur, has remained anecdotal up till now. In this work, we used GPS/GSM telemetry data from a long-term study, to examine nocturnal versus diurnal foraging of the Dalmatian pelican, across diverse wetlands in its Southeastern European range. From 2012 to 2025, we fitted a total of 79 GPS/GSM solar-powered transmitters on individuals of both sexes and of different age groups captured in Greece, Albania and Montenegro. Although tracking revealed extensive movements and use of wetlands from Montenegro to Ukraine and Turkey, in this study we focused solely on Greek and Albanian wetlands. We attached transmitters either as a 'backpack' using harnesses, or, after 2023, attached to the patagium, using a piercing mount. Transmitters operated 24 hours/day and were programmed to take a GPS location every 15 minutes. Speed and flight height were used as the basic criteria to determine behaviour and distinguish between resting/roosting, flying and active feeding. Speeds over 0 km/hour were considered to represent snapshots in an active foraging process. The total number of GPS fixes per period (day and night) in each wetland determined as 'foraging behaviour', were divided by the number of hours in this period, to obtain the number of 'foraging behaviour' fixes per hour, in order to allow for comparison between the two periods. More than 50 wetlands in this region were used for feeding, including lakes, river deltas, lagoons, and artificial reservoirs. However, we used only the results in the 20 more intensively used of them. In total, the average number of foraging fixes/hour during the night was only ca 4.4% lower than during the day, suggesting that Dalmatian pelicans, in contrast to what it was believed so far, use these wetlands with almost the same intensity for foraging in the night as in the day. This is the first time the ratio between diurnal and nocturnal activity is assessed quantitatively for any pelican species whatsoever. We suggest that the occurrence of nocturnal foraging activity may reflect the increased availability of prey, as many fish move to the surface waters at night, becoming more accessible to surface-feeding waterbirds, such as the Dalmatian pelican. Furthermore, nocturnal foraging may reflect avoidance of human disturbance at feeding sites or an adaptation to predator avoidance behaviours by fish. Future studies will have to explore possible patterns of differential occurrence of night foraging between wetlands and use the seasonal ecology of fish and other environmental factors to shed more light to the movements of these birds and the use they make of wetlands throughout the annual cycle.

Key words: waterbirds, foraging, diurnal-nocturnal

How effectively can a common habitat suitability modelling framework identify suitable habitat for forest bird species that differ in ecology, rarity and quantity/quality of available occurrence data?

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Habitat suitability models (HSMs) are increasingly used to infer species distributions from heterogeneous occurrence data, but high predictive performance does not necessarily imply that a model has recovered a robust ecological signal. We tested a common HSM framework on eight forest bird species in North Macedonia that differ substantially in ecology, distribution and availability of breeding-occurrence data: *Dendrocopos leucotos*, *D. medius*, *Dryocopus martius*, *Picus canus*, *Tetrastes bonasia*, *Ficedula parva*, *F. semitorquata* and *Aegolius funereus*. Breeding records were assigned to a 1 × 1 km UTM grid. All cells with valid breeding evidence were treated as presences, whereas absences were inferred only from sufficiently surveyed cells without detections; unsurveyed cells remained unknown. A common predictor framework incorporated climate, topography and CORINE land-cover variables measured at focal 1 × 1 km and surrounding 3 × 3 and 5 × 5 km scales. Random Forest, XGBoost, Generalized Additive Models and component-wise boosting (CWB) were evaluated using five-times-repeated five-fold nested spatial cross-validation and combined in a performance-weighted ensemble. Model credibility was assessed from predictive discrimination, stability among spatial partitions, agreement among algorithms, predictor selection and ecological plausibility of response curves and national predictions. Available information varied from 413 breeding-presence cells distributed across 119 occupied 10 × 10 km blocks for *D. martius* to only 15 cells in five blocks for *A. funereus*. Ensemble discrimination was generally high (mean repeated spatial-CV AUC 0.79–0.97), but its relationship with data availability was counterintuitive. Species with fewer occupied spatial blocks tended to have higher AUC (Spearman $\rho = -0.86$), while increasing spatial support was strongly associated with agreement among algorithms, both for continuous predictions ($\rho = 0.88$) and thresholded distributions (Jaccard agreement: $\rho = 0.98$). Thus, sparsely distributed species could be readily discriminated from surveyed non-detections while different algorithms nevertheless inferred substantially different national suitability patterns. Conversely, widespread species such as *D. medius* and *P. canus* showed only moderate AUC but strong algorithmic convergence and ecologically coherent distributions. Environmental predictors generally outperformed purely spatial CWB models, although explicit spatial structure improved the selected CWB formulation for seven species. This was biologically meaningful for range-edge or geographically restricted species such as *F. parva* and *A. funereus*. Independent RF and CWB variable selection repeatedly identified climatic conditions, deciduous forest, terrain and multiscale landscape composition, indicating that both focal habitat and surrounding forest-landscape context contribute to predicted suitability. Our results demonstrate that HSM reliability cannot be judged from discrimination statistics alone. Particularly for rare or range-restricted species, very high AUC may coexist with limited spatial information and substantial algorithmic uncertainty. Combining spatially explicit validation with model agreement, predictor stability and expert ecological interpretation provides a more defensible assessment of when heterogeneous occurrence data support robust habitat-suitability inference.

Key words: boreal owl, flycatchers, hazel grouse, woodpeckers

Wintering waterbird census on Lake Ohrid – Macedonian part in the period 2010-2017: Abundance, community, composition and conservation implications

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Lake Ohrid is one of Europe's most important freshwater ecosystems and is internationally recognized for its exceptional biodiversity and importance for waterbirds. This study examines a 17-year series of International Waterbird Census (IWC) data collected from the Macedonian side of Lake Ohrid between 2010 and 2026. The analysis describes annual total count, species composition and changes in the relative abundance of the principal wintering waterbird species, and considers the implications for the conservation status of the lake. Annual waterbird numbers varied substantially, from 10,108 individuals in 2010 to 36,631 in 2019, with a 17-year mean of 20,419 individuals, and a median of 20,435. Ten of the 17 years exceeded 20,000 birds, the threshold associated with international importance under Ramsar Criterion 5. Although total numbers showed strong interannual fluctuations without a clear monotonic trend, the species composition changed considerably. Common Coot (*Fulica atra*) was consistently dominant, but its relative abundance declined from 73.8% of the annual total in 2010 to 45.7% in 2026, following a maximum relative abundance of 80.8% in 2015 and a numerical peak in 2019. At the same time, Great Crested Grebe (*Podiceps cristatus*) and Black-necked Grebe (*Podiceps nigricollis*) became increasingly important components in several recent winters. Great Crested Grebe represented more than 22% of the annual total in both 2024 and 2025, while Black-necked Grebe represented 21.9% in 2021. Black-headed Gull (*Chroicocephalus ridibundus*) and Tufted Duck (*Aythya fuligula*) also increased their relative abundance, from 2021 onwards and 2022 onwards, respectively. Red-crested Pochard (*Netta rufina*) and Pygmy Cormorant (*Microcarbo pygmaeus*) showed pronounced interannual fluctuations. The findings demonstrate that assessment of Lake Ohrid's waterbird importance should not rely on total numbers alone: substantial changes in community structure occur even when total numbers remain broadly within the same range. The observed shift in community composition, including declining Coot dominance and increasing contributions of grebes and diving waterbirds, suggests the need to protect shallow littoral and wetland habitats and their associated feeding and resting areas, particularly for species of conservation concern, across the transboundary Lake Ohrid ecosystem. The results support the continued international importance of Lake Ohrid for wintering waterbirds and highlight the need for continued transboundary standardized monitoring, species-specific trend analysis and habitat protection.

Key words: International Waterbird Census (IWC), *Fulica atra*, Podicipedidae, Anatidae, Ramsar Convention.

ptici.mk: a citizen-science bird recording platform for North Macedonia, with sampling-event and occurrence datasets for GBIF

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North Macedonia has no national node in the Global Biodiversity Information Facility (GBIF), a common-bird monitoring scheme that does not yet contribute to the Pan-European Common Bird Monitoring Scheme and, on GBIF, no bird records from a domestic scheme: all 75,363 bird occurrence records attributed to the country are eBird rows. We describe ptici.mk, a bird recording platform operated by the Macedonian Ecological Society, and two datasets derived from it. The platform records birds in four modes spanning the effort spectrum (opportunistic records, timed searches, complete lists and line transects with perpendicular distances), is the field-data capture of the national monitoring scheme's International Census Plot transects, stores sampling events separately from occurrences, attaches an EBBA2-compatible breeding-evidence code to every record, and processes each record through grid assignment, elevation enrichment and a nightly automated plausibility screen against species and breeding-category-specific envelopes of altitude and day of year, with expert review of the records it flags. We report the screen's measured behaviour and do not claim an accuracy figure, because no reference set of records of known status exists. At the 2026-08-17 snapshot the archive held 55,570 live records of 291 species (91.5% of the national list) by 69 observers in 1,142 sessions, occupying 233 of the 256 national 10 km ETRS89-LAEA cells. Two Darwin Core archives are described: a sampling-event dataset (Event core with Occurrence and Humboldt extensions) restricted to sessions with valid effort, and an occurrence dataset of opportunistic records. Test and training sessions, sessions without a valid duration, and transects without a valid walked track are kept out of the event dataset by stated rules. Observers are pseudonymised and records of 18 sensitive species are generalised to 25 km.

Key words: biodiversity informatics; breeding bird atlas; Balkan peninsula; Darwin Core; Humboldt extension; data quality; automated screening; EuroBirdPortal; North Macedonia

Towards a national atlas, population estimates and conservation priorities for birds of North Macedonia

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The authors have been developing an integrated framework for a national bird atlas that combines heterogeneous historical and recent occurrence and count data with systematic field surveys, spatial modelling, population estimation and conservation assessment. The work builds on the national bird database, including data contributed through various field research and nature conservation projects, the ptici.mk citizen-science platform, the 2010 Important Bird Areas inventory and subsequent Natura 2000 assessments, data collected for the second European Breeding Bird Atlas, wintering waterfowl counts and other available sources. At the moment, more than 190000 observations have been digitized, which is estimated to represent >80% of all collected data since 1996. At the same time, new analytical procedures are being developed to guide the remaining atlas fieldwork and facilitate national population estimates. All records are classified using standardized breeding evidence and summarized to produce spatial (10x10 km UTM) maps and seasonal and elevation graphs, incorporating the temporal dimension, for 329 bird species. The Regional Avifauna Assessment Toolkit (RAAT) developed for data quality control integrates observation data across multiple spatial frameworks, including regional divisions, spatial-planning units and sites of conservation importance; evaluates seasonal and breeding status; flags inconsistencies for expert review; and links assessed species with national and international conservation criteria. Habitat-suitability modelling for 206 species at 1×1-km resolution currently combines Random Forest, XGBoost, GAM predictions and Component-wise Boosting in a weighted ensemble while retaining inter-model disagreement as an uncertainty measure, with potential extension towards temporal-occupancy, explicit spatiotemporal and Bayesian approaches. The framework distinguishes surveyed absences from insufficiently surveyed areas, allowing model outputs to identify priority squares for targeted field verification, instead of treating predicted suitability as confirmed distribution. Population estimation follows species ecology and data availability rather than a single analytical method. For suitable common breeding birds, line-transect distance sampling estimates territory density while incorporating complete survey effort; abundance is extrapolated only where a defensible analytical area is available, producing regional population estimates. Territory-based assessments are used for many raptors, while colony/site counts and repeated direct counts provide population information for other breeding and wintering species. The developing framework connects national distribution and population evidence with conservation planning through comparison with Important Bird and Biodiversity Areas, candidate Natura 2000 sites and nationally protected areas. It thus provides a pathway from individual observations to validated distribution, population estimates, site importance and conservation priorities. Information on population size and trends, together with Extent of Occurrence and Area of Occupancy, will also provide an important evidence base for a future National Red Data Book of Birds. The atlas remains work in progress: spatial, temporal and taxonomic coverage is heterogeneous and database quality assurance is ongoing. Identified gaps are being used to prioritize targeted surveys during the forthcoming few years. The intended outcome is a transparent national evidence base in which observed distribution, modelled habitat suitability and potential distribution, population estimates, and conservation significance remain explicitly incorporated.

Key words: distribution, distance sampling, habitat suitability modelling

Bat species activity in Serbian agricultural landscapes: The role of proximity to protected areas and linear elements

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The expansion and intensification of agricultural landscapes have profoundly altered habitat structure, reducing biodiversity and affecting the distribution and activity of many wildlife species, including bats. The structure of the landscape and the presence of managed remnant natural and semi-natural habitats in intensive agricultural landscapes can have species-specific effects on bat activity. The aim of this study was to evaluate the effects of proximity to protected areas and linear elements in intensively managed agricultural landscapes on different bat species activity. The study was conducted within an intensively managed agricultural landscape in Serbia, in the surroundings of five forested protected areas: Deliblato sands, Fruška Gora, Karadordevo, Potamišje, and the Kovilj-Petrovaradin marshes. To assess the importance of protected areas and linear elements, we used passive acoustic sampling. We placed bat detectors at three distances from the protected area boundaries: directly at the edge, 1.5 km and 3 km away. Sampling occurred in three site types: within linear elements, in adjacent fields, and in fields without nearby linear elements, allowing for a comparative analysis of bat species activity across different landscape contexts. Distance class significantly affected the activity of *Myotis* spp., *Plecotus* spp., *Barbastella barbastellus*, *Pipistrellus pygmaeus*, and *Nyctalus noctula*. Activity of *Myotis* spp. and *B. barbastellus* was significantly lower at both 1.5 and 3 km from protected areas, whereas activity of *N. noctula* was significantly lower only at 1.5 km, while *Plecotus* spp. and *P. pygmaeus* showed reduced activity only at 3 km. Site type significantly affected the activity of *Pipistrellus pipistrellus*, *Pipistrellus kuhlii/nathusii*, *Myotis* spp., *B. barbastellus*, and *N. noctula*. *B. barbastellus*, *Myotis* sp, *P. kuhlii/nathusii* and *P. pipistrellus* showed higher activity within linear elements, whereas *N. noctula* exhibited the highest activity in fields without nearby linear elements. Neither site type nor distance class significantly affected the activity of *Hypsugo savii* and *Nyctalus leisleri*. Overall, these findings demonstrate that bat species respond differently to proximity to protected areas and the presence of linear elements, highlighting the importance of maintaining landscape heterogeneity and accounting for species-specific responses when developing conservation strategies for bats in agricultural landscapes.

Key words: Chiroptera, agroecosystems, acoustic monitoring, landscape ecology

Conservation importance of caves in the Uvac Special Nature Reserve as bat hibernation sites

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Underground roosts play a crucial role in the life cycle of many temperate bat species, particularly during hibernation, when suitable and stable microclimatic conditions are essential for survival. At the same time, the concentration of large numbers of individuals in a limited number of hibernation sites makes these sites particularly vulnerable to disturbance and therefore of high conservation importance. The aim of this study was to assess bat species diversity and abundance in caves of the Uvac Special Nature Reserve, southwestern Serbia, and to evaluate their importance as hibernation sites. Field surveys were conducted in November 2021, during the early hibernation period. The Ušac cave system, Tubića cave and Baždarska cave were surveyed by visual inspection of accessible cave passages. Individuals were counted and identified to species or species-group level, with a telephoto lens used for bats roosting on high and inaccessible cave ceilings. In addition, passive acoustic monitoring was conducted at the entrance of Ušačka Cave to complement visual observations. At least nine bat species were recorded during the survey. Tubića cave supported the highest species richness and abundance, with eight species and 393 counted individuals, followed by Ledena Cave with five species and 307 individuals and Baždarska Cave with three species and 188 individuals. At least four species were recorded in Ušačka Cave, although total abundance could not be estimated because only part of the cave was visually surveyed. Passive acoustic monitoring at the entrance of Ušačka cave also revealed bat activity during the survey, indicating that bat activity was still present at this early stage of the hibernation period. The lesser horseshoe bat, *Rhinolophus hipposideros*, was by far the most abundant species, with 324 individuals recorded in Tubića Cave, 291 in Ledena Cave and 154 in Baždarska Cave. *Rhinolophus ferrumequinum* and the *Myotis myotis*/*M. blythii* species group were also recorded in notable numbers. *Barbastella barbastellus* was documented for the first time within the reserve during the 2021 survey. The high abundance and diversity of bats recorded during the early hibernation period highlight the importance of the investigated caves as hibernation sites, particularly for *R. hipposideros*, but also other recorded species. As the November counts may not represent peak winter abundance, further surveys during mid-winter (January–February) are needed to assess whether larger aggregations occur later in the hibernation period, although access to these high-elevation caves may be challenging under severe winter conditions. Minimising disturbance and establishing long-term monitoring of important hibernation sites, particularly those supporting high bat abundance and species diversity, should be prioritised to detect population changes and inform effective conservation management.

Key words: Keywords: winter monitoring; underground roosts; population counts; Serbia

Data on mammal diversity and occurrence in the Demir Kapija area, North Macedonia

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North Macedonia is a country with high biodiversity, and in this respect is one of the hotspots in Europe. The diverse natural habitats create living conditions for species with different environmental requirements. 84 species of mammals have been identified in the country, 10 of which are protected or strictly protected according to the national laws. Among the strictly protected species are the wild cat *Felis silvestris*, the otter *Lutra lutra*, and the brown bear *Ursus arctos*.

The Demir Kapija region provides diversity of natural habitats. The continuous forests and the traditional agricultural lands in the area are suitable for the presence of different wild mammals. The basins of Vardar and Boshava rivers welcome species dependent on freshwater ecosystems. The mountain villages are abandoned, which additionally provides undisturbed natural habitats. The present study was conducted in Demir Kapija and its surroundings, within a specific study area. The aim was to collect data on the species of mammals inhabiting the area, and to identify the potential and actual threats for those species. The field study was conducted from the end of May to the beginning of August, 2025. The following methods were used to record the different mammal species: a) Recording signs of the species' activity by transect walks for identifying tracks, excrements, food remains, rodent burrows, vocalization of mammals, as well as direct observations; b) Using a network of camera traps to record mammal species; c) Live capture of small mammals (Micromammalia). Altogether, 14 mammal species were recorded with certainty using the above methods (*Erinaceus roumanicus*, *Sciurus vulgaris*, *Lepus europaeus*, *Dryomys nitedula*, *Martes foina*, *Meles meles*, *Felis silvestris*, *Lutra lutra*, *Vulpes vulpes*, *Canis aureus*, *Canis lupus*, *Ursus arctos*, *Capreolus capreolus*, *Sus scrofa*). Some records of the family Gliridae, fam. Muridae and the genus *Microtus* remain undetermined to the species level. The identification of two species (*Glis glis* and *Martes martes*) remains uncertain. The occurrence of the brown bear *U. arctos* was registered by camera traps and by tracks. The species records are extended from June to August, which suggests its permanent presence in the area. These are the first validated registrations of brown bears in the study area. The wolf *Canis lupus* and the species' reproduction, the otter *L. lutra* along the Vardar river shore, as well as the wildcat *F. silvestris* were also recorded in the study area. Directly identified threats and disturbances to mammals are habitat fragmentation, collisions with vehicles, waste, and off-road tourism. Some of the potential threats for mammals were not registered during the present work. The occurrence of species like the brown bear and the wolf indicates the presence and connectivity of suitable habitats and highlights the conservation importance of the area. Implementation of a longer survey is needed to collect more data on the mammal species' presence and habitat use patterns in the area of Demir Kapija. The present study was conducted in the frames of the project „United for Rivers” initiated and led by the organization “Eko-svest” Skopje.

Key words: field study, camera traps, brown bear

Preliminary assessment of the BMCyt assay in bats in Serbia: Potential for ecotoxicological biomonitoring

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Bats (Mammalia: Chiroptera) are recognised as good bioindicators for assessing the impact of environmental pollution. The buccal mucosa micronucleus and other nuclear abnormalities (BMCyt) assay is a simple, non-invasive method for detecting DNA damage, chromosomal instability, disturbances in cell division, and cytotoxic effects in wild populations. However, to the best of our knowledge, it has only been applied to South American bat species, highlighting the need for further validation in conservation and environmental monitoring. We aimed to test the performance of the BMCyt assay in populations of the Schreiber's bent-wing bat (*Miniopterus schreibersii* Kühl, 1817) to determine whether the method is reliable enough to detect and quantify nuclear abnormalities in bats and support its potential application in environmental biomonitoring. We collected buccal cell samples using sterile swabs from 36 individuals of the Schreiber's bent-wing bat from Degurička (N = 24) and Šalitrena (N = 12) caves in western Serbia. Buccal cell smears were stained immediately before analysis with 20 µL of a 1:500 dilution of Green Safe Premium stain (NZYtech) and examined under a fluorescence microscope at 40× magnification. A minimum of 500 buccal cells per smear was required for successful BMCyt analysis in bats. As this was a pilot methodological assessment with a limited sample size, analysis was restricted to BMCyt cell scoring and descriptive statistics. The assay success rate was 58.33%, and nuclear abnormalities were quantified in 21 individuals, of which 14 were from Degurička cave and 7 from Šalitrena cave. Frequencies (%) of individual nuclear abnormalities were expressed as the proportion of affected cells among the 500 scored cells per individual. The BMCyt assay included assessment of karyolysis, karyorrhexis, pyknosis, micronuclei, nuclear buds, and binucleated cells as indicators of nuclear abnormalities. Karyolysis (mean frequency $\pm$ SD; 19.9 ± 5.36), karyorrhexis (12.4 ± 6.52), and pyknosis (6.7 ± 3.03) were the most common nuclear abnormalities in bat buccal cells. The mean frequencies of nuclear buds, micronuclei, and binucleated cells were 0.60 ± 0.42 , 0.17 ± 0.27 , and 0.09 ± 0.16 , respectively. Although the BMCyt assay is a non-invasive approach, its application in bats is constrained by several methodological challenges, including difficult sampling in small-sized species, uncertainty regarding sample adequacy before microscopic examination, and the low cellular density of buccal smears, which considerably prolongs manual scoring. Despite these limitations, the BMCyt assay appears feasible for non-invasive ecotoxicological assessment in bats, although further methodological optimisation is needed before its routine application in biomonitoring.

Key words: Chiroptera, buccal micronucleus test, *Miniopterus schreibersii*, ecotoxicology

Preliminary investigation of the endoparasite fauna in the Balkan Lynx (*Lynx lynx balcanicus*)

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Coprological analysis of large carnivore faeces provides valuable information on their health status and contributes to evidence-based conservation and management. Parasitic infections represent an important but still poorly investigated aspect of Balkan lynx ecology, with the potential to influence individual fitness and population dynamics. This study aimed to explore the occurrence of endoparasites in faecal samples collected opportunistically between 2019 and 2026, primarily near GPS-collared lynx kill sites. A total of 26 scats were analysed. Species identity was confirmed by PCR amplification and Sanger sequencing of the mitochondrial marker COX1 (cytochrome c oxidase subunit 1), verifying six samples as originating from *Lynx lynx*. Parasitological examination was performed using flotation for the detection of nematode and cestode eggs and coccidian oocysts, and the Baermann technique for detecting nematode larvae. Molecular analyses included multiplex PCR for *Taenia* spp. and *Echinococcus granulosus*, and quantitative PCR for *Cryptosporidium* spp. and *Toxoplasma gondii*. Analysis revealed the presence of protozoan cysts, cestode eggs, nematode eggs and larvae, and sarcoptic eggs. While the targeted molecular assays yielded negative results, microscopic examination of the faecal samples revealed the presence of parasites in 21 samples, with helminths detected in 17 samples, protozoan parasites observed in 6 samples, and *Sarcoptes* detected in 3 samples. Examination of the six confirmed lynx scats identified two protozoan parasites (*Giardia* sp. and *Cystoisospora* sp.) and two nematode parasites (*Toxocara* sp. and *Capillaria* sp.). These preliminary findings provide the first insights into the intestinal parasite fauna of the critically endangered Balkan lynx and contribute to understanding the health status of this fragile population. Under the Balkan Lynx Recovery Programme (BLRP), continued parasitological surveillance is warranted to evaluate the epidemiological significance of these parasites and their potential implications for the long-term conservation of the subspecies.

Key words: scat, parasites, critically endangered, BLRP

Seasonal patterns in population structure and body condition of *Miniopterus schreibersii* in Bogovina Cave, Serbia

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Bogovina Cave is one of the largest speleological sites in Serbia and represents an important roost for several bat species. However, for Schreiber's bent-winged bat (*Miniopterus schreibersii* Kuhl, 1817), the function of the cave during different periods of the year remains unclear. Understanding the seasonal use of this roost is important for effective population monitoring and site management, as *M. schreibersii* is a cave-dwelling, migratory species of high conservation concern throughout its range and is listed as Vulnerable on the IUCN Red List. The aim of this study was to investigate seasonal variation in the population structure and body condition of *M. schreibersii* in Bogovina Cave, and to determine whether these patterns differed between the 2014–2015 (first research period) and 2024–2026 (second research period). In the first period (N = 47), sampling was done once a year, while in the second (N = 75), seasonal sampling was introduced during spring, summer, and autumn. Sex, age class, body mass (BM), and forearm length were recorded for each individual, while the body condition index (BCI) was used as an indicator of body condition. Changes in sex ratio were analyzed using a chi-square test, and morphometric characteristics (BM and BCI) between periods, seasons, and sexes were analyzed using linear models. Basic population structure and condition-related parameters are broadly similar between the two sampling periods. Overall sex ratio, body mass, and BCI in the *M. schreibersii* population did not differ significantly between the two sampling periods. No significant differences were detected between sexes or seasons. However, sex ratio, body mass, and BCI varied seasonally. Sex ratio varied among seasons ($p = 0.0017$), with females predominating in spring ($p = 0.0028$) (of which approximately 20% were pregnant), males predominating in summer ($p = 0.010$), and no significant sex difference observed in autumn. Body mass and BCI varied seasonally (BM: $p = 0.035$; BCI: $p = 0.034$), and females showed higher values than males (BM: $p = 0.016$; BCI: $p = 0.0257$). Our results imply that the presence of pregnant females in spring, followed by their reduced representation and the absence of lactating females and juveniles in summer, suggests seasonally changing use of the part of the cave system surveyed in this study. Regular annual and seasonally structured monitoring covering the entire Bogovina cave system is needed to fully assess its seasonal use by *M. schreibersii*. Such comprehensive monitoring would also provide a robust basis for detecting future changes in roost use and population composition, particularly under increasing anthropogenic pressure.

Key words: bent-winged bat, conservation, Eastern Serbia, long-term monitoring

The Macedonian Pepper - historical pathways, regional traditions, and conservation of local diversity

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Pepper (*Capsicum annuum* L.) occupies a central position in the agriculture and food culture of North Macedonia, yet the historical processes through which it became established and regionally differentiated remain insufficiently synthesized. This study aimed to reconstruct the transition of pepper from an introduced crop to an important food, market commodity and locally diversified plant genetic resource, while linking its historical development with modern conservation challenges. The study combined evidence from the literature on the global and Ottoman–Balkan diffusion of *Capsicum* with geographically specific historical, ethnographic and agricultural sources concerning Macedonia. Documentary references were evaluated according to their date, locality and terminology, with particular attention to the ambiguity between *Capsicum* and black pepper. Historical evidence was then integrated with reports on twentieth- and twenty-first-century germplasm-collection activities and the conservation of farmer-maintained pepper populations. The available evidence does not support assigning the arrival of *Capsicum* in Macedonia to a precise date or single route. Introduction during the Ottoman period is plausible, but geographically specific records become reliable only in the nineteenth century. Red pepper appears in accounts of everyday food and in the commercial landscape of Bitola, while observations from 1889 provide strong indirect evidence of local cultivation through its regular sale as a fresh product and consumption as an inexpensive staple. Direct evidence from the Meglen basin in 1898 documents extensive field production, drying, milling and trade. By the beginning of the twentieth century, pepper products associated with Meglen, Prilep and Bukovo had acquired recognizable regional reputations, indicating the development of specialized production–processing–market systems. These geographical labels should be interpreted as regional product identities rather than automatically equated with formally defined varieties. Agricultural modernization after the Second World War increased pressure on farmer-maintained populations through the spread of selected cultivars and commercial hybrids. Successive collecting efforts in 1969–1973, the establishment of a university collection during 2003 and 2014–2025, preserved important snapshots of this changing diversity. The extraordinary diversity of Macedonian pepper landraces is exemplified by embroidered pepper—a distinctive type used only in Macedonia, where the characteristic cork-like striations are maintained rather than selected against—and supports the country's recognition as a secondary centre of diversity for *C. annuum*. Within this diversity, embroidered pepper retains substantial variation in fruit morphology, pungency, aroma and characteristic cork-like striations. However, accession records and ex situ seed storage cannot preserve the continued farmer selection, processing practices and local knowledge through which such populations evolved. The historical trajectory of pepper in Macedonia is therefore not a simple account of crop introduction. It is a process of cultural incorporation, regional specialization, farmer selection, modernization and conservation. Effective safeguarding of this heritage requires complementary gene-bank and on-farm approaches that maintain both the genetic material and its connection with farming communities, local uses and knowledge systems.

Key words: *Capsicum annuum*; embroidered pepper, documentary evidence; germplasm collections; genetic erosion

Microbial biodiversity of spontaneously fermented corn silage from three agricultural regions of North Macedonia

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Silage represents a dynamic microbial ecosystem in which naturally occurring microorganisms drive the fermentation process and influence forage preservation, nutritional quality, and ecological stability. The composition of these microbial communities is affected by environmental conditions, geographical location, and farming practices, making silage an important reservoir of indigenous microbial diversity. The objective of this study was to investigate the cultivable microbial biodiversity of spontaneously fermented corn (*Zea mays*) silage collected from three geographically distinct agricultural regions of North Macedonia and to compare the distribution and phenotypic characteristics of the isolated microorganisms. Silage samples were aseptically collected during March 2024 from farms near Tetovo, Prilep, and Kumanovo. Microorganisms were enriched in selective liquid media, followed by serial dilution and cultivation on selective agar media for the isolation of lactic acid bacteria (LAB), aerobic bacteria, Enterobacteriaceae, and yeasts. The recovered isolates were characterized according to Gram reaction, microscopic morphology, and macroscopic colony characteristics, including colony size, pigmentation, shape, margin, and elevation. A total of 109 cultivable microbial isolates were recovered from the silage samples, demonstrating substantial microbial diversity among the investigated locations. Tetovo exhibited the highest isolation efficiency (50 isolates), followed by Prilep (37 isolates) and Kumanovo (22 isolates), suggesting geographical variation in cultivable microbial populations. The microbial community consisted of comparable numbers of aerobic bacteria ($n = 36$), anaerobic bacteria ($n = 35$), and yeasts ($n = 31$), whereas Enterobacteriaceae were represented by only seven isolates. All bacterial isolates were Gram-positive, indicating the predominance of beneficial fermentative microorganisms typical of well-preserved silage. Microscopic examination revealed location-dependent variation in cellular morphology. Coccoid bacteria predominated in the Tetovo (62%) and Prilep (53%) samples, whereas bacillary forms were more abundant in Kumanovo (75%). Yeast isolates exhibited predominantly oval cells with noticeable differences in cell size, suggesting the presence of diverse yeast populations. Macroscopic characterization further confirmed the heterogeneity of the microbial community, with colonies differing in size, pigmentation, texture, and morphology. The observed differences in microbial abundance and phenotypic diversity among sampling locations indicate that geographical origin contributes to the structure of silage-associated microbial communities. The predominance of Gram-positive bacteria, together with the diversity of bacterial and yeast morphotypes, demonstrates that spontaneously fermented corn silage constitutes a rich reservoir of indigenous microorganisms adapted to local agroecological conditions. These findings provide valuable baseline information on the cultivable microbial biodiversity of North Macedonian silage ecosystems and support future ecological, taxonomic, and functional investigations of indigenous microbial resources.

Key words: agroecosystems; cultivable microbiota; lactic acid bacteria; phenotypic characterization; spontaneous fermentation

Discovery of *Epipactis exilis* P. Delforge (Orchidaceae) in Southeastern Serbia: New distribution records and habitat characteristics

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In Serbia, 11 taxa of the genus *Epipactis* Zinn have been recorded to date. One of them is *Epipactis exilis* P. Delforge, originally described in 1988 from Greece and also reported from Italy. Since then, its distribution has been documented across much of Europe, from France and Spain eastwards to the Balkan Peninsula, with records from Corsica, Sardinia, Italy, Croatia, Serbia, North Macedonia, Greece, Romania, and Bulgaria. In Central Europe, it has been recorded in Hungary, Slovakia, Austria, and as far north as Poland. *Epipactis exilis* is a rare species, generally occurring in small populations, and has been reported from just above sea level to approximately 1800 m a.s.l. It occurs on calcareous to slightly acidic substrates and prefers mature, shaded, most commonly beech and beech–hornbeam, but also mixed beech, spruce, and fir forests, as well as pine and holm oak stands. Its typical habitats are characterized by a very poorly developed herbaceous layer and a well-developed leaf litter layer. In Serbia, the species was first recorded in 2022, when a single individual was found at Panički Jelak on Mt Kopaonik. In 2023, it was recorded on Mt Željin and at another locality on Mt Kopaonik. All previous records were from Central Serbia. During our field campaigns in July 2026, *E. exilis* was recorded for the first time in Southeastern Serbia, at three geographically close localities in the Lake Vlasina area: two on the plateau near Vlasina Okruglica and one on the lower slopes of Mt Veliki Čemernik, near Vlasina Rid. At the locality on Mt Veliki Čemernik, only a single individual was found at the edge of a shaded, monodominant *Fagus moesiaca* [U1.1](K. Malý) Czeccott forest, with very poorly developed herbaceous vegetation and a thick layer of forest litter, near the settlement of Vlasina Rid (UTM FN03). The other two populations were found in the vicinity of the settlement of Vlasina Okruglica (UTM FN02), in marginal parts of monodominant *F. moesiaca*[U2.1] forests along the main asphalt road. Here, the herbaceous vegetation was either almost absent or extremely poorly developed, with a well-developed litter layer. A total of nine flowering ramets of *E. exilis* were recorded at these two localities. All individuals were found on north- or northeast-facing slopes, with inclinations of 5–26°, at elevations of 1282–1284 m a.s.l., on albite–chlorite–muscovite schists. Other orchids recorded at the localities were *Cephalanthera longifolia* (L.) Fritsch, *Epipactis helleborine* (L.) Crantz subsp. *helleborine*, and *Epipactis microphylla* (Ehrh.) Sw. Given that *E. exilis* was only recently discovered in Serbia and that additional localities have been identified since then, the species is likely to be more widely distributed in the country than currently documented.

Key words: orchids, chorology, Balkan Peninsula, Vlasina Lake, Mt. Veliki Čemernik

Ecological differentiation and evolutionary relationships of two recently described Balkan endemic species, *Euphorbia balcanica* and *Euphorbia skopjensis*

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Recent integrative taxonomic research within the *Euphorbia nicaeensis* alliance revealed cryptic diversity, resulting in the recognition of two new species from the central Balkan Peninsula, *Euphorbia balcanica* and *E. skopjensis*. The two species are consistently distinguished by morphological and cytogenetic evidence, including differences in relative genome size and ploidy level. *Euphorbia balcanica* is diploid and confined to the dolomitic and serpentine regions of the central Balkan Peninsula (Albania, Kosovo, North Macedonia), whereas the tetraploid *E. skopjensis* is endemic to the Skopje Basin. Despite their recent taxonomic recognition, their ecological differentiation and evolutionary relationships remain poorly understood. Preliminary analyses of restriction site-associated DNA sequencing (RADseq) data indicate that *E. balcanica* and *E. skopjensis* are more closely related to *E. hercegovina*, *E. japygica*, *E. macroclada* and *E. nicaeensis* than to the species of the *E. glareosa* group, although the latter grow in closer vicinity to *E. balcanica* and *E. skopjensis*. In addition, the populations from eastern North Macedonia that were traditionally treated as *E. glareosa*/*E. nicaeensis*, however belong to *E. pannonica*. To clarify the ecological differentiation between *E. balcanica* and *E. skopjensis*, we performed phytosociological surveys using the Braun–Blanquet approach and classification according to current syntaxonomic concepts and EUNIS habitat types. By integrating ecological and genomic evidence, this study provides new insights into the ecological and evolutionary differentiation of *E. balcanica* and *E. skopjensis*. Ecologically, *E. balcanica* and *E. skopjensis* show an apparent differentiation in habitat preferences, with the former occurring mainly in higher-elevation, nutrient-poor rocky grasslands and scrublands on serpentine and dolomite substrates, and the latter in lower-elevation subruderal habitats with deeper, more nutrient-rich soils. The results improve our understanding of their habitat differentiation, vegetation affiliation, and evolutionary relationships, contributing to a broader understanding of speciation and polyploid evolution within the *Euphorbia nicaeensis* alliance.

Key words: Phytosociology, RADseq, Balkan Peninsula, new species, polyploidy.

Floristic assessment and conservation value of Belchishko Blato (North Macedonia)

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Wetlands are among the most threatened ecosystems in Europe, making comprehensive biodiversity assessments essential for their conservation. In North Macedonia, they represent only 1.5% of the surface area, with lowlands harboring the majority of the country's wetland area. One such wetland is Belchishko Blato, which is highly valuable due to supporting diverse wetland habitats and rich vascular flora. However, both the condition and extent of wetlands are rapidly declining. Therefore, there is an urgent need for their conservation. In this regard, this study aims to present the floristic assessment of Belchishko Blato, which was conducted as part of the multidisciplinary evaluation of the area's natural values. Floristic surveys were carried out during the 2020 and 2021 growing seasons across the main habitat types, including wet meadows, marshes, reed beds, black alder forests and wetland edges. Vascular plants were recorded through field observations and collection of plant material for subsequent taxonomic identification and verification. The field data were complemented with unpublished records from previous floristic studies to compile an updated inventory of the vascular flora and assess species and habitats of conservation importance. The survey documented over 200 vascular plant taxa, confirming the high floristic diversity. Among them, 12 taxa were identified as conservation priorities and are included in the Priority Red List of the Macedonian flora for future IUCN assessment. Three species (*Carex elata* All., *Malus florentina* (Zuccagni) C.K.Schneid. and *Rumex hydrolapathum* Huds.) are protected under national legislation, while several orchid species are listed in CITES Appendix II. The wetland supports the largest and best-preserved alluvial forest dominated by *Alnus glutinosa* (L.) Gaertn. in the country, representing the priority habitat type 91E0* under the European Habitats Directive. Collectively, the occurrence of these species and habitats highlights the high floristic and ecological value of the wetland and reinforces its importance for biodiversity conservation at both national and European levels. Field observations indicated several potential pressures on the wetland, including abandonment of traditional mowing and grazing practices, changes in the hydrological regime and the spread of invasive alien species, which may negatively affect wetland habitats and plant diversity. The results of our study confirm the outstanding conservation value of Belchishko Blato and contribute to the scientific basis for its subsequent designation as a protected area. Long-term conservation of its floristic diversity will require continuous monitoring and appropriate habitat management.

Key words: wetlands; vascular flora; conservation assessment; Belchishko Blato

Floristic studies of the Malesija region and the Black Drim River valley (Republic of North Macedonia)

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The Malesija region, located in western North Macedonia and including the villages of Prisovjani, Zbzhdi, 'Rzhanovo, Selci, Burinec, and Lokov, as well as the right side of the Black Drim River Valley, is one of the least studied areas in the Republic of North Macedonia from a floristic point of view. Very little information about the flora of this region is available in the literature, highlighting the need for further floristic studies. Field surveys were carried out between 2022 and 2026 as part of an ongoing Master's thesis. The aim of the study is to document the flora of the area, record the distribution of plant species, and provide new information on the floristic characteristics of the region. The study included several localities with different habitats, altitudes, and geological conditions. Plant species were recorded in the field, and herbarium specimens were collected for further identification. The identification and analysis of the collected material are still in progress. However, the analysis of the floristic material conducted so far has revealed the presence of representatives belonging to 42 families. The most species-rich families are Asteraceae, Caryophyllaceae, Fabaceae, Poaceae, and Lamiaceae. Their representation, together with the diversity of the recorded plant species, indicates the pronounced habitat heterogeneity and floristic richness of the study area. The field surveys conducted so far indicate that the study area represents an important but poorly studied botanical region in western North Macedonia. The final results are expected to provide a detailed overview of the flora of the Malesija region and the Black Drim River Valley and contribute to a better understanding of the flora of this insufficiently explored area. The study will also provide a basis for future floristic, ecological, and conservation research.

Key words: flora, floristic studies, Malesija, Black Drim River Valley

From historical records to current populations: distribution and conservation assessment of *Sternbergia colchiciflora* Waldst. & Kit. (Amaryllidaceae) in Serbia

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Sternbergia colchiciflora Waldst. & Kit. is a bulbous, hysteroanthous, self-pollinating species native to the Mediterranean region, extending northwards to central Hungary and eastwards to northwestern Iran. It mainly occurs in open, rocky grasslands, scrub, forest edges, and field margins on well-drained calcareous soils. Although globally assessed as Least Concern, the species is rare, sporadic or insufficiently known in several European countries. Its distribution in Serbia is fragmented, and available information from Southeastern Europe is spatially and temporally uneven, possibly because of its small size and short autumn flowering period. Recent population-level data are particularly limited in several countries, hampering reliable assessment of population trends and conservation status. To reassess the distribution and current conservation status of *S. colchiciflora* in Serbia, we compiled and critically evaluated available occurrence records from herbarium collections, literature, and field observations. A total of 283 records were assessed and classified by data type (literature, herbarium or field data) and current state (extinct or living population). In addition, targeted field surveys were conducted at selected localities in SE Serbia, during which geographic coordinates, elevation, habitat characteristics, and the number of observed individuals were recorded. Voucher specimens were collected and deposited in the Herbarium of the University of Novi Sad (BUNS). The dataset revealed 74 reliable herbarium records, 133 reliable literature records, and 8 field observations, while 25 records referred to populations considered extinct and 42 were excluded due to erroneous interpretation. The available evidence therefore combines a substantial historical record base with considerable uncertainty concerning the current distribution of the species. Field surveys documented three new occurrences: two populations in the Svrlijske planine Mts. in eastern Serbia (Telovac and Klenje) and one occurrence on Fruška gora Mt (Krušedol). At Telovac, 25 individuals were recorded in calcareous steppe vegetation mixed with Sub-Mediterranean thermophilous deciduous shrubland, while 10 individuals were recorded at Klenje in calcareous steppe adjacent to a planted forest of European black pine. At Krušedol, a single individual was recorded in a steppic pasture. The results indicate that numerous historical records should not be interpreted as evidence of a currently widespread species in Serbia, as a substantial proportion refer to extinct populations or cannot be reliably verified. The occurrence of very small populations in suitable open, calcareous habitats, together with habitat succession, grassland conversion, invasion by Black locust and Tree of heaven, urbanization and recreational activities, indicates a potentially unfavorable conservation situation. Based on all available data, the IUCN conservation status for Serbia was assessed as Vulnerable, and inclusion of the species in further assessments during preparation of the Red Book of the vascular flora of Serbia is recommended. This pattern may extend beyond Serbia, as available records from Southeastern Europe are similarly uneven and geographically scattered, limiting regional comparisons of population status. Our results demonstrate the value of combining herbarium evidence, literature records, and field surveys when reassessing rare plants distribution. Continued field surveys and population monitoring are needed to improve the regional conservation assessment of the species in Southeastern Europe.

Key words: Babaluschka, Turkish Starflower, Vulnerable taxon, Assessment

New chorological data on the northward spreading of xero-thermophilous plants in Serbia

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The results of a systematic floristic investigation of urban habitats in southeastern Serbia, initiated in 2024, reveal a diverse floristic composition associated with ruderal habitats and built infrastructure. These environments are of particular importance for synanthropic species expanding from the southern Balkan Peninsula toward Central Europe, facilitated by major transport corridors. New chorological data for five xero-thermophilous plant species in the Serbian flora are presented. *Plantago weldenii* Rchb., previously known in Serbia only from the Vranje surroundings, was recorded in the City of Niš, where it was found growing in crevices between stone-paved surfaces. *Vicia melanops* Sibth. & Sm., previously restricted to the southernmost region of Serbia, was recorded in an abandoned area along a highway in the City of Niš. *Dysphania pumilio* (R. Br.) Mosyakin & Clemants, formerly known from the Pčinja River valley, the City of Vranje, and the Danubian river islet of Ada, was discovered in the urban habitats of Bujanovac and Leskovac, where it occurred on sandy and gravel substrates. *Pterocephalus plumosus* (L.) Coult., mainly known from southernmost Serbia, was recently found in the City of Niš and was recorded at several new localities, including four railway stations. The latter represents the northernmost known occurrence of the species in Serbia. Finally, *Rostraria cristata* (L.) Tzvelev, a species with a sporadic distribution in Serbia, was recorded at new localities in the cities of Niš and Leskovac, with abundant populations found in various sandy ruderal habitats. The newly documented occurrences provide evidence of the ongoing northward expansion of xero-thermophilous plants from the southern, warmer regions of Serbia, potentially supported by recent climatic fluctuations and anthropogenic influences. These range extensions contribute significantly to the chorological knowledge of the Serbian flora and highlight the role of urban and ruderal habitats as potential corridors for the establishment and dispersal of the given taxa.

Key words: urban habitats, infrastructure, flora, Serbia

Orchid diversity in Miločer park (Montenegro): floristic and conservation significance of a historic park

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Miločer Park is located behind Kraljeva and Kraljičina Beaches, near Pržno and Sveti Stefan Island, in the Municipality of Budva. It was established in the 1930s during the construction of Villa Miločer, the summer residence of Queen Marija Karadorđević. Covering 18 ha, the park was designed in the French classical style. Alongside native Mediterranean species, woody exotic plants from other continents were introduced during its establishment. In the post-World War II period, the villa, park, beaches, and Sveti Stefan Island predominantly served as an exclusive resort. Due to its outstanding values, a procedure for protecting the park as a cultural heritage site was initiated in 2026. During floristic fieldwork conducted in the Mediterranean region of Montenegro in spring 2026, the orchid flora of Miločer Park was investigated to assess its diversity. A total of 11 taxa belonging to five genera of Orchidaceae were recorded. *Ophrys* L. was the genus with the highest number of species, comprising four: *O. apifera* Huds., *O. cephalonica* (B. Baumann & H. Baumann) Devillers-Tersch. & Devillers, *O. minuscula* (G. Thiele & W. Thiele) Presser & S. Hertel, and *O. rhodostephane* Devillers & Devillers-Tersch. Particularly significant were *O. cephalonica*, first confirmed in Montenegro in 2025, and *O. minuscula*, recorded at several localities for the first time in 2026. The genus *Serapias* L. was represented by three taxa – *S. bergonii* E. G. Camus and *S. lingua* L. subsp. *lingua* and the intergeneric hybrid *S. × demadesii* Renz., which was first confirmed for Montenegro in 2025. *Anacamptis* Rich. included *A. laxiflora* (Lam.) R. M. Bateman, Pridgeon & M. W. Chase subsp. *laxiflora* and *A. morio* (L.) R. M. Bateman, Pridgeon & M. W. Chase subsp. *caucasica* (K. Koch) H. Kretzschmar, Eccarius & H. Dietr., while *Limodorum* Ludw. ex Kuntze and *Platanthera* Rich. were each represented by one taxon, *L. abortivum* (L.) Sw. var. *abortivum* and *P. chlorantha* (Custer) Rchb., respectively. Six taxa were recorded in open habitats, including forest gaps and clearings with dense herbaceous vegetation and locally encroaching maquis elements. The remaining five taxa occurred in wooded parts of the park, mainly on bare soil under deep shade and beneath the canopies of *Pinus halepensis* Mill. and *Cupressus sempervirens* L. Three of the identified taxa are restricted to the Balkan Peninsula (all *Ophrys* species except *O. apifera*). In contrast, the remaining taxa have broader East Mediterranean, Mediterranean, European, and southwestern Asian distributions. Considering the small area surveyed, relatively uniform habitat conditions, fieldwork restricted to the spring aspect of vegetation, and pronounced anthropogenic influence, the recorded orchid diversity is remarkable. Extending fieldwork into the summer and autumn may reveal additional Orchidaceae taxa. At the same time, both present and potential future discoveries further highlight the ecological, scientific, and conservation value of this important cultural-historical site and support the ongoing efforts towards its protection.

Key words: Orchidaceae, *Ophrys*, *Serapias*, Balkan Peninsula, Budva

Phorophyte type outweighs bark and tree characteristics in structuring epiphytic bryophyte diversity

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Epiphytic bryophytes are strongly influenced by the properties of their host trees, yet the relative importance of phorophyte type, bark chemistry, tree size, and vertical position remains insufficiently understood in temperate forests of southeastern Europe. We assessed the factors structuring epiphytic bryophyte diversity and composition in beech-dominated deciduous forests of Fruška Gora, northern Serbia. Sampling was conducted at five forest localities. At each locality, five 100 × 100 m plots were established, and 30 trees were randomly selected within each plot, giving a total of 750 sampled trees. Bryophyte cover was recorded in 10 × 10 cm microplots positioned on four sides of each trunk at 20 cm intervals from the tree base to a height of 2 m. Host trees were classified into eight phorophyte types corresponding to the genera *Acer*, *Carpinus*, *Fagus*, *Fraxinus*, *Quercus*, *Sambucus*, *Tilia*, and *Ulmus*. Bark pH, diameter at breast height, and sampling height were recorded. The effects of these variables were evaluated using generalized linear models, canonical correspondence analysis, forward selection, and variation partitioning. A total of 29 epiphytic bryophyte species were recorded. *Fagus* supported the highest observed richness, with 21 species, and the highest Shannon diversity, whereas *Ulmus* supported the lowest diversity. Bryophyte diversity declined significantly with increasing height on the trunk, while phorophyte type also had a significant effect. The measured explanatory variables accounted for 34.1% of total compositional variation. Individually, sampling height explained 9.9% of variation and bark pH 7.3%, whereas tree diameter had no significant effect and explained only 2.0%. Variation partitioning showed that phorophyte type independently explained 20.3% of variation, compared with 9.4% explained by bark pH, sampling height, and tree diameter combined; their shared contribution was 4.4%. These results indicate that phorophyte taxonomic type integrates ecological differences that are not fully represented by commonly measured tree characteristics. Maintaining a diverse composition of tree genera and preserving suitable lower-trunk microhabitats may therefore be essential for conserving epiphytic bryophyte diversity in temperate deciduous forests.

Key words: bark pH; bryophyte diversity; epiphytes; forest biodiversity; host trees;

Polyphenolic and biogenic volatile organic compound profiles of endemic metallophyte plants from the Allchar mining area

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Endemic metallophyte plants of Macedonia represent a distinctive component of the country's flora, growing in habitats with naturally elevated concentrations of potentially toxic elements. One metalliferous environment is the Allchar locality, recognized for its complex geochemical composition and history of mining activities. The area is characterized by elevated concentrations of thallium (Tl), arsenic (As), antimony (Sb), lead (Pb), nickel (Ni), cadmium (Cd) and other trace elements, and is notable for its remarkable diversity of endemic plant taxa. Ten local endemic (stenoendemic) taxa have been reported from the Allchar area: *Odontarrhena kavadarzensis*, *Centaurea kavadarzensis*, *Centaurea leucomalla*, *Galium kernerii*, *Knautia caroli-rechingeri*, *Onobrychis degenii*, *Thymus allchariensis*, *Viola alsharensis*, *Viola arsenica* and *Viola* × *halacsyana*. The distinctive ecological and geochemical characteristics of Allchar make its endemic flora of interest not only botanically but also with respect to its specialized chemical composition. Plant chemical profiles comprise diverse secondary metabolites, including polyphenolic compounds and biogenic volatile organic compounds (BVOCs), whose composition and abundance are influenced by species-specific characteristics and environmental conditions. However, previous studies of the Allchar flora have focused predominantly on the accumulation and distribution of potentially toxic elements, particularly Tl and As, while comparatively little attention has been given to specialized metabolites. To address this gap, three endemic metallophytes representing different genera were selected: *Thymus thracicus* var. *alsarensis* (TA), *Knautia caroli-rechingeri* (KC) and *Centaurea leucomalla* (CL). Polyphenolic compounds and BVOCs were analyzed using HPLC/DAD/MSⁿ and headspace GC-MS, respectively. The highest total polyphenolic content was observed in *K. caroli-rechingeri* (453.23 mg/g), followed by *C. leucomalla* (38.12 mg/g) and *T. thracicus* var. *alsarensis* (20.86 mg/g). Each species exhibited a distinct qualitative composition, particularly among phenolic acid derivatives. Mono- and dicaffeoyl derivatives were the most abundant in KC and CL, whereas salvianolic acids and rosmarinic acid derivatives characterized TA, with salvianolic acid K (2.06 mg/g) and isosalvianolic acid A (2.00 mg/g) being the most abundant. The largest number of BVOCs (77) was identified in the *Thymus thracicus* var. *alsarensis*, followed by *C. leucomalla* (37) and *K. caroli-rechingeri* (36). Monoterpene hydrocarbons were the most dominant class in the *Thymus*, represented by D-limonene (29.7 %) and γ -terpinene (7.8 %). In contrast, aldehydes were the most abundant in CL (44.3 %) and KC (41.6 %), with benzeneacetaldehyde among the dominant components (10.4 % and 10.6 %, respectively). Chrysanthenol and (-)-myrtenol were identified only in the KC. Notably, this study represents one of the first investigations of the BVOC profile of the genus *Knautia*, providing a basis for further comparative studies. Overall, the three endemic metallophytes exhibited clearly differentiated polyphenolic and BVOC profiles, with substantial interspecific differences in metabolite abundance and composition. The combined chemical profiling provides a valuable baseline for the specialized chemistry of endemic metallophytes from the Allchar area and supports further phytochemical and ecological investigations.

Key words: phytochemical profiling, Allchar mining area, metalliferous soils, phenolic acids, monoterpenes

Preliminary data on the rock vegetation of Suva Planina Mt., Southeastern Serbia

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Rocky habitats, due to their topographic heterogeneity, provide a wide range of microhabitats suitable for the persistence and conservation of endemic and rare plant species. Although these habitats are characterized by high plant diversity, they remain relatively understudied, largely due to their inaccessibility. In light of the above, we conducted a vegetation survey of rocky habitats on Suva Planina Mt. during 2024–2026. Based on a dataset comprising 85 relevés and 89 plant species, a UPGMA hierarchical cluster analysis was performed, identifying seven distinct communities that form three ecological groups along an elevational gradient. The first group comprises the *Silene flavescent*-*Asplenium ceterach* and *Micromeria cristata*-*Silene flavescent* communities, which occur across a broad elevational range, from 513 to 1502 m asl. and 430–1594 m asl., respectively. Both communities are confined to warm and dry habitats and predominantly occur on southern aspects. The second group comprises communities occurring between 1439 and 1688 m asl.: the *Ramonda nathaliae*-*Saxifraga paniculata* community, found exclusively on northern aspects; the *Edraianthus serbicus*-*Helianthemum oelandicum* community, occurring predominantly on southern exposures; and the *Potentilla apennina*-*Achillea ageratifolia* subsp. *serbica* and *Dianthus petraeus* subsp. *noeanus*-*Carum graecum* communities, both found predominantly on northern aspects. The third ecological group is represented by the *Heliosperma pusillum*-*Cystopteris fragilis* community, which is confined to the highest elevations (1621–1810 m asl) and occurs on northern and western slopes, indicating a preference for cold and moist conditions. These communities are rich in endemic (e.g., *Achillea ageratifolia* subsp. *serbica*, *Carum graecum*, *Micromeria cristata*, *Draba lasiocarpa*, *Dianthus petraeus* subsp. *noeanus*, *Erysimum commatum*, and *Seseli rigidum*) and protected taxa (e.g., *Kernera saxatilis*, *Edraianthus serbicus*, *Juniperus communis* subsp. *nana*, and *Potentilla apennina*). Although this study represents only the beginning of the investigation of rocky habitats on Suva Planina Mt., identifying seven distinct communities with different ecological affinities, along with numerous relict and endemic taxa, highlights the remarkable diversity of these habitats and their ecological and refugial value.

Key words: syntaxonomy, plant communities, chasmophytes

***Reynoutria × bohemica* in Mavrovo, North Macedonia: An early warning of an emerging invasive plant**

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Reynoutria × bohemica Chrtk & Chrtková (Polygonaceae), a hybrid between *R. japonica* Hoult. and *R. sachalinensis* (F. Schmidt) Nakai, is among the most aggressive invasive knotweeds in Europe. Despite its occurrence in several neighbouring Balkan countries, no confirmed records of the species, or indeed of the genus *Reynoutria*, have previously been reported from North Macedonia. During floristic observations conducted in July 2026 in the Mavrovo area, several large, well-developed clumps of *R. × bohemica* were recorded as cultivated ornamental plants in private gardens. More importantly, additional individuals were observed outside cultivation along a nearby stream, within riparian tall-herb vegetation. The recorded plants were identified based on diagnostic morphological characters, and their localities were georeferenced. The occurrence of individuals outside cultivation indicates an apparent initial stage of naturalization. Their position along a watercourse is of particular concern, since fragments of stems and rhizomes can be transported downstream and establish new populations. Experience from other parts of the western Balkan Peninsula demonstrates that initially isolated cultivated plants may become sources of extensive and difficult-to-control invasions, particularly in humid montane valleys and riparian habitats. The finding from Mavrovo represents an early warning and, to the best of our current knowledge, the first documented record of both *R. × bohemica* and the genus *Reynoutria* in North Macedonia. Immediate systematic mapping, taxonomic verification, monitoring, and removal of escaped individuals are recommended. Cultivated plants should also be gradually eradicated, accompanied by awareness-raising among residents and the relevant protected-area authorities. Rapid intervention at this stage could prevent the establishment and subsequent spread of the species throughout western North Macedonia.

Key words: alien plants, biological invasions, early detection, Mavrovo, riparian vegetation

Functional equivalence in peat-reduced and peat-free growing media

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Peat remains the principal reference constituent of horticultural growing media because it combines favourable physical, chemical and operational properties with comparatively consistent quality. However, the environmental consequences of peat extraction have created strong pressure to develop peat-reduced and peat-free alternatives. This critical narrative review, informed by a structured supplementary literature search, evaluates the functional equivalence of organic and bio-based constituents, focusing on coir, wood fibre, bark, compost, biochar and their mixtures. Functional equivalence is defined here as the capacity of a growing medium, within a defined crop-container system and under an appropriate irrigation and nutrient-management regime, to provide root-zone conditions and plant performance comparable to those of a peat-based reference medium. The evidence indicates that successful substitution cannot be assessed solely by the proportion of peat replaced or by similarity in initial bulk properties. No individual alternative consistently reproduces the full functional profile of peat. Coir can provide favourable water retention and aeration, but its chemical properties and environmental impact depend on origin, processing and transport. Wood fibre and bark primarily contribute structure and aeration, whereas compost and biochar may improve nutrient supply, buffering and biological activity; their performance remains strongly dependent on feedstock, processing and incorporation rate. Multi-component mixtures offer the greatest potential because complementary materials can compensate for one another's limitations. Plant performance, however, remains inseparable from container geometry, crop requirements, irrigation frequency and nutrient management. Environmental advantage should be assessed only among media delivering comparable plant performance and should account for processing, transport, water, fertiliser and energy requirements, biological safety and end-of-life management. Peat reduction should therefore align growing-medium composition with crop-management requirements and with the availability, processing and transport of its constituents, rather than simply replace one material with another.

Key words: multi-component mixtures; irrigation and fertigation; life-cycle assessment

Determination of the cytotoxic activities of *Cordyceps militaris* (L.) Link on A549 lung cancer, C6 glioma cancer, and DU-145 prostate cancer cell lines

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Cancer is a major health problem and one of the leading causes of mortality worldwide. The continuous increase in the number of cancer cases has made cancer a global concern and has increased the need for the development of new and effective therapeutic agents. Although conventional anticancer drugs can be effective, they may also cause undesirable side effects. Therefore, natural products, particularly medicinal mushrooms and their bioactive compounds, have attracted increasing attention as potential sources of alternative anticancer agents. *Cordyceps militaris* (L.) Link is a medicinal mushroom known to contain various bioactive compounds and has been investigated for its potential pharmacological properties. The aim of this study was to determine the in vitro cytotoxic activity of ethanol extracts obtained from *Cordyceps militaris* (L.) Link against A549 lung cancer, DU-145 prostate cancer, and C6 glioma cell lines. A mushroom specimen (A6848) collected from the Kömürlük locality in Sile, Istanbul, was used in the study. Ethanol extracts of *C. militaris* were prepared and their cytotoxic effects on A549, DU-145, and C6 cancer cell lines were evaluated using the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay. Cytotoxicity was evaluated at different extract concentrations, and IC50 values were statistically calculated. The cytotoxic activity of the *C. militaris* ethanol extract varied depending on the concentration and cell line. The highest cytotoxic activity against A549 cells was 60.61% at a concentration of 2400 µg/mL. For DU-145 cells, cytotoxicity reached 85.27% at 2400 µg/mL, while the highest activity against C6 glioma cells was 83.21% at the same concentration. The calculated IC50 values were 1343 µg/mL for A549, 723.2 µg/mL for DU-145, and 833.4 µg/mL for C6 cells. The findings demonstrate that the ethanol extract of *C. militaris* exhibits concentration-dependent cytotoxic activity against A549, DU-145, and C6 cancer cell lines, with the strongest activity observed in DU-145 cells. These results suggest that *C. militaris* may represent a promising natural source for the discovery and development of potential anticancer agents. However, further studies, including in vivo experiments and well-designed clinical studies, are required to clarify its mechanisms of action, efficacy, safety, and therapeutic potential.

Key words: *Cordyceps militaris*, Cytotoxicity, A549, DU-145, C6

Macromycetes as indicators of forest ecosystem functioning and biodiversity in the coniferous forests of the Shar Mountain

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Macromycetes represent an essential component of forest ecosystems, contributing to biodiversity maintenance, nutrient cycling, organic matter decomposition, and plant–fungus interactions. Coniferous forests of the Shar Mountain National Park in North Macedonia represent valuable habitats characterized by diverse ecological conditions and complex fungal communities. This study aims to evaluate the ecological diversity, distribution patterns, and functional roles of macromycetes within coniferous forest communities of the Shar Mountain. Field investigations were conducted in representative localities of the Shar Mountain, including Jelak, Popova Šapka, Lesnica, Adžina Reka, Orashje, and Rogačevo, covering different forest communities, altitudinal zones, and habitat conditions. Sampling was performed during several vegetation seasons, and fungal identification was carried out through macroscopic and microscopic analyses using standard mycological methods and current taxonomic literature. Most of the specimens were identified in a fresh condition, and the others, such as corticoids, as dried. Part of the samples have been preserved in the Macedonian Collection of Fungi (MCF), while the essential data about the collections are noted in the MACFUNGI database. Ecological analysis revealed the predominance of terricolous fungi, reflecting the important role of soil-associated fungal communities and ectomycorrhizal relationships with dominant coniferous tree species, including *Picea abies*, *P. peuce*, and *Abies alba*. The investigated fungal communities included several ecologically and conservationally significant taxa, such as *Cortinarius lividoviolaceus*, a species of conservation interest, as well as *Hygrocybe ceracea*, *H. reidii*, *Geastrum quadrifidum*, and *Leucopaxillus cerealis*, which highlight the high mycological value of the Shar Mountain forests. The occurrence of rare taxa and species newly recorded for North Macedonia emphasizes the importance of these habitats for fungal conservation. The results confirm that macromycetes serve not only as important functional components of forest ecosystems but also as valuable indicators of habitat quality and ecological changes. This study provides significant baseline information on fungal ecology in mountain coniferous forests and supports future biodiversity assessments, conservation strategies, ecological monitoring, and sustainable management of protected forest ecosystems in the Shar Mountain region.

Key words: ectomycorrhiza, ecological groups, biodiversity, coniferous forests, Shar Mountain, North Macedonia.

New site of *Lactarius omphaliformis*, endangered species on the Red List of Fungi in North Macedonia

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Red List assessment provides a framework for evaluating the conservation status of species and assigning them to appropriate threat categories. Red Lists are important for decision making, providing basis for proposing and implementing appropriate conservation measures. Therefore, one of the crucial steps in the assessment process is the identification of the major threats affecting the species. Monitoring known populations and incorporating new data, such as species distribution, number of individuals, are needed to follow the status of endangered species. The Red List of Fungi in North Macedonia comprises 64 taxa, assessed in consistency with the IUCN guidelines. Six species have been assigned as critically endangered, 19 as endangered, 34 as vulnerable, two as near threatened, two as least concern and one species is data deficient. *Lactarius omphaliformis* was assessed as endangered species in North Macedonia (2021), known from only two sites in well-developed alder forest, in Belchishta Wetland and in Monospitovo Swamp. Both known sites are Emerald areas, additionally Belchishta Wetland was recently proclaimed a Nature Park (2026), while Monospitovo Swamp is a protected area according to the Physical Plan of the country. During field research on the diversity of fungi in the riparian habitats of Lake Prespa in 2025-2026, numerous *Alnus* forest sites were visited, however *L. omphaliformis* was recorded at single site. At this new site, only two functional individuals of the species were registered, forming scattered and gregarious basidiomata. The new locality near Dolno Perovo is an old, well-developed alder forest with visibly higher water availability compared with other investigated *Alnus* localities in the area, where old trees are scarce and the conditions appear comparatively drier. These findings evidence that *L. omphaliformis* is a rare species that thrives in a well-preserved habitats. The species is categorized as endangered, under the criteria B1ab(i,iii,iv); B2ab(ii,iii,iv), though a reassessment of the species is needed, counting the exact number of functional individuals at the previously known sites in order to estimate whether it might meet the criteria C2a(i) for critically endangered. The major threat to this species is the continuous loss and degradation of the suitable habitats leading to decline in population trend. Wetlands with *Alnus* spp. are rare in the country and are highly impacted by anthropogenic pressures. The melioration of wetlands for land-use change to arable land, the irrigation that induces hydrological changes, and fires, are the major threats to maintaining a suitable habitat and a stable population of the species. These habitats, according to the latest Biodiversity Action Plan (2018) are estimated to be under high-intensity threat and are regarded as one of the most endangered habitats in the country. The new site near Dolno Perovo is a well-preserved alder forest, important for maintaining stable population of *L. omphaliformis*, therefore conservation measures should be discussed and proposed.

Key words: *Alnus rohlenae*, biodiversity, conservation, macrofungi, protected area

The second record of *Pogonoloma macrorhizum* in Türkiye

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Pogonoloma macrorhizum is regarded as a rare species across both Central and Southern Europe. Although *Pogonoloma macrorhizum* was first mentioned by Marchand in 1986 as existing in Türkiye, no record has been given regarding the origin of the mushroom. To date, no records of this species have been found. *P. macrorhizum* samples were collected in Manisa-Turgutlu, Güney village, in *Quercus cerris* var. *cerris* forest. After macroscopic photographs of the collected specimens were taken in their habitats, they were brought to the laboratory and dried in a drying oven at 40-45°C. After reviewing the relevant literature, the specimen was identified as *Pogonoloma macrorhizum*. This rare species is distinguished by the production of exceptionally large and fleshy fruiting bodies with fasciculate stems and possesses a very specific smell. The species description is given along with macro-micro and habitat characteristics. This species grows in Central European temperate broadleaved forests, in tufts with numerous specimens growing together, under broadleaved trees, mainly larger oaks. Spring to autumn. Although Marchand (1986) mentioned its presence in Türkiye in 1986, the lack of information regarding the collection area and sample details means that this newly discovered specimen will fill that gap.

Key words: *Pogonoloma macrorhizum*, rare species, Turkish mycota

Advancing eDNA-Based approaches in Europe: Opportunities for ecological research, biodiversity assessment and biomonitoring through eDNA^{Europe} and eDNA4Policy

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Environmental DNA (eDNA) is expanding the toolbox available for biodiversity assessment, species detection and ecological monitoring. However, the broader development and application of eDNA-based approaches across Europe in biodiversity research, environmental assessment, conservation, monitoring and policy support depend on more than technological progress alone. They also require access to expertise and infrastructure, standardised workflows, capacity building, and the active participation of countries and regions with different levels of research intensity and implementation experience. This challenge is particularly relevant for Inclusiveness Target Countries (ITCs). Their active participation in emerging European eDNA initiatives provides an opportunity to engage directly with current scientific and methodological developments, contribute to standardisation and methodological development, and build expertise in parallel with other European countries. Two complementary European initiatives provide a framework for advancing eDNA-based approach in ecological research, biodiversity assessment and policy-driven monitoring. eDNA^{Europe} is currently in the process of being established as a pan-European society dedicated to advancing the development, standardisation and implementation of eDNA-based methods, while connecting researchers, practitioners, policymakers and industry representatives across the European eDNA community. In parallel, the recently approved COST Action CA25163, eDNA4Policy, brings together expertise from across Europe to support the broader uptake of

eDNA-based approaches in policy-driven monitoring. Through coordinated activities covering methodological development, data processing, standardisation, quality assurance, policy-relevant indicators and regulatory integration, the Action aims to strengthen the links between scientific advances and their practical application in monitoring and decision-making. Active participation in these initiatives can contribute to building an inclusive European eDNA framework that reflects regional environmental diversity and supports coordinated biodiversity monitoring across countries.

Key words: Keywords: environmental DNA, eDNA^{Europe}, eDNA4Policy, COST Action, Inclusiveness Target Countries

An overlooked Natura 2000 species: The conservation potential of *Theodoxus transversalis* (Gastropoda: Cycloneritida: Neritidae) for designation of special areas of conservation (SACs) in North Macedonia

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The striped freshwater nerite *Theodoxus transversalis* (Pfeiffer, 1828) is an endangered species endemic to parts of the European river systems. This species is distributed along the Danube River system where its geographical range includes Germany, Austria, Slovakia, Hungary, Serbia, Bulgaria, Romania and Croatia. Unfortunately, the malacological surveys over the last 20 years have shown severe population declines as the species is disappearing from some parts of Romania, Hungary and Slovakia indicating towards urgent need of acting for its conservation. The known distribution of *T. transversalis* appears to extend beyond the Danube River basin, as previous studies have reported the species from the Vardar River near Skopje and from Lake Dojran in the Republic of N. Macedonia. However, as all this data is either outdated, not precise or based only on shell's specimens, the presence of this species in the country has remained debatable up until now. Namely this study presents the results from the three-year hydrobiological investigations throughout river Vardar and its tributaries. Biological samples were obtained using a 500 µm mesh hand-net, preserved in 75% ethanol and transferred to the Laboratory of invertebrate systematics for further analysis. The results showed presence of dense populations of viable specimens of the striped freshwater nerite at four localities in Demir Kapija gorge (two sampling localities on river Vardar, one sampling locality at the mouth of river Chelevechka Reka and one sampling locality at the mouth of river Mala Javorica), making these the first valid records of the presence of this species in the country. As *Theodoxus transversalis* is classified as an Endangered species on the IUCN Red List of Threatened Species, its confirmed presence further highlights the exceptional natural values of the recently designated Demir Kapija Gorge Monument of Nature, as well as the broader freshwater biodiversity of R. North Macedonia. Furthermore, *T. transversalis* is listed in Annexes II and IV of the EU Habitats Directive (92/43/EEC), emphasizing its importance for biodiversity conservation. The confirmation of the species as a component of the Macedonian freshwater fauna provides an additional criterion for improving the accuracy of the future Special Areas of Conservation (SACs) designation within the Natura 2000 network in the country. In particular, its occurrence should be considered in the identification and delineation of sites supporting populations of this threatened and habitat-specific species.

Key words: Neritidae, Vardar River, Demir Kapija, Natura 2000

Butterfly diversity of Belchishko Blato Wetland, North Macedonia

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Lowland wetlands in North Macedonia are particularly affected by historical drainage, agricultural conversion and continuing anthropogenic pressures. Although the national-scale inventory for wetlands provides the essential basis for identifying their spatial distribution, site-level biodiversity studies are needed to evaluate their ecological importance and establish appropriate conservation priorities. Butterflies are suitable indicators in this regard, due to their sensitivity to changes in vegetation structure, hydrological conditions and habitat management in wetlands. This study aimed to update the inventory of butterflies occurring in Belchishko Blato Wetland located in the southwest of North Macedonia. In addition, assessment of their conservation importance was conducted. Published and unpublished records were reviewed and supplemented by field surveys conducted during four visits in July and August 2020 and June 2021. Butterflies were recorded through non-systematic searches along variety of habitat types in the wetland. Individuals were identified by direct observation, while records were georeferenced and stored in a dedicated database. The conservation importance of the recorded species was evaluated using relevant national and international legislation. A total of 53 butterfly species belonging to five families (Hesperiidae, Pieridae, Papilionidae, Lycaenidae and Nymphalidae) were recorded during the field surveys. When these observations were combined with unpublished data, 63 species were confirmed from the wetland. Inclusion of species reported in previous publications increased the cumulative inventory to 73 species, representing approximately 35.6% of the butterfly fauna known from North Macedonia. The investigation contributed 25 species not previously documented from Belchishko Blato. The Large Copper, *Lycaena dispar* (Haworth, 1802), was identified as the principal species of conservation importance. It is included in Annexes II and IV of the EU Habitats Directive and is strictly protected under national legislation. Six records from Belchishko Blato indicate its association predominantly with wet meadows, including habitats supporting its larval host plants from the genus *Rumex*. The high species richness recorded within a relatively limited area highlights the importance of Belchishko Blato for butterfly conservation. Habitat conversion, habitat succession, drainage, agricultural intensification, pesticide use and uncontrolled grazing may adversely affect its butterfly assemblages. Protection of the wetland's hydrological regime, maintenance of wet meadows and long-term standardized monitoring are therefore essential for detecting population trends and supporting adaptive conservation management.

Key words: butterfly diversity; wetland conservation; *Lycaena dispar*.

Composition and distribution of Fulgoromorpha (Hemiptera) on the island of Crete: preliminary results

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Crete, the largest Greek island, is characterised by considerable topographic, climatic and habitat diversity, as well as a complex geological history. These factors have contributed to the formation of a distinctive insect fauna with a substantial endemic component. Nevertheless, the Fulgoromorpha (Hemiptera) of the island remain poorly studied, and available information on the distribution of many species is fragmentary. This study presents preliminary results from an ongoing revision of the composition and distribution of Fulgoromorpha on Crete. It builds upon the recently published revision of Auchenorrhyncha material deposited in the Natural History Museum of Crete, which substantially expanded the knowledge of the island's fauna and demonstrated the importance of natural history collections for documenting regional biodiversity. The present study incorporates additional material collected by the authors from various parts of Crete and deposited in the Zoological Collection of Sofia University (BFUS) and the Natural History Museum of Crete (NHMC). The material was collected from numerous localities representing all major administrative regions of Crete and a broad range of habitats and elevations, from coastal wetlands, dunes and maquis and phrygana to forests, gorges, plateaus and high-mountain grasslands. The records are being used to update the checklist of the Cretan Fulgoromorpha and to analyse the geographical and altitudinal distribution of the taxa. The new material documents the presence of Dictyopharidae and Caliscelidae on Crete for the first time. The ongoing identification and revision of the material are also revealing additional taxa not previously recorded from Crete. The emerging distributional pattern indicates that current understanding of the fauna remains strongly influenced by uneven collecting effort. Coastal and easily accessible areas are generally better represented, whereas the interior and high-altitude parts of the island remain comparatively poorly investigated. The new records demonstrate that further targeted sampling, combined with the revision of existing collections, will continue to expand knowledge of the diversity and distribution of Fulgoromorpha on Crete.

Key words: Keywords: planthoppers, species diversity, new records, Greece, museum collection

Discovery of three new subanophthalmous species of the rove beetle genus *Lathrobium* Gravenhorst from the Shar Planina and Jakupica Mountains in North Macedonia (Coleoptera, Staphylinidae, Paederinae)

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Three new subanophthalmous species of the genus *Lathrobium* Gravenhorst, 1802 from North Macedonia are described, illustrated, and distinguished from similar species: *Lathrobium* sp. 1 sp. nov. (Shar Mountains: Rudoka and Prshovska reka), *L. sp. 2* sp. nov. (Shar Mountains: Popova Shapka), and *L. sp. 3* sp. nov. (Jakupica Mountains: Marina Dupka). Important diagnostic characters of newly collected specimen of *Lathrobium jakupicense* Rambousek, 1928 are illustrated. With the new species, six subanophthalmous *Lathrobium* are known from the Shar Mountains (*L. knirschi* Rambousek, 1928, *L. graniticole* Rambousek, 1928, *L. scardicum* Nonveiller, Pavičević & Popović, 2001, *L. irenae* Pavičević, Popović & Stevanović, 2001 and the two new species described here) and two from the Jakupica Mountains (*L. jakupicense* Rambousek, 1928 and the new species). The Shar Mountains thus harbours the highest diversity of the group known from any single massif of the Balkan Peninsula. The ecology and distribution of the species are discussed in relation to the geological substrate and to the Pleistocene glaciation of both massifs, and the conservation of the group is briefly addressed. A map of distribution of subanophthalmous *Lathrobium* species of Shar and Jakupica Mountains are presented.

Key words: taxonomy, new species, Balkan Peninsula, Europe, Palearctic region, taxonomy, endemism, glacial relicts, nature protection.

Diversity and conservation significance of beetle assemblages (Coleoptera) along riparian ecosystems of the Vardar River and the tributaries (North Macedonia)

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Terrestrial beetles are valuable indicators of habitat quality, ecological continuity, and environmental changes. Riparian networks within major river corridors function as critical biogeographical connectors and microclimatic refugia, facilitating faunal exchange between Mediterranean and continental zones across the Balkan Peninsula. This study assesses the diversity, functional distribution, and conservation significance of coleopteran communities along riparian and semi-natural habitats of the Vardar River in North Macedonia, a key ecological axis within the Balkan–Aegean zone. Field sampling was conducted during spring-summer of 2026 across ten localities encompassing riparian forests, thermophilous scrubs, alluvial meadows, and agricultural land. Beetles were collected with manual sampling, pitfall traps, and air-intercept traps. A total of 62 species belonging to 23 families were recorded. High ecological integrity and conservation value were concentrated within well-preserved tributaries, specifically along the Babuna and Kadina rivers. These key zones supported highly specialized assemblages, incorporating unique saproxylic and xerothermophilous elements, notably within Elateridae, Scarabaeidae, and Tenebrionidae, dependent on microhabitat continuity and deadwood availability. Conversely, heavily modified agricultural landscapes supported homogenous beetle communities. These findings demonstrate that while the main Vardar stem is substantially fragmented by human activity, its natural tributaries function as vital biodiversity reservoirs that preserve regional ecological connectivity across the continental–Mediterranean transition zone.

Key words: Coleoptera, biogeographical transition, riverine ecosystems, ecological connectivity.

Habitat types of above timberline ecosystems in the high mountains of Central Greece as habitats for Orthoptera species

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The high mountains of Central Greece are characterized by a pronounced geomorphological relief, resulting in the formation of many microenvironments. Within these microenvironments, many vegetation types develop, providing habitat for a wide range of common as well as rare and endemic Orthoptera species. The purpose of this work is to investigate the function of above timberline ecosystems habitat types in the high mountains of Central Greece, as habitats for Orthoptera. For the vegetation survey, a total of 276 plant samplings were made in the high mountain ecosystems of Vardoussia, Parnassos, Tymfristos, Oiti and Helicon, using the Braun-Blanquet method, during July 2023, while additional bibliographic data were also considered. Within each sampling plot, the presence and relative abundance of Orthoptera species were also recorded and estimated. Six habitat types of Directive 92/43/EEC were recorded, supporting Orthoptera species of conservation interest. According to the collected data, the most important for the presence of Orthoptera seem to be (a) 4090 “Endemic oro-Mediterranean heaths with gorse”, where *Astragalus* spp. plant species dominates, and endemic (e.g. *Parnassiana parnassica*, *Oropodisma parnassica*) and rare (*Rhacocleis lithoscirtetes*) Orthoptera species occur, and (b) 6230 “Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in Continental Europe)”, where *Nardus stricta* and/or *Trifolium parnassi* plant species dominates, and endemic (e.g. *Chorthippus willemsei*, *Eupholidoptera megastyla*) and endangered (e.g. *Oropodisma tymphrestosi*) Orthoptera species occur. Orthoptera with conservation interest were also found in habitat types 4060 “Alpine and Boreal heaths” and 8140 “Eastern Mediterranean screes”, while none were identified in 6170 “Alpine and subalpine calcareous grasslands” and 6430 “Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels”.

Key words: mountain ecosystems, *Parnassiana*, endemism, conservation

Hidden in collections: updated diversity and distribution of sawflies revealed through digitisation of the Sofia University Zoological collection (Bulgaria)

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Entomological collections are invaluable repositories of historical biodiversity data. They provide essential information on species distribution over time and enable assessments of changes to ecosystems driven by climate change and anthropogenic pressure. They form a critical foundation for long-term monitoring and conservation strategies. Therefore, the maintenance and curation of such collections are of primary importance. Modern collection management increasingly relies on digitisation, supported by platforms such as Specify, a widely adopted open-access, open-source database system. It enables the integration of specimen metadata, images, molecular data, and georeferenced records — particularly crucial for historical specimens that lack precise geographic coordinates. Sofia University, the oldest higher education institution in Bulgaria, houses a zoological collection comprising over 120,000 specimens from Bulgaria, the Balkans, and the Mediterranean region. Until now, sawflies (Hymenoptera: Symphyta) within this collection had not been studied. The present study focuses on the identification, digitisation, curation, and assessment of the Symphyta collection in order to evaluate species richness and provide open-access data to the international scientific community. Using a modern taxonomic approach, 417 specimens representing 93 species from seven families were identified. Despite limitations in sampling methods (mainly by sweeping), five species are newly recorded for Bulgaria, one of which is also new to the Balkan Peninsula. The known Symphyta fauna of Bulgaria now comprises 437 species. Specific localities were documented for the first time for four species previously reported from Bulgaria without precise data. The presence of two species was also confirmed after more than a century. Additionally, second localities were established for seven species that were previously known from only one site in the country. High-quality macrophotographs of endemic and rare species were also produced. These results highlight the scientific value of historical collections and demonstrate the importance of their modern investigation for advancing biodiversity research. Financial support under the project No. 80-10-204/22.04.2026 (Scientific Research Fund, Sofia University) is gratefully acknowledged.

Key words: biodiversity; fauna; Symphyta; taxonomy;

Initial assessment of terrestrial invertebrate communities in newly exposed littoral habitats of Lake Prespa (North Macedonia)

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Climate change and anthropogenic pressures have contributed to substantial hydrological decline in Lake Prespa, exposing previously submerged littoral areas to terrestrialization. This study provides an initial assessment of terrestrial invertebrate communities developing in these newly exposed habitats. Seasonal field sampling was conducted from October 2025 to June 2026 at three localities—Stenje, Dolno Perovo, and Slivnica—covering reed belts, lacustrine sandy habitats, and poplar woodland. Invertebrates were collected using pitfall and air-intercept traps, complemented by active sampling. A total of 6,111 individuals representing 19 taxonomic groups were recorded, with Coleoptera, Hymenoptera, Myriapoda, Araneae, and Collembola comprising the dominant groups. Coleoptera included 1,423 individuals from 29 families, with Carabidae and Staphylinidae being the most abundant. The beetle assemblages showed pronounced spatial and seasonal variation, with the highest species richness recorded in the sandy littoral habitat at Slivnica during spring (41 species), followed by a marked decline during summer and autumn. In contrast, reed habitats and poplar woodland supported comparatively more stable communities across seasons. The representation of detritophagous and predatory taxa indicates the development of trophic interactions within the newly terrestrialized habitats. Although long-term monitoring is required to track succession, these findings provide baseline information for future biodiversity monitoring and management of receding lake littoral ecosystems.

Key words: Coleoptera, lake recession, terrestrialization, littoral ecotones, biodiversity monitoring

Integrated studies of alien Hemiptera in urban areas of Bulgaria: preliminary results on diversity, distribution and monitoring

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The order Hemiptera includes numerous economically important pests and vectors of plant viruses and phytoplasmas. Globalisation, trade, tourism and climate change facilitate the introduction and spread of non-indigenous species, while urban ecosystems may serve as entry points and reservoirs. During the formation of “urbanocenoses”, many indigenous species disappear, while numerous non-indigenous species are introduced and subsequently spread within cities. This process may threaten highly specialised, rare and endemic species and may consequently increase the proportion of widespread species with greater ecological plasticity. Thus, urban faunas in different cities are often more similar to one another than each is to the fauna of its surrounding non-urban areas. This pattern is particularly evident in regions of high biodiversity, such as the Balkan Peninsula. In Bulgaria, data on non-indigenous Hemiptera remain fragmented. The project aims to develop an evidence-based framework for their identification, mapping, monitoring and assessment in urban environments by integrating literature and collection data, citizen-science records, field surveys, morphological and molecular identification, GIS-based mapping, and assessments of invasion status and host associations. A consolidated checklist of the non-indigenous Hemiptera recorded in Bulgaria currently includes 107 species: 9 Psylloidea, 37 Coccoidea, 13 Auchenorrhyncha and 48 Heteroptera. The preliminary field-survey results presented here are based on material collected between June and September 2026 across a broad geographical area, including all regional centres, the southern Black Sea coast and south-western Bulgaria. The surveys revealed several taxa of scale insects, including representatives of the genera *Pulvinaria*, *Ceroplastes*, *Pseudococcus*, *Parthenolecanium*, *Gossyparia* and *Icerya*; aphids of the genus *Takecallis*; hoppers of the genera *Hishimonus*, *Acanalonia*, *Orosanga*, *Pochazia*, *Orientus*, *Japananus*, *Stictocephala*, *Macropsis*, *Arboridia* and *Erasmoneura*; and jumping plant lice of the genera *Trioza*, *Homotoma* and *Colophorina*. Some of these taxa were recorded for the first time in particular regions of the country or in Bulgaria as a whole. Representatives of Aphididae collected from bamboo in Sandanski and Tsarevo are currently undergoing taxonomic identification. For *Metcalfa pruinosa* (Say, 1830), a standardised protocol for population mapping and the collection of bionomic data was developed. The parasitoid *Neodryinus typhlocybae* (Ashmead, 1893) was collected and successfully reared under laboratory conditions; associated hyperparasitoids were also recorded. These preliminary results reveal a considerable diversity of non-indigenous Hemiptera in Bulgarian urban habitats and provide an initial basis for assessing their distribution, host associations and establishment status. Most introduced Hemiptera species currently known from the Bulgarian fauna were initially detected in anthropogenic environments. Recently established urban populations of non-indigenous species may subsequently act as sources for their further spread. Our results demonstrate the importance of urban environments and coastal areas as major entry points and initial stages in the establishment and spread of non-indigenous Hemiptera. Further morphological and molecular analyses will support species-level identification and contribute to the development of a national monitoring and early-warning framework for non-indigenous Hemiptera in Bulgaria. This research is funded by the Bulgarian National Science Fund (BNSF), grant KII-06-H91/11, dated of 04.12.2025.

Key words: biological invasions; urban ecology; scale insects; early warning; Bulgaria

Negative impact of the invasive species *Callinectes sapidus* on native species in Albanian lagoons

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The invasive blue crab *Callinectes sapidus* represents one of the most ecologically influential non-native species in Albanian lagoon ecosystems. This study assesses its distribution, population dynamics, and impact on local biodiversity, using data collected from seven Albanian lagoons, with a detailed focus on the Karavasta Lagoon. Subpopulation monitoring was conducted during the spring–summer season, analyzing population trends, and morphometric and meristic characteristics of captured individuals. The methodology was based on periodic population monitoring and biological analysis of specimens. For each individual, carapace width, carapace length, total weight, and sex were recorded in order to determine population structure and morphological variability. In addition, data on capture quantities, seasonal catch dynamics, and consumption levels were collected, allowing assessment of the relationship between exploitation of this invasive species and its population control. The results indicate a significant presence and a continuing expansion trend of *C. sapidus*, which exerts strong pressure on native fish communities, particularly eel populations. The study also evaluated the role of promoting blue crab consumption as a potential mitigation strategy. Findings suggest that increasing promoting consumption may contribute to the control of invasive populations and help reduce their negative impact on the biodiversity of Albanian lagoons.

Key words: Lagoon, ecosystem, blue crab, European eel, invasive.

Phylogenetic placement of caddisfly species (Insecta: Trichoptera) from Shar Planina Mountain (North Macedonia) using mitochondrial mtCOI gene marker

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Phylogenetic trees reconstruction presents the evolutionary relationships among taxa, while the visualization of genetic differentiation contributes to the validation of their separation and provides evidence for the monophyletic origin of clades corresponding to morphologically identified taxa, enabling higher level of confidence in species identification. The integrative taxonomy often recognizes these methods as a complementary tool especially when the genetic distances between the DNA sequences are extremely small and there is a risk that such differences may not be detected during standard DNA barcoding. Therefore, they are particularly important in the detection of cryptic species and conversely can also contribute to the synonymizing of taxa in cases where genetic variability and phenotypic plasticity may lead to incorrect taxonomic identification. In order to advance the taxonomic knowledge of the caddisfly fauna of the Shar Planina Mountain, this study investigates this group of insects from multiple complementary perspectives. In addition to conventional morphological identification, all recorded species were DNA barcoded and incorporated into a phylogenetic reconstruction, providing an integrative approach to species identification and assessment of their evolutionary relationships. For the construction of the dataset used for phylogenetic tree reconstruction, sequences from both the BOLD Systems and NCBI databases were included. The selected sequences were subsequently aligned using MAFFT software. The alignments were visually inspected and manually refined using BioEdit 7.0.1. Phylogenetic reconstruction was performed using the Bayesian inference (BI) approach implemented in MrBayes 3.2.6. Pairwise p-distance calculations among the mtCOI sequences, as well as Maximum Likelihood (ML) analysis, were performed using MEGA 12. The graphical representation of the topology of the final phylogenetic tree was aesthetically edited using Adobe Photoshop CS6. The correctly identified species were clearly applied to distinct clusters in the phylogenetic reconstruction, further validating their taxonomic assignment and establishing the phylogenetic position of each species and even contributed to the detection of misidentified or incorrectly assigned sequences in public databases such as BOLD and NCBI. Finally, the well-structured topology of the phylogenetic tree also enabled the clear separation of the suborders Integripalpia, Annulipalpia, and Spicpalpia into distinct clades. The integration of morphological taxonomy, DNA barcoding, and phylogenetic reconstruction demonstrated the potential of an integrative approach in strengthening species identification and resolved some of the taxonomic uncertainties in the caddisfly fauna of North Macedonia especially within the genera *Hydropsyche* and *Philopotamus*. These findings highlight the need to further develop integrative taxonomy in the country through the generation of reliable reference DNA libraries, critical assessment of existing database records and the integration of molecular and morphological data, providing a stronger foundation for biodiversity assessment, monitoring, and conservation.

Key words: integrative taxonomy, caddisflies, mtCOI

Population ecology and conservation dynamics of saproxylic beetles on Jablanica Mountain, North Macedonia

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Saproxylic beetles are key functional drivers of forest nutrient cycling and structural complexity, serving as bioindicators of old-growth forest integrity. While habitat loss and fragmentation have degraded their populations across Central Europe, the mountain massifs of the southern Balkan Peninsula remain important yet understudied areas for saproxylic beetle diversity. This study evaluates the community composition and conservation ecology of saproxylic beetles on Jablanica Mountain, an important core area of biodiversity in the region. Fieldwork was conducted across 14 localities, primarily in thermophilous oak, montane beech, and sweet chestnut (*Castanea sativa*) forests. The research was conducted through targeted field trips between 2019 and 2024, timed to coincide with the peak activity period of saproxylic beetles. Material was collected by hand searching, pitfall, window-intercept, and air-intercept traps. A total of 46 saproxylic species belonging to 22 families were recorded. Over 70% of the species (33), dominated by representatives of Cerambycidae, Scarabaeidae, Lucanidae, and Elateridae, hold international conservation status. Species richness peaked within thermophilous habitats near the St. Spas Monastery (17 species) and sweet chestnut stands at Cvetko (13 species). These habitats harbor key populations of threatened bioindicator species, including *Cerambyx cerdo*, *Rosalia alpina*, *Morimus asper* subsp. *funereus*, *Osmoderma barnabita*, and *Lucanus cervus*, alongside rare species such as *Gnorimus variabilis* and *Lacon querceus*. Our findings highlight Jablanica Mountain as a vital regional stronghold for European saproxylic beetle conservation. However, these populations face mounting risks from localized logging, infrastructure development, and allochthonous afforestation, emphasizing the urgent need for ecosystem-based forest management and targeted habitat protection.

Key words: Natura 2000, old-growth forest indicators, deadwood dynamics, Conservation ecology, Southern Balkan

Preliminary assessment of the effects of submerged light spectrum (LED vs. CFL) on nocturnal insect attraction and mortality

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Rapid expansion of artificial light at night (ALAN) can cause severe behavioral disruption and mortality in nocturnal insects. This study presents a preliminary assessment of submerged swimming pool illumination as a potential unintentional aquatic ecological trap for terrestrial and semi-aquatic entomofauna, addressing an overlooked aspect of ecological light pollution. A 30-day field experiment was conducted during June and July 2024 within a suburban oak forest ecotone in Skopje, North Macedonia. The experiment evaluated the attractive effects of broad-spectrum white LED and yellow CFL submerged lights, alternating with unlit phases. Experimental illumination was applied from 22:00 to 05:00, corresponding to a 7-hour nightly exposure period. Captured organisms were collected through the pool's passive surface-skimmer system, which functioned as an incidental trap, and biomass (wet and dry weight) and taxonomic composition were systematically quantified. A total of 762 specimens belonging to 15 taxonomic groups were recorded, with Hymenoptera and Coleoptera strongly dominating the catches. White LED produced the highest overall attraction and the greatest total biomass of captured insects, driven largely by massive swarming of the scarab beetle *Amphimallon* sp. Species-level analysis of Coleoptera revealed distinct spectral responses: total catches declined under yellow CFL, whereas the glow-worm *Lampyris noctiluca* was detected exclusively under this spectrum, suggesting potential spectral attraction or visual masking that warrants further research. High mortality of predatory taxa, particularly Coccinellidae and Carabidae, occurred mainly under white light. These preliminary results suggest that private swimming pools may represent an overlooked ecological trap for suburban entomofauna and that changes in lighting spectrum may have contrasting effects among insect groups. However, the observed patterns should be interpreted cautiously, and further research across multiple locations, seasons, pool types, and environmental conditions is needed before these findings can be extrapolated more broadly.

Key words: artificial light at night (ALAN), Coleoptera, spectral attraction, phototaxis

Saproxylic beetle diversity in high conservation value forests of Mavrovo National Park

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Saproxylic beetles are key bioindicators of forest ecosystem integrity and deadwood dynamics across Europe, yet spatial gaps in their distribution hamper regional conservation strategies. This study addresses a key knowledge gap in North Macedonia by providing a baseline assessment of saproxylic beetle fauna based on targeted field inventories conducted during the summer of 2023 within Mavrovo National Park. Community composition, spatial diversity, and conservation status were evaluated across key montane habitats and ecotones within the Korab and Bistra massifs. Field sampling yielded 71 taxa belonging to 16 families from thermophilic oak, mesophilic beech, and highly vulnerable riparian forests. Species richness was highest within riparian ecotones and montane beech-alder belts, particularly near the Zhirovnica mountain lodge and along the Radika, Adzina, and Tairovska rivers. The presence of eight species with global and European conservation status was confirmed, including taxa listed in the EU Habitats Directive: *Rosalia alpina*, *Cerambyx cerdo*, *Morimus asper* subsp. *funereus*, *Osmoderma barnabita*, and *Lucanus cervus*. However, these relict assemblages face high pressures from habitat fragmentation, logging, and small hydropower developments. These findings underscore Western Balkan riparian-forest ecotones as critical microclimatic refugia and biodiversity hotspots, pointing the need for targeted monitoring and protection of these vulnerable habitats.

Key words: Coleoptera, Habitats Directive, riparian ecotones, deadwood dynamics, Balkan mountain refugia

Spatial patterns of Trichoptera diversity and assemblage structure in the Shar Planina mountain (North Macedonia)

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The aquatic insects present a large group of aquatic invertebrates that inhabit rivers, streams, lakes, and reservoirs, where they are associated with various types of substrates, such as mineral sediments, detritus, macrophytes, and filamentous algae. Among them, caddisflies are considered one of the most diverse groups, currently comprising more than 17,000 extant species distributed worldwide. Due to this high taxonomic diversity, caddisfly species exhibit a broad range of specific habitat and environmental preferences. Their spatial distribution and community structure within a given area are determined by food availability and interactions with the physicochemical conditions of their habitat. Thus, caddisfly community structure is determined by complex interactions of numerous factors, including water quality, water flow, water temperature, sediment type, oxygen concentration, and environmental conditions surrounding the watercourse. Additionally, caddisfly community structure does not change solely as a result of natural environmental variability but also in response to anthropogenic impacts that lead to the loss or degradation of caddisfly habitats and represent one of the greatest threats to these organisms. This study presents the results obtained from intensive entomological investigations of the caddisfly fauna of Shar Planina mountain conducted from May 2021 to October 2022. Adult caddisflies were collected using an entomological net during daytime and UV light traps during the night. The collected biological material was taxonomically identified and, together with physicochemical measurements and field observations, analyzed using appropriate software. The Canonical Correspondence Analysis (CCA) was performed using R statistical software, Principal Component Analysis (PCA) using XLSTAT, while similarity between caddisfly communities across study sites, water body types, and climate–vegetation zones was assessed through cluster analysis using dendrograms based on the UPGMA algorithm. The structural characteristics of caddisfly communities at each study site were assessed using structural indices of species richness (d), diversity (H'), and evenness ($J(e)$). The results showed that caddisfly community structure changes spatially across climate–vegetation zones as a consequence of the specific characteristics of each locality, including microhabitat heterogeneity, the presence of riparian vegetation, physicochemical conditions of the water, anthropogenic pressure, and geographic location. The Shannon–Wiener Diversity Index (H') indicates that species diversity was highest at localities T1 (1.22), T4 (1.16), T2 (0.95), and T9 (0.95), while the lowest diversity was recorded at T12 (0.27) and T5 (0.00). The Canonical Correspondence Analysis (CCA) showed that, in addition to the subtle influence of pH and dissolved oxygen concentrations, elevation and water temperature were the main factors driving the grouping of localities according to caddisfly community composition. These results demonstrate clear spatial variation in caddisfly community structure across Shar Planina mountain, reflecting differences in local habitat characteristics and climate–vegetation zones. Elevation and water temperature were identified as the main factors influencing community composition, while pH and dissolved oxygen had a weaker effect. Overall, the findings highlight the importance of environmental gradients and local habitat conditions in shaping caddisfly diversity and distribution on Shar Planina mountain.

Key words: caddisflies, Shar Planina mountain, community structure, environmental gradients

Stoneflies (Insecta: Plecoptera) of Shar Planina Mountain (Republic of North Macedonia): Rarity and endemism

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Shar Planina, the largest mountain range in Republic of North Macedonia, harbors high biodiversity shaped by continental, mountainous, and alpine climates. The diversity of Plecoptera has historically been studied by foreign researchers and extensively by Ikonomov, who greatly expanded the national list of species. The main purpose of this study is to compare all published literature data for this group of aquatic insects with our three-year research, where the fusion of these results will provide the first complete checklist of stoneflies for Shar Planina Mountain (North Macedonia). The three-year's survey (May 2021–September 2023) across 24 localities of the Shar Planina Mountain yielded adult Plecoptera specimens collected with entomological nets and beating sheets, labeled with collection data, preserved in 96% ethanol, and morphologically identified at the Institute of Biology (Skopje) using a stereo microscope and standard keys. A comprehensive literature review indicates that the Plecoptera fauna of the Shar Planina Mountain comprises 73 species, including four Macedonian endemics (*Nemoura zwicki* Sivec, 1980; *Isoperla breviptera* Ikonomov, 1980; *Taeniopteryx fusca* Ikonomov, 1980; *Taeniopteryx stankovici* Ikonomov, 1978). Our three-year investigation at 24 localities resulted in 41 taxa, among which 12 represent new records for Shar Planina and four (*Chloroperla kisi* Zwick, 1967, *Nemoura asceta* Murányi, 2007, *Nemoura* cf. *rivorum* Ravizza & Ravizza Dematteis, 1995, and *Perla* cf. *madritensis* Rambur, 1842) are newly documented for the Macedonian fauna. The total number of species from the literature data, along with the identified specimens from material collected over three years of fieldwork, includes 81 taxa. The analysis of frequency and endemism revealed that 12 of the 20 ecologically important Plecoptera species in the Shar Planina Mountain are Balkan endemics, while several of these endemics (e.g., *Chloroperla kisi*, *Siphonoperla graeca*, *Leuctra hippopoides*, *Nemoura asceta*, *Isoperla bosnica*, *Brachyptera beali*, *Brachyptera helenica*) are simultaneously considered rare within the territory of Republic of North Macedonia. In addition, species such as *Leuctra major*, *Leuctra cingulata*, *Nemoura longicauda*, *Isoperla albanica*, and *Besdolus illyricus* exhibit restricted distribution ranges. With more than one-fourth of the total stonefly fauna considered of conservation importance, the high number of endemic and rare taxa highlights the Shar Planina Mountains as a unique refuge whose sensitive ecosystems require protection from anthropogenic impacts.

Key words: endemic taxa, rare species, distribution, conservation importance.

Terrestrial beetles as indicators of habitat complexity and conservation value of Monospitovo Marsh, North Macedonia

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Wetland ecosystems support diverse insect communities, among which terrestrial beetles (Coleoptera) execute critical ecological functions across trophic levels as decomposers, predators, and herbivores. Despite their utility as indicators of habitat quality, wetland beetle assemblages in North Macedonia remain insufficiently studied. This study provides a baseline assessment of the diversity, functional structure, and conservation value of the terrestrial beetle fauna in Monospitovo Marsh, a major relic wetland ecosystem in North Macedonia. Field sampling was conducted during April and May 2026 across six localities encompassing core marsh vegetation, riparian zones, alder woodland remnants, grasslands, and agricultural transition zones. Beetles were collected by using active sampling and air-intercept traps. We recorded 59 taxa belonging to 25 families, representing diverse functional feeding guilds, including phytophagous, predatory, saprophagous, saproxylic, and semi-aquatic species. Specialized hygrophilous species were largely restricted to core wetland habitats, whereas remnant alder fragments and riparian margins served as microclimatic refugia for saproxylic taxa reliant on mature trees and deadwood dynamics. Despite ongoing anthropogenic pressures from surrounding land use, Monospitovo Marsh maintains structural heterogeneity necessary to support a functionally diverse beetle community. Preserving core wetland areas and remnant woody vegetation, alongside sustained ecological monitoring, remains vital for safeguarding the functional integrity of this degraded wetland landscape.

Key words: Coleoptera assessment, wetland biodiversity, functional feeding guilds, saproxylic beetles, microclimatic refugia

The Macedonian endemic snail *Horatia macedonica* (Kuščer, 1937) (Gastropoda: Hydrobiidae): New insights into its distribution, morphology and taxonomy

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The Balkan Peninsula is a major evolutionary hotspot for freshwater biodiversity, yet data on the distribution of the endemic hydrobiid snails remain sparse. In R. North Macedonia, three species of the genus *Horatia* Bourguignat, 1887 have been described based solely on shell characteristics: *Horatia macedonica* (Kuščer, 1937), *H. novoselensis* Radoman, 1966, and *H. podvisensis* Glöer et Reuselaars, 2020. Since none of these taxa had previously been studied anatomically, their taxonomic identities remain unresolved. *Horatia macedonica* is an endemic species restricted to the western part of the country, historically recorded from only six karstic localities: Rašče (west of Skopje), river Treska near Matka, Bigor village (Kičevo), Izvor village (Kičevo), Staroec village (Kičevo), and the spring of river Crna Reka. To address these knowledge gaps, intensive hydrobiological investigations were conducted across springs and rivers in the western parts of Republic North Macedonia during the summer of 2026. Viable specimens of *Horatia* were collected from stones using a 500 µm mesh hand-net and preserved in 75% ethanol. All specimens were identified as *H. macedonica* based on shell morphology and deposited in the Macedonian National Collection of Invertebrates (MNCI) at the Faculty of Natural Sciences and Mathematics, Skopje. To resolve the taxonomic position of the species, detailed anatomical surveys and micro-photography were performed using a ZEISS Stemi 508 stereomicroscope. Our findings provide a considerable expansion of the known distribution of *H. macedonica*, extending its range with three new localities: the river Belicka, the river Cerska (at the mouth of the river Golemo Ilinska), and the spring of the river Treska. These cold, fast-flowing habitats support dense populations of this species, confirming the region as a crucial refugium for this crenobiotic species. Furthermore, distinct differences in the morphology of the copulatory organs between *H. macedonica* and the type species *H. klecakiana* Bourguignat, 1887 (described from the spring of the river Cetina in Croatia) are highlighted. These results emphasize that resolving the complex systematics of Macedonian *Horatia* species requires an integrative approach combining morphological and molecular data.

Key words: Hydrobiidae, freshwater biodiversity, taxonomy, distribution

The populations of *Microcondylaea bonellii* (Férussac 1827), Unionidae in Northwest Albania are facing rapid decline

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According to recent data, the freshwater bivalve *Microcondylaea bonellii* is categorized as critically endangered on the IUCN Red List and is listed in Annex V of the Habitats Directive. *M. bonellii* is a freshwater mussel that belongs to the Gonideinae, one of the least studied Unionidae subfamilies. Knowledge of the biology and ecology of this species in Albania remains poor and fragmented. This species mainly inhabits flowing waters on fine to coarse-grained sand lying in stable banks, eventually overgrown by submerged plants, but it has also been reported in the holes of loess banks. In recent years, its distribution range has further decreased (e.g., it is already extinct in Greece), drawing the species ever closer to extinction. The aim of this study is to assess the current state of the populations present in Albania, identify the local factors leading to their rapid decline, and propose proactive conservation measures. During the recent field survey at 21 sampling localities, the presence of *M. bonellii* was recorded in only 30% of the sites. These localities specifically included areas downstream of the Vau i Dejes Dam within the Drin River, Drini i Lezhës, Gjadri and associated channels, Buna River, Karkariqi small wetland and Shkodra Lake. Sampling was conducted using combined methods of hand collection and hand netting. Additionally, a systematic search for empty shells was conducted on gravel bars. The assessed degradation index of the localities confirmed that drainage, river channeling, sedimentation and pollution are among the major threats.

Key words: IUCN, Northwest Albania, habitat fragmentation, distribution, the Habitats Directive

Undiscovered gems of the Pešter Plateau

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Karst freshwater ecosystems of the Balkan Peninsula comprise springs, streams, periodically flooded areas, and sinkholes, where pronounced spatial and temporal changes in water flow and water level strongly influence aquatic biodiversity and ecosystem functioning. Despite the ecological importance of the Pešter Plateau, the Koštampolje karst system has received considerably less scientific attention than the nearby Pešter Field, leaving its aquatic ecosystems and biodiversity comparatively understudied. The Koštampolje karst system represents an ecologically significant freshwater complex, comprising diverse and hydrologically heterogeneous habitats that support valuable aquatic biodiversity. This study investigated the macrozoobenthic community and ecological dynamics of the Koštampolje karst system in southwestern Serbia, focusing on the relationships between community composition, abundance, habitat characteristics, and physicochemical and hydrological conditions. Sampling was conducted at 11 localities representing different hydrological habitats, including springs, stream sections, and sinkholes, during 2016 and 2017. Macrozoobenthos was sampled seasonally, while physicochemical and hydrological parameters were measured concurrently. Across the 11 investigated localities, a total of 70.518 macrozoobenthic individuals per m² were recorded during the study period. A total of 74 different macrozoobenthic species were recorded across the investigated localities. The community comprised a diverse assemblage of aquatic invertebrates, with differences in taxonomic composition and abundance among localities and sampling periods. Trichoptera, Ephemeroptera, Plecoptera, and other major taxonomic groups contributed substantially to the observed community structure, while the relative representation of functional feeding groups varied among habitats. Statistical analyses, including correlation and regression analyses, revealed relationships between macrozoobenthic assemblages and environmental variables, particularly flow velocity, water depth, dissolved oxygen, and other physicochemical parameters. The pronounced hydrological variability of the investigated karst system, including seasonal flow reduction and temporary drying of some sections, was reflected in differences in taxonomic richness, abundance, and community composition. These findings demonstrate that hydrologically heterogeneous karst systems provide a mosaic of habitats supporting diverse macrozoobenthic communities and that changes in hydrological conditions can substantially influence their structure and functioning. The study highlights the ecological importance of the Koštampolje karst system and provides baseline data for future biodiversity assessment, ecological monitoring, and conservation of karst freshwater ecosystems in the Balkan region.

Key words: aquatic biodiversity, hydrological variability, Koštampolje karst system, macrozoobenthos

Patterns of survival: A Capture–Mark–Recapture study of the Balkan Crested Newt

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Knowledge of the Balkan crested newt *Triturus ivanbureschi* in North Macedonia is limited. Its distribution is restricted to the central and eastern parts of the country, where it is known from only a small number of breeding sites. Recent genetic evidence has revealed a hybrid contact zone between *T. ivanbureschi* and *T. macedonicus*, further constraining the national distribution of genetically unmixed Balkan crested newt populations. Its isolated, vulnerable reproductive centres and limited distribution have contributed to its Endangered (EN) status nationally. There is an urgent need to clarify the species' national distribution and population ecology, as its effective conservation relies on such insight. We encountered a productive breeding pond of genetically unmixed (unpublished data) Balkan crested newts on Osogovo Mt. close to the Bulgarian border (vicinity of Kalin Kamen). We also established a capture-mark-recapture (CMR) study in the pond in 2023 and performed four consecutive annual surveys until 2026. Newts were captured using aquatic funnel traps. The traps were set in the evening and checked the following morning. Each captured newt's sex was determined and its ventral pattern photographed before release. Individuals were identified from their unique ventral patterns. The photographs were compared using I3S Pattern+ software, and all records were entered into a database. A total of 329 capture events were recorded, representing 315 different adult newts. These included 224 females, 90 males, and one individual whose sex could not be determined with certainty. The sex ratio of captured individuals was strongly female-biased, with a male-to-female ratio of 0.4. Thirteen individuals were recaptured at least once, giving a total of 14 recapture events. More newts were caught during some of the later sessions, which is likely a result of the use of more traps. Differences among sessions may also be related to trap type, sampling effort and weather conditions. Cormack–Jolly–Seber (CJS) open-population model was used to estimate population size and annual survival. The estimated population size was 352 (95% CI 127–6215) individuals for 2025 and 444 (139–2401) individuals for 2026. Population size could not be estimated for 2024 because no recaptures were recorded. Respective estimated adult sex ratio was more balanced than that of captured individuals: 0.8 and 0.5 for 2025 and 2026 respectively. Annual survival probability was estimated at 0.46 (95% CI, 0.13–0.83). The wide confidence intervals indicate uncertainty in the annual population estimates, most likely because of the small number of recaptures. Possible movements to other yet undetected reproductive centres in the vicinity may also have contributed. Since the intervals overlap, the difference between the 2025 and 2026 estimates should not be interpreted as evidence of population growth. The estimated annual survival was within the range reported for adult crested newts, suggesting that survival in this population is not unusually low. The model suggests that confidence intervals may narrow as more data are collected. The study should therefore be continued with greater emphasis on standardizing the field approach to obtain more reliable estimates of population trends.

Key words: *Triturus ivanbureschi*; population size; sex ratio; photographic identification

Population assessment and seasonal dynamics of the Macedonian crested newt (*Triturus macedonicus*) and the Yellow-bellied toad (*Bombina variegata*) on Galichica Mountain

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The Macedonian crested newt (*Triturus macedonicus*) and the Yellow-bellied toad (*Bombina variegata*) are amphibian species of conservation importance associated with aquatic breeding habitats, which are often temporary and highly variable in their hydrological conditions. National population-level data for both species are unavailable. This study provides baseline information on annual and/or seasonal population dynamics of these species on Galichica Mountain, where surveys were conducted in six breeding ponds: Asan Gjura, Simoncheska, Crvena Gjafa, Gjafa and Mala Gjafa for the newt and Korita for the toad. Capture-mark-recapture studies were implemented on all meta/populations by using photographs to identify individuals via a pattern-recognition software (I3S Pattern+). Population sizes were estimated by applying the Horvitz–Thompson correction on parameter estimates from open population Cormack–Jolly–Seber (CJS) models, alongside closed-population Schnabel estimations. The highest population estimates for *T. macedonicus* were recorded in May, corresponding with the peak breeding season. During this peak month, estimated populations reached approximately 1,981 (95% CI: 1249–3238) individuals in Gjafa, 2,669 (CI: 1723–4478) in Simoncheska, and 471 (CI: 236–1072) in Asan Gjura. Following this peak, population estimates in Gjafa dropped sharply in July to 188 (CI: 87–416) individuals before showing an unexpected increase in August to 748 (CI: 345–1688). In Asan Gjura, the population estimate slightly decreased to 414 (CI: 207–941) individuals in June, after which sampling became impossible due to the pond drying completely by late July and August. Crvena Gjafa dried completely early in the season, yielding minimal captures. Sampling at Mala Gjafa began later in the season following its discovery in June, resulting in population estimates of 232 (CI: 63–917) in July and 52 (CI: 14–204) individuals in August. Although population data from Simoncheska Pond are available for several years, these estimates cannot be directly used to assess population trends because of substantial inter-annual field effort variation, and consequent minimal number, or complete absence, of recaptures. Nevertheless, these continuous observations provide important evidence of connectivity between breeding ponds: one individual previously identified in Simoncheska was recorded in Asan Gjura the following year, 580 meters away. Gjafa and Mala Gjafa lay an additional 4517 and 4848 meters away from Simoncheska, respectively. The one dispersal event provides evidence that the ponds are not completely isolated and supports the hypothesis that the populations on Galichica may function as a metapopulation, although further standardized multi-year monitoring is required to confirm the extent and frequency of such movements. For *Bombina variegata*, multi-year evaluations demonstrated minor population fluctuations across sampling years. CJS models yielded baseline population estimates of 154 (CI: 65–466) individuals in 2023, 116 (CI: 63–309) individuals in 2024, and a peak estimate of 188 (CI: 55–764) individuals in 2025. These findings provide the first structured population-level assessment of both species on Galichica Mountain and demonstrate the importance of maintaining a network of breeding ponds with different hydrological characteristics. Continued multi-year monitoring is necessary to distinguish seasonal fluctuations from longer-term population trends and to support effective conservation planning.

Key words: Capture-mark-recapture; metapopulation; amphibian conservation

Population characteristics of Hermann's and Spur-thighed tortoises (*Testudo hermanni*, *Testudo graeca*, Testudinidae: Testudines) in syntopy

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Two terrestrial chelonians inhabit North Macedonia, the Eurasian Spur-thighed tortoise (*Testudo graeca iberica*) and the eastern Hermann's tortoise (*Testudo hermanni boettgeri*). They live in syntopy in parts of the Balkan Peninsula, conveniently also near the center of Skopje, in a semi-urban park-forest – Gazi Baba. It is a protected area covering about 108 hectares of degraded and planted forests and meadows, which are nevertheless heavily threatened from intense human presence. During capture-mark-recapture studies between 2019 and 2026, a total of 205 individuals of *T. g. iberica* and 738 *T. h. boettgeri* were sampled, with the primary goal of estimating their population sizes. Multievent models revealed their respective annual adult survival estimates to be 96% and 98% with no variation between the sexes or over the years. On the other hand, their capture probabilities exhibit temporal (inter-annual) variance (0.03-0.49 for *T. g. iberica*, 0.02-0.37 for *T. h. boettgeri*). Population sizes were estimated at 254 [CI 169–396] Spur-thighed tortoises and 1100 [CI 801–1531] Hermann's tortoises in 2026, with a density of 2 and 10 adult individuals per hectare, respectively. Adult sex ratios do not seem to vary significantly beyond 1:1 over the years. Fertility assessment during 2024-2025 showed that 55% of the sampled female Spur-thighed tortoises were gravid (5 to 8 eggs), vs. 76% of female Hermann's tortoises (3 to 9 eggs); correcting adult sex ratios with reproductive activity reveal operational sex ratios of 4:1 vs. 1:1 males per reproductive female, respectively. Both studied chelonian species are vulnerable (VU) in North Macedonia as well as globally. Their continuous monitoring is therefore a priority, in order to assess, among others, the sensitivity and elasticity of demographic traits to the ever-present threats they face.

Key words: demography; population dynamics; capture-mark-recapture; clutch size; operational sex ratio

Variation in growth and size within and among four tortoise populations possibly reflects adaptation

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Reptile growth is traditionally modelled using the van Bertalanffy curve, which assumes rapid neonate growth followed by gradual deceleration. However, logistic S-shaped curves may better reflect species with delayed maturity, like the Hermann's tortoise (*Testudo hermanni*), where neonates experience a two–three-year period of accelerated growth while acclimating to their environment. Capturing this phase requires long-term capture-mark-recapture (CMR) data on elusive juveniles. We analysed long-term CMR datasets from four Hermann's tortoise populations in North Macedonia across two distinct climatic zones: three cooler high-altitude (850 m) populations in Prespa (Golem Grad Island's beach and plateau, and Konjsko village) and one warmer sub-Mediterranean and lower altitude population (300 m) in Skopje. Filtering for reliably aged individuals yielded 1,064 tortoises and 2,481 total recaptures across 8–20 years of systematic field effort. Across all populations, the logistic growth model showed an overwhelmingly better fit than the van Bertalanffy curve. Sexual dimorphism in asymptotic size (SCLA) and growth rate (k) varied significantly across sites: both mainland populations exhibited classic dimorphism: females reached significantly larger asymptotic sizes ($p < 0.001$), but growth rates did not differ between sexes ($p > 0.1$). Island populations showed an inverse pattern: females reached SCLA significantly faster than males ($p < 0.001$), but nevertheless, at a similar size ($p > 0.1$). Life history theory suggests high environmental or social stress favours faster growth to reach maturity earlier. Island females face severe stress from intense male sexual coercion (sex ratios are up to 19-fold male-biased). Similarly, tortoises in the drier, resource-competitive Skopje region (co-occurring with *Testudo graeca*) and inhabiting an urban park (supposedly under high anthropogenic pressure) also exhibited elevated growth rates. Rapid growth allows tortoises to reach adult size—and a protective, fully ossified shell—more quickly, presumably promoted by high-stress environments. Finally, tortoises from the island's beaches reached smaller asymptotic sizes than all other populations ($p < 0.001$), likely reflecting additional habitat constraints such as rocky terrain and restricted resources.

Key words: Logistic growth models, Sexual size dimorphism, *Testudo hermanni*

Birds of Nidže Mountain: evidence for designation as a Special Protection Area

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Nidže Mountain, situated along the border between North Macedonia and Greece, has remained comparatively poorly studied from an ornithological perspective, and its importance for bird conservation has consequently received little attention. We compiled historical and recent bird records from the mountain and complemented them with targeted field surveys covering its principal forest, subalpine and open mountain habitats. The data were analysed using the Regional Avifauna Assessment Tool (RAAT), which integrates observations from different periods, evaluates breeding and seasonal status, and combines species occurrence and population estimates with national and international conservation criteria. We recorded 124 bird species on Nidže in the period 2002–2026, of which 116 showed evidence of confirmed, probable or possible breeding. Twenty-five species are listed in Annex I of the EU Birds Directive, of which 21 breed or possibly breed in the area. Particularly important is the assemblage of forest specialists, including Hazel Grouse *Tetrastes bonasia*, Boreal Owl *Aegolius funereus*, Grey-headed Woodpecker *Picus canus*, Black Woodpecker *Dryocopus martius*, White-backed Woodpecker *Dendrocopos leucotos* and Middle Spotted Woodpecker *Dendrocopos medius*. Estimated breeding populations include 20–40 pairs of *A. funereus*, 25–70 pairs of *P. canus*, 25–35 pairs of *D. martius*, 10–20 pairs of *D. leucotos* and 50–150 pairs of *D. medius*. Nidže also supports an estimated 1–3 pairs of Golden Eagle *Aquila chrysaetos*, while Wallcreeper *Tichodroma muraria* is a scarce breeder (0–1 pair). Griffon Vulture *Gyps fulvus* is a regular non-breeding visitor, with 10–25 individuals estimated to use the area, while Eleonora's Falcon *Falco eleonora* occurs as a non-breeding summer visitor. Cinereous Vultures *Aegypius monachus* from the reintroduction project in Bulgaria frequently visit the region. At least the Bearded Vulture *Gypaetus barbatus* has disappeared from the region. The results demonstrate that the previously limited ornithological knowledge of Nidže substantially underestimated its conservation importance. Of particular significance is the simultaneous occurrence of substantial populations of several Annex I species associated with mature and structurally diverse mountain forests, complemented by species of cliffs, montane grasslands and other open habitats. RAAT-based assessment identifies *T. bonasia*, *A. funereus* and *D. leucotos* among the species making particularly strong contributions to the conservation importance of the area. The presence of 21 breeding or potentially breeding Annex I species, together with the size and composition of several of their populations, demonstrates the importance of Nidže within the national network of sites for bird conservation. We conclude that Nidže merits designation as a Special Protection Area under the EU Birds Directive and should be included in the further development of the Natura 2000 network in North Macedonia.

Key words: bird conservation, forest bird species, Natura 2000

Birds of the Lower Vardar area: conservation importance of North Macedonia's largest lowland riverine ecosystem

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The Lower Vardar represents the largest and most important lowland riverine ecosystem in North Macedonia. Despite extensive transformation of lowland landscapes elsewhere in the country, substantial parts of its floodplain remain relatively well preserved. Together with the Stojakovo–Bogorodica area, they comprise a complex mosaic of the active river channel, gravel and sand banks, and islands, remnant alluvial forests, wet depressions and pastures, dry steppe-like grasslands and agricultural habitats. Parts of the area have previously been recognized as an Important Bird and Biodiversity Area, while recent Natura 2000 surveys and accumulated ornithological information allow a broader reassessment of its conservation importance. We compiled historical and recent observations and results of quantitative field surveys in 2023–2024, and analysed them using the Regional Avifauna Assessment Tool (RAAT), integrating breeding and seasonal evidence with spatial and species-conservation information. In total, 170 bird species have been recorded. The area supports a diverse breeding assemblage of riverine, wetland, open-country and sub-Mediterranean birds and serves as an important migration corridor. Particularly important breeding populations include White Stork *Ciconia ciconia* (95–100 pairs), Sand Martin *Riparia riparia* (1,030–1,050 nesting holes), Eurasian Thick-knee *Burhinus oedicnemus* (at least one pair), Common Tern *Sterna hirundo* (13 pairs), Little Tern *Sternula albifrons* (10–11 pairs) and Collared Pratincole *Glareola pratincola* (2–5 pairs). For the latter three species Lower Vardar is the only breeding site in North Macedonia. Other species of European conservation importance include Levant Sparrowhawk *Accipiter brevipes* (10–15 pairs), Short-toed Snake Eagle *Circaetus gallicus* (3–5), Booted Eagle *Hieraaetus pennatus* (3–4), Marsh Harrier *Circus aeruginosus* (5–8), Kingfisher *Alcedo atthis* (15–22) and Roller *Coracias garrulus* (2–4). Open habitats support substantial populations of Red-backed Shrike *Lanius collurio* (500–950), Woodchat Shrike *Lanius senator* (150–260), Black-headed Bunting *Emberiza melanocephala* (260–470), Calandra Lark *Melanocorypha calandra* (50–150) and European Turtle Dove *Streptopelia turtur* (130–270 pairs). The conservation value of the area depends strongly on maintaining natural river dynamics. River regulation, riverbed erosion and groundwater lowering with river deposit excavation, construction-material dumping and conversion of floodplain habitats threaten the formation and persistence of bare banks and islands used by colonial and ground-nesting birds. Remaining alluvial forests are highly restricted, while wet pastures around Stojakovo and Bogorodica and dry grasslands on former river islands support distinctive and complementary bird communities. Conservation priorities should therefore include maintaining the remaining floodplain free from urbanization and infrastructural development, preventing further river regulation and riverbank dumping, preserving sediment transport from natural tributaries, restoring alluvial forests, retaining traditional grazing and mowing, and preventing further conversion to intensive plantations. In particular, water management and agricultural practices must comply with international standards, given their negative downstream impact on the Greek section of the river (Axios). Migration along the Vardar remains insufficiently quantified and requires dedicated seasonal surveys. Protection of these habitats together with the processes maintaining them strongly supports designation of the Lower Vardar with Stojakovo–Bogorodica area as a Special Protection Area within the future Natura 2000 network.

Key words: Floodplains, Important Bird and Biodiversity Area, Natura 2000

Pelagonia: an exceptional farmland bird and raptor stronghold threatened by rapid changes in agricultural landscape

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Nowadays, Pelagonia reflects one of the last echo of ancient forms of European pastoralism and is one of the most extensive agricultural landscapes in North Macedonia which supports an exceptionally rich assemblage of farmland, wetland and raptor species of birds. At the same time, the landscape has undergone profound change: approximately 750 km² of actively grazed pastureland were present in the plain in 2002, whereas about 500 km² has subsequently been ploughed, subjected to intensified agricultural management or biocide use, or has undergone succession. Remaining grazed and semi-natural areas are now concentrated mainly along the margins of the plain and around the Topolchanska barrier which divides the northern and southern parts of the Pelagonian floodplain. We assessed the ornithological importance of the proposed Pelagonia Natura 2000 site (SPA) in North Macedonia by combining historical and recent occurrence data with targeted field surveys and a standardized species-status and conservation-valorisation framework. The compiled database contained 13,793 records of 214 bird species from 1938–2026. Breeding evidence was available for 176 species, while the site assessment classified 96 species as resident, 83 as summer visitors, 16 primarily as winter visitors and 19 as passage migrants. The conservation assessment identified 56 breeding species listed in Annex I of the EU Birds Directive, demonstrating an unusually high concentration of species of European conservation concern. The proposed site supports several populations of national and European importance. Particularly notable are 375–400 breeding pairs of Lesser Kestrel *Falco naumanni*, 50–80 pairs of Montagu's Harrier *Circus pygargus*, 16–22 pairs of Long-legged Buzzard *Buteo rufinus*, 12–18 pairs of Western Marsh Harrier *Circus aeruginosus* and 9–13 pairs of Short-toed Eagle *Circaetus gallicus*. The Egyptian Vulture *Neophron percnopterus* is a possible breeder. Pelagonia is also a major stronghold for White Stork *Ciconia ciconia*, with 440–450 breeding pairs. Large populations of characteristic farmland birds include 10,000–18,000 pairs of Red-backed Shrike *Lanius collurio*, 4,000–7,400 pairs of Woodchat Shrike *L. senator* and 500–750 pairs of Ortolan Bunting *Emberiza hortulana* (sampling 2023–2024). Important non-breeding concentrations include up to 1,000 Red-footed Falcons *Falco vespertinus* and several hundred pelicans and Ruffs. Pelagonia's conservation significance now depends on the persistence of a heterogeneous agricultural ecosystem rather than on individual rare species alone. Remaining pastures, low-intensity farmland, wetlands and peripheral slopes are complementary components of this system. Particularly concerning is the recent drainage of all three large fishponds: formerly an important wetland component of the IBA, they supported 10–15 breeding pairs of Ferruginous Duck *Aythya nyroca* and provided feeding habitat for Dalmatian Pelican *Pelecanus crispus*. Their loss illustrates that deterioration is occurring alongside the continuing high conservation value of the wider landscape. The current Lesser Kestrel population remains substantially below its earlier documented level of 760–850 breeding pairs. Natura 2000 designation should therefore be accompanied by maintenance and restoration of grazing, low-intensity agricultural mosaics and wetland habitats. The future of one of Europe's most important ancient landscapes, and of the birds it supports, depends on large-scale revitalisation of flooding and grazing.

Key words: grazing, land use change, revitalisation

Regional Avifauna Assessment Tool (RAAT): Integrating heterogeneous bird records into spatial, seasonal and conservation assessments

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Large historical ornithological datasets contain information collected over different periods, seasons, spatial scales and survey designs, ranging from systematic field surveys to occasional observations and citizen-science records. Their heterogeneity limits direct quantitative analysis, but they may nevertheless provide an important evidence base for assessing regional avifaunas, identifying knowledge gaps and supporting conservation planning. I developed the Regional Avifauna Assessment Tool (RAAT), an R-based analytical workflow designed to transform heterogeneous bird occurrence data into standardized, spatially explicit and reviewable assessments. RAAT operates on individual georeferenced observations retaining their original information on species, date, abundance and breeding evidence. Records are first interpreted according to breeding evidence and season and subsequently intersected with user-defined spatial frameworks. The current implementation simultaneously supports protected areas, regional divisions, existing Important Bird and Biodiversity Areas (IBAs) and potential IBAs, allowing the same national dataset to be examined from different geographical and conservation perspectives. Within each spatial unit, observations are aggregated by species to derive evidence for breeding, wintering, migration and non-breeding occurrence and to assign a regional seasonal status. This evidence-based classification is subsequently compared with independently defined national seasonal status. Importantly, national status does not determine the initial classification: discrepancies are retained as quality-assurance flags. Documented rules can provide a recommended interpretation, while uncertain or contradictory cases remain explicitly identified for expert review. Confidence is evaluated from the temporal depth and recurrence of available records. Thus, observation evidence, automated inference, prior national knowledge and expert judgement remain separate and traceable throughout the assessment. RAAT generates complementary site- and species-oriented outputs, including species inventories, seasonal and breeding assessments, records requiring review, spatial summaries, maps and seasonal-altitudinal visualisations. An independent valorisation module links the resulting assessments with species-level conservation criteria, enabling identification of species contributing most strongly to the conservation importance of individual areas and comparison among spatial units. Because valorisation is applied only after biological status assessment, conservation importance does not influence the interpretation of the underlying occurrence data. The principal purpose of RAAT is therefore not to replace standardized surveys or expert ornithological assessment, but to make maximum transparent and reproducible use of heterogeneous accumulated information and to identify where additional field verification is required. It is currently being applied to the preparation of a national bird atlas and population estimates for North Macedonia, assessment of regional avifaunas, and evaluation of areas of conservation importance. The approach provides a transferable framework for converting long-term ornithological databases into structured evidence for research, monitoring and spatial conservation planning.

Key words: bird inventories, site assessment, valorization

FORESTRY AND AGRICULTURE

Vulnerability of four oaks seedlings during the first year – morphological and physiological status in relation to drought –inputs for regeneration

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Regeneration of oak forests is highly dependent on species resilience and adaptability to climate conditions, especially in the early developmental stage - the first year after acorns germination. Differences between oak species are evident at the adult stage defined by the bioecology of each species. However, the vulnerability of different oak seedlings shortly after germination has not been sufficiently investigated or clearly presented. Their capacity to cope with climate extremes highly influences success of regeneration and the future survival of oak forests. Native oak species of hilly and mountainous regions of Balkan Peninsula are strongly differentiated in both vertical and horizontal distribution. These include dominant *Quercus frainetto* and *Quercus cerris* forests in the central Balkans, *Quercus pubescens* forests in the southern regions and at extreme sites, and *Quercus petraea* forests at higher altitudes. In recent decades there have been a lot of challenges in the regeneration of some of these native forests. Causes may be diverse, including irregular mast years, summer drought, predators, insects, and diseases. Knowledge about the vulnerability of seedlings to each of these factors, as well as to their combined effects, could be important for decision-making before and during the regeneration process. In recent decades, sporadic but extremely severe summer droughts have been recorded, causing dramatic consequences for unprotected plants in forests, especially young seedlings. In order to prescribe adequate climate adaptive measures and promote the regeneration of oak forests, it is necessary to understand the response of each species in the juvenile developmental phase. A series of field experiments and greenhouse tests were conducted in which seedlings were exposed to different levels of drought stress or water deficit. Seedling responses were assessed through physiological and morphological indicators. The results indicate the highest vulnerability of *Q. cerris* to summer drought, while *Q. frainetto* showed higher tolerance. *Q. petraea* demonstrated an unexpected tolerance to water deficit, employing a leaf-avoidance strategy for survival, in contrast to *Q. pubescens*, which retained its leaves but exhibited lower stomatal conductance and reduced photosynthetic activity under stress conditions. Different reactions of young oaks seedlings on various and extreme conditions could be key factors for appropriate approaches to the regeneration and restoration of oak forests. Taking into account predictions of the increasingly frequent occurrence of climate extremes in the southeast Europe (such as summer droughts), this kind of information could be useful for appropriate oak species selection and implementation of measures aimed at improving microclimatic conditions and ensuring stable regeneration. Acknowledgement: "This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 11096, Physiological and morphological responses of oaks seedlings to drought stress - PRO_DROAKS".

Key words: *Q. frainetto*, *Q. cerris*, *Q. pubescens*, *Q. petraea*, water deficit

A legacy at risk: Tree windbreak systems in Ovche Pole, North Macedonia

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The Ovche Pole plain, covering 649 km² in central North Macedonia, is a semi-arid, agriculturally intensive region characterised by Vertosols, low soil organic matter (~2%), and a significant water deficit (~50%). The region faces multiple degradation risks, including soil salinisation, desertification, erosion (with soil losses reaching and exceeding 10 t/ha/year in some areas), and wind erosion driven by prevailing northwest winds averaging 5.7 m/s. Agriculture, the primary economic activity, is constrained by dryness during the growing period, with precipitation averaging around 570 mm annually against evapotranspiration rates of 935–1105 mm. Tree windbreak systems were first established in the region in 1929, with large-scale planting occurring between 1946 and 1956; during this period, 556 hectares (67% of the planned 771 ha) were realised in Ovche Pole. These windbreaks, designed in a grid pattern with main and side tree lines, were planted predominantly with local species (black locust, elm, ash, almond) and have demonstrated multiple ecosystem functions, including wind speed reduction, microclimate regulation, and soil protection. Despite their historical significance and proven benefits, tree windbreak systems in the region have suffered continuous degradation due to institutional negligence, lack of explicit legal recognition, and insufficient scientific research. Only a handful of studies have addressed windbreaks in Macedonia, most dating back to the 1950s, with recent contributions limited to a few articles and project reports. This study aims to address this gap by investigating the current state, challenges, and potential of tree windbreak systems in the Ovche Pole region.

Key words: Shelterbelts, Agroforestry, Land management, Institutional neglect, Challenges

Spatial-temporal dynamics of the natural regeneration and expansion of Macedonian pine (*Pinus peuce* Griseb.) on unforested terrains of Jablanica Mountain

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The Macedonian or Molika pine (*Pinus peuce* Griseb.) is a tertiary relict and Balkan endemic of high ecological, biogeographical, and conservation value, whose natural distribution is closely linked to the specific conditions of high-mountain ecosystems on the Balkan Peninsula. Changes in the traditional use of mountain landscapes particularly the decline of livestock farming and the gradual abandonment of pastures are creating favorable conditions for natural succession and the progressive encroachment of woody vegetation onto formerly open high-mountain terrains. On Jablanica Mountain, intensive natural colonization of *Pinus peuce* has been observed in recent decades on unforested terrains and abandoned pastures, representing a representative example of contemporary dynamics of natural regeneration and spatial expansion of forest vegetation under changing traditional mountain land-use practices. Despite the significance of molika pine as a relict and endemic species, the processes underlying its natural expansion onto abandoned pasture areas on Jablanica have not yet been systematically documented or quantified. The aim of this study is to determine the spatial and temporal dynamics of the natural regeneration and expansion of *Pinus peuce* on Jablanica Mt., to characterize the structural and developmental dynamics of the newly established populations, and to assess the direction and intensity of their expansion across open terrains. The study is based on a combined field-spatial approach that integrates field measurements, georeferenced data, and multitemporal satellite image analysis. Field surveys are conducted along linear transects established in areas exhibiting different degrees of regeneration, with each individual tree georeferenced and its basic dendrometric characteristics recorded: age, height, and diameter at breast height (DBH). In addition, permanent sample plots are established to enable long-term monitoring of the regeneration process and of changes in vegetation structure. Field data are integrated with a series of multitemporal satellite images spanning different time periods, aiming to reconstruct historical changes in woody vegetation cover and to precisely determine the spatial dynamics of Macedonian pine expansion. Field observations indicate the presence of multiple age classes, ranging from recently established individuals to specimens several decades old, confirming a prolonged and continuous process of natural regeneration. The results of this study provide a scientific basis for the long-term monitoring of the natural regeneration and spatial expansion of *Pinus peuce* on Jablanica Mt. and contribute to a better understanding of how changes in traditional mountain land use affect the dynamics of forest and open high-mountain ecosystems.

Key words: Molika pine, dendrometry, natural forestation, spatial expansion, satellite imagery

When grass outlives grazing? Management–landscape decoupling as an early-warning signal for High Nature Value Farming in North Macedonia

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High Nature Value Farming (HNVF) depends not only on the persistence of semi-natural agricultural landscapes but also on the continuity of the farming practices that maintain their ecological condition. This distinction creates an important monitoring problem: land-cover statistics may remain stable after grazing, mowing, labour and livestock activity have begun to contract. We develop and apply a Management–Landscape Decoupling Framework for identifying this hidden early-warning signal in North Macedonia. Official 2023–2025 agricultural statistics were integrated across three evidence domains: landscape stock (pastures and meadows), small-ruminant and livestock-activity proxies, and agri-environmental policy design. The principal diagnostic, the Management–Landscape Decoupling Gap (MLDG), measures the difference between relative change in grassland area and relative change in a management proxy; it is interpreted as a screening metric, not as stocking density or a biodiversity-response indicator. Regional sheep–goat trajectories were further classified into expansion, substitution/rebalancing and joint-contraction pathways. Four grassland-dominated municipalities - Berovo, Pehchevo, Radovich and Tetovo - were retained as illustrative archetypes linking local structural preconditions to regional management contexts. Pastures and meadows declined by only 0.9%, from 794,993 ha in 2023 to 787,657 ha in 2025, whereas the combined sheep-and-goat population declined by 15.2%. Sheep numbers fell by 20.1%, while goat numbers increased by 18.1%, indicating a marked compositional shift within the small-ruminant population rather than a uniform decline across livestock types. The resulting small-ruminant MLDG was 14.3 percentage points, while grassland area per small-ruminant head increased by 16.8%. A log-response sensitivity test gave the same direction ($Dlog = 0.156$; $\exp[Dlog] = 1.168$). Corroborative activity proxies pointed in the same direction: the number of shorn sheep declined by 16.0%, milked sheep by 7.5%, sheep-milk output by 13.1% and wool output by 22.1%. The signal was not spatially uniform. East and Northeast followed expansion pathways; Southeast showed near-compensatory goat substitution; Vardar combined goat growth with a 65.0% contraction in total small ruminants; and Polog, Pelagonia, Skopje and Southwest showed joint contraction of sheep and goats. Overall, grassland extent remained broadly stable, total small-ruminant abundance contracted substantially, and small-ruminant composition shifted toward a greater contribution of goats, with pronounced regional variation. Policy analysis revealed a complementary asymmetry: Measure 213 directly supports livestock activity and rural labour but does not require biodiversity outcomes, whereas the EU Nature Restoration Regulation provides explicit ecological indicators without functioning as a farm-support instrument. The study therefore reframes the risk of HN VF degradation or loss as a process-decoupling problem. The conservation warning is not only loss of grassland area, but persistence of the landscape stock after the management system required to sustain biodiversity has weakened or become spatially disconnected. For EU-accession countries with incomplete biodiversity time series, the framework offers a transparent way to detect where field verification, grazing-management assessment and outcome-based agri-environmental design should be prioritized. It does not identify confirmed HN VF or measure ecological effectiveness; it identifies where apparently stable agricultural landscapes may conceal emerging management vulnerability.

Key words: HN VF; agri-environment-climate measures; Nature Restoration Regulation;

An integrated approach in identifying High Nature Value Farmland

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High Nature Value Farmland (HNVF) refers to agricultural areas where low-intensity farming practices maintain high levels of biodiversity, valuable habitats, and associated ecosystem services. In this context, High Nature Value (HNV) assessment provides an important framework for investigating the links between biodiversity, traditional land-use practices, and agricultural systems, with direct applications for biodiversity conservation and evidence-based agricultural policy. Reliable identification of HNVF is therefore essential for targeting conservation efforts and supporting traditional farming systems that maintain agrobiodiversity. This paper presents a methodological framework for identifying HNVF in North Macedonia through an integrated assessment combining ecological, landscape, and socio-cultural information. Landscape types were used as the primary spatial baseline for screening potential HNVF types, as they integrate ecological characteristics with the historical and contemporary land-use processes that have shaped them. Following, the identification of HNVF mainly relied on field surveys to identify essential land-use components, natural conditions, and threats. Biodiversity field assessments document key habitat structures, characteristic species of plants, birds, butterflies and saproxylic beetles enabling valorization of HNVF in terms of their value in supporting biodiversity and human wellbeing. To complement ecological field observations semi-structured interviews with local communities were conducted to document the role and contribution of current and historical traditional management practices and farming systems in sustaining HNVF, as well as the socioecological role of HNVF in sustaining local livelihoods and wellbeing. The framework will be tested in four representative locations selected to capture contrasting farming systems in hilly and mountainous rural landscapes. Detailed case studies will focus on characteristic HNVF biotopes and their associated biodiversity. Priority HNVF types include 'Pollard-grassland mosaics in high hilly landscapes' in thermophilus degraded forest landscape on Golak, 'Grassland-coppice mosaics' in the hilly dry grassland landscape on Plachkovica, 'Orchard farmland habitat types and microhabitats' in Maleshevo-Pijanec landscape on Vlaina Planina and 'Mountainous mosaics of grassland, fields and trees' in the mountain rural landscape of Shar Planina. Although the selected case studies cannot represent the full diversity of farming systems across North Macedonia, they provide a robust methodological basis for wider application. The proposed methodology provides a transferable framework for HNVF identification, strengthens understanding of the links between traditional land use and biodiversity, and supports agri-environmental policy, habitat classification, and conservation planning, including Natura 2000 implementation and sustainable rural development.

Key words: mosaic, grassland, fields, coppice, land-use

Beyond protected boundaries: Spatial validation of High Nature Value Farming potential against biodiversity priority networks in North Macedonia

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High Nature Value Farming (HNVF) is intended to identify agricultural systems in which farming practices sustain semi-natural habitats, heterogeneous landscapes and species of conservation concern. Yet national HNMF screening often relies on land-cover and habitat proxies, whereas conservation planning requires evidence that such proxies are spatially concordant with independently recognized biodiversity priorities and that important agricultural landscapes are not confined to formal protected areas. We developed a national landscape-ecology framework for North Macedonia that explicitly separates HNMF prediction from external biodiversity-priority assessment. CORINE Land Cover 2018 and a national Habitat Map were harmonized to a 200-m working raster and aggregated to a primary 2-km analytical grid. Type 1 potential was derived from pasture, natural-grassland and semi-natural grassland evidence; Type 2 structural potential from agricultural mosaics, compatible habitat classes and landscape heterogeneity. Scientifically based PBA, IBA, IPA, KBA and officially protected-areas, Emerald and Natura 2000 layers were withheld from predictor construction. The national analysis mask covered 25,532.9 km². High relative HNMF potential, defined as the upper 20% of structurally relevant cells, occupied 3,799.2 km². Of this area, 2,673.2 km² (70.4%) was spatially concordant with at least one PBA/IBA/IPA/KBA network, compared with 44.7% biodiversity-priority coverage across the national analysis mask. Within 20-km spatial strata, high-HNMF cells had 2.27 times the odds of biodiversity-priority occurrence compared with other structurally relevant cells (95% CI 1.82-2.84); the association remained strong outside national protected areas (OR 2.13, 95% CI 1.68-2.71). Of the biodiversity-priority-concordant high-HNMF area, 69.3% occurred outside national protected areas, while 949.2 km² (35.5%) remained outside the broader protected/Emerald/candidate-Natura 2000 network. The association remained positive after joint stratification by 20-km spatial blocks and broad non-HNV environmental context (OR 2.89, 95% CI 2.20-3.80), and was individually supported by IBA, IPA and KBA networks; the smaller PBA network showed a positive but imprecise association. Ablation analysis showed that Type 1 grassland evidence provided the strongest concordance with existing biodiversity-priority networks, whereas Type 2 structural potential was weakly represented by those designations. A supplementary farming-plausibility audit found that 92.8% of high Type 1 cells contained at least 10% cadastral or administrative farming-class support, although such registry evidence does not prove current management. Scale, block-size and biodiversity-overlap sensitivity tests retained the principal area pattern, while coarse 5-km cells and alternative score aggregation exposed important model-dependence. The results support HNMF potential as a transparent prioritization hypothesis for field verification, agri-environment-climate targeting and restoration planning rather than as an official HNMF designation or a direct measure of biodiversity condition. They also show that existing biodiversity-priority networks preferentially corroborate semi-natural grassland systems, while fine-grained agricultural mosaics require dedicated field and parcel-scale validation.

Key words: HNMF; landscape ecology; agricultural biodiversity;

Understanding biodiversity in human-shaped nature

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Ecology includes describing and explaining patterns and variation in nature. Several approaches have been developed to classify habitat types, species assemblages and vegetation, and to analyse the distribution and abundance of species. In most models and frameworks, mainly natural conditions and processes are used as explanatory variables, such as climate, altitude, soil, and natural disturbances, succession and dispersal. Human agency is usually avoided because of its unpredictability and because understanding of land-use often requires knowledge beyond ecology. If land-use is addressed, it is often as a deteriorating factor in natural ecosystems. However, in many European landscapes, anthropogenic habitat types constitute a considerable proportion of the species-rich ecosystems and hotspots for conservation. Although this is increasingly acknowledged, there is still a need for developing approaches that incorporate human activities into classification systems and analysis tools for populations and ecological functioning of habitat types and landscapes. Based on examples from a joint North Macedonian and Swedish project on high nature value farming, we discuss how land-use can be incorporated in analysis frameworks for ecosystem ecology, community ecology and population ecology. We show how farming practices constitute anthropogenic ecological processes that interact with natural environmental variables. The results highlight relationships between biodiversity and different types of traditional land-use, including agroforestry in wooded habitat types. Results also highlight the importance of landscape history for the current biodiversity patterns. The results are important for the possibilities to analyse biodiversity and landscape history in human-imprinted landscapes. The study provides a tool for identifying key factors for biodiversity in different habitat types, especially the importance of specific land-use practices for maintaining species-rich ecosystems. Such knowledge is essential, e.g., for making guidelines for habitat types and for developing agriculture, including agri-environment schemes.

Key words: land-use, habitat types, landscape history, ecosystem functioning.

High nature values hiding in old forgotten pollarded trees in North Macedonia

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Old deciduous trees are rare across Europe and harbour many rare and threatened species. The saproxylic beetles (Coleoptera) are among the most species rich group associated with old trees which were the focus of the research in two regions of North Macedonia: Maleševo Mountain and villages near Radoviš. The study covers oak (*Quercus cerris* and *Q. frainetto*) and beech trees (*Fagus sylvatica*). The traps were placed for two seasons 2025-26 on old hollow trees, most of them with a long history of being pollarded. Preliminary calculations show that the pollarding tradition in the country stopped 40-70 years ago. The identification of the beetles just started and just a small part of the material is identified, but several very rare species have been found. Six of them are included in EU Species and Habitat Directives and protected in the whole region: *Osmoderma barnabita*, *Cerambyx cerdo*, *Rosalia alpina*, *Morimus funereus*, *Lucanus cervus* and *Limoniscus violaceus*. Previous studies have proven the importance of the pollarding tradition of creating microhabitats and these preliminary results support this.

Key words: Saproxylic beetles, tree microhabitats, Natura 2000, dead wood, oak, *Quercus*

Butterfly diversity as an indicator of High Nature Value Farmland in North Macedonia

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High Nature Value (HNV) farmlands are important for maintaining biodiversity and providing suitable habitats for a wide range of species associated with traditionally managed agricultural landscapes. Butterflies are considered good indicators of habitat quality and can provide valuable information for identifying HNV farmland. As part of biodiversity research in North Macedonia, butterflies were surveyed from April to August 2026 at four locations: Vejce, Negrevo, Mitrashinci and Radovich. A total of 389 records representing 84 butterfly species were collected across 30 different habitats. Meadows supported the highest butterfly diversity, with 49 species recorded, followed by semi-natural pastures with bushes, where 32 species were recorded. Coppice forests dominated by oak and beech also supported considerable diversity, with 28 species recorded. The results highlight the importance of semi-natural and traditionally managed habitats for maintaining butterfly diversity in agricultural landscapes. In particular, meadows and semi-natural pastures, which are often associated with low-intensity farming practices, appear to be important habitats for butterflies and may represent key components of HNV farmland. The collected data provide a useful baseline for further assessment of the relationship between butterfly diversity, habitat characteristics and land management practices. Continued research of butterflies will contribute to the identification and mapping of HNV farmland and support the promotion of agricultural practices that maintain biodiversity and traditional landscape features in North Macedonia.

Key words: High Nature Value Farmland; butterflies; biodiversity monitoring; traditional landscapes

Breeding bird communities and their relevance for Natura 2000 designation in selected high-nature-value farmland and traditional pollarded oak forests in North Macedonia

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Identification of areas supporting significant populations of conservation-relevant bird species requires quantitative information on breeding assemblages, but such data remain limited for many regions of North Macedonia. During the 2026 breeding season, we surveyed birds in three areas in eastern part of North Macedonia—Ali Koch, Mitrashinci and Negrevo—using repeated line transects and distance sampling, complemented by casual observations. The main survey comprised 50.64 km of transect effort and recorded 70 breeding bird species. Detection functions were fitted separately for each species and area, with Half-normal models preferred where adequate, to provide conservative and methodologically consistent estimates. Breeding-density estimates were obtained for 40 species. Thirty-seven species of particular relevance in the Natura 2000 context occurred within the surveyed areas. Quantitative estimates were obtained for *Lanius collurio*, *Lullula arborea*, *Curruca crassirostris*, *Curruca nisoria* and *Dendrocopos medius*. *Lanius collurio* showed pronounced spatial variation, with estimated densities of 1.0, 10.3 and 22.7 territories km⁻² in Ali Koch, Mitrashinci and Negrevo, respectively, while *Lullula arborea* was widespread, with corresponding densities of 14.0, 4.4 and 8.3 territories km⁻². Local estimates were also obtained for *Curruca crassirostris* in Ali Koch (2.9 territories km⁻²), *Dendrocopos medius* in Mitrashinci (1.7 territories km⁻²) and *Curruca nisoria* in Negrevo (8.4 territories km⁻²). Several additional conservation-relevant species (i. e. including *Alectoris graeca*, *Circaetus gallicus*, *Columba oenas*, *Dendrocopos syriacus*, *Ficedula semitorquata*, *Hippolais olivetorum*, *Lanius nubicus*, *Pernis apivorus*, *Picus canus*, *Rhadina orientalis* and *Streptopelia turtur*), were detected on transects, but too infrequently for density estimation. Casual observations further increased the number of species relevant to conservation assessment. An additional area on Shar Planina, near the village of Vejce, was surveyed using a single 4-km transect and was therefore treated descriptively, supplemented by numerous casual observations. The area supported a diverse assemblage of conservation-relevant breeding birds, including *Alectoris graeca*, *Aquila chrysaetos*, *Caprimulgus europaeus*, *Curruca nisoria*, *Dendrocopos medius*, *Lanius collurio*, *Lullula arborea*, *Pernis apivorus* and *Picus canus*, among others. Its different survey effort precluded quantitative comparison with the three principal areas. Overall, the results demonstrate marked differences in breeding-bird assemblages and densities among the surveyed areas and show that relatively small survey datasets can provide both quantitative population evidence and complementary occurrence data for species of conservation concern. The concentration and, for several species, locally high densities of conservation-relevant breeding birds indicate that these areas merit further assessment in the context of the identification and designation of Special Protection Areas within the Natura 2000 network in North Macedonia.

Key words: distance sampling, rapid avifauna assessment, traditional use of natural resources

Root distribution of *Robinia pseudoacacia* for soil erosion mitigation, a case study in North Macedonia

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Soil erosion is one of the most widespread and severe forms of soil degradation, representing a major constraint to the sustainable use and management of land at local, regional, and national scales. To reduce the need for costly conventional engineering measures, bioengineering and eco-engineering approaches have increasingly been recognized as effective strategies for mitigating soil erosion, shallow landslides, and slope instability. Vegetation plays a crucial role in slope stabilization, as plant roots reinforce and bind soil particles, thereby increasing soil shear strength, while the canopy intercepts rainfall and reduces the erosive impact of raindrops. In forested hillslopes, tree root systems can substantially reduce susceptibility to rainfall-triggered shallow landslides by reinforcing the soil and, in some cases, anchoring the soil mantle to underlying bedrock through deep-rooting systems. The aim of this study was to investigate the root distribution and root tensile strength of black locust (*Robinia pseudoacacia* L.) as a potential bioengineering measure for soil erosion and slope stabilization. Field investigations were conducted at two sites in North Macedonia: Varvara and Techeni Bair. Five trees were excavated at Varvara and four at Techeni Bair to characterize root distribution. In addition, four soil samples were collected from each site at depths of 20 and 40 cm and subsequently analyzed to characterize the soil conditions. The results indicated substantially higher root frequencies at the Varvara site, approximately twice those observed at Techeni Bair, both in terms of vertical root distribution and root frequency relative to distance from the stem. These differences may be associated with contrasting soil and moisture conditions between the two sites. Techeni Bair was characterized by a clay-loam soil texture and lower soil moisture, whereas Varvara had a predominantly sandy soil texture and higher moisture availability due to its proximity to a river, approximately 5–10 m from the study area. At the 40 cm depth, however, higher root frequency was recorded at Techeni Bair, potentially reflecting the need for roots to penetrate deeper into the soil profile under less favorable moisture conditions in search of available water and nutrients. Root tensile strength was assessed through root pullout tests. The results obtained from the 40 pullout tests were not sufficiently conclusive to establish a clear relationship between root characteristics and tensile resistance, and further investigation is therefore required. Nevertheless, the tests revealed notable differences in soil–root interface conditions and friction between the two localities. Roots at Varvara were generally easier to pull out, despite having larger diameters, whereas the pullout tests at Techeni Bair were considerably more difficult to perform. These findings indicate that both root characteristics and site-specific soil conditions should be considered when evaluating *R. pseudoacacia* as a bioengineering measure for erosion and slope stabilization.

Key words: soil stabilization, root frequency, root tensile strength, Black locust

Monitoring of forest cover loss for water and soil management: A case study of Shara Mountain

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Shara Mountain is home to the recently established National Park in North Macedonia. Historically, the region has experienced severe flash floods. The most recent event occurred on 3 August 2015 in Shipkovica village, causing extensive material damage and the loss of four human lives. In response, it became necessary to identify land-cover disturbances that contributed to this event. Prior to its designation as a national park, the forests in this region were managed by the Public Enterprise "National Forests" (PENF). However, numerous undocumented (illegal) forest disturbances also affected large areas. The mountain is characterised by steep slopes, which contribute to higher runoff, and forest loss can further increase runoff in the catchments. This study aimed to identify forest loss between 2000 and 2015 using remote sensing data products and to assess its impact on water runoff production and flooding. Two time series were analysed: medium-term and short-term. The medium-term analysis used the Global Forest Change database (University of Maryland), covering 2000–2014. The short-term analysis employed Copernicus tree cover data for 2012–2015, applying a 70% change threshold. These datasets were validated using high-resolution Google Earth imagery and available aerial photographs. The analysis took in the account all forestry-related activities, both planned and undocumented. The results indicate an average annual cut of 488.8 ha. Over 2012–2015, 1,057 ha were cut without documentation (likely illegal), compared with only 72 ha of planned cuts. The proportion of annual cuts is considerable and can significantly affect the ecosystem as a whole. In several catchments—Debreshka, Gabrovica, Kamenjanska, and Ponika—annual cuts exceed 7% of the forest cover. At this rate, complete deforestation could occur within 14 years. These figures reflect only areas detectable via satellite imagery. A substantial proportion of illegal logging remains unaccounted for, as it often involves selective removal of high-quality timber that remote sensing cannot detect without field verification.

Key words: Land cover change detection, GIS, Remote sensing, water related hazard

Microbial community structure and functional diversity in apple orchard soils under contrasting management practices in the Prespa region

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Agricultural management practices strongly influence soil microbial communities and their ecological functions. In the Prespa region, where conventional and organic apple production coexist, information on the effects of different management systems on culturable soil bacteria remains limited. This study evaluated the abundance and functional diversity of culturable bacteria from conventionally managed and organically managed apple orchards and compared them with those from undisturbed forest soil. Composite soil samples were collected from a conventionally managed orchard treated with synthetic fertilizers and pesticides, an organically managed orchard fertilized with farmyard manure without pesticide application, and a natural forest soil on Galichica Mountain. Total viable bacterial counts were determined by serial dilution and plating, followed by isolation of individual strains. The isolates were screened for cellulolytic activity, nitrogen fixation, and phosphate-solubilizing capacity. The organically managed orchard exhibited the highest bacterial abundance (2.9×10^7 CFU), greatly exceeding the conventionally managed orchard (2×10^5 CFU) and the forest soil (4×10^5 CFU). Fourteen bacterial isolates were obtained from the organic orchard, compared with eight from the conventional orchard and nine from the forest soil. Functional characterization revealed markedly greater metabolic diversity in the organically managed soil. All isolates from the organic orchard exhibited cellulolytic and nitrogen-fixing activities, while half also demonstrated phosphate-solubilizing ability. In contrast, only a limited number of isolates from the conventionally managed orchard expressed these functional traits. The results indicate that organic management promotes both higher bacterial abundance and greater functional diversity than conventional agricultural practices. The application of organic amendments together with the absence of pesticide pressure appears to support metabolically versatile bacterial communities capable of contributing to nutrient cycling and soil fertility. These findings highlight the importance of sustainable orchard management for maintaining soil microbial biodiversity and ecosystem functioning within the ecologically important Prespa region.

Key words: agroecosystems; soil microorganisms; nutrient cycling; sustainable agriculture; microbial ecology

Ethnobotanical study and conservation trends of native plants with medicinal uses in mountainous Central Greece

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The use of native plants for medicinal purposes has been an integral element of the cultural heritage of local societies since ancient times. Mountainous Central Greece is characterized by a pronounced relief, a continental Mediterranean climate, and high plant diversity. These unique geomorphological and climatic conditions have preserved a significant wealth of native flora, largely utilized over time by local inhabitants. The purpose of this work is to record and evaluate traditional knowledge concerning the uses of native plants in Mountainous Central Greece, emphasizing their medicinal applications, while investigating the effects of human activity on their natural populations. To achieve this, a checklist of 1,400 indigenous plant species was compiled, alongside forty (40) semi-structured interviews conducted with permanent residents to record local practices, preparation methods, and intergenerational knowledge transfer. Initial results show that a significant number of indigenous species are still used as food or natural remedies, most notably St. John's wort (*Hypericum perforatum*), oregano (*Origanum vulgare*), mountain tea (*Sideritis raeseri*), dogwood (*Cornus mas*), blackberry (*Rubus spp.*), wild rose (*Rosa spp.*), hawthorn (*Crataegus spp.*), and coltsfoot (*Tussilago farfara*). Concurrently, indicators show a population decline for oregano (*Origanum vulgare*), mountain tea (*Sideritis raeseri*), and primrose (*Primula veris*) due to intensive, uncontrolled collection. Specifically, for the mountain tea, is illegally and unsustainably harvested (prematurely and by uprooting) for commercial purposes, violating Greek legislation. This finding highlights the urgent need for effective conservation measures and rational management, which has already prompted stricter enforcement by competent authorities. This research aspires to preserve traditional ethnobotanical knowledge and protect the native flora of Mountainous Central Greece, underscoring the importance of sustainable resource management and its connection to sustainability education.

Key words: medicinal plants, biodiversity, sustainable management, ethnobotany.

Urban woody flora of the Megara city (Prefecture of Attica, Central Greece)

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Urban greenery contributes significantly to the quality of city life, constituting a key element for sustainable living of its inhabitants. The city of Megara is located in the western part of the Prefecture of Attica (Central Greece), with a low altitude and xerothermic climate. The purpose of this work is to investigate the urban woody flora, and the assessment of its condition in Megara city. To carry out the research, all woody species were recorded, and for each individual the problems, they may have were identified. The recording was done per section of the city, in order to investigate the differentiation that may exist, depending on the year, the location, and the design that each park or row of trees may have been created. In total, more than 150 species and subspecies were recorded, while the total number of all individuals exceeds 10,000. The results show that 73% of the species are alien and only 27% are native, in Greece, species. The percentage of alien species comes mainly from Asia (23%), America (22%), Mediterranean (19%), Europe (14%), Australia (13%) and much less from other regions. Furthermore, it seems that there is a significant difference in the origin of the species between urban parks, where species of American and Mediterranean origin are dominate, and tree rows, where Mediterranean and Asian species are dominate. In the life form of the woody flora of Megara, megaphanerophytes, mesophanerophytes and microphanerophytes (trees) predominate with a percentage of 85%, while nanophanerophytes (shrubs) follow with a percentage of 15%. As far as their health is concerned, it seems that there are problems, almost only in alien species, like in their development and diseases (drying of leaves, root rot, etc.), distorted and/or dead individuals. At the same time, individuals were also identified to create problems for the smooth functioning of the city, such as tree crowns that block road signs.

Key words: woody plants, ornamental, alien taxa, urban green

Sustainable management of Fire blight in apple production using the bacterial antagonist *Paenibacillus polymyxa* AA/15

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Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora*, is one of the most destructive diseases affecting pome fruits worldwide, including apples and pears. The pathogen causes significant economic losses in orchards, particularly in the Prespa region of North Macedonia, a major apple-producing area. Current control measures rely heavily on antibiotics (streptomycin) and copper-based compounds, which face increasing limitations due to resistance development, environmental concerns, and regulatory restrictions. This study aimed to: (1) isolate and characterize *E. amylovora* from infected apple material; (2) determine optimal growth conditions for the pathogen; and (3) evaluate the antibacterial activity of bacterial antagonists against *E. amylovora* for potential biocontrol applications. *Erwinia amylovora* was isolated from infected apple branches and leaves collected from the Prespa region and identified based on colony morphology on nutrient agar. Optimal growth conditions were determined by evaluating five different media (MHB, NB, GP, TSB, LBB), three temperatures (30°C, 37°C, 44°C), and three pH values (5, 6, 7) using turbidimetric measurements. Antibacterial activity of 16 bacterial isolates (from the genera *Bacillus* and *Paenibacillus*) was evaluated using disk diffusion and pour-plate methods against two *E. amylovora* isolates. The most promising antagonist was further tested in dilution assays and in vivo on apple fruits. Results showed that Nutrient Broth (NB) was the optimal medium for *E. amylovora* growth, with maximum growth at 580 FAU. Optimal temperatures were 37°C for the 2015 isolate and 30°C for the 2025 isolate, while optimal pH was 7 for the 2015 isolate and 6 for the 2025 isolate. Among the 16 antagonists assessed, *Paenibacillus polymyxa* AA/15 demonstrated the most consistent and potent antibacterial activity, with inhibition zones of 6–20 mm against both *E. amylovora* isolates. Dilution tests confirmed AA/15 activity up to 10⁻⁶ dilution against the 2025 isolate. Importantly, in vivo tests on apple fruits demonstrated that AA/15 completely prevented symptom development when co-inoculated with *E. amylovora*, confirming its protective capacity. These findings demonstrate that *P. polymyxa* AA/15 is a highly promising biocontrol agent against fire blight. Its broad-spectrum antibacterial activity, stability across different concentrations, and effectiveness in preventing infection in vivo offer a sustainable alternative to chemical controls. The utilization of antagonistic microorganisms presents an environmentally friendly strategy for managing this economically important disease, reducing reliance on antibiotics and copper while minimizing risks to human health and ecosystem integrity. Importantly, the application of microorganisms for plant protection should be environmentally safe and should not adversely affect the local ecosystem or contribute to undesirable changes in microbial diversity and biodiversity in the surrounding environment.

Key words: biocontrol, plant disease management, apple production

Agroforestry systems of Pyxos area, Greece (Transboundary Prespa Park)

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Prespa is one of the most important regions in the Balkan Peninsula and Europe in terms of floristic diversity. In the Pyxos region, in the southwestern sector of the Transboundary Prespa Park, there are extensive agroforestry systems. These systems present a great diversity in flora and vegetation, but it seems that their use is gradually being abandoned. The purpose of this work is to investigate the differentiation between the three forms of land use (forests, grasslands and cultivations) in the region, as well as the evolution of these land uses. For the flora and vegetation research, 22 relevés were made (10 in forests, 8 in meadows and 4 in agricultural crops), during May and June 2024. For the research on evolution, the current extent of different habitat types was mapped and at the same time an attempt is made to map the changes in land uses based on orthophoto maps of 1945. From the research, 157 plant taxa were recorded from which 20 were common between grasslands and forests, 4 between grasslands and cultivations and 1 between forests and cultivations. In contrast, in forest environments 49 different species were recorded, which are not found in grasslands or cultivations. In grasslands, 84 different species were recorded, which are not found in the other systems. In cultivations, in addition to the cultivated species, ruderal species that usually grow in grasslands and roadsides are found. Regarding the habitat types of Directive 92/43/EEC, "Pannonian oak forests with *Quercus cerris* and/or *Quercus petraea*" (code 91M0) and the "Forests with *Quercus trojana*" (code 9250) forests are found, while grasslands belong to "Eastern sub-Mediterranean dry grasslands (*Scorzonera villosa*)" (code 62A0). From the orthophoto maps it appears that in the past cultivations were more extensive than today, and often appeared in discrete parcels on flat areas, while on steep slopes the use of terraces was distinct. In areas where cultivations used to exist and are now abandoned, both cultivated species and many grassland species appear. Today, there is an expansion of forest vegetation at the expense of grasslands and openings and a clear limitation of cultivated areas. At the same time small individual cultivations are not distinguished, as in the past, demonstrating the gradual abandonment of traditional practices and subsequent alterations in landscape diversity. It appears that the forests belonging to the habitat types 91M0 and 9250, are the ones that benefit from the abandonment of traditional land uses. On the other hand, grasslands (habitat type 62A0), which represent the xerothermic grasslands of the region, are the ones that receive the greatest pressure, as they are gradually "invaded" by woody species and are directly at risk from vegetation succession. It should be noted that these grasslands also present the greatest variety of plant taxa, which means that pressures on this habitat type will also lead to a reduction in floristic diversity.

Key words: agroforestry, floristic diversity, land-use change

Interannual variation in seed quality and its implications for black alder restoration in Lake Prespa

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Black alder (*Alnus glutinosa* (L.) Gaertn.) is a characteristic species of European riparian forests, where it contributes to ecosystem functioning and habitat stability. In the Lake Prespa region of North Macedonia, remnant black alder stands are threatened by a combination of anthropogenic pressures, changing environmental conditions, and the poor condition of mature trees. Active restoration efforts heavily rely on seedlings produced from locally collected seeds, making the availability and quality of reproductive material an important consideration for successful population recovery. This study evaluated interannual variation in the quality of locally collected black alder seeds over three consecutive seed crops (2023–2025) and explored its implications for restoration planning. Seed lots collected from the Prespa population were assessed for physical and physiological quality, including seed purity, thousand-seed weight, seed vitality and germination. For comparison among years, germination performance following cold stratification was used, as this treatment was previously identified as the most effective for seeds from the studied population. Seed quality differed markedly among the three years. In 2023, seed purity was 59.51%, thousand-seed weight was 1.165 g, 18.5% of examined seeds were healthy, and germination after cold stratification reached 20.7%. Seed quality was considerably higher in 2024, with 74.0% purity, a thousand-seed weight of 1.725 g, 36.25% healthy seeds and 33.4% germination. In contrast, the 2025 seed crop showed the lowest physiological quality. Despite having the highest purity (89.25%), thousand-seed weight decreased to 1.20 g, only 7.25% of examined seeds were healthy, 89% were empty, and germination following cold stratification reached only 4.25%. The pronounced differences among three consecutive seed crops demonstrate that the quality of reproductive material available for restoration can vary substantially between years. Particularly, the 2025 results show that high physical purity does not necessarily correspond to high physiological quality or propagation potential. Although the drivers of the observed variability cannot be determined from the present dataset, annual environmental conditions and the condition of mother trees represent potential contributing factors. These findings highlight the importance of integrating annual seed-quality assessment into restoration planning, together with diversification of mother trees and collection sites and adaptation of nursery sowing strategies to the quality of individual seed crops. Continued monitoring could help determine whether the observed variability represents short-term fluctuations or a more persistent constraint on the regeneration and restoration of black alder in Lake Prespa.

Key words: *Alnus glutinosa*; riparian forests; reproductive material; seed viability; germination capacity; conservation; North Macedonia

Seasonal population dynamics of *Grapholita funebrana* (Treitschke, 1835) (Lepidoptera: Tortricidae) in stone fruit orchards in North Macedonia

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One of the economically significant pests of stone fruit in North Macedonia is *Grapholita funebrana* (Treitschke, 1835), the plum moth, causing average damage ranging from 17.5 % to 38 %, depending on the crop and locality. The pest is active throughout most of the year and is the main reason for frequent insecticide applications. Successful management of this moth depends on the precise forecasting of adult emergence, the number of generations, and the beginning and duration of the flight of each generation. The study began in 2025 at two localities representing major stone fruit production regions: Sirkovo (Vardar Region) and Hamzali (Southeastern Region). The seasonal dynamics of *G. funebrana* were determined using accumulated degree-days (DD) obtained from automated meteorological stations and pheromone traps in both regions. The first adult of *G. funebrana* in Sirkovo was captured on 25/03/25, and the last on 11/11/25, with pest activity lasting 232 days. In Hamzali, the first adult was captured on 11/03/25 and the last on 21/10/25, resulting in a pest activity period of 225 days. Following the needs of degree-days for *G. funebrana* and starting with the lowest developmental threshold of 10 °C, we can say that the pest completed four generations at both localities in 2025. In Sirkovo, the first generation was the most abundant, active from 25/03/25 to 03/06/25, coinciding with the period of fruit formation, growth, and ripening. In Hamzali, the second generation was the most abundant, with flight occurring from 17/06/25 to 15/07/25, during the ripening period of stone fruit. These investigations will continue over the next two years to provide more precise data for predicting *G. funebrana* development. The primary aim is to support the implementation of an integrated pest management (IPM) system in stone fruit orchards to ensure safe fruit production and reduce environmental pollution caused by agrochemical use.

Key words: Plum moth, degree-days, number of generations, Integrated Pest Management.

Understanding mineral fertilizer use in Prespa and the potential buffering role of wet meadows

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Wet meadows are biodiversity hotspots and highly productive ecosystems that provide numerous ecosystem services, including nutrient and carbon retention and regulation of the hydrological cycle. In Prespa, their area has been significantly reduced as a result of conversion to agricultural land, most of it used for apple orchards, as well as due to successional processes that lead to changes in these habitats. At the same time, intensive apple production is associated with the regular use of mineral fertilizers, raising questions about their impact on the surrounding natural ecosystems. To assess the capacity of wet meadows to retain nutrients from mineral fertilizers used in agricultural production, a study was conducted in 2022 combining an in-situ experiment and a survey of 39 local apple growers. The survey provided an initial insight into the principles and practices of mineral fertilizer application, while also highlighting the need for a more detailed study involving a larger number of apple growers. As part of the in-situ experiment, fertilized and unfertilized lysimeters simulating different meadow management practices were established in a wet meadow within the Ezerani Protected Area. Based on the data obtained from the farmers, NPK 8:16:24 was applied to the experimental plots at an amount equivalent to approximately 700 kg ha^{-1} . Soil analysis showed statistically significant differences between the fertilized and unfertilized treatments in pH, N, Mn, P_2O_5 , and K_2O ($p < 0.05$). Following fertilizer application, we observed an increase in N, Mn, P_2O_5 , and K_2O contents and a decrease in pH value, indicating retention of part of the nutrients introduced in the soil of the wet meadows. Building on the initial survey, a more extensive survey involving 186 respondents was conducted in 2025 to better understand mineral fertilizer use practices in Prespa. Preliminary results showed that 166 of the 186 respondents owned apple orchards, of whom 95.2% used mineral fertilizers. Among respondents who used mineral fertilizers, only 26.5% performed soil analysis. Among those who used mineral fertilizers but did not perform soil analysis, 65.8% determined the fertilizer dose based on their own experience. Again, NPK with a composition of 8:16:24 was the most commonly used mineral fertilizer. The results of both studies indicate a close relationship between agricultural production and wet meadows in Prespa. The experiment showed that wet meadow soils can retain part of the nutrients introduced through mineral fertilizers, while the preliminary results of the expanded survey indicate widespread use of mineral fertilizers and limited use of soil analysis. These findings emphasize the need for better fertilizer management in apple production, alongside the conservation of the remaining wet meadows in Prespa.

Key words: apple orchards, nutrient retention, Prespa region, lysimeters, agriculture

Phytosociological analysis of forest vegetation in the Vrbas River canyon system with an assessment of wildfire as a threat factor

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Title: Phytosociological analysis of forest vegetation in the Vrbas River canyon system with an assessment of wildfire as a threat factor The research was conducted in the canyon system of the middle course of the Vrbas River, including the canyons of its tributaries, the Ugar and Crna Rijeka rivers. The aim of the study was to classify forest vegetation within the study area using a phytosociological approach and to assess the spatial vulnerability of forests to wildfires through the integration of vegetation, topographic, structural, hydrological, anthropogenic, and satellite-derived indicators. Vegetation was investigated using the classical Braun–Blanquet phytosociological approach, supported by numerical data analysis and stand classification using the Cocktail method. A total of 17 plant communities were identified and classified within the classes *Carpino-Fagetea sylvaticae*, *Quercetea pubescentis*, *Crataego-Prunetea*, and *Salicetea purpureae*. The vegetation is dominated by thermophilous forest communities of pubescent oak (*Quercus pubescens*), oriental hornbeam (*Carpinus orientalis*), and hop-hornbeam (*Ostrya carpinifolia*), while mesophilous beech (*Fagus sylvatica*), common hornbeam (*Carpinus betulus*), and noble broadleaved forest communities are restricted to more humid, shaded, and topographically sheltered canyon habitats. Of particular ecological and conservation importance are relict and refugial stands of *Tilio-Acerion* forests of slopes, screes and ravines, which support high floristic diversity and harbor rare and threatened taxa. A spatial wildfire vulnerability index was developed by integrating multiple groups of predictors, including Ellenberg indicator values, vegetation-derived parameters, topographic variables (slope, aspect, and elevation), forest structural characteristics, proximity to roads and settlements, hydrological conditions, and satellite-derived data. Vegetation indicators were spatially modeled to produce a continuous wildfire susceptibility map for the study area. The results reveal a clear spatial gradient of wildfire vulnerability. The highest index values were recorded in thermophilous, more open, and xerothermic forest and shrub communities dominated by oriental hornbeam, pubescent oak, and hop-hornbeam, particularly on warmer south-facing slopes, steeper terrain, and areas closer to anthropogenic infrastructure. The most vulnerable syntaxa include *Cruciato glabrae–Carpinetum orientalis*, *Carici hallerianae–Quercetum pubescentis*, and *Cotinus coggygia–Quercus pubescens* communities, whereas mesophilous beech, hornbeam, and noble broadleaved forests consistently exhibit lower vulnerability values. These findings highlight the combined influence of vegetation characteristics, ecological conditions, and human pressures on spatial patterns of wildfire susceptibility. The proposed methodological framework provides an integrated interdisciplinary approach for assessing forest wildfire vulnerability and has practical applications in monitoring strategies, preventive fire management, and forest ecosystem planning. The results also provide a basis for future research incorporating meteorological data, historical fire records, and more detailed analyses of fuel load and fuel structure. Mentor: Vladimir Stupar, PhD, Associate Professor, University of Banja Luka, Faculty of Forestry

Key words: phytosociology; biodiversity; Vrbas River canyon; wildfires; GIS analysis

Sibljak of *Paliurus spina-christi* in Central Macedonia (Greece)

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Habitats, such as natural ecosystems between forests, meadows or agricultural crops, are ecologically very important, as they are considered to have a high plant and animal diversity. These ecotones are usually dominated, in the central Balkan Peninsula, by deciduous shrubs such as *Paliurus spina-christi* and are called sibljak. They arise after continuous logging and overgrazing, create natural hedges between agricultural crops and spread rapidly in abandoned crops. The aim of this work is to investigate the vegetation formed by the *Paliurus spina-christi* shrublands. For the purposes of the study, 48 relevés were used, made using the Braun-Blanquet (1964) method, from May 2001 to June 2025, in central Macedonia (Greece), specifically at the foothills of the Beles and Krousia Mountains (area of Kerkini Lake), as well as in the basin of Lake Volvi. In the research area, in the sibljak of *Paliurus spina-christi*, several species that are diagnostic of *Crataego-Prunetea* and *Paliuretalia* appear (e.g. *Crataegus monogyna*, *Pyrus spinosa*). In northern Greece, the vegetation with a strong presence of *Paliurus spina-christi* seems to belong to the *Eryngio campestris*-*Paliurion spinae-christi* alliance. The alliance expresses the pre-forest shrublands and hedgerows that appear in the central Balkan Peninsula, at low altitudes, where *Paliurus spina-christi* appears with great abundance, and is accompanied by species such as *Rubus sanctus*, *Asparagus acutifolius*, *Pyrus spinosa*, *Vitex agnus-castus*. In central Macedonia (Greece), where probably are the southernmost limits of the order, two vegetation units can be distinguished: (i) *Vitex agnus-castus*-*Paliurus spina-christi* comm., which appears in the Volvi area. The community is largely dominated by *Vitex agnus-castus*, a diagnostic species of *Nerio-Tamaricetea*, as well as several diagnostic species of *Quercetea ilicis* and *Fraxino-Ostryion* (e.g. *Quercus coccifera*), which expresses the floristic affinity of the vegetation unit with the xerothermic evergreen broadleaf shrublands (pseudomaquis and maquis) and (ii) the *Rhagadiolo stellati*-*Paliuretum spinae-christii* ass. nova, which occurs in the area of Belles and Krousia, with the frequent presence of *Rhagadiolus stellatus*, *Ulmus procera* and *Celtis australis*. The association includes, with high abundance, many synanthropic or/and nitrophilous species (e.g. *Dracunculus vulgaris*, *Galium aparine*, *Arum maculatum*). These species, as well as many others that occur in the association, are also highly frequent in the low altitude azonal formations of *Platanus orientalis* and *Salix alba*, due to the high humidity. In the abandoned crops, the pre-forest shrublands are floristically related, with the presence of diagnostic species of forest plant communities (e.g. *Cornus mas*, *Fraxinus ornus*).

Key words: deciduous shrublands, Kerkini, Volvi, phytosociology

Landscape fire management approach for fire-resilient landscapes: Evidence from the Western Balkans

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Wildfires are increasingly recognized as complex socio-ecological challenges influenced by climate change, land-use changes, ecosystem dynamics and human activities. In response to these evolving challenges, wildfire management has progressively moved beyond fire suppression towards more integrated approaches. However, there remains a need to further integrate these perspectives within a landscape-scale framework that connects ecological processes, governance systems, land management practices, stakeholder and community participation. This paper conceptualizes Landscape Fire Management (LFM) as a holistic approach for building fire-resilient landscapes. Based on a synthesis of scientific literature on fire management, landscape fire management, adaptive governance and resilience, the LFM integrates the full fire management cycle while placing a focus on prevention. The approach combines landscape-based planning with preparedness, response and post-fire recovery, supported by participatory governance, cross-sectoral cooperation, landscape approach and knowledge-based adaptive management. Landscape fire management is applied as an analytical framework to assess fire management in the Western Balkans, including Albania, Bosnia and Herzegovina, Kosovo, Montenegro, North Macedonia and Serbia. The assessment combines spatial analysis of fire occurrence and burned areas using the European Forest Fire Information System (EFFIS) and national fire datasets with land-cover analysis and a comparative review of national LFM gap assessments to examine fire patterns, land-use characteristics, governance capacities and implementation gaps. The comparative assessment shows that fire vulnerability in the Western Balkans is driven by the interaction of climate change, land-use change, vegetation dynamics and fragmented governance systems. Despite differences among countries, common gaps were identified in prevention planning, integrated fire risk assessment, cross-sectoral coordination, community engagement and post-fire restoration. The findings highlight the need to shift from predominantly reactive fire suppression towards preventive, adaptive and participatory landscape governance. The proposed management of fire on a landscape level, not separated for foresters, farmers, etc., provides a transferable way for integrating ecological processes, institutional capacities and community action to strengthen landscape resilience under increasing fire risk.

Key words: wildfire prevention; adaptive governance; community participation; cross-sectoral cooperation; post-fire restoration

One decade of fire in a forest and transitioning landscape: Trends in the number of fires, burned area, and frequency in North Macedonia, 2016-2025

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North Macedonia's forests and open landscapes have burned with striking inter-annual variability over the past decade. Yet the country's own official reporting stream from the Public Enterprise "National Forests" compiles damage records across different time windows, spatial units, and classification schemes, and has never been reconciled into a single analytical series. This paper harmonizes these sources into a continuous 2016–2025 national forest fire-regime record for North Macedonia and subjects it to formal statistical analysis rather than the descriptive, year-by-year narration found in existing grey literature. We apply non-parametric trend tests (Mann-Kendall, Sen's slope), group-comparison tests (Kruskal-Wallis across branches, Friedman across seasons), regression models, and multivariate methods (PCA and K-means clustering of branches), complemented by concentration indices to characterize the spatial structure of fire risk. Using the harmonized series, we examine whether burned area, fire frequency, and economic loss (only for wood mass burnt) show upward trends once single extreme years (2017, 2019, 2021, 2024, 2025) are statistically controlled for, and how fire occurrence is distributed across the country's branches and its 30 Forest Management Units (FMUTs). We further assess whether regional heterogeneity — the Southeastern region's recurrent peak years versus Pelagonia's high cumulative total — reflects genuine spatial risk concentration or reporting artefacts arising from inconsistent regional boundaries across sources. Based on the analysis, 2,053 recorded forest fires occurred across the 30 FMUTs, comprising a total of 135,370.03 ha burned, while the estimated direct damage amounted to 13.95 billion Macedonian denars. Descriptive statistics, Spearman correlations, Mann–Kendall tests with Sen's slope, fixed-effects panel models, Kruskal–Wallis tests, multiple regression, and principal component analysis (PCA) reveal pronounced heterogeneity among branches and strong seasonality. Annual nonparametric tests found no statistically significant monotonic trend in burned area, damaged timber volume, financial damage, personnel involved, or suppression costs ($p > 0.05$), whereas the negative binomial panel model estimated an average annual decrease of 3.4% in the expected number of fires. The July–September period accounted for 48.3% of fires, 81.7% of the burned area, and 91.5% of total damage, with August emerging as the most critical month. The strongest association was observed between damaged timber volume and financial damage ($\rho = 0.931$; $p < 0.001$), while in the multiple regression model, timber volume was the dominant independent predictor. The paper's core contribution is methodological as much as empirical: a replicable protocol for harmonizing fragmented national forest fire statistics in data-limited Balkan settings, illustrated through, and directly applicable to, the North Macedonian case. Building on this harmonized record, we develop a novel statistical model of fire-occurrence probability at the level of individual Forest Management Units and sub-management units, offering the first disaggregated risk profile of the country's 30 forest management units

Key words: patial risk assessment; statistical harmonization; occurrence probability; spatio-temporal variability

Preliminary assessment of high nature value farmland (HNVF) landscapes and biodiversity in North Macedonia

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High Nature Value Farmland (HNVF) is associated with traditional, low-intensity farming systems that support a diversity of habitats and species. Semi-natural pastures, meadows and orchards, together with landscape elements such as hedges, coppices, pollarded trees and other semi-natural features, form a mosaic of habitats important for biodiversity. Traditional management practices, including mowing, grazing, coppicing and pollarding, are also an important part of these landscapes. Pollarded trees, in particular, can provide different microhabitats and may contribute to maintaining species associated with old trees and dead wood. Biodiversity and landscape characteristics were assessed in four areas in North Macedonia: Vejce on Šar Planina, Mitrašinci and Laki in the Berovo area, Negrevo near Pehčevo, and Ali Koč in the foothills of Plačkovica near Radoviš. The areas were selected to represent different types and characteristics of HNVF, including differences in geographic position, climate, geology, biodiversity and traditional land use. The main aim was to document the species and habitat diversity and relate recorded biodiversity to habitat types, landscape structures and traditional management practices. The assessment also considered species and habitats of conservation importance and the potential use of species as HNVF indicators, contributing to the development of a standardized methodology for future HNVF assessments in North Macedonia. The assessment included landscape analysis and habitat mapping, identification of habitats, microhabitats and landscape structures, as well as socio-economic information collected from available sources and interviews. Biodiversity was assessed using different approaches depending on the taxonomic group. Plant communities were studied using vegetation relevés and transects, birds through observations of breeding sites and associated microhabitats, butterflies using entomological nets, and saproxylic beetles using pitfall, flight-intercept and eclector traps placed on or near pollarded trees. A total of 3499 bird records were collected, with 58–92 species recorded per area. The highest number of species was recorded in Ali Koč (92 species) and Vejce (87 species). Particularly notable were records of several species of conservation importance, including *Ficedula semitorquata*, *Ciconia nigra*, *Circaetus gallicus*, *Coracias garrulus* and *Pernis apivorus*. A total of 389 records representing 84 butterfly species were documented across 30 habitat types. Meadows supported the highest species richness (49 species), followed by semi-natural pastures with scattered shrubs (32 species) and oak and beech dominated coppice forests (28 species). The surveyed areas support a highly diverse saproxylic beetle fauna, including numerous species of conservation significance and almost all Natura 2000 target species. Notable records included *Rosalia alpina*, *Lucanus cervus*, *Morimus funereus*, *Limoniscus violaceus*, *Cerambyx cerdo* and *Osmoderma barnabita*. Plant species of conservation and indicator value were identified, and small-scale habitat heterogeneity was found to be important for maintaining floristic diversity. Traditional landscape elements contributed to increased habitat heterogeneity and biodiversity across the studied farming landscapes and agroecosystems. The preliminary assessment indicates that the HNVF areas support a diverse range of habitats and species associated with traditional low-intensity farming practices. The findings will contribute to a better understanding of biodiversity and landscape characteristics in HNVF areas and support the development of a standard methodology for future HNVF assessments in North Macedonia.

Key words: traditional farming system, semi-natural habitats, landscape heterogeneity, pollarded trees

Support cooperation on high nature value farming for preserving biodiversity - pilot activity in North Macedonia

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Agricultural landscapes in North Macedonia support significant biodiversity and cultural heritage shaped by centuries of traditional farming practices. One aim of this collaboration project between North Macedonia and Sweden is to develop an approach that helps identifying High Nature Value Farmland (HNVF) systems, whereby agricultural practices contribute to biodiversity conservation, habitat maintenance, and landscape resilience. Field inventories will contribute to test and develop HNMF-typologies across a transect from mountain to lower elevation landscapes: Vejtsce and Bozovtse in Shar National Park, Kodžalija and Alij Koč in the Radoviš region, Mistrasinci and Budinarci in the Berovo region, and Negrevo in the Pehčevo region (these results are presented separately). The inventories include the four organism groups: birds, butterflies, beetles, and flora. In parallel, interviews with farmers, local communities, and authorities serve to document traditional land-management practices and socio-economic factors influencing their continuation. Finally, statistical data and mapping will help illustrate biodiversity hotspots. The poster illustrates examples of HNMF at three levels: from agroecological measures in a field, to connectivity between fields which leads to resilient landscapes. Preliminary results indicate two main threats to the loss of traditional farming practices, as immediate threats to HNMF and biodiversity. First, abandonment due to missing income opportunities, leading to overgrowth which alters biodiversity and may present increased risks of wildfires. Second, high intensity monoculture agriculture encroachment, which is more common at lower elevations. Potential benefits of existing and modernized HNMF are also highlighted at each level. The HNMF-types are expected to contribute recommendations for biodiversity-oriented rural development measures. This supports habitat classification and preparation for Natura 2000, which in preparation North Macedonia's EU accession process, particularly Chapter 27, and supports implementation of the Green Agenda for the Western Balkans.

Traditional livestock grazing as a driver of High Nature Value grassland conservation: Evidence from the Maleshevo Protected Landscape

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Traditional livestock grazing is widely recognized as an essential management practice for maintaining High Nature Value (HNV) grasslands across Europe. Approximately one-third of the habitat types listed under Annex I of the EU Habitats Directive depend on extensive agricultural management (known as Halada habitats), while the EU Biodiversity Strategy for 2030, the Nature Restoration Regulation, and the Common Agricultural Policy (CAP) promote sustainable grazing as a key conservation measure. Thus, well-managed grazing systems can serve as powerful tools for ecosystem restoration and enhance biodiversity. While the CAP aims to protect these landscapes via eco-schemes and agri-environment measures, critics and scientists note that generalized direct payments often favor intensive farming, leaving fragile HNV systems vulnerable to abandonment or conversion. HNV farmlands face a dual threat: fertile areas are pushed toward high-input intensive farming, while marginal pastoral areas suffer from total economic abandonment. Rural depopulation, declining livestock numbers, and inadequate policy support are also accelerating the abandonment of mountain pastures across the Western Balkans, including North Macedonia. This research evaluates the current livestock grazing system in the Maleshevo Protected Landscape and assesses its contribution to the conservation of mountain grassland landscapes. The research combined analysis of existing livestock and pasture-use data with structured interviews involving 34 livestock farmers and GIS-based spatial analysis of grazing patterns. The assessment examined livestock trends, grazing intensity, pasture utilization, farmers' perceptions, and the spatial distribution of grazing in relation to landscape types and conservation priorities. The results reveal a decline in livestock numbers, underutilization of mountain pastures, and a growing concentration of grazing around rural settlements. Approximately 65% of surveyed farmers no longer use mountain pastures, and almost half report progressive overgrowth due to pasture abandonment. Spatial analysis indicates that grazing is increasingly confined to agricultural landscapes near villages, while ecologically valuable mountain grasslands remain largely unmanaged, accelerating shrub encroachment and biodiversity loss. Farmers identify inadequate infrastructure, complex administrative procedures, and insufficient institutional support as the principal barriers to sustainable grazing. The findings highlight the need to better integrate agricultural and biodiversity policies by aligning national legislation with European environmental frameworks, strengthening agri-environmental incentives, improving pasture governance, and enhancing cooperation among protected area authorities, pasture management institutions, agricultural agencies, and livestock farmers to conserve High Nature Value grasslands.

Key words: Common Agricultural Policy (CAP), EU Habitats Directive, pasture abandonment, ecosystem restoration, policy integration, agri-environmental schemes, adaptive management

Vascular plant diversity in High Nature Value Farming landscapes of North Macedonia

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High Nature Value farming (HNVF) landscapes are shaped by centuries of low-intensity agricultural practices, including traditional grazing, mowing, crop rotation, pollarding and orchard management. These practices have created complex and highly heterogeneous landscapes in which semi-natural grasslands, cultivated fields, orchards, wooded patches, hedgerows and old trees occur in close proximity. Such landscape mosaics provide a wide range of ecological conditions and microhabitats and consequently support considerable vascular plant diversity. This study documents vascular plant diversity and examines its relationship with habitat structure, microhabitat availability and traditional land management in four HN VF villages in North Macedonia: Mitrashinci, Ali Koč, Vejce and Negrevo. The selected villages represent four characteristic farming landscapes: pollard–grassland mosaics, coppice–grassland–field mosaics, mountainous mosaics of grasslands, fields and trees, and orchard–grassland mosaics. Together, they illustrate the diversity of traditional farming systems occurring across different environmental and socioeconomic settings in the country. Field research focused on priority habitats, including semi-natural pastures, hay meadows, rotational fields in different successional stages, traditional orchards, wooded field margins, hedgerows, coppiced woodlands and pastures with old pollarded trees. Vascular plants were recorded through systematic zigzag surveys within mapped inventory areas, complemented by vegetation plots for a more detailed assessment of selected habitats. Species presence and abundance, vegetation structure, land-use status and associations with specific microhabitats were documented. Particular attention was given to bare soil, rocks, field and terrace margins, open gaps, shrubs, old trees, dead wood and areas affected by grazing, mowing or trampling. The study identified plant species of conservation and indicator value and evaluated the importance of small-scale habitat heterogeneity for maintaining floristic diversity. By linking vegetation patterns with current and historical management, the research contributed to the characterization of HN VF types in N. Macedonia and provided a scientific basis for management recommendations aimed at their long-term preservation.

Key words: High Nature Value farming, vascular plants, traditional land use

ECOSYSTEM SERVICES AND MANAGEMENT OF NATURAL RESOURCES

Beyond assessment: Translating ecosystem services into real-world decisions and transformative action

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Ecosystem service assessment has advanced substantially, providing increasingly robust evidence on how ecosystems support human well-being through services such as clean water provision, carbon sequestration, biodiversity support, and cultural benefits. Yet, a persistent gap remains between scientific assessment and its effective use in policy, business, and community decision-making. This presentation explores how ecosystem service knowledge can move beyond assessment and valuation to inform practical decisions and enable transformative action. Drawing on recent advances and real-world applications, the presentation examines the conditions required to translate ecosystem service evidence into effective management and policy. Particular emphasis is placed on interdisciplinary collaboration, scenario modelling, decision-support tools, natural capital approaches, and adaptive governance. Equally important are stakeholder participation, the recognition of diverse values, and considerations of equity and trade-offs, which are essential for avoiding oversimplified representations of complex social–ecological systems. Examples of approaches such as payments for ecosystem services, corporate natural capital accounting, and nature-based solutions illustrate how ecosystem service information can influence incentives, investment decisions, conservation outcomes, and sustainable development. These experiences demonstrate that the value of ecosystem services lies not only in measuring and communicating their benefits, but also in creating credible pathways through which evidence can shape decisions and behaviour. Moving from assessment to implementation is particularly urgent in the context of accelerating biodiversity loss, climate change, and increasing pressure on natural resources. Embedding ecosystem services and natural capital into decision-making can help align economic and societal priorities with ecological integrity while strengthening resilience and long-term human well-being. Ultimately, this transition supports a shift from predominantly reactive environmental management towards proactive, adaptive, and regenerative approaches, contributing to the objectives of the UN Sustainable Development Goals and the Kunming–Montreal Global Biodiversity Framework.

Key words: Ecosystem services; Natural capital; Decision support; Transformative change; Adaptive governance

Integrated assessment of ecosystem services in mountain and grassland ecosystems to support sustainable landscape management in North Macedonia

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The growing need for evidence-based ecosystem management requires assessment frameworks that integrate ecological, social and economic information into decision-making. Although ecosystem service assessments are increasingly applied worldwide, comprehensive integrated assessment frameworks remain scarce in the Western Balkans. This study presents the first integrated ecosystem services assessment conducted in North Macedonia, developed for mountain and grassland ecosystems across six municipalities in the north-western part of the country. The framework integrates four complementary dimensions. The biophysical assessment evaluates current ecosystem conditions following an enhanced national Mapping and Assessment of Ecosystems and their Services (MAES) methodology and historical land use changes between 1990 and 2021. The ecosystem services component identifies and evaluates ecosystem services by combining expert assessment with stakeholder knowledge on ecosystem service capacity, demand and priorities. The economic component quantifies the Total Economic Value (TEV) using the Ecosystem Services Valuation Database (ESVD) and available market data. The scenario component combines stakeholder-driven exploratory scenarios and two normative scenarios — Business-as-Usual (BAU) and Green — projecting land use and ecosystem condition to 2050, from which Net Present Value (NPV) was calculated. Ecosystem condition assessment showed a clear polarisation: broadleaf and riparian forests in protected or remote areas remained in excellent to very good condition and retained a high capacity to supply provisioning, regulating and cultural services, whereas forests and pastures under strong anthropogenic pressure were in poor to very poor condition and supplied comparatively little. This uneven capacity to deliver services aligned closely with land use pressure: land use change analysis between 1990 and 2021 revealed a net loss of grassland ecosystems (−7.15%), mainly converted to low-quality forests through natural succession (over 10 200 ha) and, to a smaller extent, into settlements (about 1 650 ha), while forest cover expanded by 6.04%, partly offset by roughly 4 150 ha reverting to grassland, with settlement expansion most pronounced in agricultural landscapes and around urbanising municipalities. Despite the relatively small decline in overall TEV (from about USD 337 million in 1990 to USD 334 million in 2021 (roughly USD 3 500/ha), these changes masked substantial trade-offs among ecosystem services. Grassland loss reduced provisioning and cultural services while forest expansion partly compensated through gains in regulating services, with pollination (USD 747/ha/yr) and existence value (USD 562/ha/yr) being the most valuable single services, despite being largely invisible in official policy. Scenario analysis confirmed that investing in nature pays off: the Green Scenario, combining a projected land cover gain of 4 411 ha with a doubling in ecosystem condition quality, yields a cumulative NPV of approximately USD 3.85 billion for 2021–2050, around USD 41 million above the BaU trajectory, which assumes a comparable land cover increase but a 10% decline in ecosystem quality. As a first integrated ecosystem services assessment in North Macedonia, the proposed framework provides a transferable methodology for the Western Balkans, supporting adaptive ecosystem management, prioritising restoration investments, and facilitating the integration of natural capital into land-use planning and policy development to strengthen ecosystem resilience and long-term human well-being.

Key words: ecosystem services; integrated assessment; ecosystem condition; land-use change; scenario analysis; Total Economic Value; Net Present Value

From local knowledge to conservation planning: Community mapping on Mountain Jablanica, North Macedonia

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Local communities possess detailed place-based knowledge of natural resources, environmental change and human–nature interactions. While international biodiversity policy, including the Convention on Biological Diversity and the Kunming–Montreal Global Biodiversity Framework, increasingly recognises the importance of such knowledge and community participation for equitable and effective conservation, these remain insufficiently integrated into formal conservation processes in North Macedonia. This study explored the application of participatory community mapping as a tool to document and spatially represent local ecological knowledge, resource-use patterns, perceived environmental changes and conservation priorities in the context of the proposed protected area of Mountain Jablanica. Five participatory workshops involving more than 95 participants were organised with local residents, complemented by a stakeholder meeting in Struga. Using topographic and thematic maps, participants identified natural and cultural values, resource-use areas, environmental changes, threats, important species and sites requiring protection. The mapping was complemented by a questionnaire completed by 64 respondents, who assessed the importance of 18 ecosystem services on a 0–5 scale. Water resources emerged as both highly valued and among the most threatened natural assets. Springs, rivers, glacial lakes and wetlands were considered important for water supply, livestock, ecosystem functioning, recreation and local identity, while participants reported declining water availability, drying of springs and pressures related to forest degradation, water abstraction and small hydropower development. Forests were valued particularly for water regulation, biodiversity conservation, erosion control, and landscape and recreational values, rather than solely as a source of timber. The ecosystem-service assessment supported the main patterns identified through the mapping process. Cultural and regulating services received the highest category-level mean scores (4.43 and 4.41, respectively, on a 0–5 scale), while drinking water, water purification, air purification and erosion control were among the highest-rated individual services. Local attitudes towards protected-area designation were generally positive, but conditional on effective protection of water and forests, respect for traditional resource use, transparent rules and meaningful participation of local communities in future management. The results demonstrate that community mapping can transform local knowledge and perceptions into spatially explicit information for conservation planning. Combined with ecosystem-service assessment, it can complement biological assessments by identifying locally recognised values, environmental pressures, potential conflicts and management priorities, providing evidence for participatory zoning and future protected-area management.

Key words: participatory approaches; ecosystem services; stakeholder engagement; environmental pressures; spatial information; natural resource use.

Physiological and biotechnological characterization of acetic acid bacteria isolated from wild medlar fruits in Shar Mountain National Park

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Acetic acid bacteria (AAB) are microorganisms of significant ecological and biotechnological interest, playing a crucial role in natural fermentation processes and the production of organic acids. The exploration of underexplored environments, such as mountainous national parks, offers promising opportunities for the discovery of novel AAB strains with unique metabolic capabilities. This study was conducted to evaluate the physiological and biotechnological potential of AAB isolates obtained from wild medlar (*Mespilus germanica*) fruits, a traditional and underutilized fruit species, collected from the Shar Mountain National Park in North Macedonia. The primary objective was to characterize selected isolates in terms of their growth and production indices on different media, their tolerance to ethanol, and their capacity for acetic acid production, in order to assess their suitability for biotechnological applications. Fruit samples were incubated in a Glucose Yeast Extract Peptone (GYE) enrichment broth at 30°C for 7 days to promote the growth of AAB. Following isolation, four distinct isolates, designated 4M, 5M, 6M, and 7M, were subjected to physiological screening. The isolates were cultured on two distinct solid media: Glucose Yeast extract CaCO₃ agar (GYC) and Glucose Yeast extract Ethanol CaCO₃ agar (GYE). The Production Index (PI), a measure of metabolic activity, was determined for each isolate on both media. The results revealed notable variability among the isolates. While isolates 4M, 5M, and 6M showed higher PI values on the standard GYC medium (4.09, 2.0, and 2.1, respectively) compared to the ethanol-supplemented GYE medium (3.0, 1.6, and 1.4), isolate 7M exhibited the highest PI overall, with a significant value of 5.0 on GYC and 3.5 on GYE. Furthermore, all isolates demonstrated alcohol tolerance up to 10% (v/v), except for isolate 7M, which showed tolerance only at 2% and 4% ethanol concentrations. The quantification of acetic acid production indicated that isolate 6M produced the highest amount (5.04 gL⁻¹), followed by isolate 7M (4.77 gL⁻¹), with all strains exhibiting a low final pH, ranging from 2.70 to 2.79, characteristic of AAB metabolism. The divergence in PI between the two media suggests that ethanol may have a repressive or less stimulatory effect on the metabolic activity of most isolates, with the exception of the highly active strain 7M. The high acetic acid production, coupled with strong acidification, highlights the potential of these strains, particularly isolate 6M and 7M, for use in vinegar production or other oxidative bioprocesses. In conclusion, the wild medlar fruits from Shar Mountain host a diversity of AAB strains with valuable physiological traits. The isolates 6M and 7M are identified as promising candidates for further biotechnological exploitation. This study underscores the importance of preserving and studying the microbial ecology of protected mountain ecosystems as a reservoir of industrially relevant microorganisms.

Key words: cetic acid bacteria, Wild medlar, Biotechnological potential, Shar Mountain, Organic acid production

Mapping fire risk zones in the Special Nature Reserve “Suva planina” and their impact on tourism

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Forest fires represent an important hazard in protected mountain areas, especially where seasonal drought, complex relief, vegetation cover and human activities overlap. In areas used for tourism, fire risk is also related to visitor safety, trail accessibility and the protection of tourism infrastructure. The Special Nature Reserve “Suva Planina” in southeastern Serbia is an important protected area with high natural value and considerable potential for hiking, recreational, rural and ecologically oriented tourism. The objective of this thesis is to map fire risk zones in the Special Nature Reserve “Suva Planina” and to analyse their impact on tourism infrastructure. The research was carried out using GIS-based spatial analysis. Five factors were included in the fire risk model: vegetation type, terrain slope, terrain aspect, distance from roads and tourist trails, and distance from settlements and buildings. All input layers were normalised, weighted and overlaid in a raster model. The final fire risk map was classified into five classes: very high, high, medium, low and very low risk. The model was validated using historical fire points from the NASA FIRMS database. In the final stage, the risk map was overlaid with point-based tourism facilities, access roads and hiking trails. The results show that the largest part of the analysed area belongs to the medium-risk zone, which covers 85.3 km², or 48.34% of the total area. The low-risk zone covers 52.3 km², while the high-risk zone covers 30.2 km². Very high risk was identified on 3.6 km², representing 2.04% of the analysed area. Model validation showed strong spatial agreement with previous fire occurrences. Out of 171 registered fire points, 84 were located within high and very high-risk zones, while 163 fire points, or 95.32%, were located within 100 m of these zones. The analysis of tourism infrastructure indicates that fire risk has direct relevance for tourism management. Out of 29 analysed tourism facilities, most were located in the medium-risk zone, but 5 facilities were located in high-risk areas and 4 in very high-risk areas. In addition, 76.589 km of access roads and hiking trails, or 29.8% of the analysed linear infrastructure, passed through high and very high-risk zones. The obtained results show that fire risk mapping can be a useful tool for managing tourism in protected areas. The map can support preventive measures, visitor information, trail maintenance and the spatial planning of tourism activities. The model provides a spatial basis for identifying priority zones for fire prevention and safer tourism management in the SNR “Suva Planina”.

Key words: Wildfire risk mapping; GIS; protected areas; tourism infrastructure; Suva Planina

Stakeholder perceptions of ecosystem services and management priorities in Shar Planina

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The concept of ecosystem services has become an important tool for supporting sustainable land-use planning and decision-making. However, stakeholders' perceptions on ecosystem service provision remains comparatively underrepresented in the literature, despite being essential for aligning conservation and land-use planning with both ecological conditions and societal needs. This study addresses that gap by assessing stakeholders' perceptions of the importance and capacity of ecosystem services provided by the Shar Planina ecosystems, together with their preferences for future management activities. A structured questionnaire was completed by 56 respondents from 40 institutions across seven stakeholder categories spanning from national to local governance levels as well as the public, private and civil society sectors. The questionnaire captured institutional profiles, perceived importance and capacity across eight major ecosystem types, and preferred management priorities. Results show that regulating and cultural services are consistently valued more highly than provisioning services across all sectors and governance levels. Stakeholders perceive Shar Planina's ecosystems as having an overall high capacity to supply these services, with deciduous forests rated highest and rocks ecosystems lowest. By service category, cultural services are perceived as having the greatest supply capacity, followed by regulating services, with provisioning again rated lowest. Regarding preferred management directions, most stakeholders across all categories consistently favour intensifying conservation and restoration oriented activities across ecosystem types. In contrast, opinions on increasing resource utilisation were more diverse. Although reducing resource exploitation was the dominant preference, a substantial proportion of stakeholders supported its intensification, indicating potential interinstitutional trade-offs between conservation and development objectives. These differences highlight the need for participatory planning approaches to reconcile divergent stakeholder perspectives by identifying areas of disagreement, fostering compromise, and promoting collaboration. By identifying shared priorities as well as divergent perspectives, the study offers practical guidance for balancing conservation objectives with sustainable resource use.

Key words: sustainable land-use planning; participatory assessment; social valuation; ecosystem management; decision support

Using DNA metabarcoding to assess mowing impacts on grassland arthropod communities

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Extensive grasslands support highly diverse arthropod communities but are increasingly threatened by changes in land use and management. Since they are important food source, the changes affect many vertebrates too. Mowing is essential for maintaining many semi-natural grasslands, yet its timing may strongly influence arthropod communities. Efficient approaches capable of characterizing community-level responses throughout the growing season are therefore needed to support biodiversity-oriented grassland management. Here, we present preliminary results from a two-year study evaluating the potential of DNA metabarcoding to track seasonal dynamics of grassland arthropod communities under contrasting mowing regimes. The study was conducted within the CarEx (Care for Extensive Grasslands) Interreg Si-Hr project over two consecutive years (2024–2025) at eight pilot grassland areas in Kozjansko Regional Park (Slovenia) and in Natural Park Žumberak – Samoborsko gorje (Croatia). Arthropod communities were sampled using standardized sweep-net sampling on four occasions during each season, from June to October. The areas were subjected to contrasting mowing regimes, enabling us to follow seasonal changes in arthropod communities and compare community patterns before and after mowing at different stages of the season. One area remained unmown throughout the sampling season, whereas the other three were mown prior to the second, third, and fourth sampling occasions, respectively. An intensively managed grassland was additionally sampled for comparison. To preserve specimens for subsequent morphological analyses, we implemented and optimized a mild-lysis, non-destructive DNA extraction protocol for unsorted bulk arthropod samples. DNA metabarcoding targeted a 456-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using primers designed to suppress plant amplification while broadly amplifying arthropods, followed by paired-end, low-depth Illumina sequencing. Bioinformatic processing included quality filtering and removal of putative pseudogenes. After quality filtering, samples yielded an average of 4,569 reads per sample. We assigned 456 ASVs to 133 arthropod species or genera, while an additional 90 ASVs were assigned to 32 subfamilies or families. Hemiptera were by far the most dominant order across samples, followed by Symphypleona (Collembola), Orthoptera, Sarcoptiformes (Acariformes), Lepidoptera, and Diptera. Despite the relatively low sequencing depth, our preliminary analyses indicated that mowing-associated changes were more evident in beta diversity, as reflected by PCoA ordination and UPGMA clustering, than in alpha diversity, assessed using the number of detected taxa, the Shannon–Weaver index, and Simpson’s reciprocal index. Shifts in community composition were particularly evident in plots mown in late June and/or July in both 2024 and 2025. These preliminary results demonstrate the potential of DNA metabarcoding for characterizing the temporal dynamics of highly diverse grassland arthropod assemblages and detecting community changes associated with grassland management. Repeated molecular community surveys may complement conventional biodiversity monitoring and contribute to the development of mowing strategies that better account for seasonal dynamics of grassland biodiversity. Future analyses will include deeper sequencing and DNA barcoding of morphologically identified Orthoptera to enrich regional reference sequence databases.

Key words: DNA metabarcoding; extensive grassland; mowing regime; conservation management; arthropod communities

Inclusion of youth in the promotion, protection and sustainable use of native fruit varieties in Maleshevo

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Native fruit varieties are an important component of agrobiodiversity, cultural heritage and local identity in the Maleshevo region. However, their long-term conservation is increasingly challenged by ageing rural populations, abandonment of traditional orchards, limited processing of old varieties and youth migration. The BESTbelt project “Protection of native fruits varieties in Maleshevo and Belasitsa regions” addresses these challenges by combining inventory, revitalization, stakeholder cooperation and education, with a specific focus on involving young people in the protection, promotion and sustainable use of native fruit varieties, including potentials for green jobs creation. This paper presents project-based evidence on how youth engagement can strengthen community-based conservation of native fruit varieties in Maleshevo. The analysis draws on field mapping, stakeholder consultations, educational workshops and practical demonstrations implemented between 2024 and 2026. More than 100 old fruit trees were mapped and documented across the transboundary Maleshevo–Belasitsa region, including traditional apple, pear and plum varieties. The project also collected local names, ecological characteristics and traditional uses, while linking scientific expertise with traditional ecological knowledge held by local residents. More than 30 youth were engaged through workshops, field-based learning and promotional activities in Pehchevo, Berovo, Negrevo and the wider cross-border area. This engagement included practical training session for identification of old fruit varieties, promotion of green entrepreneurship, value chains and preparation of traditional fruit products and activities with students focused on sustainable tourism, branding of local products, green jobs and agrobiodiversity conservation. Cross-border workshops further enabled youth, producers, experts and local stakeholders to exchange knowledge and participate in demonstrations of grafting, propagation and revitalization of old fruit trees. The interest and involvement of youth increase when conservation is presented not only as biodiversity protection, but also as a practical opportunity for local entrepreneurship, food processing, tourism, branding and community development. Young participants demonstrated strong interest in contributing to future promotion and dissemination activities, while project workshops strengthened intergenerational knowledge exchange and improved understanding of the economic and cultural value of native fruit varieties. 30% of the participants in practical field activities were youth, that confirms that youth inclusion can transform native fruit varieties from neglected genetic resources into visible elements of local identity, sustainable livelihoods and climate-resilient rural development.

Key words: native fruit varieties, youth engagement, green jobs, sustainable use, traditional knowledge, rural development

ECOSYSTEMS AND ENVIRONMENTAL CHANGE

Nature conservation in times of a climate crisis

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This presentation will (i) give an overview of the main climatic changes and impacts affecting SE Europe over the next decades and, (ii) stress implications for the future of nature conservation. This study draws on climate projections from the CMIP6 Global Climate Models used in the latest IPCC Sixth Assessment Report, covering the period up to 2100 under the SSP2-4.5 and SSP5-8.5 climate scenarios. The data were extracted and analysed by the CLIMADAPT group (<https://climadapt.meteo.noa.gr/>) of the National Observatory of Athens. The Southern Balkans are a global climate change hotspot, with mean annual temperature increasing by 3-5°C in 2071-2100 (reference period: 1971-2000). Future warming is estimated to exceed the global mean by 20% annually, and by 50% in summer. Projections indicate an increase in heatwave frequencies and magnitudes, while evapo(trans)piration will increase by 10-15% annually. Precipitation across the Southern Balkans is not expected to change considerably by 2050-2070, but regionally there may be (significant) decreases. Annual snow cover and depth will decrease substantially. There may be an increase in extreme rainfall events, leading to a higher frequency of flash floods, but also an increase in droughts. These climatic changes will have particularly severe impacts on freshwater environments and forests. Freshwater environments, often biodiversity hotspots, will become extremely threatened throughout the Balkans. Increases in evapo(trans)piration and decreases in snowmelt-fed discharge will lead to decreasing river flow and lake water volumes. These decreases are amplified in regions where there is also a reduction in annual precipitation. Water abstraction for agriculture will likely strongly increase under warming climates, amplified by projected increases in tourism / industrial demands, thus strengthening a negative feedback loop. Vegetation composition will change under the combined effects of drought, higher temperatures and wildfires; fire-, heat- and drought-resistant species will gain an advantage. Wildfires will likely expand in size and frequency up to the end of the century, with forests especially affected. The future increase in wildfires is fuelled by a combination of more frequent heat waves and intense droughts, humidity decreases and abundant biomass availability. Radical new conservation management strategies are required. During past periods of abrupt climate change, habitats and species distributions shifted, surviving in refugia with suitable (micro-)climatic and environmental conditions. However, the fragmented and organised present-day landscape does not favour species migration or creation of new habitats. To improve climate resilience, management should focus on establishment of migration corridors, creation of new habitats and re-establishing connectivity between landscape elements. River, lakes and wetlands with riparian forests are natural arteries that connect different parts of the landscape, both vertically and horizontally. These severely threatened environments are thus of great importance for supporting biodiversity under abrupt climate change. Finally, the root causes of wildfire ignition and spread is inadequate land management practice, including accumulation of dry biomass, burning of farmland waste and landscape simplification for industrial agriculture. New integrated approaches applying the principles of fire-smart landscape management and Forest Landscape Restoration (FLR) have proven a successful means of reducing the number of Mediterranean wildfires.

Key words: Climate change, SE Europe, nature, conservation

Multitemporal assessment of habitat dynamics along the Prespa Lake shoreline, North Macedonia: Integrating phytosociological habitat mapping and UAV-based remote sensing

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The area along the shoreline of Prespa Lake represents a dynamic landscape characterized by a complex mosaic of aquatic, wetland, grassland, riparian and anthropogenic habitats. Understanding temporal changes in habitat distribution is essential for detecting ecological transformations and supporting effective conservation and management. This study presents a multitemporal assessment of habitat dynamics along the Prespa Lake shoreline and adjacent areas in North Macedonia during 2018–2024.

The existing 2018 habitat map was used as the baseline spatial reference. In 2021 and 2024, high-resolution UAV orthophoto mosaics were produced from more than 20,000 aerial photographs. The 2018 habitat polygons were systematically reviewed and manually revised using the 2021 orthophoto, and the resulting 2021 map was subsequently revised using the 2024 orthophoto. Habitat delineation was based on expert visual interpretation of remotely sensed imagery, without machine-learning or automated classification methods. UAV surveys were predominantly conducted during the first half of June. The three maps were analysed within a common spatial framework to quantify habitat changes and identify major transitions. The results indicate substantial reorganisation of the habitat mosaic, with the most pronounced changes occurring within the aquatic–wetland–riparian continuum. Reedbed habitats showed particularly strong transformations: *Phragmites/Typha* communities with free-standing water declined by 98.8% between 2018 and 2024, from 549.1 to 6.6 ha, while communities without free-standing water increased by 178.6%, from 297.5 to 828.8 ha. Pioneer vegetation expanded to 219.1 ha in 2024, while *Salix alba* and *Populus alba* galleries increased by 45.0%. Alluvial forests decreased between 2018 and 2021 and increased thereafter, but remained 14.4% below the 2018 extent. Wet-meadow habitats generally declined, particularly *Calamagrostis epigejos* communities (–22.9%) and *Carex spp.* beds (–19.8%), while *Helleno-Moesian* riverine and humid *Trifolium* meadows declined by 5.1%. Approximately 21% of the wet-meadow area mapped in 2018 underwent conversion to other habitat types by 2024, with particularly pronounced transitions involving *Calamagrostis epigejos* and *Trifolium* meadows. The observed patterns indicate pronounced spatial reorganisation of aquatic, reedbed, wet-meadow, pioneer and riparian habitats. Interpretation of these changes must consider differences in data quality, as the 2018 mapping outside Ezerani relied largely on coarser satellite imagery, whereas the 2021 and 2024 assessments benefited from higher-resolution UAV orthophotos. Thus, some differences may reflect improved spatial delineation as well as actual habitat transformation. Nevertheless, the consistency of major trends demonstrates the value of repeated high-resolution habitat mapping for detecting fine-scale ecological dynamics. Continued monitoring at approximately three-year intervals, complemented by targeted field observations and environmental measurements, is recommended.

Key words: habitat dynamics; multitemporal mapping; UAV remote sensing; wetland habitats

Soil carbon dynamics as a functional indicator of climate-resilient agroecosystems under carbon farming practices

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Climate change and progressive soil degradation increasingly challenge the capacity of agricultural ecosystems to maintain their ecological functions and long-term productivity. In this context, soil carbon is emerging not only as a key component of climate-change mitigation, but also as an integrative indicator of management-driven changes in soil functioning. Identifying reproducible soil carbon responses across different cropping systems may therefore provide a measurable fingerprint of agricultural practices designed to strengthen agroecosystem resilience. This study investigates the dynamics of soil carbon under carbon farming (CF) and conventional management systems (CMS) across four contrasting crops, corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.) and chickpea (*Cicer arietinum* L.), within field experiments conducted in North Macedonia. Temporal changes in soil total carbon were evaluated across successive sampling periods to determine whether contrasting management regimes generate distinguishable and consistent carbon-response patterns. A clear management-dependent carbon fingerprint was observed across all four cropping systems. Under CF, soil carbon increased from 1.20% to 1.89% in corn, from 1.19% to 1.42% in sunflower, from 1.25% to 1.68% in barley, and from 1.18% to 1.57% in chickpea. These changes correspond to relative increases of approximately 57.5%, 19.3%, 34.4%, and 33.1%, respectively. In contrast, conventional management produced a consistent decline in soil carbon: from 1.45% to 0.93% in corn, 1.25% to 0.76% in sunflower, 1.34% to 0.89% in barley, and 1.22% to 0.91% in chickpea, representing decreases of approximately 35.9%, 39.2%, 33.6%, and 25.4%, respectively. The consistency of these divergent trajectories across crops with different physiological characteristics suggests that soil carbon response is strongly associated with management regime rather than crop type alone. The resulting carbon profiles provide a distinct biogeochemical signature capable of differentiating carbon-oriented from conventional agricultural management. These findings support the concept of a soil carbon fingerprint as a practical and scientifically measurable indicator for evaluating the effectiveness of carbon farming strategies. Beyond its role as a carbon sequestration metric, the temporal carbon trajectory may serve as a functional signal of soil conservation, management intensity and agroecosystem transition towards more climate-resilient states. Developing such measurable fingerprints could strengthen monitoring, reporting and verification frameworks for carbon farming and provide a robust scientific basis for climate-smart and climate-resilient agricultural strategies.

Key words: soil carbon, carbon farming, climate-resilient agriculture, agroecosystem resilience, climate-smart agriculture.

Multi-temporal remote sensing assessment of surface urban heat island (SUHI) dynamics in Skopje using Landsat 8/9 and Sentinel-2

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Urban surface heating is spatially heterogeneous and temporally variable, making long-term observations essential for distinguishing recurrent thermal patterns from short-lived anomalies. This study presents a multi-temporal remote sensing assessment of surface urban heat island (SUHI) dynamics in Skopje, North Macedonia, using Landsat 8/9 and Sentinel-2 observations. Summer land surface temperature (LST) and SUHI were analyzed for 2013–2025, while Sentinel-2 spectral indicators were used to examine surface controls on LST, identify persistent thermal hotspots, and refine the 2025 Landsat thermal field from 30 to 10 m spatial resolution. Mean summer LST during 2013–2025 was 36.9 °C, whereas mean annual SUHI intensity (SUHII) was 4.2 °C, with marked interannual differences demonstrating that absolute surface heating and urban–non-urban thermal contrast do not necessarily evolve in parallel. More than half of the analyzed urban surfaces (54.1%) showed a multi-year mean SUHII above 4 °C, with the strongest thermal contrasts concentrated mainly in the central and eastern urban sectors. LST was consistently positively related to BSI and NDBI and negatively related to NDVI, confirming the contrasting thermal influence of exposed or built surfaces and vegetation. Persistent and highly persistent hotspots occupied 11.7% of the analyzed built-up area, indicating that recurrent thermal stress is concentrated within a relatively limited part of the urban fabric. The 2025 spatial refinement model based on NDVI and BSI explained approximately 70% of Landsat LST variability and showed comparable performance under spatial block validation. The results demonstrate the value of combining thermal magnitude, urban–non-urban contrast, surface characteristics, temporal persistence and spatial refinement to distinguish recurrent urban thermal stress from both short-term anomalies and naturally heated terrain. The framework provides a consistent basis for neighborhood-scale interpretation and the identification of priority areas for urban heat mitigation and climate-sensitive planning.

Key words: urban climate; cloud-based assessment; satellite-based approach; North Macedonia; urban thermal environment;

Screening for metallophytes among wild species of Brassicaceae in North Macedonia

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The family Brassicaceae in North Macedonia counts about 165 wild species from 50 genera. Some species of this family are metallophytes, i.e. plants that can resist the toxicity of heavy metals in the soil. Some of these species accumulate heavy metals at higher concentrations than those present in the soil (hyperaccumulators). The present study screens for species within Brassicaceae with remarkable potential for accumulation of soil heavy metals (Cu, Zn, Pb, Ni, Cd, Cr, Mn, Fe) on the territory of North Macedonia. The materials of Brassicaceae species were collected throughout North Macedonia in the last 25 years. In total, the organs (roots, stems, leaves, seeds) of 96 species (60% of the national flora) were analysed. The concentration of heavy metals in plant organs and soils was determined by flame atomic absorption spectrometry. All plant tissue and soil samples (0.5 g) were grinded and wet digested. During the selection process, we considered metallophytes the species that have greater concentration of the heavy metal in aboveground organs (leaves, stems) than the one present in the soil (enrichment factor or ratio between plant organ and soil greater than 1). For hyperaccumulator selection, we considered species that have more than 100 mg/kg of Cd, more than 300 mg/kg of Cu, Co or Cr, more than 1000 mg/kg for Ni, more than 3000 mg/kg of Zn and more than 10000 mg/kg of Mn. Zn hyperaccumulator species are *Cardamine glauca* (3100-12200 mg/kg) and *Alyssum alyssoides* (1900-9600 mg/kg). The following species qualify for bioaccumulators of Zn: *Cardaria draba*, *Odontarrhena serpentina*, *Teesdalia coronopifolia*, *Erysimum diffusum*, *Descurainia sophia*, *Sisymbrium loeselii*, *Cardamine hirsuta*, *Calepina irregularis*, *Bunias erucago*, *Clypeola jonthlaspi*, *Thlaspi perfoliatum*, *Capsella bursa-pastoris* and *Sinapis arvensis*. The following species are bioaccumulators of Cd: *Capsella bursa-pastoris*, *Draba muralis*, *Cardamine hirsuta*, *C. glauca*, *C. graeca*, *Clypeola jonthlaspi*, *Brassica napus*, *Teesdalia coronopifolia*, *Sinapis arvensis*, *Calepina irregularis*, *C. rumelica*, *Cardaria draba*, *Bunias erucago*, *Myagrurn perfoliatum*, *Thlaspi perfoliatum*, *Arabidopsis thaliana*, *Sisymbrium orientale*, *S. loeselii*, *Descurainia sophia*, *Alyssum desertorum*, *A. umbellatum*, *A. saxatile*, *A. alyssoides*, *A. stribrnyi*, *A. minutum*, *A. transilvanicum*, *Odontarrhena skopjensis*, *O. serpentina*, *Raphanus raphanistrum*, *Erophilla verna*, *Aethionema saxatile*, *Erysimum crasystylum*, *E. diffusum* and *E. comatum*. We consider *Cardamine hirsuta* and *Cardamine glauca* as bioaccumulators of Pb. The following species are bioaccumulators of Cu: *Alyssum desertorum*, *Cardamine hirsuta*, *Clypeola jonthlaspi*, *Draba muralis*, *Teesdalia coronopifolia*, *Thlaspi perfoliatum*, *Sisymbrium orientale*, *Erophilla verna*, *Descurainia sophia* and *Erysimum diffusum*. Ni hyperaccumulator species are *Odontarrhena chalcidica*, *O. debarensis*, *O. gevgelicensis*, *O. kavadarcensis*, *O. muralis*, *O. pichleri*, *O. serpentina*, and *O. skopjensis*. The following species are bioaccumulators of Ni: *Alyssum desertorum*, *A. minutum*, *A. umbellatum*, *Arabidopsis thaliana*, *Brassica napus*, *Bunias erucago*, *Cardamine glauca*, *C. hirsuta*, *C. draba*, *Clypeola jonthlaspi*, *Descurainia sophia*, *Draba muralis*, *Lepidium campestre*, *Sisymbrium orientale*, *Teesdalia coronopifolia*.

Key words: bioaccumulation, hyperaccumulation, heavy metals, Cruciferae, enrichment

ECOLOGICAL EDUCATION

Systemic integration of environmental and climate change education

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The presentation gives an example of the systemic integration of environmental and climate change education within North Macedonia's national education system, highlighting its role in fostering curiosity, experiential learning, and competencies in natural sciences among students. It outlines a comprehensive, whole-system approach in which environmental content is embedded across curricula, learning standards, policy frameworks, teacher professional development, and high-quality teaching and learning resources. The model extends beyond formal education to include experiential learning opportunities, extracurricular programmes, and partnerships with public institutions and civil society, forming a coherent ecosystem for the development of green skills. The approach also emphasizes alignment with national priorities and international commitments related to sustainable development and climate action. Through coordinated institutional collaboration and horizontal integration across education levels, environmental education becomes a continuous and inclusive process, equipping children and adolescents with the knowledge, skills, and agency to actively engage in environmental sustainability, climate action, and informed decision-making.

Key words: experiential learning, education system, green skills

Biodiversity monitoring and local ecological knowledge as tools for climate change adaptation in semi-arid ecosystems: Evidence from Karamoja, Uganda

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Climate change is increasingly affecting semi-arid ecosystems through prolonged droughts, unpredictable rainfall patterns, land degradation and biodiversity loss. Understanding these changes requires approaches that integrate ecological observations with the knowledge and experience of local communities. This study presents findings from the Environmental and Social Impact Assessment (ESIA) conducted within the Climate Change Adaptation and Livelihoods Improvement (CALI) Programme in the Karamoja sub-region of north-eastern Uganda, with the aim of exploring the contribution of biodiversity monitoring and Local Ecological Knowledge (LEK) to climate change adaptation. ♦ESIA Report ATA.docx The assessment combined literature review, ecological field observations and participatory methods, including 240 household interviews, 18 focus group discussions, key informant interviews and stakeholder consultations across the districts of Abim, Kotido and Karenga. This approach enabled documentation of local perceptions of environmental change alongside observations of ecosystem condition, biodiversity and natural resource use. The results revealed consistent evidence of climate-related environmental changes reported by local communities, including prolonged droughts, increasingly erratic rainfall, flash floods, soil erosion and declining water availability. These pressures were associated with extensive loss of tree cover, reduced availability of wild fruits, medicinal plants and wildlife, changes in the seasonal occurrence of locally important species, and declining ecosystem services that directly support rural livelihoods. The integration of Local Ecological Knowledge with field observations provided a comprehensive understanding of ecosystem change and identified biodiversity responses as sensitive indicators of climate-related environmental change. The findings suggest that changes in biodiversity can provide locally observable indicators of climate-related ecosystem change, while Local Ecological Knowledge offers valuable complementary evidence for interpreting long-term environmental trends, particularly in regions where formal ecological monitoring is limited. Integrating community observations with standardized biodiversity and climate monitoring could strengthen the early detection of ecosystem change, improve ecosystem-based adaptation planning and support more resilient management of natural resources in semi-arid landscapes. These findings also highlight the importance of recognizing biodiversity monitoring not only as a conservation tool, but also as an essential component of climate change adaptation and sustainable ecosystem management.

Key words: Climate change adaptation; biodiversity monitoring; Local Ecological Knowledge; ecosystem-based adaptation; ecosystem services; semi-arid ecosystems; Karamoja; Uganda

Communication as a governance mechanism in conservation project delivery: A qualitative study of selected IUCN-supported projects in Albania and North Macedonia

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Introduction. Effective communication is widely recognised as a fundamental component of successful project management. In biodiversity conservation projects, however, communication is often regarded primarily as a supporting or dissemination function rather than as a mechanism that shapes governance, institutional coordination, and project delivery. This issue is particularly relevant in IUCN-supported initiatives, where implementation depends on collaboration among international organisations, national institutions, civil society organisations, donors, and local communities across multiple governance levels. Despite the increasing complexity of conservation partnerships, limited research has examined communication from the perspective of project managers responsible for delivering conservation outcomes. **Objective.** This study aims to investigate how communication functions as a governance mechanism influencing coordination, decision-making, stakeholder engagement, and strategic alignment in selected IUCN-supported conservation projects implemented in Albania and North Macedonia. The research adopts a project management perspective, focusing on communication as an operational and governance process rather than solely as a public outreach activity. **Methodology.** The research employs a qualitative exploratory design based on semi-structured interviews with project managers and senior staff from IUCN ECARO and partner organisations, including the Macedonian Ecological Society (MES), Protection and Preservation of Natural Environment in Albania (PPNEA), and the Urban Research Institute (URI). The empirical analysis is grounded in completed and ongoing conservation initiatives, including the Lake Ohrid Management Plan, Buna River–Velipoja Protected Landscape, the ADAPT Project, the Northern Forests Initiative, the Vjosa Wild River National Park Phase II project, and the IUCN Green List process in Albania. Interview data are analysed according to communication type, function, and their relationship to project effectiveness, institutional cooperation, stakeholder trust, and policy implementation. **Main Results.** Preliminary findings indicate that communication extends beyond information exchange and reporting to become a central governance mechanism that enables coordination across institutions, facilitates adaptive management, strengthens stakeholder relationships, and supports evidence-based decision-making. While formal communication structures provide accountability and transparency, informal interactions, trust-based relationships, and continuous dialogue frequently prove essential for resolving implementation challenges and maintaining effective partnerships. **Discussion and Conclusions.** The study suggests that communication should be recognised as a strategic governance capacity rather than a purely technical project function. Understanding how communication operates in practice can improve conservation project delivery, strengthen cross-institutional collaboration, and enhance the implementation of biodiversity policies and international conservation programmes. The findings are expected to contribute to both conservation practice and project management research by offering an empirically grounded framework for integrating communication into governance processes within complex environmental projects. They may also provide practical recommendations for organisations implementing the IUCN Programme 2026–2029 and similar multi-stakeholder conservation initiatives.

Key words: Keywords: conservation project management; communication; governance; stakeholder engagement; IUCN; biodiversity conservation; Albania; North Macedonia.

From classroom to nature: The experiential learning network as a model for advancing science education in North Macedonia

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Modern science education is increasingly focused on active learning methods that place students at the center of the educational process and encourage the development of scientific literacy, critical thinking, and research skills. Experiential learning, based on direct observation, experimentation, and inquiry, represents one of the most effective approaches to achieving these goals. To create a sustainable system connecting schools with scientific institutions and the country's natural heritage, the Macedonian Ecological Society is developing the Experiential Learning Network (ELN) as a national platform for education through experience. The Network brings together scientific institutions, protected areas, research centers, and organizations that jointly develop and implement educational programs for students and teachers. Activities include field and laboratory workshops, online lectures, teacher training, the development of teaching materials, and the introduction of innovative, inquiry-based working methods. Each educational program is designed to encourage students to independently pose questions, collect and analyze data, and draw conclusions based on their own experience. During its implementation phase, the Network has established partnerships with numerous scientific and educational institutions, organized numerous teacher training sessions, educational workshops, and national online lectures, involving several thousand students and hundreds of teachers from various regions of the country. In addition to direct work with students, teaching materials, methodological approaches, and new programs have been developed to integrate experiential learning into formal education. In 2026, the Network is further expanding through the introduction of the National Student Research Challenge, which aims to encourage students to independently apply the scientific method and present their results at the Congress of the Macedonian Ecological Society. To date, results show that the Experiential Learning Network successfully contributes to improving the quality of science education by forging partnerships between schools and scientific institutions, developing teacher competencies, and increasing student engagement in practical research activities. This model serves as a strong good practice that can provide a foundation for the further development of experiential and inquiry-based learning within the educational system.

Key words: experiential learning, science education, scientific literacy, inquiry-based learning, environmental education, Experiential Learning Network, teachers, students

A stewardship-based framework for science communication in socio-ecological systems

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Environmental science communication has traditionally focused on increasing public awareness and disseminating scientific knowledge. However, biodiversity loss represents a complex socio-ecological challenge in which scientific evidence alone is insufficient to stimulate collective action. Pollinator decline exemplifies this complexity, with cascading effects across all strata of the human and natural world. Such interconnected challenges require communication approaches that support systems thinking, collaborative learning, and shared responsibility across diverse stakeholder groups. This paper proposes a stewardship-based framework for science communication that reconceptualises communication as an integral component of environmental governance rather than a stereotypical, simplified dissemination activity. Anchored in the communication principles of the Horizon Europe Butterfly project, the paper examines how communication can facilitate knowledge co-production between scientists, policymakers, farmers, industry representatives, educators, civil society organisations, and citizens through participatory governance structures. It argues that communication becomes a mechanism for enabling stewardship by fostering dialogue, integrating local knowledge systems, and supporting the co-development of locally relevant solutions. The proposed framework identifies four interdependent dimensions of stewardship communication: (1) communicating socio-ecological complexity through systems-based narratives; (2) enabling knowledge co-production across disciplines and societal groups; (3) fostering stewardship identities through participatory engagement; (4) supporting adaptive governance through iterative learning and feedback. Together, these dimensions are a step ahead of the traditional linear models of science communication, in which communication functions as social infrastructure for environmental decision-making and lasting behavioural change. This framework extends beyond the standard models of public engagement towards a stewardship concept, tailored to create, transfer and adopt complex biodiversity content. Beyond pollinator conservation, the proposed framework provides a transferable approach for communicating complex sustainability challenges where lasting societal transformation depends on collaboration, participation, and the co-production of knowledge across governance scales.

Key words: pollinators, science communication, stewardship communication

SYMPOSIUM OF BIOLOGY STUDENTS
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The influence of ornithogenic nitrogen input on vegetation of the limestone cliffs of Golem Grad island (Prespa Lake)

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Cliff habitats are characterized by extreme environmental conditions, including vertical relief, shallow and nutrient-poor soils, high temperature variability, and strong exposure to wind. These conditions support distinct and highly adapted plant communities. In such habitats, nutrient inputs may originate from atmospheric deposition, biological transport and surface runoff from surrounding areas. Bird nesting colonies may represent a significant local source of nutrients through the accumulation and deposition of guano on cliff surfaces, as well as through its transport by surface runoff. Such nutrient inputs may be associated with differences in nutrient availability and vegetation composition. The aim of this study was to investigate differences in the deposition of mineral ions, including anions and cations, between cliff habitats with and without bird nests, and to assess differences in vegetation between the two habitat types. The island of Golem Grad was selected as the study area due to its pronounced limestone cliffs and the presence of a significant breeding nesting colony dominated by Great cormorants as well as grey herons, alpine swifts and yellow-legged gulls. Bird nests are distributed along the eastern and northeastern sides of the island, mainly along the cliff edges above the vertical cliff surfaces, with the vast majority of nests located on Greek juniper trees (*Juniperus excelsa*). To assess ion deposition, an anion exchange membrane (AMI-7001) and a cation exchange membrane (CMI-7000) were installed on vertical cliff surfaces. The membranes, measuring up to 5×5 cm, were placed in three replicates at 3 m intervals along each transect and remained exposed for 86 days. Three transects were established on cliff surfaces associated with bird nests, while two control transects were established on cliff surfaces without recorded nests. Vegetation sampling was conducted using 1×1 m quadrats on cliff surfaces beneath bird nests and on comparable cliff surfaces without nests. The most pronounced differences in ion deposition were recorded for ammonium (NH₄⁺) and nitrate (NO₃⁻). Mean NH₄⁺ deposition was 204.18 µg/cm²/day on cliff surfaces associated with bird nests, compared with 85.8 µg/cm²/day on cliff surfaces without nests. Mean NO₃⁻ deposition was 1.56 µg/cm²/day on cliff surfaces with bird nests and 0.20 µg/cm²/day on cliff surfaces without nests. Overall vegetation cover (approximately 18%) was similar between the two habitat types, but differences were observed in species composition. *Campanula versicolor* and *Poa bulbosa* were the most dominant species on cliff surfaces associated with bird nests, whereas *Alyssum saxatile* and *Hordeum bulbosum* were dominant on cliff surfaces without nests. *Umbilicus erectus* was recorded exclusively on cliff surfaces associated with bird nests, while *Umbilicus rupestris* was recorded only on cliff surfaces without nests. Although total vegetation cover was similar between the two habitat types, the observed differences in species composition suggest that nutrient inputs associated with bird activity may be related to changes in plant community composition. In contrast, total vegetation cover appears to be strongly influenced by the physical characteristics of the cliff environment, particularly the number and depth of cracks and the amount of soil accumulated within them. This research contributes to a better understanding of the relatively understudied cliff habitats by examining ornithogenic nitrogen inputs and their influence on vegetation structure and composition.

Key words: vegetation cover, ammonium, nitrates, bird colony, *Phalacrocorax carbo*

Tradescantia test indicates genotoxicity of air pollution in Skopje

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The *Tradescantia* test is a toxicological assay employed for the detection of genotoxic substances that induce alterations in the chromosomal structure of plant cells. Global urbanization has been accompanied by a range of environmental issues, among which air pollution represents one of the most pervasive and critical challenges, also evident in the city of Skopje. In this study, the use of the bioindicator *Tradescantia pallida* (Rose) D.R.Hunt, enabled the assessment of the genotoxic potential of PM_{2.5} particles across five urban locations and one control site outside of the city area. Descriptive analysis of the results indicated a relationship between PM_{2.5} concentrations and the frequency of abnormalities observed in the pollen mother cell of *Tradescantia pallida*. However, the dataset did not provide sufficient statistical evidence to confirm a significant correlation between these parameters. Consequently, further standardization is required, especially through improved consistency of measurements and the integration of other chemical, biological and molecular methods to enhance its reliability as a biomonitoring tool for urban genotoxicity.

Key words: *Tradescantia*, bioindicator, PM_{2.5} particles, genotoxicity, air pollution

Metallophytes in the Radusha region as potential accumulators of nickel

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Metallophytes possess biological mechanisms that enable them to tolerate heavy metal concentrations that are normally phytotoxic. Those commonly referred to as ‘excluders’ restrict the element’s entrance by retaining it in the root and minimizing shoot translocation. Another group, ‘accumulators’ actively take up, translocate and store heavy metals in above-ground organs. If tissue content exceeds 1000 µg/g dry weight for certain elements, including nickel (Ni), the plants are defined as hyperaccumulators. Scarce research on metallophytes in N. Macedonia makes Radusha, the region with the largest serpentinite massif in the country, a significant area of interest. The aim of this study is to assess the accumulation and translocation potential for Ni of 8 different plant species: *Centaurea kosaninii*, *Euphorbia glabriflora*, *Fumana bonapartei*, *Genista hassertiana* and four species from genus *Odontarrhena* that are known for their hyperaccumulating properties: *O. skopjensis*, *O. cf. chalcidica*, *O. cf. serpentina* and *O. pichleri*. A total of 28 localities within eight 5 x 5 km UTM quadrants were surveyed. 49 samples consisting of 216 plant individuals, along with 40 soil samples from the rhizosphere of the plants were collected. Soil temperature and pH were accounted for as additional environmental factors. Atomic absorption spectroscopy was used to determine Ni concentration in both soil and plant organs (root, stem, leaf). Relationships between soil properties and plant metal concentrations were assessed using redundancy analysis (RDA). Interspecific differences in metal accumulation and organ-specific partitioning were further evaluated using linear mixed-effects models (LMMs). Statistical analyses indicate a strong and species-specific relationship between soil and plant Ni. The linear mixed-effects model estimates the strongest positive soil-Ni slopes for *Odontarrhena cf. chalcidica* (slope = 3.92, 95% CI: 1.92–5.91) and *O. cf. serpentina* (3.78, 1.70–5.86), followed by *O. skopjensis* (1.73, 0.50–2.96). The confidence intervals of all three slopes exclude zero, indicating positive relationships between soil and plant Ni. In contrast, the estimated slopes for the other species were close to zero or highly uncertain indicating their biological mechanisms of tolerance align with ‘excluders’. These findings expand knowledge regarding local Ni accumulation for metallophytes on Radusha and can serve as a basis for future research in the fields of applied ecology and phytoremediation.

Key words: serpentinite, hyperaccumulation, plant-soil relations

***Rimbachia neckerae*, a rare basidiomycete, with first data records for North Macedonia**

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Rimbachia neckerae (Fr.) Redhead is a small parasitic basidiomycete fungus which is, considered as a rare species. It has wide distribution in temperate, boreal, montane, and polar habitats of both hemispheres. The fungus produces sexual fruiting basidiomata at the apices of infected moss gametophytes, often completely replacing the stem tips. Due to its diminutive size the fungus can be easily overlooked in field research. During field investigations in 2026, organized by the Biology Students Research Society *R. neckerae* was recorded for the first time in North Macedonia. The species was found on two localities, in May on mountain Jablanica and in July on mountain Shar Planina. At the first locality the specimens were collected from moss growing in a beech forest. At the second locality the fungus was found on moss growing on a rock near a river, also within a beech forest. Both, collections were found several days after periods of heavy rainfall. Based on macroscopic and microscopic characteristics, the collected specimens resemble *R. neckerae*. The specimens displayed the following diagnostic features: basidiocarps 3–12 mm in diameter, urceolate, with a wavy margin; surface whitish to cream, sparsely pubescent to glabrous; flesh thin, whitish to cream; hymenium smooth and concolorous with the outer surface; stipe absent or reduced to a rudimentary pseudostem. Basidia were four-spored. Basidiospores elliptical to almond-shaped, smooth and hyaline, with size of $8\text{--}12 \times 4\text{--}7 \mu\text{m}$. These findings represent the first documented records of *R. neckerae* from North Macedonia and contribute to the limited knowledge of its distribution.

Key words: bryoparasite, fungi, species distribution, microhabitats

Functional characterization of rhizospheric microbiota associated with *Anacamptis morio* in the Gazi Baba forest park

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The survival, germination, and vegetative development of the orchid *Anacamptis morio* are fundamentally dependent on complex symbiotic associations within the rhizosphere. In urban-influenced forest ecosystems, such as the Gazi Baba Forest Park, these microbial communities are subjected to anthropogenic stressors that potentially alter their functional landscape. Understanding the metabolic versatility of these microorganisms is essential for assessing the ecological stability of *A. morio* populations and identifying potential biotechnological applications for conservation. This study aimed to conduct a comprehensive functional characterization of 53 culturable microbial isolates obtained directly from the root zone of *A. morio* specimens in the Gazi Baba Forest Park. The research methodology involved the isolation of microbial strains using standard isolation and culturing microbiological techniques, followed by metabolic profiling to evaluate key enzymatic traits. We assessed protease activity to determine the isolates' ability to mobilize organic nitrogen and monitored ammonification rates as an indicator of nitrogen cycling efficiency. Additionally, we utilized specific indicator substrates, such as Congo red, to analyze the physiological adaptations of these isolates. The results demonstrate a high degree of metabolic versatility within the 53 analyzed isolates. Of these, 52 isolates (98%) were positive for ammonification, 50 isolates (94%) showed positive for cellulolytic activity, and 49 isolates (92%) were positive for nitrogen-fixing activity. Furthermore, 28 isolates (53%) exhibited positive proteolytic activity, reflecting the metabolic capacity of this microbial community. This elevated level of functional activity suggests that the *A. morio*-associated microbiota in the Gazi Baba Forest Park has developed specialized enzymatic and physiological adaptations ensuring nutrient mobilization despite urban environmental pressures. These findings are critical for future conservation efforts, as they highlight a reservoir of microorganisms capable of supporting orchid development in challenging soil conditions, providing a foundation for developing targeted microbial inoculants for the restoration and cultivation of *Anacamptis* species.

Key words: *Anacamptis morio*, Rhizosphere, Microbial Symbiosis, Orchid Conservation, Nitrogen Cycling

Effects of *Bacillus thuringiensis* var. *israelensis* (Bti) on diet choice of bats in lower Dalälven River

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Biological control of mosquito populations, using *Bacillus thuringiensis* var. *israelensis* (Bti), has been in practice in Sweden since 2002. Even though the treatment initially was considered to be environmentally safe, recently there have been concerns about its indirect effects on non-target organisms such as predators of mosquitoes, a topic in need of further exploration. Our study addresses this gap by exploring the effects of Bti on bats and the consequences that a shrinking mosquito population has on their diet, given that Diptera is a large part of it. Our aim was to explore differences in the bats diet between Bti treated and untreated areas, to uncover any underlying dietary compensation and changes in prey availability and prey consumption between the two areas. Through DNA metabarcoding of bat fecal pellets and insects collected from Malaise traps, we constructed the dietary composition of two bat species *Myotis brandtii* and *Myotis daubentonii* and identified the available insect community in the researched areas. PERMANOVA analyses determined that there are no differences in the community diet between the treated and untreated areas. However, significant differences arose between the sampling sites, hinting that local ecological factors might influence bat diet more than the treatment effect alone. Our results showed there is no compensation in the diet between treated and untreated sites and no difference in alpha diversity of the diet at order and family level. Contrary to expectations, there was significantly more Diptera eaten in the Treatment areas with the Jacobs D' electivity index showing the bats shift from avoidance at Control sites to selectivity for Diptera at Treatment sites. This indicated that there might be compensation at a finer taxonomic level such as genus and species level. But for now, we conclude that the trophic relationship between the bats and the mosquitoes remained unchanged, with *Myotis* bat diet being resilient and stable. However, longer monitoring is needed to further assess the effects with broadening the predator group and exploring their response together with ecosystem-level response to Bti.

Key words: Biocontrol, Bti, Chiroptera, DNA metabarcoding, food web

Taxonomic diversity and community structure of phytopathogenic micromycetes in agricultural systems as a foundation for sustainable biocontrol strategies

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Phytopathogenic micromycetes play an important role in the biodiversity of the agroecosystem, affecting plant health status and productivity. This study aimed to create a regional culture collection of phytopathogenic micromycetes isolated from symptomatic plants in fifteen agricultural areas in North Macedonia, as well as to determine their taxonomic diversity through a structured Proto-Atlas. A total of 50 phytopathogenic fungal isolates were obtained from 19 host plant species, including some economically valuable species such as grapevine, apple, maize, wheat, tomato, and cannabis. The results showed that there were 19 fungal genera, with *Aspergillus*, *Penicillium*, *Fusarium*, and *Botrytis* being the most common species. The phytopathogenic micromycetes showed a moderate to high level of heterogeneity (Shannon H' = 1.50; Simpson 1-D = 0.76). The dominance of *Aspergillus* species may be due to their ecological adaptation to varying environmental conditions. The culture collection created in this study can be a valuable tool for monitoring phytopathogenic micromycetes as well as for screening bacterial strains that possess antifungal properties.

Key words: phytopathogens; micromycetes; biodiversity; agroecosystems; biocontrol

Comparative analysis of diatom diversity in bogs/fens across Mountain Shar Planina

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Shar Planina is a mountain range located in the northwestern part of North Macedonia. It is characterized by complex geology and geomorphology and has an abundance of different types of wetlands that support a high diversity of diatom flora. The study concerns diversity of the diatom communities on Mt. Shar Planina, based on 27 samples from five main localities. Sampling was conducted in July 2026, during the summer field trip of the Biology Students' Research Society (BSRS). Samples were fixed with 4% formaldehyde, and microscope slides were prepared using acid digestion, then mounted with Naphrax. After processing, samples were stored in the Diatom Collection at the Institute of Biology (within Faculty of Natural Sciences and Mathematics in Skopje). Taxa identification and photomicrographs were performed on a Nikon Eclipse Ni, using Nomarski DIC optics, with images captured by a Canon EOS D60 camera. The photos were edited in Adobe Photoshop CC 2015 to display the valves at 1500× magnification. Study sites had different altitudes, geological substrate, size, exposure and inclination. Values for pH and conductivity ranged between 6.05 – 9.16 and 344 – 207 $\mu\text{S}\cdot\text{cm}^{-1}$, respectively. More than 200 species were recorded, with the most dominant genera being *Cymbella* sensu lato, *Eunotia* Ehrenberg, *Gomphonema* Ehrenberg, *Navicula* Bory and *Pinnularia* Ehrenberg. A large number of the species are rare; both for Shar Planina and on a national level, with some being potentially undiscovered. Taxa unidentified or potentially new to science will be further investigated using scanning electron microscopy (SEM) and molecular analyses for detailed morphological and molecular characterization. The examined bogs/fens have streams and springs as their water source and because these watercourses intersect and merge with each other, boundaries are not clearly defined. Furthermore, the size of the wetlands changes, depending on precipitation/evaporation and season. This creates various microhabitats that reflect environmental variation within the peatland and host diverse diatom communities. Hence, diversity analyses and comparisons are particularly valuable, especially amidst climate change.

Key words: Bacillariophyta, high-altitude, morphology, taxonomy, wetlands

***Heliotropium amplexicaule*: A new allochthonous species for the Croatian flora**

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The Croatian flora currently includes two recognised species of the genus *Heliotropium* L. (Boraginaceae): *H. europaeum* L. and *H. supinum* L. However, two records of a third species, *H. amplexicaule* Vahl, have been uploaded to iNaturalist in the past four years. The species differs from the two native representatives by its perennial habit, woody base, sessile stigma and, most notably, a violet corolla. Native to temperate regions of central South America, this species has been introduced as an ornamental plant to several Mediterranean countries, as well as to India, South Africa, North America and Australia. To verify *H. amplexicaule* records on iNaturalist, we conducted a field survey at one of the locations indicated on the platform. A single individual was found in the city of Dubrovnik, where it was growing in a ruderal habitat alongside a stone stairway. The plant formed a shrub with laterally spreading decumbent branches. As there was no indication of deliberate planting, this finding represents the first confirmed record of *H. amplexicaule* in Croatia. While *H. amplexicaule* is regarded as a naturalised alien species in parts of the Mediterranean, including Italy, the plant has proven to be invasive in other regions – most notably Australia. Its invasive potential is attributed to its drought tolerance, broad ecological amplitude, and the production of seeds capable of remaining dormant in the soil seed bank for several years. These characteristics facilitate its establishment and spread, enabling it to compete with and potentially displace native plant species. Consequently, the occurrence of *H. amplexicaule* in Croatia should be monitored to assess its future establishment and potential invasiveness.

Key words: Adriatic, Balkan, Boraginaceae, introduced

Metabolic adaptations of *Anacamptis morio*-associated microbiota in the Kozhuf Mountain ecosystem

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Mountainous ecosystems, such as the Kozhuf region, represent critical, high-diversity reservoirs for microbial life characterized by unique climatic conditions and lower anthropogenic impact. These environments create a distinct ecological niche for the orchid *Anacamptis morio* and its symbiotic partners. The functional traits of the microbiota associated with *A. morio* in these high-altitude habitats remain largely under-explored, yet they are essential for understanding how the species maintains its ecological fitness in nutrient-limited, thermally variable mountain soils. The objective of this research was to characterize the functional diversity of 38 microbial isolates derived from the rhizosphere of *A. morio* in the Kozhuf region. This study established a collection of 38 unique isolates through serial dilution and media-based isolation, allowing for an in-depth assessment of their metabolic phenotype. The screening focused on enzymatic activities deemed critical for survival in mountainous substrates, specifically proteolytic hydrolysis, ammonification capacity, cellulolytic activity and nitrogen fixation, which are key indicators of microbial nutrient mobilization strategies. The data reveals that the *A. morio*-associated microbiota in the Kozhuf region exhibit distinct functional characteristics. Of the 38 isolates analyzed, 38 (100%) demonstrated positive results for ammonification, 38 (100%) showed cellulolytic activity, and 37 (97%) were positive for nitrogen-fixing activity. Additionally, 32 isolates (84%) exhibited positive proteinase activity, reflecting an adaptation to the high organic matter turnover typical of mountain soils. These robust metabolic capabilities indicate that these symbionts are finely tuned to provide necessary nitrogenous compounds to the host orchid, confirming their adaptation to the nutrient-limited conditions of the Kozhuf ecosystem. This research contributes to our growing understanding of plant-microbe interactions, emphasizing the necessity of preserving functional microbial reservoirs to ensure the long-term conservation of native mountain orchids. Future studies will focus on identifying the specific fungal strains involved in these processes, further elucidating the complex symbiotic network that sustains *Anacamptis morio* in its natural habitat.

Key words: *Anacamptis morio*, Microbial Symbiosis, Functional Profiling, Mountain Ecosystems, Orchid Conservation, Enzymatic Activity

Impact of 400kV electrical transmission lines on the dimensions and distribution of earthworms

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Earthworms have an important ecological role in ecosystems through their effects on soil properties. Moreover, they are quite sensitive to various conditions/elements of their environment, including low frequency electromagnetic fields. One of the most prominent anthropogenic sources of electromagnetic fields are electrical transmission lines. Apart from having a negative impact on a number of species, electrical transmission lines have also been recognized to have a certain effect on earthworms. Therefore, this study aimed to assess the effect of electrical transmission lines on the distribution and morphological characteristics of earthworm populations. To measure how big an effect these structures have on earthworms, eight transects perpendicular to transmission lines, were set up on three points in the basin of Pelagonia, south-western Macedonia. Within each transect, soil samples were processed at a distance of 0, 25, 50, 100 and 200 m from the transmission line. The area of each sample was 0.5 m² and the soil was removed in 10 cm layers (up to 50 cm depth), after which the earthworms were separated by hand-sorting and then preserved according to the methodology of Shapkarev, 1978. Soil samples were further analyzed to determine their chemical and structural-physical properties. A total of 103 adult specimens were collected, with body length ranging from 6 to 220 mm and clitellum width from 2 to 8 mm. Soil properties were generally similar among sampling sites, with no statistically significant differences. The soil samples were classified as loamy-clay or loamy-clay sand with a neutral pH and high conductivity. The percentage humus in the sample ranged from 1.45% to 4.74%. The number of recorded earthworm specimens and earthworm body dimensions showed a moderate positive trend with increasing distance from the transmission lines. Soil humus generally increased with distance from the transmission lines, while soil density decreased with no apparent difference in other soil properties. Despite the noted moderately positive inversely proportional trend between earthworms' distribution, their morphological characteristics and the distance from the power line, additional research is needed. Although this study does contribute to the understanding of the potential impact of the electrical transmission lines on earthworms, additional research is still needed.

Key words: electromagnetic field, morphometry, bioindicators, soil properties, Lumbricidae

Insect diversity on Jablanica Mountain (North Macedonia)

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Woodland invertebrates have important roles in forest habitats; they are pollinators and decomposers, control pests, and provide food for larger animals. Their absence causes a collapse of their respective food webs, leading to a decline in ecosystem health. Jablanica Mountain is characterized by naturally heterogeneous ecosystems and structured habitats, which host a wide array of species of international conservation concern. The primary objective of this biodiversity assessment was to determine the diversity of insect species inhabiting Mountain Jablanica's ecosystems. The studied areas consist of woodlands rich in century-old trees, providing suitable habitat for diverse forest arthropod communities. The research was conducted in 2020 in the mountain's mixed deciduous forest habitats, specifically three localities characterized by thermophilous oak, montane beech, and sweet chestnut forests. In each locality, traps were placed either on, within, or in proximity to old-growth trees or fallen logs, including five pitfall traps, five air traps, as well as two intercept traps. Once per month over the span of five months, specimens were collected, identified, and higher taxon diversity was documented. The recorded diversity of different taxonomic groups emphasizes the existence of rich, structurally diverse habitats inhabited by a variety of insects living intertwined. The specimens were categorized into ten orders, out of which the most abundant was Coleoptera, followed by Lepidoptera, Hymenoptera, Orthoptera, and Blattodea. Less frequently collected were species from Dermaptera, Hemiptera, Mecoptera, Diptera, and Microcoryphia (Archaeognatha). The oak forest locality was singled out as the one with highest taxonomic richness, with all ten orders of insects represented. Highest specimen abundance was registered in the chestnut forest locality, whereas the beech forest locality had the lowest abundance and taxa richness. The beetles (Coleoptera) were the dominant insect order in each of the localities. These preliminary results highlight the importance and high conservation value of the forests of Jablanica Mountain, and support future efforts for the mountain's conservation, especially considering the current state of rising anthropogenic pressures and habitat fragmentation. Assessing arthropod diversity and monitoring their biodiversity patterns in forested habitats is crucial for the preservation of these essential ecosystems.

Key words: Insecta, forest entomology, ecological indicators, Jablanica Mountain

Inventory of pollinators (Anthophila – Hymenoptera; Syrphidae - Diptera) along a gradient of anthropogenic influence in the Slunjčica river - significant landscape in Croatia

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Pollination services, provided predominantly by insects, are essential for the functioning of terrestrial ecosystems. While global declines in pollinator populations and species richness are well established, there are very few studies that investigate the impact of land use changes, including urbanisation and agriculture along rivers and streams. Hydrological network can be important for pollinators as it provides relatively constant, often flower-rich areas near the riverbank. It might even have a role of movement corridors for these insects, but research data remains inconclusive. Because of important differences in their life histories, bees and hoverflies often show different responses to anthropogenic disturbances and that also depends on the type and level of disturbance. The objective of this research was to explore the diversity of pollinator assemblages (focusing on Anthophila and Syrphidae) along the significant landscape of the Slunjčica river, located in the Kordun region of central Croatia, and to evaluate anthropogenic influence on these taxa in the protected area. The investigated walking path (along the 6 km course of the Slunjčica, from the karstic spring region leading to the city of Slunj, where it flows into the Korana river) follows a natural-to-urban gradient. In May 2025, we conducted field sampling by netting and setting up pan traps across three pre-defined transects: natural, agricultural and urban. Each transect had three sampling locations, situated approximately 200-500 m apart. At each location, three triplets of pan traps were set up and we conducted a 15-minute transect survey using entomological nets. The collected pollinator specimens were pinned and identified to the lowest possible taxonomic level (species in most cases). We recorded 19 bee species (8 genera) and 9 hoverfly species (9 genera). The results showed that the pollinator assemblages were most abundant and had the highest species richness of both groups in natural areas. There is a 76.19% drop in bee abundance from natural to both agricultural and urban areas, while species richness of bees dropped by 62.50% and 56.25% from natural to agricultural and urban areas respectively. Because of a relatively low number of caught hoverflies, we cannot make a meaningful conclusion. However, species *Episyrphus balteatus* dominated in an agricultural habitat, making this the most abundant transect concerning hoverflies. These results are aligned with published literature on the anthropogenic effects on Hymenoptera and Diptera in temperate regions. This research is the first standardized pollinator survey conducted in this protected area of the Slunjčica valley and presents a valuable baseline data for further research and conservation management.

Key words: bees, hoverflies, riparian habitat, land use gradient, biodiversity

Invertebrate diversity along an urban-rural gradient in Skopje with an emphasis on the order Coleoptera

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Ecosystem health is reflected in species richness, abundance and community composition. Urbanisation transforms natural and semi-natural landscapes through fragmentation and habitat loss, reducing biodiversity, habitat quality and ecosystem functioning. However, generalist and highly adaptable groups such as beetles, often successfully exist within highly modified urban environments. This study aimed to assess differences in invertebrate diversity and community composition along an urban-rural gradient in Skopje, with particular emphasis on the diversity and abundance of Coleoptera. Samples were collected during spring 2026 at three localities in Skopje, representing different levels of urbanization (rural - Dolno Lisiche, suburban - Novo Lisiche, urban-Skopje Fortress). At each locality, 10 pitfall and 5 air-intercept traps were used. After sorting and identification, individuals were counted and biodiversity indices were calculated, including Shannon-Wiener diversity index, Margalef richness index, Pielou's evenness index, dominance and similarity index. A total of 4025 invertebrates representing 24 orders were recorded along the urban-rural gradient in Skopje. The suburban locality had the highest abundance (n=2111), compared with 957 individuals in both urban and rural localities. Hymenoptera was the dominant order in the urban and suburban localities, whereas Diptera was dominant in the rural locality. Across the three sites, 243 Coleoptera individuals representing 14 families and 40 taxa identified to species level were recorded, with the highest abundance recorded in the rural and suburban localities. The urban locality was characterised by a high abundance of Histeridae, particularly *Acritus minutus* (n = 15), whereas Chrysomelidae and Curculionidae were particularly abundant in the suburban locality, including *Podagrica fuscicornis* (n = 11) and an unidentified Curculionidae taxon (n = 15). In the rural locality, Staphylinidae was particularly abundant, mainly due to *Staphylinus caesareus* (n = 30). The highest number of species was recorded in the suburban locality, followed by the urban and rural localities, with several species occurring exclusively at individual localities and others shared between two or more sites. Shannon-Weiner diversity index ($H' = 1.98$) and Margalef richness index ($d = 2.19$) were highest in the rural locality, while the index of dominance was highest in the suburban locality ($DI = 0.487$). For Coleoptera, in contrast, both diversity and species richness were highest in the suburban locality and lowest in the rural locality. The values for homogeneity (Je) were relatively similar among localities, while community similarity was highest between the urban and suburban localities (66.67). Overall, the results demonstrate differences in invertebrate community structure along the urban-rural gradient. Coleoptera displayed a distinct pattern, with the highest diversity and species richness in the suburban locality, suggesting that moderately urbanised habitats may support a particularly diverse beetle community.

Key words: urbanisation, community structure, beetles

Population baseline and methodological challenges in Capture-Mark-Recapture (CMR) study for three newt species (*Ichthyosaura alpestris*, *Lissotriton graecus*, and *Triturus macedonicus*) on Mounts Galičica and Jablanica in North Macedonia

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Understanding population characteristics of European newts is critical as they and their habitats are often of very high conservation importance, yet fine-scale baseline demographic data remain scarce in North Macedonia. This study aimed to evaluate the population size and dynamics of the Alpine newt (*Ichthyosaura alpestris*), Greek smooth newt (*Lissotriton graecus*), and Macedonian crested newt (*Triturus macedonicus*) across selected ponds on Mount Galičica (Asan Gjura, Simoncheska, Crvena Gjafa, Gjafa, and Mala Gjafa) and Mount Jablanica (Labunishko Ezero, Labunishka Lokva, and Podgorechki Lokvi). Capture-Mark-Recapture (CMR) surveys were implemented in 2025, using the I3S Pattern+ software for photo-identification of individuals based on natural belly and lateral pattern variations. Unfortunately, methodological constraints and a lack of recaptures prevented any reliable population estimates. The alpine newt was only recorded on Jablanica. A total of 1,755 individuals were captured and photographed across four sessions. However, software-assisted photo-identification failed due to low contrast in lateral patterning, thus CMR datasets have not yet been built. Nevertheless, recording a single-session peak of 602 unique individuals confirms the absolute minimum population threshold that Mount Jablanica supports during the breeding period. Only 30 individuals of the Macedonian crested newt on Jablanica were captured across four sampling sessions with zero recaptures. This prevented modelling of CMR data and population estimation, and is likely a result of high post-breeding emigration, seasonal dispersion across abundant aquatic habitats, or naturally low density. For this species, the absolute minimum supported on Mount Jablanica is reflected by the maximum single-session capture of 12 unique individuals at Labunishko Ezero. On Galičica, we processed a total of 22 individuals of the Greek smooth newt. The species was recorded at Asan Gjura, Simoncheska, and for the first time at Gjafa Pond, expanding its known local distribution, while remaining undetected at Mala Gjafa. Zero recaptures were registered, likely influenced by low populations densities due to potential predation by *T. macedonicus*, or due to the early drying of key habitats like Asan Gjura (where most individuals were captured) and consequent transience. The baseline absolute minimum for Mount Galičica is set by the highest single-session count of 11 unique individuals. When CMR statistical modeling is impossible due to lack of recaptures or other constraints, the data can still prove useful. Namely, empirical field counts remain essential for conservation management. The highest number of unique individuals captured within a single session provides a reliable baseline, representing the absolute minimum population supported by the respective mountain habitat during the active season. These findings offer a vital foundation for future population monitoring and habitat protection plans, while future capture effort will most likely eventually yield recaptures and allow for reliable population estimates.

Key words: pattern variations; recaptures; amphibians

Baseline population size estimates for the yellow-bellied toad (*Bombina variegata*, Anura: Bombinatoridae) near Jankov Kamen on Jablanica Mountain

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The yellow-bellied toad (*Bombina variegata scabra*) is a widely distributed amphibian in North Macedonia, commonly found in small water bodies such as ephemeral puddles and stream pools. Nationally and globally the species is not listed as threatened, but its populations are decreasing, mainly due to habitat loss, climate change, as well as the development of small hydropower plants in the Western Balkans. During the field expedition of the Biology Students' Research Society in 2024, eight puddles and one stream were sampled along a line transect following a forest road towards the locality Jankov Kamen on Jablanica Mountain, where a man-made pond hosts a seemingly large toad population which was not sampled. In the first session (July 20), 66 individuals were captured and photographed in a Petri dish from the ventral side. In the second session (July 24), three of the puddles were dried out and 55 individuals were caught and photographed; the free software I3S Pattern+ was used to mark and compare the photos of the underside patterns, revealing that 21 individuals were newly captured, and 34 were recaptures (39% recapture rate). Based on body size (snout-vent length, SVL), the captured individuals ($n = 87$) were grouped into adults (50; $SVL > 40$ mm), subadults (29), and juveniles (8); 18 adults were sexed as male based on the presence of nuptial glands on the front legs, 12 were sexed as female, and 20 were not sexed successfully. Using the Lincoln-Petersen estimator, the population size of adults and subadults of both sexes was estimated to be 94 ± 18 individuals; the patterns on the bellies of juveniles are not fully developed and cannot be used for identification at recapture, so they were not included in the estimation. Based on our pilot study, we conclude that the beech forests around Jankov Kamen would be suitable for a long-term population assessment, which would expand the study area to include more localities where individuals could potentially migrate, allowing us to better understand the population or metapopulation dynamics of the yellow-bellied toads on the Macedonian side of Jablanica Mountain.

Key words: Amphibia; population ecology; two-source capture-mark-recapture

Population density of Red-backed shrikes (*Lanius collurio*) in the subalpine shrublands of Shar Planina Mountain

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The red-backed shrike (*Lanius collurio*) is a widespread, migratory species that breeds in temperate and Mediterranean climates. It relies on mosaic landscapes featuring scattered bushes, shrubs or low trees which provide hunting posts that overlook areas of short grass, heath or bare soil. The species can be found in low-land agricultural areas, as well as subalpine shrublands, which were the focus of our study. Through this research the first baseline population density framework for *L. collurio* on Shar Mt. was developed in two distinct mountain areas: above the village Vejce and beneath Ljuboten peak. During peak breeding season, in July 2026, eight 1 km line transects (four per site) were conducted above the treeline within *Juniperus* shrub communities. Each territory was registered as the perpendicular distance from the predetermined line to the first seen individual (male, female or juvenile), paying attention to avoid duplication. Field observations revealed unequal territory counts due to habitat heterogeneity between and along the transects. Habitats ranged from dense shrubs with small trees (*Rosa* sp.) which offered ideal hunting perches, to the dwarf shrub *Juniperus communis* subsp. *nana*, as well as transitions from dense shrubs to bare pastures or rocky scree. To account for the variance, Distance software was used to make an average landscape density estimate of 30 to 31 territories per km² (95% CI: 17.08-54.78 territories/km², %CV= 26.30%). These findings demonstrate that the successional *Juniperus* shrub habitats provide optimal breeding conditions for *L. collurio*.

Key words: habitat heterogeneity, Ljuboten peak, Vejce

Comparative analysis of the small mammal community on burnt and unburnt habitats on National Park Galičica Mountain in North Macedonia

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This study attempts to quantify the effect of wildfires on the population size and community structure of small mammals in National Park Galičica, comparing burnt and unburnt sites on Stara Galičica alongside an unburnt reference locality at Lako Signoj. Sites were selected based on equivalent altitude, exposition, and vegetation. Trapping was conducted using 60 Heslinga live-traps along 200 m transects, totaling 300 trap-nights over 5 consecutive nights per habitat on Stara Galičica and 180 trap-nights at Lako Signoj. Mark-recapture was performed via fur-clipping. A total of 10 individuals were captured across the primary sampling sites, with *Apodemus flavicollis* representing the dominant species ($n = 8$) used for population estimation. Simpson's diversity index was higher in the unburnt site ($D = 0.722$) than in the burnt site ($D = 0.625$) on Stara Galičica, while at Lako Signoj it was $D = 0.662$. Notably, the survey yielded three new species records for National Park Galičica (*Clethrionomys glareolus*, *Crocidura leucodon* and *Talpa caeca*). Using the Schnabel method, the estimated population size of *A. flavicollis* was 4.6 at the unburnt site and 2.7 at the burnt site on Stara Galičica, compared to 15.8 at the reference locality. This study represents the first pilot estimation of small mammal populations in North Macedonia evaluating habitat divergence driven by wildfires.

Key words: wildfires, population estimation, mark-recapture

Current status and monitoring of the Balkan chamois (*Rupicapra rupicapra balcanica*) in North Macedonia

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The Balkan chamois (*Rupicapra rupicapra balcanica*) is a characteristic ungulate of mountain ecosystems, primarily inhabiting rocky slopes, cliffs, and steep terrain, although some populations also occupy forested habitats and undertake seasonal movements. As an important prey species for large carnivores, including the wolf and the Balkan lynx, and a legally managed game species, the chamois represents a key component of mountain biodiversity in North Macedonia. Current populations are concentrated in Mavrovo National Park and the Jasen Multipurpose Protected Area, each supporting an estimated 800-1,000 individuals, while smaller populations occur in Shar Mountain and Galičica NPs. The species has recently been confirmed in Pelister NP, Nidže, and Kožuf mountains through indirect evidence, whereas no credible records have been obtained from Jablanica Mountain in recent years. Standardized monitoring based on autumn transect counts has been implemented across several protected areas. In Mavrovo NP, monitoring recorded an increase from 640 individuals (0.88 ind./km²) in 2018 to 749 individuals (1.02 ind./km²) in 2020, indicating a stable population with a positive trend. In Galičica NP, transboundary monitoring confirmed the continued persistence of a small population, with observations increasing from a single individual in 2023 to six individuals on the Macedonian side during the 2025 survey. The first systematic monitoring efforts have also been initiated in the Jasen Protected Multipurpose Area and Pelister NP, providing an important baseline for future population assessments. Future activities include repeated censuses in Jasen PA, Galičica and Pelister NPs, and targeted surveys in Shar Mountain NP and Jablanica. Regular monitoring is essential for improving population estimates and supporting evidence-based conservation and sustainable management of this emblematic mountain species.

Key words: population trends, occurrence, habitat use, monitoring methods, protected areas

Occurrence data and first baseline population estimates of *Dinaromys bogdanovi* in North Macedonia

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The Balkan snow vole (*Dinaromys bogdanovi*) is an endemic relict species restricted to the alpine screes of the Western Balkans, where it frequently co-occurs with the snow vole (*Chionomys nivalis*). While known historical records represented the core reference for our distributional assessment, baseline population data for the species were entirely lacking within North Macedonia. To gather additional distributional data, the Mammalogy Section of the Biology Students Research Society (BSRS) surveyed five localities across four western mountain ranges—Jablanica Mountain, Stogovo Mountain, Galičica Mountain, and Šar Mountain using Hestlinga live-traps during the 2025 and 2026 field seasons. Field sampling confirmed the presence of *D. bogdanovi* on Jablanica Mt., Stogovo Mt. and Šar Mt. (specifically in the central part at Vejce and the northern part at Ljuboten), whereas despite receiving the highest trapping effort over two seasons, the species was not detected on Galičica Mt. By integrating our results with historical records, we provide a more detailed mapping of the species' known distribution in North Macedonia. On Šar Planina, we obtained the first mark-recapture population estimates for the species in the country using the Schnabel estimator, recording an estimated population size of 10.5 individuals for *D. bogdanovi* compared to 50 individuals for co-occurring *C. nivalis* within a 400 m transect in their suitable habitat. At this same locality, habitat suitability data were also collected, though their quantitative analysis remains underway to further evaluate local spatial dynamics.

Key words: Balkan snow vole, mark-recapture, population dynamics

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