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Session 4: Young Researchers Session

DNA Barcoding of Terrestrial Isopods in Austria Integrating Museum Specimens



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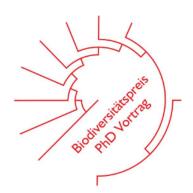
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Terrestrial isopods, a diverse group of land-dwelling crustaceans that have successfully colonized ecosystems worldwide, are key players in terrestrial ecological processes. They contribute significantly to ecosystem health by facilitating the decomposition of plant material through mechanical and chemical processes while enhancing microbial activity. In Austria, 64 terrestrial isopod species are recognized, although the precise count is challenging due to the unresolved taxonomy, marked by numerous synonyms and proposed subspecies over time. Difficulties in morphological identifications, primarily relying on male characteristics, underscore the need for supplementary molecular species identification methods such as DNA barcoding. This study utilized molecular techniques to generate DNA barcodes from 29 terrestrial isopod species, revealing intraspecific distances below 6% for most, with slight exceptions such as Armadillidium vulgare (6.5%) and Cylisticus convexus (6.3%). Notably, certain species exhibited unexpectedly high intraspecific distances, such as Porcellium collicola (13.9%), Trachelipus ratzeburgii (10.4%), and Tracheoniscus pusillus (11.7%), suggesting potential cryptic diversity. Species delimitation analyses further supported this, dividing the dataset into at least 34 putative species. Additionally, this research explored the utility of historical isopod specimens dating from 1880 to 1968, all identified by the former taxonomic expert Hans Strouhal. Despite challenges related to limited DNA content and fragmentation, 17 COI barcode sequences were successfully obtained from the historical collection of the NHM Vienna through two different approaches: mini-barcode amplification, involving short COI fragments via Sanger sequencing, and Next-Generation Sequencing (NGS). These DNA-barcode sequences, derived from historical museum specimens, are pivotal in filling taxonomic gaps in the representation of rare species, enriching our understanding of their genetic diversity. Additionally, they enable the incorporation of historic taxonomic knowledge. These sequences hold immense value for initiatives like ABOL (Austrian Barcode of Life) and biodiversity studies.

Life below the City - Groundwater fauna and impacts in Vienna



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Groundwater is one of our most important resources, making up 100 % of the drinking water in Austria as well as being heavily utilized in agriculture, industry and as a source of renewable, geothermal energy. However, groundwater is also home to a variety of organisms, microbes and fauna, that have adapted to a dark, cold, and typically energy-poor environment for thousands of years. Therefore, groundwater is a habitat with very high numbers of endemic and cryptic species as well as hidden biodiversity hotspots. Like in surface waters, these highly specialised animals are assumed to provide vital ecosystem services including water purification, are however being very susceptible to short-term (years to decades) changes in environmental conditions. In urban areas, several factors like increased temperatures, surface sealing or pollution are impacting the groundwater ecosystem with some facing a loss of biodiversity and deterioration of water quality. Focusing on groundwater warming as a main driver, over 150 groundwater wells were sampled in the City of Vienna in Autumn 2021 as well as in Spring 2022 to include seasonal variability. A large set of biotic and abiotic parameters was recorded to reveal driving factors of spatial biodiversity patterns, community composition and links between faunal richness and water quality. Our results show that the mean groundwater temperature of 14°C in Vienna is about 2°C above the natural background, with anthropogenic heat sources having a main impact on the degree of warming and groundwater fauna community composition. The absence of dissolved oxygen (DO) and NO₃₋ as well as the presence of dissolved Fe²⁺, HS⁻ and CH₄ hint at zones with reduced groundwater causing low faunal biodiversity. In progress, the application and comparison of several groundwater ecosystem health indices will contribute to the development of an integrative groundwater management strategy, combining groundwater quality aspects, its sustainable use, and conservation strategies for groundwater biodiversity in the future.

Slightly sliding communities: how plants and insects respond to landslides



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The project movement – "The moving mountain" - aims to investigate mass movements in the Austrian Alps. These natural events, often triggered by factors like heavy rainfall or droughts have the power to reshape entire landscapes. While they are known for their destructive potential to both people and infrastructure, they also provide unique geosystem services that have received limited attention so far. For example, mass movements, despite their destructive nature, likely contribute to increased landscape heterogeneity and long-term biodiversity enhancement. Our study aims to reveal the influence of these mass movements on plant- and arthropod communities and their corresponding ecosystem functions such as herbivory and predation. For this purpose, we investigated nine landslides in the UNESCO global geopark Ore of the Alps (Salzburg) and three landslides in the UNESCO global geopark Karawanken (Kärnten). Each slide was sampled in one to three plots with an additional control plot outside the landslide area. Within these plots we conducted assessments of plant and insect communities, as well as of ecosystem functions such as herbivory, predation pressure and primary productivity, using methods like pitfall traps, netting and artificial caterpillars. So far preliminary results show no significant differences in predation pressure between landslides and stable sites. While data evaluation is ongoing, our study aims to shed light on the ecological consequences of these dynamic events.

Species richness and extinction debt in dung beetles of grazed grasslands: consequences of changes in land use and agricultural practices

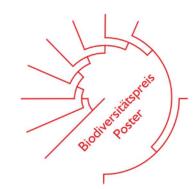


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Over the last century, land use in Central Europe underwent major changes, following intensification and structural alterations in agriculture. Within this study the influence of changing livestock farming practices and land use on dung beetles was modelled. 34 pastures within the Pannonian Region (Austria and Czech Republic) were sampled for dung beetles, information on management practices gathered and land use changes analysed by digitizing maps and aerial photograph since 1900. This revealed a threefold increase in settlement areas, a 50% increase in forest cover, and a 50% reduction in pasture areas. Hereby, historic land cover data explained current species richness the best, indicating long-lasting impacts of land use change spanning at least 150 years. Extinction debt driven by land use changes may therefore have more extensive relaxation times than assumed, even for mobile, short-lived groups as dung beetles. The models also showed varying responses of different ecological groups to land use changes, with open land- and endocoprid species being more negatively impacted than forest- and paracoprid species. Among livestock farming practices, the use of veterinary medicines emerged as the most significant predictor, having strong negative effects on overall species diversity, whereby paracoprid species appear to be more resistant against these medical substances than endocoprid species. These findings offer crucial insights for dung beetle conservation efforts. Extinction debt and long-lasting effects of land use change underline the importance of protecting and promoting extensive grazing systems in the study area. Additionally, the widespread use of veterinary medicines should be diminished.

Integrative taxonomy and DNA barcoding of Austrian turbellarians



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Freshwater biodiversity is declining at an even faster rate than biodiversity in the terrestrial and marine realms, and closing knowledge gaps on neglected taxa is seen as one of the priorities in freshwater biodiversity research (Maasri et al. 2022, WWF 2022). The study presented contributed to closing such a knowledge gap by generating DNA barcodes of turbellarians (Platyhelminthes excluding the parasitic Neodermata), a poorly studied group among freshwater invertebrates (Schockaert et al. 2008). To this end, partial sequences of the nuclear 18S and 28S rRNA genes of freshwater turbellarian specimens collected in eastern Austria were generated. Specimens were investigated alive using stereo and compound microscopes, and morphological features were photographed or drawn to allow for subsequent determination. Some specimens were then fixed in 96% ethanol for DNA analysis, and some in Bouin's fluid for histological serial sections and genital reconstructions. Amplification of both marker genes was predominantly successful, and a single primer pair per marker gene was applicable across all the high-level taxa of turbellarians collected (Catenulida, Macrostomorpha, Prorhynchida, Rhabdocoela, Tricladida, and Bothrioplanida). The resulting sequences were analyzed phylogenetically and compared with published sequences from the GenBank database. If the morphology-based identification of the species was ambiguous, it was retained at the lowest level that could be determined with certainty (e.g., the genus). Fourtytwo low-level taxa (most determined to genus or species level) were recorded. The comparison with reference sequences often confirmed the prior identifications, but reference sequences were not always available. The new records of turbellarian taxa and their DNA barcodes improve the state of knowledge of this essential component of freshwater ecosystems.

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Gluing for the future – Characterisation of the gland system of *Latia neritoides* (Mollusca; Gastropoda; Hygrophila)



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Bioadhesives derived from various organisms, including bacteria, plants, and animals, have evolved over millions of years to meet specific needs. One particularly promising biomaterial is snail mucus, which has potential benefits for human skin regeneration. The limpet-like New Zealand pulmonate Latia neritoides produces luminescent and adhesive mucus when threatened, a unique trait among freshwater gastropods. While the Latia luciferin-luciferase system is fundamentally understood, questions persist regarding the source and the composition of the adhesive mucus, and the location of the luminescent glands. There are two hypotheses concerning where the luminescent mucus is produced and released: the lateral foot region and/or the mantle cavity. Histochemical and morphological investigations in both areas revealed two distinct types of glandular cells in the lateral foot region. However, video footage indicates that the luminescent component is primarily released from within the mantle cavity. We used µ-CT and HREM stacks to examine the entire animal for mucus reservoirs and alternative glandular cell structures, which could be involved in the defence mucus system. Comparing protein profiles obtained from electrophoretic separation of the defensive and trail mucus reveals significant disparities in total protein concentration, the number and the physicochemical characteristics of proteins. We detected unknown proteins that seem to be unique to the glowing mucus. Increasing our knowledge on these unique proteins in the defence mucus not only helps us understand Latia neritoides' luminescent mucus system, but also aid in developing novel aqueous medical adhesives for use in moist environments, such as for hemostasis and tissue sealants.

Zielartenerhebung in der Quartiersplanung: Bauwerksbegrünung als Baustein zur Habitatvernetzung und Förderung von Biodiversität im Kontext eines urbanen Planungsprozesses



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Wie in der neuen Biodiversitätsstrategie Österreich 2030+ gefordert, soll das "Mainstreamen" von Biodiversität quer durch alle Sektoren erfolgen. Biodiversitätsfördernde Maßnahmen und Techniken für das Stadtgebiet sind gut bekannt, werden aber noch zurückhaltend umgesetzt. Im Bereich der Bauwerksbegrünung und Grünflächengestaltung gibt es in Österreich in einer Vorreiterrolle ein breites Regelwerk – die ÖNORMEN zur Fassaden- und Dachbegrünung werden zudem in Fachausschüssen regelmäßig auf ihre Aktualität überprüft. Die positiven Wirkungen von biodiversitätsfördernden Begrünungen sind erforscht und werden zunehmend mit neu entwickelten tools gemessen. Dennoch wird der Grünraumgestaltung im Planungsprozess eines urbanen Quartiers meist eine untergeordnete Rolle zugeteilt. Folgende Fragen leiten die Arbeit: Wie werden in einem stadtplanerischen Planungsprozess Kenntnisse der Biodiversitätsforschung umgesetzt? Welche Akteur:innen sind im Planungsprozess involviert und wie gelangt ein interdisziplinäres Planungsteam zu dem notwendigen Wissen und Übereinkunft in der Gestaltung? Welche Möglichkeiten zur Umsetzung biodiversitätsfördernder Maßnahmen gibt es und wo liegen die Hindernisse in der Umsetzung, und wie kann die Umsetzung weiter gefördert werden? Die Datenerhebung erfolgte mittels Literaturrecherche, aktiver Teilnahme in einem Forschungs-Planungsprozess, Interviews mit lokalen Expert:innen, eigener Bestandsaufnahme der Umgebung im Zielgebiet. Ergebnisse: Der Wissensstand rund um biodiversitätsfördernde Maßnahmen in der Freiraumgestaltung und Quartiersplanung ist weit fortgeschritten und wird auch durch Forschung, Verwaltung und anderen Initiativen leicht zugänglich gemacht. Die Außenraumgestaltung wird in verschiedene Expertisen dividiert. Es fehlt eine koordinierende Biodiversitätsplanungsstelle und die Verpflichtung in der Umsetzung, damit sich urbane Lebensräume und Artenschutz ergänzen können.

Combining genetics, landscape ecology and simulations to inform conservation of two alpine grouse species



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Within the current era of rapid biodiversity loss, the long-term preservation of wildlife populations and their genetic integrity stands as a main goal of conservation and sustainable development, as noted by the Convention on Biological Diversity and the United Nations Sustainable Development Goals. Thereby, indicator species are especially suited, as these species are representative for the diversity of whole ecosystems. Eastern Alpine Black Grouse (Lyrurus tetrix) and Black Forest Western Capercaillie (Tetrao urogallus) are two such species. While Eastern Alpine Black Grouse are threatened with range contradiction and already suffered from local extinctions, Black Forest Capercaillie have experienced a dramatic decline from about 8000 individuals to less than 200 left in the past decades. Both metapopulation systems therefore call for an effective design of conservation strategies. Hence, within my doctoral studies I conducted analyses of genetic diversity and population structure, paired with landscape ecological modelling and statistical simulations, to investigate burning questions of conservation genetics and derive applicable management actions. I found slight effects of isolation between subpopulations for Black Grouse and pronounced effects between subpopulations for Capercaillie. Initiated by these results, I studied whether the observed genetic structure for Black Grouse is in some way affected by the underlying landscape and found spatial genetic variation to be partially driven by the landscape's resistance. As such analyses are however snapshots in time, I further looked into genetic differentiation over time. I therefore build simulations projecting genetic differentiation driven by migration rates from past to present to future, and applied realistic yet hypothetical scenarios. By making use of newly developed approaches combined with well-established methods, I was able to disentangle landscape ecological drivers of the spatial genetic variation. The simulation built for Capercaillie can be readily applied to test any further scenario, including the effectiveness of conservation strategies. The studies within my thesis have all been mission-driven and were directly informed by practitioners' needs. As such, some results were already implemented into state-wise landscape planning. Ultimately, combating the biodiversity crisis will only be successful through a collaboration of science and practice.

Understanding Wildlife Trade: Species Determination Using DNA Barcoding in Spiders from Cambodian Wet Markets and Surrounding Areas



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The history of spiders goes back millions of years, resulting in extreme species diversity. Spiders have a large and diverse impact on their environment, which makes the protection of these animals very crucial. In my master thesis I analyzed tarantulas from Cambodia, belonging presumably to the genus Cyriopagopus, which are threatened with extinction due to heavy consumption for food, medicinal purposes, and loss of habitat structure. Still, it is unknown which species these spiders belong to, but two are suspected: Cyriopagopus albostriatus (Simon, 1886) and Cyriopagopus longipes (Von Wirth & Striffler, 2005). In my project I extended the classical DNA barcoding concept and analyzed ten DNA marker sequences with the aim to identify the number of species involved. Therefore, this project takes the first step towards protecting these spiders. Samples of 50 tarantulas were analyzed, collected in Cambodia from various wildlife markets, private homes, farmland, and forests, some of them in a fried state. Nanopore sequencing with Oxford Nanopore Technologies (ONTs) MinION was used for sequencing to demonstrate its DNA barcoding capability in tarantulas and allowing the procedure to be repeated directly in the field with minimal laboratory equipment. The results showed that this technology produces reliable data for spiders. Furthermore, DNA extraction and analysis was possible even for fried individuals. However, unambiguous species identification was not possible due to the lack of reference sequences in public data bases. Our results demonstrated, however, that at least two species are involved, one of them belonging to the genus Cyriopagopus.

Re-establishing grasslands in the agricultural landscape to promote insect diversity – How much do insects benefit from grassland strips in fields? (Project REGRASS 2)



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Land use intensification is resulting in the loss of semi-natural grasslands and their associated flora and fauna in Europe. Agri-environmental schemes seek to mitigate the trend of biodiversity loss by subsidizing the restoration of grassland. However, little is known about the colonization of newly created ecological compensation areas by insects, especially over periods of more than 1-2 years. We investigated the development of two types of newly established grassland strips over six years in an agricultural landscape in Austria: newly established grasslands (NG) were created within crop fields with a diverse, regionally adapted seed mixture in 2016 and compared to subsidized grasslands (SG; Ecological Focus Areas EFAs). Permanent semi-natural grasslands (old grassland OG) were used as reference habitat. We wanted to find out how species numbers and assemblages of wildbees, syrphids, butterflies, orthopterans and heteropteran bugs develop in the different grassland types over six years and how ecological traits affect the colonization patterns. Over the six-year period, newly established grasslands with diverse plant communities showed sustained insect diversity and high conservation value, outperforming plant species-poor SGs in promoting insect species. Therefore, we suggest that diverse seed mixtures could enhance the effectiveness of EFAs and could make re-sowing within the first six years after establishment unneccessary. Further, we found no evidence of a successional change in insect species assemblages in grassland strips, however wildbee, butterfly and orthopteran species composition differences between grassland strips and permanent grasslands were decreasing over time. Both grassland strips showed higher fluctuations in individual numbers and species ranks compared to permanent grasslands.

Subterranean Voyagers: Investigating Genetic Population Structure and Underground Migration of *Phoxinus lumaireul* (Schinz, 1840)



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The Dinaric karst on the Balkan peninsula harbors a remarkable variety of European biodiversity. Karst landscapes, known for intricate underground water systems interconnected with surface water, are prevalent in this region. A biologically-oriented approach to study these underground connections involves analyzing the genetic relationships among aquatic organisms residing in the investigated karst system. In this context, examining genetic population structure not only reveals aquatic relationships but also sheds light on the ecology of the studied organism. In our preliminary study, we sampled populations of the minnow species *Phoxinus lumaireul* in the Slovenian Dinaric karst. Genetic markers (COI, cytb, RPS7) indicated a natural population structure influenced by historical and current gene flow, not human translocations. Our ongoing research employs genome-wide ddRAD-seq data to compare populations connected through underground pathways. We aim to determine if minnows use underground water connections or if karst acts as a gene flow barrier. Additionally, we assess how paleohydrology affected present genetic structure. The rapid hydrological changes in the Dinaric karst impact species distribution and adaptation. Our findings shed light on the divergence of different genetic lineages of *P. lumaireul* in the Dinaric karst, providing valuable insights into the complex dynamics of biodiversity in this unique region. Understanding genetics and historical adaptations emphasizes karst landscape preservation, crucial due to susceptibility to human impact, highlighting the need for protective measures.

Comparing conventional and DNA-based methods for monitoring arthropod diversity in agricultural landscapes and Alpine meadows in Tyrol



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Recent shifts in land-use and effects of climate change lead to rapid declines of arthropod biodiversity in agricultural and Alpine systems. Tracking these changes with efficient and scalable methods, therefore, becomes increasingly important for monitoring the status of terrestrial ecosystems and the effects of conservation measures. Novel approaches based on DNA barcoding of arthropods caught via Malaise traps and sweep nets and the detection of species via environmental DNA deposited on plant surfaces show great potential for rapid and accurate biodiversity assessments. However, the applicability and implementation of these DNA-based monitoring approaches still needs to be validated for diverse habitats in Austria. In this study, we investigate the differences between DNA-based and conventional arthropod monitoring methods, for an agricultural landscape (720 m.a.s.l.) and an Alpine meadow (1980 m.a.s.l.) in Tyrol. Arthropods were collected using Malaise traps and net sweeping in two-week and four-week intervals, respectively, from May to September 2023. After morphological identification, a subset of specimens was metabarcoded using an Oxford Nanopore sequencing platform. Additionally, eDNA samples were obtained monthly by washing picked bundles of the vegetation and subsequent filtration. These samples will be processed via a metabarcoding approach, too, enabling the direct comparison between morphological identification, DNA barcoding, and eDNA-based data. Initial results show a dominance of Diptera in all catches and distinct seasonal trends across both locations for specific families/morphotypes exemplified by a decrease in Apocrita detections over the course of the sampling season. Our approach allows the comparative investigation of arthropod communities in Tyrol based on three powerful approaches and lays the foundation for further studies of arthropod diversity. Furthermore, this study is a first step towards standardised, rapid, and non-invasive methods for DNA-based monitoring.

Enhancing Environmental Assessment: Leveraging Advanced Technologies to Address Biodiversity Data Limitations



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Environmental Assessment (EA) as a tool of environmental planning aims to support sustainable development and preservation of biodiversity by integrating environmental considerations into decision-making on projects, plans, programs, and policies and taking environmental impacts into account (Geißler 2013). Limiting factors for the quality and effectivness of EA are data availabilty and data collection (González del Campo 2012, Cillier et al. 2022). In EA the availability and quality of baseline data are critical to be used in scoping, zero variant, and monitoring, and consequently, the availability of data for future impact assessment and gradation across planning levels (Gachechiladze-Bozhesku & Fischer 2012, Dias et al. 2022). In particular, the consideration of flora, fauna, and biodiversity in EA present some challenges (Byron et al. 2000, Mandelik et al. 2005, Cares et al. 2023). Herewith, the application of artificial intelligence (AI) combined with affordable hardware and advancing technologies in biological science might improve predictions of ecosystem dynamics by providing access to affordable long-term, high-resolution and large-scale data, while offering great potential for efficient monitoring of global biodiversity (Tabak et al. 2019, Schneider et al. 2021, Lahoz-Monfort & Magrath 2021, Jetz et al. 2022). This development raises the question of how these emerging technologies will impact EA practice. Particularly as knowledge of the uptake of advanced digital solutions within EA practice is largely unexplored (Fonseca 2022). By examining the latest technological advancements and digital tools in related fields, the study aims to identify emerging technologies relevant for EA procedural steps. Furthermore, it explores the uptake of these technologies in the field of EA on the basis of interviews and a survey among EA practitioners. The poster will outline both opportunities and challenges linked to enhanced application of advanced digital approaches such as AI supported data analysis and management. It will outline future demand for interdisciplinary research, particularly across conservation and environmental planning.